Table 1 Clinical characteristics of the patients enrolled in the phase II study

	Group A $n = 99$	Group B $n = 20$	Group C $n = 10$
Age			
Median	56 (34–69)	57 (47–68)	57 (34–69)
Histology			
Endometrioid	70 (70%)	14 (70%)	6 (60%)
UPSC	6 (6%)	4 (20%)	0 (0%)
Clear cell	5 (5%)	0 (0%)	2 (20%)
Others	18 (18%)	2 (10%)	2 (20%)
Stage			
I	36 (36%)	0 (0%)	2 (20%)
II	15 (15%)	0 (0%)	1 (1.0%)
III	44 (44%)	7 (35%)	7 (70%)
IV	4 (4%)	13 (65%)	0 (0%)

Group A: Patients with no residual tumor larger than 1 cm

Group B: Patients who had measurable disease bigger than 1 cm after a surgery, and those who received TEC therapy as the first treatment because their tumors were inoperable

Group C: Patients with recurrent disease

UPSC Uterine papillary serous carcinoma

All patients received intensive follow-up by gynecologists. The number of follow-up visits per year in the first year, the second and third years, the fourth and fifth years, and the sixth year was 12, 4–6, 2 and 1 visit, respectively. Routine physical examinations, including a pelvic-rectal examination, vaginal-vault cytology, and transvaginal ultrasonography (TV-USG), were performed every visit. A CT scan and chest X-ray was performed semi-annually in the first year and annually thereafter. We tested for tumor markers, including CA125, one to four times annually in a subset of the cases. Roughly, 90% of the patients in this retrospective study were treated by these follow-up strategies.

Analysis of efficacy of adjuvant TEC therapy (Group A) compared to radiation therapy

We evaluated the efficacy of the TEC regimen as an adjuvant therapy in Group A patients for both completely resected and optimally resected endometrial cancer cases having residual diseases equal to or less than 1 cm, with risk factors of recurrence. The prognosis of these patients was compared to that of the patients who did not agree to participate in the TEC trial, and so received radiation as an adjuvant therapy during the same study period. These patients agreed to a retrospective analysis to compare the efficacy of TEC chemotherapy and radiation therapy. The costs of chemotherapy and radiation therapy were not compared in the present study.

Statistical analysis

MedCalc (MedCalc Software, Mariakerke, Belgium) was used for statistical analysis. The frequency of adverse effects in the two groups was compared by Fisher's exact test. Distribution of patients' age, histology, and stage were analyzed by the Mann–Whitney U-test or the chi-square test. PFS and OS curves were constructed using the Kaplan–Meier method and evaluated for statistical significance by the log-rank test. Results were considered to be significant when the P value was less than 0.05.

Statements of ethics

This study was approved by the Institutional Review Board and Ethics Committee at each participating institution and all patients provided written informed consent.

Results

Phase I

During the phase I component of this study, assessable patients were enrolled to receive each dose level. Initially, three patients were tested at the starting doses of 150 mg/m^2 for paclitaxel, 50 mg/m^2 for epirubicin, and AUC 4 for carboplatin. One patient encountered a DLT at this dose level; therefore, an additional three patients were tested at the same dosage. One of these patients also encountered a DLT. Thus, 2 of the 6 patients (33%) encountered DLT at this starting dose level, and this dose level was deemed to be the MTD. We proceeded with this as the recommended phase II dosage.

Phase II

Safety

In total, 172 patients were pre-eligible for the phase II study; however, 43 of the resection patients determined to have risk factors for recurrence declined to participate in our experimental drug study and were thus excluded from our phase II analysis.

The 129 patients who did enroll in our phase II drug study were treated with the TEC MTD (150 mg/m^2 for paclitaxel, 50 mg/m^2 for epirubicin, and AUC 4 for carboplatin) and were evaluated for the safety of the TEC regimen. Four patients could not continue the TEC therapy more than 3 courses: one patient suffered anaphylactic shock in reaction to the paclitaxel during her first cycle of treatment, two others acquired grade 4 neutropenia accompanied by severe infection, and the fourth patient

refused to continue the treatment due to grade 3 nausea and emesis. In addition, the records concerning the side-effects for 2 other patients were inaccessible.

The potential adverse effects linked to the MTD of TEC therapy in our phase II study are listed in Table 2. Cardiac failure and treatment-related death did not occur. Grade 3–4 non-hematologic toxicity was infrequent, except that grade 3–4 alopecia was observed in most patients but was reversible following therapy cessation. Grade 3 nausea and vomiting occurred in 2 patients (2%). Grade 3 neutropenia was detected in 35 patients (27%) and grade 4 in 87 patients (67%); febrile neutropenia was observed in 17 patients (13%). G-CSF was used to support the immune and hematopoietic systems in 113 patients (88%). Grade 3 anemia was detected in 14 patients (11%) and grade 4 in four patients (3%). Of those with anemia, three of the patients with grade 3 and two of the patients with grade 4 endured continuous genital bleeding.

Anti-tumor effect (response), PFS, and OS

The 129 patients who received TEC therapy were divided into three risk groups: 99 in Group A, 20 in Group B, and 10 in Group C. The response rate of one Group B patient was undetermined because she refused to be examined by CT after the therapy. The anti-tumor effect was evaluated in Group B and Group C, and the response rates were 74% in Group B (CR in 3, PR in 11, SD in 1, and PD in 4 patients) and 50% in Group C (CR in 0, PR in 5, SD in 0, and PD in 5 patients) (Table 3). This difference was not statistically significant. Endometrioid tumors exhibited a 75% (15 of 20 cases) response rate and non-endometrioid tumors exhibited a 44% (4 of 9 cases) response rate. However, this difference was not statistically significant ($P = 0.39$, by Fisher's exact test).

The PFS and OS of the three groups are shown in Table 4. We obtained by TEC therapy a median OS of 37 months in resected cases with risk factors of recurrence (Group A), 26 months in advanced cases (Group B), and 19 months in recurrent cases (Group C).

Efficacy of TEC as an adjuvant therapy

While our phase II TEC study was being conducted, there were 43 eligible patients with risk factors of recurrence who had declined direct participation in our drug study. These patients opted for radiation adjuvant therapy (Group RT). The distribution of age and histology and the proportion of stage I and II disease of these cases were not different from those in Group A (supplementary Table 1). The PFS and OS of these stages were not different when compared with the RT group (supplementary Figure 1).

We next conducted subgroup analysis by stages. Only four stage IV cases received TEC; thus, a comparison between Group A and Group RT for stage IV tumors was impossible. In stages I and II, PFS and OS of all stages in Group A were not different from those in Group RT (data not shown). In stage III, the prognosis was compared between Group A and Group RT. Patients' characteristics are listed in Table 5. Distribution of age, histology, stage subclass, and sites of recurrence were not significantly different between the two groups. Both PFS and OS were demonstrated to be significantly better in Group A than Group RT (Fig. 1, $P = 0.034$ and $P = 0.040$ by the log-rank test, respectively).

Table 2 Number of patients affected (%) Grade of adverse effects were based on WHO criteria for toxicity

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia	3 (2%)	1 (0.8%)	3 (2%)	35 (27%)	87 (67%)
Thrombocytopenia	88 (68%)	22 (17%)	16 (12%)	2 (2%)	1 (0.8%)
Anemia	27 (21%)	58 (45%)	25 (19%)	14 (11%)	4 (3%)
Nausea/vomiting	24 (18%)	83 (64%)	19 (15%)	2 (2%)	0 (0%)
Diarrhea	124 (96%)	3 (2%)	2 (2%)	0 (0%)	0 (0%)
Peripheral neuropathy	40 (31%)	86 (67%)	3 (2%)	0 (0%)	0 (0%)
Fever	105 (81%)	7 (5%)	17 (13%)	0 (0%)	0 (0%)
Myalgia arthralgia	30 (23%)	92 (71%)	7 (5%)	0 (0%)	0 (0%)
Cutaneous	121 (94%)	6 (5%)	1 (0.8%)	1 (0.8%)	0 (0%)
Cardiac function	129 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cardiac rhytm	125 (97%)	4 (3%)	0 (0%)	0 (0%)	0 (0%)
Renal (Cre)	128 (99%)	1 (0.8%)	0 (0%)	0 (0%)	0 (0%)
Hepatic (AST/ALT)	124 (96%)	4 (3%)	1 (0.8%)	0 (0%)	0 (0%)
Pulmoualy	129 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Allergy	128 (99%)	0 (0%)	0 (0%)	0 (0%)	1 (0.8%)

Table 3 Anti-tumor effect (response rate) of TEC therapy

Histology	No. of patients				Response rate (%)
	CR	PR	SD	PD	
Group B					
Endometrioid	2	9	1	1	85
Non-endometrioid	1	2	0	3	75
Group C					
Endometrioid	0	4	0	3	57
Non-endometrioid	0	1	0	2	33

The anti-tumor effect of TEC was evaluated in Group B and Group C. Response rates were 74% in Group B and 50% in Group C. This difference was not statistically significant. Endometrioid tumors exhibited a 75% response rate in total; however, non-endometrioid tumors responded in 44% of cases, showing no significant difference

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

Table 4 PFS and OS of Groups A, B and C

	OS	PFS
Group A	37 (2–108)	36 (1–108)
Group B	26 (5–57)	12 (3–53)
Group C	19 (4–53)	6 (2–16)

Group A: Patients with no residual tumors larger than 1 cm

Group B: Patients who had measurable disease bigger than 1 cm after a surgery and those who received TEC therapy as the first treatment because their tumor was inoperable

Group C: Patients with recurrent disease

PFS progression-free survival (months)

OS overall survival (months)

Discussion

Systemic chemotherapy is now a standard treatment for advanced and recurrent endometrial carcinomas. The key drugs for this treatment have been the anthracycline and platinum derivatives. A previous study revealed that AP was superior to radiation as an adjuvant therapy; however, severe toxicity and treatment-related deaths were detected in the AP arm [3]. A retrospective study demonstrated that TC was a well tolerated and active regimen for the treatment of resected stages III and IV cases [8]. However, a role of a combination chemotherapy using anthracycline and platinum has been only minimally evaluated [9–11], One such study demonstrated that CAP (cyclophosphamide, doxorubicin and cisplatin) provided a higher PFS and OS rates than radiation in the subgroups of stage Ic in patients over 70 years old or with grade 3 endometrioid adenocarcinoma and stage II/IIIa [9]. In another study, TAP was shown to be superior to the previously regarded standard regimen of AP [12]. However, use of the TAP regimen is often avoided because of its potential for severe toxicity, despite its proven effectiveness for advanced and recurrent endometrial cancer. More recently, the regimen termed TEC was shown to be active in metastatic and recurrent endometrial carcinoma, with tolerable toxicity when accompanied by G-CSF support [6]. However, prior to this current study, the proper TEC dose regimen was undetermined.

Table 5 Patients' characteristics of Group A and Group RT of adjuvant therapy (Stage III)

	Group RT $n = 13$	Group A $n = 44$	*P* value
Age			
Median	53 (44–65)	57 (34–69)	0.13
Histology			
Endometrioid	9 (69%)	32 (73%)	0.82
UPSC	1 (8%)	3 (7%)	
Clear cell	1 (8%)	1 (2%)	
Others	2 (15%)	8 (18%)	
Stage			
III a	3 (23%)	16 (36%)	0.54
III b	0 (0%)	1 (2%)	
III c	10 (77%)	27 (61%)	
Site of recurrence			
Local alone	2 (33%)	2 (25%)	1.00
Distant	4 (67%)	6 (75%)	

Patients of stage III who received TEC therapy as an adjuvant (Group A) and those of stage III who received traditional radiation as an adjuvant therapy (Group RT). Distribution of age and histology was not different between the two groups

UPSC Uterine papillary serous carcinoma

Local alone: recurrence inside of the pelvis

Distant: recurrence out of the pelvis, with or without local recurrence

In our phase I prospective study of TEC therapy, a dose level of 150 mg/m^2 for paclitaxel, 50 mg/m^2 for epirubicin, and AUC 4 for carboplatin was determined to be the optimal dose to use for phase II. Our phase II study demonstrated good tolerance for TEC therapy. In a previous study that showed the safety and effectiveness of a TEC regimen for advanced or recurrent endometrial cancer, paclitaxel and epirubicin were used at the same dose level as in our present study [6]; however, their dose of carboplatin was set at AUC 5, one AUC higher than the AUC 4 used in our study. In their study, grade 3–4 neutropenia, thrombocytopenia, anemia, neuropathy, and grade 2–4 neutropenic fever occurred in 15.5, 2.0, 5.5, 5.0, and 2% of their patients, respectively, and in our study, these same side-effects occurred in 94, 2.8, 0, and 13% of our patients, respectively.

G-CSF was routinely administered from day 5 onward until the total WBC count recovered to an excess of

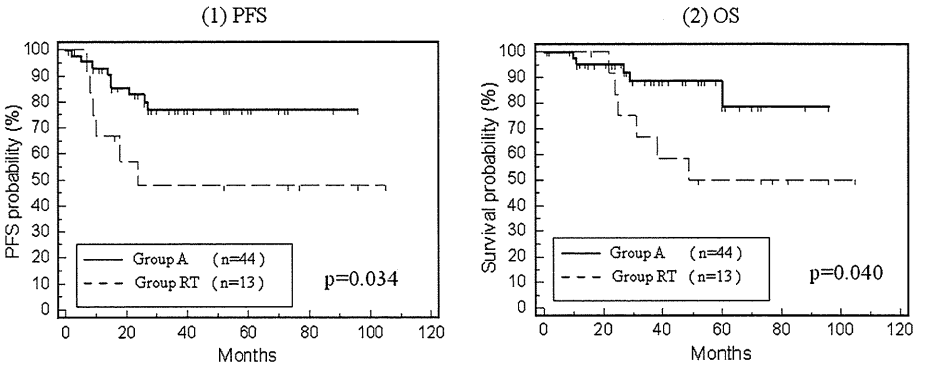

Fig. 1 PFS and OS of TEC and RT groups for adjuvant therapy (Stage III). Both PFS and OS were demonstrated to be significantly better in the TEC group than RT group ($P = 0.034$ and $P = 0.040$ by log-rank test, respectively). *Solid line*: Group A, *Broken line*: Group RT

10,000/μl in the Papadimitriou study. On the other hand, in our study, the G-CSF was administered only when total the WBC/neutrophil count decreased to under 1,000/500 per μl. We feel that this is the reason why severe neutropenia was more frequently observed in our study, despite the lower dose of carboplatin used. We compared our results to the toxicity data acquired from 126 ovarian cancer patients who received a combination of paclitaxel (175 mg/m^2) and carboplatin (AUC 5) (TC) therapy in a similar study conducted in our institute as a multi-center phase I/II study. We found no significant difference in the frequency of grade 3–4 hematologic and non-hematologic toxicities (our unpublished data). These results imply that TEC is an acceptably tolerable chemotherapeutic regimen for endometrial cancer.

The TEC response rate of 66% for advanced and recurrent endometrial carcinoma (CR in 3, PR in 11, SD in 1, and PD in 4 in the advanced group, and CR in 0, PR in 5, SD in 0, and PD in 5 in the recurrent group) was almost equal to that of Papadimitriou's study [6], and similar to the 57% reported for TAP therapy in the GOG #177 study [4]. The median PFS and OS in our study were, respectively, 12 and 26 months in advanced cases, and 6 and 18 months in recurrent cases, which was relatively longer than those in Papadimitriou's study [6]. These results indicate that TEC is an active regimen for the treatment of advanced and recurrent endometrial cancer. Pegylated liposomal doxorubicin has the potential advantage of allowing repeated administration with a lesser likelihood of cumulative cardiotoxicity and was shown to have antitumor activity against ovarian cancer [13]. However, it has only limited activity in endometrial cancer [14, 15].

A retrospective comparison of the survival rates between TEC (Group A) and radiation (Group RT) in resected cases with risk factors of recurrence demonstrated no significant difference. In particular, a subgroup analysis by tumor stages exhibited both PFS and OS of stage III was significantly better in Group A than in Group RT ($P = 0.034$ and $P = 0.040$ by the log-rank test, respectively), indicating a possible role of TEC as an adjuvant. Because less than half of the patients with stage III tumors relapsed or died in our study, the median PFS and OS were not evaluated accurately; however, the durations were estimated to be longer than 96 months at the time of evaluation. These results appeared to be better than the 13 months of PFS and 47 months of OS of the patients who received adjuvant TC therapy in a previous study [8]; however, their study included 50% stage IV cases.

In the present study, the recommended dose of TEC therapy was determined to be paclitaxel 150 mg/m^2, epirubicin 50 mg/m^2, and carboplatin AUC 4. A TEC regimen at this dose level was shown to be tolerable and effective as a remission-induction therapy for advanced and recurrent endometrial cancer. It was also demonstrated, for the first time, that adjuvant TEC therapy for optimally resected stage III cases improved their prognosis when compared to a traditional adjuvant radiation therapy. However, in this study, the effectiveness of TEC was not compared to TC in both advanced and recurrent and adjuvant cases. A further prospective randomized study is still necessary to establish a standard regimen of chemotherapy for the advanced and recurrent cases and the optimally resected cases with risk factors of recurrence.

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Conflict of interest There are no conflicts of interest.

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ONCOLOGY

Recurrent endometrial carcinoma: prognosis for patients with recurrence within 6 to 12 months is worse relative to those relapsing at 12 months or later

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OBJECTIVE: We evaluated association of prognosis of endometrial carcinoma patients and treatment-free intervals (TFIs).

STUDY DESIGN: We compared the effectiveness of second-line chemotherapy performed for patients with TFIs of 6-12 months and 12 or more months following a first-line chemotherapy based on taxane (paclitaxel) and carboplatin, with or without the anthracycline (TC).

RESULTS: Progression-free and overall survivals were significantly shorter in patients with TFIs of 6-12 months than those with TFIs of 12 or more months. Among the patients who received similar second-line chemotherapy, response rates of 15 patients with TFIs of 12 or more months and 7 patients with TFIs of 6-12 months were 67% and 43%, respectively. Progression-free survival was significantly worse in those with TFIs of 6-12 months (median, 7 months) than those with TFIs of 12 or more months (median, 12 months).

CONCLUSION: Our small retrospective analysis suggests that recurrent endometrial carcinomas with TFIs of 6-12 months can be regarded as being partially sensitive to TC-based chemotherapy.

Key words: chemotherapy, endometrial cancer, partially sensitive, prognosis, treatment-free interval

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Endometrial carcinoma is the fourth most common cancer of women in the United States, and its incidence has been increasing steadily during the last 3 decades.[1] The prognosis for early-stage endometrial carcinomas is often extremely good; however, it remains extremely difficult to completely cure the more advanced and the recurrent cases. Irradiation has been performed as the standard postoperative adjuvant therapy for cases with obvious risk factors for recurrence and for almost all advanced cases.[2] Recently, systemic chemotherapy has begun to be used as an adjuvant therapy for advanced cases because it has been reported to significantly improve the prognosis of these cases.[3,4]

Like endometrial cancer, ovarian carcinoma is also common in the United States. The gold standard of therapy for ovarian carcinomas includes aggressive cytoreductive surgery coupled with chemotherapy. The significant effect of the surgical debulking step for ovarian carcinomas has been supported by the subsequent high sensitivity of any remaining tumor to chemotherapy.[1] The length of the treatment-free interval (TFI) before tumor recurrence has been demonstrated to be a strong indicator for the likely response to the application of a second-line chemotherapy for the ovarian carcinomas. Those relapsing within 6 months of the end of first-line treatment often had disease that was likely to be highly resistant to the original first-line drugs and also usually had a low response rate to other second-line chemotherapies.

By contrast, patients with TFIs of 6 or more months had a higher chance of responding well, either to a rechallenge with the platinum-based first-line treatment (defined as platinum sensitive) or to other drugs.[5] Among them, those with a TFI of 6-12 months, although considered as partially sensitive cases, still exhibited a relatively worse response to second-line chemotherapy using carboplatin (after a standard first-line combination chemotherapy using paclitaxel and carboplatin [TC]) than those with a TFI of 12 or more months.[6] For those ovarian carcinoma patients with a 6-12 month TFI after the first-line TC therapy, a combination chemotherapy of pegylated liposomal doxorubicin and carboplatin was shown to provide a better prognosis.[7]

Recently, the effectiveness of similar types of chemotherapy for advanced endometrial cancer cases, using combinations of platinum, taxane, and adriamy-

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cin, have been reported.[8-10] We, and others, have demonstrated the beneficial role that cytoreductive surgery has in these difficult cases.[11,12] Moreover, we have demonstrated that a differential TFI of less than 6 or 6 or more months was significantly associated with the predictable response to second-line chemotherapy and survival prognosis.[13]

In our current study, the response to second-line chemotherapy, after a failure of a first-line chemotherapy of platinum and taxane (with or without adriamycin) in patients with a TFI of 6-12 months was compared with those with a TFI of 12 or more months to evaluate whether the patients with the shorter time to recurrence were able to be considered partially sensitive to chemotherapy.

Materials and Methods

Patients

All patients in the present study were diagnosed within the Departments of Obstetrics and Gynecology of the Osaka University and the Osaka Rosai Hospitals of Osaka (Japan) as having a recurrent endometrial carcinoma. Our departments used either a monthly delivered combination of taxane (paclitaxel) and platinum (carboplatin) with or without the anthracycline, epirubicin (TEC or TC, respectively) or a weekly administered TC regimen as a first-line adjuvant therapy for resected endometrial carcinomas and also as the first-line salvage-therapy for unresected and recurrent diseases.

In the first-line TEC treatment, paclitaxel (150 mg/m^2), carboplatin (area under the curve [AUC] 4), and epirubicin (50 mg/m^2) were administered intravenously every 3-4 weeks. The dose of chemotherapy drugs appropriate for our Japanese population was determined in phase I/II studies we had previously conducted (submitted). In the monthly TC therapy, paclitaxel (175 mg/m^2) and carboplatin (AUC 5) were administered intravenously every 3-4 weeks, based on published protocols.[8,14] In the weekly TC regimen, paclitaxel (80 mg/m^2) and carboplatin (AUC 2) were administered intravenously on days 1, 8, and 15 on a 4 week cycle.[15,16] We will describe both the monthly administered and weekly administered TC regimen as TC therapy in the present report; the TEC and TC regimens will be called the TC-based regimen.

Patients were enrolled in this study after providing their written informed consent. Patients were recruited if they were treated by a second-line chemotherapy for their recurrent disease, after first having an initial first-line adjuvant or salvage TEC or TC therapy. All the patients had measurable disease equal to or larger than 10 mm by a computerized tomography (CT) scan. The diseases were observed in the abdominal cavity in 24 cases (83%), retroperitoneal lymph nodes in 16 cases (55%), and other distant tissues including lung and supraclavicular and inguinal lymph nodes in 10 cases (34%).

A regimen of a second-line chemotherapy was determined by an informed choice of the patients in this retrospective study. The clinicopathological features of these cases, including the age of the patient, the histology and initial stage of the disease, and the regimen of the first and second-line chemotherapies, were retrospectively reviewed utilizing their clinical records, including physical examination notes, radiological reports, operative records, and histopathology reports. The histological diagnoses were made by authorized pathologists from the Departments of Pathology of the Osaka University and the Osaka Rosai Hospitals.

Methods

The tumors were assessed with a helical CT scan at baseline and every 3 treatment courses thereafter. Previously described standard criteria from the World Health Organization[17] and others[18-20] were used to evaluate the therapeutic effect of the second-line chemotherapy. A complete response (CR) was defined as the disappearance of all known disease, determined by 2 observations not less than 4 weeks apart. Partial response (PR) was defined as a 50% or more reduction in the summed products of the 2 largest perpendicular dimensions of bidimensionally measurable lesions, for at least 4 weeks. Stable disease (SD) was defined as a less than 50% decrease, or a less than 25% increase, of tumor size, with no new detectable lesions. Progressive disease (PD) was defined as a greater than 25% increase in tumor size or the appearance of new lesions.

Progression-free survival (PFS) was measured from the date of the last administration of chemotherapy to the date of the radiologic or pathologic relapse or to the date of the last follow-up. Overall survival (OS) was defined as the period from the start of chemotherapy to the patient's disease-specific death or the date of the last follow-up, as previously described.[21] The TFI was defined as the period between the last administration of first-line chemotherapy and the initiation of the second-line chemotherapy, as previously described.[22]

As second-line chemotherapy, some patients received TEC or TC, including weekly TC, and others were given docetaxel (30 mg/m^2) and CPT-11 (60 mg/m^2, irinotecan) (docetaxel plus CPT-11) on days 1 and 8, on a 3-4 week cycle, or daily oral medroxyprogesterone acetate (MPA) (400-600 mg/day). The dose and schedule of administration of docetaxel plus CPT-11 appropriate for our Japanese population was determined in our previous phase I/II study (to be described in detail elsewhere). We define the second-line chemotherapy after the first-line TEC or TC therapy using TEC or TC as a similar regimen to the first-line chemotherapy in the present study.

Statistical analysis of effect of second-line chemotherapy

The association of TFI with clinical characteristics was analyzed by Fisher's exact test. The association between sensitivity to second-line chemotherapy and TFI was analyzed by Fisher's exact test. PFS and OS curves determined by a TFI were constructed using the Kaplan-Meier method and were evaluated for statistical significance by the log-rank test. The multivariate Cox proportional hazards model was used to calculate the significant factors contributing to OS after second-line chemotherapy. Results were considered to be significant when the P value was less than .05.

RESULTS

Clinical characteristics of the patients

During the 10 year study period (from 2000 to 2009), 29 patients, whose disease recurred at 6 months or later from an adjuvant or salvage first-line chemotherapy using TEC or TC, underwent a second-line chemotherapy treatment. The clinicopathological characteristics of these 29 patients are shown in Table 1. The number of the patients with a TFI of 6-12 months was 12 and that of the patients with a TFI of 12 or more months was 17. Age, histology, initial stage, and regimen of the second-line chemotherapy were not significantly different in the 2 groups.

TABLE 1
Clinical characteristics of the patients

Characteristic	TFI 6–12 mo	TFI ≥12 mo	*P* value
Number	12	17	—
Age, y			1.0
<60	5	8	
≥60	7	9	
Histology			.37
Endometrioid	11	13	
Nonendometrioid	1	4	
Initial stage			.27
I/II	3	8	
III/IV	9	9	
Second-line chemotherapy			.092
TEC/TC	7	15	
Others[a]	5	2	

All patients received a first-line chemotherapy using TC/TEC.
MPA, medroxyprogesterone acetate; *TC*, anthracycline; *TEC*, epirubicin; *TFI*, treatment-free interval.
[a] MPA, oral etoposide, and a combination therapy of docetaxel and CPT-11 (irinotecan). The *P* value was calculated by Fisher's exact test.
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Prognosis of the patients after second-line chemotherapy

Survival prognosis of the 12 patients with a TFI of 6-12 months and the 17 patients with a TFI of 12 or more months were compared. The PFS was significantly shorter in those with a TFI of 6-12 months than those with TFI of 12 or more months (hazard ratio [HR], 4.359; 95% confidence interval [CI], 1.588–11.969; $P = .0001$). OS was also significantly shorter in those with a TFI of 6-12 months than those with TFI of 12 or more months (HR, 6.004; 95% CI, 2.123–16.978; $P = .0002$).

Univariate Cox proportional hazards analysis for prognostic factors

To evaluate the significance of TFI on patient survival prognosis, the univariate Cox proportional hazards model was utilized. The results are listed in Table 2. TFI was demonstrated to be a significant factor on OS. Because a regimen of a second-line chemotherapy was determined by an informed choice of the patients, multivariate Cox proportional hazards model was utilized to evaluate whether TFI was an independent prognostic factor. The adjusted HR of TFI (a TFI of 6-12 months vs a TFI of ≥12 months) on OS was 8.390 (95% CI, 2.234–31.508). TFI (a TFI of 6-12 months vs a TFI of ≥12 months) was demonstrated to be a significant factor in predicting survival prognosis ($P = .0017$, based on the multivariate Cox proportional hazards model).

Efficacy of similar TC-based second-line chemotherapy

Among the patients whose disease recurred at 6 months or later from an adjuvant or salvage first-line chemotherapy using TEC or TC, 22 patients underwent a TC-based second-line chemotherapy similar to their first-line chemotherapy. The association of sensitivity to the TC-based second-line chemotherapy and TFI was analyzed. Data from the patients with a TFI less than 6 months were updated from our previous study[13] and a new case was included in the current study. The results are shown in Table 3.

Among the 7 patients with a TFI of 6-12 months, CR or PR was achieved in 3 patients (43%). Among the 15 patients with TFI of 12 months, CR or PR was achieved in 10 patients (67%). Response to TC-based second-line chemotherapy seemed to be better in those with a TFI of 12 or more months than in those with a TFI of 6-12 months; however, this difference was not statistically significant ($P = .29$ by Fisher's exact test), probably because of the small sample size.

The response rate of the TC-based second-line chemotherapy on these patients with a TFI of 6-12 months and a TFI of 12 or more months was significantly higher than those with a TFI less than 6 months ($P = .038$ and $P = .0021$, respectively, by Fisher's exact test). The disease control rate consisting of CR, PR, and SD was 38% (3 of 8 cases), 71% (5 of 7), and 87% (13 of 15) in those with TFI less than 6 months, a TFI of 6-12 months, and a TFI of 12 months, respectively.

The disease control rate of those with a TFI of 6-12 months tended to be higher than that of patients with a TFI less than 6 months ($P = .18$) and tended to be lower than that of those with a TFI of 12 or more months ($P = .39$).

Prognosis of the patients after a similar TC-based second-line chemotherapy

The survival prognosis of the 7 patients with a TFI of 6-12 months and the 15 patients with a TFI of 12 or more months were compared. The PFS was significantly shorter in those with a TFI of 6-12 months compared with those with a TFI of 12 or more months (HR, 3.780; 95% CI, 1.046–13.802; $P = .0035$) (Figure). The OS was also significantly shorter in those with a TFI of 6-12 months than those with a TFI of 12 or more months

TABLE 2
Univariate Cox proportional hazards analysis for prognostic factors

Variable	Adjusted HR	95% CI	*P* value
Age, y			
>60	1		
≥60	0.582	0.218–1.558	.58
Histology			
Endometrioid	1		
Nonendometrioid	2.393	0.749–7.644	.17
Initial stage			
I/II	1		
III/IV	0.857	0.287–2.559	.78
TFI, mo			
≥12	1		
6–12	6.268	2.009–19.560	.0006
Second-line chemotherapy			
TEC/TC	1		
Others[a]	2.478	0.727–8.450	.17

CI, confidence interval; *HR*, hazard ratio; *MPA*, medroxyprogesterone acetate; *TC*, anthracycline; *TEC*, epirubicin; *TFI*, treatment-free interval.

[a] MPA, oral etoposide, and a combination therapy of docetaxel and CPT-11 (irinotecan).

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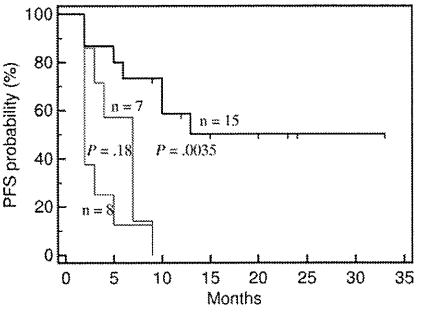
FIGURE
PFS of patients reusing a similar TC-based second-line chemotherapy



The PFS curves determined by TFI were constructed using the Kaplan-Meier method and were evaluated for statistical significance by the log-rank test. The *solid line* indicates patients with TFI of 12 or more months. The *broken line* indicates patients with a TFI of 6-12 months. The *dotted line* indicates patients with TFI less than 6 months.

PFS, progression-free survival; *TC*, anthracycline; *TFI*, treatment-free interval.

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(HR, 4.081; 95% CI, 1.124–14.816; $P = .0086$) (data not shown).

The survival prognosis of the patients with a TFI of 6-12 months was compared with those with a TFI less than 6 month. The PFS and OS tended to be longer in those with a TFI of 6-12 months than in those with a TFI less than 6 months; however, the difference was not statistically significant ($P = .18$ and $P = .55$, respectively), probably because of the small sample size.

COMMENT

In endometrial carcinoma cases such as we have examined here in this study, it has already been demonstrated that a TFI of less than 6 months or 6 or more months was significantly associated with the response to second-line chemotherapy and survival prognosis.[13] Reusing as a second line, TC-based regimens similar to the first-line chemotherapy were shown to be effective for the cases with a TFI greater than 6 months but completely ineffective for those with TFI of less than 6 months.

In our current study, we analyzed whether the patients with a TFI of 6-12 months were able to be considered partially sensitive to chemotherapy, as was the case with ovarian carcinoma. First, we compared 12 patients with a TFI of 6-12 months with 17 patients with a TFI of 12 or more months. The PFS and OS were significantly shorter in those with a TFI of 6-12 months compared with those with a TFI of 12 or more months. The multivariate Cox proportional hazards analysis revealed that TFI (a TFI of 6-12 months vs TFI of ≥12 months) was a significant factor in predicting for survival prognosis and that the adjusted HR of TFI (a TFI of 6-12 months vs a TFI of ≥12 months) on OS was 8.390 (95% CI, 2.234–31.508) (Table 2).

These results suggest that the patients with a TFI of 6 or more months can be divided into 2 groups: a better responsive group and a less responsive group. Next, sensitivity to using a TC-based second-line chemotherapy that was similar to the first-line chemotherapy used was analyzed. The response rates of 15 patients with a TFI of 12 or more months, 7 patients with a TFI of 6-12 months, and 8 patients with a TFI less than 6 months were 67%, 43%, and 0%, respectively (Table 3). The response rate of those with a TFI of 6-12 months was signifi-

TABLE 3
Association of TFI and sensitivity to TC-based second-line chemotherapy

Variable	CR	PR	SD	PD
TFI <6 mo	0 (0%)	0 (0%)	3 (38%)	5 (63%)
TFI 6–12 mo	0 (0%)	3 (43%)	2 (29%)	2 (29%)
TFI ≥12 mo	2 (13%)	8 (53%)	3 (20%)	2 (13%)

CR, complete response; *PD*, progressive disease; *PR*, partial response; *SD*, stable disease; *TC*, anthracycline; *TFI*, treatment-free interval.

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cantly higher ($P = .038$) than that of the patients with a TFI less than 6 months ($P = .038$) and tended to be lower than that of those patients with a TFI of 12 or more months ($P = .29$).

The disease control rate of those with a TFI of 6-12 months tended to be higher than that of patients with a TFI less than 6 months ($P = .18$) and tended to be lower than that of those with a TFI of 12 or more months ($P = .39$). The PFS was significantly worse in those with a TFI of 6-12 months than those with TFI of 12 or more months (HR, 3.780; 95% CI, 1.046–13.802; $P = .0035$) and tended to be better than that of TFI less than 6 months ($P = .18$) (Figure).

These results, although admittedly based on a small retrospective study, imply that endometrial carcinoma cases with a TFI of 6-12 months are still significantly more sensitive to chemotherapeutic drugs than those with a TFI of less than 6 months, and they in turn are less sensitive than those with a TFI of greater than 12 months. Our results indicate that patients with a recurrent endometrial carcinoma and with a TFI between 6 and 12 months are likely to be only partially sensitive to chemotherapy, as is found in those patients with ovarian carcinomas.

Based on these results, as for ovarian carcinoma, alternative chemotherapies should be introduced for those patients with a TFI of 6-12 months to improve their prognosis. In our previous study, no drug response was detected by using oral MPA or oral etoposide for patients with a TFI of 6-12 months; however, a combination therapy of docetaxel and CPT-11 did provide a PR in a single case.

We have recently started a prospective study to evaluate a combination chemotherapy including CPT-11 for those with a TFI of 6-12 months after a TC-based first-line chemotherapy. Further investigations are still required to establish an efficacious strategy for chemotherapy for advanced stage and recurrent endometrial cancers. ■

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ORIGINAL ARTICLE

Second-line chemotherapy for advanced or recurrent endometrial carcinoma previously treated with paclitaxel and carboplatin, with or without epirubicin

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Abstract
Purpose A combined chemotherapy of taxane and platinum, with or without anthracycline, has been used as a standard first-line regimen. The purpose of this study was to investigate the effectiveness of second-line chemotherapy for treatment of advanced or recurrent endometrial carcinoma previously treated with a combined chemotherapy of taxane and platinum, with or without anthracycline.
Methods During the 2000–2008 study period, 723 patients were diagnosed with endometrial cancer at the Departments of Obstetrics and Gynecology of the Osaka University and the Osaka Rosai Hospitals, Osaka, Japan. The subset of these cases that eventually required treatment by second-line chemotherapy was retrospectively analyzed.
Results Response rate to second-line chemotherapy was 25%. Treatment-free interval (TFI) of ≥ or <6 months was demonstrated to be significantly associated with the response to second-line chemotherapy ($P = 0.0026$), progression-free survival ($P = 0.0003$) and overall survival ($P = 0.025$). The second-line chemotherapy similar to the first-line regimen was ineffective in all the 7 cases (100%) whose TFI was shorter than 6 months. Multivariate analysis showed that TFI was the most significantly important factor predicting the effectiveness of second-line chemotherapy (the adjusted hazard ratio of TFI on PFS and OS: 3.482, 95% CI, 1.641–7.388, $P = 0.0012$, and 2.341, 95% CI, 1.034–5.301, $P = 0.042$, respectively).
Conclusions Our present study provides, for the first time, evidence that the majority of refractory or recurrent diseases, if they occur within 6 months of a first-line chemotherapy using taxane and platinum with or without anthracycline, are non-responsive to the current regimens of second-line chemotherapy.

Keywords Endometrial cancer · Second-line chemotherapy · Response · Treatment-free interval · Progression-free survival · Overall survival

Abbreviations

CR	Complete response
HR	Hazard ratio
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
RR	Response rate
SD	Stable disease
TFI	Treatment-free interval
CAP	Cisplatin, Adriamycin and cycloPhosphamide
MPA	MedroxyProgesterone Acetate

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TAP or AP	Anthracycline (doxorubicin) plus Platinum (cisplatin) (with, or without, the Taxane, paclitaxel)
TEC or TC	Taxane paclitaxel and Carboplatin (with, or without, the anthracycline, Epirubicin)
TEP	Taxane (paclitaxel), Epirubicin and the Platinum, cisplatin
TCw, TCm, TECm	TC or TEC administered on a weekly or monthly regimen

Introduction

The incidence of endometrial carcinoma, already the most common malignancy of the female pelvis and the fourth most common cancer among women in the United States, has been increasing steadily during the last three decades [1]. In approximately 75% of patients, the tumor is still confined to the uterus at diagnosis (FIGO stage I), and thus, there is a good prognosis [2]. However, the prognosis for advanced endometrial carcinomas, with extra-pelvic metastatic dissemination, is extremely poor, and the 5-year survival rate is a mere 5–32% [1]. Irradiation was usually performed as post-operative adjuvant therapy for early cases considered being at risk of recurrence and for most advanced cases [3]. Recently, systemic adjuvant chemotherapy, compared to adjuvant irradiation, was reported to significantly improve the prognosis of these cases [4, 5].

About one-fourth of those patients, treated for what was thought to be an early-stage endometrial cancer, go onto develop a recurrence. Unfortunately, 3–19 years after treatment for the recurrence, only 7.7% of the patients were alive and without evidence of disease [3]. Recurrences, restricted to the vaginal vault, are relatively better treated with radiotherapy. However, in most cases of relapse, the disease had spread to other sites, including pelvic and para-aortic lymph nodes, the peritoneum of the pelvis and abdominal cavity, and the lungs. For these cases, systemic chemotherapy is usually required.

Cisplatin and doxorubicin were shown to be the most effective drugs for both advanced and recurrent endometrial carcinomas; paclitaxel was also reported to be useful [6, 7]. A Gynecologic Oncology Group's (GOG) study showed that a tripartite regimen of the Taxane paclitaxel, plus the Anthracycline doxorubicin and the Platinum drug cisplatin (TAP), provided a better response, as measured by progression-free and overall survival rates, than without the taxane (AP). However, there were significant adverse side effects in the TAP group [8]. A modified TAP regimen, with the Taxane paclitaxel and Epirubicin (a semi-synthetic stereoisomer of doxorubicin) plus the Platinum drug cisplatin (TEP), was a more effective combination chemotherapy, with a 73% response rate for advanced endometrial carcinomas, suggesting a possible future role of TEP therapy as the first-line treatment for advanced endometrial carcinomas [9]. A combination chemotherapy of the Taxane paclitaxel and Carboplatin (TC), which is a standard regimen for ovarian carcinoma, was also shown to be a well-tolerated, active adjuvant therapy regimen for advanced endometrial carcinomas [10]. More recently, a combination chemotherapy of the Taxane paclitaxel, Epirubicin and Carboplatin (TEC) was demonstrated to be active and tolerable in patients with advanced metastatic and recurrent endometrial carcinomas [11].

Based on these findings, a combined chemotherapy of taxane and platinum, with or without anthracycline, has been used as a standard first-line regimen for both unresected and recurrent endometrial carcinomas and also as an adjuvant therapy for resected cases with a high risk of recurrence. However, a second-line regimen for the treatment of advanced or recurrent endometrial carcinoma previously treated with a combined chemotherapy of taxane and platinum, with or without anthracycline, has yet to be established. We hoped our findings would change that. In our current study, the effectiveness of second-line chemotherapy and the various clinical factors that associate with the prognosis of advanced or recurrent endometrial carcinoma previously treated with a combination chemotherapy of taxane and platinum, with or without anthracycline, were retrospectively investigated.

Materials and methods

Materials

Patients' characteristics

During the 9-year study period of 2000–2008, we diagnosed 723 endometrial carcinomas in Japanese women at the Departments of Obstetrics and Gynecology of the Osaka University and the Osaka Rosai Hospitals, Osaka, Japan. Traditionally, our department has used either a monthly delivered combination of Taxane (paclitaxel) and Carboplatin (with, or without, the anthracycline Epirubicin, TEC or TC, respectively) or a weekly administered TC regimen as the first-line adjuvant therapy for resected endometrial carcinomas and also as the first-line salvage therapy for unresected and recurrent diseases. Patients were enrolled in the present study, after obtaining their written informed consent, if they were treated by a second-line chemotherapy for their recurrent disease, after first having an initial first-line adjuvant or salvage TEC or TC therapy.

In the initial first-line monthly TEC (TECm) treatment, paclitaxel (150 mg/m^2), carboplatin (AUC = 4) and epirubicin (50 mg/m^2) were administered intravenously every 3–4 weeks. The dose of chemotherapy drugs appropriate for our Japanese population was determined in phase I/II studies we had previously conducted (to be described in detail elsewhere). In the monthly TC (TCm) therapy, paclitaxel (175 mg/m^2) and carboplatin (AUC = 5) were also administered intravenously every 3–4 weeks, based on previous reports [12, 13]. In the weekly TC regimen (TCw), paclitaxel (80 mg/m^2) and carboplatin (AUC = 2) were administered intravenously on days 1, 8 and 15 on a 4-week cycle [14, 15].

The clinicopathological features of these cases, including the age of the patient, the histology and initial stage of the disease and the regimen of the first and second-line chemotherapies, were retrospectively reviewed utilizing their clinical records, including physical examination notes, radiological reports, operative records and histopathology reports. The histological diagnoses were made by authorized pathologists from the Department of Pathology of the Osaka University and the Osaka Rosai Hospitals.

Methods

In order to evaluate the therapeutic effect of second-line chemotherapy, previously described standard criteria from the World Health Organization [16] and others (Pectasides et al. [17–19] were used. The tumors were assessed with a CT scan and/or MRI at baseline and every three treatment courses thereafter. A complete response (CR) was defined as the disappearance of all known disease, determined by two observations not less than 4 weeks apart. Partial response (PR) was defined as a 50% or more reduction in the summed products of the two largest perpendicular dimensions of bi-dimensionally measurable lesions, for at least 4 weeks. Stable disease (SD) was defined as a less than 50% decrease, or a less than 25% increase, of tumor size, with no new detectable lesions. Progressive disease (PD) was defined as a greater than 25% increase in tumor size or as the appearance of new lesions.

Progression-free survival (PFS) was measured from the date of the last administration of chemotherapy to the date of the radiological or pathological relapse or to the date of the last follow-up. Overall survival (OS) was defined as the period from the start of chemotherapy to the patient's disease-specific death or to the date of the last follow-up, as previously described [10]. Treatment-free interval (TFI) was defined as the period between the last administration of first-line chemotherapy and the initiation of the second-line chemotherapy, as previously described [20].

Second-line chemotherapy

Some patients received TECm or TCw (as described above) as second-line chemotherapy. Others were given docetaxel (30 mg/m^2) and CPT-11 (60 mg/m^2) (docetaxel + CPT) on days 1 and 8, on a 3–4-week cycles, or daily oral medroxyprogesterone acetate (MPA) (400–600 mg/day). The dose and schedule of administration of docetaxel + CPT appropriate for our Japanese population was also determined in our previous phase I/II study (to be described in detail elsewhere).

Statistical analysis of effect of second-line chemotherapy

The association between sensitivity to second-line chemotherapy and sensitivity to a TFI was analyzed by Fisher's exact test. PFS curves determined by a TFI were constructed using the Kaplan–Meier method and were evaluated for statistical significance by the log-rank test. The multivariate Cox proportional hazards model was used to calculate the significant factors contributing to PFS after second-line chemotherapy. Results were considered to be significant when the P value was <0.05.

Results

Clinical characteristics of the study cases

During the 9-year study period, 40 patients required a second-line chemotherapy treatment against a recalcitrant or recurrent disease, after having first received an adjuvant or salvage first-line chemotherapy using TECm, TCm or TCw. The clinicopathological characteristics of these 40 patients are shown in Table 1. Eighty percent (32 out of 40 cases) of the patients received first-line TECm therapy; the other eight cases underwent either monthly or weekly TC (TCm or TCw) therapy, prior to the second-line chemotherapy. No cases were canceled due to second-line chemotherapy toxicity.

Outcome of the patients after second-line chemotherapy

Among the 40 patients, 24 patients received second-line TECm, TCm or TCw, 3 received docetaxel + CPT, 7 received oral MPA therapy, and 6 received oral Etoposide therapy (Table 2). The overall response rate for second-line chemotherapy was 25% (0–38%). The PFS was 3.5 months (0–20 months), and the OS was 10 months (2–44 months). The response rate, PFS, and OS were not significantly different among the TECm/TCm/TCw, docetaxel + CPT, MPA and Etoposide groups.

Table 1 Clinical characteristics of the study cases

Characteristics	Patients ($n = 40$)	
	Number	%
Age (years)		
<60	18	45
≥60	22	55
Histology		
Endometrioid	32	80
Serous	4	10
Clear cell	1	3
Others	3	8
Initial stage		
I	10	25
II	6	15
III	19	48
IV	5	13
First-line chemotherapy		
TEC	32	80
TC	7	18
Weekly TC	1	3

All patients received first-line chemotherapy using taxane and carboplatin (with or without epirubicin, TC/TEC)

TECm monthly administration of taxane (paclitaxel), epirubicin and carboplatin, *TCm* monthly administration of paclitaxel and carboplatin, *TCw* weekly administration of paclitaxel and carboplatin

Table 2 Outcome of the patients after second-line chemotherapy

	Cases	Response rate (%)	PFS (months)	OS (months)
TEC/weekly TC	24	38	5.5 (2–20)	13 (3–44)
Docetaxel + CPT-11	3	33	4 (0–5)	6 (4–10)
MPA (oral)	7	0	1 (0–3)	5 (2–22)
Etoposide (oral)	6	0	2 (1–8)	9 (4–11)
Total	40	25	3.5 (0–15)	10 (2–44)

No significant difference was demonstrated among the four groups (TECm/TCm/TCw, docetaxel + CPT, MPA and Etoposide)

TECm monthly administration of paclitaxel, epirubicin and carboplatin, *TCw* weekly administration of paclitaxel and carboplatin, *TCm* monthly administration of paclitaxel and carboplatin, *MPA* oral daily medroxyprogesterone acetate, *Etoposide* oral daily Etoposide, *PFS* progression-free survival, *OS* overall survival

Association between TFI and sensitivity to second-line chemotherapy

The effect of a TFI, after first-line chemotherapy using TC/TEC, on the tumor's sensitivity to second-line chemotherapy was evaluated. Among the 24 patients whose TFI was equal to or longer than 6 months, CR or PR was achieved in 10 patients (42%). However, all 16 cases (100%) whose TFI was shorter than 6 months exhibited either SD or PD against the second-line chemotherapy. This association between TFI and sensitivity to a second-line chemotherapy was statistically significant ($P = 0.0026$ by Fisher's exact test). These results are tabulated in Table 3.

Association between TFI and sensitivity to second-line chemotherapy using taxane and platinum, with or without anthracycline, was, next, analyzed. Among the 17 patients whose TFI was equal to or longer than 6 months, CR or PR was achieved in 9 patients (53%). However, all 7 cases (100%) whose TFI was shorter than 6 months exhibited either SD or PD against the second-line chemotherapy. This association between TFI and sensitivity to a second-line chemotherapy was statistically significant ($P = 0.015$ by Fisher's exact test). These results are tabulated in Table 4.

PFS and OS after second-line chemotherapy by TFI

The differences by TFI in the effectiveness of second-line chemotherapy regimens were investigated. The median PFS was 7 months (1–20 months) for the 26 patients whose TFI was equal to or longer than 6 months, which

Table 3 Association between TFI and effectiveness of second-line chemotherapy

	CR/PR	SD/PD
TFI ≥ 6 months	10 (42%)[a]	14 (58%)
TFI < 6 months	0 (0%)	16 (100%)[a]

Forty-two percent (10 out of 24 cases) of the patients whose TFI was equal to or longer than 6 months exhibited sensitivity to second-line chemotherapy; however, all cases whose TFI was shorter than 6 months were resistant to second-line chemotherapy. This association was statistically significant ($P = 0.0026$ by Fisher's exact test)

TFI treatment-free interval, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

[a] $P = 0.0026$

Table 4 Association between TFI and sensitivity to second-line chemotherapy using taxane and platinum, with or without anthracycline

	CR/PR	SD/PD
TFI ≥ 6 months	9 (53%)[a]	8 (47%)
TFI < 6 months	0 (0%)	7 (100%)[a]

Fifty-three percent (9 out of 17 cases) of the patients whose TFI was equal to or longer than 6 months exhibited sensitivity to second-line chemotherapy; however, all cases whose TFI was shorter than 6 months were resistant to second-line chemotherapy. This association was statistically significant ($P = 0.015$ by Fisher's exact test)

TFI treatment-free interval, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

[a] $P = 0.015$

was significantly longer than the 2 months (0–9 months) for the 14 whose TFI was shorter than 6 months ($P = 0.0003$ by log-rank test) (Fig. 1). The median OS was 13 months (3–22 months) for the 26 patients whose TFI was equal to or longer than 6 months, which was significantly longer than the 5.5 months (2–44 months) for the 14 whose TFI was shorter than 6 months ($P = 0.025$ by log-rank test) (Fig. 1).

Multivariate Cox proportional hazards analysis for effectiveness of second-line chemotherapy on PFS and OS

In order to further support our finding that a TFI was significantly associated with the effectiveness of second-line chemotherapy, the multivariate Cox proportional hazards model was utilized. The results are listed in Table 4. The adjusted hazard ratio (HR) of TFI (≥6 months versus <6 months) on PFS was 3.482 (95% CI, 1.641–7.388) and that of TFI on OS was 2.341 (95% CI, 1.034–5.301). TFI was demonstrated to be a significant factor in predicting for PFS and OS ($P = 0.0012$ and $P = 0.042$, respectively, based on the multivariate Cox proportional hazards model) (Tables 5 and 6).

Table 5 Multivariate Cox proportional hazards analysis for effectiveness of second-line chemotherapy on PFS

Variable	Adjusted HR	95% CI	*P* value
Age (years)			0.14
<60	1		
≥60	0.573	0.274–1.197	
Histology			0.53
Endometrioid	1		
Non-endometrioid	1.331	0.546–3.247	
Initial stage			0.82
I/II	1		
III/IV	1.092	0.517–2.308	
TFI			0.0012
≥6 months	1		
<6 months	3.482	1.641–7.388	

The adjusted HR of TFI < 6 months was 3.482 (95% CI, 1.641–7.388), compared to TFI ≥ 6 months, showing statistical significance ($P = 0.0012$)

HR hazard ratio, *TFI* treatment-free interval

Conclusions

Endometrial adenocarcinoma is increasingly the most common malignancy of the female pelvis in the United States [1]. Although early-stage endometrial carcinomas have a good prognosis [2], the prognoses for advanced or recurrent cases are extremely poor (except for recurrences limited to the vaginal vault, which can generally be treated successfully with surgery and radiation therapy) [3]. Although a truly successful treatment for advanced or recurrent endometrial carcinomas has yet to be established, taxane and platinum (with or without anthracycline, TC/TEC) have demonstrated at least a partial efficacy [6–11].

Recently, based on these findings, the combined chemotherapy of TC/TEC has been used as a standard first-line regimen for unresected and recurrent endometrial carcinomas as well as an adjuvant therapy for resected cases with a high risk of recurrence. However, an effective second-line regimen for advanced or recurrent endometrial carcinomas previously treated with TC/TEC has not yet been established.

In our present study, we analyzed the outcomes of patients who, after having received a first-line TC or TEC

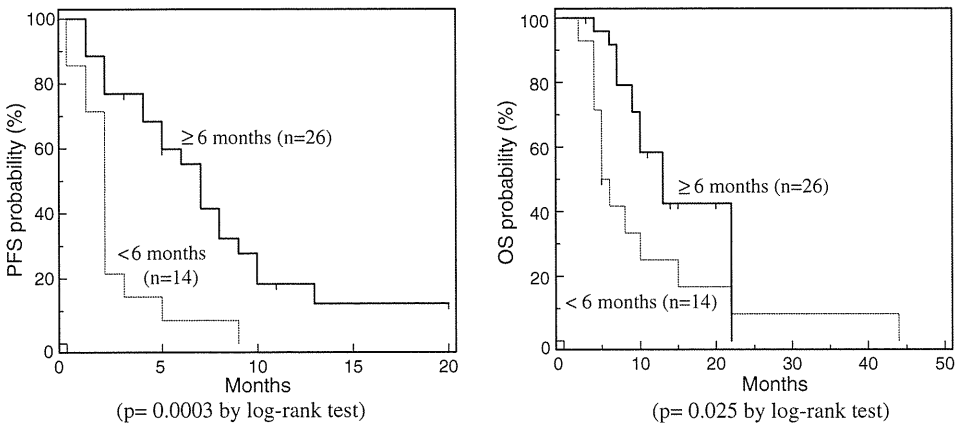

Fig. 1 PFS and OS after second-line chemotherapy by TFI. Progression-free probability and overall probability after second-line chemotherapy of the patients whose TFI was equal to or longer than 6 months were significantly longer than those whose TFI was shorter than 6 months ($P = 0.0003$ and $P = 0.025$, respectively, by the log-rank test)

Table 6 Multivariate Cox proportional hazards analysis for effectiveness of second-line chemotherapy on OS

Variable	Adjusted HR	95% CI	*P* value
Age (years)			0.081
<60	1		
≥60	0.497	0.228–1.085	
Histology			0.40
Endometrioid	1		
Non-endometrioid	1.497	0.590–3.800	
Initial stage			0.30
I/II	1		
III/IV	1.613	0.658–3.953	
TFI			0.042
≥6 months	1		
<6 months	2.341	1.034–5.301	

The adjusted HR of TFI < 6 months was 2.341 (95% CI, 1.034–5.301), compared to TFI ≥ 6 months, showing statistical significance ($P = 0.042$)

HR hazard ratio, *TFI* treatment-free interval

chemotherapy, received a variety of second-line chemotherapy against relapsed or recurrent tumors. The overall response rate to second-line chemotherapy was 25% (0–38%). The PFS was 3.5 months (0–20 months), and the OS was 10 months (2–44 months). The response rates, PFS and OS did not differ significantly by the regimen of second-line chemotherapy. Therefore, other factors associated with the response to second-line chemotherapy were investigated.

In the related field of ovarian carcinomas, a TFI has consistently been shown to be the most important factor in the prediction of the response to the second-line chemotherapy [21–24]. Patients with ovarian tumor who relapse within 6 months have a disease that is significantly more likely to be both resistant to the original drugs and to have a lower response rate to new chemotherapy; however, those who relapse after 6 months from a first-line platinum-based chemotherapy have a higher chance of responding well, either to a re-challenge with a platinum-based treatment or to other agents [21].

However, comparable predictive factors for the response to second-line chemotherapy have not been established for endometrial carcinomas. It was yet to be determined whether TFI predicts a response to second-line chemotherapy, and if so, whether 6 months was a reasonable critical threshold that would suggest the clinical outcome after second-line chemotherapy. It was also unclear which current regimen is most effective as a second-line chemotherapy for endometrial carcinoma.

In our study, a TFI greater or less than 6 months was demonstrated to be significantly associated with the tumor's likely responsiveness to a second-line chemotherapy ($P = 0.0026$ by Fisher's exact test). The current study also showed that the association between TFI and sensitivity to a second-line TECm, TCm or TCw was statistically significant ($P = 0.015$ by Fisher's exact test), indicating that second-line chemotherapy using taxane and platinum, with or without anthracycline, was not effective for those whose TFI was shorter than 6 months after initial treatment using taxane and platinum, with or without anthracycline.

PFS and OS were also shown to relate to a TFI ≥ or <6 months ($P = 0.0003$ and $P = 0.025$, respectively, by the log-rank test). As in ovarian tumors, multivariate analysis showed that the TFI for endometrial tumors was also the most significant factor for predicting the effectiveness of a second-line chemotherapy (the adjusted HR of TFI (≥6 months versus <6 months) on PFS: 3.482 (95% CI, 1.641–7.388), and that on OS: 2.341 (95% CI, 1.034–5.301)). TFI was demonstrated to be a significant factor in predicting for PFS and OS ($P = 0.0012$ and $P = 0.042$, respectively, based on the multivariate Cox proportional hazards model).

These results imply, for the first time, that the standard strategy for ovarian carcinoma second-line chemotherapy can now be applied to endometrial carcinoma as well. Endometrial carcinomas that relapse at least 6 months after first-line chemotherapy using TC/TEC have a significantly higher chance of responding to second-line chemotherapy. However, the tumors that relapse before 6 months are likely to be resistant. In fact, none of the currently popular regimens of chemotherapy (including the same as, or other than, the first-line regimen) were effective, and the PFS and OS were only 2 months (0–9 months) and 5.5 months (2–44 months), respectively (data not shown). Palliative care in these cases, rather than second-line chemotherapy, may for now be more appropriate.

Our present study provides, for the first time, good evidence that relapsed or recurrent diseases found within 6 months from a first-line chemotherapy regimen using taxane and platinum (with or without anthracycline) fail to respond to second-line chemotherapy. Further investigation is still required to establish an efficacious strategy for second-line chemotherapy for advanced or recurrent endometrial cancer.

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Expression of aldehyde dehydrogenase 1 (ALDH1) in endometrioid adenocarcinoma and its clinical implications

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Aldehyde dehydrogenase 1 (ALDH1) is expressed in stem/progenitor cells, including cancer-initiating cells (CIC) of various organs. In the present study, ALDH1 expression was immunohistochemically examined in uterine endometrioid adenocarcinoma. The ALDH1 was expressed in a small portion of tumor cells, and these ALDH1-expressing cells were less mature than ALDH1-non-expressing cells. The ALDH1-expressing (ALDH1-hi) cells were more tumorigenic, resistant to anti-cancer agents and more invasive than ALDH1-lo cells. Culture of the sorted ALDH1-hi cells yielded both ALDH1-hi and ALDH1-lo cells, whereas ALDH1-lo cells yielded ALDH-lo cells alone. Clinically, a high-level of ALDH1 expression in tumor cells was correlated with T category, lymphatic invasion, recurrence and prognosis of patients. Patients with high ALDH1 expression showed poorer prognoses than those with low expression ($P = 0.015$ for disease-free survival [DFS] and $P = 0.010$ for overall survival [OS]), and high ALDH1 expression was an independent factor for poor prognosis. Aldehyde dehydrogenase 1 is a candidate for CIC marker for uterine endometrioid adenocarcinoma. (*Cancer Sci* 2011; 102: 903–908)

Tumors consist of heterogeneous cell populations derived from a single clone. Recently, it has been demonstrated that cells with tumorigenic potential are limited to a small population among tumor cells, called cancer-initiating cells (CIC), in cancers of blood (leukemia), breast, brain and colon.[1–11] Cancer-initiating cells efficiently efflux anti-tumor agents and degrade reactive oxygen species that are related to radiation-induced apoptosis. Furthermore, CIC are in a quiescent state for cell division, and thus escape the attack of various anti-cancer drugs targeting the rapidly dividing tumor cells. These characteristics enable CIC to be resistant to anti-tumor drugs and radiation therapy.[12–15]

Endometrioid adenocarcinoma is one of the most common malignancies of the female genital system.[16,17] Despite the advances in methods for detection and treatment, prognosis of patients with endometrioid adenocarcinoma still remains unfavorable. Therapeutic strategies targeting CIC would be necessary to improve cure rates, but studies on CIC of endometrioid adenocarcinoma are limited. Gotte *et al.*[18] demonstrated that Musashi-1, highly expressed in neural stem cells, was co-expressed with Notch-1 in a subpopulation of endometrial cells and endometrioid adenocarcinoma cells. Kato *et al.*[19] demonstrated that the side-population of endometrioid adenocarcinoma, which is considered to contain CIC, possessed higher tumorigenic activities than non side-population cells. To our knowledge, the relationship of stem cell marker expression to prognosis has not been reported in endometrioid adenocarcinoma.

Aldehyde dehydrogenase 1 (ALDH1), a predominant isoform of the ALDH family in mammals, oxidizes retinol to retinoic acid in early stages of stem cell differentiation, and hematopoietic and neural stem cells show high ALDH1 activity.[20–22] Cancer-inducing cells of human multiple myeloma, acute myeloid leukemia and cancers of brain, lung and breast also show high ALDH1 activity.[23–27] The activity of ALDH1 might be a common marker for both normal and malignant stem cell populations. In the present study, ALDH1 expression was immunohistochemically examined in endometrioid adenocarcinoma and its clinical implications were evaluated.

Materials and Methods

Patients. Ninety-eight patients who underwent surgery for uterine endometrioid adenocarcinoma at Osaka University Hospital from January 1998 to January 2007 were examined. Clinicopathological findings in these 98 patients are summarized in Table 1. The age of patients ranged from 22 to 75 years (median, 55.9 years). Resected specimens were macroscopically examined to determine the location and size of the tumors. Histological specimens were fixed in 10% formalin and routinely processed for paraffin embedding. Paraffin-embedded specimens were stored in the dark room in the Department of Pathology of Osaka University Hospital at room temperature, sectioned at 4-μm thickness at the time of staining, and stained with H&E and immunoperoxidase procedures. The histological stage was determined according to the International Federation of Obstetricians and Gynecologists (FIGO) staging system. All patients were followed up with laboratory examinations including routine peripheral blood cell counts at 1- to 6-month intervals, X-ray, computed tomographic scan and pelvic examination at 6- to 12-month intervals. The follow-up period for survivors ranged from 8 to 122 months (median, 89 months). The study was approved by the ethical review board of the Graduate School of Medicine, Osaka University.

Immunohistochemistry for ALDH1, ER, PgR, CD9 and MIB-1. Expression of ALDH1, estrogen receptor (ER), progesterone receptor (PgR) and Cluster Differentiation (CD) was examined with anti-ALDH1 (BD Biosciences, Franklin Lakes, NJ, USA), anti-ER (Dako, Glostrup, Denmark), anti-PgR (Dako) and anti-CD9 (Abcam Ltd, Cambridge, UK) antibodies, respectively. The proliferative activity of cancer cells was examined with monoclonal antibody MIB-1 (Immunotech, Marseilles, France), recognizing the proliferation-associated antigen Ki67. The antigen retrieval with Pascal pressurized heating chamber (Dako) was done for the staining of ER, PgR, CD9 and MIB-1. The

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Table 1. Summary of characteristics in 98 endometrioid adenocarcinoma patients

	Number of patients
Tumor	
T1	70
T2	8
T3	20
Lymph node	
N0	73
N1	25
Tumor histological grade	
Grade 1	38
Grade 2	39
Grade 3	21
Estrogen receptor status	
Positive	40
Negative	58
Progesterone receptor status	
Positive	75
Negative	23
Ki67 labeling index	
≥ 20%	82
<20%	16
Response to chemotherapy	
Non-respond	17
Respond	32
Recurrence	
Positive	20
Negative	78
Prognosis	
Dead	15
Alive	83

sections were incubated with anti-ALDH1 (×100), -ER (×2), -PgR (×6), -CD9 antibody (×100) or MIB-1 (×50), then treated with a ChemMate EnVision kit (Dako). Diaminobenzidine (DAB) (Dako) was used as a chromogen. As the negative control, staining was carried out in the absence of primary antibody. Stained sections were evaluated independently by two pathologists (JI, EM). As described previously,[24] cases with more and <10% of cells positive for ALDH1 were regarded as ALDH1-hi and ALDH1-lo, respectively. The proportion and intensity of ER and PgR expression were evaluated as described previously.[28] The MIB-1 labeling index was defined as the percentage of stained nuclei per 1000 cells. The patients were divided into MIB-1-high and MIB-1-low groups using the median as cut-off value.

Double staining of ALDH1 and CD9, ER and PgR. Double staining of ALDH1 and CD9, ER and PgR was done with the EnVision G/2 doublestain system (Dako) according to the manufacturer's protocol. First, the ALDH1 was stained with DAB, and subsequently the staining of CD9, ER and PgR was done with Permanent Red. Since the red fluorescence is released from Permanent Red, the signal of CD9, ER and PgR was detected with a fluorescence microscope (Biozero, Keyence, Osaka, Japan).

Cell lines and isolation of ALDH1-hi population. Endometrioid adenocarcinoma cell lines HEC-1, -1A, -108, -116, -6, -88nu and -251; and SNG-M and -II were obtained from the Health Science Research Resources Bank of Osaka, Japan. Cells were cultured in DMEM (Wako, Osaka, Japan) supplemented with 10% FCS (Nippon Bio-Supply Center, Tokyo, Japan). To isolate the population with high ALDH1 enzymatic activity, the Aldefluor kit (Stem Cell Technologies, Vancouver, BC, Canada) was used according to the manufacturer's instructions. Briefly, cells were suspended in Aldefluor assay buffer containing ALDH1 substrate and BODIPY–aminoacetaldehyde (BAAA). The BAAA was taken up by living cells and converted by intracellular ALDH1 into BODIPY–aminoacetate, which causes the cells to fluoresce brightly. The brightly fluorescent ALDH1-expressing cells were detected with FACS Calibur or FACS Aria (BD Biosciences). As a negative control, cells were stained under identical conditions with the specific ALDH1 inhibitor, diethylaminobenzaldehyde (DEAB; Sigma, St Louis, MO, USA). Data were analyzed by using Cell Quest software (BD Biosciences). In endometrioid adenocarcinoma cell lines, cells with bright fluorescence were judged as ALDH-hi, and those with no or faint fluorescence as ALDH-lo. The criteria for ALDH1-hi and ALDH1-lo in cell lines were different from those for ALDH1 immunohistochemistry in clinical samples.

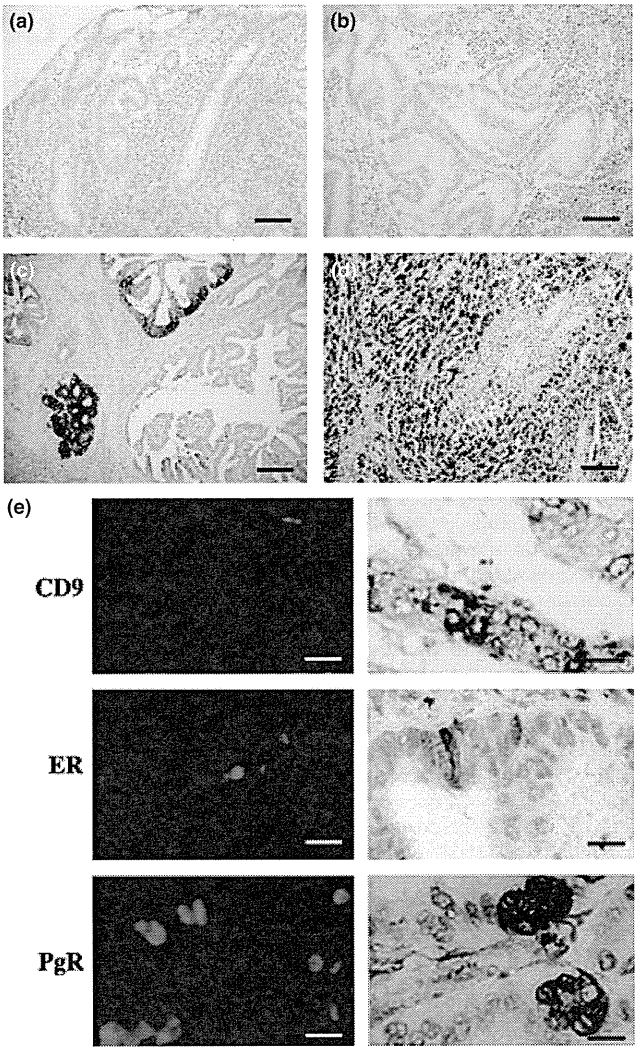

Fig. 1. Expression of ALDH1 in normal endometrium and endometrioid adenocarcinoma. Normal proliferative (a) and secretory (b) phases of endometrial epithels did not express ALDH1. (c) Small portions of endometrioid adenocarcinoma were positive for ALDH1 in some cases. (d) Most tumor cells were positive for ALDH1. (e) Double staining of ALDH1 for CD9, ER or PgR was done. Red fluorescence from CD9, ER or PgR staining signals is shown at left. Scale lines, (a–d) 200 μm, (e) 20 μm.

Effects of the anticancer drug cisplatin. Cisplatin is commonly used for the treatment of endometrioid adenocarcinoma. The effect of cisplatin on ALDH-hi cells was compared to that

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on ALDH-lo cells. Cells (1×10^4) were seeded onto cell culture plates with DMEM-10% FBS, cultured for 20 h, and various concentrations of cisplatin (0, 1, 4, 8 μg/mL) were added. After 24 h, the viability of cells was assessed with the Premix WST-1 cell assay system (Takara Bio Inc., Kyoto, Japan). The absorbance of cisplatin-treated cells at 450 nm was subtracted from the background absorbance (600 nm). The resultant value was divided by that of cells not treated with cisplatin, and the results are shown as the viability index.

Matrigel invasion assay. Invasion of tumor cells into Matrigel was examined with a BD BioCoat Matrigel Invasion Chamber (BD Biosciences). Briefly, cells were seeded in DMEM without FCS in the Matrigel invasion upper chamber and cultured for 72 h. The lower chamber contained DMEM and 10% FBS. Invading cells were stained with a Diff-quick staining kit (Siemens, Munich, Germany). The number of invading cells was counted in four microscopic fields per well at a magnification of ×20 and the extent of invasion was expressed as the average number of cells per square millimeter.

***In vitro* colony formation assay.** Cells were suspended in 0.1 mL of DMEM and 10% FBS, and 1000 cells were plated in culture dishes with 1 mL of methylcellulose-containing DMEM supplemented with 15% FBS. The number of colonies was counted on day 14.

Statistical analysis. Statistical analyses were performed using StatView software (SAS Institute Inc., Cary, NC, USA). The Chi-square and Fisher's exact probability tests were used to analyze the correlation between ALDH1 expression and clinicopathological factors in endometrioid adenocarcinoma. Kaplan–Meier methods were used to calculate overall survival (OS) and disease-free survival (DFS) rates, and differences in survival curves were evaluated with the log-rank test. Cox's proportional hazards regression model with a stepwise manner was used to analyze the independent prognostic factors. The P values of <0.05 were considered to be statistically significant.

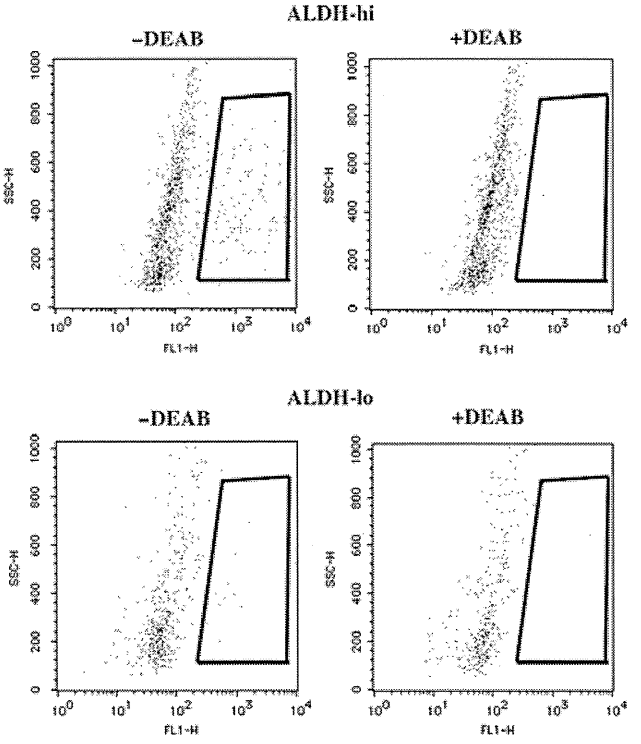

Fig. 3. ALDH1 activity of ALDH-hi and ALDH-lo HEC-1 cells after culture for 5 days. Dot–blot of Aldefluor assay without inhibitor is shown in the left side, and that with inhibitor on the right side.

Results

Immunohistochemical findings. The expression of ALDH1 was examined in normal endometrium and 98 endometrioid adenocarcinoma tissues. No signals were detected in normal proliferating and secretory phase of endometrium (Fig. 1a,b, respectively). Strong signals were found in the cytoplasm of a small portion of tumor cells in several cases (Fig. 1c), whereas signals were found in most tumor cells in other cases (Fig. 1d). The expression of ALDH1 was not detected in some cases. Based on the criteria of Jiang *et al.*,[24] cases with more than

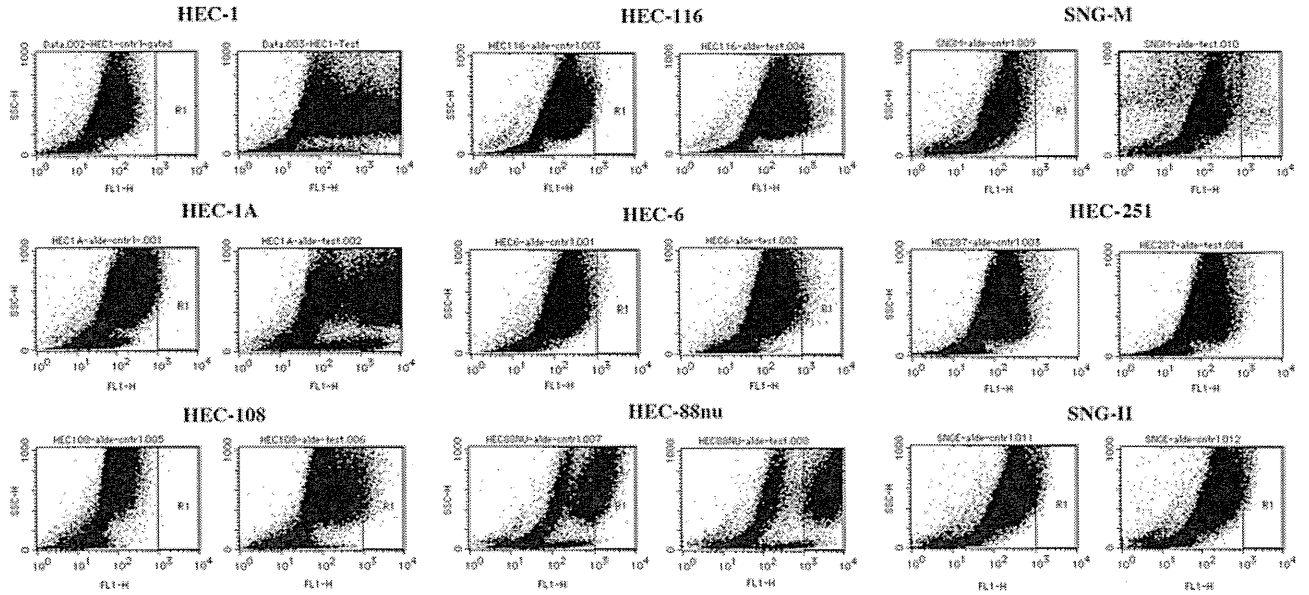

Fig. 2. Activity of ALDH1 in various endometrioid adenocarcinoma cell lines. The left side of each cell line shows dot–blot of Aldefluor assay with inhibitor (DEAB), and the right side shows dot–blot without inhibitor. The ALDH1-hi population is boxed.