A phase I/II study of cancer peptide vaccine S-288310 in patients with advanced urothelial carcinoma of the bladder


Background: S-288310, a cancer peptide vaccine composed of two HLA-A*24:02-restricted peptides derived from two oncoantigens, DEP domain-containing 1 (DEPDC1) and M-phase phosphoprotein 1 (MPHOSPH1), was investigated in urothelial carcinoma (UC) of the bladder.

Patients and methods: Thirty-eight HLA-A*24:02-positive patients with progressive UC were enrolled in this study. In the phase I part of the study, three patients each were treated with S-288310 at 1 mg or 2 mg/peptide subcutaneously once a week to evaluate safety and tolerability. In the phase II, 32 patients were randomized to receive either 1 mg or 2 mg to evaluate the difference in cytotoxic T lymphocytes (CTL) induction and safety.

Results: S-288310 was safe and well tolerated in the phase I. Of 27 patients evaluable for immune responses in the phase II, there was no difference in CTL induction rate between the 1 mg (100%) and 2 mg (80.0%) groups. Of 32 patients receiving S-288310 in the phase II, the most frequent drug-related AE was the injection site reaction that was observed in 29 patients (90.6%), but none of the patients discontinued administration due to these reactions and no dose relationship in the frequency and severity was observed. The objective response rate of the 32 patients was 6.3% and the disease control rate was 56.3%. The median overall survival (OS) rates for patients vaccinated with S-288310 after one regimen of chemotherapy, 2 regimens, or 3 or more were 14.4, 9.1 and 3.7 months, respectively, and 32.2% of patients post first-line treatment were alive at 2 years. OS of patients who showed CTL induction to both peptides was longer than that of those with CTL induction to no or one peptide.

Conclusion: S-288310 was well-tolerated and effectively induced peptide-specific CTLs, which were correlated with longer survival for patients with UC of the bladder.

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Key words: bladder cancer, DEPDC1, immunotherapy, MPHOSPH1, peptide vaccine, S-288310
Introduction

In patients with advanced or metastatic bladder cancer, first-line therapy is platinum-based therapy including conventional and dose-dense Methotrexate, Vinblastine, Doxorubicin and Cisplatin (M-VAC) or Gemcitabine and Cisplatin (GC) [1–3]. After the first-line treatment, there is no established treatment, while vinflunine is approved in some European countries as a second-line treatment [1, 4]. Currently, immunotherapy, for instance, immune check point inhibitor and adoptive T-cell therapy are drawing an attention as a promising modality for treatment of cancer in addition to surgery, radiation therapy, chemotherapy, and targeted therapy [5, 6]. Among immune checkpoint inhibitors, anti-programmed death-1 (PD-1) and programmed death-ligand-1 (PD-L1) antibodies, which restore the killing activity of cytotoxic T cells (CTLs), have demonstrated remarkable and often durable clinical responses in various types of cancer including bladder cancer [7, 8]. The efficacy of immune checkpoint inhibitors appears to be associated with the number of pre-existing CD8+ T cells which may also upregulate PD-L1 expression in cancer and/or immune cells [8], highlighting the importance of CTL tumor infiltration as a predictor of treatment efficacy.

Two tumor-associated antigens, DEP domain-containing 1 (DEPDC1) and M-phase phosphoprotein 1 (MPHOSPH1), were identified through expression profile analysis of bladder cancers [9, 10]. From these antigens, we identified HLA-A*24:02-restricted epitope peptides that have in vitro activity to induce CTLs, which are able to kill tumor cells expressing DEPDC1 and/or MPHOSPH1 in a HLA-restricted manner [9, 10]. In a translational study, the two peptides were administered to six bladder cancer patients, and were proven to be safe and well-tolerated and to have the potential to induce peptide-specific CTLs. Notably, two patients experienced significant tumor shrinkage [11].

Based on the studies, we sought to conduct a phase I/II study with a new formulation of the peptide vaccine, S-288310, composed of these two peptides. In this study, we attempted to further evaluate safety, tolerability of S-288310 in a phase I dose-escalation study, to compare the CTL induction rate for 1 mg and 2 mg groups in an open-labeled randomized phase II study, and to ascertain some efficacy of this vaccine treatment in patients with advanced UC of the bladder.

Methods

Patients

HLA-A*24:02-positive patients of 18–80 years old with histologically confirmed to be UC that was recurrent after radical cystectomy or unresectable were eligible. Patients were required to have measurable lesions after receiving at least one prior therapy with M-VAC, GC or Carboplatin and Gemcitabine modality with documented disease progression or intolerance to these chemotherapies. Eligible patients were required to have Eastern Cooperative Oncology Group performance status 0 or 1, and also adequate hematological, renal, and hepatic functions.

Study design and treatment

The phase I open-label dose ascending study was conducted according to 3 + 3 design at doses of 1 mg and 2 mg, followed by the phase II open-label randomized study to compare the immune response between the 1 mg and 2 mg groups. A peptide (EYYELFVNI) for DEPDC1 and a peptide (IYNEYIYDL) for MPHOSPH1 were separately emulsified in Montanide ISA 51 VG (Seppic) and administered subcutaneously in the axillary or inguinal regions once a week for 12 weeks and followed until unequivocal disease progression, unacceptable toxicity, or patient refusal. Primary objectives were to evaluate safety, tolerability, and immune response. Secondary objectives were to evaluate anti-tumor effect, progression-free survival (PFS), and overall survival (OS). The primary and secondary endpoints were outlined in supplementary Figure S1 (available at Annals of Oncology online). Exploratory sub-group analyses were conducted to evaluate immune response, anti-tumor effect, and OS for each patient population enrolled after first, second, and third-line or beyond treatment. In addition, OS analysis was conducted separately for patients showing CTL induction against peptides.

Tumor response was assessed by independent central reviewers using immune-related response criteria (irRC) [12] at baseline, and subsequently every 4 weeks for the first 3 months and every 4 or 8 weeks thereafter. Adverse events (AEs) were assessed during the clinical trial and graded according to NCIC common toxicity criteria version 3.0.

The study, sponsored by Shionogi, was conducted in 16 centers in Japan in accordance with the International Conference of Harmonization of Good Clinical Practice Guidelines and the Declaration of Helsinki, with approval by ethics committees/health authorities of the participating institutions. All patients provided their written informed consent.

Immune response monitoring

Peripheral blood mononuclear cells (PBMCs) were obtained from the patients at the pre-vaccination period and at 4, 8, and 12 weeks and stored at −80°C until used. Immunological responses were assessed by enzyme-linked ImmunoSpot assay after in vitro stimulation of PBMCs with peptides [13] as shown in supplementary Figure S2 (available at Annals of Oncology online).

Expression of DEPDC1 and MPHOSPH1

Mouse anti-DEPDC1 and Rat anti-MPHOSPH1 monoclonal antibodies were developed by Shionogi and OncoTherapy Science, respectively, and the expression of MPHOSPH1 and DEPDC1 was investigated by immunohistochemistry.

Sample size and statistical analysis

The phase II part of the study was designed to measure CTL induction rate within 12 weeks after the initial dose. Based on our previous publication demonstrating that four of six patients (67%) showed positive CTL response and two (33%) negative [11], the null hypothesis is that the CTL induction rate in the combined group is 33% or below. Assuming a CTL induction rate of 67%, the study requires 30 patients to have a 90% power at a one-sided 5% significance level including dropouts.

One of the primary endpoints, CTL induction rate, was analyzed using one-sided binomial test at a significance level of 5%. The comparison of CTL induction rates between the 1 mg and 2 mg groups was carried out by Fisher’s exact test. The secondary endpoints, ORR and disease control rate (DCR), were summarized using descriptive statistics. PFS and OS were estimated using Kaplan–Meier method. The median PFS and OS, and their 90% confidence interval (CIs) were calculated. Statistical analysis was carried out using SAS Version 9.2 (SAS Inc., Cary, NC).

Results

Detection of DEPDC1 and MPHOSPH1 expression in phase I and II parts of the study

Tissue samples were available from 37 of 38 patients; among them, 91.9% (34/37) were positive for both DEPDC1 and MPHOSPH1.
Patients and vaccination. Thirty two HLA-A*24:02-positive patients were enrolled in the phase II part between June 2010 and December 2011, and randomized into two groups, 1 mg (N = 15) or 2 mg (N = 17) group (intent-to-treat population, supplementary Figure S3, available at Annals of Oncology online). Patient demographics are shown in Table 1. The date of cutoff for analysis was 24 September 2014 (at the end of the study period).

All patients were given S-288310 and the mean treatment duration was 29.8 weeks (range 2.1–141.9), and 23 patients (71.9%) were treated for 12 weeks or longer; 3 months are the predetermined period to evaluate safety and immune response. The reasons of withdrawal of nine cases within 12 weeks were progression of target disease (5 patients, 15.6%) and AEs (4 patients, 12.5%) of eosinocytosis, dyspnea, metastatic pulmonary embolism, and cancer-related pain. Except for an increase of eosinophils, these AEs deemed not related to study drug. The increase of eosinophils was considered to be a drug-related event because the event was accompanied by eosinophilic pneumonitis and the patient recovered and the number of eosinophils became normal after discontinuation of vaccination without any treatment.

Safety. AEs of any grade were reported in all patients, but most of AEs were considered to be typical events of advanced bladder cancer. Drug-related AEs were reported in 30 patients (93.8%). The most frequent drug-related AE was the injection site reaction that was observed in 29 patients (90.6%); 3.1% were grade 1 (redness), 81.3% were grade 2 (induration), and 6.3% were grade 3 (ulcer). A dose relationship with these severities was not observed. Drug-related AEs of ≥ 5% of patients are shown in supplementary Table S1 (available at Annals of Oncology online). Thirty-eight serious AEs (SAEs) were observed in 23 of the 32 patients. However, except for two SAEs in the 2 mg group, causal relationships with the study drug were ruled out. In two cases (death from worsening general condition and death by unknown reason), autopsies were conducted but the causes of death could not be clarified and a causal relationship with the study drug could not be excluded.

Immune response. Analysis of immune response was conducted in 27 patients whose samples were evaluable for immune response. CTL induction rate was 88.9% (90% CI, 73.7–96.9; 24/27) in the combined group (P ≤ 0.0001, one-sided binomial test). The induction of CTL activity was observed at as early as four weeks and became higher at 12 weeks (Figure 1A). The CTL induction rate was 100% (90% CI, 77.9–100.0; 12/12) and 80.0% (90% CI, 56.0–94.3; 12/15) in the 1 mg and 2 mg groups, respectively, which was not significantly different between the groups (P = 0.2308). The CTL activities by grade for DEPDC1 and MPHOSPH1 peptides are shown in Figure 1B. The probability of grades 2 and 3 of CTL activity was increased in a time-dependent manner until 12 weeks.

Since there was no dose-related response in CTL induction, the combined data of the 1 mg and 2 mg groups has been used for the following analyses.

Clinical response. The ORR assessed by irRC was 6.3% (2/32) and DCR based on the best overall response assessed by irRC was 56.3% (18/32). Six patients (33.3%) showed durable stable disease for more than 12 weeks. As shown in Figure 2A and B, two patients showed tumor regression and reached irPR at week 8 and 20, which were maintained until week 32 and 36, respectively. The median PFS for the 32 patients receiving S-288310 was 1.9 (90% CI: 1.2–2.2) months and the median OS was 9.4 (90% CI: 4.2–11.9) months. Figure 3A shows OS according to the number of treatment regimens prior to this vaccine treatment. Median OS of patients with one-line of treatment was 14.4 months, which was numerically longer than those with two-lines of treatment (9.1 months) and with three-lines or more of treatment (3.7 months). In the group with one regimen, the 2-year survival rate was 32.2%. In contrast, there was little difference in median PFS and CTLs induction among the three groups (supplementary Table S2, available at Annals of Oncology online).

| Table 1. Patient demographics and baseline characteristics in the phase 2 part of the study (ITT population) |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | S-288310 1 mg   | S-288310 2 mg   | Total           |
|                                | n = 15          | n = 17          | n = 32          |
| Age, years (median)            | 70.0 (55–79)    | 67.6 (50–79)    | 68.5 (50–79)    |
| Sex                             |                 |                 |                 |
| Male                            | 12 (80.0%)      | 14 (82.4%)      | 26 (81.3%)      |
| Female                          | 3 (20.0%)       | 3 (17.6%)       | 6 (18.8%)       |
| Performance status (ECOG)      |                 |                 |                 |
| 0                               | 12 (80.0%)      | 11 (64.7%)      | 23 (71.9%)      |
| 1                               | 3 (20.0%)       | 6 (35.3%)       | 9 (23.4%)       |
| Hemoglobin (< 10 g/dl)          | 7 (46.7%)       | 2 (11.8%)       | 9 (28.1%)       |
| ≥ 10 g/dl                       | 8 (53.3%)       | 15 (88.2%)      | 23 (71.9%)      |
| Any visceral metastatic site    | 4 (26.7%)       | 9 (52.9%)       | 13 (40.6%)      |
| Liver                           | 0 (0.0%)        | 1 (5.9%)        | 1 (3.1%)        |
| Bone                            | 3 (20.0%)       | 3 (17.6%)       | 6 (18.8%)       |
| Lung                            | 2 (13.3%)       | 7 (41.2%)       | 9 (28.1%)       |
| Distant lymph node              | 6 (40.0%)       | 10 (58.8%)      | 16 (50.0%)      |
| Prior chemotherapy regimen     |                 |                 |                 |
| 1st-line                        | 8 (53.3%)       | 11 (64.7%)      | 19 (59.4%)      |
| 2nd-line                        | 3 (20.0%)       | 4 (23.5%)       | 7 (21.9%)       |
| 3rd-line and beyond            | 4 (26.7%)       | 2 (11.8%)       | 6 (18.8%)       |
| ECOG, Eastern Cooperative Oncology Group; ITT, intent-to-treat.
To test for correlation of CTL induction with the OS, a landmark analysis was conducted for survival curves by the number of peptides to which CTLs were induced at month 3, and in which, analysis was limited to patients who were alive for at least 3 months after the first dosing (Figure 3B). Survivors for 24 months and more were only observed in patients revealing CTL induction to both peptides.

Further analysis of OS by the levels of CTL activity for each peptide revealed that the OS of patients with the 1+ level was not clearly longer than those who have no CTL activity (supplementary Figure S4A and B, available at Annals of Oncology online). When combined the survival data of negative and 1+, the OS of patients with grade 2+/3+ was significantly longer than that of the 0/1+ patients (supplementary Figure S4C and D, available at Annals of Oncology online). Using additional criteria that patients with 2+/3+ CTL induction were defined as positive, survival rate in patients having CTL positive responses to both peptides was improved (supplementary Figure S5, available at Annals of Oncology online).

Discussion

In this study, we investigated the safety, immune response, and anti-tumor effect of S-288310 composed of two HLA-A*24:02-restricted peptides as used in the investigator-initiated study [11]. As previously reported, immunohistochemical analysis in this study confirmed high and frequent expression of DEPDC1 and MPHOSPH1 in tumor tissues with a positive rate of 97.3% and 94.6%, respectively, suggesting there is little need to select patients by antigen expression before vaccination. CTLs induction for at least one peptide was observed in all of the patients who received 1 mg and 80% of the 2 mg group for a period of 12 weeks after the beginning of vaccination, which was not statistically

Figure 1. Time-dependent dynamics of CTL induction rate in patients showing positive CTL activity to either one of the two peptides among all evaluable patients (N = 27) for the 1 mg and 2 mg groups (A) and CTL activity by grades of negative, 1+, 2+ and 3+ (B).

Figure 2. Objective response in two patients who showed irPR. (A) Chest computed tomography images of this case show durable tumor shrinkage of multiple lung metastases until 28 weeks. (B) Pelvic magnetic resonance images of this case show gradual and durable tumor shrinkage of multiple local recurrences at the subcutaneous tissue and the buttocks until 36 weeks.
notherapies generally show delayed response and are effective in prolonging survival without unequivocal tumor shrinkage, and ORR between S-288310 and vinflunine; 1.7 and 3.0 months, for median PFS and 10.5% and 8.6% for ORR, respectively. Although the patient background for the vinflunine study is not the same as that for S-288310 study, the survival rate at year 2 of the same as that for S-288310 study, the survival rate at year 2 of 32.2% in this post first-line group seems to be better than that of the same as that for S-288310 study, the survival rate at year 2 of the S-288310 study, 14.4 months, which is longer than that of vinflunine-treated patients (6.9 months) in a reported phase III study [4], although there was no difference in the median PFS and ORR between S-288310 and vinflunine; 1.7 and 3.0 months for median PFS and 10.5% and 8.6% for ORR, respectively. Although the patient background for the vinflunine study is not the same as that for S-288310 study, the survival rate at year 2 of 32.2% in this post first-line group seems to be better than that of vinflunine (around 10%). These findings suggest that S-288310 may prolong survival without unequivocal tumor shrinkage, which is consistent with previous reports indicating that immunotherapies generally show delayed response and are effective in slowing tumor growth, resulting in prolongation of survival [16]. Recently, a number of immunosuppressive pathways, referred to as ‘immune checkpoints’, have been reported to be active in the tumor microenvironment [17]. Anti-PD-1/PD-L1 antibodies which block this pathway demonstrated remarkable anti-tumor response by restoring the activity of CTLs in various tumors. In bladder cancer, anti-PD-L1 antibody, Atezolizumab currently approved by the Food and Drug Administration was reported to exert a marked anti-tumor effect particularly in patients with high expression of PD-L1 [18]. Since the efficacy of PD-1/PD-L1 therapy appears to be associated with the number of cytotoxic TILs that upregulate PD-L1 of tumor cells and tumor-infiltrating cells in response to IFN-gamma produced by activated CD8 T cells [8, 19], anti-PD-1/PD-L1 antibodies may improve its efficacy in combination with immune stimulators to activate anti-tumor T cell immune responses [5, 20]. Furthermore, the efficacy of anti-PD-1 antibodies was also reported to correlate with higher non-synonymous mutation burden and DNA repair pathway mutations in tumors, suggesting that immunogenic neoantigens may have a greater capacity for inducing tumor specific CD8+ T cells than tumor-associated overexpressing wild-type antigens [21, 22]. Ongoing and future clinical studies will further evaluate the potential of neoantigens to induce potent and durable anti-tumor immune response. However, we show here that exogenously administered non-mutated epitope peptides effectively induced the epitope-specific CTLs in cancer patients. Notably, two patients experienced durable tumor shrinkage, and some patients showed temporal tumor regression or slowdown of tumor growth following the tumor progression (data not shown). These findings suggest that repeated vaccinations of non-mutated epitopes still have potential in increasing cytotoxic T cells that have the killing activity against tumor cells. This kind of peptide vaccine treatment can be applied to a substantial proportion of patients and may have a synergistic effect when combined with an immune checkpoint inhibitor.

In conclusion, S-288310 is safe, well-tolerated, and efficiently activate CTLs in patients with UC of the bladder, which may be associated with longer survival. Our findings support the concept of cancer peptide vaccine to prime anti-tumor responses and warrant further clinical trials.

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References