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Premature ovarian failure in androgen receptor-deficient mice

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Premature ovarian failure (POF) syndrome, an early decline of ovarian function in women, is frequently associated with X chromosome abnormalities ranging from various Xq deletions to complete loss of one of the X chromosomes. However, the genetic locus responsible for the POF remains unknown, and no candidate gene has been identified. Using the Cre/LoxP system, we have disrupted the mouse X chromosome androgen receptor (*Ar*) gene. Female $AR^{-/-}$ mice appeared normal but developed the POF phenotype with aberrant ovarian gene expression. Eight-week-old female $AR^{-/-}$ mice are fertile, but they have lower follicle numbers and impaired mammary development, and they produce only half of the normal number of pups per litter. Forty-week-old $AR^{-/-}$ mice are infertile because of complete loss of follicles. Genome-wide microarray analysis of mRNA from $AR^{-/-}$ ovaries revealed that a number of major regulators of folliculogenesis were under transcriptional control by AR. Our findings suggest that AR function is required for normal female reproduction, particularly folliculogenesis, and that AR is a potential therapeutic target in POF syndrome.

male hormone | nuclear receptor | female physiology | folliculogenesis | kit ligand

Premature ovarian failure (POF) is defined as an early decline of ovarian function after seemingly normal folliculogenesis (1). Genetic causes of POF have been frequently associated with X chromosome abnormalities (1, 2). Complete loss of one of the X chromosomes, as in Turner syndrome, and various Xq deletions are commonly identified as a cause of POF. However, responsible X-linked genes and their downstream targets have not been identified so far.

The androgen receptor (*Ar*) gene, which is the only sex hormone receptor gene on the X chromosome, is well known to be essential not only for the male reproductive system, but also for male physiology. In contrast, androgens are considered as male hormones; therefore, little is known about androgens' actions in female physiology, although AR expression in growing follicles has been described (3). However, because excessive androgen production in polycystic ovary syndrome causes infertility with abnormal menstrual cycles (4, 5), it is possible that AR-mediated androgen signaling also plays an important physiological role in the female reproductive system. Recently, using Cre/LoxP system, we generated an AR-null mutant mouse line (6) and demonstrated that inactivation of AR resulted in arrest of testicular development and spermatogenesis, impaired brain masculinization, high-turnover osteopenia, and late onset of obesity in males (7–9). At the same time, no overt physical or growth abnormalities were observed in female $AR^{-/-}$ mice. Therefore, to further examine potential role of AR in female physiology, we characterized female reproductive system in $AR^{-/-}$ females. Herein we show that female $AR^{-/-}$ mice develop the POF phenotype. At 3 weeks of age, $AR^{-/-}$ females had apparently normal ovaries with numbers of follicles similar to those in the wild-type females. However, thereafter the number of healthy follicles in the $AR^{-/-}$ ovary gradually declined, with a marked increase of atretic follicles, and by 40 weeks $AR^{-/-}$ mice became infertile, with no follicle detectable in the ovary. Reflecting this age-dependent progression in ovarian abnormality, several genes known to be involved in the oocyte–granulosa cell regulatory loop were identified by microarray analysis as AR downstream target genes. These findings clearly demonstrate that AR-mediated androgen signaling is indispensable for the maintenance of folliculogenesis and implicate impaired androgen signaling as a potential cause of the POF syndrome.

Materials and Methods

Generation of AR Knockout Mice. *AR* genomic clones were isolated from a TT2 embryonic stem cell genomic library by using human *AR* A/B domain cDNA as a probe (6). The targeting vector consisted of a 7.6-kb 5′ region containing exon 1, a 1.3-kb 3′ homologous region, a single loxP site, and a neo cassette with two loxP sites (10). Targeted clones (FB-18 and FC-61) were aggregated with single eight-cell embryos from CD-1 mice (11, 12). Floxed *AR* mice (C57BL/6) were then crossed with CMV-Cre transgenic mice (6). The two lines exhibited the same phenotypic abnormalities. The chromosomal sex of each pup was determined by genomic PCR amplification of the Y chromosome *Sry* gene (13).

Western Blot Analysis. To detect AR protein expression, ovarian cell lysates were separated by SDS/PAGE and transferred onto nitrocellulose membranes (14). Membranes were probed with polyclonal AR antibodies (N-20; Santa Cruz Biotechnology), and blots were visualized by using peroxidase-conjugated second antibody and an ECL detection kit (Amersham Pharmacia Biosciences).

Morphologic Classification of Growing Follicles. Sections were taken at intervals of 30 μm, and 6-μm paraffin-embedded sections were mounted on slides. Routine hematoxylin and eosin staining was performed for histologic examination by light microscopy. Follicle numbers in 12 sections per ovary were evaluated as primary follicles (oocyte surrounded by a single layer of cuboidal granulosa cells), preantral follicles (oocyte surrounded by two or

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Abbreviations: AR, androgen receptor; DHT, 5α-dihydrotestosterone; POF, premature ovarian failure.

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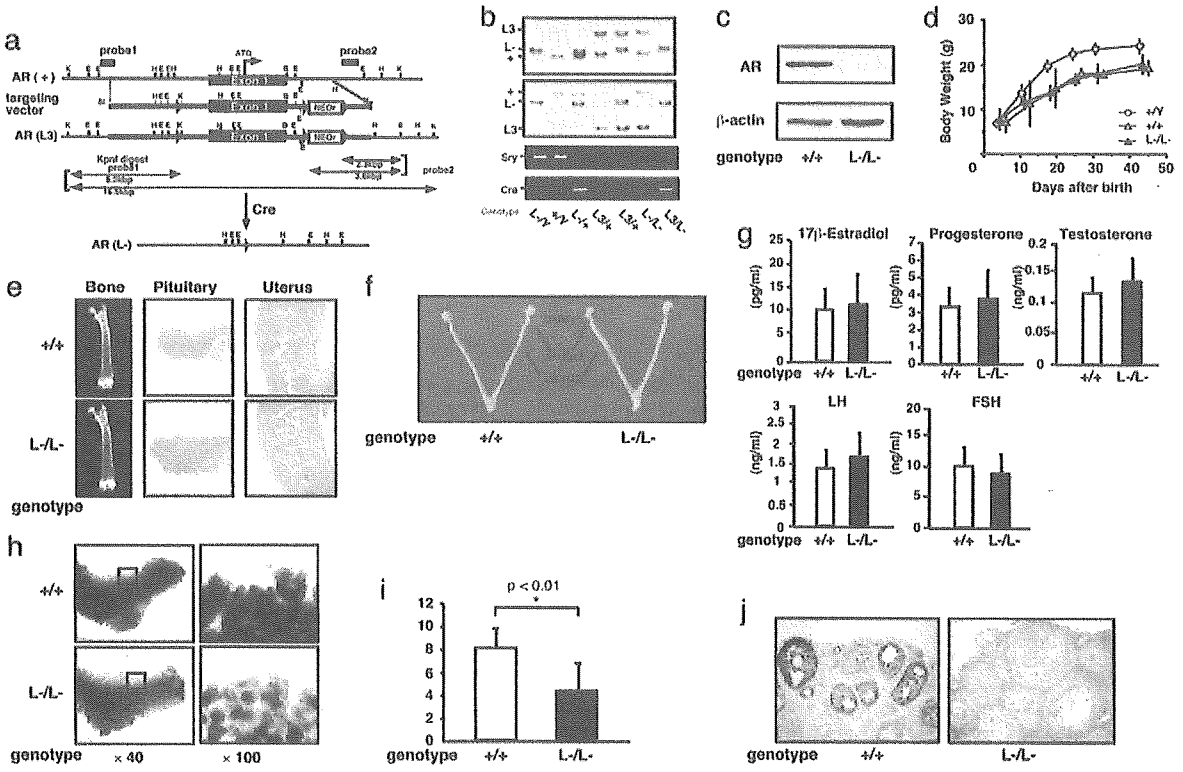

Fig. 1. Phenotypic characterization of AR knockout female mice. (*a*) Diagram of the wild-type *Ar* genomic locus (+), floxed *AR* L3 allele (L3), and *AR* allele (L-) obtained after Cre-mediated excision of exon 1. K, KpnI; E, EcoRI; H, HindIII; B, BamHI. LoxP sites are indicated by arrowheads. The targeting vector consisted of a 7.6-kb 5′ homologous region containing exon 1, a 1.3-kb 3′ homologous region, a single loxP site, and the neo cassette with two loxP sites. (*b*) Detection of the Y chromosome-specific *Sry* gene in $AR^{-/Y}$ mice by PCR. (*c*) Absence of AR protein in $AR^{-/-}$ mice ovaries by Western blot analysis using a specific C-terminal antibody. (*d*) Normal weight gain in $AR^{-/-}$ females. (*e*) Histology of pituitary, uterus, and bone tissues in $AR^{+/+}$ and $AR^{-/-}$ females at 8 weeks of age. (*f*) Female reproductive organs were macroscopically normal in $AR^{-/-}$ mice. (*g*) Serum hormone levels at the proestrus stage in $AR^{-/-}$ mice were not significantly altered. Serum 17β-estradiol, progesterone, testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels in $AR^{+/-}$ ($n = 13$) and $AR^{-/-}$ ($n = 10$) females at 8–10 weeks of age are shown. (*h*) Lobuloalveolar development is impaired in $AR^{-/-}$ mammary glands. Whole mount of inguinal mammary glands (*Left*) and its higher magnification (*Right*) were prepared on day 3 of lactation. (*i*) Average number of pups per litter is markedly reduced in $AR^{-/-}$ mice at 8 weeks of age. Data are shown as mean ± SEM and analyzed by using Student's *t* test. (*j*) AR immunocytochemistry in $AR^{+/+}$ and $AR^{-/-}$ ovaries. Sections were counterstained with eosin.

more layers of granulosa cells with no antrum), or antral follicles (antrum within the granulosa cell layers enclosing the oocyte). Follicles were determined to be atretic if they displayed two or more of the following criteria within a single cross section: more than two pyknotic nuclei, granulosa cells within the antral cavity, granulosa cells pulling away from the basement membrane, or uneven granulosa cell layers (15).

Immunohistochemistry. Sections were subjected to a microwave antigen retrieval technique by boiling in 10 mM citrate buffer (pH 6.0) in a microwave oven for 30 min (16). The cooled sections were incubated in 1% H_2O_2 for 30 min to quench endogenous peroxidase and then incubated with 1% Triton X-100 in PBS for 10 min. To block nonspecific antibody binding, sections were incubated in normal goat serum for 1 h at 4°C. Sections were then incubated with anti-AR (1:100) or anti-cleaved caspase-3 (1:100) in 3% BSA overnight at 4°C. Negative controls were incubated in 3% BSA without primary antibody. The ABC method was used to visualize signals according to the manufacturer's instructions. Sections were incubated in biotinylated goat anti-rabbit IgG (1:200 dilution) for 2 h at room temperature, washed with PBS, and incubated in avidin–biotin–horseradish peroxidase for 1 h. After thorough washing in PBS, sections were developed with 3,3′-diaminobendizine tetrahydrochloride substrate, slightly counterstained with eosin, dehydrated through an ethanol series and xylene, and mounted.

Estrus Cycles and Fertility Test. To determine the stage of the estrus cycle (proestrus, estrus, and diestrus), vaginal smears were taken every morning and stained with Giemsa solution. For evaluation of female fertility for 15 weeks, an 8- or 24-week-old wild-type or $AR^{-/-}$ female was mated with a wild-type fertile male, replaced every 2 weeks with the other fertile male. Cages were monitored daily and for an additional 23 days, and the presence of seminal plugs and number of litters were recorded.

RNA Extraction and Quantitative Competitive RT-PCR. Total ovarian RNA was extracted by using TRIzol (Invitrogen) (16). Oligo-dT-primed cDNA was synthesized from 1 μg of ovarian RNA by using SuperScript reverse transcriptase (Gibco BRL, Gaithersburg, MD) in a 20-μl reaction volume, 1 μl of which was then diluted serially (2- to 128-fold) and used to PCR-amplify an internal control gene, *cycA*, to allow concentration estimation.

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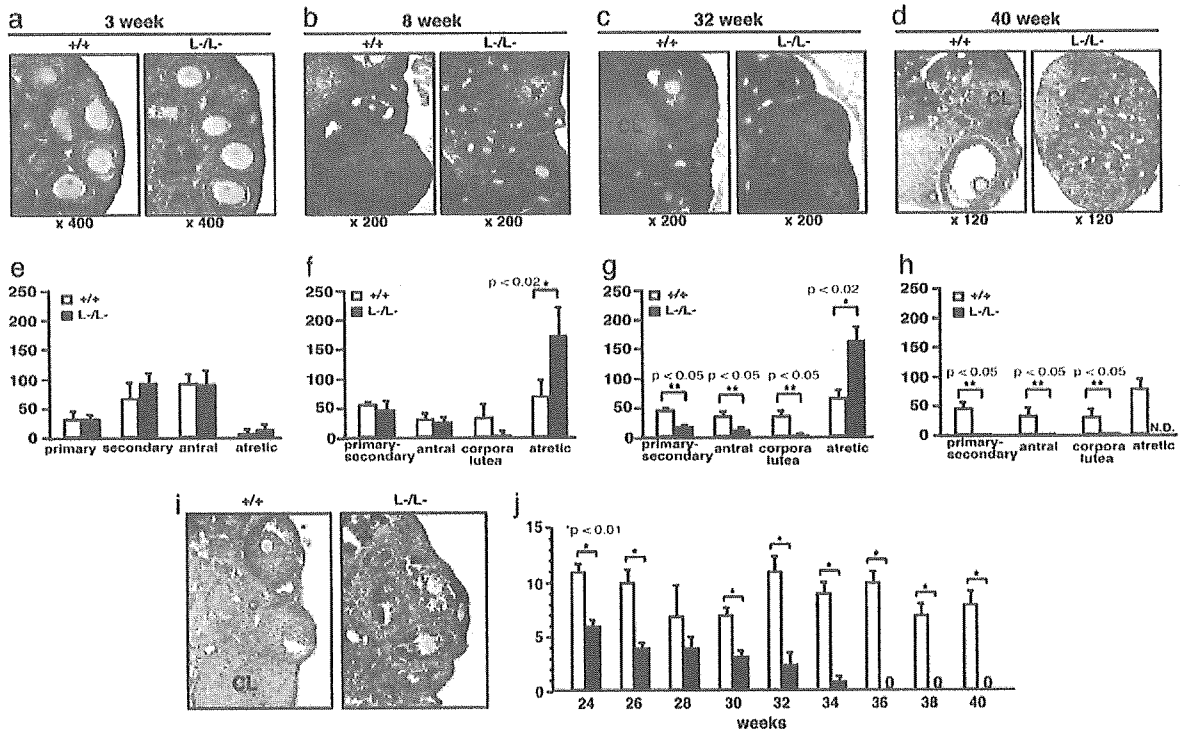

Fig. 2. POF in $AR^{-/-}$ female mice. (*a–d*) Histology of $AR^{+/+}$ and $AR^{-/-}$ ovaries at 3 weeks, 8 weeks, 32 weeks, and 40 weeks of age. All sections were stained with hematoxylin and eosin. An asterisk marks the atretic follicle. CL, corpus luteum. (*e–h*) Relative follicle counts at 3 weeks (*e*), 8 weeks (*f*), 32 weeks (*g*), and 40 weeks (*h*) of age. Numbers represent total counts of every fifth section from serially sectioned ovaries ($n = 4$ animals per genotype). (*i*) Immunohistochemical study for activated, cleaved caspase-3 revealed increased positive cells (apoptotic cells) in $AR^{-/-}$ ovaries. Sections were counterstained with hematoxylin. An asterisk marks the caspase-3-positive cell. CL, corpus luteum. (*j*) Age-dependent reduction in the number of pups per litter in $AR^{-/-}$ female mice. A continuous breeding assay was started at 24 weeks of age ($n = 6–10$ animals per genotype). For all panels, data are shown as mean ± SEM and were analyzed by using Student's *t* test.

Primers were designed from cDNA sequences of *Kitl* (M57647; nucleotides 1099–1751), Gdf9 (NM008110; nucleotides 720-1532), *Bmp15* (NM009757; nucleotides 146–973), *Ers2* (NM010157; nucleotides 1139–1921), *Pgr* (NM008829; nucleotides 1587–2425), *Cyp11a1* (NM019779; nucleotides 761-1697), *Cyp17al* (M64863; nucleotides 522–932), *Cyp19* (D00659; nucleotides 699-1049), *Fshr* (AF095642; nucleotides 625-1427), *Lhr* (M81310; nucleotides 592-1331), *Ptgs2* (AF338730; nucleotides 3–605), and *Ccnd2* (NM009829; nucleotides 150-1065) and chosen from different exons to avoid amplification from genomic DNA.

GeneChip Analysis. Ovaries were isolated and stabilized in RNA*later* RNA Stabilization Reagent (Ambion, Austin, TX) before RNA purification (17). Total RNA was purified by using an RNeasy mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. First-strand cDNA was synthesized from 5 μg of RNA by using 200 units of SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA), 100 pmol T7-$(dT)_{24}$ primer [5′-GGCCAGTGAATTGTAATACGACTCAC-TATAGGGAGGCGG-$(dT)_{24}$-3′], 1× first-strand buffer, and 0.5 mM dNTPs at 42°C for 1 h. Second-strand synthesis was performed by incubating first-strand cDNA with 10 units of *Escherichia coli* ligase (Invitrogen), 40 units of DNA polymerase I (Invitrogen), 2 units of RNase H (Invitrogen), 1× reaction buffer, and 0.2 mM dNTPs at 16°C for 2 h, followed by 10 units of T4 DNA polymerase (Invitrogen) and incubation for another 5 min at 16°C. Double-stranded cDNA was purified by using GeneChip Sample Cleanup Module (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions and labeled by *in vitro* transcription by using a BioArray HighYield RNA transcript labeling kit (Enzo Diagnostics, Farmingdale, NY). Briefly, dsDNA was mixed with 1× HY reaction buffer, 1× biotin-labeled ribonucleotides (NTPs with Bio-UTP and Bio-CTP), 1× DTT, 1× RNase inhibitor mix, and 1× T7 RNA polymerase and incubated at 37°C for 4 h. Labeled cRNA was then purified by using GeneChip Sample Cleanup Module and fragmented in 1× fragmentation buffer at 94°C for 35 min. For hybridization to the GeneChip Mouse Expression Array 430A or 430B or Mouse Genome 430 2.0 Array (Affymetrix), 15 μg of fragmented cRNA probe was incubated with 50 pM control oligonucleotide B2, 1× eukaryotic hybridization control, 0.1 mg/ml herring sperm DNA, 0.5 mg/ml acetylated BSA, and 1× hybridization buffer in a 45°C rotisserie oven for 16 h. Washing and staining were performed by using a GeneChip Fluidic Station (Affymetrix) according to the manufacturer's protocol. Phycoerythrin-stained arrays were scanned as digital image files and analyzed with GENECHIP OPERATING SOFTWARE (Affymetrix) (17).

Luciferase Assay. The *Kitl* promoter region (−2866 to −1 bp) was inserted into the pGL3-basic vector (Promega) for assay using the Luciferase Assay System (Promega) (14, 16). Cells at 40–50% confluence were transfected with a reference pRL-CMV

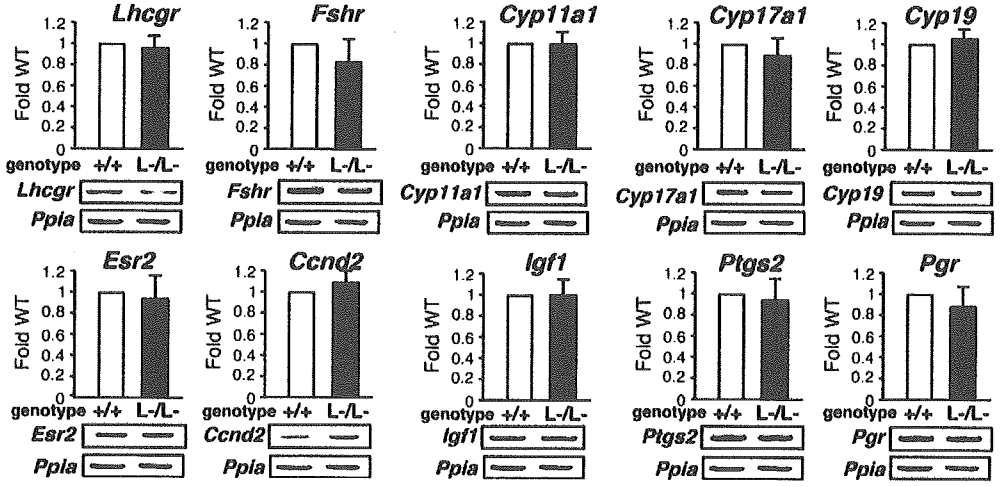

Fig. 3. No significant alterations in mRNA levels of several major regulators in folliculogenesis. Shown is semiquantitative RT-PCR of LH receptor (*Lhr*), FSH receptor (*Fshr*), p450 side chain cleavage enzyme (*Cyp11a1*), 17-α-hydroxylase (*Cyp17a1*), Aromatase (*Cyp19*), estrogen receptor-β (*Esr2*), cyclin D2 (*Ccnd2*), insulin-like growth factor 1 (*Igf1*), cyclooxygenase 2 (*Ptgs2*), or progesterone receptor (*Pgr*) gene expression in $AR^{+/+}$ and $AR^{-/-}$ ovaries. Results shown were representative (using one ovary per genotype in each experiment) of five independent experiments.

plasmid (Promega) using Lipofectamine reagent (GIBCO/BRL, Grand Island, NY) to normalize transfection. Results shown are representative of five independent experiments.

Results and Discussion

Subfertility of $AR^{-/-}$ Female Mice at 8 Weeks of Age. The *Ar* gene located on the X chromosome was disrupted in mice by using the Cre/Lox P system (6) (Fig. 1 *a–c*). Female $AR^{-/-}$ mice showed normal growth compared with the wild-type littermates (Fig. 1*d*), with no detectable bone loss (Fig. 1*e*) or obesity common for male $AR^{-/Y}$ mice (8, 9). Young (8-week-old) $AR^{-/-}$ females appeared indistinguishable from the wild-type littermates, displayed normal sexual behavior (7), and produced the first offspring of normal body size at the expected age. Macroscopic appearance of their reproductive organs, including uteri, oviducts, and ovaries, also appeared normal (Fig. 1*f*). Histological analysis showed no significant abnormality in the uterus or pituitary (Fig. 1*e*), whereas mammary ductal branching and elongation were substantially reduced, as revealed by whole-mount analysis (Fig. 1*h*). Serum levels of 17β-estradiol, progesterone, testosterone, luteinizing hormone, and follicle-stimulating hormone were also within normal range in 8-week-old mutant females at the proestrus stage (Fig. 1*g*), suggesting that the two-cell two-gonadotrophin system in female reproductive and endocrine organs (18) was intact in $AR^{-/-}$ mice at 8 weeks of age. The most obvious early sign of abnormal reproductive function in the $AR^{-/-}$ females was that their average numbers of pups per litter were only about half of those of the wild-type littermates, ($AR^{+/+}$, 8.3 ± 0.4 pups per litter; $AR^{-/-}$, 4.5 ± 0.5 pups per litter) (Fig. 1*i*).

$AR^{-/-}$ Female Mice Developed POF Phenotypes. Histological analysis of 8-week-old $AR^{-/-}$ ovaries clearly showed that numbers of atretic follicles were significantly increased, with decreased numbers of corpora lutea (Fig. 2 *b* and *f*). This finding suggests that the reduced pup numbers were due to impaired folliculogenesis in AR-deficient ovaries. Indeed, AR protein expression was readily detectable in the wild-type 8-week-old ovaries (Fig. 1*j*), with AR expressed at the highest levels in growing follicle granulosa cells at all developmental stages and at relatively low levels in corpora lutea. Thus, AR appears to play a regulatory role in granulosa cells during their maturation to the luteal phase.

To investigate this possibility, we examined the ovarian phenotype of female $AR^{-/-}$ mice at different ages. At 3 weeks, ovaries contain various stages of follicles, including primary, secondary, and antral follicles in wild-type animals (Fig. 2*a*) (19). In $AR^{-/-}$ ovaries at 3 weeks of age, the folliculogenesis appeared to be unaltered, with normal numbers and localization of primary and secondary follicles (Fig. 2 *a* and *e*). However, degenerated folliculogenesis became evident with further aging. Although follicles and corpora lutea at all developmental stages were still present, corpora lutea numbers were clearly reduced in 8-week-old $AR^{-/-}$ mutants (Fig. 2 *b* and *f*), similar to that observed in another mouse line (20). Expected apoptosis was seen in atretic follicles by activated caspase-3 immunohistochemistry assays (Fig. 2*i*). But, by 32 weeks of age, defects in folliculogenesis in $AR^{-/-}$ ovaries became profound, with fewer follicles observed and increased atretic follicles (Fig. 2 *c* and *g*), and >40% (5 of 12 mice) of the $AR^{-/-}$ females were already infertile. By 40 weeks, all $AR^{-/-}$ females became infertile, with no follicles remaining (Fig. 2 *d* and *h*); at the same age, $AR^{+/+}$ females were fertile and had normal follicle numbers. Consistent with progressive deficiency in folliculogenesis, the pup number per litter steadily decreased in aging $AR^{-/-}$ females (Fig. 2*i*). These data indicate that AR plays an important physiological role at the preluteal phase of folliculogenesis.

Alteration in Gene Expressions of Several Major Regulators Involved in the Oocyte–Granulosa Cell Regulatory Loop. To explore the molecular basis underlying the impaired folliculogenesis in $AR^{-/-}$ ovaries, we analyzed expression of several major known regulators and markers of folliculogenesis (21–23). Surprisingly, no significant alterations in mRNA levels of LH receptor (*Lhr*), FSH receptor (*Fshr*), p450 side chain cleavage enzyme (*Cyp11a1*), 17-α-hydroxylase (*Cyp17a1*), aromatase (*Cyp19*), estrogen receptor-β (*Esr2*), cyclin D2 (*Ccnd2*), or insulin-like growth factor 1 (*Igf1*) of 8-week-old $AR^{-/-}$ ovaries at the proestrus stage, and further cyclooxygenase 2 (*Ptgs2*) or progesterone receptor (*Pgr*) at the estrus stage, were detected by

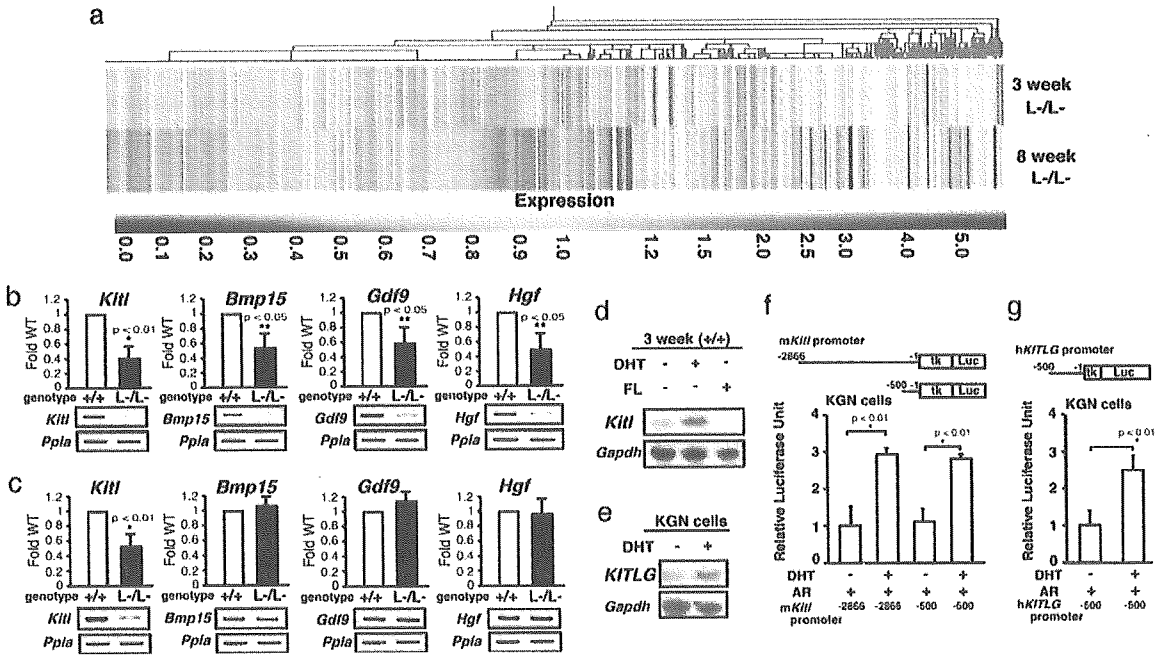

Fig. 4. Genome-wide microarray analysis and semiquantitative RT-PCR revealed that expression of the oocyte–granulosa cell regulator loop was down-regulated in $AR^{-/-}$ ovaries. (*a*) Microarray analysis of $AR^{-/-}$ compared with $AR^{+/+}$ ovaries at 3 and 8 weeks of age. Data obtained from microarray analysis as described in *Materials and Methods* were used to generate a cluster analysis. Each vertical line represents a single gene. The ratios of gene expression levels in $AR^{-/-}$ ovaries compared with wild type are presented. (*b* and *c*) Semiquantitative RT-PCR analysis of AR-regulated genes identified from the microarray study. Results shown are representative (using one ovary per genotype in each experiment) of five independent experiments. Data are shown as mean ± SEM and were analyzed by using Student's *t* test. (*d*) Comparison of *Kitl* gene expression by Northern blot analysis among placebo-, DHT-, and flutamide (FL)-treated $AR^{+/-}$ mouse ovaries. (*e*) Induction of *KITLG* gene expression by DHT treatment in KGN cells. (*f* and *g*) Androgen responsiveness in the mouse and human *kit ligand* promoters by a luciferase assay performed by using KGN cells. Data are shown as mean ± SEM and were analyzed by using Student's *t* test.

semiquantitative RT-PCR analysis (Fig. 3). Genome-wide microarray analysis (17) of RNA from 8-week-old $AR^{-/-}$ ovaries at the proestrus stage has been undertaken to identify AR-regulated genes. In comparison with $AR^{+/+}$ ovaries, expressions of 772 genes were down-regulated, whereas 351 genes were up-regulated in $AR^{-/-}$ ovaries (Fig. 4*a*; see also Tables 1 and 2, which are published as supporting information on the PNAS web site). Several genes known to be involved in the oocyte–granulosa cell regulatory loop (24) were identified as candidate AR target genes, including KIT ligand (*Kitl*) (25), morphogenetic protein 15 (*Bmp15*) (26), growth differentiation factor-9 (*Gdf9*) (27), and hepatocyte growth factor (*Hgf*) (28). Impaired folliculogenesis had been reported in mice deficient in each of these three regulators (26, 27, 29). To validate the microarray data, we performed semiquantitative RT-PCR analysis of 8-week-old $AR^{-/-}$ ovary RNA and confirmed that expression of these factors was down-regulated (Fig. 4*b*). To identify a regulator downstream of the AR signaling at an earlier stage of folliculogenesis, 3-week-old $AR^{-/-}$ ovaries that, as pointed out earlier, display no apparent phenotypic abnormality were examined. Fewer genes had altered expression levels (519 genes up-regulated; 326 genes down-regulated) (Fig. 4*a*; see also Tables 3 and 4, which are published as supporting information on the PNAS web site), and, of the four regulators tested by RT-PCR, only *Kitl* was found to be down-regulated at this age (Fig. 4*c*). Because *Kitl* is a granulosa cell-derived factor and stimulates oocyte growth and maturation (29–31), down-regulation of the *Kitl* expression in 3-week-old or even younger $AR^{-/-}$ ovaries may trigger impairment in folliculogenesis at a later age. To test for possible *Kitl* gene regulation by AR, 3-week-old wild-type females were treated with 5α-dihydrotestosterone (DHT). At 4 h after hormone injection, a clear induction of *Kitl* expression was observed in the ovaries, whereas a known antiandrogen flutamide attenuated the induction by DHT (Fig. 4*d*). The induction of endogenous human *kit ligand* (*KITLG*) gene by DHT was also observed in human granulosa-like tumor cells (KGN) in culture (Fig. 4*e*). Furthermore, androgen-induced transactivation of mouse and human *kit ligand* promoters (32) was observed by a luciferase reporter assay (33) in KGN (Fig. 4 *f* and *g*), 293T, and HeLa (data not shown) cells. However, no response to DHT was detected in the similar assay using promoters of the *Bmp15*, *Gdf9*, and *Hgf* genes (data not shown). Thus, we have shown that, in a regulatory cascade controlling folliculogenesis, *Kitl* represents a direct downstream target of androgen signaling.

As an upstream regulator, AR may also be indirectly involved in control of expression of other genes critical for folliculogenesis, because an age-dependent down-regulation of *Bmp15*, *Gdf9*, and *Hgf* gene expression was also observed in $AR^{-/-}$ ovaries. *Bmp15* and *Gdf9* are oocyte-derived factors that promote the development of surrounding granulosa cells in growing follicles (34, 35), whereas *Hgf* is secreted by theca cells and acts as a granulosa cell growth factor (36). Down-regulation of these factors, presumably due to decreased *Kitl* expression, may lead to impaired bidirectional communication between oocyte and granulosa cells (24) and, eventually, to early termination of folliculogenesis, as in POF syndrome.

Thus, we have identified AR as a novel regulator of follicu-

logenesis that apparently acts in the regulatory cascade upstream of the major factors controlling ovarian function, confirming the previous findings of the AR expression in granulose cells of growing follicles (3). Although not immediately relevant to the ovarian physiology, abnormal development of the mammary glands observed in our AR-deficient mice adds further strong evidence of an essential role of the AR not only in male, but also in female, reproductive function.

With increasing age of the first childbirth by women in the modern society, POF syndrome has become an important social and medical problem. Our findings suggest that POF syndrome may be caused by an impairment in androgen signaling and that X chromosomal mutations affecting the *AR* gene function may play a key role in hereditary POF. From clinical perspective, the present study provides evidence that AR can be a beneficial therapeutic target in treatment of POF syndrome patients.

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1. Laml, T., Preyer, O., Umek, W., Hengstschlager, M. & Hanzal, H. (2002) *Hum. Reprod. Update* **8,** 483–491.
2. Davison, R. M., Davis, C. J. & Conway, G. S. (1999) *Clin. Endocrinol. (Oxford)* **51,** 673–679.
3. Tetsuka, M., Whitelaw, P. F., Bremner, W. J., Millar, M. R., Smyth, C. D. & Hillier, S. G. (1995) *J. Endocrinol.* **145,** 535–543.
4. Ehrmann, D. A., Barnes, R. B. & Rosenfield, R. L. (1995) *Endocr. Rev.* **16,** 322–353.
5. Norman, R. J. (2002) *Mol. Cell. Endocrinol.* **191,** 113–119.
6. Kato, S. (2002) *Clin. Pediatr. Endocrinol.* **11,** 1–7.
7. Sato, T., Matsumoto, T., Kawano, H., Watanabe, T., Uematsu, Y., Sekine, K., Fukuda, T., Aihara, K., Krust, A., Yamada, T., *et al.* (2004) *Proc. Natl. Acad. Sci. USA* **101,** 1673–1678.
8. Sato, T., Matsumoto, T., Yamada, T., Watanabe, T., Kawano, H. & Kato, S. (2003) *Biochem. Biophys. Res. Commun.* **300,** 167–171.
9. Kawano, H., Sato, T., Yamada, T., Matsumoto, T., Sekine, K., Watanabe, T., Nakamura, T., Fukuda, T., Yoshimura, K., Yoshizawa, T., *et al.* (2003) *Proc. Natl. Acad. Sci. USA* **100,** 9416–9421.
10. Li, M., Indra, A. K., Warot, X., Brocard, J., Messaddeq, N., Kato, S., Metzger, D. & Chambon, P. (2000) *Nature* **407,** 633–636.
11. Sekine, K., Ohuchi, H., Fujiwara, M., Yamasaki, M., Yoshizawa, T., Sato, T., Yagishita, N., Matsui, D., Koga, Y., Itoh, N. & Kato, S. (1999) *Nat. Genet.* **21,** 138–141.
12. Yoshizawa, T., Handa, Y., Uematsu, Y., Takeda, S., Sekine, K., Yoshihara, Y., Kawakami, T., Arioka, K., Sato, H., Uchiyama, Y., *et al.* (1997) *Nat. Genet.* **16,** 391–396.
13. Gubbay, J., Collignon, J., Koopman, P., Capel, B., Economou, A., Munsterberg, A., Vivian, N., Goodfellow, P. & Lovell-Badge, R. (1990) *Nature* **346,** 245–250.
14. Yanagisawa, J., Yanagi, Y., Masuhiro, Y., Suzawa, M., Watanabe, M., Kashiwagi, K., Toriyabe, T., Kawabata, M., Miyazono, K. & Kato, S. (1999) *Science* **283,** 1317–1321.
15. Britt, K. L., Drummond, A. E., Cox, V. A., Dyson, M., Wreford, N. G., Jones, M. E., Simpson, E. R. & Findlay, J. K. (2000) *Endocrinology* **141,** 2614–2623.
16. Ohtake, F., Takeyama, K., Matsumoto, T., Kitagawa, H., Yamamoto, Y., Nohara, K., Tohyama, C., Krust, A., Mimura, J., Chambon, P., *et al.* (2003) *Nature* **423,** 545–550.
17. Fujimoto, N., Igarashi, K., Kanno, J., Honda, H. & Inoue, T. (2004) *J. Steroid Biochem. Mol. Biol.* **91,** 121–129.
18. Couse, J. F. & Korach, K. S. (1999) *Endocr. Rev.* **20,** 358–417.
19. Elvin, J. A. & Matzuk, M. M. (1998) *Rev. Reprod.* **3,** 183–195.
20. Hu, Y. C., Wang, P. H., Yeh, S., Wang, R. S., Xie, C., Xu, Q., Zhou, X., Chao, H. T., Tsai, M. Y. & Chang, C. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 11209–11214.
21. Elvin, J. A., Yan, C., Wang, P., Nishimori, K. & Matzuk, M. M. (1999) *Mol. Endocrinol.* **13,** 1018–1034.
22. Zhou, J., Kumar, T. R., Matzuk, M. M. & Bondy, C. (1997) *Mol. Endocrinol.* **11,** 1924–1933.
23. Burns, K. H., Yan, C., Kumar, T. R. & Matzuk, M. M. (2001) *Endocrinology* **142,** 2742–2751.
24. Matzuk, M. M., Burns, K. H., Viveiros, M. M. & Eppig, J. J. (2002) *Science* **296,** 2178–2180.
25. Joyce, I. M., Pendola, F. L., Wigglesworth, K. & Eppig, J. J. (1999) *Dev. Biol.* **214,** 342–353.
26. Yan, C., Wang, P., DeMayo, J., DeMayo, F. J., Elvin, J. A., Carino, C., Prasad, S. V., Skinner, S. S., Dunbar, B. S., Dube, J. L., *et al.* (2001) *Mol. Endocrinol.* **15,** 854–866.
27. Dong, J., Albertini, D. F., Nishimori, K., Kumar, T. R., Lu, N. & Matzuk, M. M. (1996) *Nature* **383,** 531–535.
28. Parrott, J. A., Vigne, J. L., Chu, B. Z. & Skinner, M. K. (1994) *Endocrinology* **135,** 569–575.
29. Driancourt, M. A., Reynaud, K., Cortvrindt, R. & Smitz, J. (2000) *Rev. Reprod.* **5,** 143–152.
30. Huang, E. J., Manova, K., Packer, A. I., Sanchez, S., Bachvarova, R. F. & Besmer, P. (1993) *Dev. Biol.* **157,** 100–109.
31. Packer, A. I., Hsu, Y. C., Besmer, P. & Bachvarova, R. F. (1994) *Dev. Biol.* **161,** 194–205.
32. Grimaldi, P., Capolunghi, F., Geremia, R. & Rossi, P. (2003) *Biol. Reprod.* **69,** 1979–1988.
33. Kitagawa, H., Fujiki, R., Yoshimura, K., Mezaki, Y., Uematsu, Y., Matsui, D., Ogawa, S., Unno, K., Okubo, M., Tokita, A., *et al.* (2003) *Cell* **113,** 905–917.
34. Otsuka, F. & Shimasaki, S. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 8060–8065.
35. Joyce, I. M., Clark, A. T., Pendola, F. L. & Eppig, J. J. (2000) *Biol. Reprod.* **63,** 1669–1675.
36. Parrott, J. A. & Skinner, M. K. (1998) *Endocrinology* **139,** 2240–2245.

A Phase I Study and Pharmacologic Evaluation of Irinotecan and Carboplatin for Patients with Advanced Ovarian Carcinoma who Previously Received Platinum-Containing Chemotherapy

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BACKGROUND. The objectives of the current study were to determine the maximum tolerated dose (MTD) of irinotecan and carboplatin in combination, to evaluate the efficacy and toxicity of the combination in patients with advanced ovarian carcinoma who previously received platinum-containing chemotherapy, and to examine the pharmacokinetics and pharmacodynamics of both drugs by using the Chatelut formula.

METHODS. Patients with advanced ovarian carcinoma who previously received platinum-containing chemotherapy were treated with a combination of irinotecan and carboplatin. Carboplatin was administered as a 60-minute intravenous infusion on Day 1 and was followed by irinotecan, which was administered as a 90-minute intravenous infusion on Days 1, 8, and 15. Six dose levels of irinotecan (in mg/m^2)/carboplatin (mg · mL/min) were planned: 50 mg/m^2/4 mg · mL/minute, 60 mg/m^2/4 mg · mL/minute, 50 mg/m^2/5 mg · mL/minute, 60 mg/m^2/5 mg · mL/minute, 50 mg/m^2/6 mg · mL/minute, and 60 mg/m^2/6 mg · mL/minute. The carboplatin dosage was calculated by using the Chatelut formula. Treatment was repeated at 28-day intervals.

RESULTS. In total, 19 patients in cohorts of 3 to 5 patients received irinotecan and carboplatin at 5 dose levels. The dose-limiting toxicities were Grade 4 neutropenia and Grade 4 thrombocytopenia. The MTD of the irinotecan/carboplatin combination was 60 mg/m^2/5 · mg mL/minute. Partial responses were observed at higher dose levels. Pharmacologic studies demonstrated that administration of the dosage estimated with the Chatelut formula instead of the Chatelut formula with adjustment for serum creatinine resulted in a slightly excessive dose of carboplatin.

CONCLUSIONS. The recommended dose for the Phase II study was irinotecan 60 mg/m^2 on Days 1, 8, and 15 with carboplatin 5 mg/mL · minute on Day 1 repeated every 4 weeks. ***Cancer* 2005;104:1204–12.** © *2005 American Cancer Society.*

KEYWORDS: irinotecan, carboplatin, ovarian carcinoma, pharmacokinetics, Chatelut formula.

An objective response is achieved in approximately 60–80% of women with advanced ovarian carcinoma who are treated with platinum plus taxane combination chemotherapy.[1,2] Nonetheless, recurrence rates remain high even with current treatments, and most women with advanced ovarian carcinoma ultimately die of their disease. Thus, it is important not only to establish effective second-line chemotherapies but to use new drugs with no cross-resistance in first-line chemotherapy to avoid expression of drug-resistant clones in the early treatment period.

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Irinotecan (CPT-11) is a plant alkaloid extract from *Camptotheca acuminate* and a potent inhibitor of DNA topoisomerase I. It exhibits excellent antitumor activity not only against experimental models of a broad spectrum of tumors but against drug-resistant tumor cell lines. No cross-resistance has been found between CPT-11 and carboplatin, and a synergistic effect has been observed when CPT-11 has been used in combination with carboplatin in preclinical studies.[3–5] Moreover, it was demonstrated recently that CPT-11 is an active agent in patients with platinum-resistant ovarian carcinoma.[6]

Carboplatin is an analogue of cisplatin with less nonhematologic toxicity, and leukopenia and thrombocytopenia are its dose-limiting toxicities (DLTs).[7,8] The area under the plasma concentration-versus-time curve (AUC) of carboplatin correlates well with the extent of myelosuppression as well as with the response rate in patients with ovarian carcinoma,[9] and the dose of carboplatin can be individualized to achieve a particular AUC by using several formulae.[10,11] The most widely accepted is the Calvert formula, which is based on the linear correlation between carboplatin clearance and the glomerular filtration rate.[10] However, because the effect of the possible pharmacokinetic interaction with coadministered drugs on carboplatin clearance is unknown, the most practical formula for routine clinical use remains a matter of controversy.[12–17] The objectives of the current study were: 1) to determine the maximum tolerated dose (MTD) and the recommended dose of CPT-11 and carboplatin in combination for patients with advanced ovarian carcinoma who previously received platinum-containing chemotherapy, 2) to investigate the pharmacokinetics and pharmacodynamics of the combination, and 3) to evaluate the utility of the serum creatinine adjustment model for the Chatelut formula by using the creatinine peroxidase-antiperoxidase (PAP) method developed in a previous study in Japanese patients.[18]

MATERIALS AND METHODS

Patient Selection

Patients were enrolled in the study if they fulfilled the following eligibility criteria: 1) histologically proven ovarian carcinoma; 2) prior platinum-containing chemotherapy, whether in platinum-sensitive or platinum-resistant patients[19]; 3) life expectancy $\geq$ 3 months; 4) age 15 years or older but younger than 75 years; 5) an Eastern Cooperative Oncology Group performance status $<$ 2; 6) adequate bone marrow and organ function (leukocytes $\geq$ 4000/μL, neutrophils $\geq$ 2000/μL, platelets $\geq$ 100,000/μL, total bilirubin $\geq$ 1.5 mg/dL, serum transaminase levels not more than 2.5 times the upper limit of normal, serum creatinine $\geq$ 1.5 mg/dL); 7) having measurable lesions was not required; and 8) written informed consent. This study was approved by the Institutional Review Board of the National Cancer Center Hospital.

Patients who had active infection, bowel obstruction, interstitial pneumonitis, severe heart disease, or a past history of hypersensitivity to antitumor drugs were excluded from the study. Patients who had pleural effusion or ascites that required drainage, brain metastasis, or active concomitant malignancy also were excluded.

Treatment Plan and Dose-Escalation Procedure

Carboplatin dissolved in 250 mL of saline or 5% glucose solution was infused over 60 minutes; subsequently, CPT-11 dissolved in 500 mL saline or 5% glucose solution was given as a 90-minute intravenous infusion. Administration of CPT-11 was planned for Days 1, 8, and 15, and administration of carboplatin was planned on Day 1 at a dose targeting a specific AUC, as determined by the Chatelut formula: dose (mg) = AUC $\cdot$ [0.134 $\cdot$ weight + (218 $\cdot$ weight $\cdot$ (1-0.00457 $\cdot$ age) $\cdot$ (1-0.314 $\cdot$ gender)/serum creatinine expressed in micromolar concentration)], with weight expressed in kilograms, age in years, and gender equal to 0 for male and 1 for female.[11] Serum creatinine was measured by the PAP method with the Serotec CRE-L kit (Serotec Company, Sapporo, Japan). CPT-11 was withdrawn on Days 8 and 15 if the leukocyte count was $<$ 3000/μL, the platelet count was $<$ 100,000/μL, or diarrhea was $\geq$ Grade 1. This chemotherapy regimen was repeated every 4 weeks. Granisetron was used routinely as an antiemetic on Days 1, 8, and 15. Prophylactic granulocyte-colony stimulating factors were not used routinely.

The starting dose of CPT-11 and carboplatin was 50 mg/m^2 and AUC 4. Dose escalation with six different dose levels was planned, and at least three patients were entered at each dose level. No interpatient dose escalation was performed.

DLT and MTD

Severe or life-threatening (Grade 3 or 4) nonhematologic toxicity, with the exception of nausea and emesis, was considered dose limiting. A leukocyte count $<$ 1000/μL or a neutrophil count $<$ 500/μL that lasted $>$ 3 days or a platelet count $<$ 25,000/μL of any duration also were considered dose limiting. The dose was escalated to the next level when none of the three patients experienced DLT in the first cycle. If one of the three patients experienced DLT in the first cycle, then three additional patients were entered at that dose level. The MTD was defined as one dose level

below the dose that induced DLT in three of six patients during the first cycle.

Assessment of Treatment

We used World Health Organization (WHO) criteria to assess the response to treatment of patients who had measurable lesions.[20] Measurable lesions were evaluated radiographically. Pleural effusion, ascites, and bone metastases were not considered measurable sites. Patients without measurable lesions were classified as not evaluable.

CA-125 response was defined as a 50% reduction in the CA-125 level below the baseline value that persisted for ≥ 4 weeks. The CA-125 response was assessed and reported separately from the response of patients with measurable disease.[21] Toxicity was evaluated according to the Japan Clinical Oncology Group Grading system.[22]

Pharmacologic Analysis

CPT and carboplatin were infused in 1 arm of each patient, and blood samples for the pharmacokinetic study were taken from each patient's other arm on Day 1 of the first course. Blood samples (1 mL) for pharmacokinetic analysis of CPT-11 were obtained before the chemotherapy; at the end of the CPT-11 infusion; and 5 minutes, 15 minutes, and 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours after the end of the infusion. The concentrations of CPT-11 and its metabolites (SN-38 and SN-38 glucuronide [SN-38G]) were measured by a modified, reverse-phase, high-performance liquid chromatography method.[23] Blood samples (2 mL) for measurement of carboplatin were obtained before chemotherapy; at the end of the infusion; and 0.5 hours, 1 hour, 2 hours, 4 hours, 6 hours, 10 hours, and 24 hours after the end of the infusion. After immediate centrifugation, the plasma was transferred to an Amicon Centrifree tube (Amicon, Inc., Beverly, MA), and the ultrafiltrates of the plasma were stored at − 20 °C until measurement of the plasma-free platinum concentration by flameless atomic absorption spectrometry.[24] The carboplatin level was calculated based on the platinum/carboplatin molar ratio. The AUC was obtained by the trapezoidal method with extrapolation to infinity using WINNONLIN version 1.1 software (Scientific Consulting, Apex, NC). The biliary index was calculated based on the method described in a previous report.[25]

In the pharmacodynamic study, we evaluated the correlations between pharmacokinetic parameters and observed hematologic toxicities in the first course. Hematologic toxicity was calculated according to the following formula: percentage decrease = 100 × (count before treatment − nadir count)/(count before treatment), and it was related to the AUC according to a sigmoid E_{max} model as follows: Effect (%) = $100 \times E_{max}(AUC)^{\kappa}/(AUC_{50})^{\kappa} + AUC^{\kappa}$. Nonlinear least-squares regression performed with WINNONLIN was used to estimate the AUC that produce 50% of the maximum effect (AUC_{50}) and the sigmoidicity coefficient (κ).

To evaluate for adjustment serum creatinine by adding 0.2 mg/dL,[18] we compared the observed carboplatin clearance with carboplatin clearance calculated with the Chatelut formula using PAP methods with or without the adjustment model. The accuracy of the estimate was measured by calculating the mean predictive error (MPE) and the root mean square error (RMSE).[26]

TABLE 1
Patient Characteristics

Characteristic	No. of patients
No. of patients entered	19
Median age in yrs (range)	58 (40–63)
Performance status	
0	6
1	13
Histology	
Serous	15
Mucinous	1
Endometrioid	1
Clear cell	1
Unclassified	1
No. of previous regimens	
1	10
2	6
3	2
4	1
Platinum-free interval	
< 3 mos	10
3–6 mos	3
≥ 6 mos	6
Disease sites	
Pelvic tumor	7
Liver metastasis	3
Lymph node metastasis	3
Ascites	5
Pleural effusion	3
Other	2
Median 24-hr creatinine clearance mL/min (range)	65.9 (16.9–98.8)

RESULTS

Patient Characteristics

In total, 19 patients were enrolled on this trial between August 1996 and July 1999, and all patients previously has received platinum-containing chemotherapy. The patient characteristics are listed in Table 1. Their median age was 58 years (range, 40–63 yrs), and the performance status was 0 in 6 patients and 1 in 13

TABLE 2
Dose-Escalation Schedule and Actual Doses Given to Patients

Level	Dose		No. of patients	Total no. of courses	CPT-11 dose intensity (mg/m^2/wk) delivered/projected	CPT-11 percentage dose delivered[a]
	CBDCA (AUC)	CPT-11 (mg/m^2)				
1	4	50	3	7	30/38	81
2	4	60	3	8	26/45	64
3	5	50	3	7	29/38	80
4	5	60	5	23	25/45	60
5	6	50	5	25	21/38	58

CBDCA: the observed area under the concentration curve (AUC) for carboplatin; CPT-11: irinotecan.
[a] Actually delivered CPT-11 dose as a percentage of the planned dose.

TABLE 3
Major Toxicities Stratified by Dose Levels (70 courses)

	Toxicity grade															
	Leukopenia		Neutropenia		Anemia		Platelets		Nausea/emesis				Diarrhea			
Level	3	4	3	4	3	4	3	4	1	2	3	4	1	2	3	4
1	0	0	1	0	1	0	0	0	2	1	0	0	0	0	0	0
2	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0
3	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
4	1	0	0	1	3	0	4	0	0	2	0	0	0	1	0	0
5	1	1	2	2[a]	2	0	1	3	2	1	1	0	2	1	0	0

Platelets: thrombocytopenia.
[a] Two patients experienced Grade 3 febrile neutropenia.

patients. Nine patients had received one or more chemotherapy regimens before this study. In total, 70 courses of the regimen used in this study were administered through 5 dose levels, and all patients were assessable for toxicity (Table 2). The median number of courses was 4 (range, 1–7 courses). One-half of the patients (58.2%) in this study actually received CPT-11 on Day 8, but only 31.3% of patients received CPT-11 on Day 15. CPT-11 was withdrawn on Day 8 or Day 15 on 72 occasions, because of thrombocytopenia in 49% of episodes and because of leukopenia in 31% of episodes. However, on 9 occasions, the thrombocyte count was $> 75{,}000/\mu L$; and, on 17 occasions, the leukocyte count was $> 2000/\mu L$. Dose intensity and the percentage of the CPT-11 dose administered that was delivered at each dose level are shown in Table 2.

Recommended Dose Level

None of the 3 patients at Dose Levels 1, 2, 3, and 4 experienced DLTs. Because 1 of the 3 patients at Dose Level 5 experienced DLT (neutropenia and thrombocytopenia), an additional 3 patients were enrolled. Two of those patients developed DLT (neutropenia and thrombocytopenia); therefore, it was concluded that Dose Level 4 was the MTD and the recommended dose.

Toxicity

Leukopenia and neutropenia were the DLTs associated with this combination chemotherapy. Major toxicities stratified by dose levels are shown in Table 3. At Dose Level 5, 2 patients required platelet infusion and developed febrile neutropenia that required intravenous antibiotics. No anemia that required blood transfusion was observed in any treatment cycle at any level. Gastrointestinal toxicities, such as nausea, emesis, diarrhea, and appetite loss, were prominent. CPT-11 caused diarrhea, but it was mild (Grade 1–2). No treatment-related deaths occurred in the current study.

Responses

Ten patients had measurable lesions, and they were assessed for responses according to WHO criteria (2 patients at Dose Level 1, 2 patients at Dose Level 2, 1 patient at Dose Level 3, 3 patients at Dose Level 4, and

TABLE 4
Objective Response and CA-125 Response

Level	No. of patients	Response				No. of CA-125 responses[a]
		PR	NC	PD	NE	
1	3	0	2	0	1	1
2	3	0	2	0	1	0
3	3	0	0	1	2	0
4	5	3	0	0	2	2
5	5	2	1	0	2	3

PR: partial response; NC: no change; PD: progressive disease; NE: not evaluable.
[a] The number of CA-125 responses means number of patients who achieved a 50% reduction in CA-125 level compared with the baseline level, which that must have persisted for ≥ 4 weeks.

3 patients at Dose Level 5). An objective response was observed in 5 patients: a partial response was seen at Dose Level 4 in 3 patients, and a partial response was seen at Dose Level 5 in 2 patients. The platinum-free interval was < 3 months in 2 of the 3 patients who achieved a partial response at Dose Level 4. CA-125 responses were observed in 6 patients (Table 4). The median time to disease progression in this study was 6.1 months (range, 0.93–19.4 mos), and the median survival was 16.2 months (range, 2.5–51.9 mos).

Pharmacologic Study of CPT-11 and CBDCA

The pharmacokinetic study was performed in only 13 patients, because the other 6 patients refused blood sampling for the pharmacokinetic analysis. A summary of the pharmacokinetic parameters of CPT-11, SN-38, and SN-38G is shown in Table 5. The concentrations of CPT-11 and SN-38 versus the time curve at each dose are shown in Figures 1 and 2, respectively. The metabolic ratios of SN-38 and the biliary indexes calculated as the AUC of CPT-11 and the AUC of SN-38/AUC of SN-38G[25] were similar at both dose levels.

A summary of the pharmacokinetic parameters of carboplatin is shown in Table 5. The measured AUCs of carboplatin were higher than the estimated AUCs (Fig. 3). The observed carboplatin clearance and the carboplatin clearance calculated using the Chatelut formula were 74.1 ± 24.6 mL/minute (range, 31.8–120.0 mL/min) and 93.7 ± 29.2 mL/minute (range 37.0–138.9 mL/min), respectively. The accuracy of the estimation evaluated on the basis of the MPE and the RMSE was 22.8% and 31.3%, respectively, using the Chatelut formula without the adjustment model and − 1.1% and 17%, respectively, on the basis of calculations with the adjustment model (Fig. 4).

The pharmacodynamic analysis was undertaken to evaluate the correlations between pharmacokinetic parameters and hematologic toxicity in the first course. The correlation between the SN-38 AUC and the percentage decrease in neutrophil count is shown in Figure 5 ($r = 0.292$), and the AUC_{50} of SN-38 was 36.0 ng/hour/mL, with κ estimated at 0.38. The correlation between the carboplatin AUC and the percentage decrease in thrombocyte is shown in Figure 6 ($r = 0.514$), and the AUC_{50} of carboplatin was 2.76 mg · min/mL, with κ estimated at 2.80.

DISCUSSION

Based on the results of the current study, the combination of CPT-11 and carboplatin was feasible for patients who previously received platinum-containing chemotherapy, and it was concluded that the recommended dose for the Phase II study in patients with advanced ovarian carcinoma was CPT-11 60 mg/m^2 on Days 1, 8, and 15 combined with carboplatin AUC 5 on Day 1. To our knowledge, this is the first report of combination therapy with carboplatin and CPT-11 in patients with ovarian carcinoma.

In Phase I trials in previously untreated patients with lung carcinoma, the recommended dose of this regimen was CPT-11 50 mg/m^2 on Days 1, 8, and 15 and carboplatin AUC 5 mg/mL · minute on Day 1, and the DLTs were neutropenia, thrombocytopenia, and diarrhea.[27,28] Although the recommended CPT-11 dose in the current study was higher than in those studies, the main DLTs were neutropenia and thrombocytopenia, as expected, and no severe nonhematologic toxicities, such as diarrhea, were observed. We believe that the reasons for this may be that the dose intensity of CPT-11 (mg/m^2 per week) and the percentage of the dose delivered in our study were lower than in the other studies. The difference of the dose intensity is attributable to the fact that our criteria for administration on Days 8 and 15 were stricter than those used in the previous studies[17,27,28] and to the difference in the number of patients in a previously untreated or heavily treated setting.

Although the sequence of administration of CPT-11 and carboplatin in the current study was different from the sequence used in the patients with lung carcinoma, the pharmacokinetic parameters of CPT-11 and SN-38 were almost the same as those in the patients with lung carcinoma who were treated with CPT-11 (50 mg/m^2 on Days 1, 8, and 15) followed by a fixed dose of carboplatin (300 mg/m^2 on Day 1).[28] The sequence of the drug administration in a previous study did not affect the pharmacodynamics or kinetics in the combination of CPT-11 and cisplatin.[29] Therefore, the drug sequence administration may have no major influence on the pharmacokinetic parameters in the combination of CPT-11 and carboplatin.

TABLE 5
Summary of Pharmacokinetic Parameters[a]

Pharmacokinetic parameter	No. of patients	Cmax	$AUC^{0-\infty}$	CL	$T_{1/2}$	Biliary index[b]
CPT-11		μ/mL	μg·hr/ml	L/m² hr	Hr	
50 mg/m²	8	0.69 ± 0.06	3.32 ± 0.25	13.21 ± 1.32	8.03 ± 0.75	—
60 mg/m²	5	1.14 ± 0.10	4.79 ± 0.44	13.00 ± 1.32	8.35 ± 1.05	—
SN-38		ng/mL	ng·hr/ml		Hr	
50 mg/m²	8	22.86 ± 2.6	225.6 ± 40.3	—	10.51 ± 1.98	—
60 mg/m²	5	28.27 ± 4.5	283.9 ± 62.7	—	11.67 ± 2.41	—
SN-38G		ng/mL	ng·hr/ml		Hr	ng·hr/ml
50 mg/m²	8	29.8 ± 3.1	464.7 ± 70.4	—	12.06 ± 1.50	1692.8 ± 843.7
60 mg/m²	5	45.6 ± 12.5	1040.2 ± 416.8	—	16.05 ± 1.41	1652.2 ± 511.4
CBDCA		mg/mL	mg·min/ml	mL/min	Hr	
AUC 4	6	13.97 ± 4.1	4.95 ± 0.99	77.7 ± 27.53	4.25 ± 0.74	—
AUC 5	4	16.4 ± 3.3	5.59 ± 0.48	77.99 ± 30.73	4.17 ± 1.23	—
AUC 6	3	21.7 ± 2.5	7.94 ± 1.88	80.69 ± 24.83	4.18 ± 0.37	—

Cmax: maximum plasma concentrations; AUC: area under the concentration curve; CL: clearance; $T_{1/2}$: elimination half-life; CPT-11: irinotecan; SN-38: 7-ethyl-10-hydoxycamptothecin; SN38G: SN-38-glucronide; CBDCA: the observed AUC of carboplatin.

[a] Data shown are the mean ± standard deviation in 13 patients.

[b] "Biliary index (ng·hr/ml)" was calculated as $AUC_{CPT-11} \times AUC_{SN-38}/AUC_{SN-38G}$.

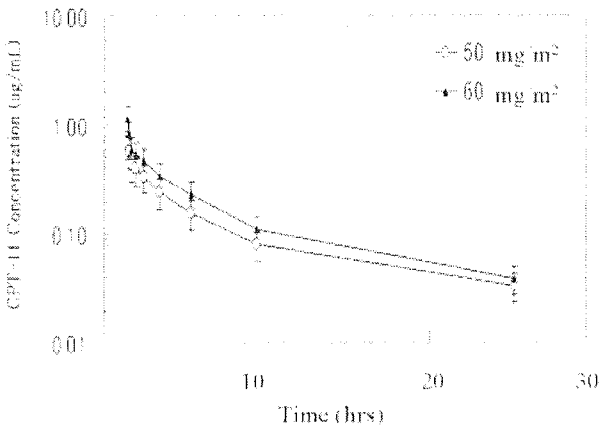

FIGURE 1. The concentrations of irinotecan (CPT-11) versus the time curve are illustrated for patients who received doses of 50 mg/m² and 60 mg/m² (n = 13 patients).

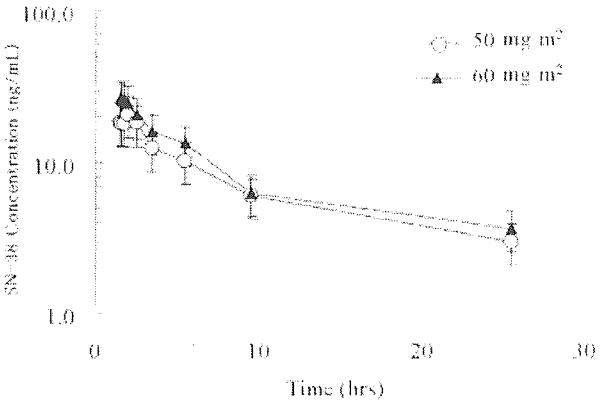

FIGURE 2. The concentrations of SN-38 versus the time curve are illustrated for patients who received in doses of 50 mg/m² and 60 mg/m² (n = 13 patients).

Although we used the Chatelut formula in the current study, several formulas are available to calculate the dose of carboplatin. The Calvert formula requires measurement of the glomerular filtration rate with a radioisotope, but creatinine clearance rates estimated by the Cockcroft–Gault or Jelliffe formula or actually measured, 24-hour creatinine clearance have been used widely instead. Several studies have compared the performance of the Chatelut formula and the Calvert formula by using several methods,[14–16] but the performance of each formula remains a matter of controversy, because previous studies have reported differences according to race, gender, method of calculating creatinine clearance, and unknown pharmacokinetic interactions between drugs in combination.[12–17] Fukuda et al. reported that a Phase I study of CPT-11 and carboplatin in 11 previously untreated Japanese patients with solid malignancies showed a significant correlation between measured carboplatin clearance and carboplatin clearance estimated by the Chatelut formula, and those authors recommended using the formula.[18] However, several clinical pharmacologic studies have shown that carboplatin clearance calculated by the Chatelut formula was higher than measured clearance and clearance calculated by the Calvert formula.[12–14] Furthermore, it has been reported that the adjusted serum creatinine value is

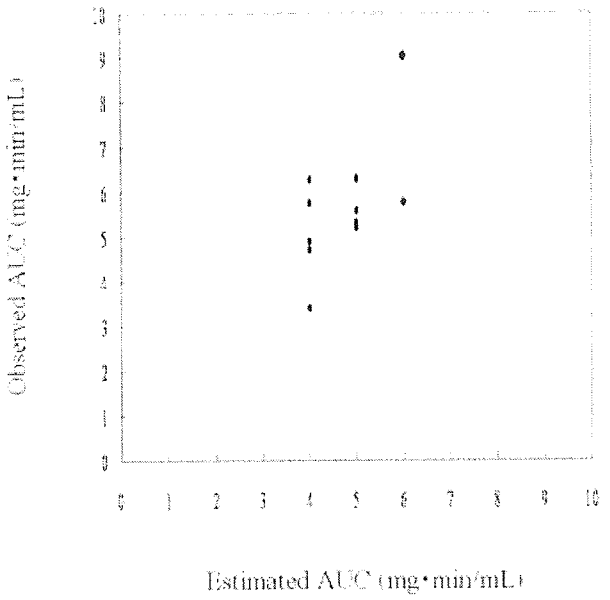

FIGURE 3. This chart illustrates the correlation between the observed area under the plasma concentration-versus-time curve (AUCs) of carboplatin (CBDCA) and the estimated AUC.

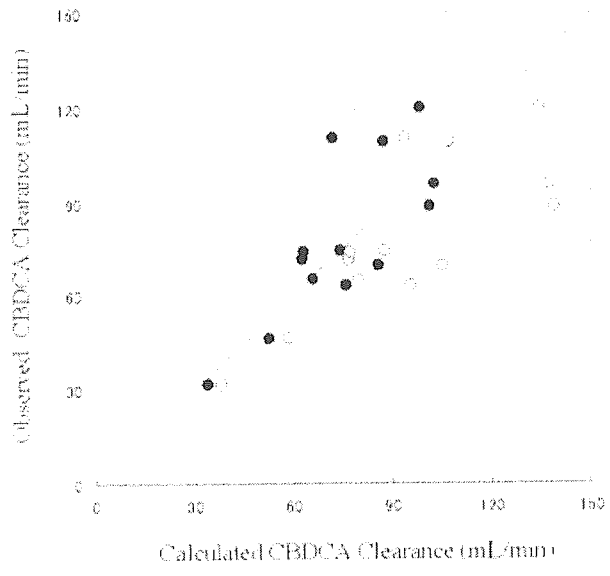

FIGURE 4. This chart illustrates the correlation between observed carboplatin (CBDCA) clearance and CBDCA clearance calculated with the Chatelut formula (open circles) and with the Chatelut formula adjusted for serum creatinine (solid circle) (see Dooley et al., 2002[17]). The line of identity (solid line) is shown. The bias and precision of each formula are expressed by the mean prediction error and the root mean square error (see Kaneda et al., 1990[24]).

appropriate for calculating the proper carboplatin clearance in Japanese patients.[18] Similar to previous reports, our study showed that the calculated carbo-

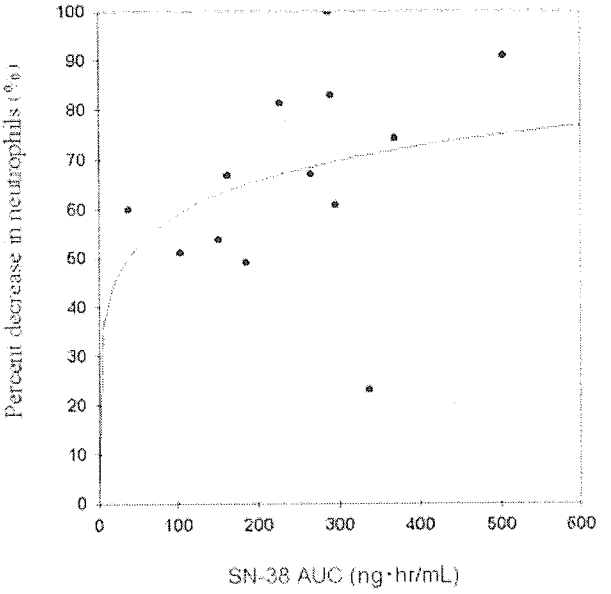

FIGURE 5. This chart illustrates the correlation between the SN-38 area under the plasma concentration-versus-time curve (AUC) and the percentage decrease in neutrophil count in Course 1 based on the sigmoid E_{max} model.

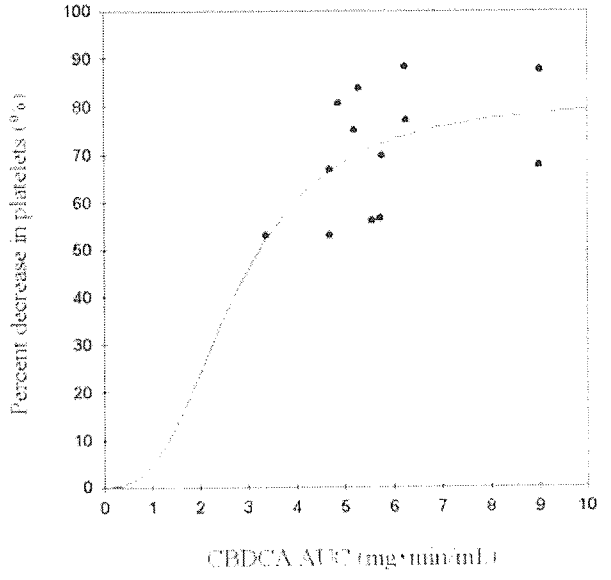

FIGURE 6. This chart illustrates the correlation between the carboplatin (CBDCA) area under the plasma concentration-versus-time curve (AUC) and the percentage decrease in platelet count in Course 1 based on the sigmoid E_{max} model.

platin clearance values were higher than the measured values, and the accuracy of estimation by using the Chatelut formula improved when adjusted serum creatinine values were used.

In recent years, it has been demonstrated that paclitaxel is highly effective for ovarian carcinoma, and paclitaxel plus platinum combination chemotherapy now is accepted widely as a standard regimen for the first-line treatment for advanced ovarian carcinoma.[1,2] CPT-11 is a topoisomerase I inhibitor that has unique antitumor action. In a Phase II trial, CPT-11 and cisplatin combination chemotherapy yielded a high response rate of 76% in previously untreated patients with advanced ovarian carcinoma.[30] CPT-11 and cisplatin also yielded an overall response rate of 40% in patients who were treated previously with platinum-containing chemotherapy and a response rate of 30% in platinum-resistant patients.[31] Kigawa et al. reported a high response rate (60%) to second-line CPT-11 and cisplatin combination chemotherapy among patients who were treated previously cisplatin, and there was no difference in the proportion of patients who had platinum-sensitive or platinum-resistant tumors between responders and nonresponders.[32] Although the current study was performed in a Phase I trial setting, it is noteworthy that half of the patients with measurable lesions achieved responses. A previous study reported that the CPT-11 response rate was 17%, even among patients with platinum-resistant tumors.[7] Thus, our regimen may be effective both in patients with platinum-sensitive disease and in patients with platinum-resistant disease who previously received paclitaxel plus platinum combination chemotherapy.

In conclusion, the recommended doses of CPT-11 and carboplatin in combination are 60 mg/m^2 and an AUC of 5 mg/mL · minute according to the Chatelut formula, respectively, and this regimen may be effective in patients with ovarian carcinoma who previously received platinum-containing chemotherapy. The results of the pharmacologic analysis in this study suggest that the carboplatin clearance rates calculated with the Chatelut formula are higher than the actually measured carboplatin clearance and that adjustment for serum creatinine may be useful in calculating the proper dose.

REFERENCES

1. McGuire WP, Hoskins WJ, Brady MF, et al. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with Stage III and IV ovarian cancer. *N Engl J Med.* 1996;334:1–6.
2. Ozols RF, Bundy BN, Greer BE, et al. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected Stage III ovarian cancer: a Gynecologic Oncology Group study. *J Clin Oncol.* 2003;21:3194–3200.
3. Misawa T, Kikkawa F, Maeda O, et al. Establishment and characterization of acquired resistance to platinum anticancer drugs in human ovarian carcinoma cells. *Jpn J Cancer Res.* 1995;86:88–94.
4. Kano Y, Akutsu M, Suzuki K, Yoshida M. Effects of carboplatin in combination with other anticancer agents on human leukemia cell lines. *Leukemia Res.* 1993;17:113–119.
5. Kano Y, Suzuki K, Akutsu M, et al. Effects of CPT-11 in combination with other anticancer agents in culture. *Int J Cancer.* 1992;50:604–610.
6. Bodurka DC, Levenback C, Wolf JK, et al. Phase II trial of irinotecan in patient with metastatic epithelial ovarian cancer or peritoneal cancer. *J Clin Oncol.* 2003;21,291–297.
7. Evans BD, Raju KS, Calvert AH, Harland SJ, Wiltshaw E. Phase II study of JM8, a new platinum analog, in advanced ovarian carcinoma. *Cancer Treat Rep.* 1983;67:997–1000.
8. Leyvraz S, Ohnuma T, Lassus M, Holland JF. Phase I study of carboplatin in patients with advanced cancer, intermittent intravenous bolus, and 24-hour infusion. *J Clin Oncol.* 1985;3:1385–1392.
9. Jordrell DI, Egorin MJ, Canetta RM, et al. Relationships between carboplatin exposure and tumor response and toxicity in patients with ovarian cancer. *J Clin Oncol.* 1992;10: 520–528.
10. Calvert AH, Harland SJ, Newell DR, et al. Early clinical studies with cisdiammine-1,1-cyclobutane dicarboxylate platinum II. *Cancer Chemother Pharmacol.* 1982;9:140–147.
11. Chatelut E, Canal P, Brunner V, et al. Prediction of carboplatin clearance from standard morphological and biological patient characteristics. *J Natl Cancer Inst.* 1995;87:573–580.
12. Fujiwara Y, Takahashi T, Yamakido M, Ohune T, Tsuya T, Egorin MJ. Re: prediction of carboplatin clearance from standard morphological and biological patients characteristics. *J Natl Cancer Inst.* 1997;89:260–261.
13. Minami H, Ando Y, Saka H, Shimokata K. Re: prediction of carboplatin clearance from standard morphological and biological patients characteristics. *J Natl Cancer Inst.* 1997;89: 968–969.
14. Okamoto H, Nagamoto A, Kunitoh H, Kunikane H, Watanabe K. Prediction of carboplatin clearance calculated by patient characteristics or 24-hour creatinine clearance: a comparison of the performance of three formulae. *Cancer Chemother Pharmacol.* 1998;42:307–312.
15. Donahue A, McCune JS, Faucette S, et al. Measured versus estimated glomerular filtration rate in the Carver equation: influence on carboplatin dosing. *Cancer Chemother Pharmacol.* 2001;47:373–379.
16. Dooley MJ, Poole SG, Rishchin D, Webster LK. Carboplatin dosing: gender bias and inaccurate estimates of glomerular filtration rate. *Eur J Cancer.* 2002;38:44–51.
17. Fukuda M, Oka M, Soda H, et al. Phase I study of irinotecan combined with carboplatin in previously untreated solid cancers. *Clin Cancer Res.* 1999;5:3963–3969.
18. Ando Y, Minami H, Saka H, Ando M, Sugiura S, Sakai S, Shimokata K. Adjustment of creatinine clearance of improves accuracy of Calvert's formula for carboplatin dosing. *Br J Cancer.* 1997;76:1067–1071.
19. Ozols RF. Treatment of recurrent ovarian cancer: increasing option-recurrent results. *J Clin Oncol.* 1997;15:2177–2180.
20. Miller AB, Hoogstraten B, Staquet M. Reporting results of cancer treatment. *Cancer.* 1981;147:207–214.

21. Rustin GJ, Nelstrop AE, McClean P, et al. Defining response of ovarian carcinoma to initial chemotherapy according to serum CA 125. *J Clin Oncol.* 1996;14:1547–1551.
22. Tobinai K, Kohno A, Shimada Y, et al. Toxicity grading criteria of the Japan Clinical Oncology Group. *Jpn J Clin Oncol.* 1993;23:250–257.
23. Kaneda N, Nagata H, Furuta T, YokokuraT. Metabolism and pharmacokinetics of the camptothecin analogue CPT-11 in the mouse. *Cancer Res.* 1990;50:1715–1720.
24. Le Roy AF, Wehling ML, Sponseller HL, et al. Analysis of platinum in biological materials by flameless atomic absorption spectrophotometry. *Biochem Med.* 1977;18:184–191.
25. Gupta E, Lestingi TM, Mick R, Ramirez J, Vokes EE, Ratain MJ. Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. *Cancer Res.* 1994;54:3723–3725.
26. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. *J Pharmacokinet Biopharm.* 1981;9:503–512.
27. Takeda K, Negoro S, Takefuji N, et al. Dose escalation study of irinotecan combined with carboplatin for advanced non-small-cell lung cancer. *Cancer Chemother Pharmacol.* 2001;48:104–108.
28. Sato M, Ando M, Minami H, et al. Phase I/II and pharmacologic study of irinotecan and carboplatin for patients with lung cancer. *Cancer Chemother Pharmacol.* 2001;48:481–487.
29. de Jonge MJA, Verweij J, Planting AST, et al. Drug-administration sequence does not change pharmacodynamics and kinetics of irinotecan and cisplatin. *Clin Cancer Res.* 1999;5:2012–2017.
30. Sugiyama T, Yakusiji M, Kamura T, et al. Irinotecan and cisplatin as first line chemotherapy for advanced ovarian cancer. *Oncology.* 2002;63:16–22.
31. Sugiyama T, Yakushiji M, Nishida T, et al. Irinotecan combined with cisplatin in patients with refractory or recurrent ovarian cancer. *Cancer Lett.* 1998;128:211–218.
32. Kigawa J, Takahashi M, Minagawa Y, et al. Topoisomerase-1 activity and response to second-line chemotherapy consisting of camptothecin-11 and cisplatin in patients with ovarian cancer. *Int J Cancer.* 1999;84:521–524.

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Secondary cytoreductive surgery for recurrent epithelial ovarian carcinoma: proposal for patients selection

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The value of secondary cytoreductive surgery (SCS) for recurrent ovarian cancer is still controversial. The aim of this study was to clarify candidates for SCS. Between January 1987 and September 2000, we performed SCS in 44 patients with recurrent ovarian cancer, according to our selection criteria, disease-free interval (DFI) >6 months, performance status <3, no apparent multiple diseases, age <75years and no progressive disease during preoperative chemotherapy, if undertaken. The variables were investigated by univariate and multivariate analyses. Of 44 patients, 26 (59.1%) achieved complete removal of all visible tumours at SCS. Secondary cytoreductive surgery outcome, complete or incomplete resection, was significantly related to overall survival ($P=0.0019$). As for variables determined before SCS, DFI >12 months, no liver metastasis, solitary tumour and tumour size <6 cm were independently associated with favourable overall survival after recurrence in the multivariate analysis. Patients with three or all four variables ($n=31$) had significantly better survival compared with the other patients ($n=13$) (47 *vs* 20 months in median survival, $P<0.0001$). In these patients, fairly good median survival (40 months) was obtained even in patients with incomplete resection. Secondary cytoreductive surgery had a large impact on survival of patients with recurrent ovarian cancer when they had three or all of the above-mentioned four factors at recurrence. These patients should be considered as ideal candidates for SCS.
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Since Griffiths (Griffiths, 1975) first demonstrated the inverse relationship between residual tumour size after primary debulking and survival of ovarian cancer patients in 1975, many investigators have reproduced and confirmed this observation (Hacker *et al*, 1983; Vogl *et al*, 1983; Delgado *et al*, 1984; Conte *et al*, 1985; Louie *et al*, 1986; Neijt *et al*, 1987; Hainsworth *et al*, 1988; Sutton *et al*, 1989). Thus, the value of debulking of large tumour masses in the primary surgery of ovarian cancer has been generally accepted, and primary cytoreductive surgery followed by chemotherapy is considered to be a standard treatment procedure for patients with advanced ovarian cancer.

The cytoreduction contributes to removal of the tumour burden and relief of symptoms caused by tumours or massive ascites. In addition, the cytoreduction has another important effect on the sensitivity to postsurgical chemotherapy. By removing bulky tumours, the decreased growth fractions should increase (Norton and Simon, 1977) and poorly perfused anoxic cells should decrease. By reducing the number of cancer cells, the chance for cancer cells to undergo spontaneous mutations resulting in drug resistance should decrease (Goldie and Coldman, 1979). All these effects are believed to enhance the sensitivity to chemotherapy.

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Theoretically, the favourable effects of cytoreduction may also be expected in patients with recurrent ovarian cancer. Recently, several investigators have reported the significant value of secondary cytoreductive surgery (SCS) in a subset of patients with recurrent ovarian cancer (Jänicke *et al*, 1992; Eisenkop *et al*, 1995, 2000; Vaccarello *et al*, 1995; Cormio *et al*, 1999; Zang *et al*, 2000, 2004; Munkarah *et al*, 2001; Scarabelli *et al*, 2001; Tay *et al*, 2002). The value of complete resection at the time of SCS for highly selected patients is in consensus in these recent reports. They reported a considerable number of factors related to good prognosis including longer disease-free interval (DFI), smaller size of residual tumour at primary cytoreductive surgery, good response to first-line chemotherapy, younger age at recurrence and smaller size of maximum tumour at recurrence. However, there is limited information regarding the ideal candidates for SCS. Although only preoperative or intraoperative variables before starting SCS should be analysed for selection of the candidate, these variables have been analysed together with SCS outcome in most previous studies. In addition, the follow-up periods of living patients were rather short (the median or average follow-up periods were between 1 and 4 years) (Jänicke *et al*, 1992; Vaccarello *et al*, 1995; Cormio *et al*, 1999; Zang *et al*, 2000, 2004; Munkarah *et al*, 2001; Scarabelli *et al*, 2001) in most of the previous reports.

Since 1987, we have performed SCS according to our criteria of patient selection in 44 out of 70 ovarian cancer patients who had recurrence after DFI. In the present study, the median follow-up

period of living patients is 60 months after the initiation of treatment, SCS or chemotherapy before SCS, for recurrence. Using univariate and multivariate analyses of variables before starting SCS, we planned to clarify the ideal candidates for SCS among patients with recurrent ovarian cancer.

PATIENTS AND METHODS

Patient selection

Between January 1984 and December 1999, we treated 236 patients with stage I to IV epithelial ovarian cancer at the Department of Obstetrics and Gynecology, University of Tokyo Hospital. Our standard surgical procedures for ovarian cancer consist of total abdominal hysterectomy, bilateral salpingo-oophorectomy, infracolic or total omentectomy, and in advanced cases, debulking of tumour masses with maximum efforts. Patients with no or small intraperitoneal residual tumours (less than 2 cm in diameter) also underwent systematic retroperitoneal lymphadenectomy. The extent of retroperitoneal lymphadenectomy is pelvic lymph nodes only (1984–1986) or both pelvic and aortic lymph nodes (1987–1999). All but stage Ia patients underwent at least six cycles of cisplatin-based chemotherapies following surgery as described previously (Onda *et al*, 1998). Of the 236 patients, 204 (86%) achieved complete clinical remission after primary treatment.

By September 2000, 70 of the 204 (34%) patients had recurrence and, from January 1987 to September 2000, 44 of the 70 (63%) patients underwent SCS prior to or following chemotherapy. Administration of chemotherapy before SCS was decided based on various clinical factors including short DFI (DFI <12 months) and poor performance status (PS 3) defined by ECOG (Eastern Cooperative Oncology Group). Our selection criteria for SCS were as follows: (1) DFI >6 months, (2) age at recurrence <75 years, (3) PS 0–2 just before the surgery, (4) absence of apparent extensive intraperitoneal dissemination or multiple distant metastases and (5) no progressive disease during presurgical chemotherapy, if undertaken. There were three exceptions to the above-mentioned criteria for SCS. One patient with DFI <6 months (5 months) underwent SCS, because the recurrent site was expected to be limited to a solitary aortic lymph node by CT. The other two patients had PS 3 at surgery. One patient with three metastatic brain tumours underwent emergent brain surgery followed by γ-knife radiosurgery to one residual tumour (Kawana *et al*, 1997), and one patient underwent ileocaecal resection because of acute bowel obstruction. Before the treatment, informed consent was obtained from all of the patients.

Chemotherapy

Of 44 patients, 21 (47.7%) received chemotherapy before SCS and all of 44 patients were treated with chemotherapy after SCS. In all, one to eight (median: 2) cycles of presurgical chemotherapy were performed in eight of 13 (61.5%) patients with DFI <12 months and 13 of 31 (41.9%) patients with DFI >12 months. In total, 44 patients received two to nine (median: 4) cycles of postsurgical chemotherapy.

In all, two to four cycles of presurgical chemotherapy were generally administered until beneficial response (partial or minor response) was observed. In two patients, second-line chemotherapy showed no beneficial response, and SCS was performed after successful third-line chemotherapy (seven and eight cycles in total). One patient received only a cycle of presurgical chemotherapy, because SCS could not be scheduled immediately after diagnosis of recurrence.

The number of postsurgical chemotherapy given was determined by SCS outcome and response to chemotherapy, evaluated by CT scan and serum level of CA125. Generally, three to four cycles of chemotherapy were planned for patients with no residual tumour and five to six cycles of chemotherapy were planned for patients with any residual disease. In principle, we gave at least two cycles of chemotherapy after the serum level of CA125 was normalised. Thus, three patients were treated with more than six cycles of chemotherapy after SCS. On the contrary, chemotherapy was discontinued before accomplishment of the planned cycles in five patients because rapid disease progression or severe adverse effects were observed during the planned cycles.

In presurgical and postsurgical chemotherapies, a platinum-based combination, CAP, EP or TJ, was used. The CAP regimen consisted of 600 mg m^{-2} of cyclophosphamide, 30 mg m^{-2} of doxorubicin and 50–75 mg m^{-2} of cisplatin. The EP regimen consisted of 80 mg m^{-2} of etoposide during days 1–5 and 75 mg m^{-2} of cisplatin. Paclitaxel was introduced in Japan in 1998 and, thereafter, a TJ regimen consisting of paclitaxel (175 mg m^{-2} over 3-h infusion) and AUC 5 of carboplatin was used as second-line chemotherapy.

Statistical methods

Survival was measured from the day of starting treatment for recurrence, that is, the day of starting presurgical chemotherapy or the day of performing SCS. The survival curves were determined by the Kaplan–Meier product limit method (Kaplan and Meier, 1958). Factors influencing survival were analysed using the log-rank test (univariate) and Cox's proportional-hazards regression analysis (multivariate). These analyses were performed using a JMP program (SAS Institute Inc., USA). Contingency table analysis was performed using the χ^2 test or χ^2 test for trend.

RESULTS

Patient characteristics

The number of patients was three in stage I, two in stage II, 36 in stage III and three in stage IV according to the International Federation of Gynecology and Obstetrics (FIGO). Histology was serous type in 35, clear-cell type in three, endometrioid type in three, transitional cell type in two and mixed epithelial type in one. Median DFI was 18.5 months with a range of 5–58 months: one patient (2.3%) had 5 months, 12 (27.3%) had 6–12 months and 31 (70.5%) had >12 months. Median age at recurrence was 52 years with a range of 37–74 years. Median follow-up period of patients, excluding those who died, was 60 months with a range of 17–199 months from the initiation of treatment for recurrence.

Surgery

Our attempt to perform SCS resulted in exploratory laparotomy in four patients (9.1%) due to the presence of unexpected extensive peritoneal tumours. Various debulking surgeries classified into four categories such as (1) gastrointestinal resection, (2) resection of other organs, (3) lymph node dissection and (4) other tumour debulking was performed with maximum efforts in the remaining 40 patients (90.9%). Among these patients, gastrointestinal resection (category 1) was required in 11 patients (25.0%), large bowel resection in nine patients (20.5%), small bowel resection in three patients (6.8%), partial gastrectomy in one patient and ileocaecal resection in one patient (2.3%), and one of the patients (2.3%) underwent sigmoid colostomy. Three patients had category 1 surgeries at two sites. Resection of other organs (category 2) was required in six patients (13.6%), splenectomy in three patients (6.8%), distal pancreatectomy in two patients (4.5%), partial liver resection in one patient, hysterectomy in one patient and brain tumour resection in one patient (2.3%). Two patients had category 2 surgeries at two sites. Regional or distant lymph node dissection (category 3) was performed in 12 patients (27.3%). Five patients

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(11.4%) underwent systematic aortic lymphadenectomy and one (2.3%) underwent both systematic pelvic and aortic lymphadenectomies. Selective dissections of the following lymph nodes were performed in six patients: aortic nodes in one patient, pelvic nodes in one patient, axillary nodes in one patient, portal nodes in one patient, inguinal nodes in one patient and mesenteric nodes in one patient (2.3%). Other tumour debulking (category 4) including removal of tumours in the remnant omentum, the diaphragmatic muscles and vaginal stump, and tumours on the visceral or parietal peritoneum including the under surface of the diaphragm, was performed in 22 patients (50.0%); omentectomy in seven patients; partial full-thickness diaphragm resection in one patient; resection of tumours around the vaginal stump in four patients (9.1%); peritoneum resection of disseminated tumours on the under surface of the diaphragm; and other peritoneal surfaces in 16 patients (36.4%). Six patients were counted twice because they underwent two types of category 4 surgeries. In all, 10 patients underwent two or three out of the above four categories of debulking surgery. No patients died within a month following SCS.

Cytoreductive outcome and survival of patients

Among a total of 44 patients, complete resection of visible tumours was achieved in 26 patients (59.1%), largest residual tumours <1 cm in diameter were left in 11 patients (25.0%) and largest residual tumours $\geqslant 1$ cm in diameter were left in seven patients (15.9%). The median survival and 5-year survival of all patients who underwent cytoreductive surgery were 32 months and 33.2% (Figure 1), whereas the median survival and 5-year survival of 26 patients who had recurrence after complete remission achieved by primary treatment and did not undergo the surgery were 11 months and 3.9%. Figure 2 shows the survival of patients after the initiation of treatment for recurrence according to the outcome of SCS (SCS outcome). The median survival and 5-year survival after recurrence of the patients with largest residual tumours 0, <1 and $\geqslant 1$ cm were 52 months and 47.6%, 23 months and 18.2% and 20 months and 0%, respectively ($P=0.0007$, log rank). The overall survival of patients with no residual tumour was much better than that of patients with residual tumours (22 months in median survival and 12.0% in 5-year survival, figure not shown) with statistical significance ($P=0.0019$). There was no statistical difference in overall survival between patients with residual tumours <1 and $\geqslant 1$ cm ($P=0.1314$).

Factors influencing survival in univariate analyses

Factors influencing overall survival after recurrence were analysed using univariate analyses. Factors analysed and the results of univariate analyses are listed in Tables 1 and 2. As for prognostic factors determined during primary therapy, univariate analyses revealed that peritoneal tumour spread ($P=0.039$), FIGO stage ($P=0.045$) and aortic lymph node metastasis ($P=0.009$) were significantly associated with overall survival after recurrence. Regarding prognostic factors determined at recurrence, univariate analyses revealed that DFI ($P=0.002$), presence of liver metastasis ($P=0.005$), number of recurrent tumours ($P=0.007$), size of maximum tumour ($P<0.001$) and SCS outcome ($P=0.002$) had significant associations with overall survival after recurrence.

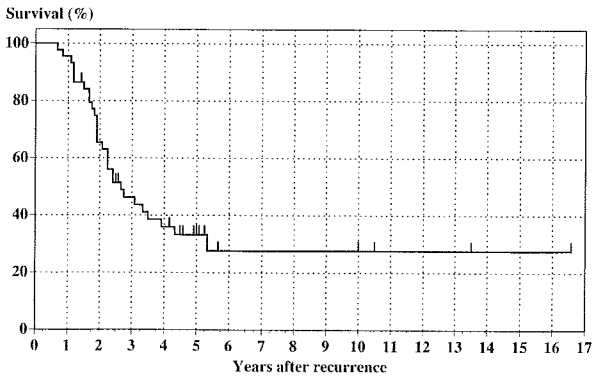

Figure 1 Survival of all 44 patients who underwent SCS.

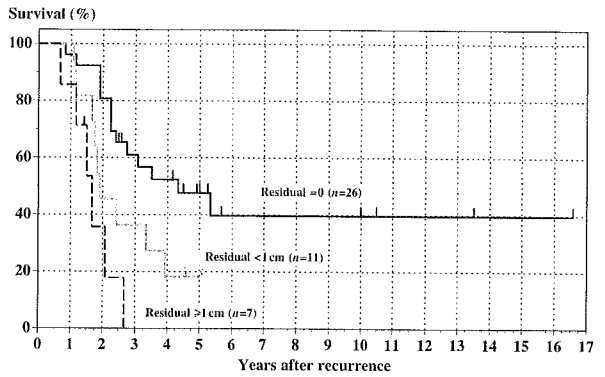

Figure 2 Outcome of SCS and survival. Survival of the patients with largest residual tumours 0, <1 and $\geqslant 1$ cm is shown in solid black, solid grey and dotted black line, respectively. The difference of survival is statistically significant ($P=0.0007$, log rank). There is no statistical difference in survival between patients with residual tumours <1 and $\geqslant 1$ cm ($P=0.1314$, log rank).

Table 1 Univariate analyses for variables during primary treatment

Variables	Number	Median survival (months)	P-value
Peritoneal tumour spread			
Localised to the pelvis	10	NA	
Extended beyond the pelvis	34	29	0.039
Stage			
I/II	5	NA	
III/IV	39	29	0.045
Aortic lymph node metastases			
Absent	25	64	
Present	14	27	
Not assessed	5	25	0.009
Pelvic lymph node metastases			
Absent	20	47	
Present	21	32	
Not assessed	3	25	0.126
Systematic lymphadenectomy			
Not performed	3	25	
Pelvic only	7	29	
Pelvic and aortic	34	33	0.296
Histology			
Serous	35	37	
Others	9	23	0.197
Residual tumour at PCS			
0	34	32	
Any	10	40	0.961

PCS = primary cytoreductive surgery; NA = not applicable.

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Table 2 Univariate analyses for variables at recurrence

Variables	Number	Median survival (months)	P-value
Age at recurrence (years)			
<50	17	29	
⩾50	27	40	0.860
Disease-free interval (months)			
⩾12	31	47	
<12	13	23	0.002
Intraperitoneal tumour			
Absent	12	64	
Present	32	27	0.117
Pelvic or aortic lymph node metastases			
Absent	34	32	
Present	10	37	0.419
Distant metastasis			
Absent	38	32	
Present	6	40	0.496
Liver metastasis			
Absent	42	33	
Present	2	20	0.005
No. of recurrent tumours			
Solitary	16	64	
Multiple	28	27	0.007
Size of maximum tumour (cm)			
<6	38	40	
⩾6	6	14	<0.001
Massive ascites (>500 ml)			
Absent	41	33	
Present	3	32	0.318
PS			
0–2	42	29	
3	2	42	0.746
Presurgical chemotherapy			
Not done	23	33	
Done	21	29	0.677
Bowel resection			
Not done	33	33	
Done	11	27	0.650
Residual tumour at SCS			
0	26	52	
Any	18	22	0.002

PS = performance status; SCS = secondary cytoreductive surgery.

Factors influencing survival in multivariate analysis

To determine patient selection for the surgery, we performed multivariate analysis using statistically significant prognostic factors in univariate analyses. Out of eight significant factors, SCS outcome was omitted in the multivariate analysis because SCS outcome is not yet known on considering indications for the surgery, although SCS outcome had a statistically significant correlation with the number of recurrent tumours ($P<0.001$, χ^2 test). The multivariate analysis using the remaining seven factors revealed that four factors determined at recurrence, specifically DFI, presence of liver metastasis, number of recurrent tumour and size of maximum tumour, were independently and significantly associated with survival after recurrence (Table 3). Additionally, the multivariate analysis using only these four factors confirmed

Table 3 Multivariate analysis using the seven prognostic variables in the univariate analyses

	Multivariate analysis	
Variables	Risk ratio (95% CI)	P-value
Peritoneal tumour spread at PCS		
Localised to the pelvis	1.00	
Extended beyond the pelvis	0.80 (0.42–1.76)	0.540
Stage		
I/II	1.00	
III/IV	0.90 (0.22–5.60)	0.893
Aortic lymph node metastases at PCS		
Absent	1.00	
Present	1.23 (0.56–2.64)	
Not assessed	1.78 (0.61–5.33)	0.088
Disease-free interval (months)		
⩾12	1.00	
<12	2.45 (1.11–5.39)	0.027
Liver metastasis		
Absent	1.00	
Present	4.00 (1.40–10.03)	0.013
No. of recurrent tumours		
Solitary	1.00	
Multiple	3.73 (1.79–9.58)	<0.001
Size of maximum tumour (cm)		
<6	1.00	
⩾6	7.43 (3.12–18.92)	<0.001

PCS = primary cytoreductive surgery.

that all four factors were independently and significantly associated with survival after recurrence. The relative risk (95% confidence interval) was 0.37 (0.20–0.68) for DFI >12 months, 0.23 (0.10–0.65) for absence of liver metastasis, 0.26 (0.12–0.48) for a solitary tumour and 0.20 (0.09–0.42) for size of maximum tumour <6 cm.

Grouping of patients determined by the number of favourable prognostic factors

According to the number of favourable statuses among the above-mentioned four prognostic factors, that is, DFI >12 months, no liver metastasis, solitary tumour and tumour size <6 cm, patients were divided into four groups as follows: patients with all four favourable factors (Group 4, $n=10$), patients with three favourable factors (Group 3, $n=21$), patients with two favourable factors (Group 2, $n=11$) and patients with only one favourable factor (Group 1, $n=2$). There were no patients with zero favourable factors. Complete resection of visible tumours was achieved in 100% (10 of 10), 62% (13 of 21), 18% (two of 11) and 50% (one of two) of patients in Group 4, Group 3, Group 2 and Group 1, respectively. Apparently, a higher rate of complete surgical resection was achieved in patients with a larger number of favourable factors, and the distribution was statistically significant by contingency table analysis ($P<0.001$, χ^2 test for trend). The 5-year survival of Group 4 was 88.9% and median survival was not reached. The 5-year survivals and median survivals of Group 3, Group 2 and Group 1 were 26.0, 0 and 0%, and 37, 20 and 10 months, respectively (figure not shown). The differences of overall survival were also statistically significant among the four groups ($P<0.001$, log rank) and between them (e.g. $P<0.007$ in Group 1 *vs* Group 2, $P<0.001$ in Group 2 *vs* Group 3 and $P<0.001$ in Group

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