と比較して-：第45回日本癌治療学会総会： 10月24-26日.京都.

18. 川上洋介、竹原和宏、他：卵巣がんにおける腫瘍抑制遺伝子 *RUNX3* のエピジェネティックな異常：第45回日本癌治療学会総会: 10月24-26日、2007、京都.
19. 島田宗昭、八重樫伸夫、竹原和宏、他：子宮頸部腺癌に対する放射線療法の意義：第45回日本癌治療学会総会： 10月24ー26日、2007、京都.
20. Shimada M, Yaegashi N, K Takehara, Hiura M, et al : The significance of radiotherapy for adenocarcinoma of uterine cervix : ESGO : 10. 28-11. 1. Berlin.
21. Harada M, Takehara K, et al: MICROINVASIVE MUCINOUS ADENOCARCINOMA OF ENDOCERVICAL TYPE WITH ADENOMA MALILGNUM-LIKE FEATURES OF UTERINE CERVIX: A CASE REPORT：第6回日韓細胞診合同会議： 11月3日、釜山市

H. 知的財産権の出願・登録状況（予定含）

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

研究成果の刊行に関する一覧表

書籍：該当なし

雑誌：

発表者氏名	論文 タイトル名	発表誌名	巻号	ページ	出版年
Onda T, Konishi I, Kamura T, Yoshikawa H, et al.	Phase Ⅲ trial of upfront debulking surgery versus neoadjuvant chemotherapy for stage Ⅲ/Ⅳ ovarian, tubul and peritoneal cancers: Japan clinical oncology group study JCOG0602.	Jpn J Clin Oncol	38(1)	74-77	2008
恩田貴志	【Neoadjuvant Therapy の適応と効用・II】 卵巣癌	癌と化学療法	34(11)	1735-0739	2007
Nishio S, Kamura T, et al.	Pilot study evaluating the efficacy and toxicity of irinotecan plus oral etoposide for platinum-and taxane - resisitant epithelial ovarian cancer.	Gynecol Oncol	106(2)	342-347	2007
Sakuma M, Yaegashi N, et al.	Promoter methylation status of the Cyclin D2 gene is associated with poor prognosis in human epithelial ovarian cancer.	Cancer Science	98(3)	380-386	2007
Takano M, et al.	Progression-Free Survival and Overall Survival of Clear Cell Carcinoma of the Ovary Treated with Paclitaxel-Carboplatin or Irinotecan-Cisplatin: Retrospective analysis.	International Journal of Clinical Oncology	12(4)	256-260	2007
Osada R, Konishi I, et al.	Expression of hypoxia-inducible factor-1α, HIF-α and von Hippel-Lindau (VHL) protein in epithelial ovarian neoplasms: Nuclear expression of hypoxia-inducible factor-1α is an independent prognostic factor in ovarian carcinoma.	Hum Pathol	38(9)	1310-1320	2007
Nakai H, Hoshiai H, et al.	Hypoxia inducible factor 1-alpha expression as a factor predictive of efficacy of taxane/platinum chemotherapy in advanced primary epithelial ovarian cancer.	Cancer Lett	251(1)	164-167	2007
Teramukai S, Ochiai K, et al.	PIEPOC: a new prognostic index for advanced epithelial ovarian cancer —Japan Multinational Trial Organization OC01-01.	J Clin Oncol	25(22)	3302-3306	2007

Jpn J Clin Oncol 2008;38(1)74–77
doi:10.1093/jjco/hym145

Phase III Trial of Upfront Debulking Surgery Versus Neoadjuvant Chemotherapy for Stage III/IV Ovarian, Tubal and Peritoneal Cancers: Japan Clinical Oncology Group Study JCOG0602

Takashi Onda[1], Koji Matsumoto[2], Taro Shibata[3], Akihiro Sato[3], Haruhiko Fukuda[3], Ikuo Konishi[4], Toshiharu Kamura[5] and Hiroyuki Yoshikawa[6]

[1]Division of Gynecologic Oncology, National Cancer Center Hospital, Tokyo, [2]Division of Medical Oncology, Hyogo Cancer Center, Akashi, Hyogo, [3]JCOG Data Center, Center for Cancer Control and Information Services, National Cancer Center, Tokyo, [4]Department of Obstetrics and Gynecology, Shinshu University, Matsumoto, [5]Department of Obstetrics and Gynecology, Kurume University School of Medicine, Kurume, Fukuoka and [6]Department of Obstetrics and Gynecology, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan

Received May 11, 2007; accepted October 2, 2007

On the basis of promising results of neoadjuvant chemotherapy (NAC) in our previous study (JCOG0206), we have been performing a Phase III study of treatment starting with NAC versus standard treatment starting with primary debulking surgery (PDS) for Stage III/IV müllerian carcinomas (ovarian, tubal and peritoneal carcinomas) since November 2006. The purposes are to prove the non-inferiority of the efficacy and to show the decrease in adverse effects resulting from reduced surgical invasiveness of treatment starting with NAC. Three hundred patients with advanced müllerian carcinomas will be randomized during 3 years. NAC arm patients undergo four cycles of NAC with paclitaxel plus carboplatin followed by interval debulking surgery and an additional four cycles of postsurgical chemotherapy. Standard arm patients undergo PDS and eight cycles of postsurgical chemotherapy with or without interval debulking surgery. The primary endpoint is overall survival. The major secondary endpoints are the incidence of adverse events and parameters representing surgical invasiveness.

Key words: ovarian neoplasms – neoadjuvant therapy – interval debulking surgery – primary debulking surgery

INTRODUCTION

The current standard treatment for advanced müllerian cancer is primary debulking surgery (PDS) followed by postsurgical chemotherapy. Better prognosis can be expected in cases in which optimal debulking can be achieved. Unfortunately, optimal debulking in the primary surgery can be achieved in only 30–60% of Stage III/IV müllerian cancers in average institutions (1,2), and the prognosis of patients with advanced müllerian cancers is poor. Neoadjuvant chemotherapy (NAC) has been recognized as a possible approach to improve the prognosis of these patients. In initial studies, NAC was chosen for patients with apparently unresectable bulky tumors or poor performance status as an alternative treatment to primary surgical debulking. Retrospective analyses (3–7) revealed that progression-free and overall survival were comparable between patients treated with NAC followed by interval debulking surgery (IDS) and those treated with PDS, though the former group had more advanced disease and poorer performance status. On the basis of these favorable results of NAC for patients with advanced disease or poor performance status, the target disease was extended to all cases of advanced disease, including patients without apparently unresectable tumors and good performance status in prospective studies. The European Organization for Research and Treatment of Cancer (EORTC) is conducting a Phase III study comparing neoadjuvant setting treatment with standard treatment for advanced müllerian cancers (8). We conducted a Phase II study of NAC with paclitaxel plus carboplatin followed by IDS and postsurgical chemotherapy as the study of the Japan

For reprints and all correspondence: Takashi Onda, Division of Gynecologic Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. E-mail: taonda@ncc.go.jp

Clinical Oncology Group (JCOG0206) (9). In the study, we assessed the safety and efficacy of NAC treatment, and also assessed whether we can accurately diagnose advanced müllerian cancer based on clinical findings, including imaging studies, cytologic findings and tumor markers. Although the final survival results of this Phase II study are awaited, we have started the Phase III trial on the basis of the efficacy and diagnostic accuracy shown in the study (10). Our study is basically similar to the EORTC study, with the aim of comparing NAC treatment with standard treatment for advanced müllerian cancer. One of the distinct points of our study is omitting the diagnostic surgical procedure, such as laparoscopy or laparotomy, based on the results of our above-mentioned previous study. This means the elimination of an extra procedure for the purpose of the clinical trial in both treatment arms and it has the advantage of making it possible to start NAC treatment earlier. In our study, it is possible to compare the two treatment protocols under clinically relevant conditions. Another distinct point is the number of cycles of chemotherapy. Since the study subjects are patients with evidently advanced disease according to clinical findings, we administer a total of eight cycles of chemotherapy in both treatment arms instead of the standard of six cycles.

The study protocol was designed by the Gynecologic Cancer Study Group (GCSG) of the Japan Clinical Oncology Group (JCOG), approved by the Protocol Review Committee of JCOG on 18 October 2006 and activated on 17 November 2006. This trial was registered at the UMIN Clinical Trials Registry as UMIN000000523 (http://www.umin.ac.jp/ctr/index.htm).

PROTOCOL DIGEST OF THE JCOG0602

Purpose

The purposes are to prove the non-inferiority of the efficacy and to show the decrease in adverse effects due to reduced surgical invasiveness of treatment starting with NAC with paclitaxel plus carboplatin compared with standard treatment starting with PDS for stage III/IV müllerian carcinomas.

Study Setting

A multi-institutional (30 centers) randomized Phase III trial.

Resources

Health Sciences Research Grants for the Third Term Comprehensive Control Research for Cancer (Nos. h16-035, h19-028) and Grants-in Aid for Cancer Research (Nos. 17S-1, 17S-5, 17-12), from the Ministry of Health, Labor and Welfare, Japan.

Endpoints

The primary endpoint is overall survival among all eligible patients. Secondary endpoints concerning the efficacy of the treatments are as follows: (i) proportion of clinical complete remission (%cCR) among all eligible patients, (ii) progression-free survival among all eligible patients, (iii) response rate to NAC among patients assigned to the NAC arm. Clinical complete remission is defined as the disappearance of all lesions by computed tomography (CT) or magnetic resonance imaging (MRI), no pleural effusions by chest radiography and normal serum CA125 level (<20 U/ml) after completion of the protocol treatment. Secondary endpoints concerning the safety and surgical invasiveness of the treatments are as follows: (i) adverse events, (ii) number of times of surgery, (iii) total duration of the surgery, (iv) total amount of blood loss, (v) amount of blood transfusion during protocol treatment, (vi) amount of blood plasma, plasma expander and albumin infusion during protocol treatment, among all treated patients.

Eligibility Criteria

Inclusion Criteria

The study subjects are patients diagnosed with Stage III or IV ovarian, tubal or peritoneal carcinoma. The diagnosis is based on both imaging studies (CT or MRI, and chest radiography) and cytology/histology of ascites, pleural effusion or fluid/tissue obtained by tumor centesis. Malignancies of other origins, such as breast and digestive tract, should be excluded by endoscopy, opaque enema, or ultrasonography when these malignancies are suspected from symptoms, physical examination or imaging diagnosis. To rule out malignancies of digestive tract origin, the criteria for tumor markers are set to be CA125 >200 U/ml and CEA <20 ng/ml.

Further inclusion criteria are (i) the patient is clinically deemed to be a candidate for debulking surgery without evidence of brain, bone or bone marrow metastases, (ii) previously untreated for these malignancies and have no history of treatment with chemotherapy or radiotherapy for other diseases, (iii) age 20–75 years, (iv) Eastern Cooperative Oncology Group (ECOG) performance status of 0–3, (v) adequate bone marrow, hepatic, renal, cardiac and respiratory functions and (vi) written informed consent.

Exclusion Criteria

Exclusion criteria are (i) synchronous or metachronous (within 5 years) malignancy other than carcinoma *in situ*, (ii) pregnant or nursing, (iii) severe mental disorder, (iv) systemic and continuous use of steroidal drugs, (v) positive for serum hepatitis B surface antigen, (vi) active infections, (vii) uncontrolled hypertension, (viii) diabetes mellitus, uncontrolled or controlled with insulin, (ix) history of cardiac failure, unstable angina, myocardial infarction within 6 months prior to registration, (x) intestinal occlusion requiring surgical treatment, (xi) hypersensitivity to polyoxyethylated castor oil and (xii) hypersensitivity to alcohol.

Treatment Methods

Standard Treatment Arm

Primary debulking surgery. PDS is performed within 4 weeks of study enrollment. Standard procedures for PDS consist of total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and maximal debulking of metastatic tumors. Systematic pelvic and/or aortic lymphadenectomies are allowed.

Postsurgical chemotherapy. Eight cycles of a combination of paclitaxel (175 mg/m^2, Day 1) and carboplatin (AUC = 6, Day 1), namely the TC regimen, are administered every 3 weeks. Postsurgical chemotherapy is initiated within 3–5 weeks after PDS, according to the invasiveness of the surgery.

Interval debulking surgery. IDS is required when any of the standard procedures is not completed at PDS. IDS is allowed, as an option, when residual tumor larger than 1 cm is left at PDS. In such cases, IDS is performed 4–7 weeks after administration of the fourth cycle of postsurgical chemotherapy unless there is disease progression. The standard goal of IDS is completion of all standard procedures of PDS and maximal debulking of metastatic tumors. Systematic pelvic and/or aortic lymphadenectomies are allowed, but not included in the standard goal of IDS. Following IDS, four additional cycles of chemotherapy (TC regimen) is administered (eight cycles of chemotherapy in total). The chemotherapy is initiated within 3–5 weeks after IDS, according to the invasiveness of the surgery.

NAC Arm

Neoadjuvant chemotherapy. Four cycles of a combination of paclitaxel (175 mg/m^2, Day 1) and carboplatin (AUC = 6, Day 1) are administered every 3 weeks. NAC is initiated within 2 weeks of study enrollment.

Interval debulking surgery. IDS is performed 4–7 weeks after administration of the fourth cycle of NAC unless disease progression occurs during NAC. Standard procedures of IDS consist of total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and maximal debulking of metastatic tumors. Systematic pelvic and/or aortic lymphadenectomies are allowed, but not included in standard procedures.

Postsurgical chemotherapy. Four additional cycles of chemotherapy (TC regimen) are administered (8 cycles of chemotherapy in total). Postsurgical chemotherapy is initiated within 3 to 5 weeks after IDS, according to the invasiveness of the surgery.

Study Design and Statistical Methods

The study is designed as a randomized Phase III non-inferiority study. Patients are randomized to each treatment arm by a minimization method with institution, Stage (III or IV), PS (0, 1 or 2, 3) and age (<60 or ≥ 60) as balancing factors at the JCOG Data Center. The planned accrual period is 3 years and the follow-up period is set as 5 years after completion of accrual. The hazard ratio between treatment arms and its confidence interval, estimated by the Cox proportional hazard model stratified with stage, PS and age is used to test the non-inferiority of the NAC treatment regarding overall survival. The significance level is set as 0.05 in a one-sided test because of the non-inferiority design of the study. Two hundred seventy-six events would provide a statistical power of 80%, to conclude that the NAC arm is not inferior to the standard arm in 3-year overall survival with a non-inferiority margin of 5%, and non-inferiority is concluded if the upper limit of the confidence interval of the hazard ratio does not exceed the limit of 1.161, which is in accord with the non-inferiority margin. A sample size of 298 patients is necessary to observe 276 events, considering the accrual and follow-up period, an estimated 3-year overall survival of 25% in the standard treatment arm and an expected 3-year overall survival of 30.3% in the NAC arm. Thus, the target sample size of 300 patients (150 patients per regimen) was set. Interim analysis is planned after half of the patients are enrolled, taking into account the multiplicity with the O'Brien Fleming type alpha spending function.

Study Monitoring

In-house interim monitoring is performed by the JCOG Data Center to ensure data submission, patient eligibility, protocol compliance, safety and on-schedule study progress according to standard JCOG procedures. Monitoring reports are submitted to the investigators in GCSG and the JCOG Data and Safety Monitoring Committee every 6 months.

Participating Institutions

Hokkaido University, Sapporo Medical University, Tohoku University, University of Tsukuba, National Defense Medical College, Saitama Cancer Center, Saitama Medical Center, National Cancer Center Hospital, The Jikei University School of Medicine, Cancer Institute Hospital, University of Tokyo, Juntendo University, Kitasato University, Niigata Cancer Center Hospital, Shinshu University, Aichi Cancer Center, National Hospital Organization Nagoya Medical Center, Kinki University, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka City General Hospital, Osaka City University, Hyogo Cancer Center, Tottori University, National Hospital Organization Kure Medical Center, National Hospital Organization Shikoku Cancer Center, National Hospital Organization Kyushu Cancer Center, University of Kurume, Kyushu University, Saga University and Kagoshima City Hospital.

References

1. Covens AL. A critique of surgical cytoreduction in advanced ovarian cancer. *Gynecol Oncol* 2000;78:269–74.

2. Dauplat J, Le Bouedec G, Pomel C, Scherer C. Cytoreductive surgery for advanced stages of ovarian cancer. *Semin Surg Oncol* 2000;19:42–8.
3. Jacob JH, Gershenson DM, Morris M, Copeland LJ, Burke TW, Wharton JT. Neoadjuvant chemotherapy and interval debulking for advanced epithelial ovarian cancer. *Gynecol Oncol* 1991;42:146–50.
4. Onnis A, Marchetti M, Padovan P, Castellan L. Neoadjuvant chemotherapy in advanced ovarian cancer. *Eur J Gynaecol Oncol* 1996;17:393–6.
5. Schwartz PE, Rutherford TJ, Chambers JT, Kohorn EI, Thiel RP. Neoadjuvant chemotherapy for advanced ovarian cancer: long-term survival. *Gynecol Oncol* 1999;72:93–9.
6. Vergote I, De Wever I, Tjalma W, Van Gramberen M, Decloedt J, van Dam P. Neoadjuvant chemotherapy or primary debulking surgery in advanced ovarian carcinoma: a retrospective analysis of 285 patients. *Gynecol Oncol* 1998;71:431–6.
7. Kayikçioğlu F, Kose MF, Boran N, Caliskan E, Tulunay G. Neoadjuvant chemotherapy or primary surgery in advanced epithelial ovarian carcinoma. *Int J Gynecol Cancer* 2001;11:466–70.
8. Vergote I, de Wever I, Tjalma W, Van Gramberen M, Decloedt J, Van Dam P. Interval debulking surgery: an alternative for primary surgical debulking? *Semin Surg Oncol* 2000;19:49–53.
9. Onda T, Kamura T, Ishizuka N, Katsumata N, Fukuda H, Yoshikawa H. Feasibility study of neoadjuvant chemotherapy followed by interval cytoreductive surgery for stage III/IV ovarian, tubal and peritoneal cancers: Japan Clinical Oncology Group Study JCOG0206. *Jpn J Clin Oncol* 2004;34:43–5.
10. Onda T, Kamura T, Hirakawa T, Kuzuya K, Hatae M, Iwasaka T, et al. Feasibility study of neoadjuvant chemotherapy (NAC) followed by interval debulking surgery (IDS) for stage III/IV ovarian, tubal and peritoneal cancers: Japan Clinical Oncology Group study JCOG0206 (Abstract 15020). *J Clin Oncol, ASCO Annual Meeting Proceedings* 2006;24:15020

● 特 集 ● Neoadjuvant Therapyの適応と効用・II

卵巣癌

恩田 貴志*

〔*Jpn J Cancer Chemother* 34(11): 1735-1739, *November*, 2007〕

Ovarian Cancer: Takashi Onda (*Division of Gynecologic Oncology, National Cancer Center Central Hospital*)

Summary

The current standard treatment for advanced ovarian cancer is primary debulking surgery (PDS) followed by post-surgical chemotherapy. We can expect a better prognosis in cases of optimal debulking (residual disease < 1 cm). Unfortunately, optimal debulking in the PDS can be achieved in only about 40% of stage III/IV ovarian cancers as a rule. Neoadjuvant chemotherapy (NAC) has been recognized as an alternative treatment to primary surgical debulking for patients with apparently unresectable bulky tumors or poor performance status. Retrospective analyses revealed that overall survival was comparable between patients treated with NAC followed by interval debulking surgery (IDS) and those treated with PDS, though the former group had more advanced disease and poorer performance status. Based on these favorable results of NAC for patients with advanced disease or poor performance status, the target disease was extended to all cases of advanced disease, including patients without apparently unresectable tumors and good performance status in prospective studies. The European Organization for Research and Treatment of Cancer (EORTC) and The Japan Clinical Oncology Group (JCOG) is now conducting a phase III study comparing neoadjuvant setting treatment with standard treatment for advanced müllerian cancer, such as ovarian, tubal or peritoneal cancer. These prospective studies are expected to reveal the role of NAC for advanced müllerian cancer. **Key words**: Neoadjuvant chemotherapy, Advanced ovarian cancer, Optimal debulking, **Corresponding author**: Takashi Onda, Division of Gynecologic Oncology, National Cancer Center Central Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

要旨 進行卵巣癌に対する現在の標準治療は，初回腫瘍縮小手術（PDS）と術後化学療法である。PDSで残存腫瘍1 cm未満のoptimal surgeryが達成できれば予後の改善が期待できるが，残念ながら一般的には進行卵巣癌の約40%程度にしか達成することができない。元々，術前化学療法（NAC）は明らかに切除不能な症例や，全身状態不良な症例に対して標準治療の代わりとして行われてきた。NAC＋腫瘍縮小手術（IDS）と標準治療の比較を行ったretrospective studyでは，明らかにNAC群のなかに進行例や全身状態不良例が含まれているのに，ほぼ同等の生存率が示された。この良好な成績を基に，NAC療法の対象は明らかに切除不能な腫瘍をもたない症例，全身状態良好な症例も含めた進行癌症例に拡大され，EORTC（The European Organization for Research and Treatment of Cancer）やJCOG（The Japan Clinical Oncology Group）などで現在，卵巣癌，卵管癌，腹膜癌などのミューラー管原発の進行癌を対象とした第III相比較試験が行われている。これらのprospective studyにより，進行卵巣癌に対するNAC療法の役割が明らかとなることが期待される。

I．卵巣癌に対する標準治療

卵巣癌の治療には，主として手術療法と化学療法が用いられる。標準的治療ではまず開腹手術を行い，後に化学療法が追加される。初回治療における手術の目的は卵巣癌の診断の確定，進行期の正確な診断，転移病巣も含めた癌病巣の可及的摘出である。この目的のために行われる術式としては，単純子宮全摘，両側付属器切除，大網切除および転移病巣の局在に応じて直腸切除，結腸切除，小腸切除，脾摘，腹膜切除，虫垂切除，骨盤および傍大動脈リンパ節郭清術などであり，初回腫瘍縮小手術（primary debulking surgery: PDS）と呼ばれる。1975年にGriffiths[1]が，初回手術後の最大残存腫瘍径が予後と関連することを報告して以来，多くの報告によりその事実が確認され，良好な予後が期待できるoptimal surgery（時代により変遷しているが，近年は残存腫瘍径＜1 cmが多く用いられる）をめざしたPDS＋術後化学療法という治療方式が卵巣癌の標準治療となった。化学療法はGriffithsらの時代以降，プラチナ製剤やタキサン系薬剤が導入され，現在ではpaclitaxel（PTX）とcarbo-

連絡先: 〒104-0045 東京都中央区築地5-1-1 * 国立がんセンター中央病院・婦人科
恩田 貴志

0385-0684/07/¥500/論文/JCLS

platin（CBDCA）の併用療法（TC療法）が標準であり，早期癌では3～6コース，進行癌では最低6コースが投与される。

Ⅱ．卵巣癌に対する術前化学療法

標準治療において，初回手術により良好な予後が期待できるoptimal surgeryが達成できるのは，一般には40%程度の症例に限られる。全身状態や合併症などのため侵襲の大きな手術が困難な症例，PDSが試験開腹に終わった症例，画像診断あるいは腹腔鏡診断により切除不能と判断される症例に対して，代替の治療として術前化学療法（neoadjuvant chemotherapy: NAC）が行われていた。以下に，NAC療法と標準治療を比較した成績を紹介する。現在までの報告は，Kuhnら[2]の報告以外はいずれもretrospective studyである。

1．腫瘍縮小手術におけるoptimal surgery達成率，治療成績の比較（表1）

Jacobら[3]は，PDSにて生検のみの試験開腹に終わり他院より紹介された患者に対して，NACの後IDS（interval debulking surgery）を行ったNAC療法群22例とすぐに再度腫瘍縮小手術を行った標準治療群18例を比較した。生存期間中央値（median survival time: MST）では有意差は認められなかったが，NAC群において77%，標準治療群において39%とNAC群において有意（$p=0.02$）にoptimal surgery（<2 cm）の割合が高かったことを報告している。

Onnisら[4]，Kayikçioğluら[5]，Loizziら[6]，Inciuraら[7]，Everettら[8]，Leeら[9]，Houら[10]はCTなどの画像診断や，全身状態によりNAC群を決定，標準治療を行った症例との比較を報告している。いずれの報告もNAC群でより進行した症例や，高齢の症例，PS不良の症例を含んだ比較である。NAC群では標準治療群と同程度あるいは有意に高率にoptimal surgeryが達成され，NAC群で標準治療群に劣らない生存率を得ることができた。

Kuhnら[2]は，多量の腹水貯留（>500 mL）を認める進行卵巣癌症例を対象にNAC療法31例と標準治療32例のnon-randomizedの第Ⅱ相比較試験を行った。NAC療法群では84%，標準療法群では63%と，NAC療法群では有意に（$p=0.04$）高率にoptimal surgery（<2 cm）が達成でき，MSTにおいてもNAC群42 M，標準治療群23 Mと有意な予後改善を認めた。

Vergoteら[11]は，進行卵巣癌症例を対象として切除可能性を試験開腹あるいは腹腔鏡により判断し，切除可能例には標準治療，不能例にはNAC療法を行うという方針で治療を行った1989～1997年の治療成績を，NAC療法導入以前の全例に標準治療を行うという治療方針で治療を行った1980～1988年の治療成績と比較した。NAC療法導入後の3年生存率は42%で，NAC療法導入以前の3年生存率26%に比して有意に予後良好であった（$p=0.0001$）。

2．腫瘍縮小手術における手術侵襲の比較（表2）

Schwartzら[12]は，全身状態/合併症により手術不能な症例，画像診断（CT）により腫瘍切除不能と評価した59例にNAC療法を施行，標準治療を行った206例と比較した。NAC療法群では，標準治療群に比較して有意に高齢でPS不良であった。腫瘍縮小手術の比較においてはNAC療法群で出血量，ICU滞在日数，入院日数などが標準治療群に比して有意に少なかった。

Kayikçioğluら[5]の報告では，腫瘍縮小手術において結腸切除や脾摘を要した割合は，標準治療群においては16,11%，NAC療法群においては2,0%であり，他臓器合併切除を要する割合がNAC療法群で有意に低かった（$p=0.01$，$p=0.02$）。

Vergoteら[11]の報告では，時代により手術手技の違いはあると考えられるが，NAC療法導入後，進行卵巣癌治療における手術関連死亡率の減少を認めた。

Moriceら[13]，Hegazyら[14]，Leeら[9]，Houら[10]の報告においても同様にNAC群において，腸切割合，重篤な合併症割合の減少，手術時間の短縮，出血量，輸血量の減少，ICU滞在日数，入院日数の短縮を認めた。

3．NAC療法の利点と問題点

上記の結果から，NAC療法は進行卵巣癌に対する新たな標準治療として，検討に値する治療と考えられている。NAC療法の利点として，①化学療法を先行することにより（手術枠の確保や他科との連携を要する手術先行に比べて），速やかに治療を開始することが可能である。②腫瘍や胸水，腹水によるPSの低下をNACにより改善し，また治療開始時にしばしばみられる血栓症の改善も期待でき，より安全に侵襲の大きな手術を行い得る。③NACによる腫瘍量，範囲の減少により他臓器合併切除の頻度が減少し，また（術式を拡大しなくても）optimal surgeryの達成が期待でき，同時に重篤な合併症の減少も期待できる，などがあり，進行卵巣癌においてはNAC療法によって治療成績および患者のQOL（quality of life）の改善が期待される。一方，NAC療法にも種々の問題点があげられる。①最初にstagingを兼ねたPDSを行わないため，対象疾患の診断が不正確となる可能性がある。したがって，消化器癌や乳癌などの転移を十分に除外した上で，CT，MRIなどの画像診断で確実にⅢ-Ⅳ期の進行卵巣癌と診断される症例をNAC療法の対象とする必要がある。他臓器癌の転移が否定できない場合や，Ⅰ-Ⅱ期癌の可能性が否定できな

表 1 NAC療法と標準治療の比較（腫瘍縮小手術における optimal surgery，生存率）

報告者（年） 治療法［症例数］	生存率の比較		腫瘍縮小手術	NAC群の選択
Jacob（1991） 標準治療 ［n＝18］ NAC療法［n＝22］	MST 18 M 16 M NS		optimal（<2 cm） 39%（7/18） 77%（17/22） p＝0.02	NAC群，標準群とも他院で生検のみ施行。標準治療群は進行期，組織型，分化度，年齢を match させた control
Onnis（1996） 標準治療 ［n＝284］ NAC療法［n＝88］	3 year 31% 27% NS	5 year 21% 19% NS	optimal（<2 cm） 29%（83/284） 42%（37/88） NA	胸水，肝転移の有無，試験開腹による切除可能性の評価によりNAC療法群を決定。NAC療法群はより進行した症例が多い
Vergote（1998） NAC導入前［n＝112］ NAC導入後［n＝173］	3 year 26% 42% p＝0.0001			試験開腹，腹腔鏡による切除可能性の評価によりNAC療法群を決定
Kayikçioğlu（2001） 標準治療 ［n＝158］ NAC療法［n＝45］	5 year 24% 30% NS	MST 38 M 34 M NS	optimal（＝0） 14%（22/158） 49%（22/45） p<0.001	胸水，肝転移，切除不能な多発転移の有無，全身状態によりNAC療法群を決定。NAC療法群は有意に高齢（p＝0.01），PS不良（p<0.001）でⅣ期症例が多い（p＝0.03）
Kuhn（2001） 標準治療 ［n＝32］ NAC療法［n＝31］	MST 23 M 42 M p＝0.007		optimal（<2 cm） 63%（20/32） 84%（26/31） p＝0.04	対象は多量の腹水（>500 mL）を有する卵巣癌ⅢC期に限定。臨床試験に同意が得られなかった症例に標準治療。標準治療群とNAC療法群の背景に有意差なし
Loizzy（2005） 標準治療 ［n＝30］ NAC療法［n＝30］	MST 40 M 32 M NS	DFI 16 M 21 M NS	optimal（<1 cm） 60%（18/30） 63%（19/30） NS	多量の胸水，腹水，全身状態，CTによる切除可能性の評価によりNAC療法群を決定。標準治療群は組織型，進行期を match させた control。NAC群は有意に高齢（p＝0.03），有意にPS不良（p＝0.02）
Lee（2006） 標準治療 ［n＝22］ NAC療法［n＝18］	MST 55 M 53 M NS	DFI 17 M 15 M NS	optimal（<2 cm） 46%（10/22） 78%（14/18） p＝0.04	CT，MRIにより切除可能性を評価し，NAC群を決定
Everett（2006） 標準治療 ［n＝102］ NAC療法［n＝98］	MST 42 M 33 M NS		optimal（<1 cm） 54%（55/102） 86%（84/98） p<0.001	肝転移，大きな上腹部転移，広範なリンパ節転移，重篤な合併症などによりNAC群を決定。NAC群は有意にⅣ期（p＝0.042），低分化（p＝0.025）症例が多い
Inciura（2006） 標準治療 ［n＝361］ NAC療法［n＝213］	MST 25 M 24 M NS	DFI 15 M 13 M NS	optimal（<2 cm） 67%（242/361） 63%（134/213） NS	多量の腹水，大きな骨盤内 or 腹部腫瘍の存在によりNAC療法群を決定
Hou（2007） 標準治療 ［n＝109］ NAC療法［n＝63］	MST 47 M 46 M NS	DFI 14 M 16 M NS	optimal（<1 cm） 71（77/109） 95（60/63） <0.001	重篤な合併症および画像診断で腹部を超えた進展，広範な腹腔内進展によりNAC群を決定。NAC群で有意にⅣ期症例が多い（<0.05），NAC群でより高齢，より低分化腫瘍であったが有意差はなし

NA: not available，MST: median survival time，DFI: disease free interval

い場合には，診断確認のための開腹術や腹腔鏡が必須である。② 化学療法の効果が得られなければ，腫瘍縮小手術の機会を逸する，optimal surgery の達成を逸する，などの可能性がある。③ 腫瘍量の多い状態で化学療法を行うため，薬剤耐性細胞の出現数が多くなり，また血流不十分な細胞の存在により，薬剤耐性の出現の可能性も高くなる。④ 腫瘍縮小手術に際して，肉眼的に腫瘍の縮小，消失が得られているため，術式を縮小しすぎて，却って根治性を損なってしまう可能性がある。

②～④の問題点に関しては，これらの問題がありながらもNAC療法が標準治療と同等あるいは優る治療成績が得られるのかを，NAC療法と標準治療の prospective な比較試験で検証する必要があると考えられる。

4．無作為比較試験による比較

retrospective study の結果を踏まえて，EORTC（European Organization for Research and Treatment of

表 2 NAC 療法と標準治療の比較（手術合併症などの比較）

報告者（年） 治療法［症例数］	手術合併症などの比較				NAC 群の選択
Vergote（1998）	手術関連死亡率				
NAC 導入前［n＝112］	6%				試験開腹，腹腔鏡による切除可能性の評価により NAC 療法群を決定
NAC 導入後［n＝173］	0%				
	NA				
Schwartz（1999）	出血量	ICU 滞在	入院期間		全身状態，合併症による手術可否の評価，CT による切除可能性の評価により NAC 療法群を決定。NAC 療法群は有意に高齢（<0.001），PS 不良（<0.001）であった
標準治療［n＝206］	1,000 mL	1.26 days	11 days		
NAC 療法［n＝59］	600 mL	1.03 days	7 days		
	p＝0.001	p＝0.01	p<0.001		
Kayikçioğlu（2001）	結腸切除	脾摘			胸水，肝転移，切除不能な多発転移の有無，全身状態により NAC 療法群を決定。NAC 療法群は有意に高齢（p＝0.01），PS 不良（p<0.001）で，Ⅳ期症例が多い（p＝0.03）
標準治療［n＝158］	16%	11%			
NAC 療法［n＝45］	2%	0%			
	p＝0.01	p＝0.02			
Morice（2003）	腸切	脾摘	重篤な合併症	輸血割合	
標準治療［n＝28］	61%	7%	36%	39%	試験開腹，腹腔鏡による切除可能性の評価により NAC 療法群を決定
NAC 療法［n＝57］	19%	5%	7%	21%	
	p＝0.01	NS	p＝0.01	NS	
Hegazy（2005）	出血量	ICU 滞在	入院期間		試験開腹，腹腔鏡による切除可能性の評価により NAC 療法群を決定。NAC 群は有意に高齢（p＝0.04）
標準治療［n＝32］	735 mL	4.4 days	15.9 days		
NAC 療法［n＝27］	420 mL	1.7 days	10.5 days		
	p＝0.02	p＝0.03	p<0.05		
Lee（2006）	出血量				CT，MRI により切除可能性を評価し NAC 群を決定
標準治療［n＝22］	1,061 mL				
NAC 療法［n＝18］	620 mL				
	p＝0.04				
Hou（2007）	出血量	手術時間	入院期間	輸血量	重篤な合併症，および画像診断で腹部を超えた進展，広範な腹腔内進展により NAC 群を決定。NAC 群で有意にⅣ期症例が多い（<0.05），NAC 群でより高齢，より低分化腫瘍であったが有意差はなし
標準治療［n＝109］	1,033 mL	276 min	8.5 days	2.4 U	
NAC 療法［n＝63］	546 mL	211 min	5.7 days	1.2 U	
	p<0.0001	p<0.0001	p<0.0001	p＝0.03	

NA: not available

Cancer）では，第Ⅲ相ランダム化比較試験として EORTC55971[15]を行っている。卵巣癌，卵管癌，腹膜癌のⅢC/Ⅳ期を対象に，診断的腹腔鏡，試験開腹，穿刺組織診のいずれかの方法で原発診断，組織診断，進行期診断の後，NAC 療法群と手術先行の標準治療群に割り付けしている。卵管癌，腹膜癌は，組織学的所見，化学療法感受性，予後が卵巣癌とほぼ同一であり，卵巣，卵管の摘出なしでは鑑別診断困難であることから対象に含めている。プロトコール治療は，NAC 療法群では 3 コースの化学療法の後，腫瘍縮小手術を行い，術後 3 コースの化学療法追加，標準治療群では PDS を行い，optimal surgery が達成できた症例では 6 コースの化学療法，suboptimal の症例では 3 コースの化学療法の後 IDS を行い，術後 3 コースの化学療法追加である。化学療法としては，白金製剤＋タキサン系薬剤のいずれの組み合わせでも可としている。この臨床試験は 2006 年 12 月で登録終了となり現在データ集積中である。

JCOG（Japan Clinical Oncology Group）の婦人科腫瘍グループでは，2003 年 1 月から，「Ⅲ/Ⅳ期卵巣癌，卵管癌，腹膜癌に対する術前化学療法の Feasibility study」（JCOG0206）[16]を行い，その結果を踏まえ，現在 EORTC と同様の第Ⅲ相比較試験「Ⅲ期/Ⅳ期卵巣癌，卵管癌，腹膜癌に対する手術先行治療 vs 化学療法先行治療のランダム化比較試験」（JCOG0602）を開始している。化学療法としては PTX と CBDCA の組み合わせの TC 療法で，NAC 群では術前 4 コース，術後 4 コースの合わせて 8 コースを行っている。これらの試験はいずれも NAC 療法が標準治療に対して，効果の点で劣らないことを検証する非劣性試験である。NAC 療法では手術に関連した侵襲の軽減（手術回数，輸血必要量など）が期待されるため，非劣性が証明されれば NAC 療法が進行卵巣癌の標準治療になると考えられる。

おわりに

retrospective studyの結果から，進行卵巣癌に対するNAC療法は治療成績およびQOLの改善が期待される治療ではあるが，診断が不正確となる可能性，手術の機会を逸する可能性，薬剤耐性の出現を助長する可能性，根治性を損なう可能性などのriskも有している。現在行われている二つのprospectiveな比較試験により，進行卵巣癌におけるNAC療法の役割が明らかとなることが期待される。

文 献

1) Griffiths CT: Surgical resection of tumor bulk in the primary treatment of ovarian carcinoma. *Natl Cancer Inst Monogr* **42**: 101-104, 1975.
2) Kuhn W, Rutke S, Spathe K, *et al*: Neoadjuvant chemotherapy followed by tumor debulking prolongs survival for patients with poor prognosis in International Federation of Gynecology and Obstetrics Stage ⅢC ovarian carcinoma. *Cancer* **92**: 2585-2591, 2001.
3) Jacob JH, Gershenson DM, Morris M, *et al*: Neoadjuvant chemotherapy and interval debulking for advanced epithelial ovarian cancer. *Gynecol Oncol* **42**: 146-150, 1991.
4) Onnis A, Marchetti M, Padovan P, *et al*: Neoadjuvant chemotherapy in advanced ovarian cancer. *Eur J Gynaecol Oncol* **17**: 393-396, 1996.
5) Kayikçioğlu F, Kose MF, Boran N, *et al*: Neoadjuvant chemotherapy or primary surgery in advanced epithelial ovarian carcinoma. *Int J Gynecol Cancer* **11**: 466-470, 2001.
6) Loizzi V, Cormio G, Resta L, *et al*: Neoadjuvant chemotherapy in advanced ovarian cancer: a case-control study. *Int J Gynecol Cancer* **15**: 217-223, 2005.
7) Inciura A, Simavicius A, Juozaityte E, *et al*: Comparison of adjuvant and neoadjuvant chemotherapy in the management of advanced ovarian cancer: a retrospective study of 574 patients. *BMC Cancer* **6**: 153, 2006.
8) Everett EN, French AE, Stone RL, *et al*: Initial chemotherapy followed by surgical cytoreduction for the treatment of stage Ⅲ/Ⅳ epithelial ovarian cancer. *Am J Obstet Gynecol* **195**: 568-574; discussion 574-576, 2006.
9) Lee SJ, Kim BG, Lee JW, *et al*: Preliminary results of neoadjuvant chemotherapy with paclitaxel and cisplatin in patients with advanced epithelial ovarian cancer who are inadequate for optimum primary surgery. *J Obstet Gynaecol Res* **32**: 99-106, 2006.
10) Hou JY, Kelly MG, Yu H, *et al*: Neoadjuvant chemotherapy lessens surgical morbidity in advanced ovarian cancer and leads to improved survival in stage Ⅳ disease. *Gynecol Oncol* **105**: 211-217, 2007.
11) Vergote I, De Wever I, Tjalma W, *et al*: Neoadjuvant chemotherapy or primary debulking surgery in advanced ovarian carcinoma: a retrospective analysis of 285 patients. *Gynecol Oncol* **71**: 431-436, 1998.
12) Schwartz PE, Rutherford TJ, Chambers JT, *et al*: Neoadjuvant chemotherapy for advanced ovarian cancer: long-term survival. *Gynecol Oncol* **72**: 93-99, 1999.
13) Morice P, Dubernard G, Rey A, *et al*: Results of interval debulking surgery compared with primary debulking surgery in advanced stage ovarian cancer. *J Am Coll Surg* **197**: 955-963, 2003.
14) Hegazy MA, Hegazi RA, Elshafei MA, *et al*: Neoadjuvant chemotherapy versus primary surgery in advanced ovarian carcinoma. *World J Surg Oncol* **3**: 57, 2005.
15) Vergote IB, De Wever I, Decloedt J, *et al*: Neoadjuvant chemotherapy versus primary debulking surgery in advanced ovarian cancer. *Semin Oncol* **27**(3): (*Suppl 7*), 31-36, 2000.
16) Onda T, Kamura T, Ishizuka N, *et al*: Feasibility study of neoadjuvant chemotherapy followed by interval cytoreductive surgery for stage Ⅲ/Ⅳ ovarian, tubal and peritoneal cancers: Japan Clinical Oncology Group Study JCOG0206. *Jpn J Clin Oncol* **34**: 43-45, 2004.

Available online at www.sciencedirect.com
ScienceDirect
Gynecologic Oncology 106 (2007) 342–347
Gynecologic Oncology
www.elsevier.com/locate/ygyno

Pilot study evaluating the efficacy and toxicity of irinotecan plus oral etoposide for platinum- and taxane-resistant epithelial ovarian cancer

Shin Nishio [a], Toru Sugiyama [b,*], Tadahiro Shouji [b], Akira Yoshizaki [b], Ryo Kitagawa [a], Kimio Ushijima [a], Toshiharu Kamura [a]

[a] *Department of Obstetrics and Gynecology, Kurume University School of Medicine, 67 Asahimachi, Kurume, Fukuoka Pretecture, 830-0011, Japan*
[b] *Department of Obstetrics and Gynecology, Iwate Medical University School of Medicine, 19-1 Uchimaru, Morioka, Iwate Prefecture, 020-8505, Japan*

Received 29 November 2006
Available online 11 May 2007

Abstract

Objectives. To evaluate the efficacy and toxicity of combination chemotherapy with intravenous irinotecan and oral etoposide in women with platinum- and taxane-resistant epithelial ovarian cancer.

Methods. Between October 2002 and September 2005, we studied 27 women with platinum- and taxane-resistant epithelial ovarian cancer. Irinotecan was administered in an intravenous dose of 70 mg/m^2 as a 90-min infusion on days 1 and 15 of a 28-day cycle, and etoposide was administered in an oral dose of 50 mg/day on days 1 to 21. For heavily pretreated patients, the initial dose of irinotecan was lowered to 60 mg/m^2. Treatment cycles were repeated until disease progression or unacceptable toxicity.

Results. All 27 patients were eligible and assessable. There were 11 partial responses and 1 complete response for an overall response rate of 44.4%. The median durations of overall response and of stable disease were 11 months and 8 months, respectively. The major toxicity was neutropenia (grade 3, 22.2%; grade 4, 37.1%). Diarrhea was infrequent and mild, and gastrointestinal toxicity was moderate and manageable. Acute myeloid leukemia (M5) developed as a secondary malignancy in 1 patient.

Conclusions. The results of our pilot study suggest that a combination of irinotecan and oral etoposide is effective and tolerable in women with platinum- and taxane-resistant epithelial ovarian cancer.

Keywords: Irinotecan; Oral etoposide; Platinum/taxane-resistant ovarian cancer

Introduction

Various agents and treatment regimens have been introduced to treat recurrent ovarian cancer resistant to platinum/taxane (PT), currently the standard first-line chemotherapy. Generally, relapse within 3 months after first-line platinum containing therapy is defined as platinum-refractory disease, relapse between 3 and 6 months after therapy is defined as platinum-resistant disease and relapse more than 6 months after therapy is defined as platinum-sensitive disease. Topotecan, gemcitabine, etoposide and liposomal doxorubicin produce response rates of 20% to 30%, but the time to progression is usually short, particularly in PT-resistant or -refractory disease [1–3]. Irinotecan is a topoisomerase-I inhibitor similar to topotecan, a drug approved by the Food and Drug Administration (FDA) for the second-line treatment of ovarian cancer [4,5]. Irinotecan has been studied in Japan for the management of ovarian cancer. In a phase II study, 55 patients received irinotecan in a dosage of 100 mg/m^2 once weekly and 150 mg/m^2 once every 2 weeks. The response rate was 23.6%. Major adverse effects were leukopenia, nausea and vomiting, diarrhea and anorexia, with incidences (grade 3 or 4 hematological toxicity and grade 2 or higher nonhematological toxicity) of 57.1%, 60.3%, 44.0% and 67.2%, respectively [6]. This compares favorably with the response to topotecan. Etoposide, a topoisomerase-II inhibitor, has high antitumor activity against various animal and human malignancies [7]. The efficacy of etoposide may be regimen-dependent, since prolonged oral administration has yielded better results than intravenous administration [8,9]. The largest

* Corresponding author. Fax: +81 19 6221900.
E-mail address: sugiyama@iwate-med.ac.jp (T. Sugiyama).

0090-8258/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2007.03.036

study to date, performed by the Gynecologic Oncology Group (GOG), reported a response rate of 8.3% [10]. Long-term treatment with oral etoposide has produced better results in women with platinum-resistant ovarian carcinoma [1,11–14]. In a study by de Wit et al. [12], 50 mg/m^2 of oral etoposide was administered for 21 days every 4 weeks to 28 patients with platinum-resistant ovarian cancer, resulting in a response rate of 16.0%. Rose et al. [1] gave oral etoposide to 41 patients with platinum-sensitive or -resistant recurrent ovarian cancer and obtained response rates of 34.6% and 26.8%, respectively.

DNA topoisomerases-I and -II are nuclear enzymes that participate in various genetic processes, including transcription, replication, recombination and chromosome segregation at mitosis [4]. These two DNA topoisomerases are functionally related and act in concert. Both seem to be essential for maintaining cell viability throughout the cell cycle. Topoisomerase-I treatment induces an increase in the S-phase cell population with an increase in topoisomerase-II mRNA expression. Thus, topoisomerase-I can modulate topoisomerase-II levels to enhance the effect of topoisomerase-II inhibitors [15,16]. Therefore, combined use of topoisomerase-I- and topoisomerase-II-targeting agents could theoretically inhibit both DNA and RNA synthesis completely, resulting in synergistic cytotoxicity.

This pilot study was undertaken to evaluate the antitumor efficacy and toxicity of a combination of irinotecan, a DNA topoisomerase-I inhibitor, and oral etoposide, a DNA topoisomerase-II inhibitor, in women with platinum- and taxane-resistant epithelial ovarian cancer.

Patients and methods

Eligibility

Patients were eligible for this study if they satisfied the following criteria: (1) histologically confirmed epithelial ovarian cancer; (2) recurrent disease after previous treatment with platinum and taxanes; (3) an Eastern Cooperative Oncology Group performance (ECOG) status of <2; (4) measurable or assessable disease. Assessable disease was defined according to the following CA-125 criteria: a CA-125 level of greater than 70 U/mL at study entry; this CA-125 level must have at least doubled from the baseline level, providing evidence of disease progression while receiving a previous treatment regimen (as confirmed by at least two separate blood samples obtained >4 weeks apart) (GCIG guidelines) [17]. (5) Age <75 years; (6) adequate laboratory values (leukocyte count >4000/μL, absolute neutrophil count >1500/μL, platelet count >100,000/μL, hemoglobin level >9.5 g/dL, total bilirubin <2.0 mg/dL and serum aspartate aminotransferase or alanine aminotransferase <2 times the upper limit of normal at the center performing the test); and (7) a signed informed consent statement confirming that the subject understood the experimental nature of the study treatment.

Patients were excluded from the study if any of the following applied: (1) Previous treatment with irinotecan or topotecan; (2) concurrent active or uncontrolled infection; (3) any psychiatric disorders potentially interfering with consent or follow-up; (4) pregnant women or nursing mothers; (5) other active malignancies; (6) clinically significant comorbidity (e.g., a history of previous myocardial infarction within the past 6 months, congestive heart failure requiring therapy, a history of seizures or uncontrolled diabetes, clinically apparent metastases to the central nervous system); (7) poor oral intake due to intestinal obstruction; (8) large amounts of pleural effusion, pericardial fluid or ascitic fluid, requiring repeated drainage; (9) previous abdominal radiation therapy; (10) Apparent pulmonary fibrosis or interstitial pneumonia; and (11) watery diarrhea or other health problems that the attending physician felt would interfere with treatment. The study protocol was approved by institutional review board of each participating center.

Platinum/taxane-refractory disease was defined as tumor progression during treatment or within 3 months after the completion of therapy. Platinum/taxane-resistant disease was defined as tumor progression between 3 and 6 months after the completion of the most recent course of therapy. Any regimen that contained a platinum/taxane drug was counted as one regimen for the purpose of this study. For example, if a patient received cisplatin with paclitaxel as first-line therapy and then received weekly carboplatin and paclitaxel after recurrence, the number of regimens was considered to be two ("cisplatin with paclitaxel" and "weekly carboplatin with paclitaxel"). If the patient then received carboplatin monotherapy after progression, the number of regimens was considered to be three ("cisplatin with paclitaxel," "weekly carboplatin with paclitaxel" and "carboplatin").

Treatment schedule

Irinotecan 70 mg/m^2 was administered as a 90-min intravenous infusion on days 1 and 15 of a 28-day cycle. Etoposide 50 mg/day was given orally on an empty stomach at bedtime with metoclopramide or domperidone for 21 days starting on day 1. These starting doses were based on the results of a phase I study [18]. For patients who were heavily pretreated and received the study treatment as third- or fourth-line therapy, the starting dose of irinotecan was reduced to 60 mg/m^2. Treatment cycles were repeated until evidence of disease progression or unacceptable toxicity. A $5HT_3$-antagonist was given before the administration of irinotecan. For etoposide, premedication was left to the discretion of the attending physicians. Routine prophylactic treatment with granulocyte colony-stimulating factor (G-CSF) was not recommended. During the first course of chemotherapy, G-CSF was used to treat grade 4 neutropenia. During subsequent courses, G-CSF could be used to treat grade 3 or 4 neutropenia in accordance with published guidelines [19]. However, etoposide was withheld on days when G-CSF was administered.

Treatment with irinotecan was withheld if the patient had a leukocyte count of less than 2000/μL, a platelet count of less than 100,000/μL, or >grade 2 diarrhea, fever, or both on the day scheduled for treatment. Before the next course was started, the leukocyte count had to be at least 3000/μL, the platelet count at least 100,000/μL and the diarrhea or fever had to have completely resolved. Subsequent doses were decided on the basis of hematologic and nonhematologic toxicity. If the criteria for resuming treatment were not met for more than 6 weeks since the last dose, the patient was withdrawn from the study. The dose of irinotecan for subsequent cycles of treatment was reduced by 10 mg/m^2 if grade 4 neutropenia persisted for more than 7 days, the platelet nadir was less than 50,000/μL or >grade 3 diarrhea occurred in the preceding cycle. The minimum dose of irinotecan was set at 40 mg/m^2. Patients who had evidence of disease progression or intolerable toxicity (grade 4 diarrhea, neutropenic fever, or both for more than 7 days, or grade 2 or higher pneumonitis) were withdrawn from the study. The dose of etoposide was reduced to 25 mg/day if grade 3 or 4 (according to The National Cancer Institute [NCI] Common Terminology Criteria for Adverse Events [Version 2]; NCI-CTC ver.2) emesis occurred despite treatment with antiemetic agents.

Study evaluations

All patients underwent a complete blood count, platelet count, serum chemical analyses to measure renal and hepatic functions, electrolyte analysis, urinalysis and toxicity assessments weekly. At the end of each 4-week cycle, the CA-125 level was determined. Antitumor effects were evaluated according to the RECIST criteria [20] on the basis of computed tomographic or magnetic resonance imaging scans in patients with measurable lesions. The GCIG CA-125 response criteria proposed by Rustin et al. [17] were used to evaluate antitumor response in patients without measurable lesions. These evaluations were performed after the completion of each cycle of treatment (4 – 6 weeks). Response in patients with measurable lesions was evaluated on the basis of symptoms or imaging findings. Response in patients with non-measurable lesions was evaluated based on elevation of CA 125.

NCI-CTC Ver. 2 was used to grade organ damage [21]. Survival was calculated from the date of starting the study treatment to the date of death, or data were censored at the time of last contact.

Table 1
Patient characteristics

Characteristic	No. of patients (%)
Age, years	
Median	58
Range	34–71
No. of previous regimens	
1	5 (18.6)
2	12 (44.4)
3	9 (33.3)
≥4	1 (3.7)
Treatment-free interval, months	
<3	19 (70.3)
3–6	8 (29.7)
Performance status (PS)	
0	13 (48.2)
1	7 (25.9)
2	7 (25.9)
Measurable sites or assessable CA-125 (=70 U/mL)	
Visceral	9 (33.3)
Soft tissue	6 (22.2)
Lymph node	2 (7.4)
CA-125	10 (37.1)
Histology	
Serous	19 (70.4)
Mucinous	3 (11.1)
Endometrioid	3 (11.1)
Clear cell	2 (7.4)
No. of cycles: median	5 (range, 1–25)
CR+PR	6 (range, 1–16)
SD	5 (range, 1–25)
PD	2 (range, 2–3)

CR, complete response; PR, partial response; SD, stable Disease; PD, progressive disease.

Statistical analysis

To evaluate toxicity, time-to-event data were analyzed with the use of Kaplan–Meier survival curves. Duration of response was measured from the date an initial response was documented to the date of disease progression, relapse or death. Time to progression was calculated from the date of starting treatment with irinotecan and etoposide to the date of first documentation of tumor progression. Survival time was calculated from the date of diagnosis to the date of death or the date of the last known contact.

This study was designed to test the hypothesis that the true response rate was <0.10 versus the alternative hypothesis that it was >0.25. A two-stage sampling plan was employed, which featured accrual of 27 patients in the first stage and additional accrual of 13 patients in the second phase if at least three responses were observed in the first stage. At least 10 responses among these 40 patients were necessary to reject the null hypothesis. This design featured a size of 0.05 and a power of 0.8.

Results

Between October 2002 and September 2005 at Kurume University Hospital and Iwate Medical University Hospital, we enrolled 27 women with platinum- and taxane-resistant epithelial ovarian cancer. All were eligible for analysis. The characteristics of the subjects are shown in Table 1. The median age was 58 years (range, 34–71 years). The treatment-free interval was less than 3 months in 19 patients and 3 to 6 months in 8. Seventeen patients had measurable lesions. The most common sites of recurrent lesions were the viscera, soft tissue and lymph nodes. Ten patients lacked measurable lesions but had high CA-125 levels (>70 U/mL), with a median value of 100 U/mL (range, 75–350 U/mL) at enrollment. Five patients received the study therapy as second line, 12 as third line, 9 as fourth line and 1 as fifth line.

Response

Of the 17 patients with measurable disease, 10 (47.6%) had objective responses (1 complete response [CR] and 9 partial responses [PR]). Of the 10 patients in whom response was evaluated according to the CA-125 criteria, 6 (42.9%) had at least a 50% decrease in the level of this tumor marker. Two of these patients met the criteria for PR. Thus, the overall rate of objective response (CR+PR according to the RECIST and CA-125 criteria) in this pilot study was 44.4% (12/27) (95% confidence interval, 30.5% to 61.8%). Eleven patients (42.8%) had stable disease (SD), and the other 4 (11.4%) had progressive disease (PD). The progression-free (CR+PR+SD) rate was 85.1%.

The median duration of response in the 12 patients who had objective responses was 11 months (range, 4–18 months). The median duration of SD in the 11 patients who had SD was 8 months (range, 4–22 months). The median time to progression (TTP) in the study group as a whole was 9 months (range, 1–28 months). The median survival was 17 months (range, 3–31 months).

Toxicity and treatment received

The 27 patients received a total of 186 cycles of therapy. The initial dose of irinotecan was 70 mg/m^2 in 19 patients. The dose was subsequently reduced to 60 mg/m^2 in 4 of these patients. Among the 8 patients who initially received irinotecan 60 mg/m^2, the dose was reduced to 50 mg/m^2 in 1. There was no difference in response between 70 mg/m^2 and 60 mg/m^2. In 179 cycles, irinotecan was administered on days 1 and 15 as scheduled. In 3 cycles the dose of irinotecan scheduled for day 1 was delayed. In 4 cycles the dose of irinotecan scheduled for day 15 was skipped.

Table 2
Adverse effects (n=27)

	Grade (%)				
Adverse effect	1	2	3	4	≥3
Leukopenia	2	10	10	4	14 (51.9)
Neutropenia	4	7	6	10	16 (59.3)
Thrombocytopenia	0	1	2	0	2 (7.4)
Anemia	1	3	10	0	10 (37.1)
Nausea	13	7	3	1	4 (14.8)
Vomiting	13	2	3	1	4 (14.8)
Diarrhea	6	1	2	0	2 (7.4)
Renal	0	0	0	0	0
Neurotoxicity	0	0	0	0	0
Infection	1	0	2	1	3 (11.1)
Febrile neutropenia	0	0	2	1	3 (11.1)
Secondary malignancy	–	–	0	1[a]	1 (3.7)

[a] Acute myeloid leukemia (AML).

The dose of oral etoposide was reduced in 8 (29.6%) of the 27 patients. All patients were in-patients during first cycle of treatment; after the first cycle 24 patients (88%) were out-patients.

Table 2 lists adverse effects according to the highest grade during treatment. The nadir of the neutrophil count was usually reached around day 15, with recovery in most patients by day 18. During the first course of treatment, G-CSF was administered to 6 patients (22.2%) who had grade 4 neutropenia, and in subsequent courses a total of 7 patients (25.9%) received G-CSF. The median duration of treatment with G-CSF was 5 days (range, 2–11 days).

One 64-year-old woman had grade 4 acute myeloid leukemia (AML; karyotype of M5) as a secondary malignancy after 10 treatment cycles. The white cell count rose to $277 \times 10^2/\mu L$ after 10 cycles, and bone marrow examination confirmed AML. Despite 2 cycles of cytarabine and idarubicin, complete remission was not achieved. The patient did not respond to subsequent treatment, including 1 cycle of mitoxantrone, etoposide and cytarabine (MEC), 2 cycles of cytarabine and aclarubicin and 1 cycle of aclarubicin, vincristine and daunorubicin, She died from acute respiratory failure of unknown cause 13 months after initial treatment.

Discussion

Despite therapeutic advances during the past 5 decades, culminating in the development of cytoreductive surgery followed by PT chemotherapy, more than 60% of patients with ovarian cancer die of recurrent disease. In patients with PT-refractory or resistant disease, the response rate remains between 15% and 20%, with median survival of only 8 months [22]. In this study, all patients received the first cycle of therapy on an in-patient basis, whereas 24 (88%) received subsequent cycles as out-patients. Thus, irinotecan plus oral etoposide maintained the patients' quality of life (QOL). Such treatments must achieve a balance between antitumor effectiveness and toxicity. The results of our pilot study, performed at 2 centers, suggest that combination therapy with irinotecan and oral etoposide produces high rates of objective responses in women with recurrent ovarian cancers, especially PT-resistant disease. Our results also demonstrated that this regimen is relatively well tolerated even in heavily pretreated patients who have received multiple chemotherapeutic agents, including platinum compounds and taxanes.

In this study, the RECIST criteria [20] were used to assess response in patients with measurable disease, and the GCIG CA-125 response criteria [17] were used in patients without measurable disease. Our overall objective response rate (44.4%) was high, given that disease resistance to prior chemotherapy was higher than that in most previous trials of second-line therapy for ovarian cancer. Of note, the non-progression (CP + PR + SD) rate was 85.1%. However, this study included seven patients who were sensitive relapse in the first-line and might produce higher response rate. Actually, the response rate might be lower in general population of platinum/taxanes resistance. A study by van der Burg et al. [23] reported response rates of 46%, 91% and 92% in patients who had progression at 0–4, 4–12 and >12 months, respectively, while receiving a combination of weekly cisplatin and oral etoposide. Meyer et al. [24] reported a response rate of 46% in patients who had progression within 6 months while receiving the same regimen.

The responses to irinotecan plus oral etoposide were durable, with a median TTP of 9 months, as compared with 2.8 to 4 months in studies of single-agent irinotecan [6,25,26]. Our results suggest that irinotecan and oral etoposide may have "supra-additive" or synergistic effects against ovarian cancer, consistent with the findings of *in vitro* studies [27].

The frequency of grades 3 and 4 neutropenia with our regimen of irinotecan plus oral etoposide was slightly higher than that of hematological toxicity reported for irinotecan alone, but all reactions could be managed successfully. The frequency of severe diarrhea, a toxic effect specific to irinotecan, was less than expected. In patients with metastatic platinum-resistant or-refractory ovarian cancer, Bodurka et al. [25] found that single-agent irinotecan at a dose of 300 mg/m^2 given every 3 weeks had an overall response rate of 17.2% and caused reversible >grade 3 neutropenia and diarrhea in 36% and 33% of patients, respectively. Matsumoto et al. [26] reported a response rate of 29% and reversible >grade 3 neutropenia and diarrhea in 17.8% and 10.7% of patients, respectively, during treatment with irinotecan 100 mg/m^2 on days 1, 8 and 15 of a 28-day cycle. The frequency of severe diarrhea caused by irinotecan can thus be reduced by modifying the treatment schedule. However, 17 patients were under 60 years old. Previous studies have found that older patients with ovarian cancer are less likely to receive intensive chemotherapy regimens [28–30], and in clinical practice there is often concern about the tolerability of cytotoxic agents in older patients. Thus, our regimen might be more toxic in the general population of women with ovarian cancer.

In 1 patient AML (M5) developed as a secondary malignancy after 10 cycles of treatment. Topoisomerase-II-related AML, initially noted as a therapy-related complication of childhood leukemia [31], is characterized by lack of a myelodysplastic phase, no dysplastic changes in diagnostic bone marrow specimens, a short latency period (usually less than 3 years), balanced chromosomal translocations involving 11q23 and variable chemosensitivity [32]. Rose et al. [1] reported that AML developed in 3 of 52 patients with ovarian cancer 16, 27 and 35 months after receiving a cumulative dose of 200 mg/m^2, 1200 mg/m^2 and 2400 mg/m^2, respectively. Rose et al. [33,34] also reported that AML developed in 1 patient with ovarian cancer after 10 courses of chemotherapy with oral etoposide (total dose, 16,550 mg) and 1 patient with uterine leiomyosarcoma after 7 courses of chemotherapy with oral etoposide (total dose, 7350 mg/m^2). These leukemias are characteristically related to the cumulative dose of etoposide and have a shorter latency period (median, 24 to 30 months) than the AMLs associated with alkylating agent therapy. Le Deley et al. [35] reported that the risk of AML was related to the cumulative dose of etoposide, with a particularly high risk at dose levels exceeding 6 g/m^2. The total dose of etoposide received by our patient who had AML was 10.5 g. Because the efficacy of continuous palliative treatment with etoposide is offset by its strong leukemogenicity when the total dose exceeds

6 g/m^2, we recommend that our regimen is not given for more than 6 cycles, even if the response is sustained.

Recurrent ovarian cancer, especially PT-resistant disease, is incurable. Single-agent therapy is therefore frequently used for disease management, attempting to maximize therapeutic response while minimizing toxicity. In patients with platinum-sensitive recurrent ovarian cancer, randomized studies have shown that carboplatin-based combination therapies are more effective than carboplatin alone [36]. In contrast, survival with combination chemotherapy has not been found to be superior to that with single-agent therapy in platinum-resistant ovarian cancer. The results of our study suggest that a combination of irinotecan and oral etoposide might extend survival and maintain the QOL of patients with chemoresistant ovarian cancer. Given that our subjects had PT-resistant recurrent ovarian cancers, the median survival time of 17 months appears very promising. However, we had initially planned to enroll 43 patients, but could not because of poor accrual. Moreover, the study was done at only two centers, and secondary leukemia developed in 1 patient after ten cycles. The small size of our study and the lack of a control group preclude us from concluding that irinotecan plus oral etoposide should be the treatment of choice for PT-resistant ovarian cancer. Well-designed phase II trials are needed to confirm the efficacy and toxicity of up to 6 cycles of irinotecan plus oral etoposide or to refute our findings. A nationwide multicenter phase II study is now being considered by the Japan Clinical Oncology Group (JCOG), a large cooperative group.

In conclusion, we believe our results, although preliminary, justify further studies of irinotecan plus oral etoposide in patients with PT-resistant ovarian cancer.

Acknowledgments

We thank Takeyuki Kato and Shinji Izuno for their helpful suggestions and fruitful discussions.

References

[1] Rose PG, Blessing JA, Mayer AR, Homesley HD. Prolonged oral etoposide as second-line therapy for platinum-resistant and platinum-sensitive ovarian carcinoma: A Gynecologic Oncology Group Study. J Clin Oncol 1998;16:405–10.

[2] Markman M. Second-line treatment of ovarian cancer with single-agent gemcitabine in platinum-paclitaxel refractory ovarian cancer. Gynecol Oncol 2003;90:593–6.

[3] Gordon AN, Fleagle JT, Guthrle D, Parkin DE, Gore ME, Lacave AJ. Recurrent epithelial ovarian carcinoma: a randomized phase III study of pegylated liposomal doxorubicin versus topotecan. J Clin Oncol 2001;19:3312–22.

[4] Wang JC. DNA topoisomerases. Annu Rev Biochem 1985;54:665–97.

[5] Kunimoto T, Nitta K, Tanaka T, Uehara N, Baba H, Takeuchi M, et al. Antitumor activity of 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin, a novel water-soluble derivative of camptothecin, against murine tumors. Cancer Res 1987;47:5944–7.

[6] Takeuchi S, Dobashi K, Fujimoto S, Tanaka K, Suzuki M, Terashima Y, et al. A late phase II study of CPT-11 on uterine cervical cancer and ovarian cancer: research groups of CPT-11 in gynecologic cancers. Gan To Kagaku Ryoho 1991;18:1681–9.

[7] van Maanen JM, Retel J, de Vries J, Pinedo HM. Mechanism of action of antitumor drug etoposide: a review. J Natl Cancer Inst 1988;80:1526–33.

[8] Maskens AP, Armand JP, Lacave AJ, De Jager RL, Hansen HH, Wolff JP. Phase II clinical trial of VP-16-213 in ovarian cancer. Cancer Treat Rep 1981;65:329–30.

[9] Eckhardt S, Hernadi Z, Thurzo L, Telekes A, Sopkova B, Mechl Z, et al. Phase II clinical evaluation of etoposide (VP-16-213, Vepesid) as a second-line treatment in ovarian cancer: results of the South-East European Oncology Group (SEEOG) Study. Oncology 1990;47:289–95.

[10] Slayton RE, Creasman WT, Petty W, Bundy B, Blessing JA. Phase II trial of VP-16-213 in the treatment of advanced squamous cell carcinoma of the cervix and adenocarcinoma of the ovary: A Gynecologic Oncology Group study. Cancer Treat Rep 1979;63:2089–92.

[11] Hoskins PJ, Swenerton KD. Oral etoposide is active against platinum-resistant epithelial ovarian cancer. J Clin Oncol 1994;12:60–3.

[12] de Wit R, van der Burg ME, van den Gaast A, Logmans A, Stoter G, Verweij J. Phase II study of prolonged oral etoposide in patients with ovarian cancer refractory to or relapsing within 12 months after platinum-containing chemotherapy. Ann Oncol 1994;5:656–7.

[13] Kavanagh JJ, Tresukosol D, De Leon CG, Edwards CL, Freeman RS, Hard M, et al. Phase II study of prolonged oral etoposide in refractory ovarian cancer. Int J Gynecol Cancer 1995;5:351–4.

[14] Markman M, Hakes T, Reichman B, Curtin J, Burakat R, Rubin S, et al. Phase 2 trial of chronic low-dose oral etoposide as salvage therapy of platinum-refractory ovarian cancer. J Cancer Res Clin Oncol 1992;119:55–7.

[15] Kim R, Hirabayashi N, Nishiyama M, Jinushi K, Toge T, Okada K. Experimental studies on biochemical modulation targeting topoisomerase I and II in human tumor xenografts in nude mice. Int J Cancer 1992;50:760–6.

[16] Masumoto N, Nakano S, Esaki T, Tatsumoto T, Fujima H, Baba E, et al. Sequence-dependent modulation of anticancer drug activities by 7-ethy-10-hydroxycamptothecin in an HST-1 human squamous carcinoma cell line. Anticancer Res 1995;14:405–9.

[17] Rustin GJ, Quinn M, Thigpen T, du Bois A, Pujade-Lauraine E, Jakobsen A, et al. Re: new guidelines to evaluate the response to treatment in solid tumors (ovarian cancer). J Natl Cancer Inst 2004;96:487–8.

[18] Yamanaka Y, Katsumata N, Watanabe T, Andoh M, Mukai H, Kitagawa R, et al. A dose finding study of irinotecan in combination with oral etoposide in patients with platium treated advanced epitherial ovarian cancer. Proc Am Soc Clin Oncol 2002;21:176 [abstract #2521].

[19] Smith TJ, Khatcheressian J, Lyman GH, Ozer H, Armitage JO, Balducci L, et al. 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. J Clin Oncol 2006;24:3187–205.

[20] Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors: European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–16.

[21] The Revised Common Toxicity Criteria: Version 2.0 [cited; Available from: http://www.ctep.info.nih.gov].

[22] Lister-Sharp D, McDonagh MS, Khan KS, Kleijnen J. A rapid and systematic review of the effectiveness and cost-effectiveness of the taxanes used in the treatment of advanced breast and ovarian cancer. Health Technol Assess 2000;4:1–113.

[23] van der Burg MEL, de Wit R, van Putten WLJ, Logmans A, Kruit WHL, Stoter G, et al. Weekly cisplatin and daily oral etoposide in highly effctive in platinum pretreated ovarian cancer. Br J Cancer 2002;86:19–25.

[24] Meyer T, Nelstrop AE, Mahmoudi M, Rustin GJS. Weekly cisplatin and oral etoposide as treatment for relapsed epithelial ovarian cancer. Ann Oncol 2001;12:1705–9.

[25] Bodurka DC, Levenback C, Wolf JK, Gano J, Wharton JT, Kavanagh JJ, et al. Phase II trial of irinotecan in patients with metastatic epithelial ovarian or peritoneal cancer. J Clin Oncol 2003;21:291–7.

[26] Matsumoto K, Katsumata N, Yamanaka Y, Yonemori K, Kohno T, Shimizu C, et al. The safety and efficacy the weekly dosing of irinotecan for platinum and taxanes-resistant epithelial ovarian cancer. Gynecol Oncol 2006;100:412–6.

[27] Kano Y, Suzuki K, Akutsu M, Suda K, Inoue Y, Yoshida M, et al. Effects of CPT-11 in combination with other anti-cancer agents in culture. Int J Cancer 1992;50:604–10.

[28] Markman M, Lewis Jr JL, Saigo P, Hakes T, Jones W, Rubin S, et al. Epithelial ovarian cancer in the elderly. The Memorial Sloan-Kettering Cancer Center experience. Cancer 1993;71:634–7.
[29] Oriel KA, Hartenbach EM, Remington PL. Trends in United States ovarian mortality, 1979–1995. Obstet Gynecol 1999;93:30–3.
[30] Hershman D, Jacobson JS, McBride R, Mitra N, Sundararajan V, Grann VR, et al. Effectiveness of platinum-based chemotherapy among elderly patients with advanced ovarian cancer. Gynecol Oncol 2004;94:540–9.
[31] Pui CH, Ribeiro RC, Hancock ML, Sandlund JT, Behm FG, Head DR, et al. Acute myeloid leukemia in children treated with epipodophyllotoxins for acute lymphoblastic leukemia. N Engl J Med 1991;325:1682–7.
[32] Pedersen-Bjergaard J, Anderson MK, Christian DH, Nerlov C. Genetic pathyways in therapy-related myelodysplasia and acute myeloid leukemia. Blood 2002;99:1909–12.
[33] Rose PG, Rodriguez M, Waggoner S, Greer BE, Horowitz IR, Fowler JM, et al. Phase I study of paclitaxel, carboplatin, and increasing days of prolonged oral etoposide in ovarian, peritoneal, and tubal carcinoma: a Gynecologic Oncology Group study. J Clin Oncol 2000;18: 2957–62.
[34] Rose PG, Blessing JA, Soper JT, Barter JF. Prolonged oral etoposide in recurrent or advanced leiomyosarcoma of uterus: a Gynecologic Oncology Group study. Gynecol Oncol 1998;70:267–71.
[35] Le Deley MC, Leblanc T, Schamsadldin A, Raquin MA, Lacour B, Sommelet D, et al. Risk of secondary leukemia after a solid tumor in childhood according to the dose of epipodophyllotoxins and anthracyclines: a case–control study by the Societe Francasise d' Oncologie Pediatrique. J Clin Oncol 2003;21:1074–81.
[36] Parmar MK, Ledermann JA, Colombo N, du Bois A, Delaloye JF, Kristensen GB, et al. ICON and AGO Collaborators. Paclitaxel plus platinum-based chemotherapy versus conventional platinum-based chemotherapy in women with relapsed ovarian cancer: the ICON 4/AGO-OVAR-2.2 trial. Lancet 2003;361:2099–106.

Promoter methylation status of the Cyclin D2 gene is associated with poor prognosis in human epithelial ovarian cancer

Michiko Sakuma,[1,3] Jun-ichi Akahira,[2] Kiyoshi Ito,[1] Hitoshi Niikura,[1] Takuya Moriya,[2] Kunihiro Okamura,[1] Hironobu Sasano[2] and Nobuo Yaegashi[1]

Departments of [1]Obstetrics and Gynecology, and [2]Pathology Tohoku University, Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku Sendai, 980-8574 Japan

(Received March 18, 2006/Revised July 28, 2006/2nd Revised November 10, 2006/Accepted November 18, 2006/Online publication January 15, 2007)

Gene silencing associated with aberrant DNA methylation of promoter CpG islands is one mechanism through which several genes may be inactivated in human cancers. Cyclin D2, a member of the D-type cyclins, implicated in cell cycle regulation, differentiation and malignant transformation, is inactivated due to aberrant DNA methylation in several human cancers. In the present study, we examined the promoter methylation status and expression of Cyclin D2 in human epithelial ovarian cancer, and then determined the relationship between methylation status and various clinicopathological variables. Twelve ovarian cancer cell lines and 71 surgical specimens were examined by methylation-specific polymerase chain reaction and quantitative reverse transcription–polymerase chain reaction to evaluate the methylation status and expression of the Cyclin D2 gene. The relationship between methylation status and various clinicopathological variables was evaluated using statistical analysis. Aberrant methylation of Cyclin D2 was present in five of 12 ovarian cancer cell lines and 16 of 71 primary ovarian cancer tissues. In five cell lines with methylation, expression of the Cyclin D2 gene tended to be lower than in cell lines without methylation. In ovarian cancer tissues, methylation bands were detected in 16 of 71 cases. The methylation status of Cyclin D2 was associated with advanced stage and a residual tumor size (>2 cm) ($P = 0.027$ and $P = 0.031$, respectively). Based on univariate analysis, patients with aberrant methylation of the Cyclin D2 promoter had a significantly worse chance of disease-free survival than those without methylation ($P = 0.021$). Our results suggest that aberrant promoter methylation of the Cyclin D2 gene is significantly associated with patient prognosis in epithelial ovarian cancer. (*Cancer Sci* 2007; 98: 380–386)

Epithelial ovarian cancer is the most common and deadliest gynecological malignancy in developed countries. Early stages of ovarian cancer are generally asymptomatic and difficult to detect. By the time clinical diagnosis is made, most patients have widespread tumor dissemination.[1] Despite a high response rate to first-line chemotherapy, the prognosis of these women is poor, with an overall 5-year survival rate of only 10–20%.[1,2]

Epigenetic alterations, changes that affect gene expression but not the gene sequence itself, are believed to be one mechanism by which tumor suppressor genes are inactivated in human cancers.[3,4] In particular, hypermethylation of cytosine residues in CpG islands leads to heritable gene silencing via the formation of a repressive chromatin structure.[5,6] Studies of DNA hypermethylation in human ovarian cancer have identified some key genes as targets for epigenetic downregulation, including some hormone receptors,[7] cytokines, cell signaling intermediates, adhesion molecules,[8] DNA damage checkpoint genes,[9] and regulators of the cell cycle.[10] The cell cycle regulators, notably the cyclins, have the potential to function as oncogenes when regulated inappropriately.

The cyclins are a family of proteins that dictate transitions between phases of the cell cycle by regulating the activity of their downstream effectors, the cyclin-dependant kinases (cdk). The D-type cyclins, D1, D2 and D3, play a critical role in early checkpoint regulation of the G_1 phase of the cell cycle. They activate cdk4 and cdk6, leading to the phosphorylation of the retinoblastoma tumor suppressor protein (Rb). This, in turn, dissociates Rb from the transcription factor E2F, thereby permitting DNA transcription. Given the critical role of the D-type cyclins in cell cycle regulation, their abnormal or untimely expression could disrupt the normal cell cycle, resulting in cell proliferation.[11] In fact, Cyclin D1 is considered by some to be a putative protooncogene, as it is overexpressed in a number of tumor types, including breast cancer, thyroid carcinoma, stomach cancer and lymphomas.[12] Aberrant expression of Cyclin D2 has also been demonstrated in human ovarian granulose cell tumors and testicular germ cell tumor cell lines.[13]

Although well known for their proliferation-promoting activity, the D-type cyclins (notably D2) also have growth-inhibitory effects. Cyclin D2 has been shown to be dramatically upregulated under conditions of growth arrest in human and murine fibroblasts. Furthermore, transient overexpression of Cyclin D2 efficiently inhibits cell cycle progression and DNA synthesis. This suggests that an alternative role for Cyclin D2 may be to promote exiting from the cell cycle and maintenance of a non-proliferative state.[14] The expression of Cyclin D2 is frequently lost in human breast cancers, gastric cancers, lung cancers and ovarian granulose cell tumors. This loss of expression is the result of promoter hypermethylation.[10,15–18]

In the present study, we examined the promoter methylation status and gene expression of Cyclin D2 in human epithelial ovarian cancer cell lines. We also evaluated the correlation between methylation status of the Cyclin D2 promoter and various clinicopathological parameters in patients with epithelial ovarian cancer.

Materials and Methods

Cell lines. Twelve ovarian carcinoma cell lines were used. OVCAR3, SKOV3 (both adenoarcinomas), Caov3, OV90 (both serous adenocarcinoma), TOV21G, ES2 (both clear cell adenocarcinoma) and TOV112D (endometrioid adenocarcinoma) were purchased from American Type Culture Collection. JHOS2, JHOS3, HTOA (all serous adenocarcinoma), OMC3 (mucinous adenocarcinoma) and JHOC5 (clear cell adenocarcinoma) were purchased from Riken Cell Bank (Tsukuba). Cell lines were maintained in DMEM/F12 medium (Invitrogen), supplemented

[3]To whom correspondence should be addressed.
E-mail: msakuma@mail.tains.tohoku.ac.jp

doi: 10.1111/j.1349-7006.2007.00394.x

with 10% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen), and incubated in a 5% CO_2 atmosphere at 37°C.

Surgical specimens and clinical data. The research protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine, Sendai, Japan. We examined 71 ovarian cancer specimens obtained from patients treated between 1988 and 2002 at Tohoku University Hospital, Sendai, Japan. All specimens were retrieved from the surgical pathology files at Tohoku University Hospital. Informed consent was obtained from each patient. Specimens were fixed in 10% formalin and embedded in paraffin. Patient age, performance status on admission, histology, stage, grade, residual tumor after primary surgery, and overall survival were obtained from a chart review. The median follow-up time for patients was 59 months (range, 4–120 months). Performance status was defined according to the WHO criteria.[19] Histology, stage and grading followed the FigO criteria.[20] Residual tumor was defined as the amount of unresectable tumor left following primary volume reductive surgery. Optimal volume reduction was achieved when the residual tumor was less than 2 cm. Patients with a residual tumor greater than 2 cm were considered to have suboptimal volume reduction. Overall survival was calculated from the time of initial surgery to death or the date of the last contact. Survival times of patients still alive or lost to follow-up were censored as of December 2002.

An ovarian tissue obtained from a 50-year-old w oman who had received surgical treatment for benign uterine tumor was used as a normal ovarian tissue for methylation-specific polymerase chain reaction (MSP) and reverse transcription–polymerase chain reaction (RT-PCR).

Methylation-specific polymerase chain reaction. The methylation status of the samples was assessed using MSP as described previously.[21] Genomic DNA from ovarian cancer cell lines was extracted using the AquaPure Genomic DNA kit (Bio-Rad). Genomic DNA from ovarian tumor specimens was extracted from paraffin blocks. For each tissue, the presence of carcinoma was confirmed on a H&E stained section. For DNA extraction, three 5-μm tissue sections from the same block were scraped from the slide and treated with Dexpat (Takara). The quality and integrity of the DNA were evaluated in terms of the $A_{260/280}$ ratio. Genomic DNA (1 μg) was treated with sodium bisulfite using a CpGenome DNA modification kit (Intergen) according to the manufacturer's protocol. Amplification was conducted in a 20-μL reaction volume containing 2 μL of 10× ExTaq buffer, 1.5 μL of 2.5 mM $MgCl_2$, 1 mM of each primer, 1.5 mL of 2.5 mM dNTPs, and 1 unit of Takara ExTaq polymerase (Takara). The reaction was cycled for 40 cycles, each of which consisted of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 45 s, followed by a 7-min extension at 72°C. The primers used were 5′-AGAGTAT-GTGTTAGGGTTGATT-3′ and 5′-ACATCCTCACCAACCCTCCA-3′ (−1431 to −1326, 106-bp) for the unmethylated reaction (U), and 5′-GGCGGATTTTATCGTAGTCG-3′ and 5′-CTCCAC-GCTCGATCCTTCG-3′ (−1404 to −1304, 101-bp) for the methylated reaction (M).[18] Universal unmethylated human genomic DNA (Intergen) was used as a positive control for the unmethylated reaction. Universal methylated human male genomic DNA (Intergen) was used as a positive control for the methylated reaction. Reaction products were separated by electro - phoresis on 3% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light.

Quantitative RT-PCR. Total RNA was isolated from cells by phenol–chloroform extraction using Isogen reagent (Nippon Gene). RNA was treated with RNase-free DNase (Roche Diagnostics; 1 μg/μL) for 2 h at 37°C, followed by heat inactivation at 65°C for 10 min. Total RNA (5 μg) was reversed transcribed using the Superscript II first-strand synthesis system (Invitrogen) with random hexamers according to the manufacturer's protocol. Quantitative polymerase chain reaction (PCR) was carried out using an iCycler system (Bio-Rad). For the determination of Cyclin D2 cDNA content, a 25-μL reaction mixture consisting of 23 μL iQSYBR Green MasterMix, 1 μL of each primer and 1 μL of cDNA template was cycled as follows: 2-min denaturation at 90°C, 30-s annealing at either 60°C (for Cyclin D2) or 62°C (for β-actin), and 1.5-min extension at 72°C. Primers for PCR reactions were as follows: Cyclin D2-F, 5′-TACTTCAAGTGCGTGCAGAAGGAC-3′ and Cyclin D2-R, 5′-TCCCACACTTCCAGTTGCGATCAT-3′;[22] and β-actin-F, 5′-CCAACCGCGAGAAGATGAC-3′ and β-actin-R, 5′-GGAAGGAAGGCTGGAAGAGT-3′.[23] β-Actin primers were utilized as an internal positive control and Cyclin D2 expression level was calculated by dividing the quantity obtained for Cyclin D2 by the quantity obtained for β-actin. Two independent RT-PCR reactions were carried out for each sample.

5-Aza-2′-deoxycitidine and trichostatin A treatment. To confirm that epigenetic change contributed to loss of Cyclin D2 gene expression, we assessed the effect of 5-aza-2′-deoxycitidine (5azaC) (Sigma), a demethylating agent, and trichostatin A (TSA) (Sigma), a histone deacetylase inhibitor, on Cyclin D2 mRNA expression and cell growth of ovarian cancer cell lines by quantitative RT-PCR and cell count, respectively.

Ovarian cancer cell lines (OMC3, OVCAR3, JHOS2, JHOC5 and SKOV3) were cultured at a point of 70% confluence in 10-cm cell dishes. They were treated with 1.0 μM 5azaC for 3 or 5 days. They were also treated with 0.5 μM TSA.[24,25] We set up TSA treatment times of 4, 8, 16 and 32 h, and the treatments for 8 and 16 h appeared the most effective for gene expression compared to control culture (data not shown). Total RNA was prepared at each time point and the expression of Cyclin D2 mRNA was analyzed by quantitative RT-PCR. Furthermore, we investigated the effects of these chemical agents on cell growth of ovarian cancer cell lines by cell count at each time point.

Immunohistochemistry. For the purpose of investigating cell proliferation we examined the immunohistochemical expression of Ki-67 in ovarian cancer tissue. Immunohistchemical analysis was carried out with the streptavidin–biotin amplification method using the NX/ES IHC system (Ventana Medical Systems). Monoclonal antibody for Ki-67 (MIB-1) was purchased from DAKO. For antigen retrieval, the slides ware heated in an autoclave at 120°C for 5 min in citric acid buffer (2 mM citric acid and 9 mM trisodium citrate dyhydrate [pH 6.0]). The dilution of primary antibody was 1:50. Scoring of Ki-67 in carcinoma cells w as counted independently by two of the authors (M. S. and J. A.), and the percentage of immnunoreactivity in at least 500 carcinoma cells (i.e. the labeling index) was determined.

Statistical analysis. Statistical analysis was carried out using Stat View 5.0 software (SAS Institute). The correlation between the Cyclin D2 mRNA expression level and methylation status was assessed using the Mann–Whitney *U*-test. The statistical significance between methylation status and various clinico-pathological parameters w as evaluated using Friedman's χ^2 *r*-test and the Mann–Whitney *U*-test. A univariate analysis of prognostic significance for prognostic factors was carried out using the log-rank test after each survival curve was obtained by the Kaplan–Meier method. Multivariate analysis was carried out using the Cox regression model to evaluate the predictive power of each variable independently. All patients who could be assessed were included in the intention-to-treat analysis. A result was considered significant when the *P*-value was less than 0.05.

Results

Methylation status of the Cyclin D2 gene in ovarian cancer cell lines and tissues. Bands corresponding to methylated Cyclin D2 were

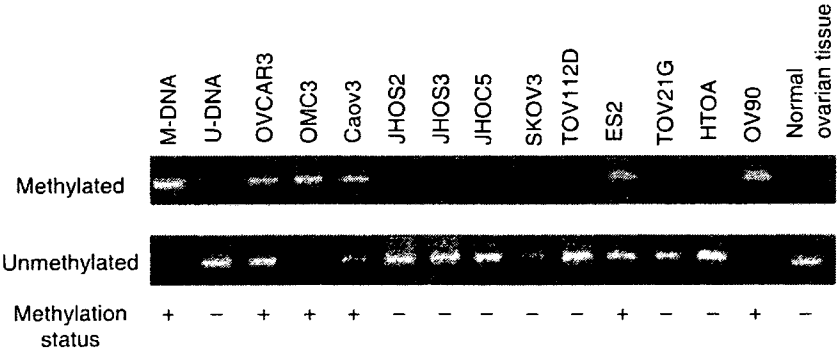

Fig. 1. Methylation status of the Cyclin D2 gene in ovarian cancer cell lines and a normal ovarian tissue. The 101-bp bands in the 'Methylated' lanes indicate the presence of methylated alleles of the Cyclin D2 gene. The 106-bp bands in the 'Unmethylated' lanes correspond to the unmethylated alleles. Methylation status is denoted as follows: +, methylated alleles with or without unmethylated alleles; −, purely unmethylated alleles. M-DNA, universal methylated human male genomic DNA, was used for positive control of methylated reaction. U-DNA, universal unmethylated fetal genomic DNA, was used for positive control of unmethylated reaction.

Table 1. Patient characteristics and cyclin D2 methylation status

Variable	*n*	Cyclin D2 methylation			*P*-value
		+	−	%	
Age (years)					
<50	29	8	21	27.6	
≥50	42	8	34	19	NS
Performance status†					
0–1	51	9	42	17.6	
2–4	19	7	12	36.8	NS
FigO stage					
I, II	35	4	31	2.9	
III, IV	36	12	24	33.3	0.027
Histological type of adenocarcinoma					
Serous	26	6	20	23.1	
Endometrioid	15	3	12	20	
Mucinous	7	3	4	75	
Clear cell	23	4	19	17.4	NS
Grade					
1	24	5	19	20.8	
2	22	7	15	31.8	
3	17	3	14	17.6	NS
Residual tumor size (cm)					
<2	47	7	40	14.9	
≥2	24	9	15	37.5	0.031
Ki-67 labeling index (median)		21.6	23.6	20.4	NS

†0, asymptomatic and fully active; 1, symptomatic, fully ambulatory, restricted in physically strenuous activity; 2, symptomatic, ambulatory, capable of self-care, more than 50% of walking hours are spent out of bed; 3, symptomatic, limited self-care, more than 50% of time is spent in bed, but not bedridden; 4, completely disabled, no self-care, bedridden

detected in five of 12 cell lines, three of which also contained the unmethylated band, as shown in Fig. 1. The methylated band was detected in two of five cell lines derived from serous adenocarcinoma (Caov3, OV90), in one of three cell lines from clear cell carcinoma (ES2), in the one mucinous adenocarcinoma (OMC3), but not in the endometrioid adenocarcinoma. The normal ovarian tissue was negative for the methylated band. The methylated band was detected in 16 of the 71 surgical specimens (6/26 serous, 4/23 clear cell, 3/15 endometrioid and 3/7 mucinous adenocarcinoma), as shown in Table 1.

Expression of the Cyclin D2 gene in ovarian cancer cell lines and normal ovarian tissue. The expression of the Cyclin D2 gene in the cell lines is presented in Fig. 2. Quantitative RT-PCR was carried out and the ratio of Cyclin D2 to β-actin was calculated to allow for comparison among the cell lines. The median value of relative Cyclin D2 gene expression in cell lines with methylation (0.015) tended to be lower than that in cell lines without methylation (0.03), although the difference was not significant ($P = 0.19$, Mann–Whitney U-test). The expression level of the Cyclin D2 gene in normal ovarian tissue was relatively high compared with ovarian cancer cell lines.

Effects of 5azaC and TSA treatment on methylated cell lines. To confirm that promoter methylation contributed to the loss of Cyclin D2 gene expression, we assessed the effect of 5azaC, a demethylating agent, on Cyclin D2 mRNA expression by quantitative RT-PCR. OMC3 and OVCAR3 cells, which were positive for the methylated band in MSP, were treated. From MSP analysis OMC3 had only methylated alleles, but OVCAR3 had both methylated and unmethylated alleles. We also assessed the effect of TSA, a histone deacetylase inhibitor, to investigate whether another epigenetic change, histone deacetylation, contributed to the silencing of Cyclin D2 gene expression. Treatment of OMC3 cells with 5azaC for 5 days led to a 2.64-fold increase in expression (Fig. 3a). Treatment of OVCAR3 cells with 5azaC for 5 days resulted in a 222-fold increase in expression (Fig. 3b). Treatment with TSA also contributed to re-expression of the Cyclin D2 gene in OMC3 and OVCAR3 cells (2.3-fold and 119-fold, respectively) (Fig. 3). These results suggested that the decreased expression of Cyclin D2 in these cell lines was related to epigenetic change, including DNA methylation or histone deacetylation.

The effects of 5azaC and TSA on cell growth are summarized in Fig. 4. Compared with cell growth in control culture, cell growth with 5azaC or TSA treatment was suppressed in each culture. These chemical agents resulted in inhibition of cell growth in these ovarian cancer cell lines simultaneous with re-expression of the Cyclin D2 gene.

Effects of 5azaC and TSA treatment on unmethylated cell lines. In the MSP and quantitative RT-PCR analyses, expression of the Cyclin D2 gene was decreased in some cell lines without promoter methylation. We assessed the effect of 5azaC or TSA treatment in these cell lines (JHOS2, JHOC5 and SKOV3) to investigate the participation of epigenetic change in the silencing of this gene. Treatment of JHOS2 cells with TSA resulted in higher re-expression than treatment with 5azaC (Fig. 5a). Treatment of JHOC5 cells with TSA for 16 h resulted in an 84.4-fold increase in expression, and treatment with 5azaC also led to a 137-fold increase in expression (Fig. 5b). As for SKOV3 cells, treatment with TSA did not increase the expression of this gene. These results suggest that histone deacetylation may contribute to silencing of the Cyclin D2 gene in JHOS2 and JHOC5 cells, but not in SKOV3.

Correlation between clinicopathological parameters and methylation status of Cyclin D2 in epithelial ovarian cancer. The clinicopathological parameters relative to the methylation status of Cyclin D2 are presented in Table 1. Methylation status was significantly associated with advanced stage and residual tumor size >2 cm.

doi: 10.1111/j.1349-7006.2007.00394.x