Table 3. Multiple regression analysis for overall survival in the endometrial carcinoma patients with suspected gross cervical involvement

Variables	Hazard ratio	95% confidence interval	*P*-value
Age (years)			0.02
<54	1		
>55	2.41	1.15; 5.07	
Performance status			0.52
0	1		
1,2,3	1.35	0.54; 3.36	
Histology			0.02
E, G1/2	1		
E, G3 + others	2.25	1.14; 4.42	
Pathological invasion of cervix			0.92
None	1		
Glandular	1.15	0.52; 2.56	
Stromal	1.18	0.54; 2.58	
Parametrial invasion			0.87
Negative	1		
Positive	1.11	0.32; 3.98	
Myometrial invasion of corpus			0.32
⩽1/2	1		
>1/2	1.50	0.68; 3.32	
Lymph node metastasis			<0.01
No	1		
Yes	3.32	1.03; 10.63	
Not available	3.24	0.99; 10.64	
Ascites/malignant washing			<0.01
Negative	1		
Positive	3.34	1.63; 6.80	
Dissemination beyond pelvis			0.09
Negative	1		
Positive	2.88	0.85; 9.71	
Lymph-vascular invasion			0.39
Negative	1		
Positive	0.73	0.35; 1.50	
Type of hysterectomy			0.39
SH	1		
mRH	1.76	0.76; 4.07	
RH	1.56	0.67; 3.62	
Postoperative treatment			0.63
None	1		
Chemotherapy (C)	0.92	0.35; 2.39	
Radiotherapy (R)	0.97	0.29; 3.26	
R followed by C	0.35	0.06; 1.90	

Abbreviations: E = endometrioid adenocarcinoma: G1/2 = grade 1 and 2; G3 = grade 3; RH = radical hysterectomy; mRH = modified radical hysterectomy; SH = simple hysterectomy.

Table 4. Adverse effects according to surgical procedures

Variables	RH (*n* = 74)	mRH (*n* = 112)	SH (*n* = 114)	*P*-value
Perioperative adverse effects				
Operative time (minutes)				0.058[a] <0.01[b]
Median	292	282	184	
Range	174–677	187–475	81–288	
Blood loss (g)				<0.01[a]
Median	1162	855	355	<0.01[b]
Range	320–6000	120–4060	30–3140	
Blood transfusion				<0.01
Yes	43	47	18	
No	31	65	96	
Deep vein thrombosis, or pulmonary embolism (grade ⩾2)				0.25
Yes	2	2	0	
No	72	110	114	
Ileus (grade ⩾2)				0.87
Yes	2	3	2	
No	72	109	112	
Late adverse effects [c]				
Lymphedema (grade ⩾2)				0.18
Yes	9	7	6	
No	65	105	108	
Urinary retention (grade ⩾2)				<0.01
Yes	11	1	0	
No	63	111	114	

Abbreviations: mRH = modified radical hysterectomy; RH = radical hysterectomy; SH = simple hysterectomy.
[a]RH *vs* mRH.
[b]mRH *vs* SH.
[c]Judged by Common Terminology Criteria for Adverse Events v4.0.

recommended for grade 3 tumours from the results of two randomized trials (Hogberg *et al*, 2010); however, the addition of chemotherapy was related with only improved PFS, and there was no effect on OS. In contrast, the effect of systematic chemotherapy as adjuvant therapy has been evaluated for the patients with intermediate–high risk endometrial cancers. The Japanese Gynecologic Oncology Group compared pelvic radiotherapy and chemotherapy with cyclophosphamide, doxorubicin, and cisplatin in patients with stage IC–IIIC endometrial cancer, and suggested a survival advantage of chemotherapy in the women from the high-to-intermediate risk group (stage IC, >70 years of age, grade 3, stage II, or positive cytology with >50% myometrial invasion) (Susumu *et al*, 2008). Moreover, The Gynecologic Oncology Group study 122 revealed that chemotherapy with doxorubicin plus cisplatin was associated with superior PFS in patients with stage III–IV endometrial cancer along with a minimal residual tumour, compared with radiation therapy (Randall *et al*, 2006). These results have had a significant impact on clinical practice in the Japanese gynaecologic oncology community, and adjuvant chemotherapy was often used for the endometrial cancer patients with high-to-intermediate risk group including stage II disease. The present study included a higher abundance of patients treated with adjuvant chemotherapy, which might be reflecting completely different preference of Japanese physicians. However, the survival data of these series were not inferior to the previous reports that investigated stage II patients (Mariani *et al*, 2001; Sartori *et al*, 2001). Chemotherapy could be potentially a candidate for adjuvant therapy for endometrial cancers with intermediate–high risk. In the present study, there were no significant differences of

adjuvant therapy on survival. Of note, radiotherapy followed by chemotherapy produced lowest hazard ratio in local recurrence-free survival (HR = 0.28) and OS (HR = 0.35), although it did not reach statistical significance. Further analyses are needed to elucidate to select adjuvant therapy for the disease.

The limitation of the present study included a retrospective investigation and multi-institutional analysis. Also, the results obtained by the present study could potentially have a bias such as selection bias, and further prospective investigation is needed to confirm the impact of operative procedures. Nevertheless, the survival improvement was not observed by RH for endometrial cancer patients with suspected cervical involvement by multivariate analyses. The necessity of RH in these patients should be evaluated in further clinical studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Akin O, Mironov S, Pandit-Taskar N, Hann LE (2007) Imaging of uterine cancer. *Radiol Clin North Am* **45**(1): 167–182.

Ayhan A, Taskiran C, Celik C, Yuce K (2004) The long-term survival of women with surgical stage II endometrioid type endometrial cancer. *Gynecol Oncol* **93**(1): 9–13.

Cohn DE, Woeste EM, Cacchio S, Zanagnolo VL, Havrilesky LJ, Mariani A, Podratz KC, Huh WK, Whitworth JM, McMeekin DS, Powell MA, Boyd E, Phillips GS, Fowler JM (2007) Clinical and pathologic correlates in surgical stage II endometrial carcinoma. *Obstet Gynecol* **109**(5): 1062–1067.

Cornelison TL, Trimble EL, Kosary CL (1999) SEER data, corpus uteri cancer: treatment trends versus survival for FIGO stage II, 1988-1994. *Gynecol Oncol* **74**(3): 350–355.

Creasman W (2009) Revised FIGO staging for carcinoma of the endometrium. *Int J Gynaecol Obstet* **105**(2): 109.

Evans T, Sany O, Pearmain P, Ganesan R, Blann A, Sundar S (2011) Differential trends in the rising incidence of endometrial cancer by type: data from a UK population-based registry from 1994 to 2006. *Br J Cancer* **104**(9): 1505–1510.

Hogberg T, Signorelli M, de Oliveira CF, Fossati R, Lissoni AA, Sorbe B, Andersson H, Grenman S, Lundgren C, Rosenberg P, Boman K, Tholander B, Scambia G, Reed N, Cormio G, Tognon G, Clarke J, Sawicki T, Zola P, Kristensen G (2010) Sequential adjuvant chemotherapy and radiotherapy in endometrial cancer–results from two randomised studies. *Eur J Cancer* **46**(13): 2422–2431.

Lee TS, Kim JW, Kim DY, Kim YT, Lee KH, Kim BG, McMeekin DS (2010) Necessity of radical hysterectomy for endometrial cancer patients with cervical invasion. *J Korean Med Sci* **25**(4): 552–556.

Manfredi R, Mirk P, Maresca G, Margariti PA, Testa A, Zannoni GF, Giordano D, Scambia G, Marano P (2004) Local-regional staging of endometrial carcinoma: role of MR imaging in surgical planning. *Radiology* **231**(2): 372–378.

Mariani A, Webb MJ, Keeney GL, Calori G, Podratz KC (2001) Role of wide/radical hysterectomy and pelvic lymph node dissection in endometrial cancer with cervical involvement. *Gynecol Oncol* **83**(1): 72–80.

Nagase S, Katabuchi H, Hiura M, Sakuragi N, Aoki Y, Kigawa J, Saito T, Hachisuga T, Ito K, Uno T, Katsumata N, Komiyama S, Susumu N, Emoto M, Kobayashi H, Metoki H, Konishi I, Ochiai K, Mikami M, Sugiyama T, Mukai M, Sagae S, Hoshiai H, Aoki D, Ohmichi M, Yoshikawa H, Iwasaka T, Udagawa Y, Yaegashi N. Japan Society of Gynecologic Oncology (2010) Evidence-based guidelines for treatment of uterine body neoplasm in Japan: Japan Society of Gynecologic Oncology (JSGO) 2009 edition. *Int J Clin Oncol* **15**(6): 531–542.

NCCN Guidelines. version 1.2013 (2013) *Uterine Neoplasm*. http://www.nccn.org/index.asp. Accessed on 11 April 2013.

Randall ME, Filiaci VL, Muss H, Spirtos NM, Mannel RS, Fowler J, Thigpen JT, Benda JA. Gynecologic Oncology Group Study (2006) Randomized phase III trial of whole-abdominal irradiation versus doxorubicin and cisplatin chemotherapy in advanced endometrial carcinoma: a Gynecologic Oncology Group study. *J Clin Oncol* **24**(1): 36–44.

Sartori E, Gadducci A, Landoni F, Lissoni A, Maggino T, Zola P, Zanagnolo V (2001) Clinical behavior of 203 stage II endometrial cancer cases: the impact of primary surgical approach and of adjuvant radiation therapy. *Int J Gynecol Cancer* **11**(6): 430–437.

Siegel R, Naishadham D, Jemal A (2012) Cancer statics, 2012. *CA Cancer J Clin* **62**(1): 10–29.

Susumu N, Sagae S, Udagawa Y, Niwa K, Kuramoto H, Satoh S, Kudo R. Japanese Gynecologic Oncology Group (2008) Randomized phase III trial of pelvic radiotherapy versus cisplatin-based combined chemotherapy in patients with intermediate- and high risk endometrial cancer: a Japanese Gynecologic Oncology Group study. *Gynecol Oncol* **108**(1): 226–233.

Wright JD, Fiorelli J, Kansler AL, Burke WM, Schiff PB, Cohen CJ, Herzog TJ (2009) Optimizing the management of stage II endometrial cancer: the role of radical hysterectomy and radiation. *Am J Obstet Gynecol* 2004: 419.e1–e7.

Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)

Checkpoint Kinase Inhibitor AZD7762 Overcomes Cisplatin Resistance in Clear Cell Carcinoma of the Ovary

Hiroaki Itamochi, MD, PhD, Mayumi Nishimura, PhD,† Nao Oumi, PhD,† Misaki Kato, PhD,† Tetsuro Oishi, MD, PhD,* Muneaki Shimada, MD, PhD,* Shinya Sato, MD, PhD,* Jun Naniwa, MD, PhD,* Seiya Sato, MD, PhD,* Akiko Kudoh, MD,* Junzo Kigawa, MD, PhD,† and Tasuku Harada, MD, PhD**

Objective: Checkpoint kinase (Chk) inhibitors are thought to increase the cytotoxic effects of DNA-damaging agents and are undergoing clinical trials. The present study was aimed to assess the potential to use the Chk1 and Chk2 inhibitor, AZD7762, with other anticancer agents in chemotherapy to treat ovarian clear cell carcinoma.

Methods: Four ovarian clear cell carcinoma cell lines were used in this study. We treated the cells with AZD7762 and anticancer agents, then assessed cell viability, cell cycle distribution, apoptosis, and the expression of protein in apoptotic pathways and molecules downstream of the Chk signaling pathways. We also investigated the effects of these drug combinations on tumor growth in a nude mouse xenograft model.

Results: Synergistic effects from the combination of AZD7762 and cisplatin were observed in all 4 cell lines. However, we observed additive effects when AZD7762 was combined with paclitaxel on all cell lines tested. AZD7762 effectively suppressed the Chk signaling pathways activated by cisplatin, dramatically enhanced expression of phosphorylated H2A.X, cleaved caspase 9 and PARP, decreased the proportion of cells in the gap 0/ gap 1 phase and the synthesis-phase fraction, and increased apoptotic cells. Combinations of small interfering RNA against Chk 1 and small interfering RNA against Chk2 enhanced the cytotoxic effect of cisplatin in both RMG-I and KK cells. Finally, treating mice-bearing RMG-I with AZD7762 and cisplatin significantly suppressed growth of tumors in a xenograft model.

Conclusions: The present study indicates that chemotherapy with AZD7762 and cisplatin should be explored as a treatment modality for women with ovarian clear cell carcinoma.

Key Words: Clear cell, Cisplatin, Resistance, Ovarian carcinoma, Checkpoint kinase

Received August 6, 2013, and in revised form August 10, 2013.
Accepted for publication August 29, 2013.

(*Int J Gynecol Cancer* 2014;24: 61–69)

Clear cell carcinoma of the ovary is recognized in the World Health Organization classification of ovarian tumors as a distinct histologic entity, and its clinical behavior is distinctly different from other epithelial ovarian cancers.[1] Clear cell carcinoma accounts for approximately 4% to 12% of epithelial ovarian cancers in the United States and, for unknown

*Department of Obstetrics and Gynecology, Tottori University School of Medicine, and †Tottori University Hospital Cancer Center, Yonago, Japan.

The authors declare no conflicts of interest.

ISSN: 1048-891X
DOI: 10.1097/IGC.0000000000000014

Address correspondence and reprint requests to Hiroaki Itamochi, MD, PhD, Department of Obstetrics and Gynecology, Tottori University School of Medicine, 36-1 Nishicho, Yonago 683-8504, Japan. E-mail: itamochi@med.tottori-u.ac.jp.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (24592517 to H. Itamochi).

reasons, more than 20% of such cancers in Japan. The poor prognosis of patients with advanced disease may reflect the resistance of clear cell carcinoma to conventional platinum-based chemotherapy.[2,3]

Several mechanisms involved in drug resistance have been proposed, including decreased drug accumulation, increased drug detoxification, increased DNA repair activity, and up-regulation of growth factor signaling pathways.[4] We previously reported that clear cell carcinoma tends to have a low proliferation rate, which could contribute to its poor prognosis and resistance to chemotherapy.[5,6] We also showed that cyclin-dependent kinase (CDK) 2 activity reduced because high p27 expression may suppress proliferation of clear cell carcinoma, and we confirmed that up-regulation of CDK2 activity enhanced the cytotoxic effects induced by DNA-damaging agents, such as cisplatin.[7] Furthermore, phorbol 12-myristate 13-acetate abrogates the cisplatin-induced activation of cell cycle checkpoint kinase (Chk) 1 and Chk2 expression and resulted in apoptosis of cisplatin-resistant ovarian serous adenocarcinoma cells.[8] Therefore, cell cycle and its checkpoint pathways can be exploited to enhance the cytotoxic effects of chemotherapeutic agents in ovarian clear cell carcinoma.

Activation of cell cycle checkpoints by DNA damage leads to transient arrest in gap 1 (G_1), synthesis (S), and G_2/mitotic (M) phases, which allows time for DNA repair and promotes cell survival.[9,10] When the DNA repair is incomplete, the cells undergo apoptosis. Thus, inhibiting Chk proteins is thought to enhance response to the DNA-damaging effects of cytotoxic drugs and radiosensitivity by abrogating DNA damage-induced S and G_2 checkpoints and the cell cycle arrest in several types of cancer.[11–15] Recently, a novel ATP-competitive and selective Chk1 and Chk2 inhibitor, (*S*)-5-(3-fluorophenyl)-*N*-(piperidin-3-yl)-3-ureidothiophene-2-carboxamide (AZD7762) was developed and has entered clinical trials.[11,12] However, the effects of Chk inhibitors combined with the cytotoxic agents have not been evaluated in ovarian clear cell carcinoma. We, therefore, conducted the present study to determine whether AZD7762 enhanced the cytotoxic effects of cisplatin in ovarian clear cell carcinoma cells. We also explored the mechanisms of synergistic interactions between AZD7762 and cisplatin.

MATERIALS AND METHODS

Cell Lines and Culture Conditions

The 4 human ovarian clear cell carcinoma cell lines used in this study were obtained as follows: RMG-I from Professor Shiro Nozawa, Keio University; KK from Dr. Yoshihiro Kikuchi, National Defense Medical College; and OVMANA from Dr. Hiroshi Minaguchi, Yokohama City University. TU-OC-1 was established by our department.[16] These cell lines were maintained in Dulbecco modified Eagle medium /Ham F-12 medium (Wako Pure Chemical Industries, Osaka, Japan) with 10% fetal bovine serum, 100-IU/mL penicillin, and 50-μg/mL streptomycin in a humidified atmosphere containing 5% carbon dioxide at 37°C.

Dose-Response Studies

The sensitivity of the cell lines to anticancer agents was determined by a cytotoxicity assay by using Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan), according to the specifications of the manufacturer. Briefly, cells were incubated with various concentrations of the anticancer agents to obtain a dose-response curve for each agent. Concentrations for each drug were 10- to 1000-nmol/L AZD7762 (Axon Medchem BV, Groningen, The Netherlands), 1- to 30-μmol/L cisplatin (Sigma-Aldrich Co, St. Louis, MO), 1- to 1000-nmol/L paclitaxel (Sigma-Aldrich Co), and 1- to 1000-nmol/L 7-ethyl-10-hydroxycamptothecin (SN-38; Yakult Honsha Co, Tokyo, Japan), which is an active metabolite of camptothecin. After being incubated for 72 hours, 20 mL of Cell Counting Kit-8 solution was added to each well, and the plates were incubated for another 1 to 2 hours. Absorbance was measured at 450 nm with a microplate reader (iMark Microplate Absorbance Reader, Bio-Rad Laboratories, Inc, Richmond, CA).

Dose-Effect Analysis

AZD7762 was combined with each of the different anticancer agents at a fixed ratio that spanned the individual half maximal inhibitory concentration (IC_{50}) of each drug. The half maximal inhibitory concentration was determined based on the dose-effect curves by a cytotoxicity assay. Median effect plot analyses and calculated combination indices (CI) were analyzed by the method of Chou and Talalay.[17] CalcuSyn software (Biosoft, Ferguson, MO) was used to analyze data from the cytotoxicity assays in which cells were exposed to agents alone or combined with cisplatin and AZD7762. CalcuSyn provides a measure of the combined agents in an additive or synergistic manner. Chou and Talalay defined CI as synergistic ($CI < 0.9$), additive ($0.9 < CI < 1.1$), or antagonistic ($CI > 1.1$).

Western Blot Analyses

Cells were lysed in lysis buffer. A total of 50-μg protein was separated by electrophoresis on a 5% to 20% or 15% polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). The specific antibodies used were mouse anti-Chk1 (1:200 dilution, Santa Cruz Biotechnology, Inc, Santa Cruz, CA), rabbit anti–phospho-Chk1 (serine 296, 1:1000 dilution, Cell Signaling Technology, Beverly, MA), rabbit anti-Chk2 (1:200 dilution, Santa Cruz Biotechnology, Inc), rabbit anti–phospho-Chk2 (threonine 68, 1:1000 dilution, Cell Signaling Technology), mouse anti-CDC25A (1:200 dilution, Santa Cruz Biotechnology, Inc.), rabbit anti–phospho-Histone H2A.X (serine 139, 1:1,000 dilution, Cell Signaling Technology), rabbit anticleaved caspase-9 (1:500 dilution, Cell Signaling Technology), rabbit anticleaved PARP (1:1,000 dilution, Cell Signaling Technology), and mouse anti-actin (1:1,000 dilution, Sigma-Aldrich Co). These were visualized with secondary antimouse or antirabbit immunoglobulin G antibody coupled with horseradish peroxidase, using enhanced chemiluminescence (Amersham Biosciences, Bath, UK) according to the manufacturer's recommendation.

Immunofluorescence Studies

Cells were grown on Labtek chamber slides at 2000 cells per well and cultured with or without reagents (15-μmol/L cisplatin and/or 50-nmol/L AZD7762) for 24 hours. The cells were fixed in 1% paraformaldehyde for 15 minutes at 4°C, followed by incubation for 10 minutes with 0.2% Tween-20/phosphate-buffered saline (PBS). After blocking with 5% bovine serum albumin in 0.1% Tween-20/PBS for 1 hour at room temperature, cells were incubated with rabbit anti–phospho-Histone H2A.X antibody (serine 139, 1:150 dilution, Cell Signaling Technology) for 90 minutes at room temperature. The cells were incubated with antirabbit immunoglobulin antibodies conjugated with Alexa Flour 488 (1:1500 dilution, Molecular Probes, Carlsbad, CA) for 45 minutes at room temperature and stained with DAPI/PBS for 10 minutes at room temperature. The cells were mounted with Fluoromount (Diagnostic BioSystems, Pleasanton, CA) and visualized with a Keyence (Osaka, Japan) BZ-8100E fluorescence microscope.

Flow Cytometry

For analysis of cell cycle distribution, the cells (2×10^6) were trypsinized, collected by centrifugation, fixed in 70% ethanol at 4°C for 1 hour, and resuspended in PBS, containing 50-μg/mL propidium iodide and 0.1-mg/mL RNase. After 30 minutes at 37°C, the cells were analyzed with a FACSAria cytofluorometer (Becton Dickinson, Franklin Lakes, NJ).

Small Interfering RNA

Cells were seeded in 6-well culture plates at 2.5×10^5 per well (30%–50% confluence) in Dulbecco modified Eagle medium/F12 medium supplemented with 10% fetal bovine serum. The next day, cells were transfected with small interfering RNA (siRNA) against Chk1 (si-Chk1) (Cell Signaling Technology), Chk2 (si-Chk2) (Cell Signaling Technology), or control siRNA (Santa Cruz Biotechnology, Inc) to a final siRNA concentration of 100 nmol/L using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA).

Ovarian Clear Cell Carcinoma Xenograft Model

This study was carried out at the Laboratory Animal Research Center under the control of the Animal Research Committee, in accordance with the Guidelines for Animal Experimentation in the Faculty of Medicine, Tottori University, Yonago, Japan. RMG-I cells (5×10^6 viable cells in 0.25-mL PBS) were inoculated subcutaneously under aseptic conditions into the left flank of female nude mice. The mice were assigned randomly to one of 4 groups (10 mice per group), and treatment was started 10 days later as follows. Group 1, intraperitoneal (IP) PBS weekly; group 2, IP AZD7762 weekly (25 mg/kg per injection); group 3, IP cisplatin weekly (1.5 mg/kg per injection) for 4 weeks; and group 4, IP cisplatin with AZD7762 weekly for 4 weeks. Tumor size was measured with a caliper twice weekly, and tumor volume was calculated as: Tumor Volume (mm^3) = $\pi / 6 \times L \times W^2$, where L and W were the longer and shorter dimensions of the tumor, respectively.

Statistical Analyses

Analyses were performed with the JMP version 9 program (SAS Institute Inc, Cary, NC). Data are presented as means ± standard deviation. Means for all data were

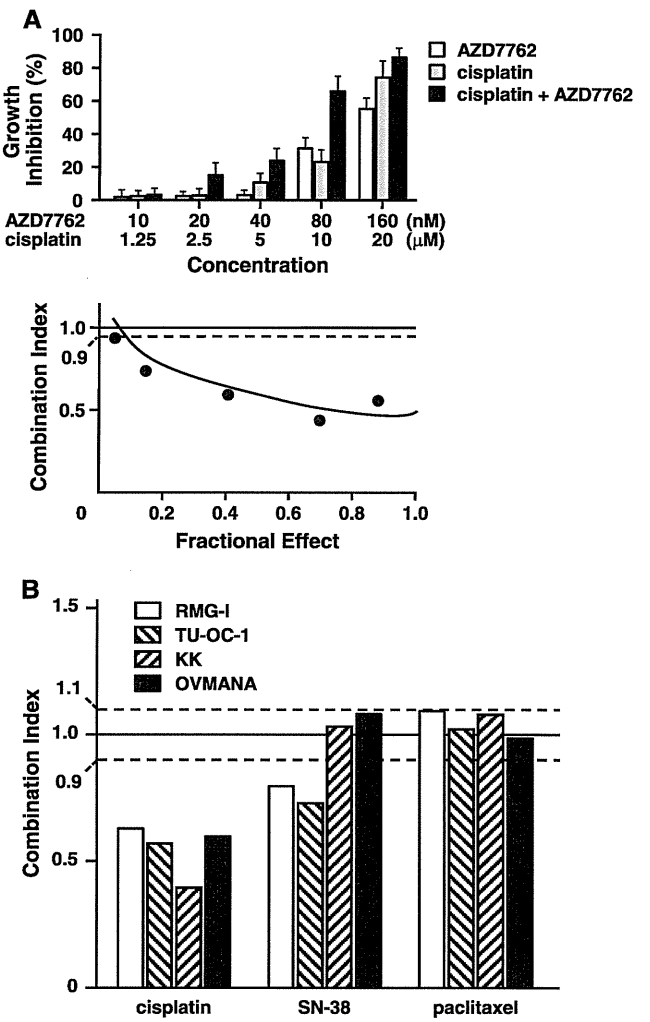


FIGURE 1. Effects of AZD7762 are synergistic with those of cisplatin. Cells were incubated with increasing concentrations of AZD7762 and cisplatin, 7-ethyl-10-hydroxycamptothecin (SN-38), or paclitaxel at a fixed ratio for 72 hours. A, Representative data from AZD7762 combined with cisplatin in RMG-I cells. Results are mean ± SD from 6 dishes. B, Data analyzed with CalcuSyn software to determine the CI. Chou and Talalay defined CI < 0.9, 0.9 < CI < 1.1, and CI > 1.1 as synergism, additivity, and antagonism of the 2 agents, respectively.

compared by one-way analysis of variance with post hoc testing. $P < 0.05$ was considered statistically significant.

RESULTS

Combination Effects of AZD7762 and Anticancer Agents

We analyzed the synergistic activity of combining AZD7762 with each anticancer agent from CI values calculated by the method of Chou and Talalay.[17] Data representative of AZD7762 combined with cisplatin in RMG-I cells are shown in Figure 1A. The CI value at an effective dose of 50 (effective dose means the percentage inhibition of cell growth using the drug combinations in the actual experiment) was less than 0.9 (synergism) for all 4 cell lines for cisplatin and 2 cell lines for SN-38 (Fig. 1B). However, the CI value was between 0.9 and 1.1 (additive) for all 4 cell lines for paclitaxel. Thus, when cisplatin was combined with AZD7762, synergistic effects were found in a greater number of cell lines.

AZD7762 Combined With Cisplatin Down-regulates Cell Cycle Checkpoints and Up-regulates the Apoptotic Pathway

We then examined whether the synergism arose from an increase in apoptosis induced by cisplatin. We confirmed that the protein expression levels of phosphorylated (p)-Chk1 at serine 296 and p-Chk2 at threonine 68 had increased and Cdc25A decreased after treatment with cisplatin alone in RMG-I and KK cells (Fig. 2A). AZD7762 inhibited

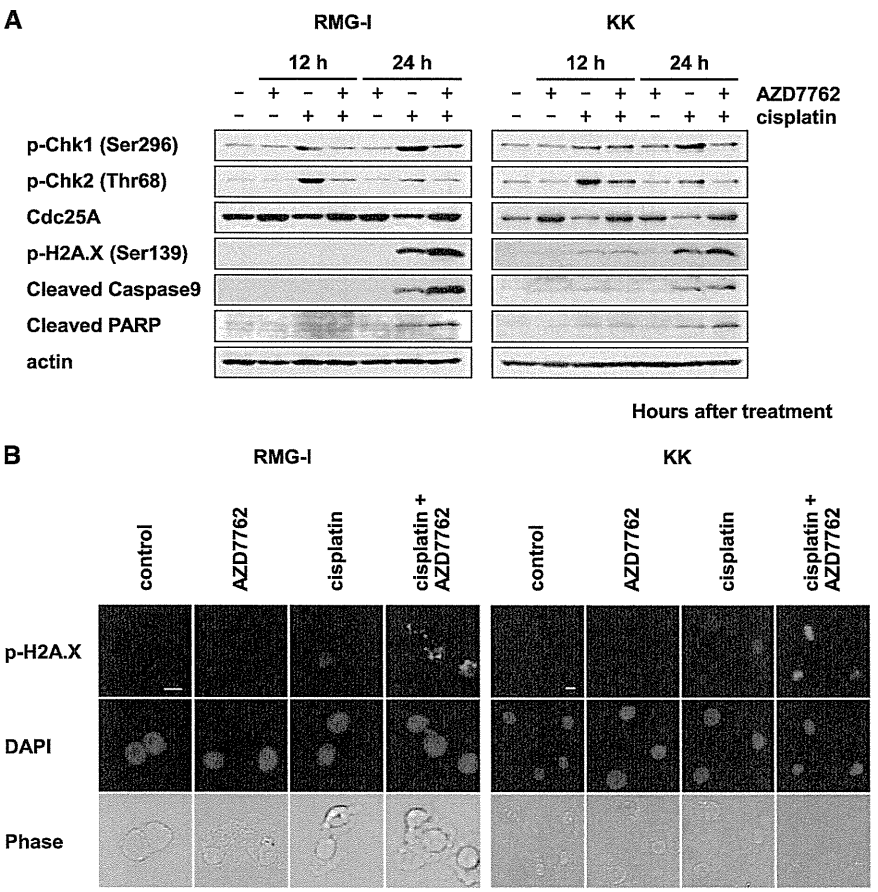


FIGURE 2. AZD7762 suppresses the cell cycle checkpoint pathways and enhances the apoptotic pathways induced by cisplatin in ovarian clear cell carcinoma cells. A, RMG-I and KK cells were treated at the indicated times with 15- or 7.5-μmol/L cisplatin and with PBS (control) and/or 50- or 150-nmol/L AZD7762, respectively. After being treated with cisplatin combined with AZD7762, the expression of p-Chk and p-Chk2 was suppressed and p-H2A.X, cleaved caspase 9, and cleaved PARP increased. The results shown represent duplicate experiments. B, RMG-I and KK cells were treated with AZD7762 and/or cisplatin for 24 hours and then fixed and immunostained for p-H2A.X. The nuclear expression of p-H2A.X increased dramatically after the treatment with AZD7762 and cisplatin in both cell lines. The results shown represent duplicate experiments. Scale bars, 10 μm.

phosphorylation of Chk1 and Chk2 effectively and stabilized Cdc25A in response to cisplatin. Interestingly, 24 hours after being treated with cisplatin and AZD7762, the protein expressions of p-H2A.X, cleaved caspase 9, and cleaved PARP were up-regulated. Immunofluorescence studies also showed that, compared to cisplatin alone, the increased formation of p-H2A.X protein in the nuclear foci was observed when cisplatin was combined with AZD7762 (Fig. 2B). Similar results were obtained in the other 2 cell lines (data not shown). These findings suggested that apoptosis induced with cisplatin in ovarian cancer cells may be enhanced by abrogating cell cycle check points, followed by up-regulation of unrepaired DNA damage by adding AZD7762.

AZD7762 Decreased S-Phase Fraction and Increased Cisplatin-Induced Cell Death

We assessed the cell cycle distribution by flow cytometry to confirm whether the combination treatment of cisplatin with AZD7762 influenced cell cycle distribution. After treatment with cisplatin alone, the proportion of RMG-I and KK cells in the S-phase fraction and the G_2/M phase were markedly increased (Figs. 3A, B). However, after treatment with cisplatin and AZD7762, the proportion of the cells in the G_0/G_1 and S phases decreased dramatically. Moreover, 48 hours after the combination treatment, the subG_1 population was significantly increased compared to treatment with cisplatin alone. Similar results were obtained in the other

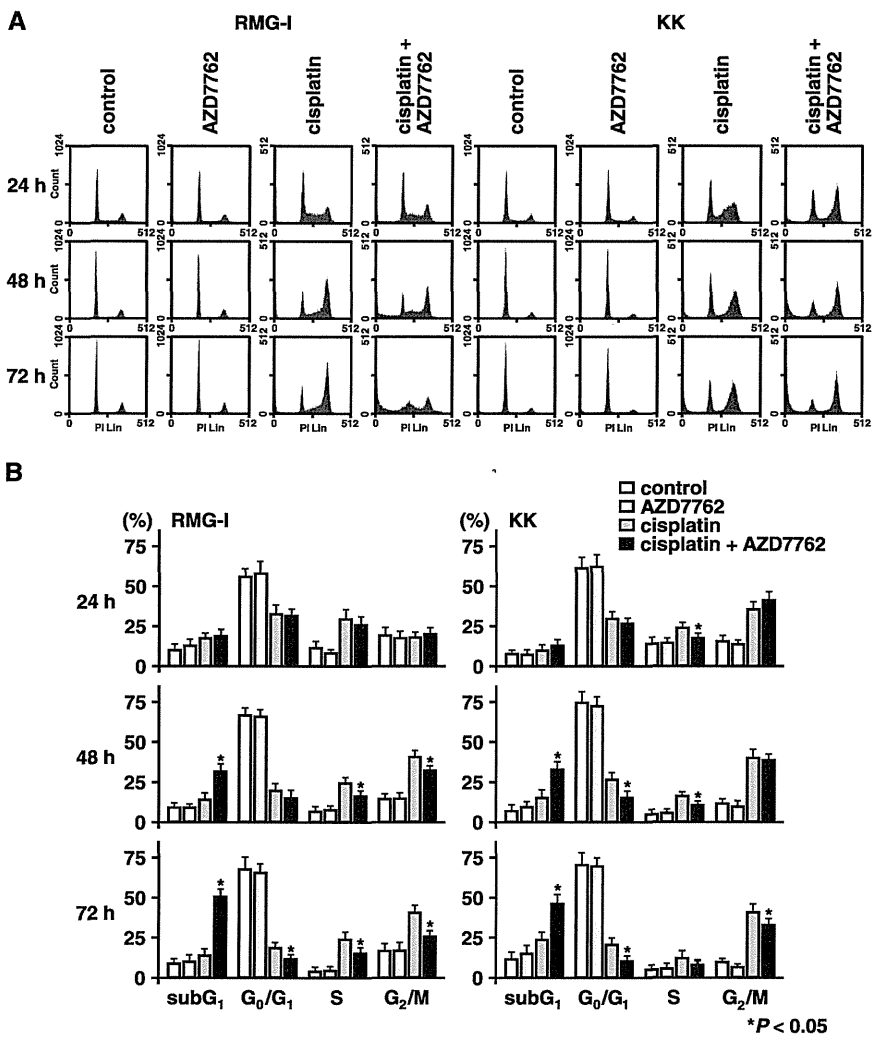


FIGURE 3. Effects of AZD7762 on the cell cycle distribution in response to cisplatin. Ovarian clear cell carcinoma cells RMG-I and KK were treated with PBS (control) or 5- or 3-μmol/L cisplatin and/or 50- or 150-nmol/L AZD7762, respectively. A, Representative data from the flow cytometry in RMG-I and KK cells are shown. Cell cycle distribution is displayed as propidium iodide (*x*-axis) versus cell number (*y*-axis). B, Cisplatin combined with AZD7762 decreased the S-phase fraction, and the cell cycle distribution was shifted to the subG1 phase for 48 and 72 hours in RMG-I and KK cells.

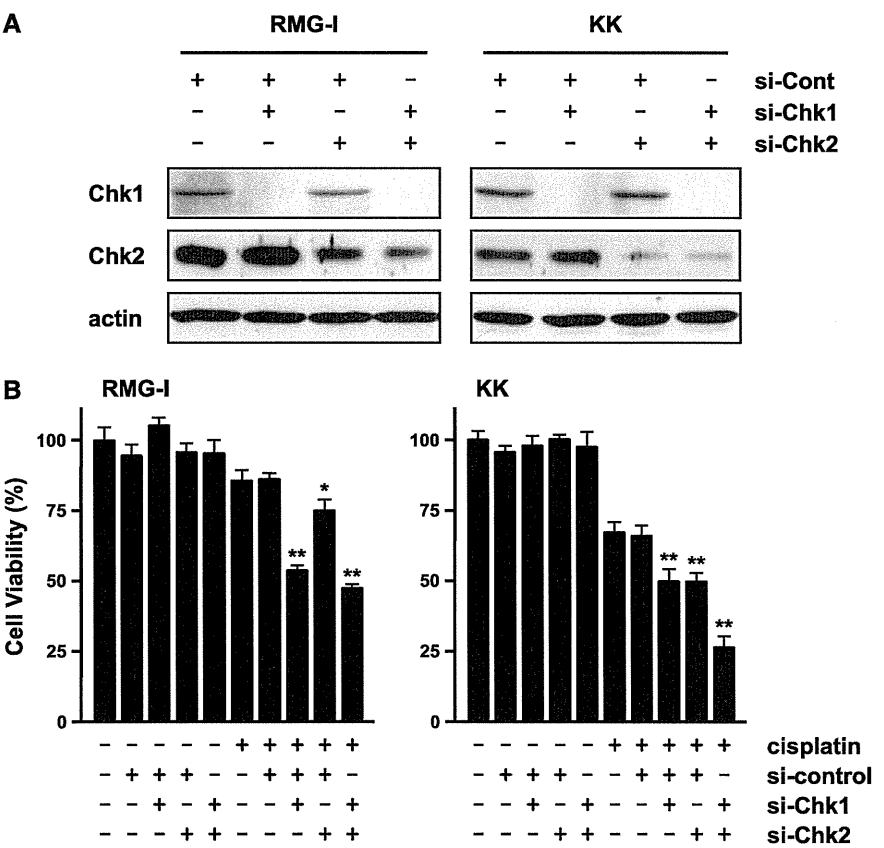
2 cell lines (data not shown). These results indicated that adding AZD7762 to cisplatin abrogated G_1- and S-phase arrest, after which the clear cell carcinoma cells died.

Cisplatin Sensitization in Clear Cell Carcinoma Cell Lines by Knockdown of Chk1 and Chk2

We next examined the relative contributions of inhibition of Chk1 or Chk2 by AZD7762 on sensitization of response to cisplatin in clear cell carcinoma cell lines by using siRNA to selectively knock down Chk1 and/or Chk2 in RMG-I and KK cells. After 24 hours of treatment with si-Chk1 or si-Chk2, expressions of Chk1 or Chk2 were down-regulated in RMG-I and KK cells, respectively (Figs. 4A, B). Sensitivity to cisplatin was increased upon treatment with si-Chk1 or si-Chk2 compared with nonspecific siRNA (si-control). Interestingly, simultaneous treatment with si-Chk1 and si-Chk2 dramatically increased sensitivity to cisplatin. Similar results were obtained in the other 2 cell lines (data not shown). These findings suggested that enhanced cisplatin sensitivity in clear cell carcinoma cells may be modulated by both Chk1 and Chk2 inhibition.

Cisplatin Combined With AZD7762 Reduced Tumor Growth in an Ovarian Clear Cell Carcinoma Xenograft Model

After confirming that AZD7762 enhanced cytotoxicity induced by cisplatin in vitro, we examined the effect of combined cisplatin and AZD7762 on the growth of subcutaneous tumors in an ovarian clear cell carcinoma xenograft. Female nude mice were given subcutaneous injections of RMG-I cells and then treated with PBS or cisplatin and/or



*** $P < 0.05$ vs. cisplatin + si-control, ** $P < 0.01$ vs. cisplatin + si-control**

FIGURE 4. Simultaneous inhibition of Chk1 and Chk2 expression by si-Chk1 and si-Chk2 increases cisplatin sensitivity in RMG-I and KK clear cell carcinoma cells. Cells were treated with 100 nmol/L si-Chk1 and/or si-Chk2 or a control siRNA (si-control) for 24 hours. A, si-Chk1 and si-Chk2 inhibited the expression of Chk1 and Chk2, respectively, in both RMG and KK cells. B, Cytotoxic effect of cisplatin was significantly enhanced by cisplatin combined with si-Chk1 and si-Chk2 in RMG-I and KK cells compared with other treatment conditions. Points represent mean ± SD from quadruplicate experiments.

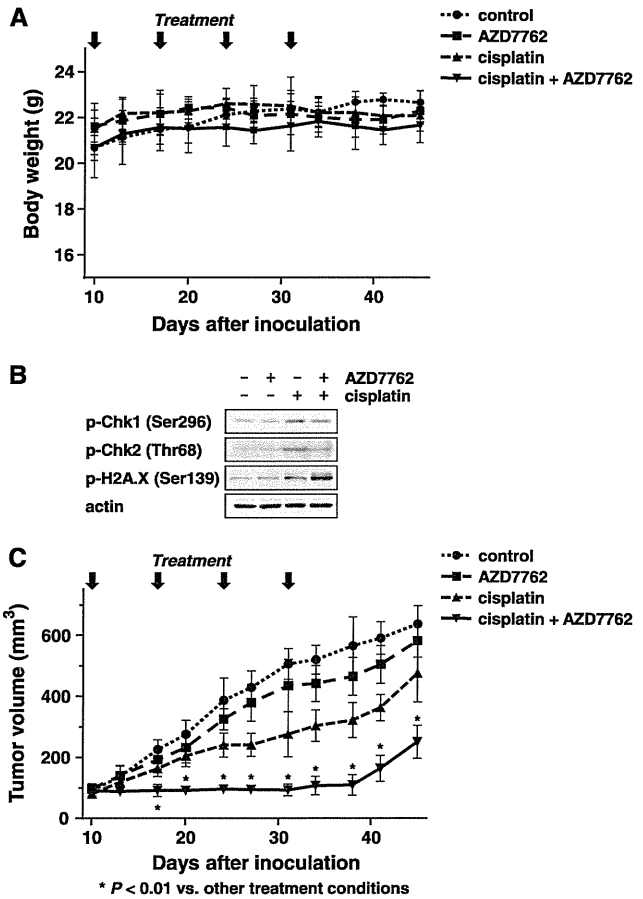


FIGURE 5. Treatments combining cisplatin and AZD7762 suppressed growth of subcutaneous tumors in mice with RMG-I cells implanted. A, Mean body weight of each treatment group. Error bars represent standard deviation. B, Levels of p-Chk1, p-Chk2, and p-H2A.X proteins were determined by Western blotting 24 hours after IP treatment with PBS (control), 25-mg/kg AZD7762, and/or 1.5-mg/kg cisplatin. The results shown represent duplicate experiments. C, In mice inoculated with RMG-I, the tumors were significantly smaller in the mice treated with AZD7762 combined with cisplatin compared with those under other treatment conditions ($P < 0.01$).

AZD7762. There were no signs of overt toxicity (weight loss or gross clinical signs) in any group (Fig. 5A).

To confirm that p-Chk1 and p-Chk2 were inhibited by AZD7762 in vivo, we performed Western blot analysis of tumor tissues (Fig. 5B). As expected, p-Chk1 and p-Chk2 proteins were up-regulated in tumors form the mice treated with cisplatin, and these were suppressed effectively in tumors from mice treated with both cisplatin and AZD7762. We also observed increased expression of p-H2A.X protein in tumors in mice treated with this combination.

In nude mice bearing RMG-I, the mean tumor volume of subcutaneous tumors in the group treated with AZD7762 combined with cisplatin was significantly smaller than that in the group treated with PBS, AZD7762, or cisplatin alone ($P < 0.01$; Fig. 5C). These findings indicated that combining cisplatin and AZD7762 suppressed tumor growth in the subcutaneous tumors of nude mice bearing RMG-I cells.

DISCUSSION

In this exploration of the combination effects of the Chk1 and Chk2 inhibitor AZD7762 with 3 cytotoxic agents used commonly to treat ovarian clear cell carcinoma, we found that AZD7762 and cisplatin had the strongest cytotoxic effects. We also showed that AZD7762 abrogated the G_1/S-phase cell cycle arrests induced by cisplatin and enhanced unrepaired damage to DNA. Thus, these findings suggest that inhibition of Chks up-regulates cisplatin-induced cytotoxicity in ovarian clear cell carcinoma cells.

Cisplatin-induced DNA damage triggers recruitment of multiprotein complexes and activates a number of pathways, including ataxia telangiectasia−mutated (ATM) and ATM and Rad3-related (ATR) signaling pathways.[9,10] Serine/threonine kinases of Chk1 and Chk2 are functionally redundant protein kinases that respond to checkpoint signals initiating ATM and ATR and play a critical role in determining cellular responses to DNA damage.[18] Checkpoint kinase 1 is mainly activated through phosphorylation mediated by ATR. Activated Chk1 phosphorylate Cdc25A leads to ubiquitin- and proteasome-dependent protein degradation and, downstream, to increased phosphorylation of CDK2. This limits its ability to drive progression from G_1 to S phase.[19] In contrast, Chk2 is activated mainly by ATM, and activated Chk2 phosphorylates Cdc25A.[18] Indeed, we confirmed that expression of p-Chk1 and p-Chk2 increased, whereas Cdc25A decreased after the cells were exposed to cisplatin. Furthermore, we observed that cells treated with cisplatin accumulated at S and G_2/M phases.

Preclinical studies have shown that AZD7762 potentiated the effects of DNA-damaging agents, such as cisplatin, gemcitabine, irinotecan, and paclitaxel, by abrogating drug-induced activation of Chk signaling pathways.[11–15] Similarly, we observed the synergistic effect of AZD7762 and cisplatin on inhibiting cell growth in clear cell carcinoma cell lines. AZD7762 also enhanced the cisplatin-induced up-regulation of p-H2A.X, reflecting a greater number of p-H2A.X molecules near sites of DNA damage, and activation of apoptotic pathways. These results suggested that AZD7762 enhanced the cytotoxicity induced by cisplatin such that this combination may be an effective treatment for ovarian clear cell carcinoma.

Although AZD7762 is an inhibitor of both Chk1 and Chk2, it has been suggested that Chk1 inhibition may play a central role in AZD7762-mediated chemosensitization.[20] Several studies have reported that knockdown of Chk1, but not Chk2, by siRNA produced sensitization to cisplatin and gemcitabine.[21,22] Additionally, the small molecule inhibitors of Chk1, PF-00477736, and PD-321852 enhanced the effects of cytotoxic agents.[20,23] In contrast, a novel small molecule inhibitor of Chk2, PV1019, had a synergistic effect

in combination with a topoisomerase I inhibitor in ovarian cancer cells.[24] We observed that simultaneous treatment with si-Chk1 and si-Chk2 dramatically increased sensitivity to cisplatin. Furthermore, recent studies have unveiled several roles for Chk1 in repairing damaged DNA (eg, homologous recombination), negatively regulated mitosis, stabilized stalled replication fork, and inhibited apoptosis.[25,26] Similar to Chk1, Chk2 also has several functions in controlling DNA repair, mitosis, and apoptosis.[27,28] Inhibiting these functions of Chk1 and Chk2, therefore, is thought to bring the potential to enhance the cell killing effects of DNA-damaging agents and radiotherapy. Thus, the chemosensitization effect of AZD7762 may be caused by not only inhibiting Chk1 but also Chk2 in clear cell carcinoma cells. Future studies may be needed to elucidate mechanisms for the synergistic interaction between Chk1/2 inhibitors and cytotoxic agents.

Finally, we confirmed the importance of cell cycle checkpoint pathways in cisplatin therapy in vivo in an ovarian clear cell carcinoma xenograft model. AZD7762 downregulated cisplatin induced activation of p-Chk1 and p-Chk2 expressions in the tumor, and combined AZD7762 and cisplatin suppressed growth of tumors in these mice compared with those treated with AZD7762 or cisplatin alone. The present study provides clear evidence that the downregulation of Chk pathways enhanced the effects of cisplatin treatment both in vitro and in vivo.

In summary, our study showed that the Chk1/2 inhibitor AZD7762 enhanced the cytotoxicity of cisplatin in clear cell carcinoma cells. We also found that the synergistic interaction of AZD7762 and cisplatin may be related to abrogation of the cisplatin-induced G_1/S-phase cell cycle arrest that induces apoptosis. Furthermore, this combined treatment suppressed growth of tumors in nude mice injected with clear cell carcinoma cells. Therefore, we concluded that combining AZD7762 with cisplatin is worth exploring as a treatment for clear cell carcinoma. We hope that this combination therapy will improve the survival of patients with advanced ovarian clear cell carcinoma.

REFERENCES

1. Scully RE. World Health Organization classification and nomenclature of ovarian cancer. *Natl Cancer Inst Monogr.* 1975;42:5–7.
2. Aure JC, Hoeg K, Kolstad P. Mesonephroid tumors of the ovary. Clinical and histopathologic studies. *Obstet Gynecol.* 1971;37:860–867.
3. Sugiyama T, Kamura T, Kigawa J, et al. Clinical characteristics of clear cell carcinoma of the ovary: a distinct histologic type with poor prognosis and resistance to platinum-based chemotherapy. *Cancer.* 2000;88:2584–2589.
4. Itamochi H, Kigawa J, Terakawa N. Mechanisms of chemoresistance and poor prognosis in ovarian clear cell carcinoma. *Cancer Sci.* 2008;99:653–658.
5. Itamochi H, Kigawa J, Akeshima R, et al. Mechanisms of cisplatin resistance in clear cell carcinoma of the ovary. *Oncology.* 2002;62:349–353.
6. Itamochi H, Kigawa J, Sugiyama T, et al. Low proliferation activity may be associated with chemoresistance in clear cell carcinoma of the ovary. *Obstet Gynecol.* 2002;100: 281–287.
7. Itamochi H, Yoshida T, Walker CL, et al. Novel mechanism of reduced proliferation in ovarian clear cell carcinoma cells: cytoplasmic sequestration of CDK2 by p27. *Gynecol Oncol.* 2011;122:641–647.
8. Nonaka M, Itamochi H, Kawaguchi W, et al. Activation of the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase pathway overcomes cisplatin resistance in ovarian carcinoma cells. *Int J Gynecol Cancer.* 2012;22:922–929.
9. Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. *Oncogene.* 2003;22:7265–7279.
10. Ashwell S, Zabludoff S. DNA damage detection and repair pathways—recent advances with inhibitors of checkpoint kinases in cancer therapy. *Clin Cancer Res.* 2008;14:4032–4037.
11. Oza V, Ashwell S, Almeida L, et al. Discovery of checkpoint kinase inhibitor (*S*)-5-(3-fluorophenyl)-*N*-(piperidin-3-yl) -3-ureidothiophene-2-carboxamide (AZD7762) by structure-based design and optimization of thiophenecarboxamide ureas. *J Med Chem.* 2012;55: 5130–5142.
12. Zabludoff SD, Deng C, Grondine MR, et al. AZD7762, a novel checkpoint kinase inhibitor, drives checkpoint abrogation and potentiates DNA-targeted therapies. *Mol Cancer Ther.* 2008;7:2955–2966.
13. Bartucci M, Svensson S, Romania P, et al. Therapeutic targeting of Chk1 in NSCLC stem cells during chemotherapy. *Cell Death Differ.* 2012;19:768–778.
14. Mitchell JB, Choudhuri R, Fabre K, et al. In vitro and in vivo radiation sensitization of human tumor cells by a novel checkpoint kinase inhibitor, AZD7762. *Clin Cancer Res.* 2010;16:2076–2084.
15. Parsels LA, Qian Y, Tanska DM, et al. Assessment of chk1 phosphorylation as a pharmacodynamic biomarker of chk1 inhibition. *Clin Cancer Res.* 2011;17:3706–3715.
16. Itamochi H, Kato M, Nishimura M, et al. Establishment and characterization of a novel ovarian clear cell adenocarcinoma cell line, TU-OC-1, with a mutation in the PIK3CA gene. *Hum Cell.* 2013;26:121–127.
17. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul.* 1984;22:27–55.
18. Bartek J, Lukas J. Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell.* 2003;3:421–429.
19. Mailand N, Falck J, Lukas C, et al. Rapid destruction of human Cdc25A in response to DNA damage. *Science.* 2000;288:1425–1429.
20. Parsels LA, Morgan MA, Tanska DM, et al. Gemcitabine sensitization by checkpoint kinase 1 inhibition correlates with inhibition of a Rad51 DNA damage response in pancreatic cancer cells. *Mol Cancer Ther.* 2009;8:45–54.
21. Carrassa L, Broggini M, Erba E, et al. Chk1, but not Chk2, is involved in the cellular response to DNA damaging agents: differential activity in cells expressing or not p53. *Cell Cycle.* 2004;3:1177–1181.
22. Morgan MA, Parsels LA, Parsels JD, et al. The relationship of premature mitosis to cytotoxicity in response to checkpoint abrogation and antimetabolite treatment. *Cell Cycle.* 2006;5:1983–1988.
23. Blasina A, Hallin J, Chen E, et al. Breaching the DNA damage checkpoint via PF-00477736, a novel small-molecule inhibitor of checkpoint kinase 1. *Mol Cancer Ther.* 2008;7:2394–2404.

24. Jobson AG, Lountos GT, Lorenzi PL, et al. Cellular inhibition of checkpoint kinase 2 (Chk2) and potentiation of camptothecins and radiation by the novel Chk2 inhibitor PV1019 [7-nitro-1H-indole-2-carboxylic acid {4-[1-(guanidinohydrazone)-ethyl]-phenyl}-amide]. *J Pharmacol Exp Ther.* 2009;331:816–826.
25. Dai Y, Grant S. New insights into checkpoint kinase 1 in the DNA damage response signaling network. *Clin Cancer Res.* 2010;16:376–383.
26. Enders GH. Expanded roles for Chk1 in genome maintenance. *J Biol Chem.* 2008;283:17749–17752.
27. Antoni L, Sodha N, Collins I, et al. CHK2 kinase: cancer susceptibility and cancer therapy—two sides of the same coin? *Nat Rev Cancer.* 2007;7:925–936.
28. Stolz A, Ertych N, Bastians H. Tumor suppressor CHK2: regulator of DNA damage response and mediator of chromosomal stability. *Clin Cancer Res.* 2011;17: 401–405.

FULL PAPER

BJC

British Journal of Cancer (2013) 108, 1957–1963 | doi: 10.1038/bjc.2013.179

Keywords: cervical cancer; neoadjuvant chemotherapy; phase III trial; radical surgery

Phase III randomised controlled trial of neoadjuvant chemotherapy plus radical surgery *vs* radical surgery alone for stages IB2, IIA2, and IIB cervical cancer: a Japan Clinical Oncology Group trial (JCOG 0102)

N Katsumata*[,1], H Yoshikawa[2], H Kobayashi[3], T Saito[4], K Kuzuya[5], T Nakanishi[6], T Yasugi[7], N Yaegashi[8], H Yokota[9], S Kodama[10], T Mizunoe[11], M Hiura[12], T Kasamatsu[13], T Shibata[14] and T Kamura[15] on behalf of the Japan Clinical Oncology Group

[1]Department of Medical Oncology, Nippon Medical School Musashikosugi Hospital, Kawasaki, Japan; [2]Department of Obstetrics and Gynecology, University of Tsukuba, Tsukuba, Japan; [3]Department of Obstetrics and Gynecology, Kyushu University, Fukuoka, Japan; [4]Department of Gynecologic Oncology, Kyushu Cancer Center, Fukuoka, Japan; [5]Kuzuya Clinic, Nagoya, Japan; [6]Department of Gynecologic Oncology, Aichi Cancer Center, Nagoya, Japan; [7]Department of Obstetrics and Gynecology, Tokyo University, Tokyo, Japan; [8]Department of Obstetrics and Gynecology, Tohoku University, Sendai, Japan; [9]Department of Gynecologic Oncology, Saitama Cancer Center, Saitama, Japan; [10]Department of Gynecologic Oncology, Niigata Cancer Center, Niigata, Japan; [11]Department of Gynecologic Oncology, Kure Medical Center, Kure, Japan; [12]Department of Gynecologic Oncology, Shikoku Cancer Center, Matsuyama, Japan; [13]Department of Gynecologic Oncology, National Cancer Center Hospital, Tokyo, Japan; [14]Japan Clinical Oncology Group Data Center, Multi-institutional Clinical Trial Support Center, National Cancer Center, Tokyo, Japan and [15]Department of Obstetrics and Gynecology, Kurume University, Kurume, Japan

Background: A phase III trial was conducted to determine whether neoadjuvant chemotherapy (NACT) before radical surgery (RS) improves overall survival.

Methods: Patients with stage IB2, IIA2, or IIB squamous cell carcinoma of the uterine cervix were randomly assigned to receive either BOMP (bleomycin 7 mg days 1–5, vincristine 0.7 mg m^{-2} day 5, mitomycin 7 mg m^{-2} day 5, cisplatin 14 mg m^{-2} days 1–5, every 3 weeks for 2 to 4 cycles) plus RS (NACT group) or RS alone (RS group). Patients with pathological high-risk factors received postoperative radiotherapy (RT). The primary end point was overall survival.

Results: A total of 134 patients were randomly assigned to treatment. This study was prematurely terminated at the first planned interim analysis because overall survival in the NACT group was inferior to that in the RS group. Patients who received postoperative RT were significantly lower in the NACT group (58%) than in the RS group (80%; $P=0.015$). The 5-year overall survival was 70.0% in the NACT group and 74.4% in the RS group ($P=0.85$).

Conclusion: Neoadjuvant chemotherapy with BOMP regimen before RS did not improve overall survival, but reduced the number of patients who received postoperative RT.

*Correspondence: Dr N Katsumata; E-mail: nkatsuma@nms.ac.jp

This study was presented in abstract form at the Annual Meeting of the American Society of Clinical Oncology, June 2006 and June 2010.

Received 30 November 2012; revised 17 March 2013; accepted 1 April 2013; published online 2 May 2013

Treatment of International Federation of Gynaecology and Obstetrics (FIGO) stages IB2, IIA2, and IIB cervical cancer remains controversial. Bulky stage IIA (tumour diameter >4 cm) cervical cancer was revised to stage IIA2 (Pecorelli *et al*, 2009) in the FIGO staging system in 2009. Major treatment options include radical surgery (RS) with or without postoperative radiotherapy (RT), neoadjuvant chemotherapy (NACT) followed by RS with or without postoperative RT, and concurrent chemoradiotherapy (CCRT). Radical surgery usually entails type III radical hysterectomy (Piver *et al*, 1974) plus pelvic or para-aortic lymphadenectomy (or both). For stage IB2 and IIA2 cervical cancer, the National Comprehensive Cancer Network (NCCN Clinical Practice Guidelines, 2012) clinical guidelines mainly recommend CCRT (category 1) and, to a lesser degree, radical hysterectomy with pelvic lymphadenectomy and para-aortic lymph node sampling (category 2b). In Japan, however, more radical procedures, such as Okabayashi's (type III or IV) radical hysterectomy plus pelvic or para-aortic lymphadenectomy (or both), remain the standard treatment of choice for stages IB2, IIA2, and IIB cervical cancer (Fujii *et al*, 2007).

Before we started this study, only one randomised controlled trial conducted at a single centre had compared NACT plus RS with RS alone. In 1997, Sardi *et al* (1997) reported the results of a randomised trial that compared NACT plus RS with RS in 205 patients with stages IB squamous cell cervical cancer. Three courses of NACT with vincristine, bleomycin, and cisplatin (VBP) were given in NACT group. Overall survival at 8 years with NACT group was superior to RS group (81% *vs* 66%, $P<0.05$). In a subgroup analysis in patients with non-bulky tumours <4 cm, there was no significant difference between the two groups (82% *vs* 77%, NS).

Thus, NACT plus RS has emerged as a valid alternative investigational treatment. In 1998, one institution affiliated with our group confirmed that combination chemotherapy with bleomycin, vincristine, mitomycin, and cisplatin (BOMP) produced a high response rate (76%) in metastatic cervical cancer (Shimizu *et al*, 1998). We decided to use the BOMP regimen as NACT.

To clarify the potential benefits of NACT before RS, we undertook a phase III, randomised controlled trial to compare NACT plus RS with RS alone in patients with stages IB2, IIA2, and IIB cervical cancer.

PATIENTS AND METHODS

Eligibility criteria. Patients who had primary, previously untreated, histologically confirmed squamous cell carcinoma of the cervix with bulky FIGO stage IB2, IIA, and IIB disease (tumour diameter >4 cm on magnetic resonance imaging (MRI)) were eligible for this Japan Clinical Oncology Group (JCOG) study (JCOG 0102). In July 2003, the criteria were amended to patients with FIGO stage IB2, IIA2 (tumour diameter >4 cm by clinical measurement), and IIB (irrespective of tumour diameter) disease and additionally required the presence of target cervical lesions (>2 cm) on MRI according to the Response Evaluation Criteria in Solid Tumours (RECIST) guidelines. Patients who were suitable candidates for radical hysterectomy as described in the treatment schedule section were eligible. Patients were also required to be between 20 and 70 years of age, to have performance status of 0 or 1, and to have normal organ functions and normal electrocardiogram. Patients with any of the following conditions were excluded: synchronous or metachronous (within 5 years) malignancy other than carcinoma *in situ* or mucosal cancer; pregnancy; psychotic disease; active infection with fever; uncontrolled hypertension or diabetes mellitus; positive hepatitis B surface antigen; a history of heart failure, unstable angina, or myocardial infarction; interstitial pneumonitis or pulmonary fibrosis; or severe obesity, liver cirrhosis, or bleeding tendency. All patients gave informed consent before enrolment in this study, which was approved by the institutional review boards at the participating institutions (UMIN-CTR No. C000000194 and clinicaltrials.gov No. NCT00190528).

Treatment schedule

Neoadjuvant chemotherapy. Patients were randomly assigned to receive either NACT followed by RS or RS alone. The BOMP regimen for NACT comprised bleomycin (7 mg) as a 30-min intravenous infusion on days 1–5, vincristine (0.7 mg m^{-2}) as a bolus intravenous injection on day 5, mitomycin (7 mg m^{-2}) as a bolus intravenous injection on day 5, and cisplatin (14 mg m^{-2}) as a 30-min intravenous infusion on days 1–5 of a 21-day cycle. Patients initially received two cycles. Patients who had a complete response (CR) or partial response (PR) after two cycles of BOMP were given two additional cycles. Treatment was administered if the white cell count was $\geqslant$2000 per μl and the platelet count was $\geqslant$75 000 per μl. Treatment could be delayed for up to 2 weeks until these minimum criteria were met.

After NACT, the patients were clinically reassessed and classified as suitable or unsuitable for radical hysterectomy. The criteria for radical hysterectomy includes adequate organ function with good performance status. The unsuitable patients received RT, including whole pelvis RT and brachytherapy.

Surgery. The standard procedure used to perform radical hysterectomy in this study was based on Okabayashi's radical hysterectomy as reported by Kyoto Imperial University in 1921. This procedure involves wide extirpation of the parametrial tissue and separation of the posterior leaf of the vesicouterine ligament (Okabayashi, 1921). With the use of this technique, the surgeon can separate the bladder with the ureter completely away from the lateral side of the cervix and the vagina. This dissection facilitates resection of all periureteral tissue and any length (more than one-third) of the vagina and paravaginal tissues. Okabayashi's radical hysterectomy is thus classified as type III or IV radical hysterectomy (Okabayashi, 1921).

In this study, radical hysterectomy require removal of at least 3 cm of the vaginal and paravaginal tissues, and if the vagina was involved, removal of the vagina and vaginal tissues with a margin of at least 2 cm from the cancer. Twenty or more pelvic lymph nodes were required to dissect. If metastases to the para-aortic nodes were suspected, the para-aortic nodes were sampled or dissected. Radical surgery was performed within 3 weeks after randomisation in the RS group and within 8 weeks after the last administration of chemotherapy in the NACT group.

Postoperative RT. The protocol required that postoperative RT was started within 6 weeks after surgery. A total dose of 4500–5040 cGy was delivered to the whole pelvis in daily fractions of 180–200 cGy if patients had pelvic lymph node metastasis, parametrial involvement, or deep stromal invasion ($\geqslant$2/3). Extended-field external beam therapy, delivering a dose of 4500 cGy by a four-field technique, was administered to patients with positive para-aortic nodes. High-dose rate brachytherapy was delivered to the vaginal stump if patients had positive surgical margins.

Response and toxicity evaluation. Tumour response in the NAC group was assessed according to the RECIST guidelines (Therasse *et al*, 2000). Target lesions, including the primary cervical tumour, were measured by MRI. An independent response review committee evaluated all tumour responses after the investigators had completed their assessments.

Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC, version 2.0) (Trotti *et al*, 2000). Surgical morbidity was defined as adverse events related to surgery that occurred between the date of surgery

1 month postoperatively. Early and late adverse events of RT were respectively defined as adverse events that occurred within the first 90 days or more than 90 days after the completion of RT. Late adverse events were evaluated according to the RTOG/EORTC Late Radiation Morbidity Scoring Scheme in Appendix IV of NCI-CTC, version 2.0 (Trotti *et al*, 2000).

Statistical considerations. This was a randomised, multicentre, nonblinded, prospective, phase III study. After confirmation of the inclusion/exclusion criteria by telephone or fax to the JCOG Data Center, the patients were randomly assigned to treatment according to a minimisation procedure. Minimisation criteria were disease stage (I; II), age ($\leqslant$50 years; $>$50 years), and institution. The primary end point was overall survival. The secondary end points were progression-free survival, surgical morbidity, compliance with radical hysterectomy, omission of postoperative irradiation, early and late radiation-related morbidity, and rate of response to chemotherapy. Overall survival was measured from the date of registration to the date of death from any cause, and data were censored at the time of the last follow-up for surviving patients. Progression-free survival was measured from the date of randomisation to the date of the first event (i.e., confirmation of disease progression or death from any cause), and data were censored at the last date on which the absence of disease progression was confirmed.

We assumed that the 5-year survival rate would be 60% in the RS group and 75% in the NACT group. The planned sample size was 100 patients in each treatment group, with a one-sided α-level of 0.05, a power of 0.8, an accrual of 5.5 years, and a follow-up of 3.5 years (Schoenfeld and Richter, 1982). Two interim analyses were scheduled. The first interim analysis was done when 100 patients had been randomly assigned to treatment, and the second was done when all patients had been assigned treatment. Multiplicity was adjusted by the method proposed by the Southwest Oncology Group (Green *et al*, 1997). The significant levels were one-sided 0.005 at each interim analysis and one-sided 0.045 at the final analysis. Survival curves were estimated with the Kaplan–Meier method, and stratified log-rank tests were used to assess differences between treatment groups, stratified according to disease stage (I *vs* II) and age ($\leqslant$50 years *vs* $>$50 years). We used a Cox proportional hazard model to estimate treatment effects. All analyses were done on an intention-to-treat basis, except for toxicity. Toxicity analyses were restricted to patients who had received at any part of their assigned treatment. Although this trial was designed for one-sided hypothesis testing, follow-up results are reported with two-sided *P*-values because of the exploratory nature of the analysis. All analyses were carried out using SAS software, version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Enrolment in this study began on 1 December 2001. The first planned interim analysis was performed in July 2005 (Figure 1). Data from 108 patients enrolled by November 2004 were analysed. On the basis of this analysis, the Data and Safety Monitoring Committee (DSMC) recommended to prematurely terminate the study because overall survival in the NACT group was inferior to that in the RS group (HR, 2.11; multiplicity-adjusted 99% CI, 0.34–13.2), and the predicted probability of significant superiority in the NACT group at the end of the study as assessed by Spiegelhalter's method (Spiegelhalter *et al*, 1993) was extremely low (6.4%). The study was therefore closed on 1 August 2005.

Between December 2001 and August 2005, a total of 134 patients (67 in the NACT group and 67 in the RS group) were randomly assigned to treatment at 28 institutions. Table 1 summarises the baseline characteristics of the patients. One patient

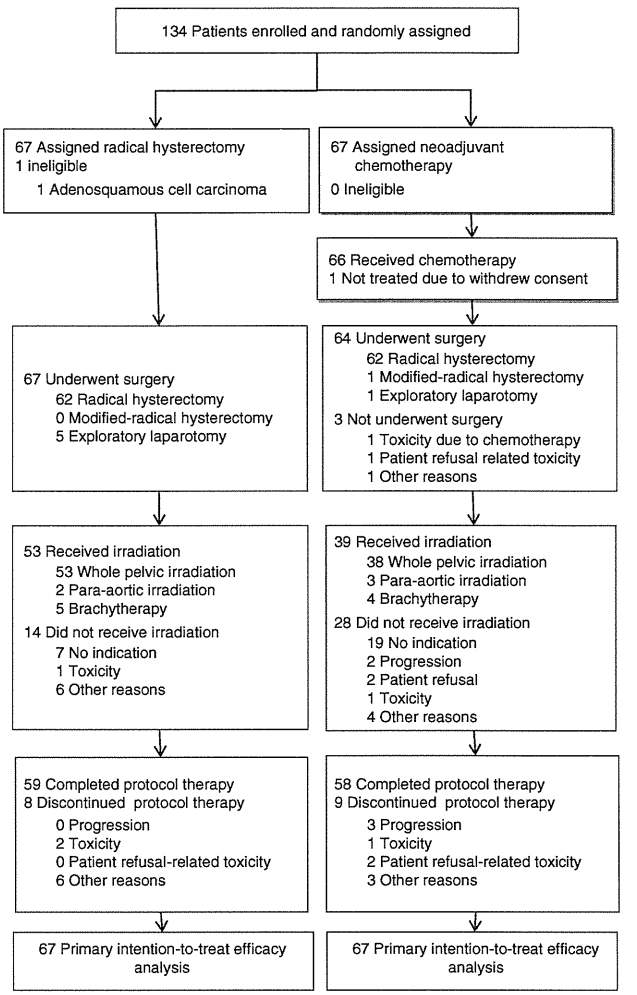


Figure 1. Trial profile.

in the RS group was ineligible because of an incorrect histopathological diagnosis of adenosquamous carcinoma on cervical biopsy before enrolment. Three patients in the NACT group who were given a diagnosis of squamous cell carcinoma on biopsy before enrolment were found to have adenosquamous carcinoma on evaluation of their surgical specimens. These patients were considered eligible.

Of the 67 patients randomly assigned to the NACT group, 66 received chemotherapy. One patient did not receive chemotherapy because of her refusal after registration. This patient underwent primary RS. The other 66 patients received at least two cycles of NACT. The overall response (CR + PR) rate was 70% (47 out of 67) on the investigators' assessment and 66% (44 out of 67) on independent central review (Table 2). Toxicity associated with chemotherapy is summarised in Table 3. Nearly all toxic effects were tolerable, and chemotherapy could be continued in all but three patients who discontinued treatment during the third or fourth cycle because of toxicity (persistent grade 3 thrombocytopenia in two patients and grade 3 skin toxicity in one patient). Grade 3 alkalosis with hypertension, thrombosis, atrial fibrillation, or skin ulceration occurred in one patient each, but these toxic effects were transient and soon resolved.

Of the 67 patients in each group, 62 (93%) underwent RS, suggesting that operability was similar in the groups. Five patients in the RS group and one in the NACT group underwent laparotomy for RS, but the procedure was terminated during surgery because of inoperable disease associated with conditions

Table 1. Patient characteristics

	Radical hysterectomy ($n=67$)		Neoadjuvant chemotherapy ($n=67$)	
	No. of patients	%	No. of patients	%
Age, years				
Median Range	46 22–67		47 28–70	
ECOG performance status				
0	59	88	62	93
1	8	12	5	8
FIGO stage				
IB2	26	39	24	36
IIA	7	10	5	8
IIB	34	51	38	57
Histology in biopsy				
Squamous cell	66	99	67	100
Adenosquamous cell	1	1	0	0

Abbreviations: ECOG = Eastern Cooperative Oncology Group; FIGO = International Federation of Gynaecology and Obstetrics surgical staging system.

Table 2. Clinical response of neoadjuvant chemotherapy

Response category	Investigator assessment ($n=67$)	Independent central review ($n=67$)
CR	9 (13)	8 (12)
PR	38 (57)	36 (54)
SD	18 (27)	20 (30)
PD	0 (0)	0 (0)
NE	1 (1)	2 (3)
Overall response	47 (70)	44 (66)
95% CI	58–81	53–77

Abbreviations: CI = confidence interval; CR = complete response; NE = not evaluable; PD = progressive disease; PR = partial response; SD = stable disease. Values are presented as *n* (%).

Table 3. Toxicity of chemotherapy ($n=66$)

	Grade 3	Grade 4	Grade 3 or 4 (%)
Leukopenia	24	3	41
Neutropenia	21	15	56
Haemoglobin	11	5	24
Thrombocytopenia	18	0	27
Hyponatraemia	3	0	5
Hyperkalaemia	1	0	2
Nausea	11	—	17
Vomiting	4	0	6
Febrile neutropenia	2	0	3
Fatigue	3	0	5
Hypersensitivity	2	0	3

Table 4. Surgical findings

	Radical hysterectomy ($n=62$)		Neoadjuvant chemotherapy ($n=62$)	
	No. of patients	%	No. of patients	%
Tumour diameter (cm)				
Median Range	5.1 2.5–13.5		3 0–10.3	
Postsurgical T classification (pT)				
0–IB1	5	8	25	40
IB2–pT2B	57	92	34	55
>2B	0	0	3	5
Positive pelvic nodes	27	44	17	27
Invasion to muscle layer ⩾2/3	52	84	38	61
Parametrial invasion	28	45	25	40

such as pelvic wall involvement, disseminated tumours, or both. Median dissected lymph nodes were 47 (range 20–119) in the RS group and 45 (range 13–95) in the NACT group. Para-aortic lymph node sampling and dissection were respectively performed in 22 and 14 patients in the RS group and 20 and 14 patients in the NACT group. Median blood loss and operation time were respectively 950 ml and 5.5 h in the RS group and 1370 ml and 5.6 h in the NACT group.

Table 4 shows the pathological findings of surgical specimens obtained from patients who underwent RS. The median tumour diameter in the NACT group was smaller than that in the RS group (3.0 *vs* 5.1 cm). On postsurgical T classification (pT), downstaging to pT0-Ib1 was confirmed in 40% of the patients in the NACT group. The proportion of patients with positive pelvic nodes was lower but statistically not significant in the NACT group than in the RS group (27% *vs* 44%, $P=0.091$), whereas parametrial involvement was similar in both groups (40% *vs* 45%, $P=0.717$). The incidence of para-aortic lymph node metastasis was 2 and 1 in the RS group and NACT group, respectively.

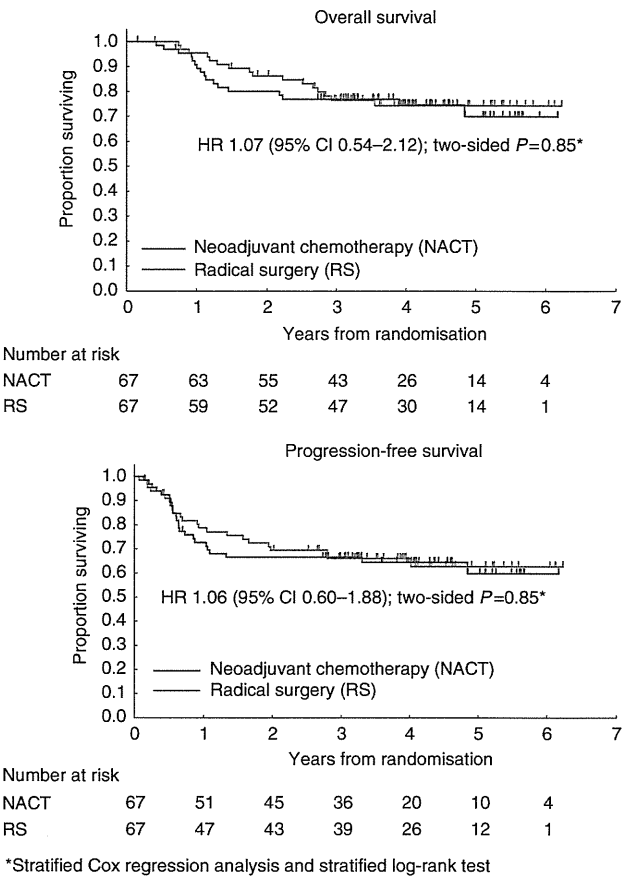
As for surgical morbidity, ureteral or bladder injuries occurred and were repaired during surgery in two patients in the RS group and two in the NACT group. A ureterovaginal fistula developed postoperatively in another patient in the RS group. Grade 3 wound infections occurred in one patient in the RS group and two patients in the NACT group. Grade 3 dysuria developed in one patient in the RS group. Grade 3 disseminated intravascular coagulation occurred in one patient in the NACT group. The incidences of pneumonia, bowel obstruction, and haemorrhage during the first month after surgery were similar in both treatment groups (0, 3, and 0 patients in the RS group *vs* 1, 2, and 1 patients in the NACT group).

The proportion of patients who met the criteria for post-operative radiation (i.e., lymph node metastasis, parametrial involvement, or deep stromal invasion >2/3) was significantly lower in the NACT group (48 (72%) of 67) than in the RS group (59 (89%) of 66; $P=0.015$), and the patients who received

Table 5. Radiation morbidity

	Radical hysterectomy ($n=66$)			Neoadjuvant chemotherapy ($n=67$)		
	Grade 3	Grade 4	Grade 3 or 4 (%)	Grade 3	Grade 4	Grade 3 or 4 (%)
Early adverse events						
Leukocytes	0	0	0	1	0	1
Haemoglobin	0	0	0	2	1	4
Thrombocytes	0	0	0	1	0	1
Diarrhoea	5	0	8	2	0	3
Nausea	0	—	0	1	—	1
Vomiting	0	0	0	1	0	1
Lymphedema	1	0	2	0	0	0
Dysuria	1	—	2	0	—	0
Urinary retention	9	0	14	5	0	7
Late adverse events[a]						
Lymphedema	2	0	3	5	0	7
Urinary retention	7	0	11	3	1	6
Vesicovaginal fistula	1	0	2	1	1	3
Bowel obstruction	6	0	9	1	2	4

[a]Late adverse events were defined as the adverse events that were observed more than 90 days after radiation therapy.



*Stratified Cox regression analysis and stratified log-rank test

Figure 2. Survival curves of all randomised patients.

radiation in the NACT group (39 (58%) of 67) were lower than those in the RS group (53 (79%) of 67; $P=0.015$). Postoperative RT to the whole pelvis, RT to the para-aortic region, and brachytherapy were respectively given to 53, 2, and 5 patients in the RS group and 38, 3, and 4 patients in the NACT group. Early adverse events (within 90 days after radiation) occurred in 70% (46 of 66) of the patients in the RS group and 55% (37 of 67; $P=0.108$) of the patients in the NACT group. Grade 3 or 4 haematologic toxicity was more common in the NACT group than in the RS group (Table 5), whereas nonhaematologic toxic effects such as diarrhoea or urinary retention were more common in the RS group than in the NACT group. Late adverse events (90 days or more after radiation) occurred in 65% (43 of 66) of the patients in the RS group and 42% (28 of 67; $P=0.009$) of the patients in the NACT group. The incidence of grade 3 or 4 lymphedema was slightly higher in the NACT group than in the RS group, whereas urinary retention and bowel obstruction were more common in the RS group than in the NACT group. One patient in the NACT group died of perforation and necrosis of the small intestine 215 days after the last dose of radiation. This death was considered treatment related.

At the time of final follow-up (May 2008), with a median follow-up of 49 months for patients with censored data, there had been 17 deaths in the NACT group and 16 in the RS group. The 5-year overall survival was 70.0% in the NACT group and 74.4% in the RS group (Figure 2; hazard ratio (HR) by Cox regression analysis, 1.07; 95% CI, 0.54–2.12; two-sided $P=0.85$, stratified log-rank test). The 5-year progression-free survival was 59.9% in the NACT group and 62.7% in the RS group (Figure 2; HR, 1.06; 95% CI, 0.60–1.88; two-sided $P=0.85$, stratified log-rank test). On subgroup analyses among patients with stage IB2 disease, the 5-year overall survival and progression-free survival were, respectively, 82.9% and 71.2% in the RS group ($n=25$) and 78.4% and 60.5% in the NACT group ($n=25$), and among patients with stages IIA2 and IIB disease, the 5-year overall survival and progression-free survival were 69.5% and 58.4% in the RS group ($n=42$) and 65.3% and 59.3% in the NACT group ($n=42$).

DISCUSSION

Our study concluded that NACT with BOMP before RS did not improve overall survival of patients with stages IB2, IIA2, and IIB cervical cancer. However, NACT was associated with a reduced proportion of patients who received postoperative RT.

The benefits of NACT followed by surgery as compared with surgery alone were addressed in a Cochrane meta-analysis (Rydzewska *et al*, 2010) of six phase III trials (FIGO stage of the subjects: Sardi's trial (Sardi *et al*, 1997), IB1 + IB2; Napolitano's trial (Napolitano *et al*, 2003), IB-IIIB; Cai's trial (Cai *et al*, 2006), IB1 + IB2; Katsumata's trial (present study) (Katsumata *et al*, 2006), IB2, IIA2, IIB; Eddy's trial (Eddy *et al*, 2007), IB2; Chen's trial (Chen *et al*, 2008), IB2-IIB) of 1036 patients, including our immature survival data, after a median follow-up of 34 months. Progression-free survival was significantly improved by NACT + RS (HR = 0.76, 95% CI, 0.62–0.94). However, the improvement in overall survival with NACT plus RS was not statistically significant (HR = 0.85, 95% CI, 0.67–1.07). Only Sardi's trial showed a statistically significant benefit of NACT in terms of overall survival (HR = 0.53, 95% CI, 0.31–0.92) (Sardi *et al*, 1997). Among the six trials, Eddy's GOG trial (Eddy *et al*, 2007) and our trial

demonstrated no survival benefit of NACT (HR = 1.01, 95% CI, 0.68–1.49 and HR = 1.12, 95% CI, 0.56–2.22). Why the results differed substantially among trials remains unclear. The meta-analysis concluded that the type of drugs used or how they were given had no effect on the overall results. Moreover, the results were similar in women with early-stage disease and those with more advanced cancer.

The clinical response rate of 67% reported in this study is lower than the rate of 84% obtained in patients with stage IB2 disease in Sardi's trial (quick VBP regimen: intravenous vincristine 1 mg m^{-2}, bleomycin 25 mg m^{-2} on days 1–3 and cisplatin 50 mg m^{-2} every 10 days for 3 cycles), but higher than the rate of 52% obtained in Eddy's GOG trial (quick VP regimen, intravenous vincristine 1 mg m^{-2} and cisplatin 50 mg m^{-2} every 10 days for 3 cycles). A previous meta-analysis of Neoadjuvant Chemotherapy for Locally Advanced Cervical Cancer Meta-analysis Collaboration (2003) reported that the timing and dose intensity of cisplatin-based NACT appear to have an important impact on the benefits of such treatment despite some unexplained heterogeneity between the trials in their design and results.

It is very difficult to compare the radicality of RS among trials in the Cochrane meta-analysis. Two of the trials (Napolitano's and Sardi's trials) reported markedly increased rates of radical resection with NACT, whereas no difference was found in the three trials (Cai's, Chen's, and Eddy's trials). In the present study, the rate of RS was similar in NACT group and RS group (93%). The 5-year survival rate of patients with stage IB2 disease in the RS group of Sardi's trial was only 60%, whereas the 4-year survival rate of patients with stage IB2 disease in the RS group of our study was 82%. Perhaps more radical surgery eliminates the survival benefits of NACT.

Concurrent chemoradiotherapy has been considered as current standard adjuvant therapy after RS for patients with high-risk factors for recurrence since 2000 (Peters *et al*, 2000). The role of NACT for high-risk patients who will receive chemoradiotherapy after RS is unclear. Radiotherapy alone was administered in previous NACT trials including our study. Therefore, concurrent chemoradiotherapy should be included when conducting the future NACT trial.

Optimal regimens for NACT have yet to be defined. Among the six trials included in the Cochrane meta-analysis, four trials used cisplatin-based chemotherapy combined with vincristine, three trials used bleomycin, and two trials used 5-fluorouracil or mitomycin because these trials were started between 1987 and 2001. Cisplatin-based chemotherapy combined with ifosphamide, paclitaxel, and topotecan may be more effective for cervical cancer (Omura *et al*, 1997; Moore *et al*, 2004; Long *et al*, 2005). Paclitaxel combined with cisplatin was associated with a higher response rate and better progression-free survival in patients with metastatic cervical cancer (Moore *et al*, 2004), and one phase III trial reported that a combination of paclitaxel, cisplatin, and ifosphamide had a significantly higher response rate than cisplatin and ifosphamide (Buda *et al*, 2005). To clarify the benefits of neoadjuvant chemotherapy, more potent regimens of chemotherapy should be explored.

In this study, the proportion of patients who received postoperative RT was significantly lower in the NACT group than in the RS group (58% *vs* 80%). In Eddy's GOG trial, the rate of postoperative RT was small, but not significantly lower in the NACT group than in the RS group (45% *vs* 52%). When we compared improvements in extrauterine pathological findings associated with NACT between these studies, the reduction in the proportion of patients with positive pelvic nodes was more apparent in the present study than in the GOG trial (from 44% to 29% *vs* from 39% to 32%). Improvements in other extrauterine pathological findings such as positive para-aortic nodes, parametrial involvement, and positive surgical margins were marginal in both studies. The decreased incidence of positive pelvic nodes in our trial most likely influenced the rate of postoperative RT in the NACT group.

Recently, Matsumura *et al* (2010) reported that NACT followed by surgery plus postoperative chemotherapy with cisplatin/irnotecan or nedaplatin/irinotecan, but not RT, is a viable option for the treatment of stage IB2-IIB cervical cancer. This treatment offers the advantage of eliminating radiation-induced morbidity

In conclusion, NACT before RS did not improve overall survival in patients with stages IB2, IIA2, and IIB locally advanced cervical cancer. However, NACT did reduce the proportion of patients who received postoperative RT. Further trials are warranted to clarify the potential benefits of NACT in locally advanced cervical cancer, once new drugs or new combination regimens are shown to be effective as NACT, postoperative adjuvant chemotherapy, or both. Two ongoing randomised phase III trials (EORTC 55994; NCT00193739) are comparing NACT followed by surgery with concurrent chemoradiation. The results of these trials may play an important role in determining whether NACT before surgery is a valid alternative to chemoradiation.

ACKNOWLEDGEMENTS

We are indebted to Dr Naoki Ishizuka for the study design, Mr Takashi Asakawa for statistical support, Ms Mika Hiroo and Ms Harumi Kaba for data management, and Dr Haruhiko Fukuda for oversight of the study. This study was supported in part by the National Cancer Center Research and Development Fund (23-A-16 and 23-A-17) and by the Grants-in-Aid for Cancer Research (14S-1, 14S-4, 17S-1, 17S-5, 20S-1, 20S-6, 10–12, 14-12) from the Ministry of Health, Labour and Welfare, Japan.

REFERENCES

Buda A, Fossati R, Colombo N, Fei F, Floriani I, Gueli Alletti D, Katsaros D, Landoni F, Lissoni A, Malzoni C, Sartori E, Scollo P, Torri V, Zola P, Mangioni C (2005) Randomized trial of neoadjuvant chemotherapy comparing paclitaxel, ifosfamide, and cisplatin with ifosfamide and cisplatin followed by radical surgery in patients with locally advanced squamous cell cervical carcinoma: the SNAP01 (Studio Neo-Adjuvante Portio) Italian Collaborative Study. *J Clin Oncol* **23**(18): 4137–4145.

Cai HB, Chen HZ, Yin HH (2006) Randomized study of preoperative chemotherapy versus primary surgery for stage IB cervical cancer. *J Obstet Gynaecol Res* **32**(3): 315–323.

Chen H, Liang C, Zhang L, Huang S, Wu X (2008) Clinical efficacy of modified preoperative neoadjuvant chemotherapy in the treatment of locally advanced (stage IB2 to IIB) cervical cancer: randomized study. *Gynecol Oncol* **110**(3): 308–315.

Eddy GL, Bundy BN, Creasman WT, Spirtos NM, Mannel RS, Hannigan E, O'Connor D (2007) Treatment of ("bulky") stage IB cervical cancer with or without neoadjuvant vincristine and cisplatin prior to radical hysterectomy and pelvic/para-aortic lymphadenectomy: a phase III trial of the gynecologic oncology group. *Gynecol Oncol* **106**(2): 362–369.

EORTC 55994. http://www.cancer.gov/clinicaltrials/search/view?cdrid=69375&version=healthprofessional.

Fujii S, Takakura K, Matsumura N, Higuchi T, Yura S, Mandai M, Baba T, Yoshioka S (2007) Anatomic identification and functional outcomes of the nerve sparing Okabayashi radical hysterectomy. *Gynecol Oncol* **107**(1): 4–13.

Green S, Benedetti J, Crowley J (1997) Interim Analysis and Data Monitoring Committee. *Clinical Trials in Oncology*, 1st edn, pp 80–99. Chapman & Hall/CRC.

Katsumata N, Yoshikawa H, Hirakawa T, Saito T, Kuzuya K, Fujii T, Hiura M, Tsunematsu R, Fukuda H, Kamura T (2006) Phase III randomized trial of neoadjuvant chemotherapy (NAC) followed by radical hysterectomy (RH) versus RH for bulky stage I/II cervical cancer (JCOG 0102). *Proc Am Soc Clin Oncol* **24**((No 18S)): abstract 5013.

Long 3rd HJ, Bundy BN, Grendys Jr EC, Benda JA, McMeekin DS, Sorosky J, Miller DS, Eaton LA, Fiorica JV (2005) Randomized phase III trial of cisplatin with or without topotecan in carcinoma of the uterine

cervix: a Gynecologic Oncology Group Study. *J Clin Oncol* **23**(21): 4626–4633.

Matsumura M, Takeshima N, Ota T, Omatsu K, Sakamoto K, Kawamata Y, Umayahara K, Tanaka H, Akiyama F, Takizawa K (2010) Neoadjuvant chemotherapy followed by radical hysterectomy plus postoperative chemotherapy but no radiotherapy for Stage IB2-IIB cervical cancer–irinotecan and platinum chemotherapy. *Gynecol Oncol* **119**(2): 212–216.

Moore DH, Blessing JA, McQuellon RP, Thaler HT, Cella D, Benda J, Miller DS, Olt G, King S, Boggess JF, Rocereto TF (2004) Phase III study of cisplatin with or without paclitaxel in stage IVB, recurrent, or persistent squamous cell carcinoma of the cervix: a gynecologic oncology group study. *J Clin Oncol* **22**(15): 3113–3119.

Napolitano U, Imperato F, Mossa B, Framarino ML, Marziani R, Marzetti L (2003) The role of neoadjuvant chemotherapy for squamous cell cervical cancer (Ib-IIIb): a long-term randomized trial. *Eur J Gynaecol Oncol* **24**(1): 51–59.

NCCN Clinical Practice Guidelines (2012) Oncology-Cervical Cancer, version I. *National Comprehensive Cancer Network*. http://www.nccn.org/professionals/physician_gls/pdf/cervical.pdf.

NCT00193739. http://clinicaltrials.gov/ct2/show/NCT00193739?term=NCT00193739.

Neoadjuvant Chemotherapy for Locally Advanced Cervical Cancer Meta-analysis Collaboration (2003) Neoadjuvant chemotherapy for locally advanced cervical cancer: a systematic review and meta-analysis of individual patient data from 21 randomised trials. *Eur J Cancer* **39**(17): 2470–2486.

Okabayashi H (1921) Radical abdominal hysterectomy for cancer of the cervixuteri, modification of the Takayama operation. *Surg Gynecol Obstet* **33**: 335–341.

Omura GA, Blessing JA, Vaccarello L, Berman ML, Clarke-Pearson DL, Mutch DG, Anderson B (1997) Randomized trial of cisplatin versus cisplatin plus mitolactol versus cisplatin plus ifosfamide in advanced squamous carcinoma of the cervix: a Gynecologic Oncology Group study. *J Clin Oncol* **15**(1): 165–171.

Pecorelli S, Zigliani L, Odicino F (2009) Revised FIGO staging for carcinoma of the cervix. *Int J Gynaecol Obstet* **105**(2): 107–108.

Peters 3rd WA, Liu PY, Barrett 2nd RJ, Stock RJ, Monk BJ, Berek JS, Souhami L, Grigsby P, Gordon Jr W, Alberts DS (2000) Concurrent chemotherapy and pelvic radiation therapy compared with pelvic radiation therapy alone as adjuvant therapy after radical surgery in high-risk early-stage cancer of the cervix. *J Clin Oncol* **18**(8): 1606–1613.

Piver MS, Rutledge F, Smith JP (1974) Five classes of extended hysterectomy for women with cervical cancer. *Obstet Gynecol* **44**(2): 265–272.

Rydzewska L, Tierney J, Vale CL, Symonds PR (2010) Neoadjuvant chemotherapy plus surgery versus surgery for cervical cancer. *Cochrane Database Syst Rev* (1): CD007406.

Sardi JE, Giaroli A, Sananes C, Ferreira M, Soderini A, Bermudez A, Snaidas L, Vighi S, Gomez Rueda N, di Paola G (1997) Long-term follow-up of the first randomized trial using neoadjuvant chemotherapy in stage Ib squamous carcinoma of the cervix: the final results. *Gynecol Oncol* **67**(1): 61–69.

Schoenfeld DA, Richter JR (1982) Nomograms for calculating the number of patients needed for a clinical trial with survival as an endpoint. *Biometrics* **38**(1): 163–170.

Shimizu Y, Akiyama F, Umezawa S, Ishiya T, Utsugi K, Hasumi K (1998) Combination of consecutive low-dose cisplatin with bleomycin, vincristine, and mitomycin for recurrent cervical carcinoma. *J Clin Oncol* **16**(5): 1869–1878.

Spiegelhalter DJ, Freedman LS, Parmar MK (1993) Applying Bayesian ideas in drug development and clinical trials. *Stat Med* **12**(15-16): 1501–1511 discussion 1513–7.

Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) 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* **92**(3): 205–216.

Trotti A, Byhardt R, Stetz J, Gwede C, Corn B, Fu K, Gunderson L, McCormick B, Morrisintegral M, Rich T, Shipley W, Curran W (2000) Common toxicity criteria: version 2.0. an improved reference for grading the acute effects of cancer treatment: impact on radiotherapy. *Int J Radiat Oncol Biol Phys* **47**(1): 13–47.

APPENDIX

Institutions that participated in this study:

Hokkaido University, Sapporo Medical University, Tohoku University, Tsukuba University, Gunma Prefectural Cancer Center, National Defense Medical College, Saitama Cancer Center, Saitama Medical Center, National Cancer Center Hospital, The Jikei University Hospital, The Cancer Institute Hospital, Tokyo University, Juntendo University, Niigata Cancer Center, Nagaoka Red Cross Hospital, Shinsyu University, Aichi Cancer Center Hospital, National Hospital Organization Nagoya Medical Center, Kinki University, Osaka Medical Center for Cancer and Cardiovascular Disease, Tottori University, Kure Medical Center, Shikoku Cancer Center, National Kyushu Cancer Center, Kurume University, Kyushu University, Saga University, Kagoshima City Hospital.

Gynecologic Oncology 131 (2013) 574–580

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Status of treatment for the overall population of patients with stage IVb endometrial cancer, and evaluation of the role of preoperative chemotherapy: A retrospective multi-institutional study of 426 patients in Japan

Takako Eto [a], Toshiaki Saito [a,*], Mototsugu Shimokawa [b], Masayuki Hatae [c], Nobuhiro Takeshima [d], Hiroaki Kobayashi [e], Takahiro Kasamatsu [f], Hiroyuki Yoshikawa [g], Toshiharu Kamura [h], Ikuo Konishi [i]

[a] Gynecology Service, National Kyushu Cancer Center, 3-1-1 Notame, Minami-ku, Fukuoka 811-1395, Japan
[b] Cancer Biostatistics Laboratory, Clinical Research Institute, National Kyushu Cancer Center, 3-1-1 Notame, Minami-ku, Fukuoka 811-1395, Japan
[c] Department of Obstetrics and Gynecology, Kagoshima City Hospital, 20-17 Kajiya-cho, Kagoshima 892-8580, Japan
[d] Department of Gynecology, Cancer Institute Hospital of the Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto-ku, Tokyo 135-8550, Japan
[e] Department of Obstetrics and Gynecology, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
[f] Department of Gynecology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
[g] Department of Obstetrics and Gynecology, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8575, Japan
[h] Department of Obstetrics and Gynecology, Kurume University School of Medicine, 67 Asahi machi, Kurume 830-0011, Japan
[i] Department of Gynecology and Obstetrics, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

HIGHLIGHTS

- Only 66% of patients with stage IVb endometrial cancer underwent primary surgery.
- Hysterectomy and chemotherapy may prolong overall survival in selected patients with stage IVb EMCA.
- Primary chemotherapy followed by surgery may be a useful treatment choice in patients not suitable for primary surgery

ARTICLE INFO

Article history:
Received 2 July 2013
Accepted 30 August 2013
Available online 7 September 2013

Keywords:
Stage IVb endometrial cancer
Prognostic factor
Extra-abdominal metastasis
Hysterectomy
Preoperative chemotherapy
Neoadjuvant chemotherapy

ABSTRACT

Objective. We previously reported on the role of cytoreduction in 248 patients with surgical stage IVb endometrial cancer (EMCA). This study aimed to evaluate the clinical characteristics, prognosis according to initial treatment, and impact of preoperative chemotherapy in the overall population of patients with clinical and surgical stage IVb EMCA.

Methods. A multi-institutional retrospective analysis was performed in 426 patients diagnosed with clinical and surgical stage IVb EMCA from 1996 to 2005. Factors associated with overall survival (OS) were identified using univariate and multivariate analyses.

Results. The median OS for all 426 patients was 14 months. Patients were divided into three groups according to their initial treatment: primary surgery group ($n = 279$), primary chemotherapy group ($n = 125$), and palliative care group ($n = 22$). The median OS times for these groups were 21, 12, and 1 month, respectively ($p < 0.0001$). Patients in the primary surgery group had better performance status (PS) and lower numbers of extra-abdominal metastases than those in the primary chemotherapy group. Multivariate analysis identified good PS, endometrioid histology, absence of clinical intra-abdominal stage IVb metastasis, hysterectomy, and chemotherapy as independent predictors of OS. In the primary chemotherapy group, 59 patients subsequently underwent surgery, and these patients had similar OS to those in the primary surgery group.

Conclusions. Hysterectomy and chemotherapy may prolong OS in selected patients with stage IVb EMCA. Our data suggest that primary chemotherapy followed by surgery may be a useful treatment choice in patients not suitable for primary surgery.

Introduction

Most cases of endometrial cancer (EMCA) present in the early stages and have a favorable prognosis, but patients occasionally present with

* Corresponding author. Fax: +81 92 551 4585.
E-mail address: tsaitou@nk-cc.go.jp (T. Saito).

0090-8258/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygyno.2013.08.036

stage IVb disease which has a very poor prognosis [1,2]. Therapeutic decision making for patients with stage IVb EMCA remains challenging because of the lack of available data for this group.

Several retrospective studies and a meta-analysis have reported that surgical cytoreduction, as performed in patients with ovarian cancer, was also useful in patients with stage IVb EMCA [3–11]. We conducted a multicenter retrospective study of patients with stage IVb EMCA who were treated at Japan Clinical Oncology Group-related institutions. We previously reported the detailed clinicopathological characteristics and role of cytoreductive surgery in 248 patients with surgical stage IVb EMCA [12].

Some patients with stage IVb EMCA do not undergo surgery for initial treatment. In patients with unresectable intra- or extra-abdominal metastases, surgery may not be the preferred initial treatment. Furthermore, some patients with stage IVb EMCA are medically inoperable. According to the International Federation of Gynecology and Obstetrics (FIGO) Annual Report [13], the 4-year survival rate is 22.3% in patients with surgical stage IVb EMCA and 7.0% in patients with clinical stage IVb EMCA. The prognosis of patients with stage IVb EMCA who do not undergo initial surgery is extremely poor, but detailed data about the treatments received by these patients are lacking.

Neoadjuvant chemotherapy (NAC) and interval debulking surgery followed by chemotherapy (NAC-setting treatment [NACT]) has emerged as an alternative treatment for patients with advanced ovarian cancer who have unresectable disease or poor performance status (PS). A phase III study found that NACT had a comparable outcome to primary debulking surgery followed by chemotherapy, but with less surgery-related adverse effects [14]. The first prospective study of NACT in patients with serous EMCA with transperitoneal spread reported that NACT resulted in a high rate of optimal debulking surgery [15].

One of the reasons for the difficulty in establishing therapeutic algorithms for stage IVb EMCA is the small numbers of patients in most series. Another reason is that few studies have evaluated treatment of the overall population of patients with stage IVb EMCA, including non-surgical patients.

In the current study, we analyzed all patients who were diagnosed with stage IVb EMCA, including non-surgical cases. The primary objectives of this study were to clarify the current status of treatment, and to analyze the clinicopathological characteristics and prognostic factors in this population. The secondary objective was to evaluate the role of preoperative chemotherapy in patients who did not undergo initial surgical intervention.

Methods

Patients

We performed a retrospective review of all patients diagnosed with clinical or surgical FIGO 1988 stage IVb EMCA from 1996 to 2005, who were treated in 30 Gynecologic Cancer Study Group of Japan Clinical Oncology Group-related institutions. Patients with sarcoma were excluded.

A case report form was developed using data software (FileMaker-pro Version 6 or 8) to obtain equivalent data from multiple institutions. The investigation protocol, including the case report form, was approved by the Institutional Review Board of each institution.

Complete clinical data were collected by reviewing inpatient charts, operative records, and outpatient records from each institution. Pathological information was collected from the pathology reports of the endometrial biopsy specimen and the hysterectomy specimen. The sites of metastases, surgical procedures, and sites and maximum diameter of residual disease after surgery were collected from radiology reports, intraoperative findings, and pathology reports. Treatment data included initial treatment, adjuvant treatment after surgery, and surgical treatment after chemotherapy. Regular follow-up was performed at each institution. Follow-up information included the date and disease status at the last follow-up, or the date and cause of death.

Patients were divided into the following three groups according to their initial treatment: primary surgery group, primary chemotherapy group, and palliative care group. Patients who underwent primary radiotherapy only were classified into the palliative care group. The primary chemotherapy group was subdivided into a group who underwent laparotomy after chemotherapy, and a group who did not. Stage IVb metastases were divided into intra- and extra-abdominal disease. Metastasis to the liver parenchyma was classified as extra-abdominal disease. Because patients with non-surgical treatment were included, we defined intra-abdominal lesions that were detectable on pretreatment imaging studies as clinical intra-abdominal stage IVb metastases. In patients of the primary chemotherapy group who subsequently underwent surgery, the response to preoperative chemotherapy was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST). Patient outcomes were analyzed by overall survival (OS), calculated from the date when initial treatment was started to the date of death or last contact.

Statistical analyses

Differences in the distributions of clinicopathological characteristics among groups were analyzed by Fisher's exact test for qualitative variables. OS curves were estimated using the Kaplan–Meier method, and were compared among groups using the log-rank test. A two-sided p value of <0.05 was considered statistically significant. Independent prognostic factors were identified using multivariate Cox proportional hazards regression analyses. All analyses were performed using SPSS statistical software (11.0.1J; SPSS Inc., Chicago, IL).

Results

Initial treatment and patient characteristics

We identified a total of 426 patients with stage IVb EMCA. Patient grouping by initial treatment was as follows: 279 patients (66%) in the primary surgery group, 125 (29%) in the primary chemotherapy group, and 22 (5%) in the palliative care group (Fig. 1).

The primary surgery group included 149 patients who had stage IVb disease detected by preoperative imaging examinations and 130 patients diagnosed as stage IVb EMCA only after they underwent laparotomy. Of the 125 patients in the primary chemotherapy group, 59 (47%) underwent subsequent laparotomy and the remaining 66 did not undergo any subsequent surgery.

Table 1 shows the clinicopathological characteristics of patients according to the initial treatment. The median age of patients was 59 years, and 84% of patients had a pretreatment Eastern Cooperative Oncology Group (ECOG) PS of 0 or 1. The mean body mass index was 23 kg/m^2 (range: 14–39 kg/m^2). Medical comorbidities included hypertension in 23% of patients and diabetes in 12%. The most common histological subtype was endometrioid, accounting for 58% of cases.

The majority of patients in the palliative care group were old, emaciated, hypertensive, and had two or more extra-abdominal metastases. Patients in the primary surgery group had a better PS ($p = 0.002$) and lower rates of comorbidities (hypertension, $p = 0.049$; diabetes, $p = 0.003$) than those in the primary chemotherapy group. Tumor histology was not significantly different among the three groups.

Extra-abdominal stage IVb disease was documented in 229 patients (54%), including 82% of patients in the primary chemotherapy group and 86% in the palliative care group. Patients in these two groups were more likely to have metastases in two or more anatomical regions than those in the primary surgery group. The most common sites of extra-abdominal metastases were the lungs (28%), liver (13%), mediastinal lymph nodes (9%), and bone (8%). Clinical intra-abdominal stage IVb disease was documented in 134 patients (31%). Of the 220 patients with intra-abdominal stage IVb disease in the primary surgery group,