ANTICANCER RESEARCH *32*: 4029-4034 (2012)

# Salvage Chemotherapy Using Gemcitabine for Taxane/ Platinum-resistant Recurrent Ovarian Cancer: A Single Institutional Experience

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**Abstract.** *Background: The purpose of this study was to report on the safety and efficacy of gemcitabine used as salvage chemotherapy for ovarian cancer. Patients and Methods: From January 2002 to October 2011, 27 patients were treated with gemcitabine for platinum-resistant recurrent ovarian cancer. Gemcitabine (800 mg/m$^2$) was given on days 1, 8, and 15 of every 28 days. The patients' medical records were retrospectively reviewed. Results: All 27 patients had previously received paclitaxel/carboplatin doublet and their disease had become platinum-resistant. The median number of previous chemotherapy regimens was 2 (range 1-7). A total of 114 cycles of single-agent gemcitabine were administered, with a median of 3 (range 1-10). No complete responses were observed. Partial response (PR) was observed in five patients (18.5%). Eight patients demonstrated stable disease (SD). The median duration of response for 5 responders was 4 months (range 2-6 months). The median survival time was 15 months. Patients with PR or SD (n=13) had significantly better survival compared with the group with progressive disease (n=14) (p=0.03, by univariate analysis). In addition, multivariate Cox proportional hazards analysis revealed that responses to gemcitabine were a significant factor for survival (hazard ratio=0.08, 95% confidence interval=0.0138 to 0.5614, p=0.01). Cases with hematological toxicity included 10 patients (37.0%) with grade 3/4 neutropenia, 3 patients (11.1%) with grade 3 thrombocytopenia, and 3 patients (11.1%) with grade 3 anemia. Non-hematological toxicity was well-tolerated. Conclusion: Gemcitabine (800 mg/m$^2$) used for recurrent ovarian cancer possesses a modest activity and a well-tolerated toxicity.*

The golden-standard of therapy for epithelial ovarian cancer (EOC) includes maximal surgical debulking followed by chemotherapy with a taxane/platinum doublet. Although this therapy has resulted in some improvement in survival rates of patients with advanced ovarian cancer, the majority (70%) will eventually experience disease relapse and succumb to their disease (1). The recurrence of ovarian cancer remains the foremost formidable clinical problem, which will have to be resolved by better control of this malignant disease in order to improve survival. It is therefore critically important to develop new non-cross-resistant drugs for use after taxane/platinum doublet failure.

Gemcitabine (2', 2'-difluorodeoxycytidine), a synthetic nucleoside analog of cytidine, has been demonstrated to be an active agent for various types of solid tumors, such as non-small cell lung cancer and pancreatic, genitourinary, and breast cancers (2). As described in the pioneering work of Plunkett *et al.*, gemcitabine is a pro-drug, which is metabolized to gemcitabine diphosphate and triphosphate, whose incorporation into DNA results in chain termination by inhibiting DNA polymerase activity (3). Consequently, tumor cells are blocked in the $G_1$ phase of the cell cycle. The gemcitabine triphosphate metabolite can also be incorporated into RNA, thus inhibiting RNA production (4).

Clinical use of gemcitabine for ovarian cancer was first reported in 1994 by Lund *et al.* (5). In their report, gemcitabine (800 mg/m$^2$) was given to patients with recurrent ovarian cancer, intravenously, once a week for three consecutive weeks, followed by one week of rest. A partial response was observed in 8 out of the 42 patients (19%), with a median response duration of 8.1 months. Seven out of the eight responders were resistant to first-line platinum-containing combination chemotherapy. Median overall time to progression was 2.8 months, and median overall survival (OS) was 6.2 months.



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*Key Words:* Salvage chemotherapy, recurrent ovarian cancer, gemcitabine, taxane resistance, cisplatin resistance.

Leukocytopenia and thrombocytopenia were the main toxic effects that caused dose omissions (27% and 14%, respectively) and dose reductions (37% and 21%, respectively). Non-hematological toxicity was mild and tolerable.

Matsuo *et al.*, recently carried out a systematic literature review of clinical studies published between January 2005 and March 2010 to analyze which systemic agents were being employed for platinum-resistant ovarian cancer. They found that gemcitabine was the most common drug used in the clinical trials reporting the highest response rates. Gemcitabine-based combination therapy had an average response rate of 27.2%, with relatively better progression-free survival (more than 4.1 months) (6).

In the present retrospective study, we evaluate the antitumor response and toxicity profile of single-agent gemcitabine (800 mg/m$^2$) and report our experience in using it for taxane/platinum-resistant recurrent ovarian cancer.

## Patients and Methods

*Patients.* We retrospectively reviewed the medical records of all patients with recurrent ovarian cancers treated with the single agent gemcitabine, who underwent such a treatment between January 2002 and October 2011 while at the Osaka University Hospital, Japan. Eligible patients were required to have histologically confirmed EOC.

*Gemcitabine therapy.* Gemcitabine (800 mg/m$^2$) was given on days 1, 8, and 15 of every 28 days. Courses were repeated until either the disease progressed or an unacceptable toxicity appeared. The initial doses of gemcitabine were reduced in subsequent courses, depending on toxicity. The minimum dose of gemcitabine was 650 mg/m$^2$.

*Response criteria.* Patients were evaluated for their response to treatment after they completed at least one 28-day treatment cycle. Reevaluation procedures included serial computed-tomography (CT) visualization of measurable disease. Response categories were assigned when patients had measurable disease fulfilling the revised RECIST guidelines (version 1.1) (7).

*Safety assessment.* All patients who received at least one cycle of gemcitabine were included in the toxicity analysis. Both hematological and non-hematological toxicities were assessed through review of laboratory reports, including standard variables, such as hemoglobin, hematocrit, neutrophil, leukocytes and platelet counts, and medical records for clinical history. Toxicity was assessed using the National Cancer Institute's Common Toxicity Criteria (v. 4.0, Common Terminology Criteria for Adverse Events, 2009) (8).

*Statistical analysis.* The treatment-free interval (TFI) was defined as the time (months) from completion of the previous therapy to the start of gemcitabine treatment. OS was defined as the time elapsed between the start of gemcitabine treatment and date of death, or the date of last follow-up. The Kaplan and Meier statistical method was used for the calculation of overall survival times. The log-rank test was employed to assess the statistical significance; $p$-values less than 0.05 were considered to indicate statistical significance.

Table I. *Patients' characteristics.*

| Characteristic | No. (n=27) | % |
|---|---|---|
| Median age (range), years | 57 (26-75) | |
| FIGO stage | | |
| I | 3 | 11.1 |
| II | 5 | 18.5 |
| III | 17 | 62.9 |
| IV | 2 | 7.4 |
| Histology | | |
| Serous | 13 | 48.1 |
| Clear cell | 7 | 25.9 |
| Endometrioid | 3 | 11.1 |
| Other | 4 | 14.8 |
| Number of prior chemotherapy regimens | | |
| 1 | 1 | 3.7 |
| 2 | 14 | 51.6 |
| 3 | 7 | 25.9 |
| 4 | 4 | 14.8 |
| 7 | 1 | 3.7 |
| Median TFI (months, range) | 1 (1-11) | |

TFI, Treatment-free interval; FIGO, International Federation of Gynecology and Obstetrics.

The Mann-Whitney *U*-test was used to compare toxicity and efficacy. Univariate and multivariate proportional-hazards models (Cox) were fitted to the data to determine the importance of recognized explanatory variables. Selected factors were included in the multivariate Cox proportional-hazards analysis, namely, age (≤57 *vs.* >57 years), FIGO stage (I/II *vs.* III/IV), type of histology (clear cell *vs.* non-clear cell), TFI (<3 *vs.* ≥3 months), number of gemcitabine courses (≤3 *vs.* >3), the number of previous regimens (≤2 *vs.* >2), maximum response to gemcitabine [(PR) + (SD) *vs.* (PD)] and hematological toxicity (grade 1/2 *vs.* 3/4). Statistical analyses were performed using MedCalc for Windows (version 11.3.3.0; MedCalc Software, Mariakerke, Belgium).

## Results

*Patients' characteristics.* Twenty-seven patients treated with gemcitabine for recurrent ovarian cancer were identified in our hospital archive. Clinical characteristics of the 27 patients are summarized in Table I. The median age was 57 years (range 26-75). FIGO stage for these patients were: 3 patients (11.1%) at stage I, 5 patients (18.5%) at stage II, 17 patients (62.9%) at stage III and 2 patients (7.4%) at stage IV. Histological diagnoses revealed serous adenocarcinoma in 13 (48.1%), clear cell carcinoma in 7 (25.9%), endometrioid adenocarcinoma in 3 (11.1%), and other types in 4 (14.58%). The median number of prior chemotherapy regimens was 2 (range 1-7). All 27 patients had platinum-resistant recurrences and all had received paclitaxel/ carboplatin doublet previously. Their median TFI was one month (range 1-11 months).

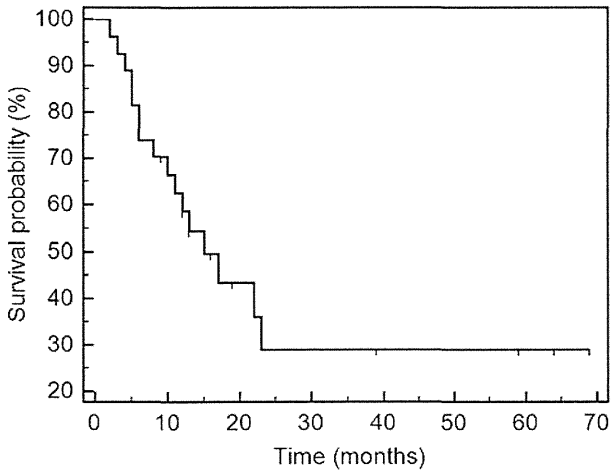


Figure 1. *Kaplan–Meier curve showing overall survival time of 27 patients with recurrent ovarian cancer treated with gemcitabine. The median survival time was 15 months.*

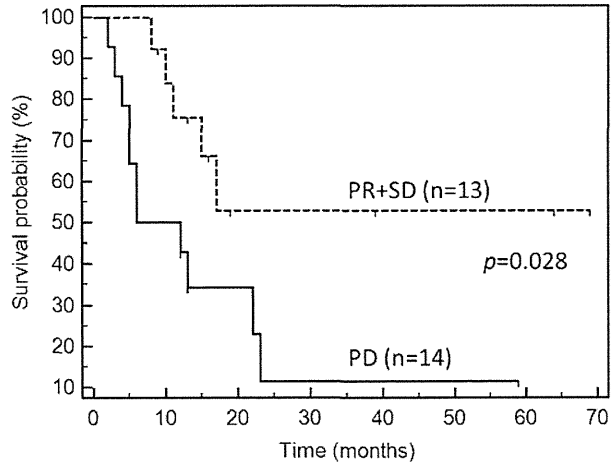


Figure 2. *Kaplan–Meier curve showing overall survival time stratified by maximum responses to gemcitabine. OS was significantly better in the group of patients who had PR or SD when compared with the group of PD (p=0.028).*

*Efficacy.* The responses of the platinum-resistant recurrences to gemcitabine are summarized in Table II. Twenty-seven patients received at least two cycles of gemcitabine treatment and all of them fulfilled the RECIST criteria. The overall response rate was 18.5% [no CRs; 18.5% (5/27) PRs] and SD was found in 29.6% (8/27), whereas PD was noted in 51.9% (14/27) patients. When comparing between different histologies, PR was observed more frequently in clear cell carcinoma and endometrioid adenocarcinoma. However, there was no significant difference between the groups ($p$=0.66, chi-square test). The disease control rate (CR+PR+SD) was 53.8% (7/13) for serous adenocarcinoma, 57.1% (4/7) for clear cell carcinoma, 33.3% (1/3) for endometrioid adenocarcinoma and 25.0% (1/4) for other histologies. Figure 1 shows the OS, which was a median of 15 months. As shown in Figure 2, OS was significantly better in the group of patients who had PR or SD when compared with the group of PD ($p$=0.028). Meanwhile, age (≤57 *vs.* >57 years), FIGO stage (I/II *vs.* III/IV), type of histology (clear cell *vs.* non-clear cell), TFI (≤3 *vs.* >3 months), number of gemcitabine courses (≤3 *vs.* >3), the number of previous regimens (≤2 *vs.* >2) and hematologic toxicity (grade 1/2 *vs.* 3/4) had no impact on OS by univariate analysis.

Table III shows the results of multivariate analysis, in which maximum response to gemcitabine (PR+SD *vs.* PD) has been defined as the independent prognostic factor for OS in patients with recurrent ovarian cancer treated with gemcitabine (hazard ratio=0.08, 95% confidence interval=0.0138-0.5614, $p$=0.01); whereas as observed in univariate analysis, none of the other parameters had any impact on OS.

Table II. *Antitumor effect of single-agent gemcitabine for recurrent ovarian cancer.*

| Histology | PR | PR+SD |
|---|---|---|
| Serous | 15.3% (2/13) | 53.8% (7/13) |
| Clear cell | 28.5% (2/7) | 57.1% (4/7) |
| Endometrioid | 33.3% (1/3) | 33.3% (1/3) |
| Other | 0/4 | 25.0% (1/4) |
| Total | 18.5% (5/27) | 48.1% (13/27) |

PR, Partial response; SD, stable disease.

*Toxicity.* All 27 patients were evaluated for safety and tolerability. Collectively, a total of 118 cycles of gemcitabine were administered. The starting dose for all patients was 800 mg/m$^2$ of gemcitabine, which was given on days 1, 8, and 15 of every 28 days. The median number of cycles of gemcitabine was 3 (range 1-10). In 3.7% (1/27) of patients, a dose reduction, to 650 mg/m$^2$ was necessary due to hematological toxicity. Discontinuation of the gemcitabine chemotherapy was required for two patients (7.4%) due to thrombocytopenia and neutropenia. There was no death associated with the gemcitabine treatment. The main toxicities are shown in Table IV. Hematological toxicity included 10 patients (37.0%) with grade 3/4 neutropenia and three patients (11.1%) with grade 3 thrombocytopenia and three patients (11.1%) with grade 3 anemia. Non-hematological toxicity was well-tolerated, with the exception of a grade 3 urticaria observed in one patient, which disappeared within three days.

Table III. *Multivariate Cox proportional hazards-analysis for recurrent ovarian cancer treated with gemcitabine.*

| Variables | Hazard ratio | 95% CI | *p*-Value |
|---|---|---|---|
| Age, years | | | |
| ≤57 (n=15) | 1 | | 0.14 |
| >57 (n=12) | 0.17 | 0.0171-1.7481 | |
| Stage | | | |
| I/II (n=8) | 3.57 | | 0.09 |
| III/IV (n=19) | 1 | 0.8165-15.6056 | |
| Histology | | | |
| Clear cell (n=7) | 0.60 | | 0.52 |
| Non-clear cell (n=20) | 1 | 0.1328-2.7509 | |
| Treatment-free interval | | | |
| <3 months (n=16) | 0.59 | 0.0781-4.5217 | 0.62 |
| ≥3 months (n=11) | 1 | | |
| Number of courses of gemcitabine | | | |
| ≤3 (n=13) | 1 | 0.4246-10.4723 | 0.36 |
| >3 (n=14) | 2.11 | | |
| Number of previous regimens | | | |
| ≤2 (n=15) | 4.02 | 0.9098-17.7512 | 0.07 |
| >2 (n=12) | 1 | | |
| Maximal response to gemcitabine | | | |
| PR or SD (n=13) | 0.08 | 0.0138-0.5614 | 0.01 |
| PD (n=14) | 1 | | |
| Hematological toxicity | | | |
| Grade 1/2 (n=17) | 1 | 0.1917-4.5447 | 0.93 |
| Grade 3/4 (n=10) | 0.93 | | |

PR, Partial response; SD, stable disease; PD, progressive disease; CI, confidence interval.

## Discussion

It is well-recognized that salvage therapy in ovarian cancer strongly depends upon the primary chemotherapy results. When the recurrence occurs more than 6 months after completion of the initial therapy, a re-administration of the platinum-containing doublet can be effective in many cases, resulting in extended survival times. However, if the recurrence occurs before 6 months pass, most chemotherapeutic agents are no longer effective (9, 10). Second-line treatment for patients with platinum-resistant disease relies on medication with a single-agent of chemotherapy, such as topotecan, liposomal pegylated doxorubicin, oral etoposide, paclitaxel and gemcitabine. All of these agents have a similar response rate of 10%. Among these agents, gemcitabine is currently the most commonly used drug because of its tolerable toxicity, although the antitumor activity of all these second-line agents is quite similar (6).

The use of gemcitabine for ovarian cancer was first reported by Silver *et al.* (11), where it was used at a dose of 800 mg/m$^2$ on days 1, 8, and 15 of every 28 days. Gemcitabine has been subsequently used at up to 1250 mg/m$^2$, as reviewed by Lorusso *et al.* (12). In Japan, Watanabe *et al.* (13) reported on 27 patients with recurrent ovarian cancer of similar condition to the ones of the present study. In their report, gemcitabine was used at 1000 mg/m$^2$ on days 1, 8 and 15, every 28 days. In contrast, we administered 800 mg/m$^2$ of gemcitabine for days 1, 8 and 15, every 28 days. The antitumor effects of our treatment course (800 mg/m$^2$) and that of Watanabe *et al.* (1000 mg/m$^2$) was similar, as response rates were 18.5% and 17.9%, respectively. The median survival times were 15 and 11 months, respectively. Regarding hematological toxicity, grade 3/4 neutropenia was observed in 37.0% and 39.3% of the patients, respectively, and grade 3/4 thrombocytopenia was observed in 11.1% and 46.4% of patients, respectively. Non-hematological toxicities were mild and tolerable in both studies.

Table IV. *Adverse events of gemcitabine therapy (n=27).*

| Toxicity | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|
| Hemoglobin | 8 (29.6%) | 3 (11.1%) | 0 |
| Neutropenia | 7 (25.9%) | 9 (33.3%) | 1 (3.7%) |
| Platelet reduction | 2 (7.4%) | 3 (11.1%) | 0 |
| Urticaria | 0 | 1 (3.7%) | 0 |

The results of both univariate and multivariate analyses showed that our patients with a response to gemcitabine of SD or PD had better OS compared with these with PD. This result supports the idea that the survival benefit following second-line chemotherapy for platinum-resistant disease, if a complete remission is not obtained, is similar for PR and SD, as described by Cesano *et al.* (14). Thus, disease stabilization is important for patients whose life expectancy is generally short.

It should be noted that having a histology of clear cell carcinoma was not a significant factor for OS. Extremely low response rates for first-line platinum-based (15) and platinum/taxane doublet (16) chemotherapy for ovarian cancer have been reported. In addition, recurrent clear cell carcinoma has been reported by Takano *et al.* (17) and Yoshino *et al.* (18) to be particularly chemoresistant.

Although not statistically significant in our series, clear cell carcinoma had a better response rate compared to serous adenocarcinoma. The disease control rate and OS were similar between these groups. Thus, patients with recurrent clear cell carcinoma did not have an inferior prognosis when gemcitabine was used. Benefits of gemcitabine administration for recurrent ovarian cancer have been reported by other groups. Ferrandina *et al.* described a case of multidrug-resistant clear cell carcinoma of the ovary showing a selective susceptibility to gemcitabine at first administration and again at re-challenge. Moreover, they showed that the tumor expressed a certain molecular profile that likely made it highly sensitive to gemcitabine (19). Komiyama *et al.* reported successful control of massive

ascites due to *peritonitis carcinomatosa* with gemcitabine in a patient with recurrent clear cell carcinoma (20).

In conclusion, our results suggest that administration of gemcitabine at 800 mg/m$^2$ to platinum-resistant disease is as valuable as the commonly used 1000 mg/m$^2$ dose, irrespectively of tumor histology. However, our data include only a relatively small number of patients in this retrospective study, whereas the importance of this subject warrants a prospective randomized trial for full validation. In addition, due to its low toxicity, gemcitabine might be useful in combination chemotherapy, to overcome platinum-resistant recurrent ovarian cancer.

## References


1 Heintz AP, Odicino F, Maisonneuve P, Quinn MA, Benedet JL, Creasman WT, Ngan HY, Pecorelli S and Beller U: Carcinoma of the ovary. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet *95(Suppl 1)*: S161-192, 2006.

2 Ozols RF: The current role of gemcitabine in ovarian cancer. Semin Oncol *28*: 18-24, 2001.

3 Plunkett W, Huang P, Searcy CE and Gandhi V: Gemcitabine: preclinical pharmacology and mechanisms of action. Semin Oncol *23*: 3-15, 1996.

4 Mackey JR, Mani RS, Selner M, Mowles D, Young JD, Belt JA, Crawford CR and Cass CE: Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. Cancer Res *58*: 4349-4357, 1998.

5 Lund B, Hansen OP, Theilade K, Hansen M and Neijt JP: Phase II study of gemcitabine (2',2'-difluorodeoxycytidine) in previously treated ovarian cancer patients. J National Cancer Institute *86*: 1530-1533, 1994.

6 Matsuo K, Lin YG, Roman LD and Sood AK: Overcoming platinum resistance in ovarian carcinoma. Expert Opinion Investigational Drugs *19*: 1339-1354, 2010.

7 Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D and Verweij J: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer *45*: 228-247, 2009.

8 National Cancer Institute: Common Terminology Criteria for Adverse Events v4.0. http://ctep.cancer.gov/reporting/ctc.html

9 Markman M, Rothman R, Hakes T, Reichman B, Hoskins W, Rubin S, Jones W, Almadrones L and Lewis JL Jr.: Second-line platinum therapy in patients with ovarian cancer previously treated with cisplatin. J Clin Oncol *9*: 389-393, 1991.

10 Harries M and Gore M: Part II: chemotherapy for epithelial ovarian cancer-treatment of recurrent disease. Lancet Oncol *3*: 537-545, 2002.

11 Silver DF and Piver MS: Gemcitabine salvage chemotherapy for patients with gynecologic malignancies of the ovary, fallopian tube, and peritoneum. American J Clinical Oncol *22*: 450-452, 1999.

12 Lorusso D, Ferrandina G, Fruscella E, Marini L, Adamo V and Scambia G: Gemcitabine in epithelial ovarian cancer treatment: current role and future perspectives. Int J Gynecol Cancer *15*: 1002-1013, 2005.

13 Watanabe Y, Koike E, Nakai H, Etoh T and Hoshiai H: Phase II study of single-agent gemcitabine in heavily pretreated Japanese patients with recurrent ovarian cancer. International journal of clinical oncology/Japan Society of Clinical Oncology *13*: 345-348, 2008.

14 Cesano A, Lane SR, Poulin R, Ross G and Fields SZ: Stabilization of disease as a useful predictor of survival following second-line chemotherapy in small cell lung cancer and ovarian cancer patients. Int J Oncol *15*: 1233-1238, 1999.

15 Sugiyama T, Kamura T, Kigawa J, Terakawa N, Kikuchi Y, Kita T, Suzuki M, Sato I and Taguchi K: Clinical characteristics of clear cell carcinoma of the ovary: a distinct histologic type with poor prognosis and resistance to platinum-based chemotherapy. Cancer *88*: 2584-2589, 2000.

16 Enomoto T, Kuragaki C, Yamasaki M, Sugita N, Ohtsuki Y, Ikegami H, Matsuzaki N, Yamada T, Wakimoto A and Murata Y: Is clear cell carcinoma and mucinous carcinoma of the ovary sensitive to combination chemotherapy with paclitaxel and carboplatin? Proc Am Soc Clin Oncol *22*: 447, 2003.

17 Takano M, Sugiyama T, Yaegashi N, Sakuma M, Suzuki M, Saga Y, Kuzuya K, Kigawa J, Shimada M, Tsuda H, Moriya T, Yoshizaki A, Kita T and Kikuchi Y: Low response rate of second-line chemotherapy for recurrent or refractory clear cell carcinoma of the ovary: a retrospective Japan Clear Cell Carcinoma Study. Int J Gynecol Cancer *18*: 937-942, 2008.

18 Yoshino K, Enomoto T, Fujita M, Ueda Y, Kimura T, Kobayashi E and Tsutsui T: Salvage chemotherapy for recurrent or persistent clear cell carcinoma of the ovary: a single-institution experience for a series of 20 patients. International journal of clinical oncology / Japan Society of Clinical Oncology 2011.

19 Ferrandina G, Legge F, Mey V, Nannizzi S, Ricciardi S, Petrillo M, Corrado G and Scambia G: A case of drug resistant clear cell ovarian cancer showing responsiveness to gemcitabine at first administration and at re-challenge. Cancer Chemother Pharmacol *60*: 459-461, 2007.

20 Komiyama S, Nakamura M, Murakami I, Kuwabara Y, Kurahashi T, Tanaka K and Mikami M: A heavily pretreated patient with recurrent clear cell adenocarcinoma of the ovary in whom carcinomatous peritonitis was controlled successfully by salvage therapy with gemcitabine. Arch Gynecol Obstet *278*: 565-568, 2008.


*Received May 15, 2012*
*Revised July 19, 2012*
*Accepted July 23, 2012*

Review

Cancer Epidemiology, Biomarkers & Prevention

# Biomarkers for Screening, Diagnosis, and Monitoring of Ovarian Cancer

Eiji Kobayashi, Yutaka Ueda, Shinya Matsuzaki, Takuhei Yokoyama, Toshihiro Kimura, Kiyoshi Yoshino, Masami Fujita, Tadashi Kimura, and Takayuki Enomoto

## Abstract

Serum tumor markers have a major role in the screening, diagnosis, and monitoring of most of the gynecologic cancers. Ovarian cancer is one of the deadliest of the group because it is so frequently asymptomatic until it has advanced to an untreatable stage. Even serum cancer antigen-125 (CA-125), clinically one of the most reliable serum markers for ovarian cancer, is elevated in only half of early-stage still-treatable tumors. Because of the very low prevalence of ovarian cancer in the general population, at present, there is no cost-effective imaging or simple microscopic screening test for ovarian cancer as there is for breast and cervical cancers. However, recent proteomics and nucleic acid–based analyses have shown great promise for the discovery of new and more useful serum biomarkers, which cumulatively might provide such a screening tool. In this review, we will discuss both the currently used serum tumor markers for screening, diagnosis, monitoring of ovarian cancer, and the novel biomarkers that are now under investigation and validation. *Cancer Epidemiol Biomarkers Prev; 21(11); 1902–12. ©2012 AACR.*

## Introduction

Endometrial, cervical, and ovarian cancers are 3 of the most common malignancies of the female reproductive tract. Of the 3, ovarian cancer, although rare in occurrence, is the deadliest; in 2008 alone, 224,747 women were diagnosed with ovarian cancer worldwide, and a heartbreaking 62% of these women died from the disease (1). This is primarily because roughly three-quarters of ovarian cancer cases present at an advanced stage, with the disease spread well beyond the ovaries (2). The cancer is insidious, patients usually have their first symptoms only in the advanced-stage of the disease, and these are often related to the presence of a grossly enlarging tumor and extensive ascites fluid; in the early- and midstage disease, most patients are largely asymptomatic (3). Serum cancer antigen-125 (CA-125) levels and transvaginal ultrasonography (TV-USG) screening have contributed to an earlier detection of ovarian cancer; however, the value of tumor markers and USG to screen for epithelial ovarian cancer (EOC) is yet to be clearly established by prospective studies (3, 4).

For any hope of curing ovarian, endometrial, and cervical cancers, it is critical to detect these diseases at the earliest possible stage. These tumors are phenotypically and genetically heterogeneous, so no single tumor marker will detect all variations; therefore, the discovery of additional useful serum biomarkers for the early detection of gynecologic cancers has thus been highly sought after. Such tumor markers will be molecules arising from the presence of a tumor, which can appear in the surrounding tissues, blood, and excretions because they are secreted or shed by the tumor in excess of the normal tissue or cell phenotype. Sometimes, the marker will be uniquely specific to a tumor subtype, for example, as embryonic, fetal, undifferentiated, or stem-cell phenotypes. Tumor markers can occur as reexpression of genes silenced during differentiation or as anomalous alternative mRNA splicing products of a currently expressed gene. Glycoproteins produced by cancer cells can have detectably altered glycan structures, although the core proteins themselves are ubiquitous (5). Tumor markers might be unique extracellular matrix or cell adhesion molecules, or they can be receptors, growth factors, cytokines, or products of abnormal metabolism. Rarely, the marker molecules can be released by other tissues and organs in response to signals from the tumor. Even the body's autoantibodies against tumor antigens can be markers.

Tumor markers can be associated with patient diagnosis, prognosis, clinical management, and follow-up. In an ideal world, tumor markers would be highly tumor-specific, would always be produced in sufficient amounts to allow fast, easy, cheap, and noninvasive detection of minimal disease, and would quantitatively reflect tumor burden. These idealistic tumor markers would enable their use in screening, diagnosis, monitoring response to therapy, and detecting earlier recurrence during follow-up.

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**doi:** 10.1158/1055-9965.EPI-12-0646

Recent advances in clinical proteomics and serum miRNA analysis have propelled us into an exciting period of discovery of new cancer biomarkers, although the available technologies still have their limitations. The principles of serum marker technology require stringent guidelines for the collection of clinical material, the application of analytic techniques, and for interpretation of the data.

In this review, we will present an overview of the currently used serum tumor markers for the screening of ovarian cancer. Also, we will discuss novel biomarkers that have given us great hope for the future of better detection and management of ovarian cancers.

## Serum Markers for Ovarian Cancer

Roughly, three-quarters of all cases of ovarian cancers are diagnosed only after the disease has progressed to stage III or IV, and have involved the peritoneal cavity or other organs. The ultrapoor prognosis for this cancer results directly from the lack of reliable, sensitive screening tests and our limited understanding of the mechanisms of its chemoresistance and relapse. Thus, establishment of an appropriate earlier stage screening test for ovarian cancer has long been sought.

The symptoms that are commonly associated with early to midstage ovarian cancer are typically nonspecific, and the association is often not clinically recognized until the disease is irretrievably advanced (6). Previous studies have shown that USG can provide some degree of high sensitivity; however, its specificity and positive predictive values (PPV) were found to be unsatisfactory (7, 8).

Given the low prevalence of ovarian cancer in the general population, an effective and acceptable screening strategy must have not only a high sensitivity for early-stage disease (>75%), but must also have a very high specificity (99.6%) so as to prompt no more than 10 exploratory operations for each actual case of ovarian cancer diagnosed; that is, it must have a PPV of 10%, even in postmenopausal women more than 50 years of age, who are at a significantly higher risk than younger women (9). At present there is no highly effective screening test for ovarian cancer (such as for breast and cervical cancer). However, the serum markers for ovarian cancers that are currently being used, and those novel biomarkers under investigation, will be discussed later.

### Usefulness of CA-125 for screening and surveillance of ovarian cancer

*Early detection of ovarian cancer.* To date, CA-125 is the serum marker that has received the most use and is the most trusted as an identifying method for ovarian cancer early detection (Table 1). CA-125 was originally developed to monitor patients previously diagnosed with an ovarian cancer but not for its screening. When used as an individual marker on a single occasion, CA-125 is not sufficiently sensitive to detect most cases of early-stage ovarian cancer. Serum CA-125 levels do become more frequently elevated in patients as the disease progresses; elevations are detected in 50% and 92% of ovarian cancers in early and late stages, respectively (10). Nossov and colleagues (11) found that PPV of CA-125 assay for early detection of ovarian cancer was 57%. Unfortunately, for identifying the source of this tumor marker, elevated CA-125 occurs in other cancers as well, such as endometrial, breast, pancreatic, gastrointestinal, and lung cancers. Elevated CA-125 levels can also be found in patients with benign gynecologic conditions, such as during menstruation, pregnancy, endometriosis, and pelvic inflammatory disease, and even in nongynecologic conditions, such as hepatitis and pancreatitis (12). The physician, therefore, has to always consider the possibility that this tumor marker is creating a false positive case due to another pathologic condition. A one-time determination of CA-125 is thus neither sufficiently sensitive nor specific enough to be used as a biomarker for screening the general population.

Table 1. Clinical significance of CA125 level for ovarian cancer

| |
|---|
| Screening of ovarian cancer |
| Differential diagnosis between primary ovarian cancer and metastatic ovarian cancer[a] |
| Prediction of prognosis |
| Surveillance of recurrence |

[a]In combination with CEA.

To augment its usefulness for screening, CA-125 has been combined with TV-USG. Various combinations of CA-125 and imaging screening, both concurrent testing as well as sequentially, are being tested. There are currently 4 major ovarian cancer screening trials, 2 of which are still ongoing and 2 that have been completed (Table 2). The prostate, lung, colorectal, and ovarian (PLCO) trial in the United States was a randomized control trial of 78,216 women, ages 55 to 74 years, assigned either to annual screening ($N = 39,105$) or usual care ($N = 39,111$; ref. 13). The "intervention group" received annual screening with CA-125 for 6 years and TV-USG for 4 years at 10 medical centers throughout the country. The control "usual care" group was not offered this advanced screening for 6 years but did receive their usual medical care. Twenty-two percent of patients with screening-detected cancers had stage I or II disease, versus 22% in the control group, and there was no evidence of a shift to early-stage disease associated with screening. There was equivalent ovarian cancer mortality in both groups.

The second completed study, a multicenter screening trial in Japan, was a prospective randomized trial conducted between 1985 and 1999, in which asymptomatic postmenopausal women were assigned either to a screening group ($N = 41,688$) or a control group ($N = 40,799$; ref. 14). Women in the screening arm received an annual pelvic examination, a serum CA-125 test, and an ultrasound examination. Ovarian cancers were detected by

Table 2. Results from major ovarian cancer screening trials

| Screening trial | Years | Study design | Screening test | Nonscreened | Cancers detected | Stage I and II | Stage III and IV | Survival benefit |
|---|---|---|---|---|---|---|---|---|
| PLCO (USA) | 1993–2001 | Randomized control | Ultrasound C125 vs. usual care | 34,253 | 212 | 22% | 77% | (–) |
| UKCTOCS (UK) | 2001–2005 | Randomized control | Ultrasound C125 or ultrasound alone vs. usual care | 101,279 | 58 | 48% | 52% | Analysis pending[a] |
| SCSOCS (Japan) | 1985–1999 | Randomized control | Ultrasound C125 vs. usual care | 41,688 | 27 | 67% | 33% | Analysis pending[a] |
| University of Kentucky (USA) | 1987–2011 | Population control | Ultrasound | 37,293 | 47 | 70% | 30% | (+) |

[a]Not reported until present. PLCO, The prostate, lung, colorectal, and ovarian trial; UKCTOCS, The United Kingdom Collaborative Trial of Ovarian Cancer Screening; SCSOCS, The Shizuoka Cohort Study of Ovarian Cancer Screening.

screening in 27 women, of which 67% had a stage I or stage II disease. Thirty-two women in the control group developed ovarian cancer, 44% of whom had stage I or II disease. Analysis of site-specific ovarian cancer mortality in the screening and control groups has not yet been reported.

The largest ongoing screening trial is the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS; ref. 15). From 2001 to 2005, 202,638 postmenopausal women, ages 50 to 74 years, were randomly assigned to annual TV-USG screening ($N = 50,639$), multimodal screening with sequential serum CA-125 testing and ultrasound ($N = 50,640$), or no treatment ($N = 101,359$). Fifty-eight invasive ovarian cancers were detected by screening, 28 patients (48%) had stage I or II disease, versus 26% in the control population, and 22% in the prevalence screen of the PLCO trial. This trial is ongoing, therefore, the effect of the screening program on ovarian cancer mortality awaits further analysis.

The University of Kentucky Ovarian Cancer Screening Trial has been in progress from 1987 to the present time, and 37,293 women have been screened (16). To date, 47 EOCs have been detected, with 70% of patients having stage I or II disease. Twelve women developed detectable ovarian cancers within 12 months of a negative screen. The stage at detection and the site-specific ovarian cancer mortality in women with screen-detected cancers have been compared with women from the same geographic area whose cancers were detected clinically during the same time period. Screening produced a stage shift, in which 70% of women with screening-detected ovarian cancers had stage I or II disease versus 27% in the unscreened control group ($P < 0.01$). The 5-year survival of all women whose EOCs were detected by this screening study, including the interval cancers, was 74.8% ± 6.6%, as compared with 53.7% ± 2.3% for women with routine clinically detected ovarian cancers treated at the same institution with the same surgical and chemotherapy protocols ($P < 0.01$).

Although in several of the trials described earlier, screening seems to have allowed for detection of the tumor at an average of an earlier stage, the effects of screening on ovarian cancer mortality has varied significantly, and disappointingly, in the different trials, and that itself is the subject of further investigations. In addition, these tests (combined TV-USG and CA-125) are not cost-effective as currently conducted and are thus still not used routinely to screen for ovarian cancer.

*Differentiation from other malignancies.* The differentiation of a primary ovarian cancer from a tumor metastatic to the ovary is still tremendously challenging. In a previous study, Yedema and colleagues (17) described the preoperative discrimination of ovarian cancer from colorectal cancer. They reported that the specificity increased significantly when using a combination of a CA-125 positive score (>35 U/mL) and a simultaneous negative tumor marker CEA; carcinoembrionic antigen score (5 ng/mL; specificity 100%, sensitivity 81%). A CA-125/CEA serum ratio of more than 25 resulted in the highest discriminative power, with a specificity of 100% and a sensitivity of 91%, resulting in an overall test accuracy of 94%. They concluded that a combination of CA-125 and CEA are helpful in the preoperative differential diagnosis between a primary ovarian cancer and a colorectal origin.

Sørensen and Mosgaard (18) also reported the ability of CEA in combination with CA-125 to differentiate between malignant ovarian and malignant nonovarian disease. They reported that, among the patients with CEA levels of more than 5 ng/mL, 68% had nonovarian malignancies. In patients with a CA-125/CEA ratio of more than 25, ovarian cancer was found in 82%. The specificity increased to around 85% when the cut-off value of the CA-125/CEA ratio was increased from 25 to 100 (18). From these results, a combination of CA-125 and CEA may be helpful in the preoperative differential diagnosis between ovarian cancer and another originated cancer.

*Prediction of prognosis and surveillance of recurrence.* The predictive value of pretreatment CA-125

levels for prognosis is controversial. While some studies did not find preoperative CA-125 levels to be an independent prognostic factor (19–21), others reported that it could identify poor prognostic subgroups, independent of stage (22, 23). However, changes in CA-125 levels can also correlate with regression, stability, and progression of the disease in 87% to 94% of instances (12).

Elevation levels in CA-125 can be used to document progressive disease in patients who achieve a normal CA-125 after primary treatment. Rustin and colleagues (24) found that a doubling of CA-125 level from the upper limit of normal had a sensitivity of 86% and a specificity of 91% for detecting progression. A second confirmatory value reduces the false-negative rate to less than 2%. Similarly, a doubling of CA-125 from baseline in patients with persistently elevated CA-125 following primary treatment accurately predicts progression (25). Increase in CA-125 levels tend to precede symptomatic relapse by a median of 4.5 months (range 0.5–29.5 months), and there is considerable debate about whether additional treatment should be commenced on the basis of increasing CA-125 alone. In the recent Medical Research Council/European Organisation for Research and Treatment of Cancer (MRC/EORTC) trial, Rustin and colleagues showed no difference in overall survival (HR, 1.00) between patients who received chemotherapy based on increasing CA-125 and those who did not receive chemotherapy until they were symptomatic (26). Thus, whether or not early reintroduction of treatment produces a survival advantage remains unclear.

Although a high probability exists that some tumor response can be achieved with chemotherapy, a complete cure of these patients is rarely possible. Potential advantages of early treatment of relapse include delaying cancer-related symptoms, providing psychologic reassurance, and possibly improved survival. Potential disadvantages include loss of time without treatment and associated toxic effects. Patients should be counseled on these advantages and disadvantages before deciding whether to have their CA-125 concentrations routinely measured during follow-up.

*Other tumor markers.* Serum levels of CA 19-9 (a monosialoganglioside antigen widely used in gastrointestinal adenocarcinoma diagnostics) are elevated in 68% to 83% of mucinous ovarian cancers but in only 28% to 29% of nonmucinous types, whereas CA-125 is elevated in 80% of nonmucinous ovarian tumors (27–30) providing a differential diagnostic tool for nonmucinous versus mucinous subtypes. Other markers, alone or in combination, have also been used; serum CA 15-3, CA 72-4, and CEA levels are elevated, respectively, in 50% to 56%, 63% to 71%, and 25% to 50% of patients with ovarian cancer (27, 31–38; Table 3). According to Gadducci and colleagues, the levels of the markers CA 19-9, CA 15-3, and CA 72-4 were poorly correlated with the clinical course of the disease, when compared with CA-125, and thus these markers did not offer additional clinical benefit for monitoring ovarian cancer. However, the serial measurement of these markers may still play an important role in the management of the relatively large group of patients with a CA-125 negative tumor (12). This would be similar to monitoring Her-2-negative/estrogen receptor-negative breast tumors with other breast tumor markers.

There are additional serum markers for ovarian cancer that are under active investigation (Table 3). For example, HE4 has recently been accepted by the U.S. Food and Drug Administration (FDA) as a monitoring method for patient management with EOC. In a review by Li and colleagues, they found that HE4 displayed the highest sensitivity (72.9%) among all single markers, including CA-125, in the detection of ovarian cancer, in both the early (62%–83%) and late (75%–93%) stages

Table 3. Diagnostic serum markers for ovarian cancer

| | Cut-off | Ref. No. | SE (%) | SP (%) | PPV | NPV |
|---|---|---|---|---|---|---|
| CA125 | >35 U/mL | 26 | 82.2 | 67.3 | 47.1 | 91.4 |
| | >65 U/mL | 26 | 75.6 | 86.6 | 66.7 | 90.9 |
| CA19-9 | >40 U/mL | 26 | 35.6 | 81.1 | 40 | 78 |
| CA15-3 | >32 U/mL | 26 | 57.1 | 93.9 | 75.9 | 86.7 |
| CA72-4 | >3.8 U/mL | 26 | 70.7 | 91.8 | 75.7 | 89.6 |
| CEA | >3 ng/mL nonsmoke, >5 ng/mL smoker | 37 | 16 | 93 | 37 | 83 |
| HE4 | >70 pmol/L | 41 | 72.9 | 95 | NA | NA |
| LPA | 1.3 μmol/L | 41 | 98 | 90 | NA | NA |
| IAP | 482 μg/mL | 34 | 93.3 | 91 | NA | NA |
| HP-α | 65 μg/mL | 44 | 64 | 90 | NA | NA |
| OVX-1 | 7.2 μ/mL | 49 | 70 | 95 | NA | NA |
| Methothelin | – | 43 | 60 | 98 | NA | NA |

Abbreviations: IAP, immunosuppressive acidic protein; NA, not assessed; NPV, negative predictive value; Ref. No., reference number; SE, sensitivity; Spec, specificity; –, not shown.

Table 4. Level of circulating exosome in patients with ovarian cancer by tumor stage

| Stage | Number of patients | Level of circulating tumor-derived exosomes |
|---|---|---|
| I | 10 | 0.320 ± 0.056 mg/mL |
| II | 10 | 0.640 ± 0.053 mg/mL |
| III | 20 | 0.995 ± 0.084 mg/mL |
| IV | 10 | 1.42 ± 0.228 mg/mL |

NOTE: Table modified from ref. (71).

(39). In addition, serum levels of HE4 are elevated in at least a third of the patients with EOC who do not have tumors that overexpress CA-125, suggesting a complementary application of the 2 tests would be useful (40, 41).

Elevated serum lysophosphatidic acid (LPA) levels, another potentially useful marker, were found in 90% and 98% of ovarian cancer in early and late stages, respectively; however, serum levels of LPA do not correlate well with the stage of the disease, and nonspecific elevation of LPA was detected in healthy and benign gynecologic conditions (11, 42, 43).

Significantly, elevated sFas levels are detected in some patients with ovarian cancer as compared with healthy women, and serum sFas level was shown to be a statistically significant indication factor for survival, as well as histologic grade, in ovarian carcinomas (44). Another antigen marker, mesothelin (41), is a protein of unknown biologic function, which is present in normal mesothelium and has been detected at elevated levels in the serum of patients with mesothelioma, ovarian cancer, and some squamous cell carcinomas. Through transcriptional profiling, Mesothelin was found to be elevated in the serum of 76% of patients with ovarian cancer and was also found to be informatively complementary to CA-125 in early detection of ovarian cancer (45).

Haptoglobin-α (HP-α) is a liver glycoprotein (with α-electrophoretic mobility on a gel) that binds to free hemoglobin released from red cells. Using surface enhanced laser desorption and ionization (SELDI) and mass spectrometric (MS) protein profiling, HP-α has been identified as being a potential tumor marker having a 64% sensitivity and a 90% specificity (46).

Bikunin is a glycosylated protease (glycoprotein) that inhibits tumor cell invasion and metastasis. Preoperative plasma bikunin levels have been reported to be a strong prognostic marker for ovarian cancer. A large study showed that low plasma level of bikunin were associated with late-stage disease, probable suboptimal debulking with a large residual tumor (>2 cm) outcome, low response to chemotherapy, and reduced survival time (47).

OVX1 is an epitope of a high molecular weight mucin-like glycoproteins, which can be detected by radioimmunoassay. OVX was found to be elevated in 67% of patients with ovarian cancer who were CA-125 negative (48, 49).

Other novel biomarker panels have also been investigated for early detection of ovarian cancers. Zhang and colleagues identified a panel of markers that consisted of 3 proteins, including apolipoprotein A-I (apoA-I), a truncated form of transthyretin (TTR), and a cleavage fragment of H4 (inter-α-trypsin inhibitor heavy chain) to detect early-stage ovarian cancer with a sensitivity of 83% and a specificity of 94% (50). Su and colleagues used a multiple logistic regression model (MLRM), with values for CA-125, ApoA-I, transferrin (TF), and TTR for early detection of ovarian cancer (51). This model provided a sensitivity of 89% and a specificity of 97% for detection of early-stage ovarian cancer. The sensitivity and the specificity in distinguishing normal and mucinous ovarian cancer samples were 95% and 92%, respectively. Nosov and colleagues applied this same MLRM model and marker panel to analyze serous and endometrioid histologic types of ovarian carcinomas; they showed a sensitivity of 94% and a specificity of 94% for serous ovarian carcinoma in its early stage, and a sensitivity of 98% and a specificity of 98% for endometrioid ovarian carcinoma in its early stage (52).

Visintin and colleagues proposed a panel of serum biomarkers that consisted of leptin, prolactin, osteopontin, insulin-like growth factor II (IGF-II), macrophage inhibitory factor (MIF), and CA-125 to discriminate between patients with ovarian cancer and healthy women. The panel had a sensitivity of 95% and a specificity of 99% (53). Not surprisingly, this panel provided a significant improvement over CA 125 alone. However, these studies had similar methodologic limitations of excessive numbers of tumor cases versus small numbers of matched population controls.

Still, with all this said, novel proteomics-based investigations and bioinformatics analysis provide great promise for finding ever more accurate and useable biomarkers for these gynecologic cancers.

## miRNAs

miRNAs (or miR) are a class of small (18–25 nt) non-protein-coding gene-regulatory RNA molecules that are emerging as immensely important diagnostic and potentially therapeutic tools. miRNAs play important roles in a variety of human biologic processes, including development, organogenesis, metabolism, and homeostasis. miRNAs negatively regulate mRNA translation into protein of a large number of important target genes, either by translational repression or by degradation of the messenger RNA transcript, after targeting, by sequence complementarity, the 3′-untranslated region of the mRNA.

Similar to other cancers, the initiation and development of ovarian cancer is characterized by disruption of oncogenes and tumor suppressor genes by both genetic and epigenetic mechanisms (54). It is now well known that altered or deregulated miRNA expression can also be a determinant of disease development and/or progression

in a host of pathologic conditions. Importantly, for the purposes of this review, miRNAs are functionally involved in the pathogenesis of many tumors (including our subject, ovarian cancer), in which miRNAs can have important roles as regulatory molecules, acting as oncogenes (oncomirs) or tumor suppressors. A variety of miRNA candidates are differentially or aberrantly expressed in ovarian carcinomas, or by adjoining stromal tissues, and even by other tissue in the host body in response to the tumor.

Changes in tumor miRNA expression patterns occur through a variety of mechanisms, such as genetic alterations, epigenetic regulation, or altered expression of transcription factors, which target the miRNA genes. For example, in cancer cells, transcriptional gene silencing has frequently been associated with epigenetic defects. miR-125b1 has been suggested to be an miRNA with tumor suppressor activity, and it has been shown to be deregulated in various human cancers. DNA methylation at its regulatory-region–associated CpG island can reduce miR-125b1 expression, and these effects have been observed in several gynecologic cancers, including ovarian and cervical tumors (55).

RNases are abundant in the bloodstream. Therefore, to be stabile, some secretory miRNAs are contained in apoptotic bodies, microvesicles, or bound to the RNA-binding proteins (56). However, the vast bulk of the miRNA in serum and saliva is found in tiny membrane vesicles known as exosomes (57), which are cell-derived extracellular vesicles of endosomal origin. In addition to miRNAs, exosomes can contain proteins and mRNAs, and thus exosomes have been shown to constitute a mode of intercellular communication selectively transmitting several types of information between cells. These "bioactive shuttle vesicles" are known to transfer these various molecules, including the miRNAs, to recipient cells, and to promote cell–cell communication and immunoregulatory functions (58, 59).

Cancer cells can secrete excessive amounts of exosomes as compared with normal cells (60). A new aspect of cancer research is being revealed by the emergence of these "secretory miRNA." The molecular composition and functional role of tumor cell–derived exosomes in tumorigenesis, metastasis, and response to therapy are slowly being decrypted (60). Inappropriate release of miRNAs via exosomes may cause significant alterations in biologic pathways that affect disease development. Their active secretion has functional implications, albeit, it is often still unknown whether they are tumor promoting or suppressing. Notably, the interplay via the exchange of exosomes between cancer cells and between cancer cells and the tumor stroma may promote the transfer or expression of oncogenes (e.g., β-catenin, CEA, HER2, Melan-A/Mart-1, and LMP-1) and onco-miRNAs (e.g., let7, miR1, miR15, miR16, and miR375) from one cell to another, leading to the reprogramming of the recipient cells (60).

Some miRs exert negative control over the expression of numerous oncoproteins in normal cells, and consequently, their deregulation is believed to be an important mechanism underlying cancer development and progression (61). miRNAs have distinct patterns of expression associated with specific cancer types, and once secreted by the cancer cells, they have remarkable stability in blood and other body fluids (61).

Because of the amount of signal amplification possible with nucleic acid serum markers, the identification of "miR signatures" associating cancer cell phenotypes with disease outcome and specific risk factor exposures will open new avenues for early diagnosis of cancer, as well as for the development of novel strategies for cancer prevention and therapy (61). Because these miRNA signatures can appear in the body fluids in exosomes, they can serve as relatively stable circulating diagnostic biomarkers, and have been shown to do so for ovarian cancer (62). Isolation of an exosome fraction also improves the sensitivity of miRNA amplification from human biologic fluids and reduces the probability of false negative results involving low abundance miRNAs that may be missed by using unfractionated serum or saliva (57).

Moving from merely being biomarkers for ovarian cancer to being targets for therapy, the development of strategies that might block the expression or mimic the functions of miRNAs could represent new therapeutic strategies for any of the aforementioned gynecologic disorders. Exosome vesicles can also be used as gene therapy vehicles for delivery of miRNAs and siRNA with therapeutic effects. The ability to do so has already been shown in mice (59). It thus seems that exosomal RNA has the potential to play important roles in the diagnosis, prognosis, and treatment of such diseases in the future.

Using well-characterized examples from other tumors, clinicians can begin to understand some of the functions of tumor miRs. Some miRNAs, such as let-7 in lung cancer and miRs-15/16 in leukemia, normally act as tumor suppressor genes, in these cases suppressing the expression of the oncogenes *Ras* and *BCL2*, respectively (63, 64). When they are underexpressed, tumor growth is permitted. Tumor overexpressed miRNAs, such as miR-21, and the cluster miR-17–92, can act as oncogenes (oncomirs), targeting tumor suppressors PTEN and E2F1 in solid and hematologic malignancies, respectively (65, 66).

miRNA research in the gynecologic malignancies is now progressing quite rapidly, as the miRNA signature profiles of ovarian cancer were first published in 2007 and 2008 (67–69). The use of miRNA signatures of tumor-derived serum exosomes as a diagnostic biomarker for ovarian cancer was first convincingly showed by Taylor and Gercel-Taylor (70; Table 4). The authors showed that the level of tumor-derived miRNA-containing exosomes in serum is strongly increased in women with invasive ovarian cancer as compared with women with benign ovarian tumors or healthy controls. In addition, the levels of circulating, tumor-derived exosomes increased in parallel to the stage of disease. Furthermore, they showed, by miRNA microarray profiling, that the 218 miRNAs that were identified in tumor samples were also identified in

Table 5. Differently expressed miRNAs in the serum

| | *P*-value |
|---|---|
| Overexpressed | |
| miRNAs-21 | 0.0002 |
| miRNAs-29a | 0.0003 |
| miRNAs-92 | 0.0001 |
| miRNAs-93 | 0.0003 |
| miRNAs-126 | 0.007 |
| Underexpressed | |
| miRNAs-127 | 0.0001 |
| miRNAs-155 | 0.0003 |
| miRNAs-99b | 0.0001 |

NOTE: Table modified from ref. (72).

circulating exosomes and that some miRNAs are even more overexpressed in the circulating exosomes than in the original tumor samples.

Differences in serum miRNAs between healthy controls and patients with ovarian cancer were also reported by Resnick and colleagues (71; Table 5). They were seeking an alternative or complementary diagnostic approach to TV-USG and serum CA-125 levels for women at high risk for ovarian cancer, knowing that this would be of great importance because CA-125 remains such a poor marker for early-stage disease, with a documented sensitivity of only 40%. Thus, it was hoped that miRNAs might serve as early detection biomarkers in patients with normal CA-125 levels. They identified 21 miRNAs that were differentially expressed between normal and patient sera with ovarian cancer. Analyzing these miRNAs in more detail, 5 miRNAs were found to be overexpressed and 3 miRNAs were decreased in the serum of patients with ovarian cancer, as compared with controls, establishing a possible set of miRNAs as biomarkers for ovarian cancer.

The Cancer Genome Atlas (TCGA) Network has recently catalogued the most extensive set to date of molecular aberrations in ovarian cancers. Patterns of miRNA expression in 487 high-grade serous tumors revealed multiple tumor subtypes and a set of 34 miRNAs predictive of overall patient survival (72). The miR-29 family and predicted target genes were among the most strongly anticorrelated miR: mRNA pairs, meaning the mRNA targets were suppressed when the miRs were active. In the standard test for miR functionality, overexpression of miR-29a *in vitro* repressed several anticorrelated genes (including *DNMT3A* and *DNMT3B*) and substantially decreased ovarian cancer cell viability. Mining the TCGA microarray database has also shown that the expression level of RAD51AP1 was found to be strongly anticorrelated with the expression of hsa-miR-140-3p, which was significantly downregulated in the tumor samples (73). Other pairs of potentially biologic relevance included: hsa-miR-145/E2F3, hsa-miR-139-5p/TOP2A, and hsa-miR-133a/GCLC (73).

The interplay between various families of miRs is quite complex, resulting in researchers finding "signatures" of expression in which no single component is essential, but overall patterns are consistent. For example, Bentink and colleagues (74) identified a previously undescribed patient stratification based on an "angiogenesis signature" of miRNA expression profiles. These pathways are probably determined early in tumorigenesis. Recent recognition of (HG-SOC) high grade serous ovarian cancer precursor lesions, defined as serous tubal intraepithelial carcinoma (STIC) in fimbria, provides a new venue for the study of early genetic changes in HG-SOC. Using miRNA profiling analysis, Liu and colleagues (75) found that miR-182 expression was significantly higher in STIC than in matched normal Fallopian tube. Further study revealed that miR-182 was significantly overexpressed in most HG-SOC cases. miR-182 overexpression resulted in increased tumor transformation *in vitro*, and enhanced tumor invasiveness *in vitro* and metastasis *in vivo*. Mechanistically, they showed that the oncogenic properties of miR-182 in ovarian cancer were mediated in part by its impaired repair of DNA double-strand breaks and negative regulation of breast cancer 1 (BRCA1) and metastasis suppressor 1 (MTSS1) expression, as well as its positive regulation of the oncogene high-mobility group AT-hook 2 (HMGA2).

Chang and colleagues (76) have suggested that miR-148b may be one of the dysregulated miRs involved in the early stage of ovarian carcinogenesis. They found that miR-148b was overexpressed in 92.21% (71/77) of the ovarian cancer samples they examined, and the overexpression was not associated with any of the clinicopathologic features of patients with ovarian cancer (meaning it correlated with the causation and not the symptoms of the disease).

The human kallikreins are a cluster of 15 kallikrein-related peptidases (KLK). Evidence shows the involvement of KLKs in a wide range of pathologic processes and their potential contribution to cancer. Recently, epigenetic changes (including methylation and miRNA regulation) were shown to control KLK expression. Target prediction showed that KLK mRNAs are potential targets of miRNAs that are dysregulated in tumors, including ovarian cancers, with downstream effect on tumor proliferation (77).

Malignant ovarian disease is characterized by high rates of mortality arising from high rates of recurrent chemoresistant disease due to the chemoresistant properties of cancer stem cells (CSC). Microarray analysis showed a 90% difference between gene expression events involved in early regulation of differentiation in murine EC (mEC) and embryonic stem cells (41). Gene list comparisons have identified a signature set of genes for 'cancer stemness' in data from primary versus recurrent tumors, a subset of which are known to be p53–p21 regulators. Gallagher et al. (78) have proposed that this tumor signature of miRNA expression may, at least partially, differentially regulate the p53–p21 mechanism in

ovarian disease. Targeting CSCs within ovarian cancer via miR expression targeting represents another potential therapeutic avenue.

In ovarian cancer, unique $CD44^+/CD117^+$ stem cells, also known as cancer-initiating cells (CIC), are highly proliferative, have a low degree of differentiation, and are resistant to chemotherapeutics. Therefore, the $CD44^+/CD117^+$ subpopulation is thought to be an important target for novel therapeutic strategies. $CD44^+/CD117^+$ ovarian CICs were enriched from human primary ovarian tumor tissues and studied for miRNA expression and responses to miRs. When miR-199a was cloned and transfected into ovarian CICs, it significantly increased the chemosensitivity of the ovarian CICs to cisplatin, paclitaxel, and Adriamycin, and reduced mRNA expression of the multidrug resistance gene *ABCG2* as compared with miR-199a mutant–transfected and -untransfected cells (79). The expression of "stemness markers" was also significantly reduced. Furthermore, xenograft experiments confirmed that miR-199a suppressed the growth of xenograft tumors formed by ovarian CICs *in vivo*. Thus, expression of an endogenous mature miR-199a may prevent tumorigenesis in human ovarian cancer, via regulating expression of its target gene, *CD44*.

Mesothelin, the aforementioned differentiation antigen present in a series of malignancies, such as ovarian, mesothelioma, lung, and pancreatic cancer, has been studied as a marker for diagnosis and a target for immunotherapy. Wang and colleagues (80) have been evaluating the effects of direct targeting of mesothelin on the viability of cancer cells as the first step toward developing a novel therapeutic strategy. They have shown that the gene-specific silencing for mesothelin by distinct methods (siRNA and miRNA) decreased viability of ovarian cancer Skov3 and Ovcar-5 cell lines. In addition, the invasiveness of these cancer cells *in vivo* was also significantly decreased upon such treatment. Mesothelin-silencing revealed a significant decrease in phospho-ERK1 and PI3K/AKT activity. The molecular mechanism of reduced invasiveness was connected to the reduced expression of β-catenin, an important marker of epithelial–mesenchymal transition (EMT). Ero1, a protein involved in clearing unfolded proteins and a member of the ER stress (endoplasmic reticulum-stress) pathway, was also markedly reduced (80).

Tiam1 has been implicated in the aggressive invasive phenotype of ovarian cancer, as Tiam1 expression was remarkably increased in both primary and metastatic ovarian cancer tissues. Li and colleagues (81) showed that miR-22, miR-183, and miR-31 expression had negative regulatory effects on Tiam1 expression and that downregulation of Tiam1 in SKOV-3ip and HO-8910PM ovarian cancer cells lead to reduced cell migration and invasion and to growth inhibition, without significantly affecting cell apoptosis, suggesting that the differential expression profiles of these miRs may contribute to the dysregulation of Tiam1 abundance, which contributes to the invasive, migratory, and viability properties of ovarian cancer cells.

## miRNA in Prognosis

Recently, miR-100 was reported to be significantly downregulated in human ovarian carcinoma; however, the clinical significance and functional roles of miR-100 expression in human EOC were unclear. Peng and colleagues (82) now report that low miR-100 expression was found to be closely correlated with advanced Federation Internationale des Gynaecologistes et Obstetristes (FIGO) stage, higher serum CA-125 expression levels, and lymph node involvement. Also, low miR-100 expression is correlated with shorter overall survival of patients with EOC, and multivariate analysis showed that the status of miR-100 expression was an independent predictor of overall survival in EOC. In addition, they show that miR-100 could affect the growth of EOC cells by posttranscriptionally regulating polo-like kinase 1 (PLK1) expression. Together, these results suggest that low miR-100 expression may be an independent poor prognostic factor and miR-100 can function as a tumor suppressor by targeting PLK1 in human EOCs.

Bagnoli and colleagues (83) delineated a miRNA signature associated with early relapse in advanced-stage patients with EOC. Thirty-two differentially expressed miRNAs in early versus late relapsing patients were identified; 8 of these, belonging to a cluster located on chrXq27.3, were downmodulated in early relapsing patients. Forced expression of the chrXq27.3-cluster selected miRNAs in human EOC cellular models was associated to reduction of cell proliferation and increased sensitivity to cisplatin.

## Drug Resistance

miR-93 is significantly upregulated in cisplatin-resistant ovarian cancer cells and inversely correlates with PTEN expression in cisplatin-resistant and -sensitive human ovarian cancer tissues (84). They used *in vitro* assays to show that overexpression and knockdown of miR-93 regulates apoptotic activity, and thereby cisplatin chemosensitivity, in ovarian cells. Furthermore, they found that miR-93 could directly target PTEN, and participated in the regulation of the AKT signaling pathway.

The miR-34 family has a strong role in regulating the genotoxic-response p53 pathway in ovarian cancer. Zhang and colleagues (85) have shown that the miR-449a, miR-449b, and miR-192 family of miRNAs may play the same role. They have shown that the expressions of miR-449a/b, miR-34b, and miR-34c were 19- to 21-fold elevated after p53 activation by genotoxic agent. Ectopic expression of miR-449b, as well as miR-34c, resulted in cell-cycle arrest in SKOV3.ipl cells. Thus, as tumor-suppressor miRNAs, miR-449a/b, miR-34b, and miR-34c cooperate and play important roles in p53 pathway. Their inactivation may contribute to the carcinogenesis and progression of serous ovarian carcinomas.

## Conclusions and Future Directions

For gynecologic cancers, only a small handful of tumor-associated antigens, such as SCC and CA-125, have been routinely used as tumor markers. Some markers are useful not only as a diagnostic tool but also as a predictive marker for the prognosis and clinical course after treatment. Some newer serum markers being recently investigated seem to be clinically useful, such as HE4 for endometrial and ovarian cancers. The future of tumor marker research is being rapidly expanded because of the recent technologic advances in genomics and proteomics. While a large amount of information has been gained about the roles and possible therapeutic use of miRNAs in ovarian carcinoma, much remains to be done. In particular, more thorough miR expression profiling will be necessary to understand the intricacies of their expression in ovarian carcinoma of various grades, stages, or drug resistance status. The next step, the identification of relevant therapeutic miRNA targets, will likely be a tedious task, complicated by the fact that miRs can have multiple functional targets and that these targets may be dependent on several factors, including the expression of other miRs. Once relevant miRs and their functional targets are identified, the investigation of possible clinical use for these molecules will represent the next frontier in cancer research, and may, ultimately, lead to novel strategies for ovarian cancer detection and therapy.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Authors' Contributions

**Conception and design:** E. Kobayashi, Y. Ueda, T. Kimura, M. Fujita, T. Enomoto

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**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** E. Kobayashi, S. Matsuzaki, T. Yokoyama, M. Fujita

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** E. Kobayashi, Y. Ueda, S. Matsuzaki, T. Yokoyama, M. Fujita

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Received June 4, 2012; revised July 19, 2012; accepted August 14, 2012; published OnlineFirst September 7, 2012.

## References


1. World Health Organization [Internet]. GLOBOCAN 2008. [updated 2011 Dec; cited 2012 Feb 23]. Available from: http://globocan.iarc.fr/factsheets/populations/factsheet.asp?uno=900.
2. Scully RE, Young RH, Clement PB. Tumors of the ovary, maldeveloped gonads, fallopian tube, and broad ligament. Atlas of Tumor Pathology, AFIP Washington, DC: 1998.
3. Berek JS, editor. Novak's gynecology. 13th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002.
4. Ueda Y, Enomoto T, Kimura T, Miyatake T, Yoshino K, Fujita M, et al. Serum biomarkers for early detection of gynecologic cancers. Cancers 2010;2:1312–27.
5. Narimatsu H, Sawaki H, Kuno A, Kaji H, Ito H, Ikehara Y. A strategy for discovery of cancer glyco-biomarkers in serum using newly developed technologies for glycoproteomics. FEBS J 2010;277:95–105.
6. Clarke-Pearson DL. Clinical practice. Screening for ovarian cancer. N Engl J Med 2009;361:170–7.
7. Kupesic S, Plavsic BM. Early ovarian cancer: 3-D power Doppler. Abdom Imaging 2006;31:613–9.
8. Lerner JP, Timor-Tritsch IE, Federman A, Abramovich G. Transvaginal ultrasonographic characterization of ovarian masses with an improved, weighted scoring system. Am J Obstet Gynecol 1994; 170:81–5.
9. Bast RC Jr. Status of tumor markers in ovarian cancer screening. J Clin Oncol 2003;21:200s–5s.
10. Jacobs I, Bast RC Jr. The CA 125 tumour-associated antigen: a review of the literature. Hum Reprod 1989;4:1–12.
11. Nossov V, Amneus M, Su F, Lang J, Janco JM, Reddy ST, et al. The early detection of ovarian cancer: from traditional methods to proteomics. Can we really do better than serum CA-125? Am J Obstet Gynecol 2008;199:215–23.
12. Gadducci A, Cosio S, Carpi A, Nicolini A, Genazzani AR. Serum tumor markers in the management of ovarian, endometrial and cervical cancer. Biomed Pharmacother 2004;58:24–38.
13. Buys SS, Partridge E, Black A, Johnson CC, Lamerato L, Isaacs C, et al. Effect of screening on ovarian cancer mortality: the prostate, lung, colorectal and ovarian (PLCO) cancer screening randomized controlled trial. JAMA 2011;305:2295–303.
14. Kobayashi H, Yamada Y, Sado T, Sakata M, Yoshida S, Kawaguchi R, et al. A randomized study of screening for ovarian cancer: a multicenter study in Japan. Int J Gynecol Cancer 2008;18:414–20.
15. Menon U, Gentry-Maharaj A, Hallett R, Ryan A, Burnell M, Sharma A, et al. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). Lancet Oncol 2009; 10:327–40.
16. van Nagell JR Jr, Miller RW, DeSimone CP, Ueland FR, Podzielinski I, Goodrich ST, et al. Long-term survival of women with epithelial ovarian cancer detected by ultrasonographic screening. Obstet Gynecol 2011;118:1212–21.
17. Yedema CA, Kenemans P, Wobbes T, Thomas CM, Bon GG, Mulder C, et al. Use of serum tumor markers in the differential diagnosis between ovarian and colorectal adenocarcinomas. Tumour Biol 1992;13:18–26.
18. Sørensen SS, Mosgaard BJ. Combination of cancer antigen 125 and carcinoembryonic antigen can improve ovarian cancer diagnosis. Dan Med Bull 2011;58:A4331.
19. Makar AP, Kristensen GB, Kaern J, Børmer OP, Abeler VM, Tropé CG. Prognostic value of pre- and postoperative serum CA 125 levels in ovarian cancer: new aspects and multivariate analysis. Obstet Gynecol 1992;79:1002–10.
20. Scholl SM, Bascou CH, Mosseri V, Olivares R, Magdelenat H, Dorval T, et al. Circulating levels of colony-stimulating factor 1 as a prognostic indicator in 82 patients with epithelial ovarian cancer. Br J Cancer 1994;69:342–6.
21. Venesmaa P, Lehtovirta P, Stenman UH, Leminen A, Forss M, Ylikorkala O. Tumour-associated trypsin inhibitor (TATI): comparison with CA125 as a preoperative prognostic indicator in advanced ovarian cancer. Br J Cancer 1994;70:1188–90.
22. Nagele F, Petru E, Medl M, Kainz C, Graf AH, Sevelda P. Preoperative CA 125: an independent prognostic factor in patients with stage I epithelial ovarian cancer. Obstet Gynecol 1995;86:259–64.
23. Parker D, Bradley C, Bogle SM, Lay J, Masood M, Hancock AK, et al. Serum albumin and CA125 are powerful predictors of survival in epithelial ovarian cancer. Br J Obstet Gynaecol 1994;101:888–93.

24. Rustin GJ, Nelstrop AE, Tuxen MK, Lambert HE. Defining progression of ovarian carcinoma during follow-up according to CA 125: a North Thames Ovary Group Study. Ann Oncol 1996;7:361–4.
25. Rustin GJ, Marples M, Nelstrop AE, Mahmoudi M, Meyer T. Use of CA-125 to define progression of ovarian cancer in patients with persistently elevated levels. J Clin Oncol 2001;19:4054–7.
26. Rustin GJ, van der Burg ME, Griffin CL, Guthrie D, Lamont A, Jayson GC, et al. Early versus delayed treatment of relapsed ovarian cancer (MRC OV05/EORTC 55955): a randomised trial. Lancet 2010;376: 1155–63.
27. Gadducci A, Ferdeghini M, Prontera C, Moretti L, Mariani G, Bianchi R, et al. The concomitant determination of different tumor markers in patients with epithelial ovarian cancer and benign ovarian masses: relevance for differential diagnosis. Gynecol Oncol 1992;44:147–54.
28. Koelma IA, Nap M, Rodenburg CJ, Fleuren GJ. The value of tumour marker CA 125 in surgical pathology. Histopathology 1987;11:287–94.
29. Kudoh K, Kikuchi Y, Kita T, Tode T, Takano M, Hirata J, et al. Preoperative determination of several serum tumor markers in patients with primary epithelial ovarian carcinoma. Gynecol Obstet Invest 1999;47: 52–7.
30. Negishi Y, Furukawa T, Oka T, Sakamoto M, Hirata T, Okabe K, et al. Clinical use of CA 125 and its combination assay with other tumor marker in patients with ovarian carcinoma. Gynecol Obstet Invest 1987;23:200–7.
31. Castelli M, Battaglia F, Scambia G, Panici PB, Ferrandina G, Mileo AM, et al. Immunosuppressive acidic protein and CA 125 levels in patients with ovarian cancer. Oncology 1991;48:13–7.
32. Castelli M, Romano P, Atlante G, Pozzi M, Ferrini U. Immunosuppressive acidic protein (IAP) and CA 125 assays in detection of human ovarian cancer: preliminary results. Int J Biol Markers 1987;2:187–90.
33. Fioretti P, Gadducci A, Ferdeghini M, Prontera C, Malagnino G, Facchini V, et al. The concomitant determination of different serum tumor markers in epithelial ovarian cancer: relevance for monitoring the response to chemotherapy and follow-up of patients. Gynecol Oncol 1992;44:155–60.
34. Negishi Y, Iwabuchi H, Sakunaga H, Sakamoto M, Okabe K, Sato H, et al. Serum and tissue measurements of CA72-4 in ovarian cancer patients. Gynecol Oncol 1993;48:148–54.
35. Sawada M, Okudaira Y, Matsui Y, Shimizu Y. Immunosuppressive acidic protein in patients with ovarian cancer. Cancer 1983;52:2081–5.
36. Yedema C, Massuger L, Hilgers J, Servaas J, Poels L, Thomas C, et al. Pre-operative discrimination between benign and malignant ovarian tumors using a combination of CA125 and CA15.3 serum assays. Int J Cancer Suppl 1988;3:61–7.
37. Onsrud M. Tumour markers in gynaecologic oncology. Scand J Clin Lab Invest Suppl 1991;206:60–70.
38. Roman LD, Muderspach LI, Burnett AF, Morrow CP. Carcinoembryonic antigen in women with isolated pelvic masses. Clinical utility? J Reprod Med 1998;43:403–7.
39. Li J, Dowdy S, Tipton T, Podratz K, Lu WG, Xie X, et al. HE4 as a biomarker for ovarian and endometrial cancer management. Expert Rev Mol Diagn 2009;9:555–66.
40. Rosen DG, Wang L, Atkinson JN, Yu Y, Lu KH, Diamandis EP, et al. Potential markers that complement expression of CA125 in epithelial ovarian cancer. Gynecol Oncol 2005;99:267–77.
41. Moore RG, Brown AK, Miller MC, Skates S, Allard WJ, Verch T, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. Gynecol Oncol 2008; 108:402–8.
42. Urban N. Specific keynote: ovarian cancer risk assessment and the potential for early detection. Gynecol Oncol 2003;88:S75–9;discussion S80–3.
43. Xu Y, Shen Z, Wiper DW, Wu M, Morton RE, Elson P, et al. Lysophosphatidic acid as a potential biomarker for ovarian and other gynecologic cancers. JAMA 1998;280:719–23.
44. Konno R, Takano T, Sato S, Yajima A. Serum soluble fas level as a prognostic factor in patients with gynecological malignancies. Clin Cancer Res 2000;6:3576–80.
45. McIntosh MW, Drescher C, Karlan B, Scholler N, Urban N, Hellstrom KE, et al. Combining CA 125 and SMR serum markers for diagnosis and early detection of ovarian carcinoma. Gynecol Oncol 2004;95: 9–15.
46. Ye B, Cramer DW, Skates SJ, Gygi SP, Pratomo V, Fu L, et al. Haptoglobin-alpha subunit as potential serum biomarker in ovarian cancer: identification and characterization using proteomic profiling and mass spectrometry. Clin Cancer Res 2003;9:2904–11.
47. Matsuzaki H, Kobayashi H, Yagyu T, Wakahara K, Kondo T, Kurita N, et al. Plasma bikunin as a favorable prognostic factor in ovarian cancer. J Clin Oncol 2005;23:1463–72.
48. Xu FJ, Yu YH, Li BY, Moradi M, Elg S, Lane C, et al. Development of two new monoclonal antibodies reactive to a surface antigen present on human ovarian epithelial cancer cells. Cancer Res 1991;51:4012–9.
49. Xu FJ, Yu YH, Daly L, DeSombre K, Anselmino L, Hass GM, et al. OVX1 radioimmunoassay complements CA-125 for predicting the presence of residual ovarian carcinoma at second-look surgical surveillance procedures. J Clin Oncol 1993;11:1506–10.
50. Zhang Z, Bast RC Jr, Yu Y, Li J, Sokoll LJ, Rai AJ, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. Cancer Res 2004;64: 5882–90.
51. Su F, Lang J, Kumar A, Ng C, Hsieh B, Suchard MA, et al. Validation of candidate serum ovarian cancer biomarkers for early detection. Biomark Insights 2007;2:369–75.
52. Nosov V, Su F, Amneus M, Birrer M, Robins T, Kotlerman J, et al. Validation of serum biomarkers for detection of early-stage ovarian cancer. Am J Obstet Gynecol 2009;200:639.e1–5.
53. Visintin I, Feng Z, Longton G, Ward DC, Alvero AB, Lai Y, et al. Diagnostic markers for early detection of ovarian cancer. Clin Cancer Res 2008;14:1065–72.
54. Cheh H, Hardy TM, Tollefsbol TO. Epigenomics of ovarian cancer and its chemoprevention. Front Genet 2011;2:67.
55. Soto-Reyes E, González-Barrios R, Cisneros-Soberanis F, Herrera-Goepfert R, Pérez V, Cantú D, et al. Disruption of CTCF at the miR-125b1 locus in gynecological cancers. BMC Cancer 2012; 12:40.
56. Kosaka N, Ochiya T. Unraveling the mystery of cancer by secretory microRNA: horizontal microRNA transfer between living cells. Front Genet 2011;2:97.
57. Gallo A, Tandon M, Alevizos I, Illei GG. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. PLoS ONE 2012;7:e30679.
58. Hu G, Drescher KM, Chen XM. Exosomal miRNAs: biological properties and therapeutic potential. Front Genet 2012;3:56.
59. Lässer C. Exosomal RNA as biomarkers and the therapeutic potential of exosome vectors. Expert Opin Biol Ther 2012;12 Suppl 1:S189–97.
60. Kharaziha P, Ceder S, Li Q, Panaretakis T. Tumor cell-derived exosomes: a message in a bottle. Biochim Biophys Acta 2012;1826: 103–11.
61. Krutovskikh VA, Herceg Z. Oncogenic microRNAs (OncomiRs) as a new class of cancer biomarkers. BioEssays 2010;32:894–904.
62. Kuhlmann JD, Rasch J, Wimberger P, Kasimir-Bauer S. microRNA and the pathogenesis of ovarian cancer—a new horizon for molecular diagnostics and treatment? Clin Chem Lab Med 2012;50: 601–15.
63. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 2005;102:13944–9.
64. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, et al. RAS is regulated by the let-7 microRNA family. Cell 2005; 120:635–47.
65. Mendell JT. miRiad roles for the miR-17-92 cluster in development and disease. Cell 2008;133:217–22.
66. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007;133: 647–58.
67. Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, et al. MicroRNA signatures in human ovarian cancer. Cancer Res 2007;67: 8699–707.

68. Nam EJ, Yoon H, Kim SW, Kim H, Kim YT, Kim JH, et al. MicroRNA expression profiles in serous ovarian carcinoma. Clin Cancer Res 2008;14:2690–5.
69. Zhang L, Volinia S, Bonome T, Calin GA, Greshock J, Yang N, et al. Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer. Proc Natl Acad Sci U S A 2008;105:7004–9.
70. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol 2008;110:13–21.
71. Resnick KE, Alder H, Hagan JP, Richardson DL, Croce CM, Cohn DE. The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol 2009;112:55–9.
72. Creighton CJ, Hernandez-Herrera A, Jacobsen A, Levine DA, Mankoo P, Schultz N, et al. Integrated analyses of microRNAs demonstrate their widespread influence on gene expression in high-grade serous ovarian carcinoma. PLoS ONE 2012;7:e34546.
73. Miles GD, Seiler M, Rodriguez L, Rajagopal G, Bhanot G. Identifying microRNA/mRNA dysregulations in ovarian cancer. BMC Res Notes 2012;5:164.
74. Bentink S, Haibe-Kains B, Risch T, Fan JB, Hirsch MS, Holton K, et al. Angiogenic mRNA and microRNA gene expression signature predicts a novel subtype of serous ovarian cancer. PLoS ONE 2012;7:e30269.
75. Liu Z, Liu J, Segura MF, Shao C, Lee P, Gong Y, et al. MiR-182 overexpression in tumourigenesis of high-grade serous ovarian carcinoma. J Pathol 2012 Feb 9. [Epub ahead of print].
76. Chang H, Zhou X, Wang ZN, Song YX, Zhao F, Gao P, et al. Increased expression of miR-148b in ovarian carcinoma and its clinical significance. Mol Med Report 2012;5:1277–80.
77. Pasic MD, Olkhov E, Bapat B, Yousef GM. Epigenetic regulation of kallikrein-related peptidases: there is a whole new world out there. Biol Chem 2012;393:319–30.
78. Gallagher MF, Heffron CC, Laios A, O'Toole SA, Ffrench B, Smyth PC, et al. Suppression of cancer stemness p21-regulating mRNA and microRNA signatures in recurrent ovarian cancer patient samples. J Ovarian Res 2012;5:2.
79. Cheng W, Liu T, Wan X, Gao Y, Wang H. MicroRNA-199a targets CD44 to suppress the tumorigenicity and multidrug resistance of ovarian cancer-initiating cells. FEBS J 2012;279:2047–59.
80. Wang K, Bodempudi V, Liu Z, Borrego-Diaz E, Yamoutpoor F, Meyer A, et al. Inhibition of mesothelin as a novel strategy for targeting cancer cells. PLoS ONE 2012;7:e33214.
81. Li J, Liang S, Jin H, Xu C, Ma D, Lu X. Tiam1, negatively regulated by miR-22, miR-183 and miR-31, is involved in migration, invasion and viability of ovarian cancer cells. Oncol Rep 2012;27:1835–42.
82. Peng DX, Luo M, Qiu LW, He YL, Wang XF. Prognostic implications of microRNA-100 and its functional roles in human epithelial ovarian cancer. Oncol Rep 2012;27:1238–44.
83. Bagnoli M, De Cecco L, Granata A, Nicoletti R, Marchesi E, Alberti P, et al. Identification of a chrXq27.3 microRNA cluster associated with early relapse in advanced stage ovarian cancer patients. Oncotarget 2011;2:1265–78.
84. Fu X, Tian J, Zhang L, Chen Y, Hao Q. Involvement of microRNA-93, a new regulator of PTEN/Akt signaling pathway, in regulation of chemotherapeutic drug cisplatin chemosensitivity in ovarian cancer cells. FEBS Lett 2012;586:1279–86.
85. Zhang Q, He XJ, Ma LP, Li N, Yang J, Cheng YX, et al. [Expression and significance of microRNAs in the p53 pathway in ovarian cancer cells and serous ovarian cancer tissues]. Zhonghua Zhong Liu Za Zhi 2011;33:885–90. [Article in Chinese].

# Prediction of Progression-Free Survival and Response to Paclitaxel Plus Carboplatin in Patients With Recurrent or Advanced Cervical Cancer

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**Objective:** The aim of this study was to identify predictors of the response to paclitaxel-carboplatin chemotherapy (TC) in recurrent or patients with advanced cervical cancer.

**Methods:** The records of 61 consecutive women with recurrent or advanced cervical cancer who were treated with TC were retrospectively reviewed. Data regarding their primary disease, follow-up, recurrence, and the activity and toxicity of TC were collected. Multivariate analysis was performed using the Cox proportional hazards regression model to identify predictors of the response to TC. Survival was calculated using the Kaplan-Meier method and compared using the log-rank test.

**Results:** Overall, TC was well tolerated and displayed a response rate of 60.7% (19 complete response and 18 partial response). The median progression-free survival was 14 months for all patients and 20 months for the responders. Grade 3 to grade 4 toxicities were observed in 51 patients (83.6%). Multivariate analysis revealed that performance status, symptom status, and prior chemotherapy were independent prognostic predictors of a poor response. Patient survival was inversely correlated with the number of these prognostic factors. When the patients were divided into 2 prognostic groups (low risk: patients with no or one poor prognostic factor; and high-risk: patients with 2 or more poor prognostic factors), the patients in the high-risk group had a significantly shorter progression-free survival than those in the low-risk group (4 vs 16 months, log-rank; $P < 0.0001$).

**Conclusions:** The combination of paclitaxel and carboplatin is effective in patients with recurrent or advanced cervical cancer. Our prognostic model composed of 3 clinical variables might enable physicians to identify patients who would not derive clinical benefit from TC and offer them the opportunity to receive other types of treatment.

**Key Words:** Paclitaxel-carboplatin, Prognostic factors, Recurrent cervical cancer, Survival

Received August 9, 2011, and in revised form November 5, 2011.
Accepted for publication December 18, 2011.

(*Int J Gynecol Cancer* 2012;22: 623–629)

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ISSN: 1048-891X
DOI: 10.1097/IGC.0b013e3182473277

This work was supported in part by Grant-in-aid for General Scientific Research No 23592446 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.
The authors declare that there are no conflicts of interest.
Supplemental digital content is available for this article. Direct URL citation appears in the printed text and is provided in the HTML and PDF versions of this article on the journal's Web site (www.ijgc.net).

Recurrent cervical cancer is a difficult clinical problem because of its poor outcome, with a reported 1-year survival rate of between 15% and 20%.[1] Chemotherapy is the main treatment for this patient group, excluding patients in whom long-term survival can be achieved with surgery or radiotherapy. Despite a strong effort being made to improve survival in the past few decades, chemotherapy still plays a palliative role in this subset of patients.[2]

Historically, cisplatin has been the most active single agent for recurrent cervical cancer, with response rates varying from 17% to 38%.[2] The addition of paclitaxel[3] or ifosfamide[4] to single-agent cisplatin has improved outcomes in the response rate and progression-free survival (PFS) in patients with advanced or recurrent cervical cancer. In a subsequent phase 3 study (protocol GOG 179), it was demonstrated that the addition of topotecan to cisplatin resulted in improved overall survival.[5] Based on these phase 3 clinical trials, cisplatin-containing combination chemotherapy has become the standard treatment for recurrent cervical cancer.[2]

In a Gynecologic Oncology Group (GOG) phase 3 trial (protocol GOG 204), the activity of 4 different cisplatin-based doublets containing paclitaxel, topotecan, vinorelbine, or gemcitabine were evaluated.[6] In this 4-arm trial, none of the experimental regimens were found to be superior to the control arm of cisplatin plus paclitaxel with regard to the response rate, survival, or treatment-related toxicities. According to the results of this trial, paclitaxel-cisplatin has become the most widely used regimen for patients with recurrent cervical cancer.

Considering renal, gastrointestinal, and neurological toxicities, as well as the necessity of hospitalization associated with the paclitaxel-cisplatin regimen, the use of paclitaxel-carboplatin instead of paclitaxel-cisplatin might be beneficial for patients with recurrent cervical cancer. Different from cisplatin, the dose of carboplatin can be tailored according to the patient's renal function.[7] In addition, carboplatin has a more favorable nonhematologic toxicity profile.[8] Moreover, paclitaxel-carboplatin can be administered in the outpatient setting.

The use of paclitaxel-carboplatin chemotherapy (TC) in patients with cervical cancer was first reported in 1996 by Termrungruanglert et al.[9] So far, 8 retrospective studies have evaluated the clinical activity of TC. These studies included a total of 192 patients and indicated an overall response rate of 65.1%.[10–17]

Owing to the short life expectancy of patients treated with platinum-based combination chemotherapy,[6] in addition to using a less toxic regimen, it is also important to identify the independent predictors of the response to salvage chemotherapy. Identifying patients who would not derive clinical benefit from the current treatment modalities would allow physicians to offer them the opportunity to receive other types of treatment including best supportive care.

In the current study, we retrospectively investigated the predictors of the response to paclitaxel-carboplatin TC among patients with recurrent or advanced cervical cancer and then used these prognostic factors to establish an appropriate predictive model.

## MATERIALS AND METHODS

### Patients

Permission to proceed with the data acquisition and analysis was obtained from Osaka University Hospital's institutional review board. Sixty-one patients who were treated with carboplatin and paclitaxel for recurrent or advanced cervical cancer at Osaka University Hospital from 2005 to 2010 were identified through the institutional pharmacy database and retrospectively reviewed. Patients with small cell carcinoma were excluded. Clinical data on the following characteristics were collected for all patients: initial disease stage, cell type, primary treatment, site of recurrent disease, disease-free interval (DFI), the presence or absence of symptoms, response, and PFS. Disease-free interval was defined as the time from the primary diagnosis to the detection of recurrence or disease progression. Progression-free survival was measured from the start of TC to disease progression. At the time of this study, of the 61 patients, 33 (54.1%) are still alive, and 20 patients (32.8%) had not displayed disease progression after a median follow-up of 26 months. Therefore, we did not include overall survival as an end point in the current study. Of a total of 61 patients, 35 were included in previous clinical studies.[15,16]

### Treatment Protocol

Paclitaxel-carboplatin was administered on a monthly basis (monthly TC) in 47 patients: carboplatin (area under the curve, 5) and 175-mg/m$^2$ paclitaxel given as a 3-hour intravenous infusion every 28 days. Fourteen patients were treated on a weekly basis (weekly TC): 80-mg/m$^2$ paclitaxel and carboplatin (AUC, 2) on days 1, 8, and 15 of each 28-day cycle. A median of 4 courses of TC was administered (range, 2–9 courses).

### Response and Toxicity Evaluations

The patients' response was considered to be evaluable for if they had received at least 2 cycles of chemotherapy or had demonstrated significant disease progression after one course of treatment. The response to treatment was assessed according to the Response Evaluation Criteria in Solid Tumors after every 3 cycles of each regimen. A complete response (CR) was defined as the disappearance of all target and nontarget lesions and the absence of new lesions on 2 consecutive assessments performed at least 4 weeks apart. A partial response (PR) was defined as at least a 30% decrease in the sum of the longest dimensions of the target lesions on consecutive 2 assessments performed at least 4 weeks apart. Progressive disease was defined as a 20% increase in the sum of the longest dimensions of the target lesions or the development of new lesions. Stable disease was defined as when none of the above applied. Treatment-related toxicity was graded according to the NCI Common Terminology Criteria for Adverse Events, version 3.0.

### Statistical Analysis

The response rate was compared with the patients' characteristics using the $\chi^2$ test and multivariate logistic regression.

The PFS curve was estimated using the Kaplan-Meier method and compared using the log-rank test. Furthermore, PFS was analyzed using the multivariate Cox proportional hazards regression. $P < 0.05$ was considered statistically significant. All analyses were performed using JMP® software, version 8.0.2 (SAS Institute, Cary, NC).

## RESULTS

### Patients

Sixty-one consecutive women with recurrent or advanced cervical cancer who were treated with TC were identified. The clinicopathologic characteristics of these patients are shown in Table 1. The median age of the patients at the time of their TC treatment was 56 years. Of these patients, 51 had recurrent disease and 10 had stage IVB disease. Forty-five women (74%) had squamous cell carcinoma, 16 women (26%) displayed nonsquamous histology. Thirty-one patients (51%) had performance statuses of 1 to 2. Eleven patients (18%) had been treated with platinum-based combination chemotherapy. Twenty patients (33%) received radiosensitizing chemotherapy. Twenty-four patients (39%) displayed symptomatic disease, and 37 (61%) had asymptomatic disease. Twenty-two (36.1%) displayed pelvic recurrence. The mean DFI of the patients was 15 months.

### Treatment Outcomes

Generally, paclitaxel-carboplatin was well tolerated. There were no treatment-related deaths. Grade 3 or 4 toxicity was observed in 51 patients (83.6%) as their worst toxicity. Of these, 47 patients had neutropenia, and 4 had a combination of neutropenia and thrombocytopenia. There were no nonhematologic toxicities. The overall response rate was 60.7% (37/61). Nineteen patients achieved a CR, 18 patients achieved a PR, and 12 patients had stable disease. The median PFS was 14 months in the entire group and 20 months in the responders (PR + CR). Then, the response rate was classified according to the patients' characteristics (Table 2). As shown, performance status (2) and prior chemotherapy were found to be significant predictors of a poor response. The patients with asymptomatic disease showed a higher response rate than those with symptomatic disease; however, the difference did not reach statistical significance ($P = 0.056$). To identify the independent prognostic predictors of the response to TC, we next performed multivariate analysis (Table 3). As shown, Cox multivariate analysis identified 3 independent prognostic factors that were predictive of a poor response: performance status ($P = 0.025$), prior chemotherapy ($P < 0.001$), and symptom status ($P = 0.006$). The response rate was inversely correlated with the number of poor prognostic factors the patients displayed. The response rates in patients with 0, 1, 2, and 3 poor prognostic factors were 92%, 50%, 13%, and 0%, respectively (Fig. 1). To establish a model capable of predicting life expectancy in this patient population, PFS was assessed according to the number of the aforementioned poor prognostic factors. As shown in Figure 2, when the patients were divided into 2 prognostic groups (the low-risk group and high-risk group), the patients in the high-risk group were found to have significantly shorter PFS (median, 4 months) than those in the low-risk group (median, 16 months; log-rank; $P < 0.0001$).

**TABLE 1.** Patients' characteristics

| | No. Patients | Percent |
|---|---|---|
| Age, yrs | | |
| Median (range): 56 (28.0–79.0) | | |
| <60 | 36 | 59.0 |
| ≥60 | 25 | 41.0 |
| Stage | | |
| Advanced (IVB) | 10 | 16.4 |
| Recurrent | 51 | 83.6 |
| Histology | | |
| SCC | 45 | 73.8 |
| A or AS | 16 | 26.2 |
| Performance status | | |
| 0 | 30 | 49.2 |
| 1 | 19 | 31.1 |
| 2 | 12 | 19.7 |
| Prior chemotherapy | | |
| Yes | 11 | 18.0 |
| No | 50 | 82.0 |
| Prior radiosensitizer | | |
| Yes | 20 | 32.8 |
| No | 41 | 67.2 |
| Symptom status | | |
| Yes | 24 | 39.3 |
| No | 37 | 60.7 |
| Site of disease | | |
| Pelvic | 22 | 36.1 |
| Distant/pelvic and distant | 39 | 63.9 |
| DFI, mos | | |
| Mean | 15 | |
| <6 | 36 | 59.0 |
| ≥6 | 25 | 41.0 |

Prior chemotherapy is chemotherapy excluding radiosensitizing agent.

A, adenocarcinoma; AS, adenosquamous carcinoma; SCC, squamous cell carcinoma.

## DISCUSSION

In patients with ovarian cancer, the combination of paclitaxel and carboplatin (TC) was demonstrated to be equally efficacious to paclitaxel-cisplatin (TP) and to display less toxicity.[8] However, experience with the use of this combination in uterine cervical cancers is limited. It is known that when combined with cisplatin, paclitaxel should be given as a 24-hour infusion to reduce neurologic toxicity.[3] However, when combined with carboplatin, paclitaxel can

**TABLE 2.** Response rate according to patients' characteristics

| | No. Patients | Patients With Response | Response Rate | *P* |
|---|---|---|---|---|
| Age, yrs | | | | |
| <60 | 36 | 20 | 56% | 0.328 |
| ≥60 | 25 | 17 | 68% | |
| Stage | | | | |
| Advanced (IVB) | 10 | 7 | 70% | 0.508 |
| Recurrent | 51 | 30 | 59% | |
| Histology | | | | |
| SCC | 45 | 27 | 60% | 0.860 |
| A or AS | 16 | 10 | 63% | |
| Performance status | | | | |
| 0–1 | 49 | 34 | 69% | 0.004 |
| 2 | 12 | 3 | 25% | |
| Prior chemotherapy | | | | |
| Yes | 11 | 2 | 18% | 0.001 |
| No | 50 | 35 | 70% | |
| Prior radiosensitizer | | | | |
| Yes | 20 | 14 | 70% | 0.297 |
| No | 41 | 23 | 56% | |
| Symptom status | | | | |
| Yes | 24 | 11 | 46% | 0.056 |
| No | 37 | 26 | 70% | |
| Site of disease | | | | |
| Pelvic | 22 | 11 | 50% | 0.201 |
| Distant/pelvic and distant | 39 | 26 | 67% | |
| DFI, mos | | | | |
| <6 | 36 | 24 | 67% | 0.249 |
| ≥6 | 25 | 13 | 52% | |

A, adenocarcinoma; AS, adenosquamous carcinoma; SCC, squamous cell carcinoma.

be administered as a 3-hour infusion.[18] Given the advantages of this regimen with regard to the patient's convenience and tolerance, we believe that TC is a reasonable treatment option in this patient population. To address the clinical benefit of TC compared to TP in advanced, persistent, or recurrent cervical cancers, the Japan Clinical Oncology Group (JCOG) is currently conducting a randomized phase 3 trial (protocol JCOG 0505).[19]

In the current study, as predicted, the administration of TC was well tolerated without any significant treatment delays or dose reduction. The overall response rate was 60.7%, which was similar to those found in previous retrospective studies.[10–14] Importantly, TC showed significant clinical activity in patients with both squamous cell carcinoma (response rate of 60%) and adenocarcinoma histology (response rate of 63%).

The prognostic factors for the response to cisplatin-based chemotherapy in recurrent or advanced cervical cancer have been reported previously. Among these factors, the site of recurrence (pelvic), young age, poor performance status, a short DFI, race (black), and the prior use of radiosensitizers were reported to be significant predictors of a worse response.[4,20] In the current study, performance status, prior chemotherapy, and symptom status were found to be independent prognostic factors that are predictive of the response to TC. The use of radiosensitizers as a part of the initial treatment was also associated with a worse response; however, the difference was not statistically significant ($P = 0.134$). Although patients with pelvic recurrence tended to show a worse response to TC, multivariate analysis did not identify the disease site as an independent predictor of a poor outcome, which can be explained by the small number of patients included in the current study. We further determined whether the treatment-free interval (the time from the end of primary treatment to the detection of recurrence or disease progression) is an independent predictor of response to TC in a separate multivariate analysis in which DFI was not included as a prognostic variable (Supplemental Digital