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Feasibility study of personalized peptide vaccination for hepatocellular carcinoma patients refractory to locoregional therapies

Shigeru Yutani,[1] Takahisa Shirahama,[2] Daisuke Muroya,[2] Satoko Matsueda,[1] Rin Yamaguchi,[3] Michi Morita,[3] Shigeki Shichijo,[1] Akira Yamada,[4] Tetsuro Sasada[5] and Kyogo Itoh[1]

[1]Cancer Vaccine Center, Kurume University; [2]Department of Surgery, Kurume University School of Medicine; [3]Division of Pathology, Medical Center of Kurume University; [4]Cancer Vaccine Division, Research Center for Innovative Cancer Therapy, Kurume University, Kurume; [5]Cancer Vaccine Center, Kanagawa Cancer Center, Yokohama, Japan

Key words

Cancer immunotherapy, hepatocellular carcinoma, immune response, overall survival, personalized peptide vaccine

Correspondence

Shigeru Yutani, Cancer Vaccine Center, Kurume University, 155-1 Kokubu-machi, Kurume, Fukuoka 839-0863, Japan.
Tel: +81-942-27-5210; Fax: +81-942-27-9305;
E-mail: yutani@med.kurume-u.ac.jp

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Overall survival of patients with hepatocellular carcinoma (HCC) refractory to locoregional therapy is dismal, even following treatment with sorafenib, a multikinase inhibitor. To develop a more efficacious treatment, we undertook a feasibility study of personalized peptide vaccination (PPV) for HCC, in which the peptides were selected from 31 peptide candidates based on the pre-existing immunity. Twenty-six HCC patients refractory to locoregional therapies (cohort 1) and 30 patients refractory to both locoregional and systemic therapies (cohort 2) were entered into the study. There were no severe adverse events related to PPV except for one injection site reaction. At the end of the first cycle of six vaccinations, successful CTL or IgG boosting was observed in 57% or 46% of patients in cohort 1 and in 54% or 52% of patients in cohort 2, respectively. Successful IgG boosting at the end of the second cycle was observed in the majority of patients tested. Median overall survival was 18.7 months (95% confidence interval, 12.2–22.5 months) in cohort 1, and 8.5 months (95% confidence interval, 5.9–12.2 months) in cohort 2. Based on the higher rates of immune boosting and the safety profile of PPV, further clinical studies of PPV would be warranted for patients with HCC refractory to not only locoregional therapy but also both locoregional and systemic therapies. The protocol of this study was registered with the UMIN Clinical Trials Registry (UMIN000001882 and UMIN000003590).

Hepatocellular carcinoma is the third leading cause of cancer-related death.[1] Although sorafenib, a multikinase inhibitor, has been approved for HCC, it has shown only modest efficacy, achieving a median survival time of 10.9 months.[2–4] Therefore, there is urgent need to develop a new treatment method.[5,6] Immunotherapy could be a candidate approach based on the promising results of early phase clinical studies.[7,8] As sorafenib has been reported to affect the function of dendritic cells and suppress the induction of primary immune responses,[9] careful scheduling would seem to be critical to achieve productive immunotherapy when sorafenib is combined with a therapeutic vaccine.[10,11]

We previously developed a novel regime known as PPV that is affordable for cancer patients with many different HLA-class IA types.[12–15] Recently, we reported that PPV has the potential to prolong the OS of patients with advanced prostate cancer or bladder cancer based on randomized clinical studies.[16–18] One of the prominent features of PPV is the capability to stimulate secondary immune responses, which thereby could bypass the potential suppression of primary immune responses by sorafenib even if PPV is combined with sorafenib. We undertook a feasibility study of PPV for patients with HCC refractory to locoregional therapy and for those refractory to both locoregional and systemic therapies in order to develop a new treatment method.

Materials and Methods

Patients. Pathologically confirmed HCC patients were entered into this study if they were not eligible for or had disease progression after locoregional therapies alone, including HAIC (cohort 1) or both locoregional and systemic therapies (cohort 2). Eligibility criteria were: positive IgG responses to at least 2 of the 31 peptide vaccine candidate peptides, age between 20 and 80 years, an ECOG performance status of 0 or 1 at the time of first visit, positive status for the HLA-A2, -A24, or -A3 super types (A3, A11, A31, or A33), or the HLA-A26 type, life expectancy of at least 12 weeks, and adequate hematologic, hepatic, and renal function as reported previously.[13–18]

Clinical protocol. This study was carried out based on two phase II protocols approved by the Ethical Committee of Kurume University (Kurume, Japan) and registered with the UMIN Clinical Trials Registry (UMIN000001882 and UMIN000003590). Primary and secondary end-points were immunological responses and safety and biomarkers (correlation between OS and peptide-specific IgG levels), respectively.

Thirty-one peptides were used for vaccination (12 peptides for HLA-A2, 14 for HLA-A24, 9 for HLA-A3 super types, and 4 for HLA-A26) as reported previously[13–18] (Table S1). These peptides were prepared under conditions of Good Manufacturing Practice by Polypeptide Laboratories (San Diego, CA, USA) and American Peptide Company (Vista, CA, USA). Peptides for vaccination (two to four peptides) were selected in consideration of the HLA typing and pre-existing host immunity before vaccination, as assessed by the titers of IgG specific to each of the 31 different vaccine candidates. The selected peptides (3 mg each) were injected s.c. with incomplete Freund's adjuvant (Montanide ISA-51VG; Seppic, Paris, France) once a week for six consecutive weeks as the first cycle. Thereafter, up to four antigen peptides, which were reselected according to the titers of peptide-specific IgG at every cycle of six vaccinations, were given every 2 weeks. During the PPV, patients were allowed to receive combination therapies (locoregional therapies alone for cohort 1; locoregional and/or systemic therapies for cohort 2). All patients were given a full explanation of the protocol and provided their informed consent before enrollment.

Adverse events were monitored according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. Complete blood counts and serum biochemistry tests were carried out before and after each cycle of vaccinations. Tumor assessments by computed tomography or MRI scans were carried out before and after every cycle (six vaccinations) of PPV, and best clinical responses were evaluated according to RECIST version 1.1.

Measurement of IgG and CTL responses. The IgG responses specific to each of the 31 peptide candidates were determined by measuring peptide-specific IgG using the Luminex system (Luminex, Austin, TX, USA), as previously reported.[13–18] If the titers of peptide-specific IgG to at least one of the vaccinated peptides in the post (6th or 12th)-vaccination plasma were more than twofold higher than those in the prevaccination plasma, the changes were considered to be significant, as previously reported.[13–18]

The CTL responses specific to the mixture of HLA-A-matched peptides were evaluated by IFN-γ ELISPOT assay using pre- and post- (6th) vaccination PBMCs. Fresh PBMCs were separated by density gradient centrifugation from peripheral blood (30 mL) with Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden), followed by cryopreservation until analysis. After thawing, PBMCs (2×10^5 cells/well) were washed twice and incubated in 96-well microculture plates (Thermo Fisher Scientific, Rochester, NY, USA) with 200 μL of RPMI-1640 medium (Life Technologies, Carlsbad, CA, USA) containing 10% FBS (MP Biologicals, Solon, OH, USA), interleukin-2 (20 IU/mL; AbD Serotec, Kidlington, UK) in the presence of a mixture of HLA-matched peptides (14 peptides for HLA-A24, 12 for A2, 8 for A3 super type, and 4 for A26) (final concentration of each peptide, 4.8 μg/mL), a mixture of 23 different virus-derived CTL epitopes (CEF peptide pool; Mabtech, Cincinnati, OH, USA), or the medium alone without any peptides. On day 3 of culture, PBMCs were harvested and tested for their ability to produce IFN-γ by ELISPOT assay in response to a mixture of HLA-matched peptides, CEF peptides, or the medium alone, in accordance with the manufacturer's instructions (MBL, Nagoya, Japan). All the assays were carried out in quadruplicate, and the results were analyzed with an ELISPOT reader (CTL-ImmunoSpot S5 Series; Cellular Technology, Shaker Heights, OH, USA). Student's *t*-test was used to determine statistical differences between the numbers of spots produced in response to a mixture of HLA-A-matched peptides and those produced in response to the medium alone, and the values of $P < 0.05$ were considered to be positive for peptide-specific CTL responses. The same evaluation was carried out for CTL responses to CEF peptides. If the spot numbers after the 6th vaccination were more than twofold higher than those before vaccination, the changes were considered to be significant, as previously reported.[13–18]

Immunohistochemical analysis. Tissue specimens were collected at the time of hepatectomy from 20 HCC patients who did not receive PPV therapy. Paraffin-embedded tissue samples were sectioned at 4-mm thickness and labeled on a BenchMark XT (Ventana Medical Systems, Tucson, AZ, USA) with each of 15 different antibodies to the 15 tumor-associated antigens that encoded 31 peptides provided for PPV, as reported previously.[13,17,18] The DAB (Ventana iVIEW DAB Detection Kit; Ventana Medical Systems) was used for the detection of antigens.

Statistical analyses. Comparisons among groups were carried out by ANOVA test. Overall survival was calculated from the first day of peptide vaccination until the date of death or the last date when the patient was known to be alive. The survival analysis was undertaken with the Kaplan–Meier method, and a comparison of the survival curves was undertaken with the log–rank test. All statistical analyses were carried out using JMP version 10 (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics. Twenty-six HCC patients (3, 16, 1, and 2 patients with stage II, III, IVa, and Ivb disease, respectively) refractory to locoregional therapies (cohort 1) and 30 HCC patients (1, 9, 4, and 22 patients with stage II, III, IVa, and Ivb disease, respectively) refractory to both locoregional and systemic therapies (cohort 2) were enrolled in this study between January 2009 and October 2015 (Table 1). The patients in cohort 1 did not receive any systemic therapy (sorafenib or chemotherapy) during PPV, but received the following locoregional therapies: transarterial chemoembolization or transcatheter arterial infusion in 10, radiation in 3, and HAIC in 2 cases. The median number of vaccinations was 14 (range, 6–45). The patients in cohort 2 received PPV alone because of intolerance to either sorafenib or systemic chemotherapy ($n = 11$), both PPV and sorafenib ($n = 12$), both PPV and systemic chemotherapy ($n = 5$), or PPV, sorafenib, and systemic chemotherapy ($n = 2$). They also received the following locoregional therapies: transarterial chemoembolization or transcatheter arterial infusion in 4, radiation in 3, and HAIC in 3 cases. The median number of vaccinations was 12 (range, 3–22).

Adverse events. The most frequent AEs in all 56 patients were dermatologic reactions at the injection sites (93%), hypoalbuminemia (42%), and anemia (41%) (Table S2). Two grade 5 (pneumonia and hepatic failure), two grade 4 (lymphopenia and rupture of HCC), and 16 grade 3 AEs were noted. However, according to the evaluation by the independent safety evaluation committee for this trial, none of these severe AEs, except for the one skin reaction, were directly associated with the vaccinations.

Immune responses. Peptide-specific CTL responses reactive to the mixture of HLA-matched peptides in prevaccination PBMCs were detectable in only 5 of 23 (22%) patients tested in cohort 1, but were boosted in 13 of 23 (57%) patients tested after the sixth vaccination (Table S3). In contrast, CTL

Table 1. Characteristics of patients with hepatocellular carcinoma refractory to locoregional therapies (cohort 1) or both locoregional and systemic therapies (cohort 2) treated with personalized peptide vaccination

Factor	Whole (n = 56)	Cohort 1 (n = 26)	Cohort 2 (n = 30)
Age, median (range)	64.5 (27–85)	72 (50–85)	63 (27–84)
Gender, male/female	48/8	21/5	27/3
Cause of disease			
HBV only	16	5	11
HCV only	28	20	8
Both HBV and HCV	1	0	1
Neither HBV nor HCV	11	1	10
ECOG performance status, 0/1	51/5	24/2	27/3
HLA type			
A2	24	10	14
A24	31	15	16
A3 super	19	12	19
A26	12	8	4
Clinical stage			
II	4	3	1
III	25	16	9
IVa	5	1	4
IVb	22	2	22
JIS score			
1	3	2	1
2	18	9	9
3	16	8	8
4	18	3	15
5	1	0	1
BCLC stage			
A	5	5	0
B	21	12	9
C	28	3	25
D	2	2	0
Previous treatment			
Hepatectomy	25	10	15
Radiation	10	5	5
HAIC	13	4	9
TAI	14	6	8
TACE	39	18	21
RFA or PEIT	36	21	15
MCT	4	3	1
Sorafenib	28	0	28
Chemotherapy	16	0	16
Number of vaccinations, median (range)	12 (3–45)	14 (6–45)	12 (3–22)
Combination therapy			
None	35	24	11
Sorafenib	12	0	12
Chemotherapy	5	0	5
Sorafenib and chemotherapy	2	0	2

BCLC, Barcelona Clinic liver cancer; HAIC, hepatic artery infusion chemotherapy; HLA, human leukocyte antigen; JIS, Japan integrated staging; MCT, microwave coagulation therapy; PEIT, percutaneous ethanol injection therapy; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization; TAI, transcatheter arterial infusion.

responses specific to CEF peptides consisting of a mixture of virus-derived CTL epitopes were consistently detectable in 17 of 22 (77%) patients tested both in pre- and post-vaccinations. The mean peptide-specific IFN-γ spot numbers reactive to HLA-matched peptides per 10^5 PBMCs in the post-vaccination samples were 18.5 if the numbers of prevaccination samples were set as 1.0 ($P = 0.01$) (Fig. 1a). Such a significant increase, however, was not observed in CTL responses to the CEF peptides. Representative results of ELISPOT assays are shown in Figure 1(b). The IgG responses were boosted in 12 of 26 (46%) and all 21 patients tested after the 6th or 12th vaccinations in cohort 1, respectively (Table S4). Regarding the rate of change in the peptide-specific IgG titers, 3.7- or 65.1-fold increases of IgG response were observed after the 6th or 12th vaccinations when the titers before vaccination were set as 1.0 ($P = 0.06$, $P < 0.001$), respectively (Fig. 1c).

Peptide-specific CTL responses reactive to the mixture of HLA-matched peptides in pre-vaccination PBMCs were detectable in 7 of 28 (25%) patients tested in cohort 2, and were boosted in 13 of 24 (54%) patients tested after the 6th vaccination. The CTL responses specific to the CEF peptides were detectable in 18 of 28 (64%) patients tested, and were not suppressed or boosted after the 6th vaccination (Table S3). The number of median peptide-specific IFN-γ spots reactive to HLA-matched peptides per 10^5 PBMCs in the post-vaccination samples was 12.1 when the numbers of prevaccination samples were set as 1.0 ($P = 0.002$) (Fig. 1a). Such a significant increase, however, was not observed in CTL responses to the CEF peptides. The IgG responses were boosted in 14 of 27 (52%) and 14 of 17 patients (82%) tested after the 6th and 12th vaccinations in cohort 2, respectively. Regarding the rate of changes of peptide-specific IgG titers, a 3.1- or 34.8-fold increase of IgG response was observed in the samples after the 6th or 12th vaccinations when the titers before vaccination were set as 1.0 ($P = 0.01$, $P = 0.001$), respectively (Fig. 1b). Among the 30 patients in cohort, 2, 11, 12, 5, and 2 patients received PPV alone, PPV and sorafenib, PPV and chemotherapy, and PPV, sorafenib, and systemic chemotherapy, respectively. Successful CTL boosting in each of these four groups was observed in 5 of 10, 4 of 9, 3 of 4, and 1 of 2 patients tested, respectively. Similarly, peptide-specific IgG responses were observed in 6 of 10, 5 of 11, 3 of 4, and 0 of 2 patients tested, respectively.

Clinical responses. The median OS of 26 patients in cohort 1 was 18.7 months (95% CI, 12.2–22.5 months) (Fig. 2a). A decrease of tumor marker (AFP) after PPV compared to the prevaccination levels was observed in 4 of 26 (17%) patients. The median OS of these four patients with AFP decrease and the remaining 19 patients was 49.4 months and 18.3 months, respectively ($P = 0.013$, log–rank test). There were no cases of complete or partial response, but 16 cases of stable disease and 10 of progressive disease.

The median OS of 30 patients in cohort 2 was 8.5 months (95% CI, 5.9–12.2 months) (Fig. 2b). Among them, the OS was 6.2 months in 11 patients under PPV alone, 10.2 months in 12 patients under PPV and sorafenib, 6.6 months in five patients under PPV and systemic chemotherapy, and 8.4 months in two patients under PPV, sorafenib, and systemic chemotherapy ($P = 0.92$, log–rank test). A decrease of tumor marker (AFP) after PPV compared to the prevaccination levels was observed in 2 of 29 (7%) patients tested. Best clinical responses were one patient with partial response, 13 with stable disease, and 10 with progressive disease. There were no complete responses.

Immune responses and OS. Among the 23 of 26 patients in cohort 1, whose samples were available for the CTL assay, the median OS of the patients with (n = 13) or without CTL boosting (n = 10) after the 6th vaccination was 21.4 months or

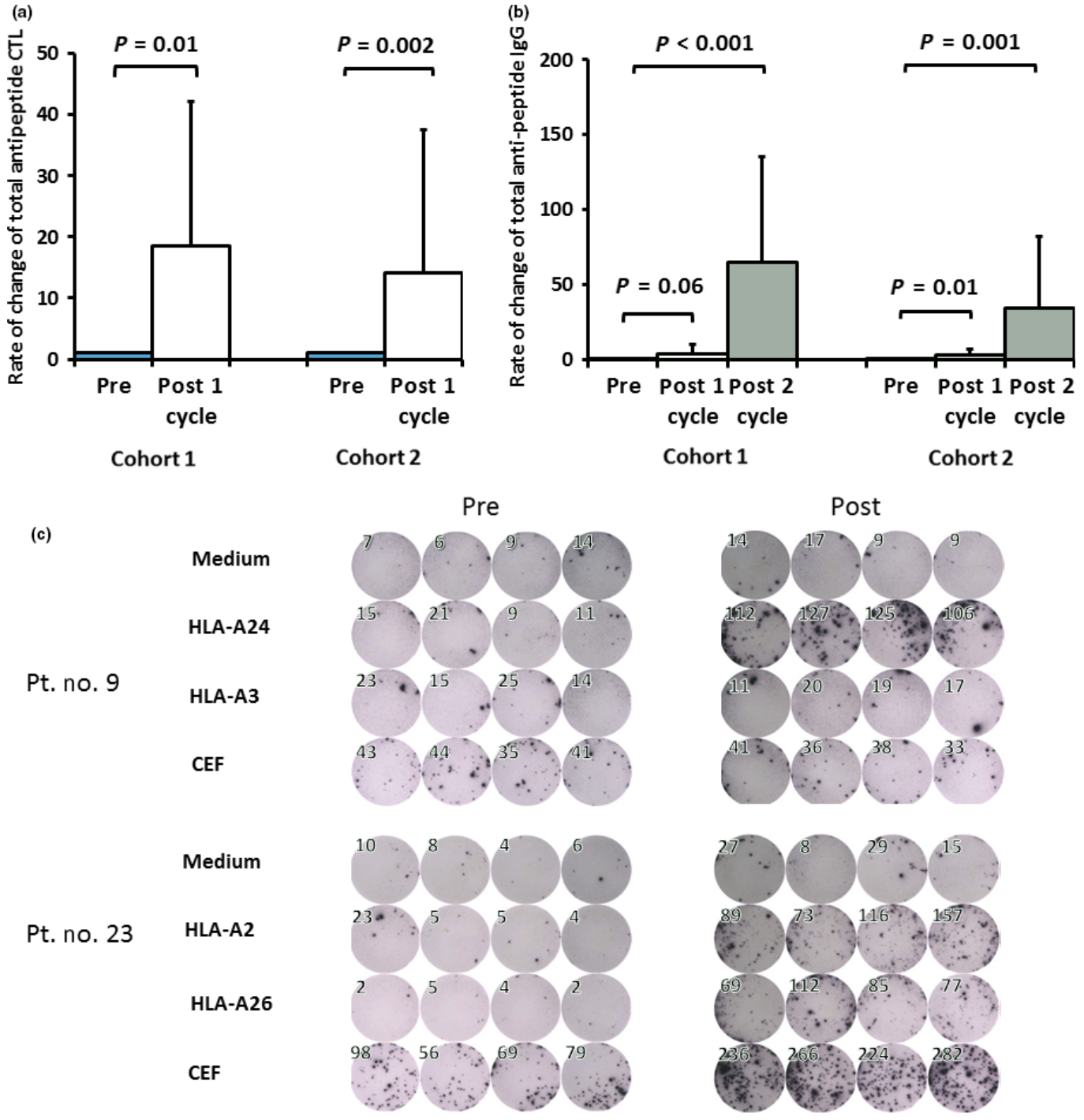

Fig. 1. Peptide-specific immune responses in patients with hepatocellular carcinoma refractory to locoregional therapies (cohort 1) or both locoregional and systemic therapies (cohort 2) treated with personalized peptide vaccination (PPV). (a) Rates of changes in peptide-specific interferon-γ spot numbers in response to vaccinated peptides after the first cycle of vaccinations were calculated by setting those before vaccination as 1.0. Under this definition, the median rates of change after vaccination were 18.5 and 12.1 in cohorts 1 and 2, respectively. (b) Rates of changes in peptide-specific IgG titers after the first and second cycles of vaccinations were measured by setting those before vaccinations as 1.0. Under this definition, 3.7- and 65.1-fold increases of IgG responses were observed after the first and second cycles of vaccinations in cohort 1, respectively. Similarly, 3.1- and 34.8-fold increases of IgG responses were observed after the first and second cycles of vaccinations in cohort 2, respectively. (c) Representative results of ELISPOT assay are shown in patient (Pt.) no. 9 (increase in spot numbers for human leukocyte antigen (HLA)-A24 mix peptides after PPV) and no. 23 (increase in spot numbers for HLA-A2, -A26, and CEF mix peptides after PPV).

18.4 months, respectively ($P = 0.464$). The median OS of the patients with ($n = 12$) or without IgG boosting ($n = 14$) after the 6th vaccination was 16.6 months or 19.1 months, respectively ($P = 0.872$).

Among 25 of the 30 patients in cohort 2, whose samples were available for the CTL assay, the median OS of patients with ($n = 14$) or without CTL boosting ($n = 11$) after the 6th vaccination was 8.4 months or 9.9 months, respectively

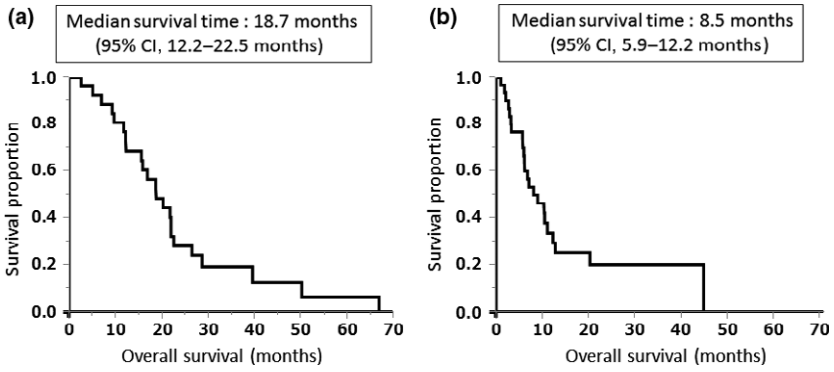

Fig. 2. Survival analysis in patients with hepatocellular carcinoma refractory to locoregional therapies (cohort 1) or both locoregional and systemic therapies (cohort 2) treated with personalized peptide vaccination. The survival analysis was undertaken using the Kaplan–Meier method, and the survival curves was compared with the log–rank test. The median survival times for 26 patients in cohort 1 (a) and 30 patients in cohort 2 (b) were 18.7 months (95% confidence interval [CI], 12.2–22.5 months) and 8.5 months (95% CI, 5.9–12.2 months), respectively.

(P = 0.968). Similarly, that of patients with (n = 14) or without IgG boosting (n = 13) after the 6th vaccination was 6.0 months or 12.0 months, respectively (P = 0.385).

Immunohistochemical analysis. Twenty HCC tissues, harvested at the time of hepatectomy from patients with non-advanced HCC without PPV treatment, were provided for the immunohistochemical analysis of 15 vaccine antigens. Samples from advanced-stage HCC were not available for the study. Representative results are shown in Figure 3. Cyclophilin B was expressed in the cytoplasm of both tumor cells and non-malignant hepatocytes from 18 of 20 samples, with lower levels in the latter cells. Epidermal growth factor receptor was expressed in the cell membrane of tumor cells from 16 of 20 samples, and also slightly expressed in non-malignant cells from 4 of 20 samples. Parathyroid hormone-related protein was expressed in both the cytoplasm and nucleus of tumor cells from 15 of 20 samples, and also slightly expressed in non-malignant cells from 5 of 20 samples. We detected SART2 expression in the cytoplasm of tumor cells from 13 of 20 samples, and also slight expression in non-malignant cells from 5 of 20 samples. ppMAPkkk was expressed in the cytoplasm of tumor cells from 13 of 20 samples, and also slightly expressed in non-malignant cells from 10 of 20 samples. The WHSC2 protein was expressed in both the cytoplasm and nucleus of tumor cells from 6 of 20 samples, and also slightly expressed in non-malignant cells from 3 of 20 samples. HNRPL was expressed in the cytoplasm of tumor cells from all 20 samples, and also slightly expressed in non-malignant cells from 17 of 20 samples. SART3 was expressed in the nuclei of tumor cells from 19 of 20 samples, and also weakly expressed in non-malignant cells from 16 of 20 samples. UBE-2V was expressed in the cytoplasm of tumor cells from 19 of 20 samples, and also weakly expressed in non-malignant cells from 15 of 20 samples. EZH2 was expressed in the nucleus of tumor cells from 15 of 20 samples, and also slightly expressed in non-malignant cells from 3 of 20 samples. MRP3 was expressed in the cytoplasm of tumor cells from 6 of 20 samples, but was not expressed in non-malignant cells from any of 20 samples. In contrast, none of the three prostate-related antigens, PSA, PAP, and PSMA, were expressed in any of the 20 samples tested, with the exception of PSA, expressed in the cytoplasm of tumor cells from one sample, and PSMA, expressed in the nucleus of tumor cells from one sample (data not shown). LCK was not expressed in any of the tumor cells tested, but was expressed in the cytoplasm of lymphocytes from all 20 samples, and in the cytoplasm of a portion (20%–60%) of Kupffer cells from 7 of 20 samples.

Discussion

It is important to better understand the tumor immunity in the HCC patients enrolled in this study, as it has been reported that the antitumor responses of HCC patients are deeply suppressed.[19] In addition, the molecules involved in T-cell checkpoints have been suggested to inhibit CTL responses against tumor cells in these patients.[20] Furthermore, sorafenib has been reported to suppress the induction of primary immune responses.[9] We showed that CTL responses to the mixed HLA-matched peptides in prevaccination PBMCs from patients refractory to locoregional therapies (cohort 1) and those refractory to both locoregional and systemic therapies (cohort 2) were observed in 22% and 25% of patients, respectively. In contrast, CTL responses to the CEF peptides were observed in 77% and 64% of patients, respectively. These results suggest that antitumor immunity, but not antivirus response, of these HCC patients was depressed. However, PPV boosted the CTL responses to the HLA-matched peptides in PBMCs from 57% of the patients in cohort 1 and 54% in cohort 2, whereas it did not affect CTL responses to the virus-derived peptides. Immunoglobulin G responses were boosted in 46% and 52% of patients after the 6th vaccination, and in 100% and 84% of patients after the 12th vaccination in cohorts 1 and 2, respectively. Severe PPV-related AEs were not observed in any of the 56 patients except for one injection site reaction, in agreement with previous reports.[12–18, 21] These results indicated that PPV successfully boosted specific immunity against the vaccinated peptides in the majority of the HCC patients refractory to not only locoregional therapies but also both locoregional and systemic therapies. These immunological and safety features could warrant further clinical studies of PPV for HCC patients refractory to not only locoregional therapies but also both locoregional and systemic therapies.

We reported that nine tumor-associated antigens (CypB, EGFR, EZH2, HNRPL, ppMAPkkk, PTH-rP, SART2, SART3, and UBE-2V) were expressed in the majority (≥65%) of HCC tissues tested. The MRP3 antigen, a protein preferentially expressed in chemotherapy-resistant tumor cells,[22] and WHSC2 were expressed in 6 of 20 samples. None of the three prostate-related antigens (PAP, PSA, and PSMA) were detectable in HCC samples, with a few exceptions. However, as these three antigens have been reported to be expressed in tumor cells other than prostate cancers,[23–26] we cannot exclude the possible expression of these prostate-related antigens in advanced HCC samples. Therefore, peptides derived from these three antigens were selected only for patients who had no IgG responses to other peptides in this study as reported previously.[12] The LCK antigen, a unique vaccine

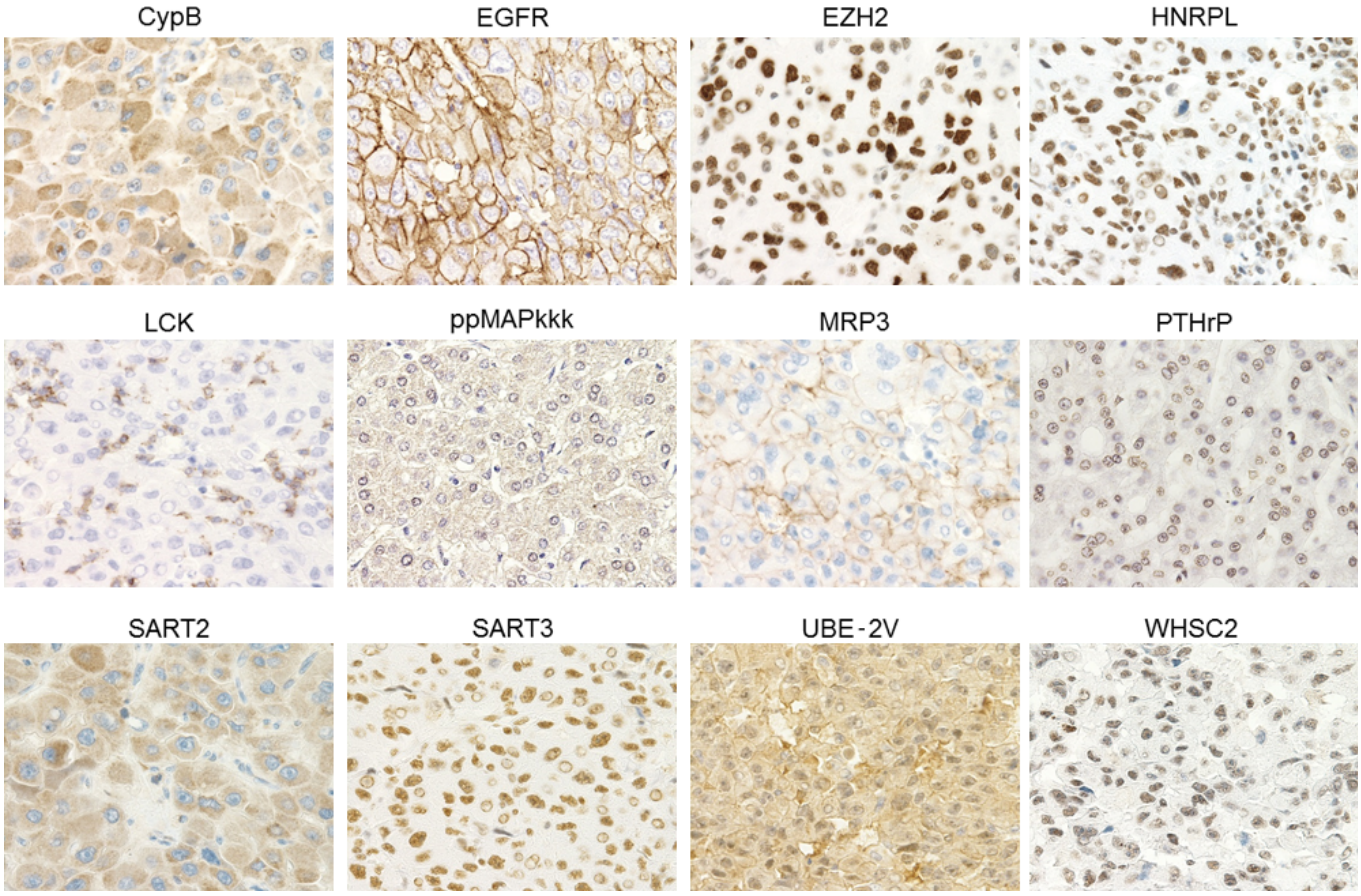

Fig. 3. Immunohistochemical analysis of the expressions of 15 vaccine antigens in tumor tissues from 20 patients with hepatocellular carcinoma without personalized peptide vaccination treatment. Representative data of 12 antigens are shown (all sections, ×200). Data on prostate-related vaccine antigens (prostate-specific antigen, prostatic acid phosphatase, and prostate-specific membrane antigen) are not shown. CypB, cyclophilin B; EGFR, epidermal growth factor receptor; EZH2, enhancer of zeste homolog 2; HNRPL, heterogeneous nuclear ribonucleoprotein L; MRP3, multidrug resistance-associated protein 3; PTHrP, parathyroid hormone-related protein; SART, squamous cell carcinoma antigen recognized by T cells; UBE-2V, ubiquitin-conjugating enzyme E2 variant; WHSC2, Wolf–Hirschhorn syndrome candidate 2 protein.

antigen that was previously reported to be preferentially expressed in both T cells and metastatic tumor cells,[27] was expressed in a small fraction of Kupffer cells in certain tissues. To the best of our knowledge, expression of the LCK antigen in Kupffer cells has not previously been reported. We recently found that antibody against LCK-486 peptide inhibited tumor growth in a mouse model in association with the inhibition of T cells at the tumor site (S. Matsueda, K. Itoh, S. Shichijo, manuscript in preparation), suggesting that LCK peptide vaccination could inhibit the activity of not only LCK-positive tumor cells but also LCK-positive T cells and Kupffer cells.

It might be important to discuss whether PPV could improve OS in patients with HCC refractory to locoregional or systemic therapy as treatment methods for advanced HCC have been very limited. We reported that the median OS of 26 patients refractory to locoregional therapies (cohort 1) was 18.7 months (95% CI, 12.2–22.5 months). That of advanced HCC patients treated with sorafenib as a first-line systemic therapy after failing various locoregional therapies was reported to be 11.6 months in the Analysis of the Kurume Liver Cancer Study Group[28] and 10.9 months in the final analysis of a global investigation involving 3202 patients.[4] This study also showed that the median OS of 30 patients refractory to both locoregional and systemic therapies (cohort 2), for whom no standard treatments have been developed, was 8.5 months (95% CI, 5.9–12.2 months). Based on the higher rate of immune boosting and such potential prolongation of OS by PPV, further clinical studies to precisely evaluate the clinical benefits of PPV for HCC patients refractory to not only locoregional therapies but also both locoregional and systemic therapies could be warranted.

Disclosure Statement

Akira Yamada is a board member and has stock ownership of Green Peptide Co., Ltd. Kyogo Itoh received research fund from Taiho Pharmaceutical Co., Ltd. The other authors have no conflict of interest.

Abbreviations

CypB	cyclophilin B
EZH2	enhancer of zeste homolog 2
HAIC	hepatic artery infusion chemotherapy
HLA	human leukocyte antigen
HNRPL	heterogeneous nuclear ribonucleoprotein L
MRP3	multidrug resistance-associated protein 3
PAP	prostatic acid phosphatase
PPV	personalized peptide vaccination
PSA	prostate-specific antigen
PSMA	prostate-specific membrane antigen

PTH-rP	parathyroid hormone-related protein
SART	squamous cell carcinoma antigen recognized by T cells
UBE-2V	ubiquitin-conjugating enzyme E2 variant
WHSC2	Wolf–Hirschhorn syndrome candidate 2 protein

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Supporting Information

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

Table S1. Peptide candidates for personalized peptide vaccination in patients with hepatocellular carcinoma refractory to locoregional therapies.

Table S2. Adverse events during personalized peptide vaccination in hepatocellular carcinoma patients refractory to locoregional therapies.

Table S3. Peptide-specific CTL responses in hepatocellular carcinoma patients treated with personalized peptide vaccination.

Table S4. Peptide-specific IgG responses in hepatocellular carcinoma patients treated with personalized peptide vaccination.