Clinical Cancer Research

# An Open-Label, Randomized Phase II Trial of Personalized Peptide Vaccination in Patients with Bladder Cancer that Progressed after Platinum-Based Chemotherapy

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## Abstract

**Purpose:** The prognosis of platinum-based chemotherapy–resistant metastatic urothelial cancer of the bladder remains poor. Personalized selection of the right peptides for each patient could be a novel approach for a cancer vaccine to boost anticancer immunity.

**Experimental Design:** In this randomized, open-label, phase II study, patients ages ≥18 years with progressive bladder cancer after first-line platinum-based chemotherapy were randomly assigned (1:1) to receive personalized peptide vaccination (PPV) plus best supportive care (BSC) or BSC. PPV treatment used a maximum of four peptides chosen from 31 candidate peptides according to human leukocyte antigen types and peptide-reactive IgG titers, for 12 s.c. injections (8 injections, weekly; 4 injections, bi-weekly). The primary endpoint was progression-free survival (PFS). Secondary endpoints were overall survival (OS), immune response, and toxicity.

**Results:** Eighty patients were randomly assigned to receive either PPV plus BSC ($n = 39$) or BSC ($n = 41$). No significant improvement in PFS was noted [HR, 0.7; 95% confidence interval (CI), 0.4–1.2, $P = 0.17$]. For the secondary endpoints, PPV plus BSC significantly prolonged OS compared with BSC (HR, 0.58; 95% CI, 0.34–0.99, $P = 0.049$), with median OS of 7.9 months (95% CI, 3.5–12.0) in the PPV plus BSC and 4.1 months (95% CI, 2.8–6.9) in the BSC. PPV treatment was well tolerated, without serious adverse drug reactions.

**Conclusions:** PPV could not prolong PFS, but OS appeared to be improved with low toxicity and immune responses. Further large-scale, randomized trials are needed to confirm these results.
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## Introduction

Urothelial cancer of the bladder is known to be sensitive to chemotherapy. The two first-line chemotherapy regimens for advanced or metastatic bladder cancer that have been widely adopted combine either cisplatin and gemcitabine or methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC; refs. 1, 2). Unfortunately, the vast majority of this population treated with platinum-based regimens develop progressive disease within 8 months of treatment, and the median survival is reported to be only 13 to 15 months (2). Multiple second-line options are available for patients with advanced disease, but most have limited activity in patients with platinum-refractory disease, with response rates below 20%, and none are considered in the United States (3). Recently, vinflunine has been approved outside of the United States on the basis of a large phase III trial ($n = 370$), which showed an extension of median overall survival (OS) compared with best supportive care (BSC) in the predefined eligible population (6.9 vs. 4.3 months) instead of intention-to-treat (ITT) population (4). Many phase II studies have been conducted to seek activity of alternative agents, and further study is needed to identify options in the second line.

There is remarkable progress in cancer immunotherapy with anti–programmed death-1 (PD-1) or anti–programmed death-ligand 1 (PD-L1) antibody for advanced stages of cancers,

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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## Translational Relevance

The aim of this study was to assess the potential of personalized peptide vaccination (PPV) to improve outcomes in patients with progressive bladder cancer after first-line platinum-based chemotherapy. To our knowledge, this is the first randomized phase II trial investigating the efficacy of a cancer vaccine in patients with metastatic bladder cancer after failure of platinum-based regimens. Although PPV led to a significant improvement in overall survival, progression-free survival (PFS) was not improved. However, PPV might extend PFS in patients with positive immune responses. The safety profile of PPV was predictable and manageable. The results suggest that PPV might be an important treatment option for advanced bladder cancer after failure of platinum-based regimens.

including melanoma, lung cancer, ovarian cancer, and bladder cancer (5–7). Presence of PD-L1$^+$ tumor-infiltrating lymphocytes (TIL) was a favorable predictive biomarker for these therapies. In contrast, the complexity and diversity of tumor cell characteristics and host immune repertoires seemed to limit the clinical efficacy of conventional therapeutic cancer vaccines (8). To overcome this limitation, we have developed a novel immunotherapeutic approach of personalized peptide vaccination (PPV) in which a maximum of four human leukocyte antigen (HLA)–matched peptides are selected for vaccination from a pool of 31 peptides on the basis of both HLA type and the preexisting host immunity before vaccination (9, 10). Our previous phase I study of PPV in patients with advanced bladder cancer who failed MVAC treatment showed some promising data (11). 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.

On the basis of these promising results and an unmet therapeutic need in this patient population, we conducted a multicenter, randomized phase II trial of PPV plus BSC in second-line treatment of patients with metastatic bladder cancer who had experienced progression after platinum-based regimens.

## Materials and Methods

### Patient population

Eligible patients had histologically proven metastatic urothelial carcinoma of the bladder and were documented within 12 months after first-line platinum-containing chemotherapy. Patients were also required to have measurable disease to be eligible for the trial. Eligible patients were ages $\geq$18 years, and had Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1, life expectancy of at least 12 weeks, and adequate bone marrow function, hepatic function, and renal function. Exclusion criteria included an acute infection, a history of severe allergic reactions, pulmonary, cardiac or other systemic diseases, and other inappropriate conditions for enrollment as judged by clinicians.

The study was compliant with the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice guidelines, and was conducted in an outpatient setting. The protocol was approved by institutional review boards or ethical committees at all of the institutions. All patients were Japanese and provided written informed consent before participating in this study.

### Study design and treatment

This was a multicenter, open-label, randomized phase II trial of PPV plus BSC in patients with advanced urothelial cancer of bladder progressing after platinum-containing chemotherapy (clinical trial registration, UMIN000003157). The primary endpoint was progression-free survival (PFS), and secondary endpoints were OS, safety, and immune responses to PPV. Patients were randomly assigned 1:1 to either PPV plus BSC (study arm) or BSC alone (control arm) using a minimization technique with the following factors: age (<60- or $\geq$60-years-old) and PS (0 or 1). BSC was including palliative radiotherapy, antibiotics, analgesics, corticosteroids, and transfusion. Patients who were allocated to the BSC arm received BSC and the safety was assessed at 2-week intervals. The administration schedule of PPV in the PPV plus BSC arm comprised 8 doses at 1-week intervals followed by 4 doses at 2-week intervals; total administration was 12 doses. After the protocol treatment, participants were offered continuous PPV treatment or additional palliative chemotherapy options include single-agent cisplatin, carboplatin, doxorubicin, 5-FU, ifosfamide, methotrexate, and vinblastine as subsequent therapies. Under PPV treatment, 2 to 4 peptides were selected by HLA typing and a high titer level of peptide-specific IgG to 31 candidate peptides in pretreatment serum. These 31 candidate peptides were applicable for patients with positive HLA-A2, -A3, -A11, -A24, -A26, -A31 or -A33 alleles, which cover the majority of the global population (Supplementary Table S1). The safety and immunologic effects of these 31 peptides had been confirmed in previous clinical trials (10). Each of the selected peptides was mixed with incomplete Freund's adjuvant (Montanide ISA-51VG; Seppic) and emulsified in a 5 mL plastic syringe, and a maximum of four peptides of 1.5 mL emulsion (3 mg/peptide) were injected s.c. into the lateral thigh area. All peptides were prepared under conditions of Good Manufacturing Practice using a Multiple Peptide System.

### Efficacy, safety, and immunologic assessment

The primary endpoint of PFS was defined as the time in months from randomization until objective disease progression, according to the RECIST 1.1 criteria (12), or death, whichever occurred first. Preregistration assessments included a detailed medical history, chest, and abdominal CT scan, and physical examination. Patients were monitored at each visit by history and physical examinations. CT and routine laboratory studies were performed every 2 months for efficacy assessments. The secondary efficacy endpoint of OS was calculated as the time in months from the date of randomization to death for noncensored observations (events) or to the date of last contact for censored observations. Analyses of primary and secondary efficacy endpoints of PFS and OS were based on an ITT population that included all randomly assigned patients.

Safety was assessed throughout the study by the monitoring of adverse events (assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 [NCI-CTC Ver. 4]), biochemical laboratory tests, vital signs, and physical examinations.

For a prespecified exploratory analysis, immune responses to the vaccinated peptides were assessed at pretreatment, and 7 and

15 weeks during the vaccination; peptide-specific IgG titers were measured using a Luminex system (13) and T-cell responses specific to peptides were evaluated by the IFNγ ELISPOT assay, as described elsewhere (14). In brief, 30 mL of peripheral blood was obtained before and after vaccination, and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Conray density gradient centrifugation. For measurement of IgG levels, plasma was incubated with 100 μL of peptide-coupled color-coded beads for 1.5 hours at 30°C on a plate shaker. After incubation, the mixture was washed with a vacuum manifold apparatus and incubated with 100 μL of biotinylated goat anti-human IgG (gamma chain-specific; Vector Laboratories) for 1 hour at 30°C. The plate was then washed, followed by the addition of 100 μL of streptavidin-PE (Life Techinologies) into wells, and was incubated for 30 minutes at 30°C on a plate shaker. The bound beads were washed three times followed by the addition of 100 μL of Tween-PBS into each well and detection of fluorescence intensity unit (FIU) on the beads using the Luminex system. The cutoff values of anti-peptide IgG were set to 10 FIU in 100-time diluted samples. For measurement of CTL responses, PBMCs ($1 \times 10^5$ cells/well) were incubated in 96-round-well microculture plates (Nunc) with 200 μL of medium (OpTmizer T Cell Expansion SFM; Invitrogen) containing 10% FBS (MP Biologicals), IL2 (20 IU/mL; AbD Serotec), and each peptide (10 μg/mL) for 5 days. After incubation for 5 days, the cells were harvested and tested for their ability to produce IFNγ in response to either the corresponding peptides or a negative control peptide from human immunodeficiency virus (HIV) sequence (SLYNTVATL for HLA-A2; RYLRQQLLGI for HLA-A24; RLRDLLLIVTR for HLA-A3 super-type; EVIPMFSAL for HLA-A26). Antigen-specific IFNγ secretion after 18 hours of incubation was determined by the ELISPOT assay, in accordance with the manufacturer's instructions (MBL). All assays were carried out in quadruplicate, and spots were counted by an ELISPOT reader (CTL-immunoSpot S5 Series; Cellular Technology, Ltd.). Antigen-specific T-cell responses were evaluated by the difference between the numbers of spots produced in response to each corresponding peptide and those produced in response to the control peptide. If the IgG titers to vaccinated peptides at week 7 were 10-fold higher than those in the prevaccination plasma, or more than 500 spots to the corresponding peptides were detected by the IFNγ ELISPOT assay in the PBMCs at week 7, these changes were considered to be positive immune responses.

### Statistical design and analysis

The estimated number of events was based on the following clinical hypothesis: Median PFS time of 4 months for the study arm and 2 months for the control arm. A total of 72 patients would be needed for the detection of survival superiority with a type I error rate of 5% and power of 80%, using a two-sided log-rank test and a 1:1 random assignment. Sample size estimation also took into account projected accrual time and losses to follow-up; thus, 80 patients were planned for inclusion. Time-to-event endpoints were analyzed using the Kaplan–Meier method, and between-treatment comparisons for PFS and OS were conducted using the log-rank test with a two-sided significance level of 5%. Cox proportional hazards analysis was used to calculate HRs and 95% confidence interval (CI). Data were entered into an online database with a security system by two research nurses using the electric data capturing system (System Laboratory), and statistical analyses were performed using SAS software version 9.1 (SAS Institute).

## Results

### Baseline demographics, disposition, and disease characteristics

Of the 93 patients identified and screened at the nine medical centers in Japan, 80 patients (13 patients were excluded for not meeting inclusion criteria) were enrolled and randomly assigned to receive either PPV plus BSC ($n = 39$) or BSC ($n = 41$) between February 5, 2010 and November 15, 2013. Randomization was performed centrally at the clinical research unit of Kurume University in Kurume, Japan. Demographic and baseline disease characteristics of the ITT population were generally well-balanced between the treatment arms (Table 1). Prior therapy included surgery, radiotherapy, or local bladder instillations of either chemotherapy or BCG and all had received systemic platinum-based chemotherapy. Mean courses of the platinum-based chemotherapy before enrollment to the study in the PPV plus BSC and the BSC alone were 1.44 (95% CI, 1.60–1.27) and 1.59 (95% CI, 1.84–1.33), respectively, and these difference were not significant ($P = 0.37$). All patients enrolled in the study had clear progressive disease and metastatic lesions within 1 year after first-line chemotherapy, and 46% in the PPV plus BSC and 44% in the BSC had two or more metastatic lesions at entry, including liver or bone metastasis (18% in the PPV plus BSC and 15% in the BSC). Patient disposition and compliance to allocated treatments are shown in Fig. 1. After randomization, patients who were assigned to the PPV plus BSC treatment were measured for HLA status and IgG levels, and all patients assigned to the PPV plus BSC had matched HLA and a positive status of IgG levels to ≥2 peptides

**Table 1.** Patient demographics and baseline characteristics

| | PPV + BSC ($n = 39$) | BSC ($n = 41$) |
|---|---|---|
| Age, y | | |
| Median | 65 | 65 |
| Range | 51–84 | 46–81 |
| Sex | | |
| Male | 28 (72%) | 33 (80%) |
| Female | 11 (22%) | 8 (20%) |
| ECOG PS | | |
| 0 | 31 (79%) | 33 (80%) |
| 1 | 8 (21%) | 8 (20%) |
| Hemoglobin | | |
| <10 g/dL | 10 (26%) | 9 (22%) |
| ≥10 g/dL | 29 (74%) | 32 (78%) |
| Lymphocyte count | | |
| <1,300/mm$^3$ | 19 (49%) | 14 (34%) |
| ≥1,300/mm$^3$ | 20 (51%) | 27 (66%) |
| Number of metastatic sites | | |
| 1 | 21 (54%) | 23 (56%) |
| >1 | 18 (46%) | 18 (44%) |
| Metastatic sites | | |
| Lymph node only | 15 (39%) | 16 (39%) |
| Lung and lymph node | 7 (18%) | 10 (24%) |
| Lung only | 6 (15%) | 7 (17%) |
| Others with liver or bone | 7 (18%) | 6 (15%) |
| Others without liver or bone | 4 (10%) | 2 (5%) |
| Prior cystectomy | 18 (46%) | 19 (46%) |
| Prior irradiation | 9 (23%) | 5 (12%) |
| Prior chemotherapy setting | | |
| Neoadjuvant/adjuvant only | 13 (37%) | 11 (38%) |
| Neoadjuvant and adjuvant | 3 (9%) | 3 (10%) |
| One for advanced bladder cancer | 10 (28%) | 10 (35%) |
| ≥Two for advanced bladder cancer | 9 (26%) | 5 (17%) |

NOTE: The Student $t$ test and the $\chi^2$ test were used to compare quantitative and categorical variables between the treatment arms, respectively. There was no difference in patient characteristics between the two arms.

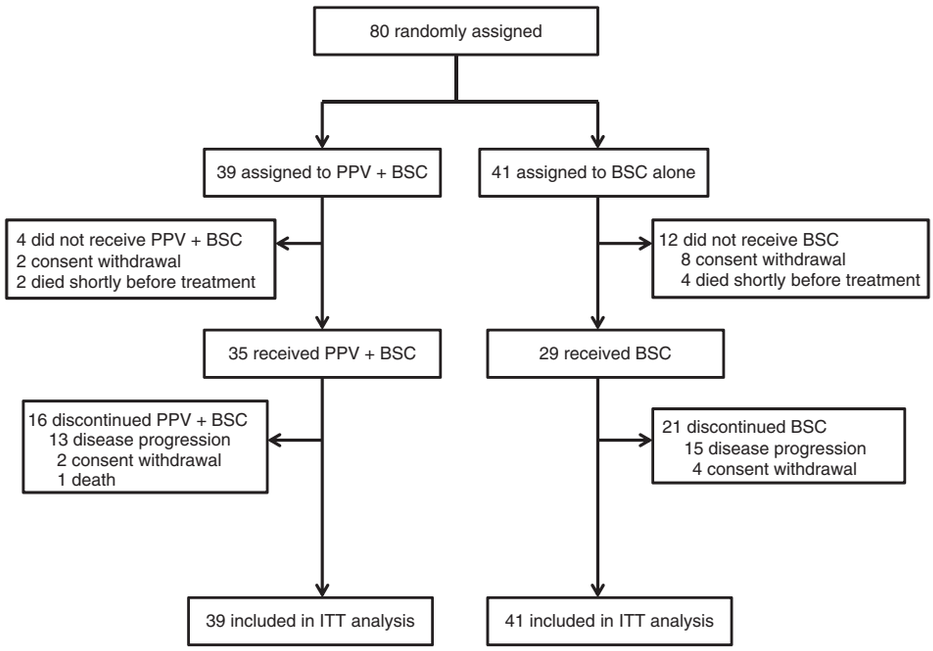


**Figure 1.**
Study flowchart.

from the 31 candidate peptides. Mean number of selected peptides in the PPV plus BSC arm was 3.8 (range, 2 to 4). Thirty-five (90%) patients received PPV plus BSC treatment and 29 (71%) patients were treated by BSC alone. Four (10%) patients in the PPV plus BSC withdrew consent ($n = 2$) or died ($n = 2$) shortly before treatment, and 12 (29%) in the BSC withdrew consent ($n = 8$) or died ($n = 4$) shortly before treatment.

### Efficacy outcomes

At the data cutoff date of April 20, 2014, 67 (84%) of 80 patients [34 (87%) in the PPV plus BSC and 33 (80%) in the BSC] had progressed or died. On the basis of investigator-derived assessment of disease response and progression using RECIST criteria, partial response (PR) was observed in 9 (23%) patients (5 patients with lung metastasis and 4 patients with lymph node metastasis) in the PPV plus BSC, but no patients in the BSC. Two of these 9 PR patients who had lung or lymph node metastasis after cystectomy had been alive without any progression until the data cutoff. No complete responses were observed in both arms. After the study treatment, 2 patients received PPV treatment and 2 patients received single-agent carboplatin. The remaining patients were treated by BSC alone. The median durations of follow-up was 6.8 months (IQR, 2.8–13.1) in the PPV plus BSC and 3.2 months (1.5–7.3) in the BSC. Median PFS times were 2.0 months (95% CI, 1.8–3.4) for PPV plus BSC and 1.8 months (95% CI, 1.3–2.3) for BSC (Fig. 2A), but this difference was not significant (HR, 0.7; 95% CI, 0.4–1.2; $P = 0.17$). At the data cutoff, 59 (74%) of 80 patients had died due to their disease, 29 (74%) of 39 in the PPV plus BSC and 30 (73%) of 41 in the BSC. For the OS, the objective of a median 3.8 months survival advantage favoring PPV plus BSC versus BSC was achieved [7.9 months (95% CI, 3.5–12.0) vs. 4.1 months (95% CI, 2.8–6.9)], and the risk of death was reduced by 42% in the study arm versus the control arm (HR, 0.58; 95% CI, 0.34–0.99; Fig. 2B). The overall $P$ value for comparing the 2 arms was $P = 0.049$ (two-sided unstratified log-rank test). Because patients were stratified before randomization according to age and PS, we recalculated the $P$ value using the stratified log-rank test; with this test, the two-sided $P$ value was 0.046, which also supports the hypothesis that the addition of PPV to BSC increases the OS in these patients. We have done per-protocol analysis, where 4 and 12 subjects (non-eligible) were removed from PPV plus BSC and BSC arm, respectively, and the results were similar to the ITT analysis as follows: Median OS time was 8.2 months (95% CI, 3.97–13.07 months) on PPV plus BSC versus 4.2 months (95% CI, 2.33–6.3) on BSC alone (HR, 0.53; $P = 0.042$), and median PFS time was 2.0 months (95% CI, 1.9–3.5 months) for PPV plus BSC and 1.8 months (95% CI, 1.4–2.3 months) for BSC alone (HR, 0.62; 95% CI, 0.36–1.1; $P = 0.081$).

### Immune responses

Peptide-specific IgG or CTL during the PPV plus BSC treatment were measured in 35 patients at pretreatment, in 29 patients at 7 weeks and in 21 patients at 15 weeks. Average total IgG titers at prevaccination, and at 7 and 15 weeks were 1,138 (SD 1,986), 4,898 (SD 9,898) and 26,260 (SD 29,450) FIUs, respectively. The average total IgG titers at 7 weeks ($P = 0.001$) and 15 weeks ($P = 0.002$) were significantly higher than that at prevaccination (Fig. 3A). Average spot numbers to the vaccinated peptide in the PBMCs by the IFNγ ELISPOT assay at the same points were 8.3 (SD 33.2), 530 (SD 987) and 300 (SD 612), respectively, and the average number of spots at 7 weeks was significantly higher than that at prevaccination ($P = 0.017$; Fig. 3B). Total IgG titers at 15 weeks significantly correlated with the CTL activity at the same point ($r = 0.6$; $P = 0.023$). Positive immune responses at 7 weeks were observed in 13 (45%) patients of the evaluable 29 patients, and 5 (71%) of 7 patients with PR showed positive immune responses at 7 weeks. To reduce the biases in the statistical analysis for comparisons of PFS or OS between immune response positive and negative groups, we have used the landmark time analysis in which the survivals from week 7 was evaluated by immune response status at week 7 (15). Week 7 was considered to be the

end of the boosting phase of the PPV treatment. Two patients who progressed before week 7 have been excluded from the evaluable 29 patients for PFS analysis. Patients with positive immune responses showed longer PFS compared with those with negative immune responses, and this difference was significant (HR, 0.32; 95% CI, 0.12–0.83; $P = 0.019$; Fig. 3C). In addition, patients with positive immune responses showed significantly longer OS compared with those with negative immune responses with median OS time of 11.3 months versus 2.2 months (HR, 0.29; 95% CI, 0.11–0.76; $P = 0.012$; Fig. 3D).

### Safety

The overall adverse events due to any cause are listed in Table 2. The most frequently reported adverse events in the PPV plus BSC were dermatologic reactions at injection sites (91%), abdominal pain (34%), increased creatinine (34%), and anemia (29%). In the BSC, the most frequently reported adverse events were abdominal pain (41%), peripheral edema (38%), anemia (38%), appetite loss (34%), fever (31%), lymphocytopenia (31%), increased creatinine (31%), and hypoalbuminemia (31%). Almost all adverse events were of grade 1 or 2 and resolved in 2 to 3 days. Thirty-two grade 3 adverse events were reported, 16 in each study arm, and all were considered unrelated to the treatment. There were no grade 4 adverse events and no treatment-related deaths.

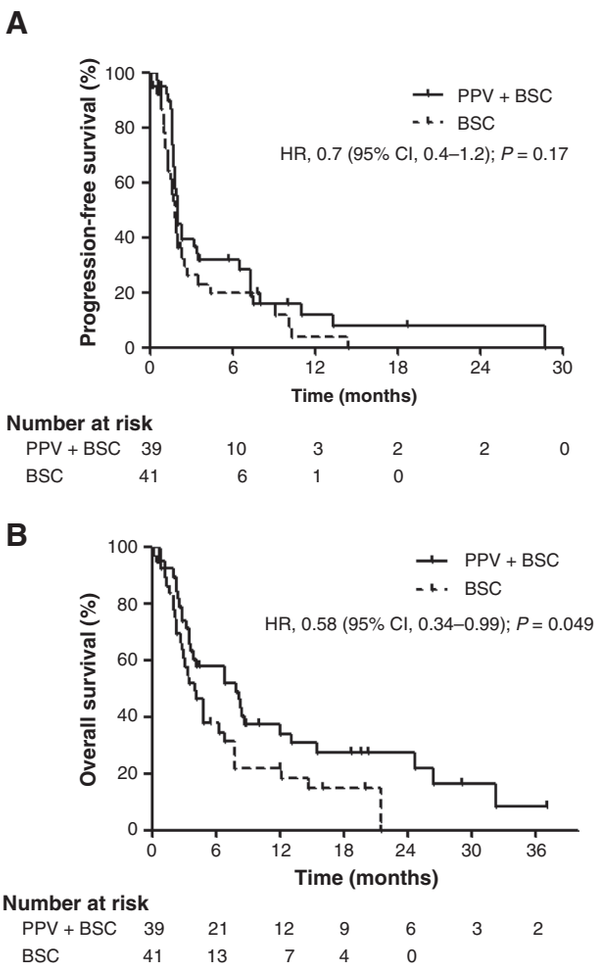


**Figure 2.**
Kaplan–Meier estimates of PFS (A) and of OS (B).

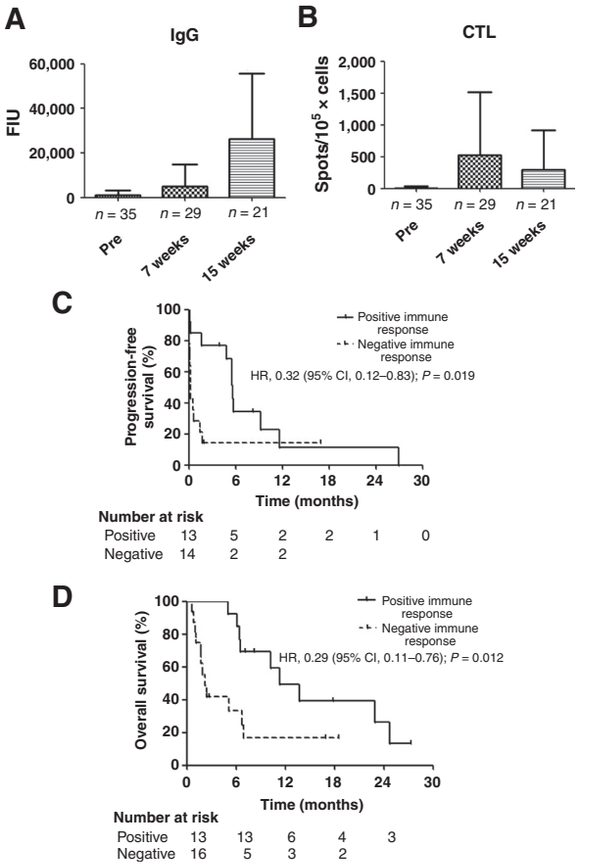


**Figure 3.**
Averages of total IgG titers (A) and of spot numbers by the IFNγ ELISPOT assay (B) before vaccination, and at 7 and 15 weeks. Evaluation of PFS (C) and OS (D) for patients with positive immune response versus those with negative immune response by the landmark method with landmark at week 7.

## Discussion

To date, only few pilot clinical trials have been conducted to evaluate vaccine candidates for bladder cancer. These trials were conducted on a limited number of study patients and only in the context advanced invasive bladder cancer (11, 16–18). This study is, to our knowledge, the first randomized phase II study investigating the efficacy of PPV treatment in patients with metastatic bladder cancer after failure of platinum-based regimens, and showed longer survival after cancer vaccination than BSC treatment.

The PPV treatment was found to be well tolerated. The most common adverse events were dermatologic reactions at injection sites of grade 1 or 2, consistent with previous studies of PPV for patients with other advanced cancers (10). No immune-related serious adverse events were observed. In the present study, the late divergence of both the PFS and the OS curves attests to a slow onset of clinical benefit in several patients, some of whom initially

**Table 2.** Adverse events

| | PPV + BSC ($n = 35$) | | | | BSC ($n = 29$) | | | |
|---|---|---|---|---|---|---|---|---|
| | **Any grade** | **Grade 1** | **Grade 2** | **Grade 3** | **Any grade** | **Grade 1** | **Grade 2** | **Grade 3** |
| Systemic symptoms | | | | | | | | |
| Injection site reaction | 32 (91%) | 30 (86%) | 2 (6%) | 0 | 0 | 0 | 0 | 0 |
| Abdominal pain | 12 (34%) | 5 (14%) | 7 (20%) | 0 | 12 (41%) | 7 (24%) | 5 (17%) | 0 |
| Edema peripheral | 8 (23%) | 4 (11%) | 4 (11%) | 0 | 11 (38%) | 5 (17%) | 6 (21%) | 0 |
| Hematuria | 8 (23%) | 2 (6%) | 4 (11%) | 2 (6%) | 7 (24%) | 3 (10%) | 2 (7%) | 2 (7%) |
| Fatigue | 7 (20%) | 3 (9%) | 4 (11%) | 0 | 8 (28%) | 3 (10%) | 5 (17%) | 0 |
| Fever | 7 (20%) | 3 (9%) | 1 (3%) | 3 (9%) | 9 (31%) | 3 (10%) | 3 (10%) | 3 (10%) |
| Nausea | 7 (20%) | 4 (11%) | 3 (9%) | 0 | 7 (24%) | 4 (14%) | 3 (10%) | 0 |
| Appetite loss | 6 (17%) | 5 (14%) | 0 | 1 (3%) | 10 (34%) | 7 | 1 (3%) | 2 (7%) |
| Diarrhea | 4 (11%) | 3 (9%) | 1 (3%) | 0 | 4 (14%) | 3 (10%) | 1 (3%) | 0 |
| Urinary tract obstruction | 2 (6%) | 0 | 0 | 2 (6%) | 3 (10%) | 0 | 0 | 3 (10%) |
| Blood/bone marrow | | | | | | | | |
| Anemia | 10 (29%) | 3 (9%) | 5 (14%) | 2 (6%) | 11 (38%) | 4 (14%) | 5 (17%) | 2 (7%) |
| Lymphocytopenia | 8 (23%) | 4 (11%) | 4 (11%) | 0 | 9 (31%) | 5 (17%) | 4 (14%) | 0 |
| Neutropenia | 2 (6%) | 1 (3%) | 1 (3%) | 0 | 3 (10%) | 2 (7%) | 1 (3%) | 0 |
| Thrombocytopenia | 3 (9%) | 1 (3%) | 2 (6%) | 0 | 4 (14%) | 2 (7%) | 2 (7%) | 0 |
| Laboratory | | | | | | | | |
| Creatinine increased | 12 (34%) | 2 (6%) | 4 (11%) | 6 (17%) | 9 (31%) | 2 (7%) | 3 (10%) | 4 (14%) |
| Hypoalbuminemia | 6 (17%) | 4 (11%) | 2 (6%) | 0 | 9 (31%) | 4 (14%) | 5 (17%) | 0 |
| ALP increased | 2 (6%) | 2 (6%) | 0 | 0 | 5 (17%) | 4 (14%) | 1 (3%) | 0 |
| AST increased | 2 (6%) | 2 (6%) | 0 | 0 | 3 (10%) | 3 (10%) | 0 | 0 |
| ALT increased | 2 (6%) | 2 (6%) | 0 | 0 | 2 (7%) | 2 (7%) | 0 | 0 |
| Hyponatremia | 1 (3%) | 1 (3%) | 0 | 0 | 2 (7%) | 2 (7%) | 0 | 0 |

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

experienced disease progression. A hypothesis to explain this observation could involve the time required to mount an effective immune response, in contrast with chemotherapy or small molecules, which can cause immediate tumor shrinkage. Delayed clinical benefit has been described for other immunotherapeutic approaches as well, including ipilimumab and sipuleucel-T (19, 20).

We had previously showed that PPV induced quicker and stronger immune responses with certain clinical benefits as compared with the conventional peptide vaccine with rare clinical benefits (10). One of the mechanisms involved in PPV-induced quicker and stronger immune responses could be explained by its ability to induce rapid infiltration of $CD45RO^+$ activated/memory lymphocytes into tumor cites (21). Furthermore, PPV thereafter recruited $CD45RA^+$ effector T cells into tumor sites to efficiently eliminate tumor cells (21). Therefore, one of the advantages of PPV could be the ability to stimulate the effector T cells for quicker and stronger immune responses as compared with the conventional peptide vaccine in which repeated vaccinations are required to prime antigen–specific naïve T cells to the functional effector T cells. Indeed, this study showed that PPV plus BSC significantly prolonged OS of metastatic bladder cancer that progressed after platinum-based chemotherapy as compared with BSC alone, and the 13 (45%) of the evaluable 29 patients with positive immune responses in this study demonstrated longer PFS and OS as compared with the remaining 16 patients without immune responses. On the other hand, the failure of inducing positive immune responses to these 16 patients could be in part due to the immune suppression primarily by myeloid-derived suppressor cells and their products, including IL6 (10, 11). In addition, the failure might be in part due to by inhibition through immune check point molecules expressed on TILs and also tumor cells. Therefore, one of the approach to overcome this type of suppression might be a sequential use of PPV and anti–PD-1 or anti–PD-L1 antibody, primarily because PPV could promote rapid infiltration of $CD45RO^+$ activated/memory T cells into tumor cites, which in turn facilitate the increase of PD-$L1^+$ TILs (7, 21).

Because pretreatment prognostic factors are known to affect objective response rate and OS in patients with urothelial cancer, the prevalence of favorable or unfavorable prognostic factors in patients enrolled onto a second-line urothelial cancer clinical trial may profoundly affect trial outcome. In the present study, patients were stratified by age and PS, and the well-known prognostic factors, such as hemoglobin levels, PS, and metastatic status were well balanced between the two arms, although these subgroups are limited by small numbers in both arms. All of patients enrolled in this study progressed after platinum-based chemotherapy, and had a good PS status and adequate lymphocyte counts. Because this study is a phase II study with a limited number of patients, clinical utility of the PPV treatment should be further verified in large-scale trials conducted in defined patient population with or without receiving the PPV treatment.

There is a debate about BSC as the control arm in randomized studies, in which lack of rigor in the BSC practices has contributed to a generation of research with ethical flaws and methodologic shortcomings that may have contributed to biased outcomes (22). Among the nine centers in the present study, site to site variability was small, and institutional treatment for the BSC was similar.

This study also has several limitations. First, we failed to detect a significant difference in PFS in the unselected population, which might be in part due to the small sample size. Second, the trial was non-blinded, and the primary endpoint of PFS was assessed by the individual investigator at each institution. Third, 20% (16/80) of randomized patients did not receive treatment. As there is no blinding, it is possible that more patients withdrew and did not receive allocated intervention (4 vs. 12), because they were randomized to the unexpected treatment. To avoid these limitations, we are preparing a further large-scale, double-blinded, placebo-controlled randomized trial in the second-line setting.

In conclusion, the results of the present study demonstrate that PPV could not prolong PFS but OS appeared to be improved with low toxicity and immune responses in the treatment of patients with urothelial cancer of the bladder who have failed prior platinum-based chemotherapy. Because this study is a small scale of randomized clinical study, further investigation of PPV in advanced or metastatic urothelial cancer is warranted.

## Disclosure of Potential Conflicts of Interest

M. Eto reports receiving speakers bureau honoraria from Bayer, GlaxoSmithKline, Novartis, Pfizer, and Sanofi. A. Yamada is an employee of Green Peptide Co. Ltd (Japan). K. Itoh reports receiving a commercial research grant from and is a consultant/advisory board member for Taiho Pharmaceutical Company Ltd. No potential conflicts of interest were disclosed by the other authors.

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