induce a hypersensitivity reaction (HSR), and the risk of HSR increases in proportion to the number of carboplatin cycles [8, 9]. HSR symptoms vary from skin rash, dyspnea, hypotension, and anaphylactic shock to death.

In several reports, patients who experienced platinum-related HSRs were successfully desensitized to carboplatin [10–15]. However, the desensitization methods differed somewhat between these reports, although almost all were accomplished through a planned chemotherapy schedule. In Japan, there are relatively few reported cases of desensitization. We therefore used a 4-step, 4-h desensitization protocol at our institution and reviewed its safety, the number of cycles, effects, and toxicity [16].

Materials and methods

Patients

We retrospectively assessed our experience with carboplatin desensitization in patients with gynecological malignancies who experienced HSRs in previous chemotherapy. We reviewed the patients who started the desensitization between January 2010 and December 2013. The initial HSR symptoms varied and included skin rash, hypotension, dyspnea, nausea, diarrhea, palpitations, and tachycardia. Those who developed symptoms during or immediately after carboplatin infusion were examined by a primary physician. If the symptoms were consistent with a diagnosis of HSR, we presented the treatment choice of carboplatin desensitization, other monotherapy, or best supportive care (BSC) alone to patients who had experienced HSRs in previous carboplatin-based chemotherapy. All patients were explained the risk (in the worst case, death) and benefits of carboplatin desensitization and signed written informed consent if they agreed to undergo the desensitization. This retrospective analysis was approved by the Institutional Review Board of Hyogo Cancer Center.

Desensitization protocol

The 4-h desensitization protocol included infusion of 4 carboplatin solutions of varying dilutions. This protocol was based on the report by Confino-Cohen et al. [11]. In that study, they used a 4-step 6-h protocol and only 1 patient experienced mild HSR. The problem with their protocol was that the desensitization procedure was too long. In another report, desensitization was performed within 3.8-h [12]. Nevertheless, that 12-step protocol was complicated. To simplify and shorten the procedure with the same degree of safety, we devised a 4-step 4-h protocol.

Carboplatin was initially dissolved in 250 mL of 5 % glucose, and a 25-mL aliquot was serially diluted with 225 mL of 5 % glucose to obtain final dilutions of 1/10, 1/100, and 1/1000. Each solution, starting with the 1/1000 dilution, was infused over a period of 1 h. Before desensitization, all patients were premedicated with granisetron, dexamethasone, ranitidine, and diphenhydramine (Table 1).

All patients was received the treatment in the room near the nurse station, and an emergency cart was standing by the room for any possible complications. All patients stayed overnight and left our hospital on the next day after desensitization if they had no adverse events.

Clinical assessments

Performance status was assessed on the basis of the Eastern Cooperative Oncology Group criteria and clinical state according to the International Federation of Gynecology and Obstetrics staging criteria. Adverse effects were analyzed using the National Cancer Institute Common Toxicity Criteria for Adverse Events (ver. 4.0). Treatment efficacy was assessed according to the Response Evaluation Criteria in Solid Tumors (ver. 1.1).

Results

From January 2010 to December 2013, a total of 482 patients with gynecological malignancies were treated with carboplatin at Hyogo Cancer Center, of whom 31 (6.4 %) were diagnosed with HSR.

The selection of patients is described in Fig. 1. Of the 31 patients, 13 experienced HSRs while being treated in our

Table 1 The desensitization regimen (with paclitaxel)

①	Normal saline 50 mL + dexamethasone 24 mg + ranitidine 50 mg	15 min
②	Normal saline 100 mL + granisetron 1 mg	15 min
③	Normal saline 500 mL + paclitaxel 175 mg/m^2 [a,b]	3 h
④	5 % glucose 250 mL (0.1 % solution)	1 h
	Remove 25 mL of 5 % glucose and put in 25 mL of ⑤	
⑤	5 % glucose 250 mL (1 % solution)	1 h
	Remove 25 mL of 5 % glucose and put in 25 mL of ⑥	
⑥	5 % glucose 250 mL (10 % solution)	1 h
	Remove 25 mL of 5 % glucose and put in 25 mL of ⑦	
⑦	5 % glucose 250 mL (100 % solution)	1 h
	Carboplatin area under curve 6 (AUC 6)	
⑧	Normal saline 50 mL	Flush

[a] Patients take 50 mg of diphenhydramine 30 min before infusion of paclitaxel

[b] 5 % glucose 250 mL + docetaxel 75 mg/m^2 or liposomal doxorubicin 30 mg/m^2 in an hour were also used

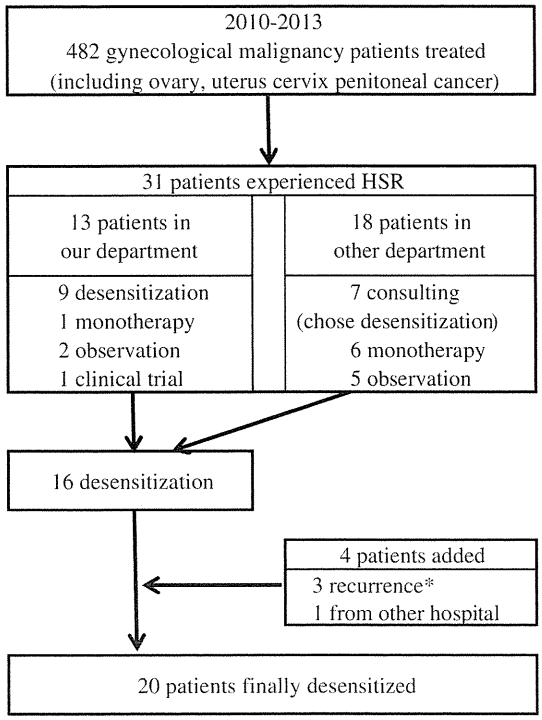

Fig. 1 Schematic showing how the patients who received desensitization were chosen and enrolled. *Asterisk* indicates the patients who experienced HSRs before 2010 and were not included in the 31 patients who experienced HSRs between January 2010 and December 2013

department. 1 patient was not eligible for the desensitization because of criteria specified in clinical trial protocols. The other 12 patients were all presented with the choice of desensitization, other monotherapy and BSC; 9 patients selected desensitization, 1 chose other monotherapy, and the last 2 patients chose BSC. The other 18 experienced HSRs while receiving treatment in other department. 7 patients were referred to us, consulted with us, and chose desensitization. Separately from the 482 patients who experienced HSRs between 2010 and 2013, 3 patients experienced HSRs before 2010, and were referred to our department for desensitization after recurrence. The last patient experienced HSR in another hospital, and was referred to our hospital. Thus, a total of 20 patients (median age 62 years, range 43–74 years) underwent carboplatin desensitization.

The patients' characteristics are listed in Table 2. 17 patients had a diagnosis of ovarian cancer or a primary peritoneal cancer and 3 had a diagnosis of uterine corpus cancers (endometrioid carcinoma, serous adenocarcinoma, or carcinosarcoma) according to postoperative histopathological examination. 3 patients experienced carboplatin HSRs during initial treatment, whereas 17 experienced HSRs after recurrence. Each patient received a median of 11 carboplatin cycles (range 2–16 cycles). The initial HSR Grade varied from 1 to 3 (Grade 1, $n = 15$; Grade 2, $n = 4$; and Grade 3, $n = 1$). In total, secondary episodes of HSR were observed during 16 cycles in 10 patients. The details of each HSR episode are listed in Table 3; the treatment results are listed in Table 4. In the first cycle of desensitization, 19 (95 %) of 20 patients successfully completed treatment, although 3 patients experienced some side effects. Two of these 3 patients had a pruritic skin rash and paresthesia during infusion (One with the 1/100 diluted solution and the other one with the 1/10 diluted and undiluted solutions). These symptoms were relieved only by suspending the infusion, followed by antihistamine agent administration and restarting treatment, and these patients eventually completed the initial desensitization protocol. Nevertheless, 1 patient developed cold sweat, palpitations, fecal incontinence, and hypotension after infusion with the 1/1000 diluted solution; she therefore decided to discontinue treatment because more severe symptoms were anticipated as a result of continuing the desensitization and because the treatment was palliative rather than curative. Her symptoms abated after taking only D-chlorpheniramine maleate and hydration, without epinephrine.

Overall, 83 desensitization cycles were administered, of which 67 were completed without any adverse effects. Although 12 patients experienced some adverse effects, they completed treatment with only temporary interruption or some changes in medication. The chemotherapy regimens associated with HSRs were carboplatin desensitization with paclitaxel ($n = 11$) and liposomal doxorubicin ($n = 1$). 4 cycles were also accompanied with some other adverse effects; treatment was therefore discontinued. All 4 of these patients received carboplatin desensitization with paclitaxel. Finally, 16 (80 %) of the 20 patients completed a planned schedule.

Toxicity

Although 1 patient experienced Grade 3 toxicity (hypotension), she quickly recovered without epinephrine after the infusion was stopped and experienced no sequela. 6 patients developed skin rash, itching, paresthesia, blushing, and cough, assumed to be cytokine-release syndrome which required either stopping treatment or initiation of some medication (Grade 2), 1 patient developed hypoxia (Grade 2), and another patient developed abdominal pain (Grade 2). Skin rash, itching, and paresthesia were observed in 7 patients but did not require either treatment interruption or some additional medication (Grade 1). Diarrhea, upper limb edema, pharyngeal discomfort, cold sweat, palpitations, and fecal incontinence were also each observed in 1 patient (Grade 1). There were no treatment-related deaths.

Table 2 Initial HSRs

ID	Age	Diagnosis/ malignancy	Stage		CBDCA cycle	Regimen[c] (number)[d]	Initial HSR symptoms	HSR Grade
1	69	Peritoneal	IIIc	Recurrence (PFI 11 M)	16	TC (3)	Palpitation, tachycardia	1
2	68	Peritoneal	IIIc	Recurrence (PFI 21 M)	7	TC (2)	Dyspnea, hypotension	3
3	64	Ovarian	IIIc	Recurrence (PFI 39 M)	16	TC (3)	Cold sweat, hypoxia, diarrhea, nausea	2
4	70	Ovarian	IIIc	Initial[a] (recurrence)	12	TC (2)	Itching, paresthesia, abdominal discomfort	2
5	62	Corpus (carcinosarcoma)	IIIa	Recurrence (PFI 13 M)	8	TC (2)	Nausea, vomiting, dizziness, skin rash	2
6	62	Ovarian	IIIc	Initial	5	ddTC (1)	Feeling of warmth, dyspnea, abnormal ECG	1
7	47	Peritoneal	IIIb	Recurrence (PFI 12 M)	16	TC (5)	Palpitation, nausea, hypoxia, skin rash	2
8	43	Ovarian	IIIc	Recurrence (PFI 7 M)	15	CBDCA (3)	Skin rash	1
9	61	Ovarian	IIIc	Recurrence (PFI 10 M)	11	TC + α[b] (2)	Hypotension, hypoxia, tachycardia	2
10	53	Corpus (endometrioid)	IV	Recurrence (PFI 18 M)	12	TC (1)	Cold sweat, palpitation, hypoxia, skin rash	2
11	58	Ovarian	IIIc	Recurrence (PFI 18 M)	12	TC (2)	Abdominal pain, nausea, flushing	1
12	74	Ovarian	IIIc	Recurrence (PFI 10 M)	11	CBDCA (3)	Skin rash	1
13	70	Ovarian (clear cell)	Ib	Recurrence (PFI 113 M)	3	DC (1)	Skin rash	1
14	67	Ovarian (clear cell)	IIIc	Recurrence (PFI 37 M)	8	CBDCA (4)	Skin rash, itching, paresthesia	2
15	52	Ovarian	IV	Recurrence (PFI 45 M)	9	CBDCA + PLD (2)	Skin rash, itching, paresthesia	2
16	52	Ovarian	IIIc	Initial	6	ddTC (1)	Skin rash, itching, paresthesia	2
17	67	Corpus	IIIc	Recurrence (PFI 25 M)	2	TC (2)	Dyspnea, hypoxia	2
18	59	Ovarian	IIc	Recurrence (PFI 110 M)	15	TC (3)	Epigastric distress, itching, paresthesia, vomiting	2
19	54	Ovarian	IIIc	Recurrence (PFI 73 M)	10	TC (4)	Skin rash, itching, paresthesia	2
20	60	Ovarian	IV	Recurrence (PFI 23 M)	15	TC + α[b] (3)	Skin rash, itching, paresthesia	2

TC carboplatin + paclitaxel, *DC* carboplatin + docetaxel, *CBDCA* carboplatin monotherapy, *PLD* pegylated liposomal doxorubicin, *ddTC* dose-dense TC, *PFI* platinum-free interval, *M* month

[a] This patient twice experienced platinum-sensitive relapse after desensitization administration

[b] Combination chemotherapy with carboplatin, paclitaxel, and investigating drug

[c] *Regimen* means the chemotherapy which patients received when HSRs occurred

[d] *Regimen (number)* means the number of prior regimens before desensitization

Outcomes

16 (80 %) of the 20 patients completed a planned schedule, 11 died (10 patients died from primary cancer and 1 patient from infection), 5 are receiving other treatments, 1 developed brain metastases and chose palliative care, and 3 were observed. The overall response rate [complete remission (CR) + partial remission (PR))/(CR + PR + stable disease (SD) + progressive disease (PD)] was 68.75 % (patients who discontinued desensitization were excluded), and the disease control rate (CR + PR + SD)/(CR + PR + SD + PD) was 93.75 %.

Table 3 Second HSRs

Patient ID	Cycle	Dilution	Symptoms	Grade	Result
2	1	1/1000	Cold sweat, palpitation, fecal incontinence, hypotension	3	Discontinued
3	3	1/1000, undiluted	Cold sweat, skin rash, itching, paresthesia	2	Discontinued
4	4	After drip	Skin rash	1	Completed
5	2	Undiluted	Abdominal pain, diarrhea	2	Discontinued
7	2	Undiluted	Skin rash	1	Completed
7	3	1/1000	Discomfort of pharynx, upper limb edema	1	Completed
7	4	Undiluted	Skin rash	1	Completed
7	5	Undiluted	Itching, paresthesia	2	Completed
10	2	1/1000, 1/100	Skin rash, hypoxia	2	Discontinued
11	1	1/10, undiluted	Skin rash, itching, paresthesia	2	Completed
13	3	1/10, after drip	Skin rash, itching, paresthesia	1	Completed
14	2	Undiluted	Itching, paresthesia	1	Completed
14	4	Undiluted	Skin rash, itching, paresthesia, dry cough	1	Completed
14	5	Undiluted	Cough, flushing	2	Completed
14	6	1/10	Cough, flushing	2	Completed
20	1	1/100	Skin rash, itching, paresthesia	2	Completed

Table 4 Treatment results

Patient ID	Regimen	Cycles	Result	Best response (PFI months)	Recurrence	After treatment	Outcome
1	PTX[b]	2	Completed	CR (6)	Yes	Brain Meta → WBRT	Deceased
2	PTX[b]	1	Discontinued	SD	Yes	DP, PLD, GEM, TPT	Deceased
3	PTX[b]	3	Discontinued	SD	Yes	No treatment	Deceased
4-1[a]	PTX[b]	4	Completed	PR (14)			
4-2	PTX[b]	2	Completed	PR (11)			
4-3	PTX[b]	2	Completed	PR (–)	No	No treatment	Deceased[c]
5-1[b]	PTX[b]	4	Completed	PR (8)			
5-2	PTX[b]	2	Discontinued	SD	Yes	PTX	Deceased
6	PTX[b]	3	Completed	CR (30)	Yes	CBDCA + PLD (desensitization)	Alive
7	PTX[b]	6	Completed	PR (9)	Yes	CPT-11, GEM	Deceased
8	CBDCA	5	Completed	PR (4)	Yes	PLD	Deceased
9	PTX[b]	3	Completed	CR (11)	Yes	Brain Meta → WBRT	Alive
10	PTX[b]	2	Discontinued	SD	Yes	PTX	Alive
11	PTX[b]	2	Completed	SD	Yes	GEM, PLD	Deceased
12	PTX[b]	3	Completed	PR (2)	Yes	GEM	Deceased
13	DTX[b]	3	Completed	PD	Yes	PLD	Alive
14	PTX[b]	6	Completed	SD	Yes	GEM	Deceased
15	PLD[b]	6	Completed	CR (14)	Yes	GC (desensitization)	Alive
16	PTX[b]	3	Completed	PR (5)	Yes	PLD	Deceased
17	PTX[b]	6	Completed	CR (–)	No	No treatment	Alive
18	PTX[b]	3	Completed	SD	Yes	PLD	Alive
19	PLD[b]	6	Completed	PR (–)	No	Observation	Alive
20	PLD[b]	6	Completed	SD	No	Observation	Alive

PTX paclitaxel, *DTX* docetaxel, *CBDCA* carboplatin monotherapy, *PLD* pegylated liposomal doxorubicin, *PFI* progression-free interval, *GC* carboplatin + gemcitabine, *Meta* metastasis (or metastases), *WBRT* whole brain radiotherapy, *DP* cisplatin + docetaxel, *GEM* gemcitabine, *TPT* topotecan, *CPT-11* irinotecan

[a] This patient twice experienced a platinum-sensitive relapse after desensitization

[b] This patient once experienced a platinum-sensitive relapse after desensitization

[c] This patient died because of infection (not because of cancer)

Discussion

In this study, we present the results of our 4-step, 4-h carboplatin desensitization protocol. In our patient cohort, the majority completed the desensitization cycles without any severe adverse events. The response rate was much higher compared with single-agent non-platinum chemotherapy for platinum-sensitive ovarian cancer. Overall, the majority (>80 %) of patients were able to complete the planned cycles, and of these, >80 % patients could control the diseases and gain relief from chemotherapy at least once.

Currently, there is no standard protocol for carboplatin desensitization because only a handful of reports on slightly more than 200 cases are available (Table 5). Rose et al. [13], Confino-Cohen et al. [11], Gomez et al. [14], and the authors of the present study used a 4-step protocol beginning with a 1/1000 dilution of a platinum-based drug. In addition, Lee et al. [12] and Castells et al. [10] used a 12-step protocol at the same institution where Hesterberg et al. [15] modified this protocol so that it had 8 or 10 steps according to skin test results. Nevertheless, treatment duration varied between the protocols.

Compared with the protocols used in other studies, the strengths of our protocol include simplicity, short duration, and a similar HSR rate. Moreover, the total duration of desensitization with paclitaxel was 7.5 h; complete desensitization could therefore be accomplished in a day. If the desensitization treatment requires a longer period, overnight administration remains an option, although night-time treatment may require additional hospital staff.

Some reports state that cisplatin [17] and nedaplatin [18] are a treatment of choice for patients with a history of HSRs. In contrast, 1 report shows that a patient who experienced carboplatin-induced HSR and received cisplatin treatment died [19]. The cause of death was not reported in a patient treated with nedaplatin; however, the effects of treatment were worse than those of carboplatin with paclitaxel or cisplatin with paclitaxel. Furthermore, patients who experience HSRs with carboplatin also do so with nedaplatin [20], and the response rate is lower than that reported in our study.

Although the treatment completion rate in our study was slightly lower than that in some previous reports, we did not restart the desensitization cycles once HSRs occurred and oxygen, corticosteroids, and/or epinephrine were required. In most cases, we discontinued treatment because more severe adverse events were anticipated. As a result, no patient even needed β_2-agonists, corticosteroids, or epinephrine. Lee et al. [12] and Castells et al. [10] (both from the same institution) restarted desensitization treatment in 3 patients after symptom resolution in the 2 reports, even if epinephrine, corticosteroids, β_2-agonists, or

Table 5 Comparison with other reports

References	*n*	Initial dilution degree	Outpatient or inpatient	No. of steps	Duration (h)	Completed initial desensitization	Cycles accompanied by HSRs	Completed cases	Completion rate (%)	Objective response rate (%)	Epinephrine use
Rose et al. [13]	33	1/1000	Inpatient[a]	4	16.5	32/33	–	29/33	88	79	No
Confino-Cohen et al. [11]	23	1/1000	–	4	6	19/20	1/81[c]	19/20	95	–	–
Lee et al. [12]	44	1/100	Both[b]	12	5.8 3.8	–	19/127	44/44	100	38.5	○[d]
Castells et al. [10]	60	1/100	Both[b]	12	5.8	–	134/413	60/60	100	–	○[d]
Hesterberg et al. [15]	30	1/100	Inpatient	8–10	11	30/30	20/106	29/30	96	–	Δ[e]
Gomez et al. [14]	7	1/1000	–	4	2	5/7	–	4/7	57	–	–
This report (2014)	20	1/1000	Inpatient	4	4	19/20	16/83	16/20	80	68.8	No

– Data were not presented in the reports

[a] With cardiovascular team support

[b] Initial desensitization was administered in medical intensive care unit (MICU)

[c] Only severe events were reported

[d] The use of epinephrine was allowed (and it was used for HSR treatment)

[e] The use of epinephrine was allowed (but not used)

oxygen were administered. In fact, the incidence of HSRs in their study was similar to ours. Standard guidelines for discontinuation of desensitization treatment are therefore needed to compare the effectiveness and completion rates between desensitization protocols.

Our study has some limitations. Because this is a retrospective study, the patients' characteristics were not controlled, and disease, stage, and the number of treatments varied; the treatment effects are therefore difficult to compare with other studies. Moreover, the number of treatment cycles and the decision to discontinue treatment were both dependent on a primary physician's judgment. Finally, the carboplatin skin test is not reimbursed by the Japanese national health insurance system.

The HSR rate in previous reports was 6.5 % in cycle 6 and 27 % in cycle 7 and beyond [21]. The HSR rate of 6.4 % observed at our institution is therefore similar to past reports and this result showed that our diagnoses would not be overdiagnosed or underdiagnosed.

Our findings suggest that desensitization is a good option for patients with a platinum-sensitive relapsed ovarian cancer and a history of carboplatin-induced HSRs. Furthermore, the response rate to desensitization is higher than that to single-agent nonplatinum chemotherapy, and, with a well-trained staff, desensitization could be performed safely. Nevertheless, to accurately evaluate treatment efficacy and safety, a prospective study should be conducted in the future to address the following 3 points. First, diagnostic criteria of HSRs must be drawn up because the symptoms vary; an examination to rule out HSRs is therefore warranted. Second, a standardized protocol is needed for comparison, but the number of cases remains limited. Finally, the criteria for resumption of treatment must be established. We believe that the resumption of desensitization after epinephrine use is too aggressive, considering the palliative nature of treatment of a recurrent cancer. However, it must be noted that the symptoms of HSR vary. Therefore, if nonhematological toxicity is of Grade 3 or higher or is refractory to supportive treatment, desensitization should be discontinued.

It should be noted that this report does not show that cisplatin and oxaliplatin can be desensitized in the same manner. This question should be examined as a separate treatment.

Our 4-step, 4-h protocol is safe and effective for patients with a history of carboplatin-induced HSRs. A prospective evaluation including criteria for discontinuation of treatment would be warranted. Desensitization should be performed by trained staff because it carries a risk of HSRs.

Conflict of interest The authors declare that they have no conflict of interest.

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Phase II trial of oral etoposide plus intravenous irinotecan in patients with platinum-resistant and taxane-pretreated ovarian cancer (JCOG0503) ☆

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HIGHLIGHTS

- A phase 2 study with oral etopocide and IV irinotecan for 60 pts (14 elderly) with platinum-resistant ovarian cancer
- The response rate, PFS, and OS was 21.7% (less than boundary), 4.1 and 11.9 months, respectively.
- Febrile neutropenia and possible TRDs occurred in 11 (4 elderly) and 3 (2 elderly) pts, respectively.

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ABSTRACT

Objective. To assess the safety and efficacy of the combination of oral etoposide and intravenous irinotecan in patients with platinum-resistant and taxane-pretreated ovarian cancer.

Methods. Eligible patients (age, 20–75 years; platinum-free interval, ≤28 weeks) with an adequate organ function received oral etoposide (50 mg/m^2 once a day) from day 1 to day 21 and intravenous irinotecan (70 mg/m^2) on days 1 and 15. The regimen was repeated every 28 days up to 6 cycles. The primary endpoint was the response rate (RR) with a threshold of 20%. The response was evaluated according to RECIST 1.0 and Gynecologic Cancer Intergroup CA-125 Response Definition, and toxicities were evaluated according to CTCAE version 3.0. This trial was registered at UMIN-CTR as UMIN000001837.

Results. Between April 1, 2009 and January 20, 2012, 61 patients were enrolled. Sixty patients were eligible. 1 CR and 12 PRs were confirmed; RR was 21.7% ($p = 0.42$, the exact binomial test). PFS and OS were 4.1 and 11.9 months, respectively. Major toxicities of ≥grade 3 were neutropenia (60%), anemia (36.7%), thrombocytopenia (11.7%), febrile neutropenia (18.3%), fatigue (13.3%), anorexia (11.7%), and nausea (11.7%). Three patients died from treatment related death (interstitial pneumonia, a pulmonary embolism, and DIC due to infection). Two of these patients were aged ≥65 years.

Conclusions. Oral etoposide and intravenous irinotecan had a moderate RR but did not meet the primary endpoint. Because of toxicity, we do not recommend this regimen outside of clinical trials. In particular, when considering this regimen for elderly patients, extreme caution is advised.

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Introduction

Ovarian cancer is the most lethal gynecological cancers in Japan. The standard first-line chemotherapy regimen is carboplatin plus paclitaxel [1,2]. Although the first-line chemotherapy is effective, more than 60% of the patients with advanced-stage cancer die of recurrent disease. After relapse, the choice of second-line chemotherapy depends on the platinum-free interval (PFI), which is a predictive factor of the effect of repeating platinum agents. The cutoff point of PFI is generally 6 months. Patients who experience recurrence within 6 months after previous chemotherapy are regarded as platinum resistant and receive subsequent line chemotherapy with a single agent, such as pegylated liposomal doxorubicin [3], topotecan [3], or gemcitabine [4]. When administered as monotherapy, many cytotoxic agents have shown activity against recurrent ovarian cancer; however, response rates (RRs) are generally low, such as 6–12% [3,4], and the responses last for a short duration because of resistance to monotherapy. Combination chemotherapy may circumvent this resistance and halt disease progression because a lower dose of two drugs with different mechanisms may reduce toxicity and enhance efficacy [5].

Irinotecan, a semisynthetic derivative of camptothecin, is a prodrug with little inherent inhibitory activity against topoisomerase I and is converted by carboxylesterases to its more active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin). In vitro, SN-38 is 250–1000 times more potent than irinotecan as a topoisomerase inhibitor. For platinum-resistant patients, irinotecan shows modest activity [6–8] as monotherapy when administered once a week, once every 2 weeks, and once every 3 weeks.

Etoposide is a semisynthetic glucosidic derivative of podophyllotoxin [9]. Intravenous etoposide has been tested in two phase II trials and has shown a relatively low RR (0% and 8.3%) [10,11] in patients with recurrent ovarian cancer. In contrast, oral etoposide has shown better efficacy, with RR of 26.8% in patients with a platinum-resistant relapse of ovarian cancer [12].

Topoisomerase I inhibitor treatment induces an increase in the S-phase cell population with an increase in topoisomerase II mRNA expression. Thus, topoisomerase I inhibitor can modulate topoisomerase II levels to enhance the effect of topoisomerase II inhibitors [13,14].

Eder et al. reported the results of an in vivo study. They showed that a combination of irinotecan and etoposide has a synergistic effect according to both a tumor excision assay and a tumor growth delay assay [15]. A phase I trial of topotecan and oral etoposide revealed severe myelosuppression but promising efficacy against platinum- and taxane-pretreated ovarian cancer [16].

The dose limiting toxicity of irinotecan is diarrhea, different from that of topotecan (myelosuppression). Accordingly, combining etoposide with irinotecan may improve the risk–benefit balance of dual inhibition of topoisomerase. The results of a phase I trial of this combination in patients with platinum-treated advanced epithelial ovarian cancer were reported at ASCO 2002 [17]. The recommended dose for a further study was as follows: oral etoposide 50 mg/m^2/day on days 1–21 and intravenous irinotecan 70 mg/m^2 on days 1 and 15. The regimen was repeated every 4 weeks.

In this phase I trial, four objective responses [2 complete responses (CRs) and 2 partial responses (PR)] were achieved among 24 patients, including 1 PR in clear cell carcinoma. Nishio et al. reported the results of a feasibility study in patients with platinum- and taxane-resistant ovarian cancer; the study was conducted by selected hospitals in Tohoku and Kyushu districts in Japan [18]. RR, time to progression, and overall survival (OS) were 44%, 9 months, and 17 months, respectively. This promising result led us to undertake a nationwide phase II trial.

Methods

Patients

Eligible patients (age, 20–75 years) had progressive or recurrent epithelial ovarian cancer, tubal cancer, or peritoneal cancer, with PFI (measured from the most recent platinum-containing regimen) of ≤28 weeks and a history of taxane treatment. The eligibility criteria included a measurable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.0) or a non-measurable disease meeting the GCIG CA-125 response definition [19]. Measurable lesion was defined as maximum tumor diameter of 20 mm or larger in CT with a slice of 6–10 mm or that of 10 mm or larger in CT with a slice ≤5 mm. Patients must be able to eat and drink without requiring parenteral nutrition. Other criteria included ECOG performance status, 0–2; absolute neutrophil count, ≥2000/μL; platelet count, ≥100,000/μL; serum creatinine, ≤1.5 mg/dL, total bilirubin, ≤1.5 mg/mL; and aspartate aminotransferase (AST), ≤100 IU/L. The patients were excluded if they had prior irinotecan, topotecan, or etoposide treatment; prior radiation; uncontrolled hypertension; a history of myocardial infarction or heart failure within 6 months; current unstable angina; mental illness or mental symptoms that would affect the participant's decision to participate; pregnancy or lactation; bowel obstruction; chemotherapy or a surgical procedure within 28 days; continuous systemic steroid; an active bacterial or fungal infection with a fever of ≥38.5 °C; hormonal or biological therapy within 14 days; malignancy within 5 years (except carcinoma in situ or intramucosal cancer); drainage of effusion, or ascites within 28 days; effusion or ascites to be drained at registration; pulmonary embolism or a history of pulmonary embolism with deep vein thrombosis requiring treatment.

Treatment

The patients received oral etoposide at 50 mg/m^2 (for patients with body surface area <1.0, 1.0–<1.5, 1.5–<2.0, or ≥2.0 m^2: 25, 50, 75, or 100 mg/day, respectively) once a day from day 1 to day 21, and received intravenous irinotecan (70 mg/m^2 over 90 min) on days 1 and 15. The regimen was repeated every 28 days up to 6 cycles until disease progression, unacceptable toxicity, or patient refusal occurred.

To begin the subsequent cycle, the pretreatment absolute neutrophil cell and platelet counts, AST, total bilirubin, and serum creatinine were ≥1000/μL, 10 × 10^4/μL, ≤100 IU/L, ≤1.5 mg/dL, and ≤1.5 mg/dL, respectively. Other criteria to begin the subsequent cycle included non-hematological toxicities (nausea, vomiting, anorexia, diarrhea, fatigue, fever, febrile neutropenia, and infection) ≤grade 1, constipation ≤grade 2, and no G-CSF within the last 2 days. Treatment modification criteria are listed in Appendix A1–2.

Endpoints

The primary endpoint was RR in all eligible patients. In patients with a measurable lesion, the response was evaluated according to RECIST 1.0 [20] and reviewed by independent radiology review. In patients with a non-measurable lesion, the response was assessed according to Gynecologic Cancer Intergroup CA-125 Response Definition [19]. To calculate RR, the sum of the number of responders was divided by the number of all eligible patients. The secondary endpoints were progression-free survival (PFS), OS, and adverse events. OS is defined as days from registration to death from any cause. OS was censored on the last day of follow-up when a patient was alive. PFS is defined as days from registration to disease progression (radiological, CA-125, or symptomatic) or death from any cause. PFS was censored on the latest day when the patient was alive without any evidence of progression.

Study design and statistical analysis

This study was a phase II trial with a two-stage design according to the Southwest Oncology Group (SWOG) [21]; we intended to evaluate this regimen as a test arm for a subsequent phase III trial. We assumed that the expected value of the primary endpoint was 35% and the threshold value was 20%. In this situation, the sample size ensuring at least 80% power with a one-sided alpha of 0.05 was 55 participants.

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Considering the likelihood of some ineligible patients among those enrolled, the total number of patients was set to 60.

Primary endpoint, RR, was tested by the exact binomial test and confidence interval of proportion was calculated by the exact method. According to the SWOG's two-stage design, preplanned interim analysis for futility was done after 30 patients enrolled, setting the threshold of the number of minimum responders as four. Then final analysis was conducted with one-sided alphas of 0.02 and 0.055, respectively. OS and PFS curves, median PFS and OS were estimated by Kaplan–Meier method, and confidence intervals for proportion were calculated with Greenwood's formula and median OS and PFS with Brookmeyer and Crowley's method. Exploratory analyses for RR were carried out by Fisher's exact test. All statistical analyses were conducted using SAS software, version 9.2 (SAS Institute, Cary, NC, USA).

Interim monitoring

In-house monitoring was to be performed every 6 months by the Japan Clinical Oncology Group (JCOG) Data Center to evaluate the study progress and to improve study quality.

Ethical considerations

The Protocol Review Committee of JCOG approved the study protocol in January 2009, and the study was initiated in April 2009. The protocol was reviewed and approved at all the participating hospitals. Every patient signed a written informed consent form. This trial was registered at UMIN-CTR as UMIN000001837 (http://www.umin.ac.jp/ctr/).

Results

Patient characteristics

From April 1, 2009 to July 5, 2010, 30 patients were enrolled and patient accrual was suspended for interim analysis. After the planned interim analysis, the study was resumed on November 22, 2010, and a total of 61 patients were enrolled until January 20, 2012. One patient was ineligible and excluded from this analysis because the days from surgery to registration were shorter than the eligibility criteria. Patient characteristics are summarized in Table 1. There were 14/60 (23.3%) elderly patients, defined as ≥65 years. Eleven of 60 (18.3%) patients had clear cell carcinoma, who were mostly (10 of 11) enrolled in the study after the interim analysis. Among 39 patients with serous carcinoma, two of them (5%) were diagnosed as low grade serous carcinoma. Nine of 60 patients (15%) received ≥3 prior chemotherapy regimens. Twenty-seven of 60 patients (45%) had platinum-refractory disease that progressed during or within 3 months after previous chemotherapy with a platinum-based drug.

Treatment administration

The median number of delivered treatment cycles was 4 (range, 1–6). Twenty-one patients completed 6 cycles of treatment. Thirty-nine patients did not complete treatment because of the following reasons: disease progression ($n = 29$), patient refusal ($n = 5$), adverse event ($n = 3$), intercurrent death ($n = 1$), and earthquake ($n = 1$).

Three treatment-related deaths (TRDs) were reported: interstitial lung disease (judged as a *probable* TRD by the Data and Safety Monitoring Committee), DIC due to infection (judged as a possible TRD), and a recurrent pulmonary embolism (judged as a possible TRD). The first 2 patients listed above were aged ≥65 years.

For etoposide, a median total dose, median dose intensity, and median relative dose intensity were 2852.3 mg/m^2, 179.3 mg/m^2/week, and 88.9%, respectively. For irinotecan, the median total dose, median dose intensity, and median relative dose intensity were 452.8 mg/m^2, 30.7 mg/m^2/week, and 88.0%, respectively.

Table 1
Patient characteristics.

Characteristics		Number of patients (%)	Median	Range
Age, years			58	31–75
	<65	46 (77)		
	≥65	14 (23)		
PS	0	51 (85)		
	1	8 (13)		
	2	1 (2)		
Histology	Serous	39 (65)		
	(LGS)	2 (5)		
	Clear cell	11 (18)		
	Endometrioid	5 (8)		
	Other	5 (8)		
Lesion	Measurable	52 (87)		
	Non-measurable	8 (13)		
Prior chemo regimens	1	34 (57)		
	2	17 (28)		
	≥3	9 (15)		
PFI	<3 months	27 (45)		
	≥3 months	33 (55)		

Abbreviations. PS: performance status, PFI: platinum-free interval, chemo: chemotherapy, LGS: low grade serous.

Toxicity

Toxicities are summarized in Table 2. Only treatment-related adverse events (definite, probable, or possible) were counted as toxicities. Grades 3–4 hematological toxicities were: neutropenia (60%), anemia (36.7%), and thrombocytopenia (11.7%). Grades 3–4 non-hematological toxicities were: febrile neutropenia (FN; 18.3%), fatigue (11.7%), anorexia (11.7%), and nausea (11.7%). FN was more frequent in patients aged ≥65 years (28.6%) or those with ≥3 prior chemotherapy regimens (44.4%) compared with patients aged <65 years (15.2%) or those with 1 or 2 prior chemotherapy regimens (13.7%). One patient was diagnosed with acute myeloid leukemia 234 days after completing 6 cycles of the present regimen. She received carboplatin plus paclitaxel for 6 cycles and PLD for 6 cycles before the study entry, and gemcitabine for 3 cycles after this regimen.

Efficacy

One patient achieved CR and 12 patients achieved PR (Table 3); accordingly, RR was 21.7% (13/60) [design-based 89% confidence interval (CI) 13.5–31.9%; 95% CI 12.1–34.2%]. This RR did not exceed the preplanned threshold (one-sided $p = 0.42$ by the exact binomial test for the null hypothesis that RR ≤20%). RR was 30.3% (10/33) in patients with PFI of ≥3 months, while it was 11.1% (3/27) in patients

Table 2
Grade 3/4 toxicities affecting >5% of the patients.

	G1	G2	G3	G4	% G3–4
Leukopenia	7	17	26	10	60
Anemia	7	29	12	10	36.7
Thrombocytopenia	4	2	5	2	11.7
Neutropenia	7	17	15	21	60
Hypoalbuminemia	30	11	5	–	8.3
Hyponatremia	13	–	4	0	6.7
Hypokalemia	18	–	1	3	6.7
Febrile neutropenia	–	–	11	0	18.3
Fatigue	23	9	7	0	11.7
Anorexia	23	13	7	0	11.7
Nausea	20	15	7	0	11.7
Vomiting	13	8	4	0	6.7
Diarrhea	14	4	3	0	5

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with PFI of <3 months (Fisher's exact test, p = 0.12). RR was 26.5% (13/49) in patients with a non-clear cell histology, while it was 0% (0/11) in patients with a clear cell histology (p = 0.10). Age and the number of prior chemotherapy regimens did not seem to affect RR (21.7 (10/46), 21.4 (3/14), 23.5 (12/51), and 11.1 (1/9) % in young patients, elderly patients (p = 1.00), patients received <3 prior regimen, and patients received ≥3 prior chemotherapy regimens (p = 0.67), respectively).

Median PFS was 4.1 months (95% CI 3.5–4.9 months), and 33.3% of patients (95% CI 21.8–45.2%) survived without progression at 6 months (Fig. 1A). Median PFS was 5.6 months in patients with PFI of ≥3 months, while it was 3.6 months in patients with PFI of <3 months (Fig. 1B). Median PFS was 4.3 months in patients with a non-clear cell histology, while it was 3.6 months in patients with a clear cell histology.

One patient was progression-free at last follow-up (PFS, >1221 days). She was diagnosed with stage 3c ovarian serous adenocarcinoma and was treated with carboplatin plus paclitaxel for 5 cycles. After 16.6 months, she had a recurrent tumor and received carboplatin plus docetaxel for 5 cycles. After 1 month, she experienced platinum-resistant recurrence and was treated with the present regimen; she showed CR.

Median OS was 11.9 months (95% CI 9.4–14.6 m) (Fig. 2A). Median OS was 16.9 months in patients with PFI of ≥3 months, while it was 8.1 months in patients with PFI of <3 months (Fig. 2B). Median OS was 12.4 months in patients with a non-clear cell histology, while it was 10.4 months in patients with a clear cell histology.

Discussion

This is the first phase II trial evaluating this combination regimen in patients with platinum-resistant ovarian cancer. This study demonstrates that the combination of oral etoposide and intravenous irinotecan has moderate efficacy in patients with platinum-resistant ovarian cancer. The overall RR was 21.7%. Disappointingly, this result does not meet the preplanned criteria for proceeding to a further phase III trial.

Preceding randomized controlled trials of combination chemotherapy against platinum-resistant ovarian cancer are summarized in Table 4. As for efficacy, our study shows a better RR, including CR lasting more than 3 years, compared with OVATURE [22], OVA301 [23] and ASSIST-5 studies [24], although PFS is in the same range. The CARTAXHY trial [25] shows a better RR and PFS compared with other studies, even in a paclitaxel single-agent arm. Nonetheless, this efficacy may not be reproduced in Japan, because weekly paclitaxel has already been adopted as a component of first-line treatment according to the results of JGOG3016 [2]. In addition, an Italian collaborative phase 3 study comparing epidoxorubicine plus paclitaxel with paclitaxel alone for patients with PFI ≤12 months, did not prove the efficacy of cytotoxic doublets in terms of neither PFS nor OS [26]. All these preceding studies concluded that combination chemotherapy utilizing two cytotoxic agents is not effective strategy. Combination chemotherapy utilizing one cytotoxic agent with one biologic agent is a promising strategy. AURELIA [27] has proved the efficacy of bevacizumab for patients with platinum resistant ovarian cancer, showing almost doubled RR and PFS, comparing with monotherapy such as weekly paclitaxel, PLD, or topotecan. Another study, TRINOVA-1 [28], also proved the efficacy of trebananib for patients with PFI ≤12 months.

Table 3
Overall response.

	RECIST (%)	CA-125 (%)	Total (%)
CR	1 (2)	–	1 (2)
PR	10 (19)	2 (25)	12 (20)
SD	21 (40)	2 (25)	23 (38)
PD	16 (31)	4 (50)	20 (33)
NE	4 (8)	0 (0)	4 (7)
Total	52	8	60

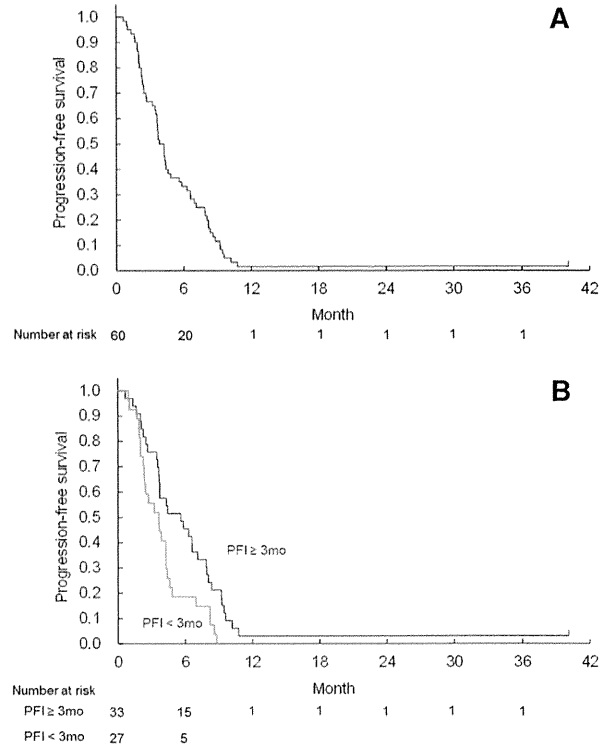

Fig. 1. A depicts PFS of all the patients. B depicts PFS by PFI <3 m (pink curve) or ≥3 m (blue curve). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

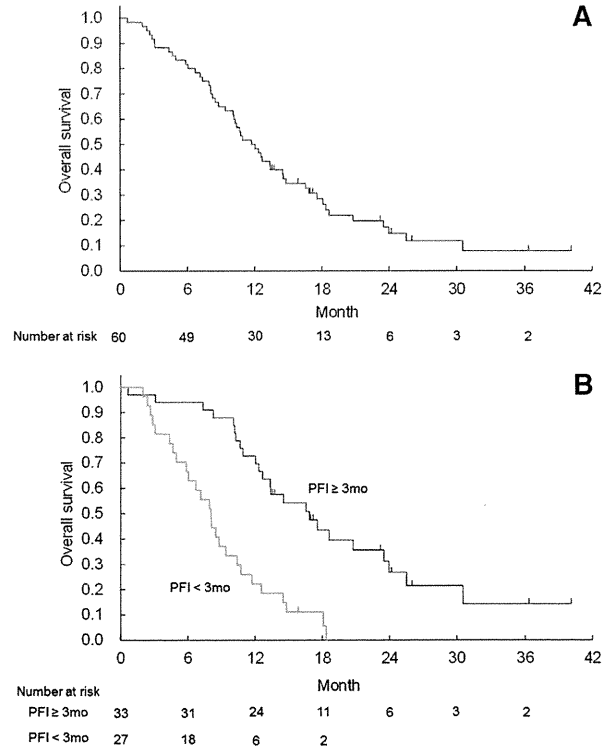

Fig. 2. A depicts OS of the patients. B depicts OS by PFI <3 m (pink curve) or ≥3 m (blue curve). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Table 4
Combination chemotherapy for platinum resistant ovarian cancer.

Study	Rx	% of 1 prior Rx	RR (%)	PFS (months)
OVATURE	Cb vs CbPXD	2.8–4.3	1 vs 0	4.7 vs 3.6
OVA301[22]	pD vs pDTr	100	12 vs 13	3.7 vs 4
CARTAXHY[24]	wP vs wPCb vs wPTp	71–74	35 vs 37 vs 39	3.7 vs 4.8 vs 5.4
ASSIST-3[23]	pD vs pDCan	60	8.3 vs 12	3.7 vs 5.6
JCOG0503	E + I	57	21.7	4.1
Buda et al.	P vs PEp	100	37[a] vs 47[a]	6[a] vs 6[a]
AURELIA	wP/pD/Tp vs +BV	57–60	13 vs 31	3.4 vs 6.7
TRINOVA-1	wP vs wPTre	38–41	30[a] vs 38[a]	5.4[a] vs 7.2[a]

Abbreviations. Rx: regimen, Cb: carboplatin, PXD: phenoxidiol, pD: liposomal doxorubicin, Tr: trabectidine, wP: weekly paclitaxel, Tp: topotecan, Can: canfosfamide, E: etoposide, I: irinotecan, P: paclitaxel (every three weeks), Ep: epidoxorubicine, BV: bavacizumab, Tre: trebananib.

[a] Data for patients with platinum free interval less than 12 months.

Regarding toxicity, FN was much more frequent in our study, especially in heavily pretreated patients or elderly patients. Even among patients aged <65 years or those with 1 or 2 prior regimens, FN was still approximately 15%. Therefore, we think that the present regimen is too toxic and cannot be recommended as an option for heavily pretreated patients or elderly patients. Moreover, even when we excluded heavily pretreated patients or elderly patients, RR was similar. Eventually, we decided to discontinue the development of this regimen for patients with platinum-resistant ovarian cancer.

In the OVA301 subset analysis, patients with PFI of 6–12 months are considered good candidates for non-platinum combination chemotherapy [29], and the hypothesis is that platinum chemotherapy after a non-platinum combination can be more effective because of an artificially prolonged PFI. This hypothesis is being tested in the INOVATYON study, which compares trabectedin plus PLD with carboplatin plus PLD in patients with ovarian cancer with PFI of 6–12 months. If the results are positive, then the combination of oral etoposide and intravenous irinotecan, which shows RR of 30.3% in patients with PFI of 3–6 months, can be promising for further investigation for that purpose.

The present study had some limitations. First, pretreatment UGT1A1 assessment was lacking. This issue was discussed at the beginning of this study. Because the dose of irinotecan used in this study is low (140 mg/m^2 per cycle) and because of the negative results of a meta-analysis of the usefulness of such low doses [30], we decided not to use the UGT1A1 assessment. Second, the eligibility criteria allowing heavily pretreated patients are relatively broad compared with those in other trials. This situation can produce a negative bias in both efficacy and safety results. On the other hand, the number of heavily pretreated patients in this study is small, and the subgroup analysis strongly suggested that the conclusions will not change.

In conclusion, this study demonstrates that the combination of oral etoposide and intravenous irinotecan has moderate efficacy in patients with platinum-resistant ovarian cancer. The overall RR was 21.7%. This result did not meet the primary endpoint for a further phase III trial. Because of toxicity, we do not recommend this regimen outside of clinical trials. If such a trial is planned, heavily pretreated patients and elderly patients should be excluded.

Role of funding source

The study was supported in part by the National Cancer Center Research and Development Funds (23-A-16, 23-A-17 and 26-A-4), the Grant-in-Aid for Clinical Cancer Research (17S-1, 17S-5, 18-6, 20S-1, and 20S-6) from the Ministry of Health, Labor and Welfare of Japan.

Conflict of interest statement

Koji Matsumoto participates in the investigation trials and receives clinical investigation expense from Astra Zeneca, Japan Boehringer Ingelheim, Pfizer, and Sanofi. Noriyuki Katsumata receives honorarium from Chugai Pharmaceutical Co. Ltd. and Ono Pharmaceutical Co Ltd. Mayu Yunokawa receives clinical investigation expense from Yakult Honsha Co. Ltd. and Sawai Pharmaceutical Co. Ltd. Taro Shibata, Toyomi Satoh, Motoaki Saitou, Tadao Takano Kenichi Nakamura Toshiharu Kamura and Ikuo Konishi have no relevant financial relationships to disclose.

Participating Hospitals

Iwate Medical University, Tohoku University, Tsukuba University, Jikei Kashiwa Hospital, National Cancer Center Hospital, Tokyo Metropolitan Cancer and Infectious Disease Center Komagome Hospital, Jikei University Hospital, Cancer Institute Hospital of Japanese Foundation for Cancer Research, The University of Tokyo Hospital, Kitasato Univerisity School of Medicine, Niigata Cancer Center Hospital, Aichi Cancer Center Hospital, Kyoto University Hospital, Osakacity University Hospital, Osaka Prefectural Hospita Organization Osaka Center for Cancer and Cardiovascular Disease, Osaka City General Hospital, Hyogo Cancer Center, National Hospital Organization Kure Medical Center Chugoku Cancer Center, National Hospital Organization Shikoku Cancer Center, National Kyushu Cancer Center, Kurume University School of Medicine, Kyushu University Hospital, Faculty of Medicine, Saga University, Kagoshima City Hospital, Faculty of Medicine, University of the Ryukyus.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ygyno.2014.10.026.

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RESEARCH ARTICLE

Feasibility study of personalized peptide vaccination for recurrent ovarian cancer patients

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Abstract

Context: To develop a personalized peptide vaccine (PPV) for recurrent ovarian cancer patients and evaluate its efficacy from the point of view of overall survival (OS), Phase II study of PPV was performed.

Patients and methods: Forty-two patients, 17 with platinum-sensitive and 25 with platinum-resistant recurrent ovarian cancer, were enrolled in this study and received a maximum of four peptides based on HLA-A types and IgG responses to the peptides in pre-vaccination plasma.

Results: Expression of 13 of the 15 parental tumor-associated antigens encoding the vaccine peptides, with the two prostate-related antigens being the exceptions, was confirmed in the ovarian cancer tissues. No vaccine-related systemic severe adverse events were observed in any patients. Boosting of cytotoxic T lymphocytes or IgG responses specific for the peptides used for vaccination was observed in 18 or 13 of 42 cases at 6th vaccination, and 19 or 29 of 30 cases at 12th vaccination, respectively. The median survival time (MST) values of the platinum-sensitive- and platinum-resistant recurrent cases were 39.3 and 16.2 months, respectively. The MST of PPV monotherapy or PPV in combination with any chemotherapy during the 1st to 12th vaccination of platinum-sensitive cases was 39.3 or 32.2 months, and that of platinum-resistant cases was 16.8 or 16.1 months, respectively. Importantly, lymphocyte frequency and epitope spreading were significantly prognostic of OS.

Discussion and conclusion: Because of the safety and possible prolongation of OS, a clinical trial of PPV without chemotherapy during the 1st to 12th vaccination in recurrent ovarian cancer patients is merited.

Keywords

Cytotoxic T-lymphocytes, epitopes, ovarian cancer, peptide vaccine, personalized medicine

History

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Introduction

Ovarian cancer is the leading cause of mortality among patients with gynecologic malignancies[1]. Although the majority of patients respond to a first-line chemotherapy with platinum and taxane agents, most patients experience relapse and develop resistance to platinum and subsequent chemotherapeutic agents[2,3]. Thus, it is important to develop new therapeutic approaches including cancer vaccines and molecular targeting therapy.

We and other groups previously reported the existence of tumor-reactive cytotoxic T lymphocytes (CTLs) among the tumor-infiltrating lymphocytes (TILs) in ovarian cancers[4–6]. In addition, a correlation between TILs and clinical outcome was reported in several studies[7–9]. These findings and several clinical trials of immunotherapy in patients with ovarian cancer indicated that ovarian cancers are responsive to immunotherapy[10–15]. We developed and clinically tested a novel regimen of personalized peptide vaccine (PPV) in which the vaccine antigens are selected and administered based on the pre-existing host immunity before vaccination[16–19]. The results suggested that PPV could prolong overall survival (OS) but not progression-free survival in advanced cancer patients who fail to respond to standard chemotherapy. Moreover, a randomized clinical trial of PPV in advanced prostate cancer patients showed a favorable clinical outcome in the vaccinated group[20]. In this study, we examined whether PPV would be feasible as a cancer vaccine for the treatment of recurrent ovarian cancer from the viewpoint of OS.

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Materials and methods

Immunohistochemical analysis

Tissue specimens were collected from 22 ovarian cancer patients, including three patients who were enrolled in a

clinical trial of PPV and another 19 who were not receiving PPV therapy. Paraffin-embedded tissue samples were cut into 4-μm sections and labeled on a BenchMark XT (Ventana Medical Systems, Tucson, AZ) with antibodies to the tumor antigens. The DAB (Ventana iVIEW DAB Detection Kit; Ventana Medical Systems) was used for the detection of antigens.

Patients

Patients with histological diagnosis of ovarian, fallopian tubal or primary peritoneal cancer were eligible for inclusion in this study. They also had to show positive IgG responses to at least two of the HLA-class I-matched vaccine candidate peptides. The other inclusion criteria as well as exclusion criteria were not largely different from those of other previously reported clinical studies[17–20]: an age between 20 and 80 years; an Eastern Cooperative Oncology Group performance status (PS) of 0 or 1; positive status for HLA-A2, -A3, -A11, -A24, -A26, -A31, or -A33; life expectancy of at least 12 weeks; and adequate hematologic, renal and hepatic function (>2500/μL of white blood cells, ≥1000/μL of lymphocytes, >80 000/μL of platelets, <1.5 mg/dL of serum creatinine and <2.5 mg/dL of total bilirubin). Patients with lymphocyte counts of <1000 cells/μL were excluded from the study, since we previously reported that pre-vaccination lymphopenia is an unfavorable factor for OS in cancer patients receiving PPV[21,22]. Other exclusion criteria included pulmonary, cardiac or other systemic diseases; an acute infection; a history of severe allergic reactions; pregnancy or nursing; and other inappropriate conditions for enrollment as judged by the clinicians. The protocols were approved by the Kurume University Ethical Committee and were registered in the UMIN Clinical Trials Registry (UMIN#3083 for 40 patients and UMIN#1482 for 2 patients). After a full explanation of the protocol, written informed consent was obtained from all patients before enrollment.

Clinical protocol

The aim of this study was to investigate the feasibility of PPV as a therapeutic cancer vaccine from the viewpoint of OS of recurrent ovarian cancer patients, along with prognostic factors for OS, safety and immunological response in ovarian cancer patients under PPV. Thirty-one peptides, whose safety and immunological effects had been confirmed in previously conducted clinical studies[17–20], were employed for vaccination (Table 1). The peptides were prepared under the conditions of Good Manufacturing Practice by the PolyPeptide Laboratories (San Diego, CA) and American Peptide Company (Vista, CA). The appropriate peptides for vaccination in individual patients were selected in consideration of the HLA-type and pre-existing host immunity before vaccination, as assessed by IgG levels against each of the 31 different vaccine candidates as described previously[23]. Similarly, the concomitant chemotherapy was permitted during the vaccination for patients who could tolerate it. A maximum of four peptides (3 mg/each peptide) were subcutaneously administrated with Montanide ISA51VG (Seppic, Paris, France) once a week for six consecutive

Table 1. Vaccine candidate peptides used for PPV.

Peptide name	Original protein	Position	Sequence	HLA-IA restriction	References
CypB-129	Cyclophilin B	129–138	KLKHYGPGWV	A2/A3 supertype	Jpn J Cancer Res 2001;92:762–767.
Lck-246	$p56^{lck}$	246–254	KLVERLGAA	A2	Int J Cancer 2001;94:237–242.
Lck-422	$p56^{lck}$	422–430	DVWSFGILL	A2/A3 supertype	Int J Cancer 2001;94:237–242.
ppMAPkkk-432	ppMAPkkk	432–440	DLLSHAFFA	A2/A26	Cancer Res 2001;61:2038–2046.
WHSC2-103	WHSC2	103–111	ASLDSDPWV	A2/A26/A3 supertype	Cancer Res 2001;61:2038–2046.
HNRPL-501	HNRPL	501–510	NVLHFFNAPL	A2/A26	Cancer Res 2001;61:2038–2046.
UBE2V-43	UBE2V	43–51	RLQEWCSVI	A2	Cancer Res 2001;61:2038–2046.
UBE2V-85	UBE2V	85–93	LIADFLSGL	A2	Cancer Res 2001;61:2038–2046.
WHSC2-141	WHSC2	141–149	ILGELREKV	A2	Cancer Res 2001;61:2038–2046.
HNRPL-140	HNRPL	140–148	ALVEFEDVL	A2	Cancer Res 2001;61:2038–2046.
SART3-302	SART3	302–310	LLQAEAPRL	A2	Int J Cancer 2000;88:633–639.
SART3-309	SART3	309–317	RLAEYQAYI	A2	Int J Cancer 2000;88:633–639.
SART2-93	SART2	93–101	DYSARWNEI	A24	J Immunol 2000;164:2565–2574.
SART3-109	SART3	109–118	VYDYNCHVDL	A24/A24/A3 supertype	Cancer Res 1999;59:4056–4063.
Lck-208	$p56^{lck}$	208–216	HYTNASDGL	A24	Eur J Immunol 2001;31:323–332.
PAP-213	PAP	213–221	LYCESVHNF	A24	J Urol 2001;166:1508–1513.
PSA-248	PSA	248–257	HYRKWIKDTI	A24	Prostate 2003;57:152–159.
EGF-R-800	EGF-R	800–809	DYVREHKDNI	A24	Eur J Cancer 2004;40:1776–1786.
MRP3-503	MRP3	503–511	LYAWEPSFL	A24	Cancer Res 2001;61:6459–6466.
MRP3-1293	MRP3	1293–1302	NYSVRYRPGL	A24	Cancer Res 2001;61:6459–6466.
SART2-161	SART2	161–169	AYDFLYNYL	A24	J Immunol 2000;164:2565–2574.
Lck-486	$p56^{lck}$	486–494	TFDYLRSVL	A24	Eur J Immunol 2001;31:323–332.
Lck-488	$p56^{lck}$	488–497	DYLRSVLEDF	A24	Eur J Immunol 2001;31:323–332.
PSMA-624	PSMA	624–632	TYSVSFDSL	A24	Cancer Sci 2003;94:622–627.
EZH2-735	EZH2	735–743	KYVGIEREM	A24	Prostate 2004;60:273–281.
PTHrP-102	PTHrP	102–111	RYLTQETNKV	A24	Br J Cancer 2004:287–296.
SART3-511	SART3	511–519	WLEYYNLER	A3 supertype	Cancer Immunol Immunother 2007;56:689–698.
SART3-734	SART3	734–742	QIRPIFSNR	A3 supertype	Cancer Immunol Immunother 2007;56:689–698.
Lck-90	$p56^{lck}$	90–99	ILEQSGEWWK	A3 supertype	Br J Cancer 2007;97:1648–1654.
Lck-449	$p56^{lck}$	449–458	VIQNLERGYR	A3 supertype	Br J Cancer 2007;97:1648–1654.
PAP-248	PAP	248–257	GIHKQKEKSR	A3 supertype	Clin Cancer Res 2005;11:6933–6943.

weeks. After the first cycle of six vaccinations, peptides were administered every two weeks. Adverse events were monitored according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (CTCAE Ver4, Bethesda, MD). Complete blood counts and serum biochemistry tests were performed at every sixth vaccination. The clinical responses were evaluated using the Response Evaluation Criteria in Solid Tumors in the vaccinated patients, whose radiological findings by computed tomography scan or magnetic resonance imaging were available before and after vaccinations.

Measurement of humoral and cellular responses

The IgG levels to each of the 31 peptide candidates were measured using the Luminex system (Luminex, Austin, TX), as previously reported[23]. If the titers of peptide-specific IgG to at least one of the vaccinated peptides in the post-vaccination plasma were more than twofold higher than those in the pre-vaccination plasma, the changes were considered to be significant. In addition, if the numbers of HLA-A-matched peptides reactive to peptide-specific IgG increased or decreased at the sixth vaccination, this was considered epitope spreading (ES) or epitope decline (ED), respectively.

Cellular responses were evaluated by INF-γ ELISPOT assay as previously described[23]. Antigen-specific T cell responses were evaluated by the difference between the numbers of spots produced in response to each corresponding peptide and that produced in response to the control HIV peptide; a difference of at least 30 spots per 10^5 PBMCs was considered positive or detectable and the subtracted spot numbers are shown. In negative cases, spot numbers are shown as ''zero''. If the post-vaccination values were more than twofold higher than the pre-vaccination values, this was considered an augmented response. If the pre-vaccination values were ''zero'', then post-vaccination values of more than 30 were considered an augmented response.

Flow-cytometric analysis of PBMCs

For the analysis of MDSCs, PBMCs were stained with the following antibodies as previously described[24]: anti-CD3-FITC, anti-CD56-FITC, anti-CD19-FITC, anti-CD33-APC, anti-HLA-DR-PE/Cy7 and anti-CD14-APC/Cy7 antibodies. In the cell subset negative for the lineage markers (CD3, CD19, CD56 and CD14) and HLA-DR, MDSCs were identified as $CD33^+$. The samples were analyzed on a FACSCanto II with Diva software (BD Biosciences, San Diego, CA). Antibodies were purchased from Biolegend (San Diego, CA) and BD Biosciences.

Statistical analysis

A two-sided Wilcoxon test was used to compare differences between pre- and post-vaccination measurements. p Values <0.05 were considered statistically significant. OS time 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. Predictive factors for OS were evaluated by univariate analysis with the Cox proportional hazards regression model. All statistical analyses were conducted using the JMP version 8 or SAS version 9.1 software (SAS Institute Inc., Cary, NC).

Results

General tumor expression of parental proteins of vaccine peptides

To confirm the general expression of the 15 different parental TAAs of the vaccine candidate peptides shown in Table 1, tumor specimens from 22 ovarian cancer patients, including three patients (FOV-019, -028 and -030) who were enrolled in a clinical trial of PPV and 19 patients who were not being treated with PPV, were subjected to immunohistochemical analysis. The results showed that 13 TAAs were detectable in the ovarian cancer cells tested. Nine of them were expressed in the majority of cancer cells tested, whereas MRP3, EGF receptor, PAP and lck were expressed in only a portion of the cancer cells. Representative staining patterns are shown in Figure 1. In contrast, the two prostate-related vaccine antigens (PSMA and PSA) were not detectable in any tissues tested, as expected from the previous studies listed in Table 1.

Patient characteristics

Between January 2009 and December 2012, 37 patients with epithelial ovarian cancer, three with fallopian tube cancer and two with primary peritoneal cancer were enrolled in this study. All patients had recurrence and persistence of disease. The characteristics of the 42 patients are listed in Table 2. Serous adenocarcinoma was the most common histology (52.2%). Seventeen patients had platinum-sensitive and 25 had platinum-resistant recurrence. All patients had achieved a documented response to initial platinum-based treatment and had been off therapy until recurrence. Platinum sensitivity or resistance was defined as an off therapy period of longer or shorter than six months after initial platinum-based treatment, respectively. Before enrollment, all the patients underwent additional chemotherapy against recurrent tumor. The median duration from the first recurrence to the PPV was 14.5 months, ranging from 1 to 89. PS at the time of enrollment was grade 0 ($n=33$) or grade 1 ($n=9$). During the PPV, 22 patients underwent concomitant chemotherapy, and the remaining 20 patients did not tolerate concomitant chemotherapy (Table 2).

Toxicities

Grade 1 or 2 dermatological reaction at the injection sites was observed in all cases (Table 3). The high grade adverse events (more than grade 3) were anemia (grade 3: $n=2$; grade 4: $n=1$), leukocytopenia (grade 4: $n=1$), neutropenia (grade 3: $n=2$; grade 4: $n=1$), lymphopenia (grade 3: $n=1$), hypoalbuminemia (grade 3: $n=1$) and infection of the injection site (grade 3: $n=1$). Except for infection of the injection site, all of these severe adverse events were concluded to be associated with chemotherapy, rather than directly associated with the vaccinations, based on the assessment of an independent safety evaluation committee. However, infection of the injection site (a lower limb) was concluded to be a vaccination-related adverse event.

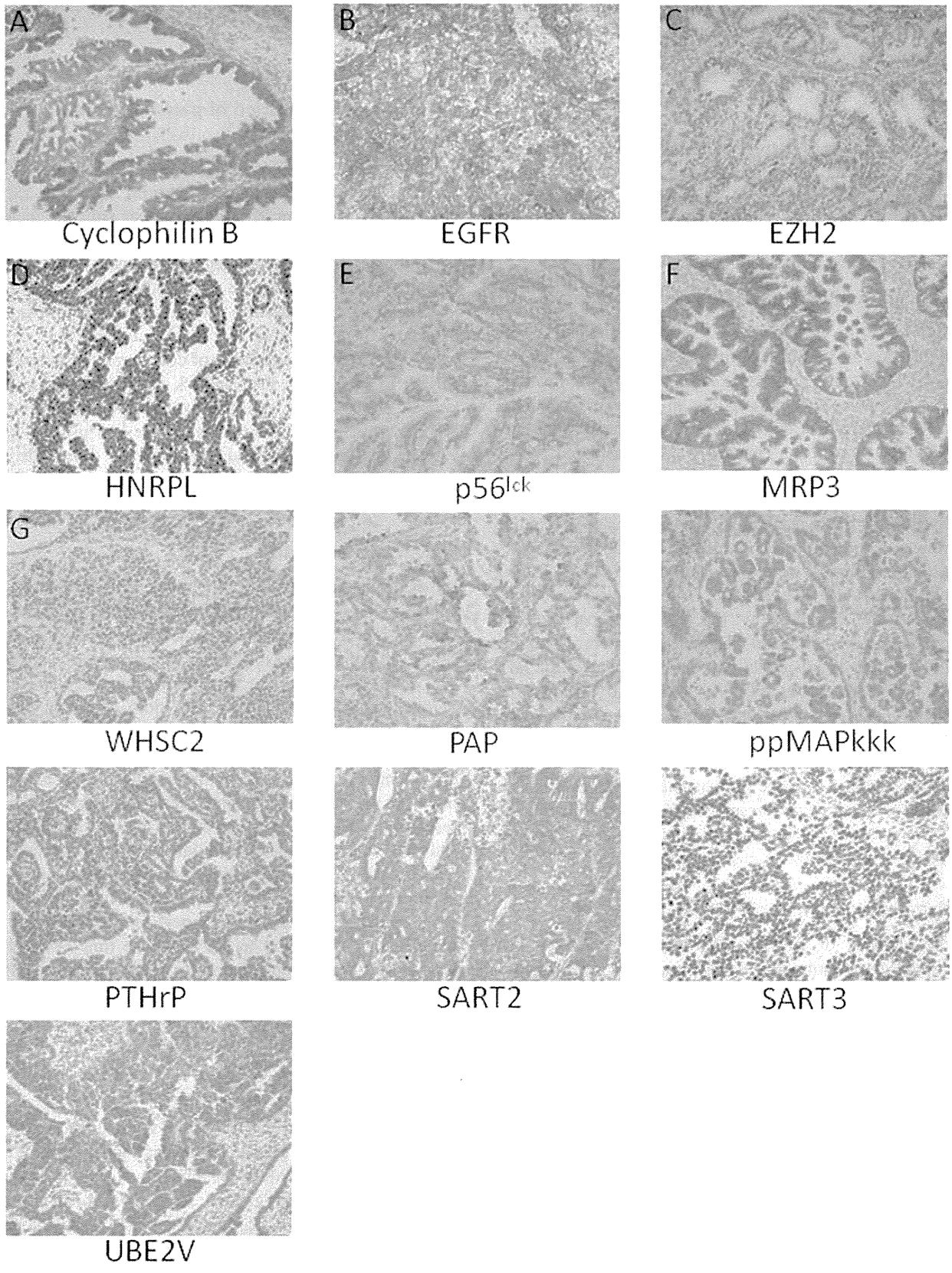

Figure 1. Expression profile of parental proteins of vaccine peptides. Tumor specimens from 22 ovarian cancer patients, including three patients who were enrolled in a clinical trial of PPV and 19 patients who were not being treated with PPV, were subjected to immunohistochemical analysis.

Table 2. Characteristics of the enrolled patient with recurrent ovarian cancer ($n = 42$).

Parameters	n
Age	
median (range)	57.5 (22–80)
Origin	
Ovary	37
Fallopian tube	3
Periosteum	2
Histology	
Serous	22
Endometrioid	7
Mucinous	3
Clear	3
Others	7
HLA	
A2	10
A24	30
A3 superfamily	26
A26	5
Performance status	
0	33
1	9
Number of prior regimen	
1	4
2	10
3	14
≥4	14
Platinum sensitivity	
Sensitive	17
Resistant	25
Combined chemotherapy	
Yes	22
No	20

Table 3. Toxicities.

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Injection site reaction	0	10	32		
Blood/Bone marrow					
Anemia	23	6	10	2	1
Leukocytopenia	32	8	1		1
Neutropenia	38	1		2	1
Lymphopenia	24	13	3	2	
Thrombocytopenia	39	1	2		
Laboratory					
AST elevation	37	5			
ALT elevation	39	3			
Hypoalbuminemia	20	16	5	1	
Creatinine elevation	33	9			
Renal/genitourinary					
Obstruction: ureter	41		1		
Intestine					
Intestinal bleeding	41			1	
Pain					
Tumor	40		2		
Leg edema	40	2			
Infection					
Injection site	41			1	

Immune responses to the vaccinated peptides

Both humoral and cellular immune responses specific for the peptides used for vaccination were analyzed in blood samples of the patients collected at pre-vaccination and at the 6th and 12th vaccinations (Table 4). Due to disease progression, 12 patients failed to complete the second cycle of vaccinations (12th vaccination), while one patient decided to withdraw from the study before the 12th vaccination. Peptide-specific IgGs reactive to each of 31 different peptides, including both vaccinated and non-vaccinated peptides, were measured.

Augmentation of the IgG responses specific for at least one of the vaccinated peptides was observed in 16 of 42 recurrent cases at the time of the 6th vaccinations. The 12th vaccination induced the augmentation in 29 of 30 recurrent cases tested. In addition, the numbers of HLA-A-matched peptides reactive to peptide-specific IgG increased in 16 cases, whereas it decreased in the other 16 cases at the 6th vaccination. In this study, the former phenomenon was referred to as ES and the latter phenomenon as ED.

CTL responses to the vaccinated peptides were measured by IFN-γ ELISPOT assay. Representative well images of ELISPOT assay are shown in Figure 2. Antigen-specific CTL responses were detectable in only 12 of 42 patients before vaccination. Augmentation of CTL responses specific for at least one of the vaccinated peptides was observed in 18 of 42 and 19 of 30 cases at the time of the 6th and 12th vaccinations, respectively. Interestingly, ES was well correlated with the augmentation of IgG and CTL responses ($p = 0.014$, $p = 0.044$), but no correlation was observed between the augmentation of IgG and CTL responses ($p = 0.101$).

Cytokines and inflammation markers

Significant increases of IL-6, CRP and SAA levels were observed after the sixth vaccination ($p = 0.0012$, $p = 0.001$ and $p = 0.010$, respectively) (Figure 3A). Furthermore, plasma CRP levels before vaccination were higher in the group that showed an augmentation of IgG response at the sixth vaccination ($p = 0.031$).

Flow-cytometric analysis of PBMCs

Immune cell subsets in both pre- and sixth vaccination PBMCs were examined by flow cytometry. No significant difference was found between the frequencies of pre- and sixth vaccination of MDSC and $CD3^+CD26^+$ cells. The median frequency of MDSC in pre-vaccination PBMCs was lower in the group that showed an augmentation of CTL response after vaccination ($p = 0.005$, Student's t-test) (Figure 3B). The frequencies of $TNFRSF14^+$ cells in both the $CD11^+$ and $CD11^-$ subsets were not changed between before and after vaccination (data not shown). However, the frequency of $CD11^+TNFRSF14^+$ before vaccination was higher in the group that showed an augmentation of IgG response after vaccination ($p = 0.019$, Student's t-test).

Relationship between clinical findings or immunological responses and OS

The median number of vaccinations was 12, with a range of 6 to 33. Among the 25 vaccinated patients whose radiological findings were available both before and after the vaccination, 1 patient (FOV-027) had a complete response (CR), and this patient was treated with a combination of PPV

Table 4. Antigen expression, immunological responses and clinical outcome of each patient.

ID (HLA-A type)	Peptides for vaccination	Antigen expression in tumor tissue	IgG response (FIU)			CTL response (IFN-γ producing cells/10^5 cells)			Numbers of IgG positive peptides			Best clinical response	Overall survival (months)	Prognosis
			Pre	6th	12th	Pre	6th	12th	pre	6th	12th			
F-018 (A2/A24)	ppMAPkkk-432	na	1492	1310	na	0	0	na	6	5	na	PD	19.1	DOD
	WHSC2-103		1875	1904	na	0	0	na						
	HNRPL-140		897	840	na	0	0	na						
	MRP3-503		230	149	na	0	0	na						
F-040 (A11/A24)	PAP-213	na	3501	2667	na	0	371	na	7	8	na	PD	48.5	DOD
	MRP3-503		173	59	na	0	774	na						
	SART3-511		108	79	na	0	268	na						
	WHSC2-103		208	196	na	0	0	na						
FOV-001 (A11/A24)	Lck-208	na	164	85	249	0	0	158	14	10	na	PD	16.2	DOD
	Lck-486		0	243	1236	0	0	0						
	Lck-488		111	106	79 293	0	0	1729						
	PSMA-624		145	96	18 990	0	0	0						
	PTHrP-102		284	233	28 873	0	221	0						
FOV-002 (A24/A31)	EGF-R-800	na	0	178	78	0	0	0	13	14	9	SD	39.3	DOD
	MRP3-503		27	20	44 357	0	710	256						
	MRP3-1293		47	50	0	0	0	0						
	Lck-488		201	159	782	0	0	0						
	EZH2-735		0	91	0	0	0	0						
	PTHrP-102		55	85	25 414	0	424	0						
FOV-003 (A24)	Lck-208	na	77	75	23 700	0	0	0	9	9	14	PD	20	DOD
	PAP-213		0	68	9029	0	0	0						
	EGF-R-800		58	36	137	0	0	0						
	EZH2-735		34	26	22	223	0	0						
	PTHrP-102		47	40	12	0	0	779						
FOV-004 (A26/A33)	SART3-109	na	0	52	na	0	0	na	3	4	na	PD	5.3	DOD
	SART3-511		47	39	na	0	0	na						
	Lck-449		81	75	na	0	0	na						
	HNRPL-501		56	236	na	0	113	na						
FOV-005 (A2/A11)	HNRPL-501	na	15	0	1661	0	65	158	5	2	16	PD	11.4	DOD
	UBE2V-85		11	0	61	0	0	0						
	SART3-302		22	0	815	0	0	0						
	SART3-511		24	16	42	0	0	0						
	Lck-449		36	31	51 851	0	104	0						
FOV-006 (A11/A31)	SART3-109	na	0	22	138	0	0	0	2	3	6	SD	34.7	DOD
	SART3-511		13	14	569	0	0	0						
	SART3-734		0	0	180	0	0	0						
	Lck-449		41	37	72 533	107	107	199						
FOV-008 (A24)	SART2-93	na	63	35	0	0	0	54	8	4	0	PD	14.1	DOD
	Lck-208		69	13	0	0	0	0						
	EGF-R-800		70	58	0	0	0	74						
	SART2-161		65	0	0	0	0	0						
	PTHrP-102		60	24	0	0	0	0						
FOV-009 (A24/A26)	SART2-93	na	271	255	0	0	0	133	8	12	9	PD	8.1	DOD
	SART3-109		0	335	6242	0	0	0						
	Lck-208		264	236	13	0	0	0						
	EGF-R-800		455	428	0	0	0	0						
	SART2-161		441	475	882	0	0	0						
FOV-010 (A2/A24)	CypB-129	na	11	0	146	0	0	0	4	24	22	PD	39.9	AWD
	Lck-246		0	109	55 146	0	0	0						
	HNRPL-140		15	16	11 185	236	233	1080						
	SART3-109		0	45	923	0	0	0						
	PAP-213		14	14	4559	0	0	615						
	SART2-161		17	21	31	67	0	362						
	Lck-486		0	50	2961	0	0	0						
	Lck-488		0	76	67 229	0	0	0						
FOV-012 (A2/A11)	Lck-422	na	33	43	0	0	0	0	7	6	5	PD	16.1	DOD
	ppMAPkkk-432		44	0	29	0	0	57						
	HNRPL-501		0	112	128	0	0	0						
	UBE2V-43		147	0	0	0	0	0						
	SART3-109		3376	5187	44 517	0	0	0						
	SART3-511		169	146	156	0	0	0						
FOV-013 (A24)	SART3-109	na	561	602	na	0	65	na	6	6	na	PD	4	DOD
	Lck-208		96	0	na	0	0	na						
	MRP3-503		19	21	na	0	0	na						
	SART2-161		44	43	na	0	0	na						
	Lck-486		403	312	na	0	0	na						

(*continued*)

Table 4. Continued

ID (HLA-A type)	Peptides for vaccination	Antigen expression in tumor tissue	IgG response (FIU)			CTL response (IFN-γ producing cells/10^5 cells)			Numbers of IgG positive peptides			Best clinical response	Overall survival (months)	Prognosis
			Pre	6th	12th	Pre	6th	12th	pre	6th	12th			
FOV-014 (A24)	SART3-109	na	1692	1899	1298	0	0	0	4	5	4	PD	32.2	DOD
	PAP-213		11	0	23	0	0	806						
	SART2-161		17	20	0	0	0	200						
	Lck-486		1528	1550	1423	0	0	0						
	Lck-488		0	18	0	0	0	0						
	PTHrP-102		0	0	11	0	0	0						
FOV-015 (A2/A31)	CypB-129	na	16	16	0	0	0	0	7	7	3	SD	10.7	DOD
	Lck-422		49	0	0	0	0	0						
	ppMAPkkk-432		29	24	0	0	0	0						
	HNRPL-501		108	81	168	0	0	0						
	SART3-109		1811	1934	148773	na	na	na						
	SART3-511		100	106	16	na	na	na						
FOV-016 (A24/A31)	SART3-109	na	1419	635	0	0	0	68	6	3	4	PD	29.5	DOD
	Lck-486		353	537	271127	0	0	217						
	Lck-488		11	0	423	0	0	0						
	SART3-511		40	0	0	0	0	0						
	PAP-248		24	12	0	0	0	0						
	Lck-486		85	na	na	0	na	na						
FOV-019 (A24/A33)	SART3-109	3+	37	0	0	0	0	0	3	2	5	PD	32.8	AWD
	Lck-486	0	1474	1528	12953	0	0	0						
	PAP-248	0	27	0	15	0	0	0						
FOV-022 (A24/A33)	SART2-93	na	39	32	6554	0	341	274	12	9	11	PD	22	DOD
	PAP-213		69	0	17580	0	502	0						
	SART3-511		82	59	76	0	0	0						
	CypB-129		289	276	811	182	0	0						
	WHSC2-103		138	143	9025	0	0	0						
FOV-023 (A24)	PAP-213	na	25	464	na	0	361	na	2	3	na	PD	16.8	DOD
	Lck-486		312	5553	na	0	0	na						
FOV-024 (A2/A24)	HNRPL-501	na	823	241	na	0	0	na	7	3	na	PD	8.7	DOD
	PAP-213		11	0	na	0	0	na						
	PSA-248		14	23	na	0	0	na						
	Lck-486		18	20	na	0	0	na						
FOV-026 (A2/A11)	Lck-246	na	662	3179	23675	0	1119	1993	16	17	19	PD	17.6	AWD
	WHSC2-141		63	5223	52601	0	2863	1214						
	SART3-302		351	26824	27041	0	928	143						
	SART3-309		48	295	4421	0	2233	506						
FOV-027 (A2/A24)	ppMAPkkk-432	na	121	127	162	0	0	0	17	18	21	CR	15.2	AWD
	SART3-302		474	4297	15968	481	314	0						
	PAP-213		104	96	37274	0	0	109						
	EGF-R-800		34	34	882	0	0	0						
	Lck-488		42	47	502	0	0	202						
FOV-028 (A24)	SART2-93	3+	56	58	65	0	0	179	12	10	10	PD	14.2	AWD
	EGF-R-800	1+	126	120	3832	0	355	140						
	Lck-486	0	28	33	106	0	0	0						
	Lck-488	0	57	63	2425	0	68	541						
	PSMA-624	0	16	17	72	0	0	0						
	PTHrP-102	3+	50	54	58	0	0	900						
FOV-030 (A24/A31)	SART2-93	3+	49	65	356	0	0	86	16	19	20	PD	16.5	AWD
	PAP-213	1+	190	335	10874	0	0	216						
	PSA-248	0	41	7738	9876	0	0	0						
	Lck-488	0	68	97	9767	0	0	1555						
FOV-031 (A11/A26)	SART3-734	na	704	807	914	0	0	0	7	7	8	PD	15.5	AWD
	Lck-449		303	344	577	0	0	0						
	PAP-248		711	728	759	0	0	0						
	WHSC2-103		443	498	1771	0	0	0						
	ppMAPkkk-432		416	364	337	0	0	0						
FOV-032 (A11/A24)	SART2-93	na	36	34	225	0	0	315	15	13	19	PD	11.6	AWD
	Lck-208		39	0	28	0	0	0						
	Lck-488		27	29	139	0	0	0						
	PSMA-624		188	193	10643	0	0	0						
FOV-033 (A24)	SART2-93	na	483	642	11561	109	357	0	8	10	9	PD	10.7	AWD
	PAP-213		125	10222	16907	0	0	0						
	Lck-486		127	146	11828	0	0	0						
	Lck-488		239	1296	1374	0	0	0						
FOV-034 (A31/A33)	SART3-511	na	49	41	298	0	0	0	5	5	7	PD	10.5	AWD
	SART3-734		4398	26909	26716	0	0	0						
	PAP-248		112	355	322	0	0	0						

(*continued*)