Personalized peptide vaccination as second-line treatment for metastatic upper tract urothelial carcinoma

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This study investigated the applicability of personalized peptide vaccination (PPV) for patients with metastatic upper tract urothelial cancer (mUTUC) after failure of platinum-based chemotherapy. In this single arm, open-label, phase II clinical trial, patients with mUTUC received PPV at a single institution. Personalized peptide vaccination treatment used a maximum of four peptides chosen from 27 candidate peptides according to human leukocyte antigen types and peptide-reactive IgG titers, for six s.c. injections weekly as one cycle. The safety of PPV, as well as its influence on host immunity and effect on overall survival were assessed. Forty-eight patients were enrolled in this study. Personalized peptide vaccinations were well tolerated without severe adverse events. Median survival time was 7.3 months (95% confidence interval [CI], 5.3–13.1) with 13.0 months for patients receiving combined salvage chemotherapy (95% CI, 5.7–17.5) and 4.5 months for patients receiving PPV alone (95% CI, 1.7–10.1) ($P = 0.080$). Patients with positive CTL responses showed a significantly longer survival than patients with negative CTL responses (hazard ratio, 0.37; 95% CI, 0.16–0.85; $P = 0.019$). Multivariate Cox regression analysis showed that lower numbers of Bellmunt risk factors and lower levels of B-cell activating factor were significantly associated with favorable overall survival for patients under PPV treatment. This study indicated that PPV for patients with mUTUC after failure of platinum-based chemotherapy induced substantial peptide-specific CTL responses without severe adverse events and has the potential to prolong survival when combined with salvage chemotherapy.
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Upper tract urothelial carcinomas (UTUCs) are relatively uncommon compared to bladder cancer, accounting for only 5–10% of urothelial cancers (UC), and are aggressive urological cancers with a propensity for multilocality, local recurrence, and metastasis.[1] Upper tract urothelial carcinomas have a peak incidence in people aged 70–90 years, and 60% of UTUCs are invasive at diagnosis.[1–3] For advanced or metastatic disease, chemotherapy would be the main choice.[4,5] Although there are currently insufficient data recommendations, platinum-based chemotherapy including cisplatin plus gemcitabine or the combination of methotrexate, vinblastine, doxorubicin, and cisplatin is expected to have similar efficacy as in bladder cancer.[6] However, the vast majority of UC patients treated with these regimens develop progressive disease within 8 months, and survival is very short after the platinum-based chemotherapy failure.[7–9] Many second-line regimens have been tested for advanced or metastatic UC in the past decade, but most have shown limited activity in patients with platinum-based chemotherapy refractory disease. Furthermore, chemotherapy-related toxicities often require treatment cessation, and may reduce survival in patients with postoperative renal dysfunction. Newer, safer, and more effective agents are urgently required.

We have recently reported that personalized peptide vaccination (PPV), in which peptides for vaccination were selected from 31 candidate peptides derived from various cancer antigens based on human leukocyte antigen (HLA) class I typing and pre-existing host immunity, was beneficial for advanced bladder cancer of UC refractory to platinum-based first-line chemotherapy in a randomized phase II trial.[9] In that study, patients treated with PPV plus best-supportive care (BSC) showed a significantly longer overall survival (OS) compared with those of BSC alone (hazard ratio [HR], 0.58; 95% confidence interval [CI], 0.34–0.99, $P = 0.049$), with the median survival time (MST) being 7.9 months (95% CI, 3.5–12.0) in the PPV plus BSC group and 4.1 months (95% CI, 2.8–6.9) in the BSC group.

To address the applicability of PPV to patients with metastatic UTUC (mUTUC), we undertook a phase II clinical trial of PPV in patients with mUTUC refractory to platinum-based chemotherapy. We report the outcomes with respect to the safety of PPV, as well as its influence on host immunity and effect on OS compared with matched patients as historical controls.

Materials and Methods

Patients. Patients with pathologically confirmed mUTUC refractory to at least one platinum-based chemotherapy regimen were eligible for this study. Eligible patients were aged ≥20 years, and had ECOG performance status (PS) 0 or 1, life expectancy of at least 12 weeks, and adequate bone marrow function, hepatic function, and renal function. Other inclusion criteria were: positivity for HLA-A02, -A24, -A03 super types (A03, A11, A31, or A33), or -A26 type; IgG positivity for at least two of the 27 candidate peptides in pretreatment serum (Table S1). Exclusion criteria included pulmonary, cardiac, or other systemic diseases, acute infection, a history of severe allergy, pregnancy or breastfeeding, and other inappropriate conditions for enrollment as judged by clinicians.

All patients were given a detailed explanation of the protocol and provided informed consent before enrollment. The study protocol was approved by the Kurume University Ethics Committee (Kurume, Japan) and the trial was registered with the UMIN Clinical Trials Registry (UMIN000001854).

Study design and treatment. This was a single-arm, open-label phase II study that was designed to investigate the feasibility of PPV, immune response to PPV, and safety of PPV in patients with mUTUC. Twenty-seven candidate peptides, for which safety and immunologic effects have been confirmed in previous clinical studies,[10,11] were prepared under Good Manufacturing Practice conditions by the PolyPeptide Laboratories (San Diego, CA, USA) and American Peptide Company (Vista, CA, USA). Based on pre-existing host immunity, two to four HLA-matched peptides were selected for each patient by assessing the titers of specific IgG against each peptide, as described previously.[10,11] The selected peptides (3 mg/each peptide) were given s.c. with incomplete Freund's adjuvant (Montanide ISA51; Seppic, Paris, France) once a week for six consecutive weeks in the first cycle of vaccination followed by six times at 2- to 4-week intervals until unacceptable toxicity or withdrawal of consent in this study. Combination therapy with salvage chemotherapy was allowed during the vaccination for the patients who were expected to be tolerable.

Measurement of immune responses and laboratory markers. Peripheral blood (30 mL) was obtained from the patients before and after each cycle of six vaccinations. Plasma was separated by centrifugation and stored frozen until analysis, while PBMC were separated by density gradient centrifugation with Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden) and stored frozen until analysis. Specific humoral immune responses to the vaccine peptides were assessed by determining the peptide-specific IgG titers using a bead-based multiplex assay and the Luminex 200 system (Luminex, Austin, TX, USA). The cut-off values of IgG titers were set to 10 fluorescence intensity units in 100-times diluted samples. Cytotoxic T lymphocyte activity specific to the vaccinated peptide was evaluated by γ-interferon (IFN-γ) ELISPOT assay using PBMC, and analyzed with an ELISPOT reader (CTL-ImmunoSpot S5 Series; Cellular Technology, Shaker Heights, OH, USA). If total IgG titers to vaccinated peptides at six vaccinations were higher than those in the prevaccination plasma or more than 30 total spots to the corresponding peptide in the PBMC at six vaccinations was observed by IFN-γ ELISPOT assay, then these changes were considered to be positive immune responses.

The pre- and post-vaccination plasma level of interleukin-6 (IL-6) was examined by ELISA using kits from R&D Systems (Minneapolis, MN, USA), Life Technologies (Carlsbad, CA, USA), and eBioscience (Vienna, Austria), respectively. Bead-based multiplex assays and the Luminex 200 system were used to measure various cytokines, including IL-4, IL-13, IL-21, IFNγ-induced protein 10, B-cell activating factor belonging to the tumor necrosis factor family (BAFF), and transforming growth factor-β. Frozen plasma samples were thawed, diluted, and assayed in duplicate according to each manufacturer's instructions. Mean values obtained from the duplicate samples were used for statistical analysis.

Assessment of toxicity and clinical activity. Toxicity was assessed by the NCI Common Terminology Criteria for Adverse Events version 4.0, and patients were monitored at each visit by history and physical examinations. Routine laboratory studies were carried out every six vaccinations. All patients underwent relevant computed tomography and bone scans every 6 months or at the progression of symptoms. Progressive disease (PD) was defined as radiographic progression evaluated by RECIST version 1.1 (The Response Evaluation Criteria in Solid Tumors Group version 1.1, Elsevier Ltd., Amsterdam, the Netherlands), or cancer specific death.

Statistical analysis. Analyses of OS were based on population of a full analysis set that included all treated patients. Overall survival was defined as the interval from the date of starting peptide vaccination until the date of death and was censored at the last date of contact for patients who were still alive at final follow-up. Survival data for OS were determined by the Kaplan–Meier method with Greenwood variance estimates. Cox proportional hazards analysis was used to calculate HRs and 95% CI. In addition, exploratory analyses were carried out to examine associations among historical control, immune responses, biomarkers, and OS, and comparisons for OS were carried out using the log–rank test with a two-sided significance level of 5%. Associations between prevaccination biomarkers and OS were evaluated by univariate and multivariate analyses with the Cox proportional hazards regression model. All analyses were conducted using JMP version 12 or SAS version 9.4 software (SAS Institute Inc, Cary, NC, USA).

Results

Patient characteristics. Between April 2009 and January 2016, 48 patients with mUTUC refractory to platinum-based chemotherapy were enrolled. Among them, 28 patients received both PPV and salvage chemotherapy based on the choice of the attending physicians (PPV plus salvage chemotherapy group), while the remaining 20 patients received PPV alone due to potential intolerance of chemotherapy (PPV group). Table 1 summarizes the demographics and baseline characteristics of these two groups. There were no significant differences in age, gender, performance status, clinical stage, or primary tumor site between the two groups. The majority of patients received more than two platinum-based chemotherapy regimens, with no significant between-group difference. Lung metastasis was more frequent in patients receiving both PPV and salvage chemotherapy than in patients given PPV alone ($P = 0.0095$), whereas liver metastasis ($P = 0.1366$) and lymph node metastasis ($P = 0.0736$) were less frequent. The percentage of patients with low hemoglobin (<10 g/dL) was lower in PPV alone group ($P = 0.0238$). In addition, the former group had fewer Bellmunt risk factors (PS, hemoglobin, and liver metastasis: three adverse risk factors that predict OS in patients with advanced UC refractory to platinum-based standard therapy)[1] ($P = 0.1083$). Furthermore, the total of numbers 2 and 3 of Bellumunt risk factors was significantly lower in the former group (4/28, 14.3%)

Table 1. Characteristics of the enrolled patients

	PPV alone (n = 20)	PPV + chemotherapy (n = 28)	P-value
Age, years			0.5764†
Median (range)	66.5 (40–82)	63 (32–79)	
Gender			0.1977‡
Male	13	23	
Female	7	5	
Performance status			0.7502‡
0	5	9	
1	15	19	
Hemoglobin, g/dL			0.0238‡
<10	7	2	
≥10	13	26	
Site of primary tumor			0.1429‡
Ureter	12	10	
Renal pelvis	8	18	
Numbers of Bellmunt risk factors			0.1083‡
0	3	9	
1	8	15	
2	7	3	
3	2	1	
Clinical stage			–
IV	20	28	
Metastatic sites			
Liver	6	3	0.1366‡
Lung	5	18	0.0095‡
Urinary bladder	1	3	0.6309‡
Bone	3	6	0.7161‡
Lymph node	16	15	0.0736‡
Other	1	1	1.0000‡
Number of previous chemotherapy regimens			0.6452†
1	6	7	
2	8	11	
≥3	6	10	
Number of vaccinations			0.0049†
Median (range)	7 (1–23)	13 (5–29)	
Combination salvage chemotherapy			–
Gemcitabine	–	5	
Gemcitabine + cisplatin	–	4	
UFT/fluorouracil	–	14	
Others	–	11	

†Student's t-test. ‡Fisher's exact test. –, not applicable. UFT, uracil–tegafur.

compared to the latter group (9/20, 45%) ($P = 0.0247$). Moreover, the number of vaccinations was significantly higher in the former group (mean, 13; range, 5–29) than in the latter group (mean, 7; range, 1–23) ($P = 0.0049$). These differences could help to explain why only one patient from the PPV plus salvage chemotherapy group dropped out before the end of the first vaccination cycle (6th vaccination) because of disease progression and 12 patients dropped out before the end of the second vaccination cycle (12th vaccination), whereas 7 and 16 patients from the PPV group dropped out before the end of the first and second cycles, respectively. The regimens for salvage chemotherapy combined with PPV were gemcitabine ($n = 5$), gemcitabine and cisplatin ($n = 4$), tegafur-uracil and fluorouracil ($n = 14$), and others ($n = 11$).

Adverse events. Adverse events (AEs) are shown in Table 2. The most frequent AEs in all 48 patients were dermatologic reactions at the injection site (39/48, 81%), hypoalbuminemia (23/48, 48%), anemia (18/48; 38%), and lymphopenia (15/48; 31%). Among the serious AEs (SAEs), there was one grade 4 event (aspartate aminotransferase increased) and three grade 3 events (two cases of increased γ-glutamyl transpeptidase and one case of tumor pain, anemia, thrombocytopenia, lymphopenia, increased alanine aminotransferase, increased bilirubin, increased alkaline phosphatase, hyponatremia, and dyspnea). According to the independent safety evaluation committee, the SAEs were not directly associated with vaccination and were associated with other causes, such as combined chemotherapy, targeted therapies, or cancer progression. The incidence of anemia, increased alkaline phosphatase, and hypoalbuminemia was significantly higher in the PPV plus salvage chemotherapy group than in the PPV group.

Table 2. Adverse events during personalized peptide vaccination (PPV) as second-line treatment for metastatic upper tract urothelial carcinoma

	Total (PPV alone [*n* = 20] *vs* PPV + chemotherapy [*n* = 28])				
	Grade 1	Grade 2	Grade 3	Grade 4	*P*-value†
Injected site reaction	22 (8 *vs* 14)	17 (6 *vs* 11)	0	0	0.2737
General disorders and administration site conditions					
Tumor pain	3 (1 *vs* 2)	9 (5 *vs* 4)	1 (1 *vs* 0)	0	0.5756
Malaise	3 (1 *vs* 2)	2 (2 *vs* 0)	0	0	0.2827
Edema limbs	2 (0 *vs* 2)	0	0	0	0.5035
Investigations					
Anemia	7 (2 *vs* 5)	10 (1 *vs* 9)	1 (1 *vs* 0)	0	*0.0412*
Platelet count decreased	6 (1 *vs* 5)	3 (0 *vs* 3)	1 (0 *vs* 1)	0	0.1414
White blood cell decreased	3 (1 *vs* 2)	2 (0 *vs* 2)	0	0	0.6309
Neutrophil count decreased	0	1 (0 *vs* 1)	0	0	1.0000
Lymphocyte count decreased	8 (3 *vs* 5)	6 (3 *vs* 3)	1 (0 *vs* 1)	0	1.0000
Creatinine increased	4 (1 *vs* 3)	7 (2 *vs* 5)	0	0	0.6778
Aspartate aminotransferase increased	4 (0 *vs* 4)	0	0	1 (1 *vs* 0)	0.0727
Alanine aminotransferase increased	4 (0 *vs* 4)	0	1 (1 *vs* 0)	0	0.0727
GGT increased	4 (1 *vs* 3)	1 (0 *vs* 1)	2 (2 *vs* 0)	0	0.3285
Blood bilirubin increased	1 (0 *vs* 1)	0	1 (1 *vs* 0)	0	0.6649
Alkaline phosphatase increased	5 (0 *vs* 5)	2 (2 *vs* 0)	1 (1 *vs* 0)	0	*0.0180*
Metabolism and nutrition disorders					
Hyperkalemia	4 (0 *vs* 4)	0	0	0	0.1301
Hypokalemia	2 (2 *vs* 0)	0	0	0	0.1684
Hyponatremia	4 (2 *vs* 2)	0	1 (0 *vs* 1)	0	1.0000
Hypoalbuminemia	19 (3 *vs* 16)	4 (2 *vs* 2)	0	0	*0.0075*
Hypercalcemia	1 (0 *vs* 1)	0	0	0	1.0000
Hypocalcemia	2 (1 *vs* 1)	0	0	0	1.0000
Hypertriglyceridemia	1 (0 *vs* 1)	0	0	0	1.0000
Hyperuricemia	3 (1 *vs* 2)	0	0	0	1.0000
Hyperglycemia	2 (0 *vs* 2)	1 (0 *vs* 1)	0	0	0.5035
Anorexia	1 (1 *vs* 0)	2 (2 *vs* 0)	0	0	0.0659
Gastrointestinal disorders					
Constipation	0	2 (1 *vs* 1)	0	0	1.0000
Nausea	2 (1 *vs* 1)	0	0	0	1.0000
Renal and urinary disorders					
Hematuria	1 (1 *vs* 0)	2 (0 *vs* 2)	0	0	0.3141
Respiratory, thoracic and mediastinal disorders					
Cough	2 (1 *vs* 1)	0	0	0	1.0000
Dyspnea	0	0	1 (0 *vs* 1)	0	0.6649
Atelectasis	1 (1 *vs* 0)	0	0	0	0.4167

The italic *P*-values are less than 0.05. †Fisher's exact test.

Clinical outcomes. In the best clinical response evaluated by RECIST criteria, there was no complete response or partial response. Ten patients had stable disease and 38 patients had PD. Among 48 patients, 41 patients (85%) have died with a median follow-up of 6.6 months (95% CI, 7.9–16). The MST was 7.3 months (95% CI, 5.3–13.1) with a 1-year survival rate of 40% (Fig. 1a). Interestingly, the MST of the PPV plus salvage chemotherapy group was 13.0 months (95% CI, 5.7–17.5 months) and the 1-year survival rate was 51%, whereas the MST of the PPV group was only 4.5 months (95% CI, 1.7–10.1 months) and the 1-year survival rate was 25% ($P = 0.080$) (Fig. 1b).

Immune responses and OS. Peptide-specific IgG reactive to HLA-matched peptides were detectable in all 48 patients: the numbers of peptides used for the first cycle of vaccinations were four peptides in 42 patients, three peptides in four patients, and two peptides in two patients. Before the vaccination, peptide-specific IFN-γ spots were only detected in three

of 43 patients (7%) tested, whereas responses to non-vaccinated control CEF peptides reactive to viral antigen were detectable in 30 of 43 patients (70%). Among 48 patients, 37 patients were analyzed for IgG and CTL responses at the end of six vaccinations. Immunoglobulin G and CTL responses specific to the vaccinated peptides were increased in 19 of 37 patients (51%) and in 17 of 37 patients (46%), respectively. Averages of IgG titers and IFN-γ spot numbers after six vaccinations were 14-fold ($P = 0.015$) and 140-fold ($P < 0.001$) higher than those at pre-vaccination, respectively, when those levels at pre-vaccination were set as 1.0.

We also performed an OS subgroup analysis based on groups stratified by the status of positive immune responses. To reduce the biases in the statistical analysis for comparisons of OS between immune response-positive and -negative groups, we used the landmark time analysis in which the survival after six vaccinations was evaluated by immune response status after six vaccinations.[12] In the landmark time analysis,
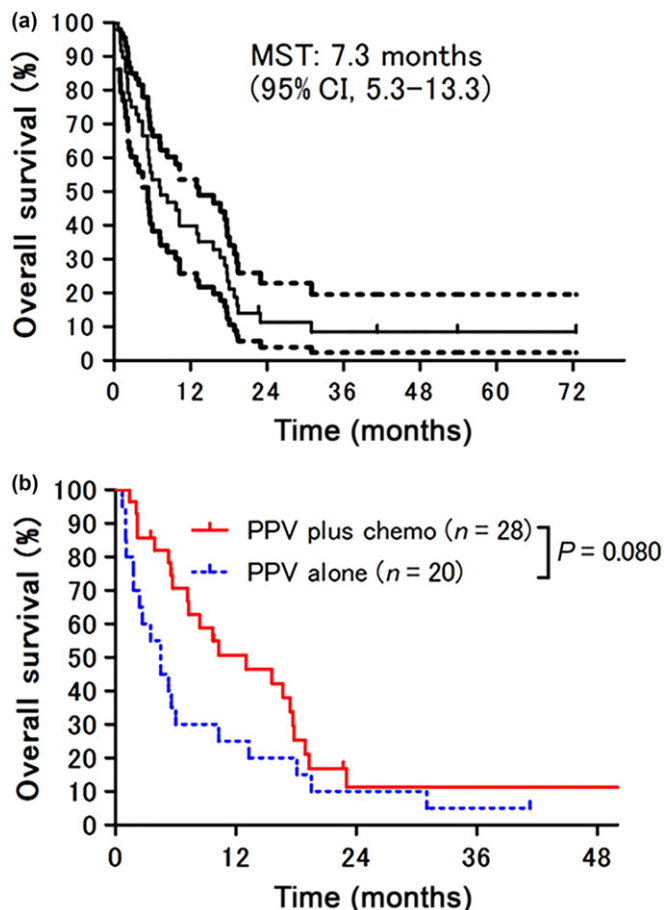
Fig. 1. Survival analysis of patients with metastatic upper tract urothelial carcinoma who received personalized peptide vaccination (PPV) as second-line treatment. (a) Overall survival after PPV treatment was estimated by the Kaplan–Meier method in all 48 enrolled patients; dotted lines show 95% confidence intervals (CI). (b) Median survival time (MST) was also estimated for the PPV plus salvage chemotherapy group (*n* = 28) and the PPV only group (*n* = 20).
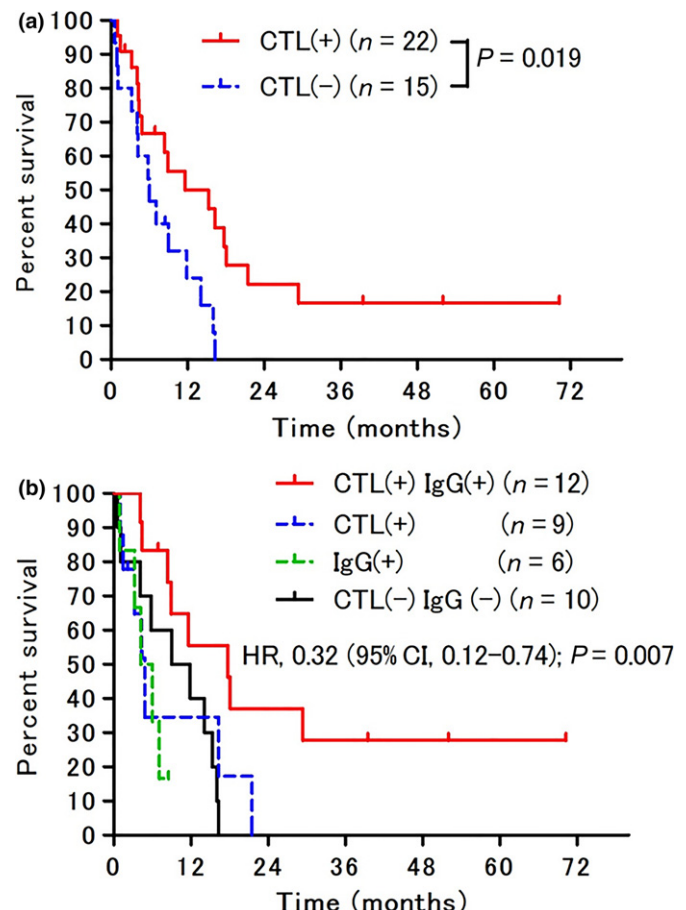

Fig. 2. Prognostic significance of increased peptide-specific CTL or IgG in patients with metastatic upper tract urothelial carcinoma who received personalized peptide vaccination (PPV) as second-line treatment. (a) Patients treated with PPV were divided into two subgroups according to CTL responses. (b) In addition, patients were divided into four subgroups: positive CTL and IgG responses; positive CTL alone; positive IgG alone; or negative CTL and IgG. HR, hazard ratio.

the MST for patients with positive and negative CTL responses were 11.6 months (95% CI, 4.4–18.1) and 6 months (95% CI, 1.1–11.9), respectively. Patients with positive CTL responses showed a significantly longer survival than patients with negative CTL responses (HR, 0.37; 95% CI, 0.16–0.85; *P* = 0.019) (Fig. 2a). The MST for patients with positive and negative IgG responses were 8.9 months (95% CI, 4.4–18.1) and 5.8 months (95% CI, 3.2–15.3). In addition, patients with both positive CTL and IgG responses showed significantly longer survival than those with positive CTL alone, positive IgG alone, or negative CTL and IgG (HR, 0.32; 95% CI, 0.12–0.74; *P* = 0.007) (Fig. 2b).

Relationship between baseline clinical findings or laboratory data and OS. To identify baseline factors significantly associated with OS from among prevaccination clinical findings or laboratory data, the Cox proportional hazards model was used. As shown in Table 3, univariate analysis of prevaccination findings showed that the number of Bellmunt risk factors (*P* = 0.0182), the number of previous chemotherapy regimens (*P* = 0.0480), albumin (*P* = 0.0470), BAFF (*P* = 0.0003), haptoglobin (*P* = 0.0130), and IL-6 (*P* = 0.0359) were significant prognostic factors for OS. None of the other factors examined were significantly correlated with OS. Multivariate Cox regression analysis was undertaken to evaluate the influence of each

factor that showed a significant association with OS in the univariate analysis with *P*-value <0.1. As indicated in Table 3, a lower number of Bellmunt risk factors (HR, 0.379; 95% CI, 0.151–0.895; *P* = 0.0265) and a lower BAFF level in prevaccination plasma (HR, 0.249; 95% CI, 0.094–0.616; *P* = 0.0024) were significantly predictive of favorable OS. Relationships between the increase in peptide-specific CTL responses after PPV and other potential prognostic factors, including prevaccination number of chemotherapy regimens, number of Bellmunt risk factors, and albumin, BAFF, haptoglobin, IFN-γ-induced protein 10, and IL-6 levels, were examined by multivariate logistic regression analysis (Table 4). The level of BAFF and haptoglobin were predictive of the increase in peptide-specific CTL responses (BAFF: OR, 0.088; 95% CI, 0.013–0.612; *P* = 0.014; haptoglobin: OR, 15.513; 95% CI, 1.455 to 165.363; *P* = 0.023), whereas other factors were not predictive.

Discussion

As expected, the most frequent AEs in all 48 patients were dermatologic reactions at the injection site (39/48, 81%), but the SAEs were not directly associated with vaccination and

were associated with other causes, such as combined chemotherapy, targeted therapies, or cancer progression, in agreement with the our previous reports on PPV.[9-11,13] However, the incidence of anemia, increased alkaline phosphatase, and hypoalbuminemia were significantly higher in the PPV plus salvage chemotherapy group than in the PPV group. This could be mainly due to the combined chemotherapy. Higher fever was rarely observed during the PPV treatment in UTUC patients.

We have shown that survival in UTUC patients with positive CTL responses is significantly longer than in patients with negative CTL responses. Patients with positive IgG responses showed a trend of longer survival (data not shown). Collectively, the patients with both positive CTL and IgG responses showed significantly longer survival than those with positive CTL alone, positive IgG alone, or negative CTL and IgG ($P = 0.037$). We also analyzed the relationship between PPV-induced CTL responses and OS by multivariate Cox regression model. Several prevaccination factors were also provided as the control. However, the positive CTL response was not significantly prognostic factor for OS, although it was the case for BAFF (Table S2).

We have been considering the causal relationship between peptide-specific CTL responses and longer survival benefit in patients who receive PPV. This causal relationship has been repeatedly reported in our published work on PPV for various advanced cancers,[14-16] in agreement with the results in UTUC shown in this study.

We previously reported IgG boosting for non-vaccinated peptides in advanced ovarian cancer patients, in which the IgG boosting (so-called antigen spreading) well correlated with favorable clinical benefits.[17] We then examined the frequency of antigen spreading in UTUC patients treated with PPV, and found that it was observed in 19 of 40 tested patients. The MST of these 19 patients was somewhat longer than that of the remaining 21 patients without antigen spreading (13.3 months vs 7.3 months; $P = 0.310$).

In this phase II study for 48 patients with mUTUC that progressed after platinum-based chemotherapy, the MST was 7.3 months (95% CI, 5.3–13.1) with 13.0 months for patients receiving combined salvage chemotherapy (95% CI, 5.7–17.5) and 4.5 months for patients receiving PPV alone (95% CI, 1.7–10.1) ($P = 0.080$). These differences in survival could have been partly due to the differences in Bellmunt risk

Table 4. Multivariate logistic analysis for predicting peptide-specific CTL after personalized peptide vaccination in patients with metastatic upper tract urothelial carcinoma

Factor	CTL response	
	OR (95% CI)	P-value
Number of previous chemotherapy regimens	1.755 (0.155–19.881)	0.649
Number of Bellmunt risk factors	0.550 (0.050–6.090)	0.626
Albumin	1.171 (0.175–7.842)	0.871
BAFF	0.088 (0.013–0.612)	0.014
Haptoglobin	15.513 (1.455–165.363)	0.023
IP-10	1.071 (0.125–9.169)	0.950
IL-6	0.438 (0.066–2.928)	0.395

BAFF, B-cell activating factor belonging to the tumor necrosis factor family; CI, confidence interval; IL, interleukin; IP-10, interferon-γ-induced protein 10; OR, odds ratio.

Table 3. Univariate and multivariate analyses for overall survival, with prevaccination clinical findings or laboratory data, in patients with metastatic upper tract urothelial carcinoma treated with personalized peptide vaccination

Factor	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age	1.698 (0.881–3.263)	0.1127		
Number of previous chemotherapy regimens	0.497 (0.231–0.994)	0.0480	0.781 (0.327–1.714)	0.5501
Lymphocytes	1.523 (0.816–2.816)	0.1838		
Number of Bellmunt risk factors	0.429 (0.194–0.871)	0.0182	0.379 (0.151–0.895)	0.0265
Albumin	1.877 (1.009–3.504)	0.0470	1.523 (0.776–2.981)	0.2192
BAFF	0.282 (0.138–0.560)	0.0003	0.249 (0.094–0.616)	0.0024
TGF	0.684 (0.363–1.279)	0.2329		
Haptoglobin	0.449 (0.236–0.843)	0.0130	0.388 (0.108–1.331)	0.1327
IL-21	1.041 (0.555–1.966)	0.9011		
IP-10	0.564 (0.293–1.072)	0.0802	0.577 (0.235–1.418)	0.2282
IL-1β	0.828 (0.436–1.559)	0.5569		
IL-10	0.665 (0.336–1.261)	0.2143		
IL-6	0.506 (0.268–0.956)	0.0359	0.554 (0.216–1.445)	0.2229
GM-CSF	0.968 (0.512–1.807)	0.9199		
IL-5	1.048 (0.539–1.967)	0.8871		
IFN-γ	1.071 (0.555–2.017)	0.8332		
TNF-α	1.015 (0.542–1.902)	0.9630		
IL-2	0.597 (0.305–1.141)	0.1187		
IL-4	0.904 (0.473–1.693)	0.7536		
IL-8	0.667 (0.355–1.247)	0.2039		

BAFF, B-cell activating factor belonging to the tumor necrosis factor family; CI, confidence interval; GM-CSF, granulocyte/macrophage colony-stimulating factor; HR, hazard ratio; IFN-γ, γ-interferon; IL, interleukin; IP-10, IFN-γ-induced protein 10; TGF, transforming growth factor; TNF-α, tumor necrosis factor-α.

factors,[1] as the PPV plus salvage chemotherapy group had a significantly smaller number of risk factors than the PPV group (Table 1). This could also be largely responsible for the differences in early dropout from the PPV trial. Namely, one patient from the PPV plus salvage chemotherapy group ($n = 28$) dropped out before the end of the first cycle and 12 dropped out before the end of the second cycle because of disease progression, whereas the corresponding numbers were 7 and 9 in the PPV group ($n = 20$). Only one of the 13 patients with two or three Bellmunt risk factors completed the second vaccination cycle, suggesting a close relation between early dropout and a higher number of risk factors.

In addition, MST of 48 patients treated with PPV after the start of first-line chemotherapy was significantly longer than the control patients who matched to Bellmunt risk factors in our institution (27.1 months *vs* 11.2 months). Of note, the MST in patients with UC after the start of first-line chemotherapy has been reported to be 12–15 months.[18] Personalized peptide vaccinations were well tolerated without SAEs; immune-related AEs with PPV were mostly dermatologic reactions at the injection site with grade 1 or 2 severity. The safety profile of PPV is important, considering that patients with refractory mUTUC are generally older and have poor PS, impaired renal function, and multiple coexisting conditions. These data indicate that PPV for platinum-based chemotherapy refractory patients with mUTUC has the potential to prolong survival with a high proportion of patients maintaining a quality of life when combined with salvage chemotherapy.

Immunotherapy for the treatment of cancer has made significant progress over past the two decades. There is remarkable progress in cancer immunotherapy with immune checkpoint inhibitors, such as anti-CTL antigen 4, anti-programmed death-1 (PD-1) or anti-programmed death-ligand 1 (PD-L1) antibody for advanced stages of cancers, including melanoma, lung cancer, renal cell carcinoma, ovarian cancer, and bladder cancer.[19–22] Checkpoint inhibition involves targeting T-cell regulatory pathways to reduce inhibitory signaling and promote T-cell activation and enhanced antitumor activity. After the long void of no advances for advanced UC, the FDA approved atezolizumab, a PD-L1 antibody, for use in advanced UC patients who have progressed to platinum-based chemotherapy, in May 2016. This approval was based on data from a single-arm, multicenter, phase II study with 315 patients that showed significant objective response rate and durability of responses.[23] The primary outcome of ORR was obtained 15% of patients, with 5% obtaining a complete response, and presence of PD-L1$^+$ tumor-infiltrating lymphocytes was a favorable predictive biomarker for this treatment. Moreover, an anti-PD-1 antibody, pembrolizumab, has been approved as the second-line therapy for advanced UC based on the results from an open-label, international, phase III trial for patients with advanced UC undertaken by J. Bellmunt and the KEYNOTE-045 investigators (MST, 10.3 months; 95% CI, 8.0–11.8 [pembrolizumab group] *vs* 7.4 months; 95% CI, 6.1–8.3 [chemotherapy group]) (HR, 0.73; 95% CI, 0.59–0.91; $P = 0.002$).[22]

Several novel immunotherapy agents with unique mechanisms of action are currently being explored. One of them is PPV treatment, and we had previously reported that PPV induced quicker and stronger immune responses with certain clinical benefits compared to the conventional peptide vaccine with rare clinical benefits.[11] The quicker and stronger PPV-induced immune responses could be explained by its ability to induce rapid infiltration of CD45RO+ activated/memory lymphocytes into tumor sites, and PPV thereafter recruited CD45RA+ effector T cells into tumor sites to efficiently eliminate tumor cells.[24] Our previous phase I study of PPV in patients with advanced bladder cancer who failed treatment with methotrexate, vinblastine, doxorubicin, and cisplatin, showed some promising data.[13] In that trial, 10 patients received PPV treatment in the second-line setting. The disease control rate was 40% and the median OS time was 8.9 months with good immune response and minimal toxicity. Subsequently, we undertook a randomized phase II study of PPV for patients with advanced bladder cancer who failed platinum-based chemotherapy, comparing BSC. Patients treated with PPV plus BSC showed a significantly longer OS compared with those who received BSC alone, with an MST of 7.9 months in the PPV plus BSC group and 4.1 months in the BSC group.[9] In this study, the disease control rate was obtained in 21% (10/48) of patients. Personalized peptide vaccination also resulted in successful boosting of CTLs, with longer survival after the start of first-line chemotherapy than reported historical controls. These data suggest that PPV is an option in platinum-based chemotherapy refractory patients with mUTUC, as a second-line treatment.

In this study, both a lower number of Bellmunt risk factors and a lower BAFF level in prevaccination plasma were significantly associated with favorable OS. The lower number of Bellmunt risk factors was reported to be favorable for the OS of advanced UC patients regardless of PPV.[1] We reported in this study that lower levels of BAFF was favorable for the OS of advanced UTUC patients under PPV, which was consistent with the results for PPV in advanced colorectal cancer patients.[16] These results suggest that lower BAFF might be favorable for the OS of various types of advanced cancers under PPV. We newly found that the patients with positive CTL responses had lower levels of prevaccination BAFF. Of note, BAFF is a member of the tumor necrosis factor superfamily and was originally identified as an important factor responsible for B cell survival and maturation,[25,26] suggesting that higher BAFF levels could promote B cell activation rather than T cell activation. Therefore, peptide-specific CTL boosting might be promoted in patients with lower BAFF levels. Higher levels of haptoglobin seemed to be associated with positive CTL responses by multivariate analysis, but no significant differences were found in the analysis of the average score, as shown in Figure S1. This discrepancy with haptoglobin might be due, in part, to the small numbers of samples, as the OR was too high (15.5) and with a wide 95% CI (1.4–165.3) These issues, however, shall be confirmed by the relatively large numbers of patients participating in studies in near future.

In summary, this study showed that PPV for platinum-based chemotherapy refractory patients with mUTUC induced substantial peptide-specific CTL responses without severe AEs and has the potential to prolong survival. Nevertheless, this study had several limitations, including the small sample size and the single arm setting of this study without a control group. Another limitation was that combined chemotherapy during PPV treatment might affect the occurrence of immune responses and results of the prospective or predictive factors. To avoid these limitations, we are preparing a further large-scale, double-blinded, placebo-controlled randomized trial in the second-line setting.

Disclosure Statement

Masanori Noguchi is an advisory board consultant of Green Peptide Co., Ltd. Kyogo Itoh has stock ownership of Green Peptide Co., Ltd. and received research funding from Taiho Pharmaceutical Company. Takuto Yamashita is an employee of Green Peptide Co., Ltd. The other authors have no conflict of interest.

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Supporting Information

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

Fig. S1. B-cell activating factor belonging to the tumor necrosis factor family (BAFF), haptoglobin, and interleukin (IL)-6 scores in each CTL and IgG response in patients with metastatic upper tract urothelial carcinoma who received personalized peptide vaccination.

Table S1. Peptide candidates for personalized peptide vaccination.

Table S2. Multivariate analysis for overall survival (OS) with post-vaccination CTL responses in patients with metastatic upper tract urothelial carcinoma who received personalized peptide vaccination.