Table 2. Institutional Costs for Cancer Treatment Trials

Ethics review and local competent authority review of proposed trials, open trials, adverse events, amendments
Time of local investigators, research nurses, pharmacists, and data managers
Time and resources for related studies (pathology, imaging) over and above that which is standard of care
Research pharmacy
Quality control efforts

clinical trials through research grants to clinical investigators and trials units.[40,41] The estimated yearly budget for academic cancer clinical trials in the United Kingdom, including support for network infrastructure is about £55 million. The Ireland–Northern Ireland National Cancer Institute Cancer Consortium, with financial support from the Republic of Ireland, the United Kingdom government, and the NCI, established a clinical trials network covering the Republic of Ireland and Northern Ireland.[42] In France, the Ministry of Health and INCa (Institut National du Cancer) have established support for clinical trials through competitive requests for applications as well as support for data management centers, including those of specialized networks. The governments of Japan and Korea have undertaken steps to support infrastructure for and encourage academic clinical trials in cancer. A similar effort is underway in the Middle East. The government of Australia, through Cancer Australia, has recently undertaken support and expansion of existing trials networks, which had previously been funded through a variety of means including fundraising and charitable donations, peer-reviewed grants for individual trials, and infrastructure support for some groups by the New South Wales Cancer Institute. Funds raised by charity (the Canadian Cancer Society) have been used for many years to support the core activity of the NCIC-CTG. Professional medical societies in China, India, Japan, Korea, and other countries have undertaken to start cooperative groups to run clinical trials for cancer patients. Local institutions also have generously contributed their own funds, as well as funds raised through charitable appeals, to help support the infrastructure for clinical trials, such as the costs listed in Tables 1 and 2.

We note that limitation of funding has hindered clinical trial research in many instances. In the United States, for example, the per-patient cost to support research nurses, data managers, and physician time for a hypothetical phase III cancer treatment trial has been estimated at $6,000 (US$) in 2003.[43] NCI funds are only sufficient to underwrite a per-patient payment of $2,000 (US$). Clinical trials groups outside the United States that lack substantive support from charity, industry, or government often must decline participation in promising phase III studies unless separate industry funding is available.

PHARMACEUTICAL INDUSTRY INVOLVEMENT IN INTERNATIONAL TRIALS

Pharmaceutical companies may run international trials on their own, or in conjunction with established clinical trials cooperative groups. Effective collaboration between industry and clinical trials groups has resulted in the successful completion of many important cancer trials. Not surprisingly, however, there may well be tensions between the objectives of the pharmaceutical company, which generally wants to support trials that provide data appropriate for a licensing application, and those of the cooperative group, which wants to evaluate the additive benefit of that new agent to standard treatment. In some cases, the cooperative group may also want to combine or compare agents from two different companies. In addition, in many instances, a trial addressing a question of great importance to oncologists and patients may be of no interest to the pharmaceutical industry. An international consortium of academic breast cancer trialists have recently proposed a model template for successful partnership between academia and industry.[44]

Pharmaceutical support for trials may include the supply and/or distribution of experimental drugs, per-patient payments to participating institutions, and support of central activities, such as investigator education, laboratory assays, statistical analysis, data management, quality control/quality assurance, and audits. The provision of study drug and financing across international boundaries may be complicated due to the variation in licensing arrangements across the globe. Recently, the Chief Executive Officer Roundtable on Cancer, working in partnership with the NCI and academic institutions in the United States, developed a set of common contract clauses designed to shorten the length of time required for legal agreements.[45]

CURRENT REPORT CARD ON GLOBAL COLLABORATION

How should we characterize the current state of global collaboration in cancer treatment trials? Ideally, clinical trials groups for each cancer site should have a regular mechanism for the exchange of ideas about current science and proposed trials. Such a structure would facilitate the design and conduct of complementary trials, avoid unnecessary duplication, and stimulate collaboration on meta-analyses of similar studies. Where appropriate, groups can work together on the design and management of joint global trials.

Regional international networks have been established for decades both in Europe and in North America. For example, leading European oncologists set up the EORTC in 1962. Today, EORTC's top 35 accruing institutions are located in 11 European countries, as well as Turkey and Egypt. Similarly, cancer researchers in Canada and the United States have worked together for many years through such collaborative groups as the National Surgical Adjuvant Breast and Bowel Project, the Radiation Therapy Oncology Group, and the Children's Oncology Group. The NCIC-CTG has worked closely with investigators in the United States, Europe, and Australia. Global networks for cancer treatment trials in the developing world have been set up by both the International Network for Cancer Treatment and Research and the International Atomic Energy Agency. In addition, many groups of trialists have established ongoing collaborations to perform meta-analyses based on data from individual patients accrued to clinical trials. A partial list of recent key cancer treatment trials made possible through effective international collaboration is presented in Appendix Table A1 (online only).

Effective interchange between clinical trials groups has most often been accomplished under the umbrella of international intergroup committees. A list of the activities which we would expect from an effective international intergroup is presented in Table 3. One of the best examples of effective intergroup activities is in breast cancer. Globally, the Breast International Group and the International Breast Cancer Study Group bring together 41 member groups from Europe,

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Table 3. Expectations for Functional Global Intergroup Committees

Required participation by member groups in at least some intergroup trials
Required participation by groups in intergroup activities
Dues to support intergroup infrastructure and meetings
Attendance at meetings and conference calls
Regular face-to-face meetings, conference calls, and trial-specific workshops
Routine exchange of information about active and planned studies
Joint development of concepts for new trials
Development of joint trials as appropriate and feasible, ideally to include:
Single protocol with country-specific appendices
Common case report forms
Single data base
Development of complementary trials as appropriate and feasible
Routine engagement with industry as an intergroup
Individual-patient date meta-analyses as appropriate

Table 4. Advantages and Disadvantages of International Collaboration in Cancer Treatment Trials

Advantages	Faster accrual from more sites for patients with common cancers and with all stages of disease
	Faster accrual for patients with uncommon and rare tumors, specific molecular defects, and less common histologic subtypes
	Broader applicability of research results
	Fewer duplicative trials
	More complementary trials
	More rapid dissemination of innovations in cancer treatment
Disadvantages	Differing regulations between countries
	Differing levels of infrastructure support for cancer clinical trials between countries
	Differing processes and schedules for scientific review by funding bodies between countries
	Longer lead time for concept and trial development
	Differing licensing arrangements for specific drugs between countries
	Contractual issues with pharmaceutical companies in different countries
	Drug distribution issues in different countries

Canada, Latin America, Australia/New Zealand, and Asia, in addition to those from North America. The Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization trial (NCT 00490139), sponsored by NCI, the Breast Intergroup, and GlaxoSmithKline is an example of a worldwide trial made possible through international collaboration and industry partnership.[46]

In brain cancer, the EORTC, NCIC-CTG, the Trans-Tasman Radiation Oncology Group (based in Australia and New Zealand), and the United States–based Radiation Therapy Oncology Group and North Central Cancer Treatment Group have developed a joint disease strategy for high-grade gliomas. This work follows up on the joint international temozolamide trial previously mentioned.

In gynecologic cancer, the Gynecologic Cancer Intergroup, formed in 1997, brings together 16 cooperative groups that conduct cancer treatment trials for women with gynecologic cancer. Under the auspices of the Gynecologic Cancer Intergroup, cooperative groups from Australia/New Zealand, Italy, the United Kingdom, and the United States quickly completed accrual of 4,000 women to Gynecologic Oncology group 182/International Collaboration in Ovarian Neoplasia 5, the largest ovarian cancer treatment trial to date.[47]

In addition, there are numerous instances of academic and industry-led trials conducted across the developing and developed worlds. To date, however, global integration of academic cancer treatment trials remains the exception, rather than the norm.

CONCLUSION

The scientific imperative for international collaboration in cancer treatment trials is clear. Our ability to establish international collaborations will result in maximization of our resources and patients, permitting us to complete definitive trials in a timely manner. Regulatory, logistical, and financial hurdles, however, often hamper the conduct of joint trials. The advantages and disadvantages of such international collaboration are listed in Table 4. Ongoing efforts on the part of cancer investigators, cooperative groups, national research institutions, national governments, competent authorities, ethics committees, and pharmaceutical companies are needed to strengthen global collaboration so that we may identify effective treatments for our patients more quickly. In addition, integration of investigators and cooperative groups in China, India, Japan, Korea, Latin America, and other countries in Asia, Africa, the Middle East, and Europe into the existing intergroups and clinical trials networks will make our trials more representative of cancer patients from around the globe and the results from our trials more broadly applicable to those patients.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Appendix

Table A1. Partial List of Key Academic Cancer Treatment Trials Made Feasible via International Collaboration Published 2003 to 2009 (by disease)

Cancer	Reference
Brain	Gorlia T, van den Bent MJ, Hegi ME, et al: Lancet Oncol 9:29-38, 2008; Hegi ME, Diserens AC, Gorlia T, et al: N Engl J Med 352:997-1003, 2005; Stupp R, Mason WP, van den Bent MJ, et al: N Engl J Med 352:987-996, 2005; Stupp R, Hegi ME, Mason WP, et al: Lancet Oncol 10:459-466, 2009
Breast	Van den Bent MJ, Afra A, De Witte O, et al: Lancet 366:985-990, 2005; Breast International Group (BIG) I-98 Collaborative Group, Thurlimann B, Keshaviah A, et al: N Engl J Med 353:2747-2757, 2005; Forbes JF, Cuzick J, Buzdar A, et al: Lancet Oncol 9:45-53, 2008; Goss PE, Ingle JN, Martino S, et al: N Engl J Med 349:1793-802, 2003; Pestalozzi BC, Zahrieh D, Mallon E, et al:. J Clin Oncol 26:3006-3014, 2008; Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al: N Engl J Med 353:1659-1972, 2005; Romond EH, Perez EA, Bryant J, et al: N Engl J Med 353:1673-1684, 2005
Colorectal	Nordlinger B, Sorbye H, Glimelius B, et al: Lancet 371:1007-1015, 2008; Quirke P, Steele R, Monson J, et al: Lancet 373:821-828, 2009; Bosset JF, Collette L, Calais G, et al: N Engl J Med 355:1114-1123, 2006; Sebag-Montefiore D, Stephens RJ, Steele R, et al: Lancet 373:811-820, 2009
Endometrial	ASTEC/EN.5 Study Group, Blake P, Swart AM, et al: Lancet 373:137-146, 2009
Genitourinary	Bolla M, van Poppel H, Collette L, et al: Lancet 366:572-578, 2005; Oliver RT, Mason MD, Mead GM, et al: Lancet 366:293-300, 2005
Head and neck	Bernier J, Domenge C, Ozsahin M, et al: N Engl J Med 350:1945-1952, 2004; Lefebvre JL, Rolland F, Tesselaar M, et al: J Natl Cancer Inst 101:142-152, 2009; Vermorken JB, Remenar E, van Herpen C, et al: N Engl J Med 357:1695-1704, 2007; Vermorken JB, Mesia R, Rivera F, et al: N Engl J Med 359:1116-1127, 2008
Leukemia	Fielding AK, Rowe JM, Richard SM, et al: Blood 113:4489-4496, 2009; Goldstone AH, Richards SM, Lazarus HM, et al: Blood 111: 1827-1833, 2008
Lung	Gilligan D, Nicolson M, Smith I, et al: Lancet 369:1929-1937, 2007; Slotman BJ, Faivre-Finn C, Kramer GWPM, et al: New Engl J Med 357:664-672, 2007; Winton T, Livingston R, Johnson D, et al: N Engl J Med 352:2589-2597, 2005
Lymphoma	Aleman BM, Raemaekers JM, Tireli U, et al: N Engl J Med 348:2396-2406, 2003; Ferme CH, Eghbali H, Meerwaldt JH, et al: N Engl J Med 357:1916-1927, 2007; Meyer R, Gospodarowicz M, Connors J, et al: J Clin Oncol 23:4634-4642, 2005; Van Oers MHJ, Klasa A, Marcus RE, et al: Blood 108:3295-3301, 2006
Melanoma	Eggermont AMM, Suciu S, Mackie R, et al: Lancet 366:1189-1196, 2005; Eggermont AMM, Sciu S, Santinami M, et al: Lancet 372: 117-126, 2008
Ovarian	Bookman et al[47]; Pfisterer J, Plante M, Vergote I, et al: J Clin Oncol 24:4699-4707, 2006; Parmar MK, Lederman JA, Colombo N, et al: Lancet 361:2099-2106, 2003; Trimbos JB, Parmar M, Vergote I, et al: J Natl Cancer Inst 95;105-112, 2003
Pediatric oncology	Arndt CA, Hawkins DS, Meyer WH, et al: Pediatr Blood Cancer 50:33-36, 2008; Lange BJ, Smith FO, Feusner J, et al: Blood 111: 1044-1053, 2008; Matloub Y, Lindemulder S, Gaynon PS, et al: Blood 108:1165-1173, 2006; Matthay KK, Quach A, Huberty J, et al: J Clin Oncol 27:1007-1013, 2009; Miser JS, Goldsby RE, Chen Z, et al: Pediatr Blood Cancer 49:894-900, 2007; Seibel NL, Steinherz PG, Sather HN, et al: Blood 111:2548-2555, 2008
Sarcoma	Vervweij J, Casali PG, Zalcberg J, et al: Lancet 364:1127-1134, 2004

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Phase II Study of Intraperitoneal Carboplatin With Intravenous Paclitaxel in Patients With Suboptimal Residual Epithelial Ovarian or Primary Peritoneal Cancer

A Sankai Gynecology Cancer Study Group Study

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Purpose: To assess the antitumor efficacy and safety of 2 treatment modalities: intraperitoneal carboplatin combined with intravenous (IV) paclitaxel.

Patients and Methods: Eligible patients were those with epithelial ovarian carcinoma or primary peritoneal carcinoma stages II to IV who underwent initial surgery and had a residual tumor size of 2 cm or larger. Patients received IV paclitaxel 175 mg/m^2 followed by intraperitoneal carboplatin AUC6. The primary end point was a response. Secondary end points were toxicity, progression-free survival, and overall survival.

Results: Twenty-six patients were enrolled, and 24 patients were eligible for assessment. The response rate was 83.3% (95% CI, 62.6%–95.3%; Table 4). The median progression-free survival was 25 months. The median overall survival had not been reached. Incidences of grade (G) 3/4 hematological toxicities were absolute neutrophil count, 96%; hemoglobin, 29%; and thrombocytopenia, 16%. Nonhematological toxicities included G2 liver function, 4%; G3 sensory neuropathy, 8%; and G3 myalgia and arthralgia, 4%.

Conclusions: Intraperitoneal administration of carboplatin combined with IV paclitaxel was well tolerated and showed satisfactory response in the patients with bulky residual tumor. Large-scale phase III trial comparing with IV carboplatin is warranted in this patient population.

Key Words: Intraperitoneal chemotherapy, Carboplatin, Ovarian cancer, Suboptimal residual disease, Phase II study

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Despite the development of new anticancer agents such as platinum and taxane, ovarian cancer remains the most lethal of gynecologic malignancies. One strategy to treat ovarian cancer is intraperitoneal (IP) chemotherapy, and because the most distinct characteristic of ovarian or peritoneal cancer is early intra-abdominal dissemination of the disease, this seems to be a reasonable approach. The IP modality has been investigated for years, including several phase I and II studies using various anticancer agents.[1] Intraperitoneal cisplatin and IP paclitaxel are now considered the choice of treatments based on a series of randomized phase III trials conducted in the United States that evaluated the survival benefit of IP over intravenous (IV) administration of these agents. Three randomized trials[2–4] showed that IP cisplatin-based chemotherapy significantly improved survival compared with cisplatin chemotherapy administered intravenously. A meta-analysis showed a 22% reduction of hazards ratio to death, prompting the US National Cancer Institute to

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recommend IP chemotherapy for patients with small residual tumors.

Rationale for Using Intraperitoneal Carboplatin

Intraperitoneal chemotherapy has not been accepted in the international gynecologic oncology community despite these positive reports. One reason is that IP cisplatin-based chemotherapy has not been tested against the current standard chemotherapy, IV paclitaxel plus IV carboplatin. In addition, significant toxicity occurred in one study that demonstrated the best survival rate using IP cisplatin plus IV paclitaxel.[4] Investigators are developing protocols for an optimal IP regimen that are superior to the current standard regimen but are less toxic than previous regimens. Carboplatin is the most feasible platinum agent to reduce cisplatin-based toxicities. A relatively large retrospective study showed the efficacy and toxicity of IP carboplatin-based chemotherapy in patients with ovarian cancer.[5]

Rationale for Investigating IP Chemotherapy in Patients With Suboptimal Residual Tumors

Usually, IP chemotherapy is given to patients with optimally debulked tumors (usually ≤1 cm) because direct penetration of the anticancer agents is limited to a few millimeters.[6–9] However, when platinum agents were administered intraperitoneally, the area under the curve (AUC) of these agents in the serum is known to be equal to the AUC after IV administration.[10,11] Therefore, IP platinum therapy is hypothesized to be a systemic chemotherapy route that should have a heightened regional effect because it can deliver an extremely high concentration of anticancer agents. An interesting clinical observation supports this hypothesis. In the Gynecologic Oncology Group (GOG) 104 trial, the hazard ratio for the risk of death in the IP group compared with the intravenous group was 0.76 (95% CI, 0.61–0.96; $P = 0.02$) in patients with residual tumors of 2 cm or less.[2] When the hazard ratio was calculated only for patients with tumors of 0.5 cm or less, the hazard ratio was 0.8. Therefore, the therapeutic gain of IP therapy in reducing death hazard was slightly greater in patients with residual tumors between 0.5 and 2 cm than in patients with smaller residual tumors (<0.5 cm). This observation implies that IP therapy in patients with larger residual tumors may be more effective than, or as effective as, in patients with smaller residual disease tumors. Two retrospective studies showed that IP carboplatin-based chemotherapy was considerably efficacious in suboptimally debulked ovarian cancer patients.[5,15]

Based on these observations, we conducted a phase II trial to evaluate the therapeutic response of IP carboplatin-based chemotherapy in patients with suboptimally debulked disease.

TABLE 1. Characteristics of patients enrolled in the study

	n = 26 (%)
Diagnosis	
Ovarian	23 (89)
Primary peritoneum	3 (11)
Histology	
Serous	18 (69)
Mucinous	1 (4)
Endometrioid	4 (15)
Undifferentiated	0
Others	3 (12)
Performance status	
0	16 (62)
1	8 (29)
2	2 (9)
FIGO stage	
II	3 (12)
III	17 (65)
IV	6 (23)
Residual disease	
<5 mm	0
5–10 mm	0
10–20 mm	0
<20 mm	26 (100)
Second or interval debulking	
Yes	16 (62)
No	10 (38)

FIGO, International Federation of Gynecology and Obstetrics.

TABLE 2. Toxicity of IP carboplatin plus IV paclitaxel combination chemotherapy

	n = 26
Ineligible for assessment of combination chemotherapy	2
Paclitaxel anaphylaxis	1
Catheter obstruction at the first cycle	1
No. patients eligible for assessment of combination chemotherapy	n = 24 (%)
ANC	
G3	6 (25)
G4	17 (71)
Febrile neutropenia	0
Hemoglobin	
G3	6 (25)
G4	1 (4)
Platelet	
G3	2 (8)
G4	2 (8)
GOT	
G2	1 (4)
GPT	
G2	1 (4)
ALP	
G2	1 (4)
Bilirubin	0
Creatinine	0
Neurotoxicity	
Sensory G3	2 (8)
Motor	0
Myalgia/arthralgia	
G2	1 (4)
Gastrointestinal	
G2	1 (4)

ALP, alkaline phosphatase; ANC, absolute neutrophil count; GOT, glutamic-oxaloacetic transaminase; GPT, glutamic-pyruvic transaminase.

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PATIENTS AND METHODS

This is a phase II study to assess the efficacy and safety of carboplatin administered intraperitoneally in combination with IV paclitaxel in patients with epithelial ovarian cancer or primary peritoneal cancer who had suboptimal residual tumor after initial debulking surgery.

Patients

Patient inclusion criteria included histologically confirmed epithelial ovarian or peritoneal cancer, stages II, III, and IV, with radiographically measurable residual tumor 2 cm or larger and adequate hematological (absolute neutrophil count $\geq 2000/mm^3$, and platelet count $\geq 100,000/mm^3$), renal (serum creatinine $\leq 1.5 \times$ the institutional upper limit of normal), and hepatic (serum bilirubin ≤ 1.5 mg/dL and both aspartate aminotransferase and alkaline phosphatase $\leq 2 \times$ the institutional upper limit of normal) laboratory values.

Exclusion criteria consisted of a history of invasive carcinoma of any other organs, excluding nonmelanoma skin cancer, and concomitant severe heart disease, cerebrovascular disease, uncontrollable diabetes, hypertension, severe infection, pulmonary fibrosis, interstitial pulmonitis, and symptomatic brain metastasis.

The study protocol was reviewed by the institutional review board, and written informed consent was obtained from the patients before registration.

Treatment

Patients had IP ports placed immediately before the abdomen was closed at the initial surgery. Chemotherapy was started by IV administration of paclitaxel at 175 mg/m^2 for 3 hours followed by IP administration of carboplatin at AUC6. During the IV paclitaxel administration, approximately 1000 mL of 5% glucose or normal saline was infused through the IP port, and then the designated dose of carboplatin was infused as a bolus immediately after IV paclitaxel administration was completed. These treatments were repeated every 3 weeks for 6 to 8 cycles. Interval debulking surgery was allowed after 3 to 5 cycles and then followed by chemotherapy, using the same regimen.

END POINTS

The primary end point was the response rate, and secondary end points were safety, progression-free survival, and overall survival.

Evaluation

Response was assessed using the Response Evaluation Criteria in Solid Tumors, and toxicity was assessed using National Cancer Institute Common Toxicity Criteria version 2.

TABLE 3. Completion of protocol treatment

No. Protocol Treatment Received	n = 26
0	2
1	0
2	0
3	0
4	1
5	1
6	13
7	2
8	7

TABLE 4. Clinical response

Clinical Response	n = 24
Complete response	6
Partial response	14
Response rate	83.3%
95% CI	62.6%–95.3%
No change	4
Progressive disease	0

Sample Size

The sample size was calculated to be 37, so that the response rate was expected to be 75%; threshold response, 55%; and alpha error, 0.05 with a power of 80%.

RESULTS

From December 2001 to January 2005, 26 patients were enrolled. The study was closed early because of slow accrual due to conflicting clinical trials.

Characteristics of patients enrolled in the study are summarized in Table 1. Of 26 patients, 2 patients were excluded from toxicity analysis because one had paclitaxel anaphylaxis at the first cycle and the other had IP port obstruction at the first cycle. Therefore, 24 patients were eligible for toxicity analysis. All 24 patients were eligible for evaluation of response and survival.

Toxicity

Table 2 lists grades 3 to 4 hematological and grade 2/3 nonhematological toxicities after the protocol treatment. The data showed that there were no specific toxicities related to the IP chemotherapy.

Completion of Protocol Treatment

The total number of protocol therapy cycles and the number of patients are shown in Table 3. Scheduled protocol treatment was completed in 22 (85%) patients. Reasons for terminating the protocol treatment in 4 patients were: disease progression (2), catheter complication (1), and paclitaxel anaphylaxis (1). There was no discontinuation of IP chemotherapy because of excessive toxicity or patient refusal.

Clinical Efficacy

Clinical response for 24 patients is described in Table 4. The response rate was 83.3% (95% CI, 62.6%–95.3%). As of the median follow-up of 31 months, median progression-free survival was 25 months. Median overall survival was not reached.

DISCUSSION

The basic concept of IP chemotherapy is that it is regional therapy. Ideally, anticancer drugs should stay in the intraperitoneal cavity for a long time and not enter systemic circulation, thus minimizing systemic toxicity. Unfortunately, however, because anticancer drugs do not penetrate more than a few millimeters, the optimal patient for IP chemotherapy is presumed to have minimal residual tumor after surgery. This study challenges that hypothesis.

The response rate, which was the primary objective of this study, was satisfactory in patients who received IP carboplatin-based chemotherapy. In addition, the median progression-free survival and overall survival seemed long enough after IP chemotherapy. Although it is not shown that IP carboplatin therapy is superior to

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IV carboplatin or IP cisplatin therapy, these observations highly warrant using IP carboplatin-based chemotherapy and justify the inclusion of suboptimally debulked patients in future trials of IP chemotherapy, although current inclusion criteria for the IP trial was only for optimally debulked patients.

Because the IP cisplatin-based chemotherapy regimen used in GOG172 was too toxic,[4] the expectation for using IP carboplatin now has become increasingly of interest. However, the use of carboplatin-based IP chemotherapy has been ignored, and the problem with the hypothesis has been discussed in previous literature.[12] One animal study[13] and one small retrospective clinical study[14] suggested that IP carboplatin-based therapy was inferior to IP cisplatin-based chemotherapy. An animal study showed that tissue platinum concentration after IP carboplatin administration was considerably lower than that after IP cisplatin administration.[13] The antitumor response in the clinical study was shown to be less effective after IP carboplatin-based chemotherapy than after IP cisplatin-based chemotherapy.[14] However, in the animal study, the author did not take into consideration the difference in the doses of these 2 platinum agents in determining the difference in biological activity. Usually, carboplatin needs to be administered in higher doses (6-8 times more milligrams per patient body) compared with cisplatin. A similar problem was found in the clinical study in which a higher dose of cisplatin (100 mg/m^2) was given, but the dose of carboplatin was considerably lower (200 mg/m^2) than the standard. The present study clearly showed that IP carboplatin-based chemotherapy, administered in sufficient dose, was efficacious and well tolerated, and a phase III trial comparing IP cisplatin and IP carboplatin is warranted to elucidate whether IP carboplatin is less toxic without compromising antitumor efficacy.

A pharmacological study[11] and 2 retrospective studies[5,15] suggested that IP carboplatin-based chemotherapy would be feasible for ovarian cancer patients with bulky residual disease. Although the size is small and the study was closed prematurely, this prospective phase II study confirmed those results. Because IP carboplatin-based chemotherapy has the ability to expose a high concentration of the drug to the tumor surface while it provides the similar AUC of platinum in the systemic blood circulation, it may provide better clinical outcome in the ovarian cancer patients.

In conclusion, our study clearly indicates that a large-scale randomized phase III trial to test the value of IP carboplatin compared with current standard IV carboplatin chemotherapy or IP cisplatin-based chemotherapy is warranted. Including patients with suboptimal residual disease is also justified in a future trial using IP carboplatin.

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Is the Adjustment of Serum Creatinine Level from < 0.6 mg/dL to 0.6 mg/dL Justified in Estimating Carboplatin Clearance Calculated by the Jelliffe Formula?

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Abstract

Background: In current Gynecologic Oncology Group studies, serum creatinine level is adjusted to 0.6 mg/dL in patients with levels < 0.6 mg/dL (Adjusted-Jelliffe formula). The purpose of this study is to evaluate whether this adjustment is suitable. **Patients and Methods:** Carboplatin clearance was estimated in 115 patients with serum creatinine < 0.6 mg/dL who received carboplatin-based chemotherapy for gynecologic malignancies between January 1996 and August 2004. The clearance was estimated using the Cockroft-Gault, Jelliffe, and Adjusted-Jelliffe formulae. The 3 estimations were then compared with each other using the post hoc Wilcoxon signed rank test. Bias was assessed by mean percentage error (MPE), and precision was assessed by mean absolute percentage error (MAPE). The relationships between body surface area (BSA) and ratios of estimated carboplatin clearance (Jelliffe formula/Cockroft-Gault formula and Adjusted-Jelliffe formula/Cockroft-Gault formula) were evaluated by simple regression analysis. **Results:** The carboplatin clearance calculated by the Jelliffe formula was significantly larger than the other 2 formulae ($P < .0001$). Although MPE was reduced from +20 to +6 by adjustment of serum creatinine, MAPE was barely reduced from 21 to 14. The simple regression line represents correlation between BSA and ratios of estimated carboplatin clearance was merely translated to below by adjusting the serum creatinine level, and the bias by BSA was not corrected. **Conclusion:** Despite adjusting the serum creatinine level, the Adjusted-Jelliffe formula overestimates the creatinine clearance when compared with the Cockcroft-Gault formula.

Keywords: Adjusted-Jelliffe formula; Body surface area; Calvert formula; Carboplatin dose; Glomerular filtration rate

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Introduction

The Calvert formula has been used most frequently for calculating the carboplatin dose.[1] It relies on the area under the curve (AUC) of the plasma carboplatin concentration-time curve and the glomerular filtration rate (GFR). AUC correlates well with the degree of myelosuppression and response rate in patients with ovarian cancer.[2,3] In these studies, GFR is measured by the clearance of [^{51}Cr]-ethylenediaminetetraacetic acid because it highly correlates with carboplatin clearance. However, the use of this radioisotopic method is not common because of its inconvenience and expense.[1,4]

Creatinine clearance (CrCl) has been widely used as a substitute for GFR. Several simpler methods using a single serum creatinine measurement to calculate CrCl have been proposed. They include the Cockroft-Gault, Wright, Jelliffe and Modified-Jelliffe formulae. We defined the original formula for estimation of CrCl advocated by Jelliffe as the 'Jelliffe formula' and the Jelliffe formula corrected by body surface area (BSA) as the 'Modified-Jelliffe formula.'[5-7] On the other hand, Chatelut et al proposed a formula to estimate carboplatin clearance directly using the serum creatinine concentration and patient characteristics, including sex, weight, and age (Chatelut formula).[8]

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Among these formulae, the Jelliffe formula does not consider BSA or body weight to adjust for body size. Previously, we demonstrated that in patients with small BSA, estimates of carboplatin clearance by the Jelliffe formula tend to have a greater positive bias compared with the other formulae.[9] The Jelliffe formula has been used to estimate carboplatin clearance in Gynecologic Oncology Group (GOG) studies. The serum creatinine level is now adjusted to 0.6 mg/dL in patients with levels < 0.6 mg/dL. However, adjustment of serum creatinine level might not decrease the bias because this method also does not consider BSA for estimating carboplatin clearance. The purpose of this study is to evaluate if the previously demonstrated bias for the Jelliffe formula is retained despite this correction in the Adjusted-Jelliffe formula.

Patients and Methods

The clinical characteristics of the patients are shown in Table 1. A total of 115 patients were included in this analysis. Mean age was 54.6 years, and mean weight, height, and calculated BSA were 51.5 kg, 153.1 cm, and 1.43 m^2, respectively.

The records of patients with serum creatinine < 0.6 mg/dL during the first cycle of carboplatin-based chemotherapy for gynecologic malignancies at Kawasaki Medical School Hospital between January 1996 and August 2004 were used in this analysis. Mean level of serum creatinine was 0.52 mg/dL (range, 0.38-0.59 mg/dL). Carboplatin in combination with cyclophosphamide was administered as first-line chemotherapy for patients with gynecologic cancer until April 2001. Thereafter, carboplatin in combination with paclitaxel was administered only to patients with epithelial ovarian cancer, and those with cancer of the endometrium, cervix, vagina, or vulva received combination chemotherapy including carboplatin and docetaxel as first-line chemotherapy from July 2001. Written informed consent was obtained from all patients before chemotherapy. This study was approved by the Institutional Review Board at Kawasaki Medical School Hospital. We reviewed the medical records and extracted pertinent information. Medical records were reviewed for age, actual body weight, height, and serum creatinine levels at the start of chemotherapy. Clinical diagnosis and chemotherapy regimen were also reviewed. Serum creatinine levels were measured using an enzymatic assay by AUTO L "MIZUHO" CRE kit (Mizuho Medy Co., Tosu, Japan). In clinical practice at our institution, we use the Calvert formula with CrCl estimation by the Cockroft-Gault formula to calculate the carboplatin dose.

Bias and precision were assessed by comparing the carboplatin clearance estimated by the Jelliffe formula or the Adjusted-Jelliffe formula with that of the Cockroft-Gault formula because we used the latter for estimating CrCl in our clinical practice. The 3 estimations of carboplatin clearance were then compared with each other using the post hoc Wilcoxon signed rank test, as described previously.[9] Bias was evaluated by mean percentage error (MPE):

(clearance calculated by the Jelliffe formula or the Adjusted-Jelliffe formula; clearance calculated by the Cockroft-Gault formula/ clearance calculated by the Cockroft-Gault formula) × 100,

and precision was assessed by mean absolute percentage error (MAPE):

(clearance calculated by the Jelliffe formula or the Adjusted-Jelliffe formula; clearance calculated by the Cockroft-Gault formula/ clearance calculated by the Cockroft-Gault formula) × 100.

Table 1
Clinical Characteristics of Patients the (N = 115)

Characteristic	Mean (Range)
Age, Years	54.6 (17-84)
Weight, kg	51.5 (31.8-91.2)
Height, cm	153.1 (135-171)
Calculated BSA, m^2	1.43 (1.12-1.84)
Serum Creatinine, mg/dL	0.52 (0.38-0.59)
Diagnosis, n	
Ovarian	72
Fallopian tube	5
Peritoneal	4
Corpus	12
Cervical	17
Vagina	2
Vulva	1
Others	2
Chemotherapy Regimen, n	
Carboplatin + paclitaxel	53
Carboplatin + docetaxel	20
Carboplatin + cyclophosphamide	42

Abbreviation: BSA = body surface area

The relationships between BSA and ratios of estimated carboplatin clearance (Jelliffe formula/Cockroft-Gault formula and Adjusted-Jelliffe formula/Cockroft-Gault formula) were evaluated by simple regression analysis. The formulae used in this analysis were as follows:

(1) Calvert formula: carboplatin dose (mg) = AUC (mg/mL/min) × (GFR [mL/min] + 25) carboplatin clearance (mL/min) = GFR + 25
(2) Cockroft-Gault formula: CrCl (mL/min) = (140 age [years]) × (weight [kg] × 0.85)/(serum creatinine [mg/dL] × 72)
(3) Jelliffe formula: CrCl (mL/min) = 0.9 × 98 − (0.8 × [age {years} − 20}])/serum creatinine (mg/dL)
(4) Adjusted-Jelliffe formula: CrCl (mL/min) = 0.9 × 98 − (0.8 × [age {years} − 20}])/0.6*

*Serum creatinine level in every patient in this analysis was < 0.6 mg/dL; therefore, it was adjusted to 0.6 mg/dL in all patients.

Results

The carboplatin clearance calculated by the Cockroft-Gault, Jelliffe, or Adjusted-Jelliffe formulae are shown in Table 2. The carboplatin clearance calculated by the Jelliffe formula was significantly larger than the other 2 formulae ($P < .0001$ by the post hoc Wilcoxon signed rank test; Table 2). Although MPE was reduced from +20 to +6 by adjusting serum creatinine level, the reduction of MAPE was considerably smaller (from 21 to 14).

There was a significant correlation between BSA and ratios of estimated carboplatin clearance (Jelliffe formula/Cockroft-Gault

Table 2
The Calculated CaCl of Three Formulae and MPE/MAPE Between CaCl Calculated by Cockroft-Gault Formula and Other Two Formulae

Formula	Cockroft-Gault	Jelliffe	Adjusted-Jelliffe
CaCl, mL/min	126.7 ± 27.7	148.2 ± 20.5*	130.5 ± 14.3
MPE, %	–	+20	+6
MAPE, %	–	21	14

*$P < .0001$ by post hoc Wilcoxon signed rank test.
Abbreviations: CaCl = carboplatin clearance; MAPE = median absolute percent error; MPE = median percent error

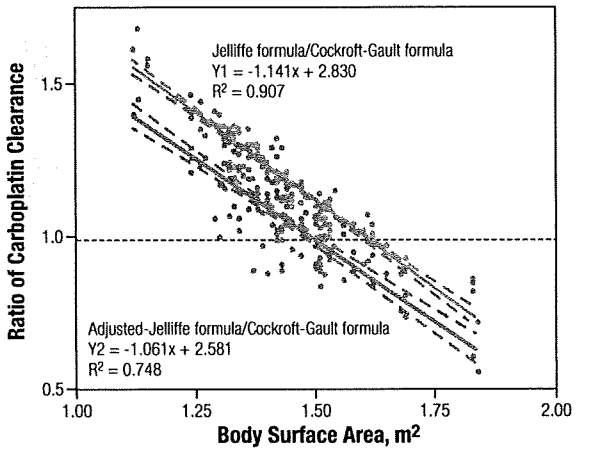
formula and Adjusted-Jelliffe formula/Cockroft-Gault formula) with Pearson correlation coefficients of 0.953 and 0.865, respectively (Figure 1). Two simple regression lines were virtually parallel. The simple regression line represents correlation between BSA and ratios of estimated carboplatin clearance (Jelliffe formula/Cockroft-Gault formula) and was merely translated to below by adjusting the serum creatinine level, and the bias by BSA was not corrected. The Adjusted-Jelliffe formula continued to overestimate carboplatin clearance in patients with smaller BSA while underestimating it in patients with larger BSA.

Discussion

In our earlier study, we demonstrated that carboplatin clearances calculated by the Cockroft-Gault, Modified-Jelliffe, Wright, or Chatelut formulae had good correlation and small bias, whereas those calculated by the Jelliffe formula had significant bias compared with the other 4 formulae.[9] The correlation of GFR with BSA has been demonstrated in a number of experimental studies.[10,11] Because the Jelliffe formula (CrCl = 0.9 × [98 – 0.8 × {age, 20}]/serum creatinine) does not include BSA or body weight, the body size is not taken into consideration. This should be considered as a cause of greater positive bias in calculating carboplatin clearances by the Jelliffe formula. Although the absence of data using the [^{51}Cr]-ethylenediaminetetraacetic acid method might weaken the validity of the conclusion of our previous study, it is evident that only the Jelliffe formula has a large bias among all formulae. Recently, Wright et al revealed that obese patients with ovarian cancer receiving chemotherapy, including carboplatin, experience substantially less toxicity than lean women when carboplatin clearance was calculated by the Jelliffe formula.[12] The lower toxicity in obese patients whose carboplatin dose was based on GFR calculated by the Jelliffe formula could have thus received a smaller dose than that required.

The Jelliffe formula has been used to estimate carboplatin clearance in GOG studies. In current GOG studies, serum creatinine level is adjusted to 0.6 mg/dL in patients with levels < 0.6 mg/dL to avoid overdose of carboplatin in patients with small BSA or higher performance status (PS). Patients with higher PS tend to have lower serum creatinine levels. We demonstrated in a previous study that adjustment of serum creatinine can reduce the positive bias of carboplatin clearance but cannot improve precision.[9] Despite adjusting the serum creatinine level, carboplatin dose continued

Figure 1
Correlation Between Body Surface Area and Ratios of Estimated Carboplatin Clearance



Jelliffe formula/Cockroft-Gault formula (green line) and Adjusted-Jelliffe formula/Cockroft-Gault formula (red line). Solid or broken line represents the regression line. Dotted line represents the 95% confidence interval.

to be overestimated in patients with smaller BSA, whereas it was underestimated in patients with larger BSA than when derived by the Cockroft-Gault formula. In patients with BSA < 1.6 m^2 (103 patients [89.6%]), the carboplatin dose tends to be overestimated when CrCl is estimated by the Jelliffe formula, whereas in patients with BSA < 1.49 m^2 (78 patients [67.8%]), the carboplatin dose tends to be overestimated when serum creatinine is adjusted to 0.6. Therefore, a considerable number of patients might have received a higher dose of carboplatin despite the creatinine level being adjusted to 0.6 mg/dL. It thus appears that although the adjustment of serum creatinine level is effective to decrease the difference of average of carboplatin dose estimated by Jelliffe formula with other formulae, it does not improve the accuracy.

In this study, for comparison, carboplatin clearance was estimated using the Cockroft-Gault, Jelliffe, and Adjusted-Jelliffe formulae. We compared carboplatin clearance estimated by Cockroft-Gault, Wright, Jelliffe, Modified-Jelliffe, and Chatelut formulae in a previous study.[9] Because carboplatin clearance estimated by these formulae, with the exception of the Jelliffe formula, had a good correlation and small bias in that study, we decided to use the Cockroft-Gault formula as the representative of these 4 formulae. Our primary purpose of this study is to determine whether the Adjusted-Jelliffe formula is equivalent to some other formulae, except the Jelliffe formula, not to find the best formula. Therefore, it is appropriate to compare carboplatin clearance estimated by Adjusted-Jelliffe formula with Cockroft-Gault formula instead of the isotopic method.

Conclusion

The Jelliffe formula remains the outlier at small BSA (unless adjusted for BSA using the Modified-Jelliffe) and results in the calculation of higher doses of carboplatin in this setting when compared with the other formulae. In international collaboration studies, the body size will vary considerably when women from various

countries are enrolled. Therefore, it is recommended to use either the Cockroft-Gault, Modified-Jelliffe, Wright, or Chatelut formula to estimate CrCl or carboplatin clearance when a carboplatin-based chemotherapy trial is conducted. The clinical significance of this observation warrants evaluation in order to determine the most appropriate formula for use in clinical trials.

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Disclosures

The authors have no relevant relationships to disclose.

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Overexpression of Class III β-Tubulin Predicts Good Response to Taxane-Based Chemotherapy in Ovarian Clear Cell Adenocarcinoma

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Abstract **Purpose:** Of the various microtubule-associated molecules, β-tubulin III has been reported to be closely associated with the therapeutic efficacy of taxane-based chemotherapy against ovarian cancer. Stathmin and microtubule-associated protein 4 (MAP4) have been reported to play an important role in microtubule stabilization. In this study, we investigated whether expression of these microtubule-associated factors affects the therapeutic efficacy of taxane-based chemotherapy in ovarian clear cell adenocarcinoma.

Experimental Design: Drug sensitivity of paclitaxel or cisplatin was assessed in ovarian cancer cell lines treated with small interfering RNA of tubulin isoforms, MAP4, and stathmin. We examined 94 surgically resected ovarian clear cell adenocarcinoma specimens from patients treated with taxane-containing regimens ($n = 44$) and with taxane-free regimens ($n = 50$), using immunohistochemistry to detect expression of β-tubulin III, stathmin, and MAP4.

Results: Knockdown of β-tubulin III and IV specifically conferred drug resistance to paclitaxel in one ovarian cancer cell line, but not to other molecules. Estimated overall survival revealed a significant synergistic effect between taxane and β-tubulin III in patients with ovarian clear cell adenocarcinoma. Of three microtubule-related molecules, among the taxane-based chemotherapy group, cases with higher β-tubulin III expression were associated with a significantly more favorable prognosis compared with those having lower β-tubulin III expression. By contrast, there was no statistical significance in the synergistic relationships between stathmin and taxane or between MAP4 and taxane.

Conclusions: Taxane-based chemotherapy was effective for patients with ovarian clear cell adenocarcinomas who were positive for β-tubulin III but not for those who were negative for these proteins.

Microtubules are the principal target of a large and diverse group of natural-product anticancer therapeutic drugs, particularly of two major classes of antimicrotubule agents: the vinca alkaloids and the taxanes (1). Microtubules are composed of polymers of heterodimers that consist of two closely related polypeptides, α-tubulin and β-tubulin, which in turn contain α- or β-subunits and at least six isotypes encoded by different genes. Isotype composition influences the intrinsic dynamics of microtubules, and the sensitivity of microtubules to depolymerizing and polymerizing agents is related to the composition of tubulin isotypes or microtubule-associated proteins (MAP; ref. 2). MAPs, important components of the tubulin and microtubule system, can bind to the microtubule wall and stabilize microtubules (3). MAP2 and MAP-τ are abundantly expressed in mature neurons, and MAP4 is ubiquitously expressed in both proliferating and differentiated cells (4). Stathmin is also the founding member of the microtubule-destabilizing family of proteins, which regulate the dynamics of microtubule polymerization and depolymerization. Stathmin is expressed at high levels in a variety of human cancers

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Translational Relevance

It has been reported that β-tubulin III, one of microtubule-associated molecules, was expected to be a useful biomarker for the clinical efficacy of taxane-based chemotherapy against human ovarian cancer. These studies have been conducted in serous adenocarcinoma of ovarian cancer. Previous reports show, however, that ovarian clear cell adenocarcinoma constituted about 20% of ovarian adenocarcinoma in Japan, although only 2% to 5% of cases of ovarian cancer worldwide were clear cell adenocarcinoma. Clear cell adenocarcinoma, which is a rare variant in western counties, has been recognized as a chemoresistant phenotype compared with serous adenocarcinoma, which is the most widespread ovarian cancer. This study aimed to identify a predictive marker for the clinical efficacy of taxane-based chemotherapy against ovarian clear cell adenocarcinoma. In this study, we found that a taxane-based regimen was effective for patients with ovarian clear cell adenocarcinomas who were positive for stathmin or β-tubulin III.

and also plays a role in altered drug sensitivity in human cancer cells, including ovarian cancer cells (5, 6).

The antitumor drug taxane stabilizes microtubules and reduces their dynamics, promoting mitotic arrest and cell death. Paclitaxel, a representative anticancer agent of the taxanes, was initially defined by Horowitz and colleagues, and its binding sites are distinct from those of colchicine, podophyllotoxin, and the vinca alkaloids (7, 8). Paclitaxel initially received regulatory approval for the treatment of patients with ovarian cancer after failure of first-line or subsequent chemotherapy (9). In a Gynecologic Oncology Group study (GOG-111), it was thus determined to be the primary induction therapy in suboptimally debulked stage III and IV ovarian cancer, which mainly consists of serous adenocarcinoma (10). This study first compared the therapeutic efficacy of paclitaxel/cisplatin and cyclophosphamide/cisplatin in patients with ovarian cancer (10). The paclitaxel arm showed a distinct advantage in terms of progression-free survival (PFS) as well as overall survival (OS). A clinical trial by the European Organization for Research and Treatment of Cancer and the National Cancer Institute of Canada also showed that a paclitaxel/cisplatin regimen improved both PFS and OS (11). Another clinical trial study, however, reported that survival in the paclitaxel arm was similar to that seen in the control arm that received either carboplatin or cisplatin, doxorubicin, and cyclophosphamide (12). It remains unclear whether paclitaxel-cisplatin (or carboplatin) therapy is superior to cyclophosphamide/cisplatin (or carboplatin) therapy.

Of the various molecular markers related to drug sensitivity to taxanes, class III β-tubulin is expected to be a useful biomarker for the clinical efficacy of paclitaxel-based chemotherapy. Class III β-tubulin is hypothesized to counteract suppression of microtubule dynamics (13). Ferlini et al. reported that a novel taxane targeting class III β-tubulin overcame paclitaxel resistance, suggesting close involvement of this tubulin isotype in drug sensitivity to paclitaxel (14). Mozzetti et al. reported that class III β-tubulin overexpression represented a prominent mechanism of resistance to paclitaxel-platinum treatment in ovarian cancer (15). Moreover, class III β-tubulin overexpression could be useful in identifying poor clinical outcome in patients with advanced ovarian cancer who are treated with platinum/paclitaxel, those mainly affected with serous adenocarcinoma (16). These studies have been conducted mainly in serous adenocarcinoma of ovarian cancer. It remains unknown, however, whether class III β-tubulin overexpression is also predictive of poor outcome in clear cell adenocarcinoma, which is a rare variant in western countries, where it is reported to constitute 5% to 10% of ovarian carcinomas (17–19). Clear cell adenocarcinoma has been recognized as a chemoresistant phenotype (20, 21).

Japanese investigators have reported that clear cell adenocarcinoma constitutes about 20% of ovarian carcinomas in Japan (20, 22), although clear cell adenocarcinoma of the ovary accounts for only 2% to 5% of cases enrolled in large-scale randomized trials worldwide (22, 23). Thus, it is unclear whether carboplatin/paclitaxel therapy, which was introduced broadly as a standard regimen for epithelial ovarian cancer based on the results of such trials, can be readily applied for clear cell adenocarcinoma. Development of novel treatment strategies based on molecular biological characteristics is further required for clear cell adenocarcinoma.

In the present study, we addressed whether expression of β-tubulin III, MAP4, and stathmin could affect the efficacy of taxane-based therapeutic regimens against clear cell adenocarcinoma. Using immunohistochemical analysis of surgically resected clinical samples of clear cell adenocarcinoma, we examined expression levels of the above three biomarkers. In comparison with ovarian cancer patients treated with taxane-free regimens, we observed a significant and specific association of β-tubulin III expression with therapeutic outcomes of ovarian cancer treated with taxane-based regimens. We discuss whether the expression of β-tubulin III could be a predictive marker for the clinical efficacy of taxane-based chemotherapy against ovarian clear cell adenocarcinoma.

Materials and Methods

Cells and reagents. The human ovarian cell lines OVCAR-3 and SKOV-3, which expressed β-tubulins (I, II, III, and IV), MAP4, and stathmin, were obtained from the American Type Culture Collection. Cells were grown in Ham's F-12 Medium (Nissui Seiyaku Co.) with 10% fetal bovine serum (FetalClone III; Hyclone), 100 IU/mL penicillin, and 100 μg/mL streptomycin (Life Technologies, Inc.) in a humidified atmosphere of 5% CO_2 at 37°C. Paclitaxel (Taxol injection) and cisplatin (Briplatin injection) purchased from Bristol-Myers Squibb were clinically used. The polyclonal antistathmin was obtained from Calbiochem. The monoclonal class III β-tubulin antibody (clone 5G8) was obtained from Promega. The monoclonal MAP4 antibody (clone 18) was purchased from BD Transduction Laboratories.

Silencing of β-tubulins (I, II, III, IV), MAP4, and stathmin genes. To reduce the expression of some genes, we used Stealth RNAi (Invitrogen Life Technologies) to knock down the expression of β-tubulin I (NM_030773_stealth_706), β-tubulin II (NM_001069_stealth_1444), β-tubulin III (NM_006086_stealth_233), β-tubulin IV (NM_006087_stealth_352), MAP4 (NM_002375_stealth_2042), and stathmin (STMN1-HSS142799). Subconfluent human ovarian cells were cultured overnight in Opti-MEM I medium (Invitrogen Life

Technologies) without antibiotics, then 40 nmol/L small interfering RNA (siRNA) and Lipofectamine RNAiMax (Invitrogen) were applied according to the manufacturer's instructions. After 32 h, cells were detached from the culture plates and seeded into 96-well plates in F-12 medium with 10% fetal bovine serum. After a further 16-h incubation, paclitaxel or cisplatin was applied and cells were cultured for 3 d more. The numbers of cells were estimated by WST-8. The IC_{50} value was estimated from the regression line of log-logit plots of T/C (%) value versus drug concentration. The assays were carried out in quadruplicate.

Quantitative real-time PCR. RNA was reverse-transcribed from random hexamers using AMV reverse transcriptase (Promega). Real-time quantitative PCR was done using the Real-Time PCR system 7300 (Applied Biosystems). In brief, the PCR amplification reaction mixtures (20 μL) contained cDNA, primer pairs, the dual-labeled fluorogenic probe, and TaqMan Universal PCR Master Mix (Applied Biosystems). The thermal cycle conditions included maintaining the reactions at 50°C for 2 min and at 95°C for 10 min, and then alternating for 40 cycles between 95°C for 15 s and 60°C for 1 min. The primer pairs and probes were obtained from Applied Biosystems. The relative gene expression for each sample was determined using the formula $2^{(-\text{delta Ct})} = 2^{(\text{Ct (GAPDH)-Ct (target)})}$, which reflected the target gene expression normalized to GAPDH levels.

Patients. Ninety-four patients with primary ovarian clear cell adenocarcinoma, who had undergone debulking surgery at Keio University Hospital from 1983 to 2005, were examined. The histopathologic diagnoses of the all cases were confirmed according to the most recent WHO classification (WHO 2003). Patients were staged according to the International Federation of Obstetrics and Gynecology (FIGO) classification (24). Forty-four patients underwent chemotherapy using regimens containing taxanes [paclitaxel plus carboplatin (n = 39), paclitaxel plus cisplatin (n = 3), docetaxel plus cisplatin (n = 2); paclitaxel, 180 mg/m^2 body surface/day 1, docetaxel, 70 mg/m^2 body surface/day 1, cisplatin, 60 mg/m^2 body surface/day 1, and carboplatin, area under the curve 6/day 1]. Fifty patients received taxane-free regimens [CAP groups (n = 36): cisplatin (60 mg/m^2 body surface/day 1), epirubicin (50 mg/m2 body surface/day 1), and cyclophosphamide (500 mg/m^2 body surface/day 1); CAP plus fluorouracil (n = 1), CAP plus tegafur-uracil (n = 2), cisplatin plus cyclophosphamide (n = 11)]. The doses of carboplatin were calculated using Calvert's formula.

The effect of chemotherapy was evaluated approximately every 6 mo by computed tomography after 6 cycles of administration of chemotherapy. After chemotherapy, all patients were followed up every 2 mo for the first year, every 3 to 4 mo for the next 2 y, and every 6 mo

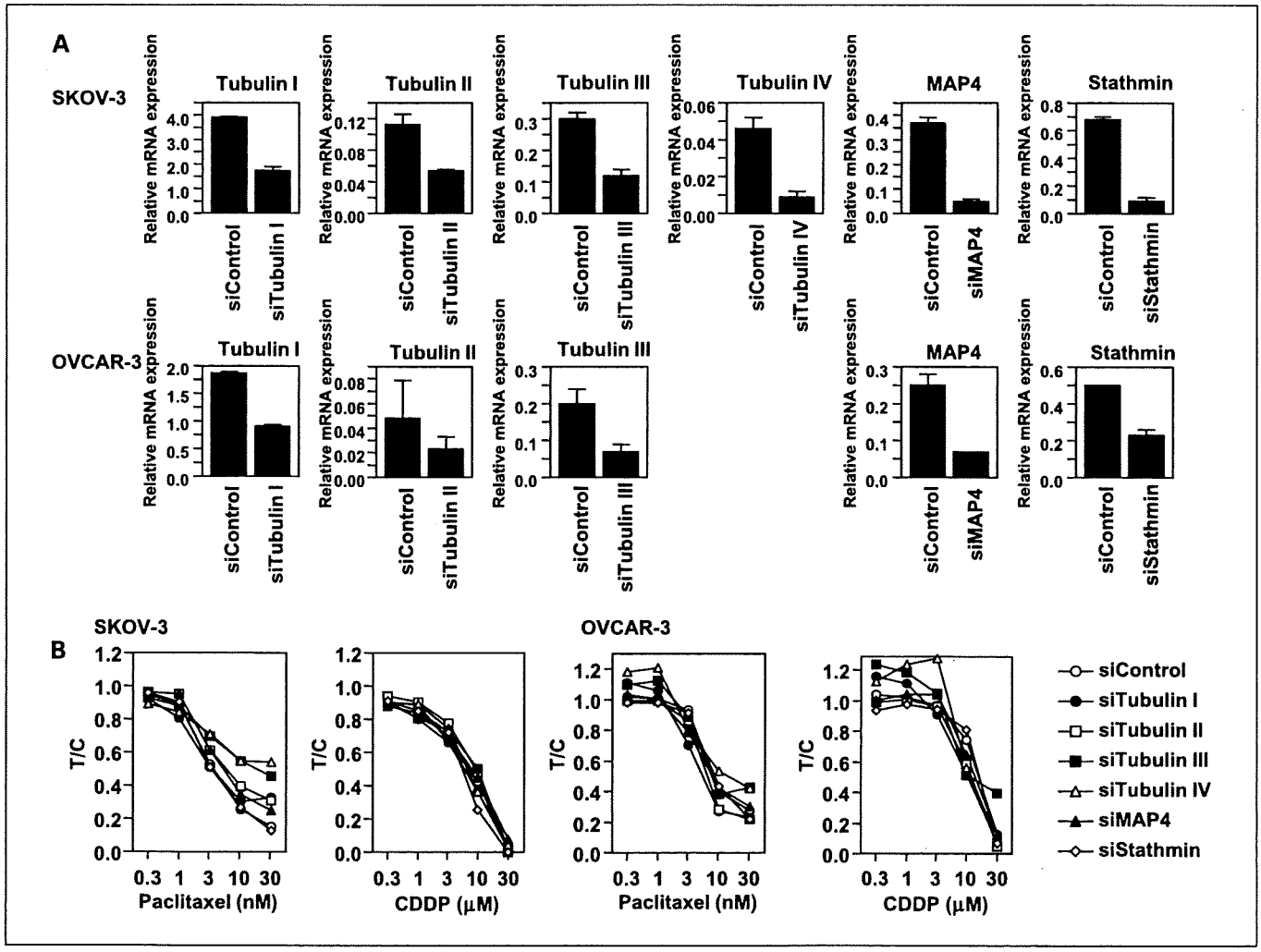

Fig. 1. Drug sensitivity to paclitaxel or cisplatin in human ovarian cancer cells treated with siRNA for β-tubulin isoforms, MAP4, and stathmin. *A*, mRNA expression of β-tubulin isoforms (I, II, III, IV), MAP4, and stathmin after treatment with respective siRNA for 48 h were determined by real-time PCR analysis. The expression of β-tubulin IV mRNA in OVCAR-3 cells was not detected. *B*, cells treated with respective siRNA were seeded into 96-well plates at 2 × 10^3 cells/0.1 mL/well and incubated overnight. On the following day, a 100-μL aliquot containing paclitaxel or cisplatin was added to the wells and cultured for a further 3 d. The number of viable cells was estimated using the WST-8 assay. The assays were carried out in quadruplicate.

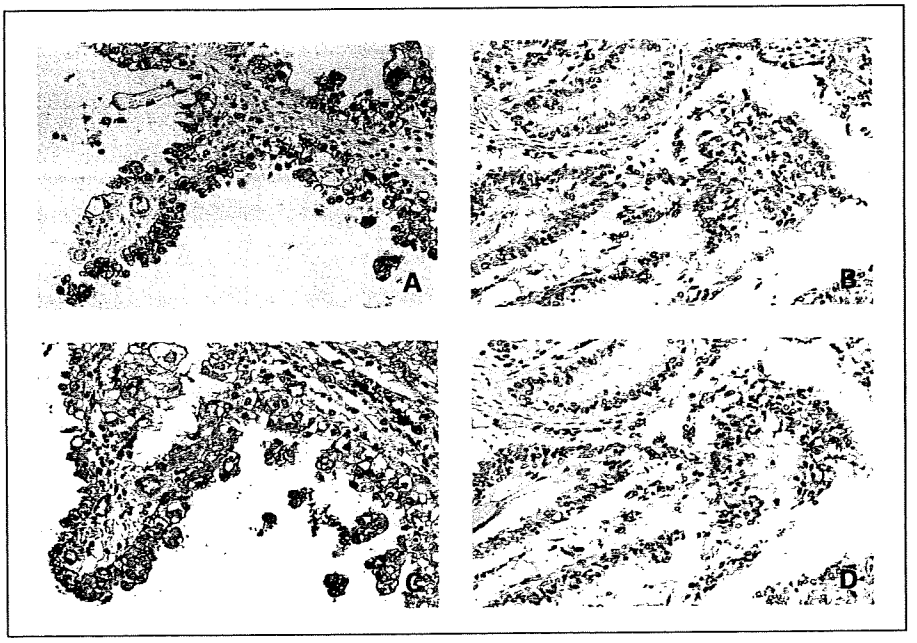

Fig. 2. *A* and *C*, stage IIc clear cell adenocarcinoma of a 55-year-old woman treated with the paclitaxel-carboplatin regimen. Cytoplasmic strong expression of stathmin (*A*, >15%) and β-tubulin III (*C*, score 7) can be diffusely observed in the tumor cells. The patient currently shows no evidence of disease 2,487 d (83 mo) after surgery. *B* and *D*, stage IIc tumor of a 56-year-old woman treated with the paclitaxel-carboplatin regimen. A few tumor cells (<15%) show only faint immunoreactivity for stathmin in the cytoplasm or nuclei, which was judged as negative (*C*). β-Tubulin III can be recognized in 10% of tumor cells with intermediate intensity and was interpreted as negative (score 4; *D*). This patient died of disease 447 d (15 mo) after initial surgery.

thereafter. Clinical outcome was measured by PFS and OS. PFS was defined as the interval from the date of first treatment (laparotomy or the first administration of neoadjuvant chemotherapy) to the date of the diagnosis of progression. We obtained informed consent from all patients, and personal information was removed from all samples before analysis.

Immunohistochemistry. Surgically resected specimens were fixed with 10% formalin and embedded in paraffin. Sections 4-μm thick on silane-coated slides were stained using the streptavidin-biotin-peroxidase method with a Histofine SAB-PO kit (Nichirei) according to the manufacturer's instructions. At least one representative section without degenerative change or necrosis was examined in each tumor. After deparaffinization, rehydration, and inhibition of endogenous peroxidase, sections were exposed to the primary antibodies at 4°C overnight. The dilutions of the primary antibody were as follows: MAP4, 1:1500; stathmin, 1:1000; and β-tubulin III, 1:200. After incubation of the secondary antibody and the streptavidin-biotin complex at room temperature, the sections were then incubated in 3 3′- diaminobenzidine, counterstained with hematoxylin, and mounted. For all antibody staining, sections were pretreated with microwave irradiation for antigen retrieval.

Immunohistochemical results were evaluated and scored by three pathologists (Y. Oda, K. Taguchi, and Y. Ohishi) without knowledge of patient clinical data. MAP4 and stathmin immunoreactivity was scored by estimating the percentage of labeled tumor cells. When >80% of the tumor cells showed immunoreactivity for MAP4, we judged the case to be positive. For stathmin expression, the cutoff value was 15%, based on a previous study (25). For class III β-tubulin expression, we evaluated the proportion and intensity of the immunoreactive cells following the protocol used to evaluate estrogen/progesterone receptors in breast cancer, proposed by Allred et al. (26, 27). Cases with a total score of ≥7 were regarded as positive.

Statistical analysis. Statistical analysis was conducted for OS and PFS to examine the effects of MAP4, stathmin, and β-tubulin III on taxane efficacy. Product-limit estimators of survival functions were obtained, respectively, relative to positivity and negativity of each marker in the patients to investigate the relationship between regimens and markers. To adjust for possible confounding factors, Cox proportional hazards models were applied. The covariates considered were a treatment indicator (0, taxane-free regimen; 1, taxane-based regimen), marker (0, negative; 1, positive), their interaction, age, two dummy variables representing FIGO stage and peritoneal cytodiagnosis (FIGO stage I-II with peritoneal cytodiagnosis negative, FIGO stage I-II with peritoneal cytodiagnosis positive, and FIGO stage III-IV) and size of residual tumor (0, <1 cm; 1, ≥1 cm).

Taking into account the size of the dataset, the latter four covariates were summarized into a propensity score (28, 29) by fitting logistic regression models with those variables to the data. The primary interest

Table 1. Correlation between positive or negative expression of MAP4, stathmin, and β-tubulin III and tumor stage or residual tumor

	FIGO stage		Residual tumor	
	I/II (*n* = 67)	III/IV (*n* = 27)	No (*n* = 74)	Yes (*n* = 20)
	No. of patients (%)	No. of patients (%)	No. of patients (%)	No. of patients (%)
MAP4 (-)	36 (54)	12 (44)	39 (53)	9 (45)
MAP4 (+)	31 (46)	15 (56)	35 (47)	11 (55)
Stathmin (-)	29 (43)	11 (41)	33 (45)	7 (35)
Stathmin (+)	38 (57)	16 (59)	41 (55)	13 (65)
β-tubulin III (-)	30 (45)	11 (41)	33 (45)	8 (40)
β-tubulin III (+)	37 (45)	16 (59)	41 (55)	12 (60)

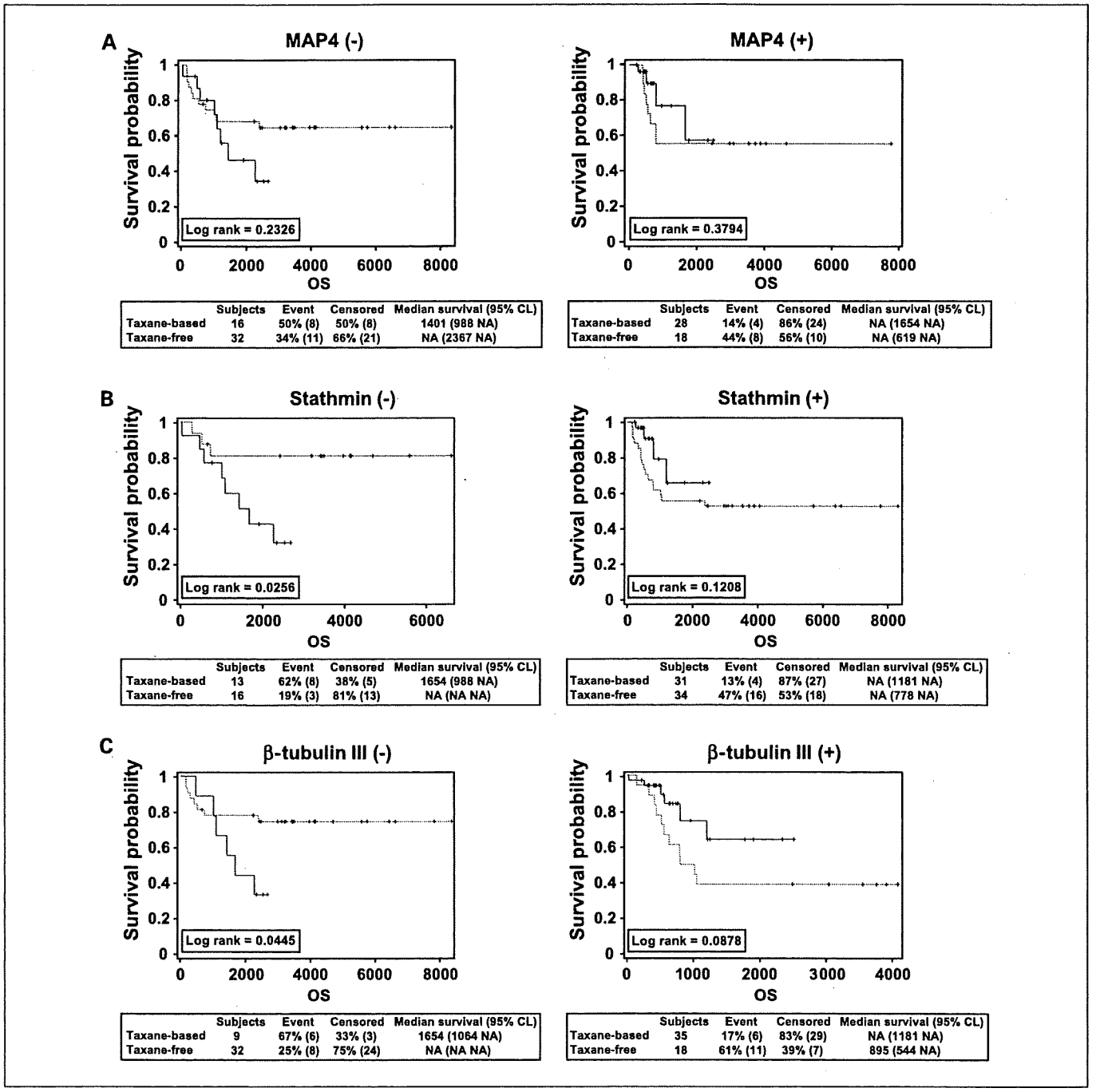

Fig. 3. The product-limit estimator of OS by regimens and microtubule-associated molecules. *A*, the product-limit estimators of OS for MAP4-negative patients (*left panel*) and for positive patients (*right panel*). *B*, the product-limit estimators of OS for stathmin-negative patients (*left panel*) and for positive patients (*right panel*). *C*, the product-limit estimators of OSfor β-tubulin III negative patients (*left panel*) and for positive patients (*right panel*). Solid lines, survival functions of patients receiving the taxane-based regimen; broken line, taxane-free regimen.

was the effect of the interaction between treatment and marker. With the supposition that the effect of a taxane-based regimen for marker-negative patients equals *A* and that of the taxane-free regimen for positive patients equals *B*, the significance of the interaction shows that the effect of the taxane-based regimen for the marker-positive patients is greater than *A+B* (i.e., it is synergistic). Evidence of a synergistic effect indicates that the effect of taxane is dependent on the status of the marker, showing the marker plays an important role in the effect of taxane. The cutoff points that determined positive and negative for each marker were chosen by the Akaike's information criterion so that the Cox model fitted best to the data (30).

Results

Effects of reducing expression of β-tubulin isoforms, MAP4, and stathmin on drug sensitivity to paclitaxel and cisplatin in ovarian cancer cells. We first examined whether gene silencing of

Table 2. Summary of interaction terms for the Cox regression

	MAP4	Stathmin	β-tubulin III
OS			
Regression coefficient (95% CI)	-0.77 (-2.50 to 0.96)	-1.37 (-3.17 to 0.43)	-1.68 (-3.16 to 0.21)
P	0.383	0.135	0.026
PFS			
Regression coefficient (95% CI)	-0 40 (-1.91 to 1.12)	-0.85 (-2.43 to 0.72)	-1.52 (-2.90 to -0.15)
P	0.608	0.288	0.030

Abbreviation: 95% CI, 95% confidence interval.

β-tubulin isoforms, MAP4, and stathmin could affect drug sensitivity to paclitaxel and cisplatin in the cultured human ovarian cancer cell lines SKOV-3 and OVCAR-3. Cellular mRNA expression levels of these genes in two human ovarian cancer cell lines were all markedly down-regulated when treated with respective siRNA (Fig. 1A). We then examined the drug sensitivities of paclitaxel or cisplatin in ovarian cancer cells treated with siRNA of the tubulin isoforms, MAP4, and stathmin (Fig. 1B). When β-tubulin III or β-tubulin IV was silenced, the IC_{50} values of paclitaxel increased to 16.8 nmol/L and 14.3 nmol/L, respectively, from the control IC_{50} value of 3.9 nmol/L in SKOV-3 cells (Fig. 1B). By contrast, down-regulation of β-tubulins I and II, MAP4, and stathmin did not influence the sensitivity to paclitaxel in SKOV-3 cells. Down-regulation of β-tubulins I, II, III, and IV, MAP4, and stathmin did not influence sensitivity to cisplatin in either cell line (Fig. 1B). Two independent experiments consistently showed the acquisition of drug resistance to paclitaxel in SKOV-3 by knockdown of β-tubulins III and IV.

Immunohistochemistry of MAP4, stathmin, and β-tubulin III in human ovarian clear cell adenocarcinomas. Clinical and pathologic characteristics at diagnosis are summarized in Supplementary Table S1. The median age of the patients was 52 years (range, 29-74 years). Sixty tumors were considered to be stage I, 7 stage II, 20 stage III, and 7 stage IV. Sixteen patients who had residual tumors more than 1 cm in maximum diameter were classified into the suboptimal group, whereas 78 patients were placed in the optimal group with a residual tumor ≤1 cm, including 74 complete resections. The median follow-up for PFS for all 94 patients was 749 days (range, 23-8,318 days), whereas the median follow-up for OS was 995 days (range, 23-8,318 days). The median follow-up of those patients who are currently progression-free is 2,399 days (range, 212-8,318 days).

Table 3. Hazard ratios (marker positive/marker negative) for subpopulations of taxane-based regimen and taxane-free regimen by the Cox proportional hazards models; two-tailed 95% confidence intervals are given in parenthesis

Marker hazard ratio (95% CI)	Taxane-based therapy	Taxane-free therapy	*P*
Overall survival			
MAP4	0.42 (0.11-1.66)	0.91 (0.35-2.40)	0.383
Stathmin	0.96 (0.26-3.53)	3.78 (1.07-13.34)	0.135
β-tubulin III	0.72 (0.22-2.44)	3.91 (1.49-10.23)	0.026
Progression-free survival			
MAP4	0.53 (0.17 -1.69)	0.79 (0.31-2.02)	0.608
Stathmin	1.11 (0.36-3.41)	2.60 (0.85-7.96)	0.288
β-tubulin III	0.77 (0.26-2.31)	3.52 (1.37-9.01)	0.030

NOTE: Age, FIGO stage, peritoneal cytodianosis, and size of residual tumor were adjusted by the propensity scores representing the four covariates.
P values are based on Wald tests for interaction of taxane with the marker.

The cytoplasmic positive expression of MAP4 was detected in 46 tumors (49%). Positive immunostaining for stathmin was found in 54 tumors (57%), predominantly as cytoplasmic staining (Fig. 2A). β-Tubulin III immunostaining was positive in 53 (56%) tumors with total scores of 7 or 8 (Fig. 2C). Positive MAP4 and β-tubulin III expression was frequent in tumors treated with taxane-containing regimens, compared with tumors treated with taxane-free regimens (Supplementary Table S1). Stathmin-positive tumors were also more frequent in patients with the taxane-based regimen, although the difference failed to reach statistical significance. There were no measurable differences in immunoreactivities for these proteins with respect to either tumor stage or residual tumor (Table 1).

Effects of β-tubulin III expression on survival in human ovarian clear cell adenocarcinomas. In Fig. 3A, the product-limit estimators for OS of patients administered the taxane-free and taxane-based regimens are shown for the MAP4-negative group (*left panel*) and for the MAP4-positive group (*right panel*). The survival outcome seemed to be less favorable for the taxane-based regimen than for the taxane-free regimen in the MAP4-negative group, although the difference was not statistically significant ($P = 0.23$); there was no difference in survival between the two regimens in the MAP4-positive group ($P = 0.38$). Paclitaxel treatment was also associated with a poorer survival in the stathmin-negative patients ($P = 0.03$); there was a trend to a better survival in the group of stathmin-positive patients ($P = 0.12$), as shown in Fig. 3B.

Survival associated with paclitaxel treatment was more evidently differential based on β-tubulin III status. In the absence of β-tubulin III expression, survival was significantly shorter in patients with the taxane-based regimen compared with those with the taxane-free regimen ($P = 0.04$), and the opposite was the case in the presence of β-tubulin III expression ($P = 0.09$; Fig. 3C). Table 2 gives the estimates, confidence intervals, and *P* values for the hazard ratios of the interaction. The table shows that for β-tubulin III, *P* values were 0.026 for OS and 0.030 for PFS. Thus, β-tubulin III seems to determine the efficacy of the taxane-based regimen. Table 2 also shows that for stathmin, *P* was 0.135 and the hazard ratio was 0.25 (95% confidence interval, 0.04-1.53) for OS, and 0.288 and 0.43, respectively (95% confidence interval, 0.09-2.06), for PFS.

Thus, stathmin may also determine the efficacy of the taxane-based regimen, but the effect was not statistically significant. Furthermore, Table 2 shows that for MAP4, the estimated hazard ratios were far from 1 but were not statistically significant (0.383 for OS and 0.673 for PFS).

The statistical significance of the interaction of taxane with β-tubulin III shown in Table 2 indicates that the efficacy of taxane depends on β-tubulin III positivity or negativity. To interpret this interaction precisely, we give the hazard ratio of the taxane-based regimen relative to the taxane-free regimen separately for β-tubulin III–positive and -negative patients. Table 3 gives the hazard ratios for patients who were positive for β-tubulin III relative to patients who were negative; these ratios are given separately for the taxane-based and taxane-free regimens. The table shows that the hazard ratio for OS was 3.91 for the taxane-free regimen but was 0.72 for the taxane-based regimen. This outcome indicates that being positive for β-tubulin III is related to a poor prognosis in the taxane-free regimen group, but that the taxane-based regimen may prolong OS for patients who are β-tubulin III–positive.

Discussion

Class III β-tubulin overexpression has been reported to be a marker of poor clinical outcome in patients with advanced ovarian cancer mainly containing serous type adenocarcinoma. With treatment using platinum/paclitaxel therapy (16), expression of class III β-tubulin also predicts response and outcome in patients with non–small cell lung cancer and in those with breast cancer who are treated with taxane-based chemotherapy (31, 32). In this study, we investigated which targets could be responsible for the therapeutic efficacy of taxane-based chemotherapy against ovarian clear cell adenocarcinoma patients when treated with either cisplatin/cyclophosphamide or cisplatin/taxane. Immunohistochemical staining was done for the surgically resected specimens using antibodies against class III β-tubulin, MAP4, and stathmin. Of these three targeting molecules, expression of class III β-tubulin was significantly associated with therapeutic efficacy of taxane-based chemotherapy, but not with taxane-free chemotherapy. Moreover, our present study showed that increased expression of class III β-tubulin significantly affected outcome for patients with ovarian clear cell adenocarcinoma in the taxane-treated patient group.

Our present finding is not consistent with those of previous studies identifying a close association of class III β-tubulin overexpression with poor therapeutic efficacy of taxane-based chemotherapy against ovarian cancers, including most non–clear cell adenocarcinomas (14–16). Of β-tubulin isoforms, microtubules containing tubulin III or IV were more dynamic and less stable than microtubules containing other tubulin types (13, 33), suggesting that cellular expression of β-tubulin isotype III or IV plays a critical role in drug sensitivity to paclitaxel *in vitro*. Paclitaxel-selected drug-resistant cancer cell lines derived from human lung, breast, pancreas, and prostate cancers and glioblastoma often exhibit enhanced expression of β-tubulin III (34). Kavallaris et al. have previously reported increased mRNA expression of β-tubulins III and IV in taxane-treated ovarian tumor samples as compared with primary untreated ovarian tumors (35). However, Nicolletti et al. have reported no correlation between tubulin expression and paclitaxel sensitivity in mouse xenografts of human ovarian carcinomas (36).

In our present study, knockdown of class III and IV β-tubulin genes but not of other tubulin isoforms specifically decreased drug sensitivity to paclitaxel in one ovarian cancer cell line, indicating the possible involvement of these tubulin isoforms in the dynamics of microtubules. At present, it remains unclear why decreased expression of type III β-tubulin differentially modulates drug sensitivity to paclitaxel among various cancer cell lines *in vitro*, and this finding requires further study. A complex network system among microtubule-related factors, including tubulin isoforms, operates in limiting drug sensitivity to taxanes; however, the results of our present study together with those of previous reports could present a novel notion that expression levels of class III β-tubulin might thus predict the therapeutic efficacy of taxane-based therapy. This effect would depend on differences in pathologic subtype between serous adenocarcinoma and clear cell adenocarcinoma.

We also found that OS of patients with lower expression of MAP4, stathmin, and β-tubulin indicated better therapeutic efficacy with non–taxane-based chemotherapy compared with taxane-based treatment. In patients with higher expression of stathmin and MAP4, these relationships were reversed but not statistically significant. Although these appeared during follow-up periods of the taxane-based therapy group for as long as 3,000 days, low expression of these three targeting molecules might predict poor prognosis for patients with ovarian clear cell adenocarcinoma.

Altered expression of proteins that regulate microtubule dynamics also mediates paclitaxel resistance in cancer cells *in vitro* through interaction with tubulin dimers or polymerizing microtubules. These proteins include stathmin, a microtubule destabilizer, and MAP4, a microtubule stabilizer (34). Altered expression of stathmin (5, 6) and MAP-4 (37) induces marked changes in drug sensitivity of cancer cells to taxanes. Further study is required to understand whether the above mechanisms *in vitro* underlie the poor therapeutic efficacy of taxane-based chemotherapy for patients with low expression of stathmin and MAP4, as well as β-tubulin. On the other hand, increased expression of stathmin also was associated (but not significantly) with an improved therapeutic efficacy of taxane-based chemotherapy in comparison with that of taxane-free therapy. Further study with a larger number of patients as well as longer follow-up periods may predict whether stathmin can be a marker for therapeutic efficacy of taxane-based therapy against ovarian clear cell adenocarcinoma.

In conclusion, our present study showed that overexpression of type III β-tubulin was a predictive marker of better prognosis for patients with ovarian clear cell adenocarcinoma when they are treated with taxane-based chemotherapy. This finding is not consistent with those involving patients with other serous type carcinoma treated by taxane-based chemotherapy, suggesting that association of β-tubulin expression with therapeutic efficacy by taxane-based chemotherapy depends on the pathologic characteristics of ovarian cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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