The other secondary end points were the response rate to NAC, the proportion of patients who underwent IDS, progression-free survival (PFS) among patients whose clinical diagnosis was confirmed by laparoscopy, the operative morbidity, the adverse events, and the overall survival (OS) among all the enrolled patients. The response to NAC was assessed according to the RECIST (Response Evaluation Criteria In Solid Tumor) [12]. Grading of the adverse events was performed based on NCI-CTC (National Cancer Institute-common toxicity criteria) ver. 2.0.

Study design and statistical methods

The study was planned as a single-stage safety and efficacy study. Sample size calculation was primarily based on the binominal test for the primary end point. Forty-four patients were required when expected %cCR of 40% and an acceptable lowest %cCR of 20% with a one-sided alpha error of 0.05 and a beta error of 0.1. Additionally, the PPV of overall prelaparoscopic diagnoses was to be sufficiently confident to enable the omission of laparoscopy in the subsequent phase III study. Thus, Bayesian monitoring of PPV was planned, and it required 56 patients to have a 10% or lower Bayesian posterior probability that PPV is less than 90% in case of 3 false-positive patients assuming the prior distribution of Beta (9,1). The target sample size was determined to be 56, which is also sufficient for the primary end point. The planned accrual period was 1 year, and the follow-up period was 3 years. All analyses were performed using the SAS software release 9.1 (SAS Institute, Cary, NC).

Results

Patient characteristics

Fifty-six women were entered between January 2003 and February 2004. All but one patient were eligible for the study. The ineligible patient once fulfilled the eligibility 1 week before enrollment. However, the blood examination just before enrollment showed a slightly lower WBC and ANC than the eligibility. This patient was included in the following analysis, though this patient dropped out of the study during NAC due to myelo-suppression. The PS of all 56 patients at enrollment was 0 in 28 patients, 1 in 18 patients, 2 in 7 patients, and 3 in 3 patients. The median age at enrollment was 55 (range, 33–73) years. The median follow-up period of the living patients was 39 (range, 34–46) months at the data cutoff in February 2007.

Accuracy of the clinical diagnosis

DLS was performed in all enrolled patients. Laparoscopic findings and histological findings revealed all 56 patients had MC with a histology corresponding to epithelial ovarian carcinoma. Concerning the stage of the disease, the diagnosis was stage III/IV in 53 patients by laparoscopic findings in combination with prelaparoscopic findings of the presence of distant metastases, malignant pleural effusion, and lymph node metastases. The PPV of prelaparoscopic diagnosis concerning the origin and histology was 100% (56/56), and both the PPVs of prelaparoscopic diagnosis concerning the stage and overall diagnosis were 95% (53/56). The histology of the diseases misdiagnosed in stage were endometrioid adenocarcinoma in 2 and serous adenocarcinoma in 1. Table 1 shows the prelaparoscopic and laparoscopic diagnoses of the disease.

Compliance to the treatment

The compliance to the treatment protocol is depicted in Fig. 1. Six patients successfully completed the treatment algorithm once they were off protocol due to toxicities. In one patient, after the discontinuation criteria were fulfilled during NAC, a similar treatment consisting of 3 cycles of chemotherapy as NAC, IDS and 6 cycles of postoperative chemotherapy was performed. In the other 5 patients, the same treatment was administered at a reduced dose and/or a delayed schedule.

Table 1
Prelaparoscopic and laparoscopic diagnosis of the disease

	Prelaparoscopic diagnosis		Laparoscopic diagnosis	
Origin[a]	Ovary	48	Ovary	47
	Tube	4	Tube	7
	Peritoneum	10	Peritoneum	12
Histology[b]	Adenocarcinoma (not specified)	56	Adenocarcinoma (not specified)	18
			Serous	29
			Mucinous	2
			Endometrioid	5
			Undifferentiated	2
T classification	T1c	0	T1c	1
	T2c	4	T2c	5
	T3	52	T3a	0
			T3b	12
			T3c	38
Stage	III	38	IC	1
	IV	18	IIC	2
			IIIA	0
			IIIB	4
			IIIC	31
			IV	18

[a] Selection of 2 or 3 sites from among the ovary, fallopian tube, and peritoneum was allowed in both prelaparoscopic and laparoscopic diagnosis.
[b] Histology by prelaparoscopic diagnosis has been estimated from cytological findings.

Safety of the treatment

The mean number of cycles of chemotherapy was 7.0 (range, 1–9) cycles. Dose reductions of chemotherapy were performed in 42% (22/53) of patients and 11% (39/371) of cycles. Discontinuation of treatment due to toxicities or patients' refusal in relation to toxicities occurred in 9 patients except 6 patients who continuously received the treatment by deviation. Table 2 shows the major toxicities of the chemotherapy. Grade 4 hematological toxicities, particularly neutropenia (>70%) and anemia (≥15%), were frequently observed during both neoadjuvant and postoperative chemotherapy. Concerning neutropenia, 74% (39/53) of patients and 49% (182/371) of cycles required G-CSF support. Although more than 10% of patients experienced grade 3 neutropenic fever, grade 4 was not observed. Other grade 3 non-hematological toxicities were rarely observed except for gastrointestinal toxicities. As a non-typical adverse event, grade 4 cerebral infarction was observed in 1 patient.

In 47 patients who underwent IDS, the median duration of the surgery and median blood loss were 330 (130–735) min and 1284 (280–4565) ml, respectively. Gastrointestinal resection excluding appendectomy was performed in 9% (4/47) of patients, and splenectomy was performed in 6% (3/47) of patients. Repair of the ureter or the colon because of operative injury was performed in 6% (3/47) of patients. Grade 3 or 4 toxicities observed during and/or after surgery were grade 3 hypotension in 19% (9/47), grade 3 bleeding without thrombocytopenia in 77% (36/47), and grade 3 ileus in 4% (2/47). Blood transfusion other than autotransfusion was required in 72% (34/47); only autotransfusion was performed in 4% (2/47).

There was no treatment-related mortality. There were 2 unexpected events: a primary aldosteronism and a metachronous lung cancer after the treatment protocol. Both events were judged as unlikely to be related with the treatment protocol by the Data and Safety Monitoring Committee of JCOG.

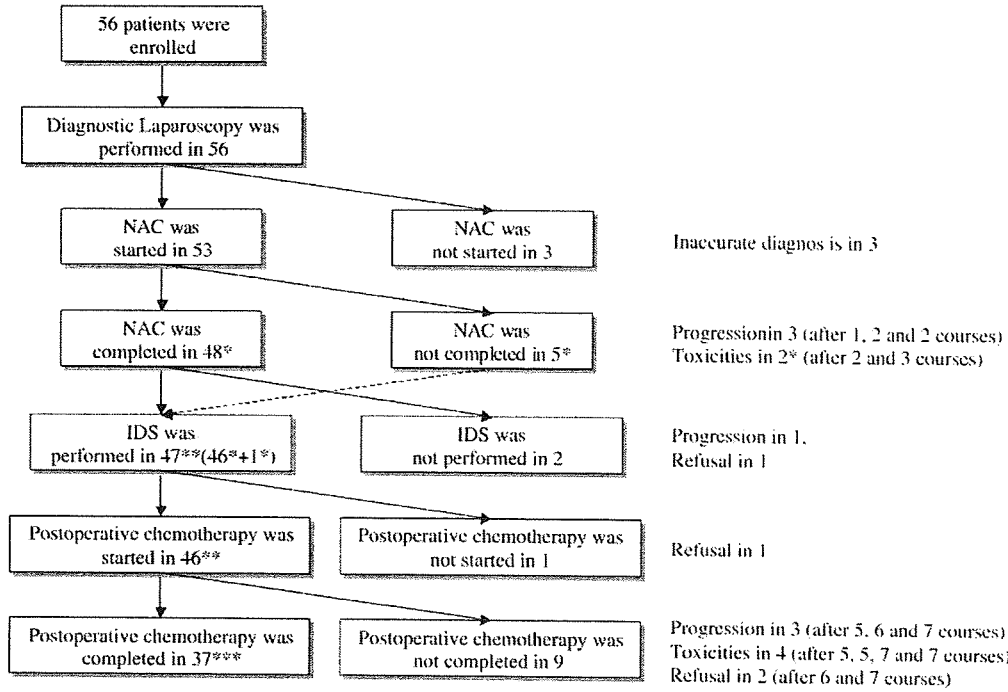

Fig. 1. Compliance of protocol treatment. Including 1 patients (*), 2 patients (**) and 6 patients (***) who deviated from the criteria for discontinuation. NAC, neoadjuvant chemotherapy; IDS, interval debulking surgery.

Efficacy of the treatment

Responses to chemotherapy after 4 cycles of NAC were evaluated in 48 patients who completed NAC. Partial response or CR was obtained in 41 patients (77% of 53 patients), SD was observed in 6 patients (11%), and PD was observed in 1 patient (2%) according to RECIST criteria.

IDS was performed in 47 patients (89% of 53 patients), including a patient who underwent IDS after 3 cycles of NAC. Complete resection of all tumors was obtained in 29 patients (55% of 53 patients), residual disease became <1 cm in 9 patients (17%) and ≥1 cm was left in 9 patients (17%).

The entire treatment protocol was completed by 37 patients and cCR was obtained in 22 patients (42% of 53 patients), including 6 and 3 patients who deviated from the discontinuation criteria. The primary end point of %cCR was 42% [95% CI: 28%–56%].

The median and 3-year PFS of 53 patients was 14 months and 19% (Fig. 2). The median and 3-year OS of 53 patients was 45 months and 60% (Fig. 3).

Discussion

The purpose of this study was to assess the safety and efficacy of NAC and to determine whether advanced MC can be accurately diagnosed on the basis of imaging studies, cytological findings, and tumor markers.

As far as the safety is concerned, treatment initiation with NAC is well known as a safe treatment [4–7,13,14]. There was no treatment-related mortality, the drug-induced toxicities were easily manageable, and surgical toxicities or severe complications were rare. In this study, the safety of NAC was reconfirmed by a prospective study.

Table 2
Drug-induced toxicities

Toxicities	Neoadjuvant chemotherapy ($n=53$)				Postoperative chemotherapy ($n=46$)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Hematological								
Leukopenia	4%	34%	53%	9%	2%	17%	72%	9%
Neutropenia	0%	6%	19%	75%	2%	0%	24%	73%
Thrombocytopenia	13%	26%	23%	2%	17%	22%	43%	2%
Anemia	9%	49%	26%	15%	11%	52%	20%	17%
Non-hematological								
Neutropenic fever	–	–	15%	0%	–	–	11%	0%
Allergy/Hypersensitivity	9%	2%	0%	0%	4%	0%	0%	0%
Fatigue	42%	9%	4%	0%	41%	9%	0%	0%
Alopecia	11%	89%	–	–	11%	84%	–	–
Arthralgia	32%	11%	0%	0%	37%	2%	2%	0%
Neuropathy (sensory)	55%	9%	0%	0%	52%	15%	0%	0%
Myalgia	38%	13%	2%	0%	26%	2%	2%	0%
Nausea	43%	23%	11%	–	46%	13%	2%	–
Vomiting	13%	6%	9%	0%	9%	9%	2%	0%
Diarrhea	15%	4%	6%	0%	11%	2%	2%	0%

Regarding the efficacy of NAC, cCR according to our definition was achieved in 22 patients (42%). It is difficult to compare our results with those of the previous studies targeting surgical stage III/IV ovarian cancer because our target was clinically diagnosed stage III/IV disease. In addition, our definition of cCR is stricter than general definition. We set the CA125 titer at <20 U/ml rather than <35 U/ml. Taking into account these differences, we set, at the beginning of the study, the expected %cCR as 40% and an acceptable lowest %cCR of 20% for the statistical analysis of the primary end point, based on the results of previous studies [15–19]. According to the calculation of the exact binominal distribution, the 95% confidence interval of the %cCR of the target population was 28%–56%. Even if we omit 3 patients with deviation from the discontinuation criteria, the 95% confidence interval of %cCR of the target population would be 23% to 50%. In either case, the null hypothesis "the true proportion of cCR is <20%" was rejected. Furthermore, the median PFS and OS of 53 patients with stage III/IV disease (14 and 45 months, respectively) in the present study also represent promising results comparable with the results of treatments consisting of PDS and postoperative chemotherapy in the previous reports [15,16,20]. Although two Gynecologic Oncology Group studies showed much better PFS and OS with PDS and postoperative intravenous chemotherapy (21 and 57 months) [21] and with PDS and postoperative intra-peritoneal chemotherapy (23 and 66 months) [22], the subjects of both studies were only patients who had undergone optimal surgery. Thus, our results may be comparable to those of the other reports. From the analysis, we confirmed that NAC for advanced MC is sufficiently effective to be compared with current standard treatment.

With regard to the accuracy of clinical diagnosis, the overall diagnosis of the tumor origin, histology, and stage was confirmed by DLS in 53/56 patients (95%). According to the Bayesian method, the Bayesian posterior probability that PPV is <90% was 9.96%, indicating that the appropriate target diseases for NAC can be diagnosed with >90% accuracy without the need for DLS. Although misdiagnosis may occur in <10% cases, the most probable misdiagnosis is the stage of disease. Misdiagnosis of the stage is acceptable rather than the misdiagnosis of the origin or histology because the treatment strategy for stage IC/IIC MC is primarily the same as that for stage III/IV MC. Thus, we concluded that we could omit the staging procedure in a phase III study. Owing to this omission, both treatment arms of the phase III trial would become more practical.

Based on our promising results, we have already started the phase III study, JCOG0602 [23], for comparing NAC followed by IDS with PDS followed by postoperative chemotherapy, on the same subjects of this study. A similar phase III study has already been conducted by EORTC and CTU-MRC. Our study and EORTC study have been designed to prove the non-inferiority of NAC as compared to the standard treatment. Because of the expected lower surgical morbidity and mortality associated with NAC, NAC should become the new standard

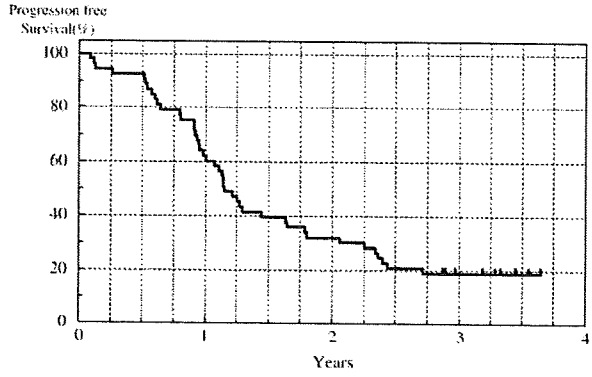

Fig. 2. Progression free survival of patients who received protocol treatment ($n=53$).

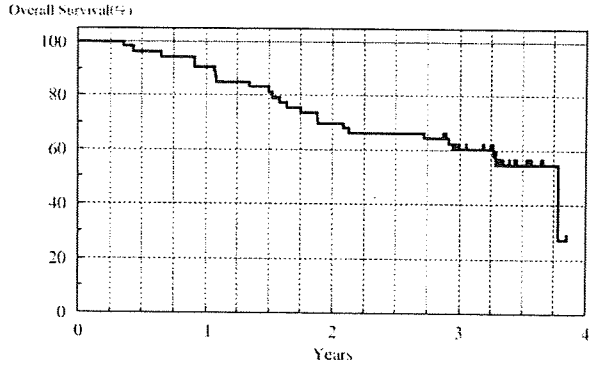

Fig. 3. Overall survival of patients who received protocol treatment ($n=53$).

treatment for patients with advanced MC if the non-inferior OS and lower treatment related morbidity and mortality are proven. The distinctiveness of our new study is that it omits the staging procedures, such as DLS, required in this feasibility study, implying the deletion of an extra procedure in both treatment regimens; thus, our new study highlights the advantage of NAC. Our ongoing phase III study should make it possible to compare both treatments in a more practical setting. From the results of ongoing phase III studies including our study, it is hoped that a new standard treatment regimen is established.

Conflict of interest statement

All authors declare that there are no conflicts of interest.

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Dose-dense paclitaxel once a week in combination with carboplatin every 3 weeks for advanced ovarian cancer: a phase 3, open-label, randomised controlled trial

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Summary

Background Paclitaxel and carboplatin given every 3 weeks is standard treatment for advanced ovarian carcinoma. Attempts to improve patient survival by including other drugs have yielded disappointing results. We compared a conventional regimen of paclitaxel and carboplatin with a dose-dense weekly regimen in women with advanced ovarian cancer.

Methods Patients with stage II to IV epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer were eligible for enrolment in this phase 3, open-label, randomised controlled trial at 85 centres in Japan. Patients were randomly assigned by computer-generated randomisation sequence to receive six cycles of either paclitaxel (180 mg/m^2; 3-h intravenous infusion) plus carboplatin (area under the curve [AUC] 6 mg/mL per min), given on day 1 of a 21-day cycle (conventional regimen; n=320), or dose-dense paclitaxel (80 mg/m^2; 1-h intravenous infusion) given on days 1, 8, and 15 plus carboplatin given on day 1 of a 21-day cycle (dose-dense regimen; n=317). The primary endpoint was progression-free survival. Analysis was by intention to treat (ITT). This trial is registered with ClinicalTrials.gov, number NCT00226915.

Findings 631 of the 637 enrolled patients were eligible for treatment and were included in the ITT population (dose-dense regimen, n=312; conventional regimen, n=319). Median progression-free survival was longer in the dose-dense treatment group (28·0 months, 95% CI 22·3–35·4) than in the conventional treatment group (17·2 months, 15·7–21·1; hazard ratio [HR] 0·71; 95% CI 0·58–0·88; p=0·0015). Overall survival at 3 years was higher in the dose-dense regimen group (72·1%) than in the conventional treatment group (65·1%; HR 0·75, 0·57–0·98; p=0·03). 165 patients assigned to the dose-dense regimen and 117 assigned to the conventional regimen discontinued treatment early. Reasons for participant dropout were balanced between the groups, apart from withdrawal because of toxicity, which was higher in the dose-dense regimen group than in the conventional regimen group (n=113 *vs* n=69). The most common adverse event was neutropenia (dose-dense regimen, 286 [92%] of 312; conventional regimen, 276 [88%] of 314). The frequency of grade 3 and 4 anaemia was higher in the dose-dense treatment group (214 [69%]) than in the conventional treatment group (137 [44%]; p<0·0001). The frequencies of other toxic effects were similar between groups.

Interpretation Dose-dense weekly paclitaxel plus carboplatin improved survival compared with the conventional regimen and represents a new treatment option in women with advanced epithelial ovarian cancer.

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Introduction

Paclitaxel and carboplatin given every 3 weeks is currently considered standard first-line chemotherapy for advanced epithelial ovarian cancer. The consensus statements on the management of ovarian cancer at the 3rd International Gynecologic Cancer Consensus Conference in 2004 recommended intravenous paclitaxel (175 mg/m^2 over 3 h) plus intravenous carboplatin (area under the curve [AUC] 5·0–7·5 mg/mL per min) given every 3 weeks for six cycles for first-line chemotherapy.[1] Paclitaxel and carboplatin have been combined with other drugs, given either concurrently or sequentially, in the hope of prolonging survival in women with advanced ovarian cancer, but the results of several randomised trials have been disappointing.[2–4] In particular, the recently reported randomised trial of the Gynecologic Oncology Group, an international collaborative study enrolling more than 4500 patients, showed that the addition of new cytotoxic drugs to paclitaxel plus carboplatin did not improve progression-free or overall survival.[2]

Dose-dense weekly administration of paclitaxel is another strategy to enhance antitumour activity and prolong survival. Preclinical studies have suggested that duration of exposure is an important determinant of the cytotoxic activity of paclitaxel.[5] Adequate cytotoxicity can be achieved at fairly low concentrations of the drug provided that exposure is extended.[5,6] Several phase 2 clinical trials of dose-dense weekly paclitaxel and carboplatin have shown promising efficacy and favourable tolerability in women with ovarian cancer.[7–9]

We undertook a phase 3, randomised controlled trial to compare conventional paclitaxel and carboplatin given every 3 weeks with dose-dense paclitaxel given every week plus carboplatin (every 3 weeks) as first-line treatment in women with advanced ovarian cancer.

Methods

Patients

Patients from 85 centres in Japan were eligible for enrolment in this phase 3, open-label, randomised trial if they had a histologically or cytologically proven diagnosis of stage II to IV epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer. If only the results of cytological examinations were available, patients needed to have the following criteria: (1) a cytological diagnosis of adenocarcinoma; (2) an abdominal mass more than 2 cm in diameter on abdominal images; and (3) a CA125/carcinoembryonic antigen (CEA) ratio[10] of more than 25, or no evidence of gastrointestinal cancer if CA125/CEA ratio was less than or equal to 25. Previous chemotherapy was not allowed. Patients needed to be aged 20 years or older, to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0–3,[11] and to have adequate organ functions, defined as absolute neutrophil count 1·5 cells×10^9 per L or more, platelet count 100×10^9 per L or more, serum bilirubin 25·7 μmol/L or less, serum aspartate aminotransferase 100 IU/L or less, and serum creatinine 132·6 μmol/L or less. Patients were excluded if they had an ovarian tumour with a low malignant potential, or synchronous or metachronous (within 5 years) malignant disease other than carcinoma in situ.

All patients gave written informed consent before enrolment in this study. The study protocol was approved by the institutional review boards at all participating centres. The protocol was coordinated by the Japanese Gynecologic Oncology Group (protocol number 3016).

Randomisation and masking

Patients were randomly assigned to receive paclitaxel and carboplatin in either a conventional regimen (control) or a dose-dense regimen (intervention). Randomisation was by telephone or fax from a central registration centre located at University of Toyama (Toyama, Japan), and the random allocation table was computer-generated by use of the SAS PROC PLAN. Randomisation was stratified by residual disease (≤1 cm *vs* >1 cm), International Federation of Gynecology and Obstetrics (FIGO) stage (II *vs* III *vs* IV),[12] and histological type (clear-cell or mucinous tumours *vs* serous or other tumours), with adequate balancing within each institution. Patients and clinicians were not masked to treatment assignment.

Procedures

Both study groups received carboplatin at a dose calculated to produce an AUC of 6 mg/mL per min on day 1 of a 21-day cycle. Carboplatin was given as an intravenous infusion over 1 h. The control group also received paclitaxel given as a 3-h intravenous infusion at a dose of 180 mg/m^2 on day 1. In the dose-dense group, paclitaxel was given as a 1-h intravenous infusion at a dose of 80 mg/m^2 on days 1, 8, and 15. The dose of carboplatin was calculated with the formula of Calvert and colleagues,[13] by use of creatinine clearance instead of glomerular filtration rate. Creatinine clearance was calculated with the formula of Jelliffe.[14] Standard premedication was given to prevent hypersensitivity reactions to paclitaxel. The treatments were repeated every 3 weeks for six cycles. Patients with measurable lesions who had a partial response or complete response received three additional cycles of chemotherapy.

Patients needed to have an absolute neutrophil count of 1·0×10^9 cells per L (amended from 1·5×10^9 cells per L on April 11, 2005, because of frequent occurrence of delaying) or more and a platelet count of 75×10^9 per L or more to receive subsequent cycles of therapy in both groups. Patients in the dose-dense regimen group also had to have an absolute neutrophil count of 0·5×10^9 cells per L or more and a platelet count of 50×10^9 per L (amended from 75×10^9 per L on April 11, 2005) or more before they received paclitaxel on days 8 and 15. Treatment was delayed for a maximum of 3 weeks (amended from 2 weeks on April 11, 2005).

The dose of carboplatin was reduced for haematological toxicity, and paclitaxel was reduced for non-haematological toxicity with dose reduction levels as follows: carboplatin AUC 5 mg/mL per min (level 1) or AUC 4 mg/mL per min (level 2) in both groups; paclitaxel 135 mg/m^2 (level 1) or 110 mg/m^2 (level 2) in the conventional treatment group, and paclitaxel 70 mg/m^2 (level 1) or 60 mg/m^2 (level 2) in the dose-dense treatment group. The carboplatin dose was reduced when febrile neutropenia occurred, an absolute neutrophil count less than 0·5×10^9 cells per L persisted for 7 days or more, the platelet count was less than 10×10^9 per L, the platelet count was between 10×10^9 per L and 50×10^9 per L with bleeding tendencies, or the treatment was delayed for haematological toxicity for more than 1 week. In general, patients did not receive prophylactic granulocyte-colony stimulating factor (G-CSF) unless they had treatment delays or neutropenic complications after treatment. The dose of paclitaxel was reduced in patients who had grade 2 or higher peripheral neuropathy.

Interval debulking surgery after two to four cycles of chemotherapy, secondary debulking or second-look surgery after six cycles of chemotherapy, or both, were allowed. These procedures were done within 6 weeks after chemotherapy, and subsequent chemotherapy was restarted within 6 weeks after surgery.

The primary endpoint of this trial was progression-free survival, defined as the time from the date of randomisation to the date of the first occurrence of any of the following events: death from any cause; appearance of any new lesions that could be measured or assessed clinically;

or CA125 criteria of disease progression.[15] The CA125 criteria of disease progression were defined as (1) patients with raised CA125 concentration before treatment with a return to normal after treatment needed to show re-elevation of CA125 greater than or equal to two times the upper normal limit; (2) patients with raised CA125 before treatment that did not return to normal needed to show evidence of CA125 greater than or equal to two times the nadir value; or (3) patients with CA125 in the normal range before treatment needed to show evidence of CA125 greater than or equal to two times the upper normal limit, with raised CA125 recorded on two occasions at least 1 week apart. In patients with measurable disease, clinical or radiographical tumour measurements had priority over CA125 concentration, and progression during treatment could not be declared on the basis of CA125 alone.

Secondary endpoints were overall survival, response rate, and adverse events. The planned analyses of progression-free survival and overall survival included data on eligible patients according to the intention-to-treat (ITT) principle. Clinical response was assessed in eligible patients with lesions that could be measured in two dimensions. The assessment of response had to be confirmed on two occasions at least 4 weeks apart. A complete response was defined as the complete disappearance of all measurable and assessable lesions, determined by two observations not less than 4 weeks apart. A partial reponse was defined as a 50% or greater decrease in the sum of the products of the perpendicular diameters of measurable lesions, determined by two observations not less than 4 weeks apart. Stable disease was defined as a steady state of response less than a partial response or as an increase of less than 25% in the sum of the products of the perpendicular diameters of measurable lesions, lasting at least 4 weeks. Progressive disease was defined as an unequivocal increase of at least 25% in the sum of the products of the perpendicular diameters of measurable lesions. The appearance of new lesions also constituted progressive disease. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0.[16]

Radiological studies to record the status of all measurable lesions noted at baseline were repeated after two, four, and six cycles of chemotherapy. Once patients discontinued the protocol therapy, disease status was assessed every 3 months for the first 2 years and every 6 months thereafter. Follow-up monitoring included clinical examinations and CA125 concentration estimation; routine CT scans were not required, but were requested if CA125 concentration rose, symptoms of relapse developed, or both.

Statistical analysis

Our hypothesis was that the dose-dense regimen would prolong progression-free survival compared with the conventional regimen. At the beginning of the study in April, 2003, a sample size of 380 patients with no interim analysis was initially planned to detect a 37·5% improvement in median progression-free survival in the conventional regimen group (from 16 months to 22 months) with 80% power, two-sided log-rank test, and alpha level of 0·05. In January, 2005, the sample size was increased to 600 patients during the trial to account for the higher accrual of patients and to detect a shorter prolongation of progression-free survival. This amendment of the protocol was made without interim analysis and was approved by the data and safety monitoring committee. The increased sample size would enable the detection of a 31·3% improvement (from 16 months to 21 months) in median progression-free survival with 80% power, two-sided log-rank test, at an alpha level of 0·05, an accrual of 3 years, and a follow-up of 1·5 years. Following the data safety monitoring committee's instructions, interim analysis was planned after 380 patients had been randomly assigned to treatment, and multiplicity by multiple look was adjusted with the

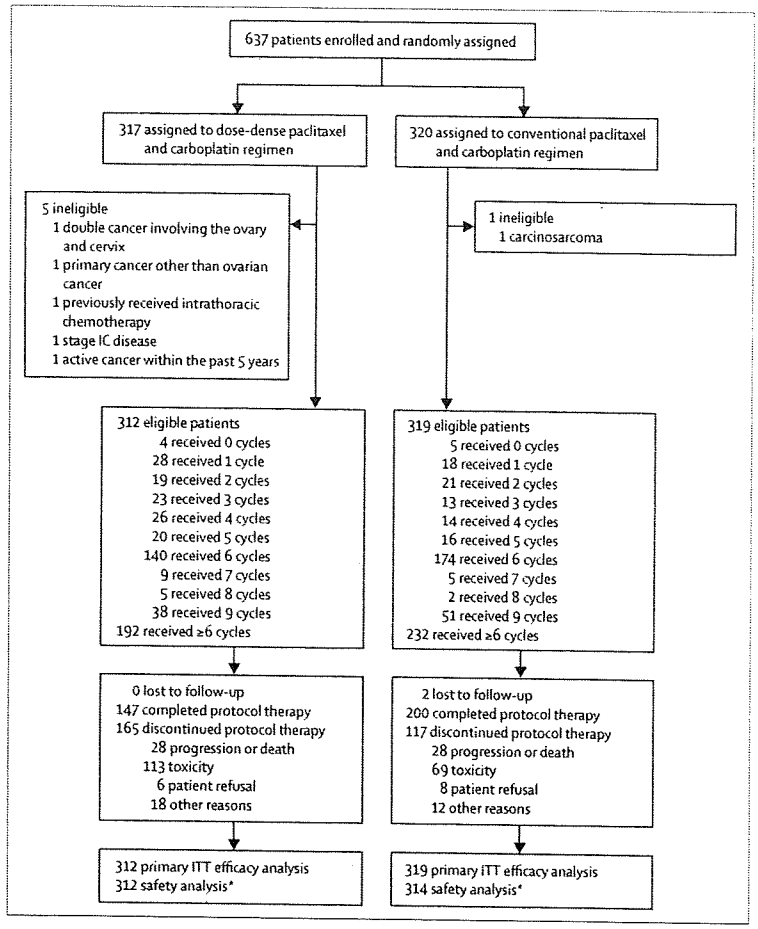

Figure 1: **Trial profile**

ITT=intention-to-treat. *Analysis of safety includes all randomised women who had received at least one cycle of treatment (one ineligible patient in each group did not receive treatment).

	Dose-dense regimen group (n=312)	Conventional regimen group (n=319)
Age (years)	57 (25-87)	57 (25-84)
FIGO stage		
II	62 (20%)	54 (17%)
III	202 (65%)	215 (67%)
IV	48 (15%)	50 (16%)
ECOG performance status		
0 or 1	283 (91%)	287 (90%)
2	23 (7%)	20 (6%)
3	6 (2%)	12 (4%)
Disease		
Ovarian	260 (83%)	276 (87%)
Fallopian tube	14 (4%)	18 (6%)
Primary peritoneal	38 (12%)	25 (8%)
Surgery		
Cytology only	35 (11%)	35 (11%)
Primary debulking	277 (89%)	284 (89%)
Interval debulking	34 (11%)	29 (9%)
Secondary/second-look	38 (12%)	56 (18%)
Residual disease		
≤1 cm	144 (46%)	145 (45%)
>1 cm	168 (54%)	174 (55%)
Histological type		
Serous adenocarcinoma	173 (55%)	182 (57%)
Endometrioid adenocarcinoma	38 (12%)	39 (12%)
Clear-cell carcinoma	31 (10%)	37 (12%)
Mucinous adenocarcinoma	23 (7%)	11 (3%)
Other types	47 (15%)	50 (16%)
Histological grade		
Well differentiated	42 (13%)	40 (13%)
Moderately differentiated	60 (19%)	71 (22%)
Poorly differentiated	79 (25%)	72 (23%)
Unknown/not applicable	131 (42%)	136 (43%)

Data are n (%) or median (range). FIGO=International Federation of Gynecology and Obstetrics. ECOG=Eastern Cooperative Oncology Group.

Table 1: **Baseline characteristics of study patients**

O'Brien-Fleming alpha-spending function. At the first interim analysis in December, 2005, the data safety monitoring committee reviewed the results and approved continuation of the planned follow-up.

The cumulative survival curve and median progression-free survival time were estimated by use of the Kaplan-Meier method. Adverse events were analysed in all randomised women who had received at least one cycle of treatment. Proportions of adverse events were compared between the groups by the use of two-sided χ^2 tests or two-sided Fisher's exact tests. Responses were compared by the use of Fisher's exact test. All analyses were performed with SAS software, version 8.2. This trial is registered with ClinicalTrials.gov, number NCT00226915.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between April, 2003, and December, 2005, 637 patients were enrolled at 85 centres. Figure 1 shows the trial profile. Table 1 shows the baseline characteristics of the 631 eligible patients whose data were included in the ITT analysis.

The median number of treatment cycles was six in both groups (figure 1). The proportion of patients who received six or more cycles of treatment was higher in the conventional regimen group (232 [73%] of 319) than in the dose-dense regimen group (192 [62%] of 312). The main reason for discontinuing treatment was toxicity. Haematological toxicity was the most common form of toxicity leading to the discontinuation of treatment (68 [60%] of 113 patients assigned to the dose-dense regimen *vs* 30 [43%] of 69 assigned to the conventional regimen; p=0·03). The proportions of patients who discontinued treatment because of neurotoxicity were low in both groups (three [3%] *vs* five [7%]). Other reasons for discontinuation of treatment because of toxic effects were patient refusal (13 [12%] *vs* 12 [17%]), allergic reaction (four [4%] *vs* seven [10%]), and other toxic effects (25 [22%] *vs* 15 [22%]).

At least one treatment cycle was delayed in a higher proportion of patients in the dose-dense treatment group (236 [76%] of 312) than in the conventional treatment group (213 [67%] of 319; p=0·02). The dose of the study drugs was reduced in a higher proportion of patients assigned to the dose-dense regimen (150 [48%] of 312) than in those assigned to the conventional regimen (112 [35%] of 319; p=0·001). The mean delivered dose intensity of carboplatin was lower in the dose-dense regimen group (AUC per week 1·54 mg/mL per min [SD 0·37]) than in the conventional regimen group (AUC per week 1·71 mg/mL per min [SD 0·36]), and the mean delivered dose-intensity of paclitaxel was higher (63·0 mg/m² per week [SD 13·0] *vs* 51·7 mg/m² per week [SD 10·6]). The mean relative dose intensities of carboplatin and paclitaxel were both lower in the dose-dense regimen group (77% [SD 18] and 79% [SD 15], respectively) than in the conventional regimen group (85% [SD 18], and 86% [SD 18], respectively).

At the time of last follow-up (December, 2007), with a median duration of follow-up of 29 months, there had been 160 disease progression events in the dose-dense treatment group and 200 in the conventional treatment group. Median progression-free survival was 28·0 months (95% CI 22·3–35·4) in the dose-dense treatment group and 17·2 months (15·7–21·1) in the

conventional treatment group (figure 2: unadjusted hazard ratio [HR] 0·71, 95% CI 0·58–0·88; p=0·0015, log-rank test). When the analysis was done with data from all 637 patients who were randomly assigned to treatment, the result was similar (p=0·0019). After adjustment for FIGO stage, residual disease, and histological type according to the preplanned analysis, the HR was 0·65 (0·53–0·80; p=0·0001). We subsequently undertook unplanned sensitivity analyses. The differences between groups were still significant when only clinical progression was defined as progression (p=0·0018), when data on patients who received second-line therapy before progression were censored (dose-dense regimen, n=3; conventional regimen, n=5; p=0·0018), or when data on patients who underwent interval or secondary surgery, or both, were censored (dose-dense regimen, n=71; conventional regimen, n=85; p=0·0092).

Analysis of overall survival was done in December, 2007, at the same time as the analysis of progression-free survival. The overall survival at 2 years was 83·6% in the dose-dense treatment group and 77·7% in the conventional treatment group (p=0·049). We updated the overall survival analysis in December, 2008, with median follow-up period of 42 months. Although median overall survival had not been reached in either group, overall survival at 3 years was higher in the dose-dense treatment group (72·1%) than in the conventional treatment group (65·1%; unadjusted HR 0·75, 0·57–0·98; p=0·03 log-rank test; figure 2).

A Cox proportional-hazards model was used to examine the effect of baseline clinical characteristics and conventional prognostic factors on the treatment effect (figure 3). Progression-free survival was longer in the dose-dense treatment group than in the conventional treatment group across all subgroups of patients apart from in those with clear-cell or mucinous tumours. In this subgroup of patients, the HR in the dose-dense treatment group was similar to that in the conventional treatment group.

Clinical response was assessed in 282 patients who had measurable disease at study entry. The overall response rate was similar between groups (conventional regimen, 72 [53%] of 135 patients; dose-dense regimen, 82 [56%] of 147 patients; p=0·72; table 2). Because patients who underwent suboptimally debulked surgery (>1 cm of residual disease) were allowed to undergo interval debulking surgery in this study, response sometimes could not be confirmed on repeated imaging. If these unconfirmed responses are taken into account (44 patients), the overall response rate was 70% (94 of 135 patients) in the conventional treatment group compared with 71% (104 of 147 patients) in the dose-dense treatment group (p=0·90).

Treatment-related adverse events were analysed in patients who received at least one cycle of the study treatment (table 3). The frequency of grade 3 or 4 anaemia was higher in the dose-dense treatment group than in the conventional treatment group (p<0·0001). Recombinant erythropoietin was not used to treat anaemia because it was not approved in Japan. G-CSF was used in 187 (60%) patients assigned to the dose-dense regimen and in 214 (67%) assigned to the conventional regimen. The frequency of neuropathy did not differ between study groups.

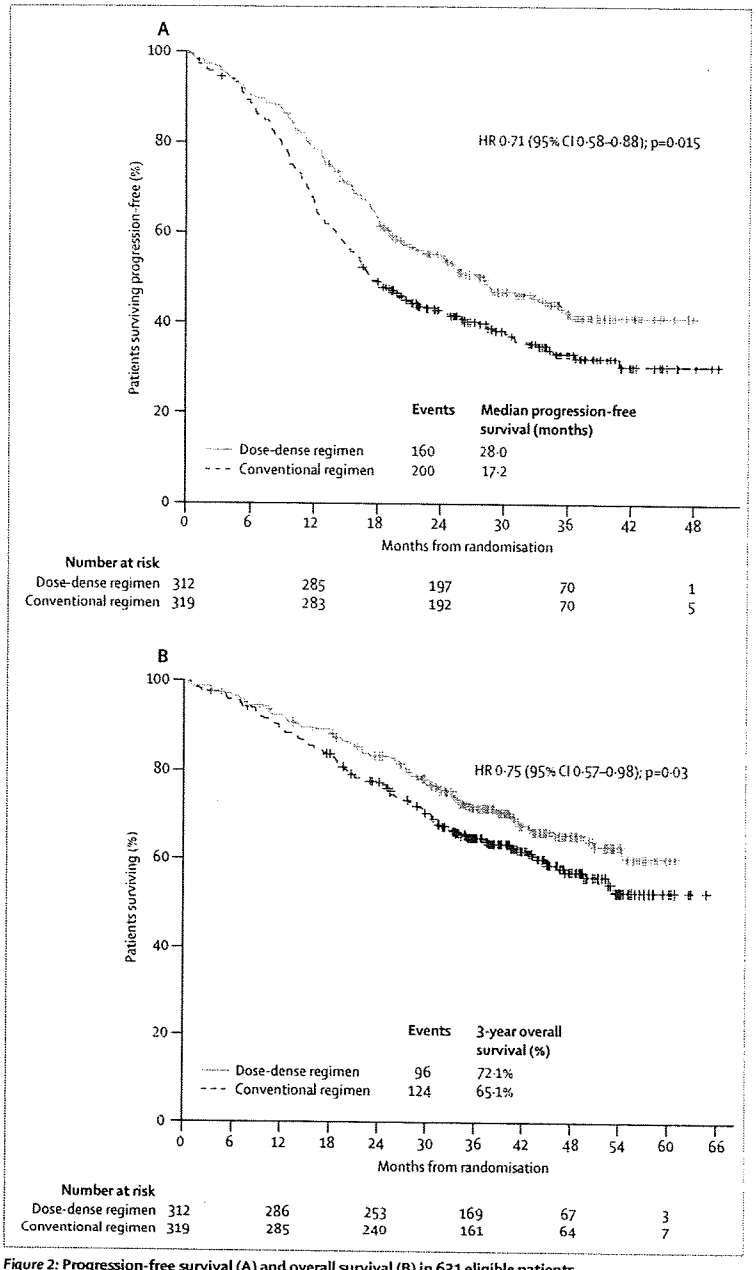

Figure 2: **Progression-free survival (A) and overall survival (B) in 631 eligible patients**
HR=hazard ratio.

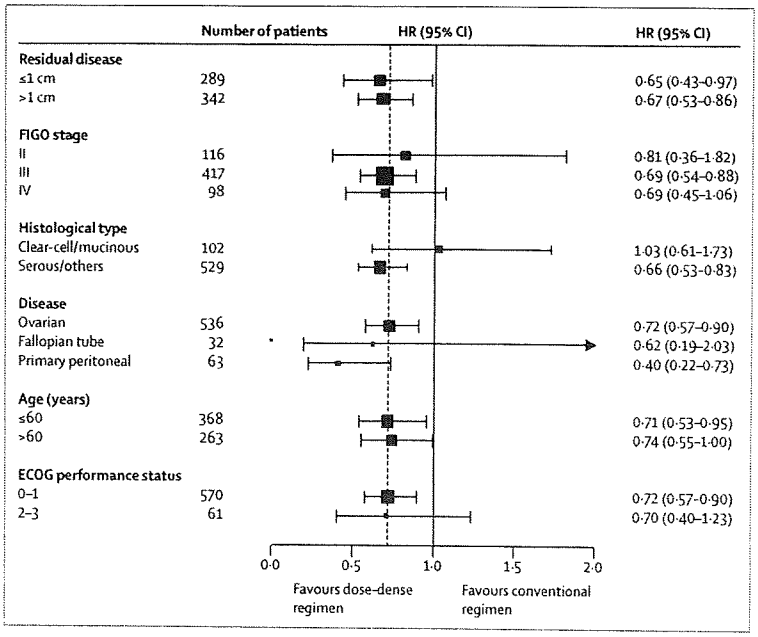

Figure 3: **Progression-free survival according to baseline characteristics**
FIGO=International Federation of Gynecology and Obstetrics. ECOG=Eastern Cooperative Oncology Group. The hazard ratios (HRs; 95% CIs) are for patients assigned to conventional paclitaxel and carboplatin, compared with those assigned to dose-dense paclitaxel and carboplatin, and were obtained from the unadjusted Cox model. The dashed vertical line indicates a hazard ratio of 0·71, which is the value for all patients, and the solid vertical line indicates a hazard ratio of 1·00, which is the null-hypothesis value.

	Dose-dense regimen group (n=147)	Conventional regimen group (n=135)	p value
Complete response	29 (20%)	21 (16%)	0·44
Partial response	53 (36%)	51 (38%)	0·81
Stable disease	43 (29%)	42 (31%)	0·80
Progressive disease	4 (3%)	9 (7%)	0·16
Not evaluable	18 (12%)	12 (9%)	0·44

See Methods section for definitions of responses.

Table 2: **Clinical response in patients with measurable lesions**

	Dose-dense regimen group (n=312)	Conventional regimen group (n=314)	p value
Neutropenia	286 (92%)	276 (88%)	0·15
Thrombocytopenia	136 (44%)	120 (38%)	0·19
Anaemia	214 (69%)	137 (44%)	<0·0001
Febrile neutropenia	29 (9%)	29 (9%)	1·00
Nausea	32 (10%)	36 (11%)	0·70
Vomiting	9 (3%)	11 (4%)	0·82
Diarrhoea	10 (3%)	8 (3%)	0·64
Fatigue	15 (5%)	8 (3%)	0·14
Arthralgia	3 (1%)	5 (2%)	0·72
Myalgia	2 (1%)	4 (1%)	0·69
Neuropathy (motor)	15 (5%)	12 (4%)	0·56
Neuropathy (sensory)	21 (7%)	20 (6%)	0·87

Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0.[16]

Table 3: **Frequency of grade 3 or 4 adverse events**

Discussion

Our study showed that compared with a conventional regimen, dose-dense treatment with paclitaxel and carboplatin improved progression-free survival in women with newly diagnosed, stage II to IV ovarian cancer. Women assigned to dose-dense paclitaxel and carboplatin had a 29% lower risk of disease progression and a 25% lower risk of death than did patients assigned to the conventional regimen. Benefits of this magnitude have been rare in women with advanced ovarian cancer, including those with suboptimally debulked stage III and IV disease, since the approval of paclitaxel for the indication of ovarian cancer.

The concept of dose density is based on the hypothesis that a shorter interval between doses of cytotoxic therapy would more effectively reduce tumour burden than would dose escalation.[17] In breast cancer, recently published phase 3 trials have shown that paclitaxel given every week improves response and survival.[18,19] Consistent with these findings, our study showed that progression-free survival and overall survival were significantly longer in the dose-dense regimen group than in the conventional regimen group. Increased doses of paclitaxel of 225 mg/m² or 250 mg/m² given every 3 weeks have been compared with the standard dose (ie, 175 mg/m²) in women with ovarian cancer, but showed no benefit in survival.[20,21] Our study showed a survival advantage with an increased total dose of 240 mg/m², given in three divided doses during a 21-day cycle, suggesting that dose density is more important than increased dose intensity.

There was greater haematological toxicity in the dose-dense treatment group than in the conventional treatment group, which resulted in more delays and dose modifications. The optimum dose and schedule of dose-dense paclitaxel and carboplatin have not yet been established. Rose and colleagues[8] reported that weekly paclitaxel at a dose of 60 mg/m² in combination with carboplatin at an AUC of 5 mg/mL per min was tolerated and active in patients with recurrent ovarian cancer. An alternative schedule of dose-dense treatment is to give both paclitaxel and carboplatin every week. Sehouli and co-workers[9] showed that weekly paclitaxel at a dose of 100 mg/m² and weekly carboplatin at an AUC of 2 mg/mL per min showed substantial activity and tolerability in patients with primary ovarian cancer. A treatment delay occurred in only 2·8% of cycles and the frequency of grade 3 neurotoxicity (2% [three of 129 patients]) was lower than that reported in our study. Additionally, weekly carboplatin of AUC 2 mg/mL per min and weekly paclitaxel of 60 mg/m² on days 1, 8, and

15 every 4 weeks showed a favourable toxicity profile in elderly ovarian cancer patients.[22]

The response rate did not differ between groups. Virtually all previous randomised trials in ovarian cancer that showed an improvement in progression-free survival and overall survival also had a higher response rate for the more effective treatment. A lower dose of paclitaxel had antiangiogenic activity in a xenograft model.[23] Antiangiogenic agents might promote tumour dormancy by maintaining tumour size and preventing outgrowth.[24] Vascular endothelial growth factor (VEGF) is frequently expressed in ovarian cancer, and might be an important therapeutic target. Longer survival in the dose-dense regimen group without an improved response rate might be attributed to the antiangiogenic effect of paclitaxel. Anti-VEGF agents such as bevacizumab combined with the dose-dense treatment will be assessed in future trials.

Neurotoxicity is the adverse reaction of greatest concern in patients who receive a combination of paclitaxel and carboplatin. In breast cancer trials, the incidence of neurotoxicity was higher in patients given paclitaxel every week than in patients given paclitaxel every 3 weeks.[19] In our study, however, the frequency of neurotoxicity was similar in both groups. This finding might be because patients in the dose-dense treatment group discontinued treatment more often than did those in the conventional treatment group.

Fewer than half the patients assigned to the dose-dense regimen completed treatment according to the study protocol. When designing the protocol, we debated whether patients who responded to six cycles of chemotherapy should receive three more cycles. However, this study was not designed to assess the relation between the duration of treatment and clinical outcomes, and there is little evidence to suggest that more than six cycles of chemotherapy would prolong survival. About 60% of patients in the dose-dense regimen group received six or more cycles of chemotherapy. Treatment cycles were more frequently delayed in the dose-dense treatment group than in the conventional treatment group, mainly because of neutropenia.

Clear-cell and mucinous adenocarcinoma of the ovary is associated with low sensitivity to chemotherapy and poor survival.[25,26] In our study, neither dose-dense nor conventional treatment seemed effective against clear-cell or mucinous ovarian carcinoma, which suggests that other treatment strategies are needed.

Thus, our study showed that a dose-dense regimen of paclitaxel once a week plus carboplatin every 3 weeks is associated with longer progression-free and overall survival than a conventional regimen of paclitaxel and carboplatin given every 3 weeks in women with advanced epithelial ovarian cancer.

Contributors

NK, MY, FT, SI, TS, EK, and KO conceived and designed the study with the Japanese Gynecologic Oncology Group. MY was the coordinating principal investigator for the study. NK and FT analysed and interpreted the results. NK drafted the report. KN was responsible for the overall planning and conduct of the study. NK, MY, SI, TJ, DA, HT, TS, SK, EK, and KO were involved in the provision of study material or patients, or data acquisition. NK, MY, TS, EK, and KO were members of the steering committee. All authors were involved in writing the report and approved the final version of the manuscript.

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Conflicts of interest

SI and DA have received honoraria from Bristol-Myers Squibb. DA and HT have received grant support from Bristol-Myers Squibb. All other authors declare that they have no conflicts of interest.

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Clinical Significance of UDP-Glucuronosyltransferase 1A1*6 for Toxicities of Combination Chemotherapy with Irinotecan and Cisplatin in Gynecologic Cancers

A Prospective Multi-Institutional Study

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Key Words
Cisplatin · Gynecologic cancer · Irinotecan · UDP-glucuronosyltransferase 1A1 · UGT1A1 · UGT1A1*6

Abstract

Background: To investigate the effects of UDP-glucuronosyltransferase 1A1 (UGT1A1) *28, *6 and *27 in patients with gynecologic cancer who received chemotherapy with irinotecan and cisplatin. ***Methods:*** Patients eligible for this study had cervical or ovarian cancer treated with chemotherapy; a course of the regimen consisted of 60 mg/m^2 of irinotecan on days 1, 8 and 15, and 60 mg/m^2 of cisplatin on day 1 every 4 weeks. UGT1A1 polymorphisms and toxicities were analyzed. ***Results:*** From March 2007 to December 2007, 30 Japanese patients were enrolled; 24 ovarian carcinoma patients and 6 cervical cancer patients. The following genotypes of UGT1A1 were found: wild type in 17 patients (57%), *28 in 4 patients (13%), *6 in 8 patients (27%), *28*6 in 1 case (3%) and no case of *27 (0%). Grade 3/4 neutropenia, thrombocytopenia and diarrhea were significantly more frequent in *6 patients compared with wild-type patients. Also, in *6 patients irinotecan administration on days 8 or 15 was significantly more often omitted due to toxicities. In patients with *28 or *28*6, side effects were similar to those in patients with *6. ***Conclusion:*** In addition to UGT1A1*28, UGT1A1*6 might also be a key candidate to determine the dose of combination chemotherapy with irinotecan and cisplatin.

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Introduction

Irinotecan hydrochloride is widely used for a multiplicity of carcinomas, including colorectal and lung cancers. Irinotecan is often used for relapsed gynecologic cancer in combination with platinum [1, 2]. Recently, combination therapy with irinotecan and cisplatin has been ascribed a potential therapeutic effect for clear cell carcinoma of the ovary [3–5], and the efficacy of this

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therapy is being explored in the worldwide, prospective, randomized trial GCIG/JGOG 3017, a study designed to compare the survival of patients with ovarian clear cell carcinoma treated either with the paclitaxel-carboplatin combination or the irinotecan-cisplatin combination.

It is well recognized that the dose-limiting toxicities of irinotecan-based therapy are severe neutropenia and diarrhea [6]. The active metabolite of irinotecan, SN-38 (7-ethyl-10-hydroxycamptothecin), is glucuronidated by uridine diphosphate glucuronosyltransferase 1As (UGT-1As) including UGT1A1, and inactivated by forming SN-38 glucuronide (SN-38G) [7, 8]. In these UGT1A enzymes, the UGT1A1 protein has the highest ability to glucuronidate SN-38. Genetic variations in the promoter and coding regions of UGT1A1 have been reported to decrease enzyme activity. A polymorphism of UGT1A1, UGT-1A1*28, is a repeat polymorphism in the TATA box of the promoter, and homozygosity for UGT1A1*28 is associated with Gilbert's syndrome, which is characterized by elevated serum levels of unconjugated bilirubin [9]. Patients bearing the UGT1A1*28 allele have a decreased ability to form SN-38G, and the UGT1A1*28 allele was significantly correlated with irinotecan-induced toxicities [10–14]. In Asians, the allele frequency of UGT1A1*28 is lower, and UGT1A1*6, another polymorphism of UGT1A1, is much higher in Asians compared with Caucasians or African-Americans [15]. Both UGT1A1*6, a G→A transition at codon 71 (G71R), and UGT1A1*27, a C→A transition at codon 229 (P229Q), are polymorphisms of the UGT1A1 gene located on exon 1 [16] and related with reduced SN-38 glucuronidation activity [17, 18]. Recently, a significant association between UGT-1A1*6 and severe adverse effects following irinotecan-based chemotherapy has been reported [19, 20].

On the other hand, a meta-analysis of 10 patient cohorts treated with medium/high-dose irinotecan demonstrated that the risk of grade 3/4 hematologic toxicity was higher in patients with the UGT1A1*28/*28 genotype than in those with the UGT1A1*1/*1 and UGT-1A1*1/*28 genotype [21]. This significant difference was not observed in patients treated with low-dose of irinotecan (100–125 mg/m^2), a commonly used therapeutic range. However, limitations of the study are possibly the heterogeneity of the patients and the treatment schedule, e.g. supplementation of platinum agents.

In the present study, the effect of UGT1A1 genotypes on toxicity profiles was prospectively investigated in gynecologic cancer patients who received the irinotecan-cisplatin combination therapy at the dose and schedule employed in the GCIG/JGOG 3017 trial.

Patients and Methods

Patients and Tumors

Patients meeting the following criteria were eligible for the study: histologically confirmed diagnosis of ovarian or uterine cervical carcinoma; age between 20 and 75 years; a performance status between 0 and 2 on the Eastern Cooperative Oncology Group scale; a life expectancy of at least 3 months; a treatment-free period of at least 4 weeks from the previous chemotherapy or irradiation, and adequate hematological (total white blood cell count ≥3,000/μl; absolute neutrophil count ≥1,500/μl; platelet count ≥100,000/μl and hemoglobin level ≥9 g/dl), hepatic (total bilirubin level ≤1.5 mg/dl, and aspartate and alanine aminotransferase levels ≤3 times the upper limit of normal) and renal (creatinine level ≤1.5 mg/dl and/or creatinine clearance ≥60 ml/min) function. The protocol included the following exclusion criteria: massive ascites and/or massive pleural effusion; serious infectious diseases or other complications such as uncontrollable diabetes, intestinal pneumonitis or bowel obstruction; active bowel bleeding or colitis; active concurrent malignancies; symptomatic brain metastasis; lacting or pregnant women; medical record of hypersensitivity reaction to irinotecan or platinum agents, or other medical problems severe enough to prevent compliance with the present protocol. The study protocol was approved by each institutional review board; the National Defense Medical College, the Saitama Cancer Center, the International Medical Center of the Saitama Medical University and the Nishisaitama Chuo Hospital. All study participants gave informed consent prior to the enrollment in the study.

Drug Administration

The enrolled patients received chemotherapy consisting of 90-min intravenous infusions of irinotecan (60 mg/m^2) on days 1, 8 and 15 and subsequently 120-min intravenous infusions of cisplatin (60 mg/m^2) on day 1 every 4 weeks. Treatment with irinotecan was withheld on days 8 or 15 if the patient experienced hematologic toxicities of grade 3 or more or non-hematologic toxicities of grade 2 or more. A subsequent cycle of chemotherapy was initiated if the patients showed adequate hematological, hepatic and renal function according to the criteria for patient enrollment and did not meet the exclusion criteria. The worst toxicity profiles of the initial two chemotherapy courses and adherence to the study protocol were investigated for the present study.

UGT1A1 Genotyping

Serum samples of the study patients were analyzed for polymorphisms of UGT1A1 using the Invader UGT1A1 Molecular Assay (BML, Kawagoe, Japan), which enabled genotyping of UGT1A1*28, *6, and *27 [22]. Wild-type and non-wild-type UGT1A1 was determined by the assay.

Response Evaluation

Response was evaluated with CT or MR images after two cycles of chemotherapy in the patients with measurable disease. Tumor response was assessed using Response Evaluation Criteria in Solid Tumors [23]. Responses were confirmed by CT at least 4 weeks later. Response evaluation of chemotherapy was not done by serum levels of CA125 in patients with ovarian carcinoma in the present study.

Table 1. Characteristics of the patients

Characteristics	Patients	Median (range)
Total patients	30	
Ovarian cancer	24 (80%)	
Cervical cancer	6 (20%)	
Age, years		58 (37–75)
Weight, kg		51 (42–75)
Performance status (ECOG)		
0	25 (83%)	
1	5 (17%)	
2	0 (0%)	
Previous chemotherapy		
≥2 regimens	14 (47%)	
1 regimen	13 (43%)	
No	3 (10%)	
Previous pelvic radiotherapy		
Yes	3 (10%)	
No	27 (90%)	

ECOG = Eastern Cooperative Oncology Group.

Table 2. UGT1A1 genotype and total bilirubin level prior to therapy and the highest value during chemotherapy

UGT1A1 genotype	Bilirubin prior mean (range)	Bilirubin highest mean (range)	p value[1]
Wild type (n = 17)	0.49 (0.2–0.7)	0.78 (0.3–2.1)	<0.01
Non-wild type (n = 13)	0.61 (0.3–1.2)	0.79 (0.3–1.2)	0.14
UGT1A1*28 (n = 4)	0.60 (0.4–1.0)	0.80 (0.4–1.2)	0.52
UGT1A1*6 (n = 8)	0.56 (0.3–1.2)	0.78 (0.3–1.2)	0.83
UGT1A1*6*28 (n = 1)	1.0	0.9	–
p value[2]	0.02	0.72	

[1] Comparing the bilirubin levels noted prior to chemotherapy with the highest value obtained during chemotherapy.

[2] Comparing serum bilirubin levels of cases with UGT1A1 wild-type with those with the non-wild-type genotype (UGT1A1*28, *6 and *6*28).

Toxicity Profiles and Statistical Analysis

Physical examination and serum blood tests were carried out on days 1, 3, 8, 15 and 21 for toxicity evaluation. Toxicity was assessed using the National Cancer Institute Common Toxicity Criteria (version 3). The χ^2 test and Student's t test for unpaired data were used for statistical analyses. Multivariate logistic regression analyses of toxicities were performed using StatView (version 5.0; SAS Institute, Cary, N.C., USA). A p value <0.05 was considered statistically significant.

Results

A total of 30 patients who fulfilled the inclusion criteria but not the exclusion criteria were investigated. Table 1 summarizes the characteristics of the patients enrolled in the study. No case was excluded due to insufficient liver and renal function. All the 30 patients were Japanese. UGT1A1 genotyping using the Invader UGT1A1 Molecular Assay revealed the wild type in 17 patients (57%), UGT1A1*28 in 4 (13%), UGT1A1*6 in 8 (27%) and UGT1A1*28*6 in 1 (3%) patient; UGT1A1*27 was not detected in any of the 30 study patients. All UGT1A1 polymorphisms were heterozygous alterations, and no homozygous polymorphisms were observed in the present study.

Possible correlations between the total serum bilirubin level prior to and the highest serum bilirubin level during the chemotherapy and UGT1A1 genotypes were assessed. The results are summarized in table 2. Serum levels of bilirubin were higher in patients with UGT1A1 non-wild type genotypes in comparison with those with the wild type (p = 0.02). Serum bilirubin levels were significantly increased after chemotherapy in the UGT1A1 wild-type cases. In UGT1A1 non-wild-type patients, serum bilirubin levels were slightly increased after chemotherapy, but the differences were not significant.

Response was assessable in 13 of 17 UGT1A1 wild-type patients, and 10 of 13 patients with UGT1A1 non-wild-type genotype. The patients with UGT1A1 wild type included 3 (23%) patients with partial responses, 6 (54%) with stable disease and 3 (23%) with progressive disease. The UGT1A1 non-wild-type group comprised 3 (30%) patients with partial responses, 4 (40%) with stable disease and 3 (30%) with progressive disease. A complete response was not observed in the present study cohort. Overall response amounted to 23% in the wild-type group and to 30% in the non-wild-type group, with no significant difference between both groups (p = 0.71).

The overall toxicity profiles according to UGT1A1 genotypes are shown in table 3. There was a marked increase in grade 3/4 toxicities in the UGT1A1 non-wild-type group. Neutropenia (p = 0.04), thrombocytopenia (p = 0.04) and diarrhea (p = 0.005) were more frequently observed in the UGT1A1 non-wild-type group. Other non-hematologic grade 3/4 toxicities including renal function were not observed. Toxicity-induced discon-

Table 3. Associations between UGT1A1 genotypes and grade 3–4 toxicities or discontinuation (days 8 and 15)/delay of chemotherapy

UGT1A1 genotype	Leukopenia	Neutropenia	Thrombocytopenia	Nausea	Vomiting	Diarrhea	Discontinuation of irinotecan	Delay of the second cycle
Wild type (n = 17)	1 (6%)	4 (24%)	0 (0%)	2 (12%)	5 (29%)	0 (0%)	5 (29%)	2 (12%)
Non-wild type (n = 13)	4 (31%)	8 (62%)	3 (23%)	5 (38%)	6 (46%)	5 (38%)	10 (77%)	4 (31%)
UGT1A1*28 (n = 4)	1 (25%)	1 (25%)	1 (25%)	2 (50%)	3 (75%)	1 (25%)	3 (75%)	1 (25%)
UGT1A1*6 (n = 8)	2 (25%)	6 (75%)*	2 (25%)*	3 (38%)	3 (38%)	4 (50%)*	6 (75%)*	3 (38%)
UGT1A1*28*6 (n = 1)	1 (100%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)
p value[1]	0.07	0.04	0.04	0.09	0.35	0.005	0.01	0.19

* $p < 0.05$, UGT1A1*6 vs. wild type.
[1] Comparing the frequencies of events in cases with UGT1A1 wild-type and those with UGT1A1 non-wild-type genotypes.

Table 4. Multiple logistic regression analysis of the occurrence of grade 3/4 neutropenia in all cases and cases with UGT1A1*6 and wild-type genotype[1]

Variables	Hazard ratio	95% confidence interval	p value
All cases			
Age			0.23
≤55 years	1		
≥56 years	0.66	0.11; 4.11	
Previous chemotherapy			0.50
≤1 regimen	1		
≥2 regimen	3.43	0.40; 29.33	
Previous pelvic radiotherapy			0.017
No	1		
Yes	7.80	1.72; 35.37	
UGT1A1 genotype			0.007
Wild type (n = 17)	1		
Non-wild type (n = 8)	7.85	2.05; 57.40	
*Cases with UGT1A1*6 and wild-type genotype*			
Age			0.67
≤55 years	1		
≥56 years	0.55	0.04; 7.24	
Previous chemotherapy			0.48
≤1 regimen	1		
≥2 regimen	2.08	0.21; 27.02	
Previous pelvic radiotherapy			0.13
No	1		
Yes	3.04	0.89; 24.44	
UGT1A1 genotype			0.03
Wild type (n = 17)	1		
UGT1A1*6 (n = 8)	10.06	1.14; 88.98	

[1] Worst toxicities in the initial two courses.

Table 5. Multiple logistic regression analysis of the occurrence of grade 3/4 diarrhea in all cases and cases with UGT1A1*6 and wild-type genotype[1]

Variables	Hazard ratio	95% confidence interval	p value
All cases			
Age			0.15
≤55 years	1		
≥56 years	1.05	0.20; 5.12	
Previous chemotherapy			0.46
≤1 regimen	1		
≥2 regimen	3.42	0.41; 27.40	
Previous pelvic radiotherapy			0.03
No	1		
Yes	6.00	1.58; 22.77	
UGT1A1 genotype			0.002
Wild type (n = 17)	1		
Non-wild type (n = 13)	6.54	1.44; 29.60	
*Cases with UGT1A1*6 and wild-type genotype*			
Age, years			0.19
≤55 years	1		
≥56 years	1.21	0.18; 5.56	
Previous chemotherapy			0.71
≤1 regimen	1		
≥2 regimen	2.00	0.05; 38.46	
Previous pelvic radiotherapy			0.14
No	1		
Yes	3.65	0.78; 30.11	
UGT1A1 genotype			0.001
Wild type (n = 17)	1		
UGT1A1*6 (n = 8)	7.45	1.44; 29.78	

[1] Worst toxicities in the initial two courses.

tinuation of irinotecan administration on days 8 and/or 15 was significantly higher in the non-wild type group ($p = 0.01$). Compared with the wild-type patients, UGT1A1*6 patients showed a significantly higher rate of grade 3/4 neutropenia ($p = 0.014$), thrombocytopenia ($p = 0.03$) and diarrhea ($p < 0.001$), and irinotecan treatment was more often modified ($p = 0.03$). There was no statistically significant relationship between response and the frequency of grade 3/4 toxicity (data not shown).

Multiple logistic regression analysis of the occurrence of grade 3/4 neutropenia (table 4) or grade 3/4 diarrhea (table 5) revealed a significant, independent association with the UGT1A1 non-wild type. In addition to the UGT1A1 genotype, previous pelvic radiotherapy was also identified as an independent factor for neutropenia and diarrhea in the analysis of all cases (upper part of table 4, 5). There was no significant relationship with the UGT1A1 genotype and other toxicities including thrombocytopenia by multivariate analysis (data not shown).

Further, excluding 4 patients with UGT1A1*28 and 1 with UGT1A1*28*6, using multiple logistic regression, the incidence of grade 3/4 toxicities was analyzed using age, previous chemotherapy, previous pelvic radiotherapy and UGT1A1 genotype (UGT1A1*6 vs. wild type) as variables. The UGT1A1*6 genotype was identified as an independent risk factor for grade 3/4 neutropenia (hazard ratio, 6.54; 95% confidence interval, 1.44–29.60) and grade 3/4 diarrhea (hazard ratio, 7.45; 95% confidence interval, 1.44–29.78; lower part in table 4, 5). Age, previous chemotherapy and previous pelvic radiotherapy were not significant risk factors in these analyses.

Discussion

There have been reports of more than hundred polymorphisms in the UGT1A1 gene [24]. UGT1A1*6, a UGT polymorphism, is one of the single nucleotide polymorphisms on the exon 1 coding region of the UGT1A1 gene. There is marked ethnical difference in the allele frequencies of UGT1A1*28 and UGT1A1*6 [15, 19]. The allele frequency of UGT1A1*28 is 30–40% in Caucasians and approximately 10% in Asians. Conversely, the UGT1A1*6 allele is observed in ~20% of Asians, but it is rarely detected in Caucasians. It is suggested that UGT1A1*6 is not negligible in clinical studies using irinotecan-based regimens in Asian patients. In the present study, UGT1A1*6 was noted in 8 cases (27%) and UGT1A1*28 in 4 cases (13%), supporting the higher incidence of the UGT1A1*6 polymorphism in Asians.

A previous report described the significant increase in the basal level of serum total bilirubin in UGT1A1*28 or UGT1A1*6 patients [20]. In our cases, serum total bilirubin was significantly higher in non-wild-type patients compared with wild-type cases, but the levels did not exceed the upper limit of normal. The present data support the potential capability of distinguishing patients with the UGT1A1 non-wild type genotype by their serum total bilirubin level. The serum total bilirubin level was elevated in both patient groups during chemotherapy, being in line with previous observations [10]. However, only in wild-type patients the difference was significant. The lack of significance in non-wild type patients might be due to the small number of cases.

In the present study, response rates to the irinotecan-cisplatin combination were similar in both UGT1A1 wild-type and non-wild-type patients. A significant correlation between homozygous UGT1A1*6 and lower tumor response to irinotecan-based chemotherapy has already been reported in Korean patients [19]. Although a significant correlation between UGT1A1 polymorphisms and tumor response was not observed in the present study, further investigations in a larger patient cohort are needed to evaluate the association between the UGT1A1 genotype and tumor response.

In the present study, previous pelvic radiotherapy was found to be a significant predictor of grade 3/4 neutropenia or diarrhea in multivariate analysis: 3 cases of cervical cancer had been treated with whole pelvic irradiation at a total dose of 50 Gy. Two of the 3 cases included patients with UGT1A1 non-wild type genotypes; a case with UGT1A1*6 and another with UGT1A1*28. Additionally, all 3 cases had received previous chemotherapy consisting of more than two regimens. Grade 3/4 neutropenia was observed in all 3 cases, and grade 3/4 diarrhea occurred in 2 cases with UGT1A1 non-wild-type genotypes. Possibly, previous heavy treatment with radiotherapy and chemotherapy affected the results in these 3 cases. However, multivariate analysis demonstrated that previous pelvic radiotherapy was significantly associated with severe toxicities.

The second significant predictor of severe toxicities in the present study was the UGT1A1 non-wild-type genotype. The relationship between the UGT1A1*28 genotype and toxicity resulting from irinotecan-based chemotherapy has already been established. On the other hand, a previous large retrospective investigation had failed to find a significant association between UGT1A1*6

and irinotecan-induced toxicity [9], but recent studies have described a positive relationship of the UGT1A1*6 genotype and toxicities. Han et al. [19] first reported that the UGT1A1*6 genotype was related with lower SN-38 glucuronidation and a higher frequency of grade 3–4 toxicities in Korean patients who were treated with the irinotecan-cisplatin combination. Sai et al. [20] also documented that the incidence of grade 3/4 neutropenia was significantly higher in patients with the UGT1A1*6 genotype who were treated with irinotecan-based chemotherapy. In line with these observations, the present study revealed a significant association of UGT1A1*6 not only with hematological toxicity but also with life-threatening diarrhea. The results of our case series were in contrast to a previous large meta-analysis that found no significant contribution of UGT1A1 genotypes to the toxicity profiles in cases treated with low-dose (100–125 mg/m^2) irinotecan monotherapy [21]. The effects of platinum-containing drugs, irinotecan and/or SN-38 on P-glycoprotein expression and function and on human intestinal and biliary transport may enhance toxicity, exceeding possible effects of UGT1A1 polymorphisms and metabolic drug pathways. In a recent study, irinotecan induced P-glycoprotein expression and function in human intestinal epithelial cells [25]. Inversely, administration of cisplatin, which increases P-glycoprotein expression, could modulate the secretory transport of irinotecan and SN-38 [26].

To confirm these observations, studies including pharmacogenomics are needed. A recent study revealed other UGT1A1 polymorphisms, UGT1A1*7 and UGT1A1*9, associated with tumor response [27] and decreased glucuronosyltransferase activity for SN-38 [28]. Additionally, UGT1A1 genotypes affecting the pharmacokinetics of irinotecan-based chemotherapy may also exist. However, the present study demonstrated a significant association of UGT1A1*6 with grade 3/4 neutropenia and grade 3/4 diarrhea in patients treated with cisplatin and low-dose irinotecan. In the clinical setting, genotyping of UGT1A1*6 in addition to UGT1A1*28 is recommendable for patients treated with irinotecan and cisplatin, especially in Asian patients.

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ORIGINAL ARTICLE

Clinical significance of the NKG2D ligands, MICA/B and ULBP2 in ovarian cancer: high expression of ULBP2 is an indicator of poor prognosis

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Abstract

Objective To investigate the clinical significance of the expression of the NKG2D ligands MICA/B and ULBP2 in ovarian cancer.

Methods Eighty-two ovarian cancer patients and six patients without ovarian cancer from Department of Obstetrics and Gynecology of Kyoto University Hospital were enrolled in this study between 1993 and 2003. Expression of MICA/B, ULBP2, and CD57 in ovarian cancer tissue and normal ovary tissue was evaluated by immunohistochemical staining, and the relationship of these results to relevant clinical patient data was analyzed. Expression of MICs, ULBP2, and HLA-class I molecules in 33 ovarian cancer cell lines and two normal ovarian epithelial cell lines, as well as levels of soluble MICs and ULBP2 in the culture supernatants, were measured.

Results Expression of MICA/B and ULBP2 was detected in 97.6 and 82.9% of ovarian cancer cells, respectively, whereas neither was expressed on normal ovarian epithelium. The expression of MICA/B in ovarian cancer was highly correlated with that of ULBP2. Strong expression of ULBP2 in ovarian cancer cells was correlated with less intraepithelial infiltration of T cells and bad prognoses for patients, suggesting that ULBP2 expression is a prognostic indicator in ovarian cancer. The expression of NKG2D ligands did not correlate with the levels of the soluble forms of the ligands.

Conclusions High expression of ULBP2 is an indicator of poor prognosis in ovarian cancer and may relate to T cell dysfunction in the tumor microenvironment.

Keywords Ovarian cancer · Tumor immunology · NKG2D ligand · MICA/B · ULBP2

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Introduction

Ovarian cancer is the leading cause of death among malignant gynecological tumors. Although a combination of surgery and chemotherapy has significantly improved patient survival, a majority of patients with advanced disease eventually die due to recurrence and progression. It is thus necessary to develop novel therapeutic approaches to treat ovarian cancer.

In the last decade, much research has focused on immunological aspects of malignant tumors. We have reported that $CD8^+$ T lymphocyte infiltration in ovarian cancer is associated with good prognoses [13]. Natural killer (NK) cell infiltration has also been reported to correlate with