Therapy effect of either paclitaxel or cyclophosphamide combination treatment in patients with epithelial ovarian cancer and relation to *TP53* gene status

B Smith-Sørensen[1], J Kærn[2], R Holm[3], A Dørum[4], C Tropé[2] and A-L Børresen-Dale[1]

[1]Department of Genetics. Institute for Cancer Research. The Norwegian Radium Hospital. 0310 Oslo. Norway: [2]Department of Gynecological Oncology. The Norwegian Radium Hospital. 0310 Oslo. Norway: [3]Department of Pathology. Institute for Cancer Research. The Norwegian Radium Hospital. 0310 Oslo. Norway: [4]Unit of Medical Genetics. Department of Oncology. The Norwegian Radium Hospital. 0310 Oslo. Norway

Summary Cell death after treatment with chemotherapy is exerted by activation of apoptosis. and the p53 protein has been shown to actively participate in this process. This recent focus on *TP53* status as a possible determinant of cancer therapy response has raised the question of whether or not mutations in the *TP53* gene have an influence on paclitaxel therapy. The *TP53* status has been analysed at the DNA level in tumours from 45 ovarian cancer patients randomized to treatment with paclitaxel and cisplatin or cyclophosphamide and cisplatin. Therapy response was obtained for 38 patients with clinically evaluable disease after initial surgery. The positive response rate to the paclitaxel/cisplatin therapy was 85°o vs 61°o for the patients who received the cyclophosphamide/cisplatin regimen. A significant difference in relapse-free survival in favour of paclitaxel/cisplatin chemotherapy was found ($P = 0.001$). A total of 33 tumour samples (73°o) had detectable sequence alterations in the *TP53* gene. When relapse-free survival was estimated for all patients with *TP53* alterations in their tumours. a significant better outcome for the paclitaxel/cisplatin group was found compared with the patient group receiving cyclophosphamide and cisplatin therapy ($P = 0.002$). We did not observe an association between *TP53* tumour status and prognosis for patients who received paclitaxel/cisplatin combination treatment. indicating that the effect of this therapy is not influenced by this parameter.

Keywords: *TP53*: paclitaxel: ovarian cancer

Two-thirds of patients with epithelial ovarian cancer are diagnosed with advanced stages. Treatment includes surgical resection of the primary tumour and metastases to reduce tumour volume. followed by cytotoxic chemotherapy. As many as 60–73% of these ovarian carcinomas are responsive to drug regimens. including the DNA-binding agents cyclophosphamide and cisplatin. or the microtubuli-stabilizing agent paclitaxel together with cisplatin (McGuire et al. 1996). The effectiveness of cancer chemotherapy is restricted by failure of some tumours to respond and by the appearance of resistant cell populations in patients who relapse after initial response. Understanding resistance to chemotherapy is dependent on the elucidation of the molecular mechanisms by which anti-cancer drugs induce cell death (Kerr et al. 1972: Vaux. 1993). It has been shown in several types of carcinomas that cell death after treatment with chemotherapy involves activation of apoptosis (Eastman. 1990: Hickman. 1992). Exposure of the cells to both platinum analogues or taxanes is associated with morphological changes in apoptosis (Bhalla et al. 1993: Havrilesky et al. 1995).

Apoptosis is a genetically controlled process. and a variety of genes have been identified coding for inducers or inhibitors of this process. The tumour-suppressor protein p53 has been shown to exert marked effects on apoptosis in some cells in addition to its involvement in the control of the cell cycle and in DNA repair. Multiple studies have revealed that wild-type p53 protein and

members of the Bcl-2 protein family (including Bcl-2 and Bax) undergo complex regulative interactions during the modulation of programmed cell death. Wild-type p53 has been shown to induce an up-regulation of *Bax* mRNA (Miyashita et al. 1994) and a concomitant down-regulation of *Bcl-2* mRNA (Haldar et al. 1994) that correlates to the induction of programmed cell death (Miyashita et al. 1994). It has been reported that cells functionally deficient in p53 or with elevated levels of either Bcl-2 or Bcl-xL are relatively resistant to cytotoxic agents. such as platinum analogues (Lowe et al. 1993a. 1994: Dole et al. 1995).

Alterations of the *TP53* gene. either as a result of point mutations. are the most frequent abnormalities described in human cancers. Abnormal p53 protein has been reported to be associated with shorter disease-free survival and poor clinical outcome of ovarian cancer patients (Levesque et al. 1995: van der Zee et al. 1995). A significant association has been reported between *TP53* missense mutations and tumours resistance to cisplatin-based therapy (Righetti et al. 1996).

During the last years. the inclusion of paclitaxel in the treatment of advanced ovarian cancer has extended the disease-free survival period. The fact that more than 50% of stage III and IV epithelial ovarian cancers are reported to have *TP53* sequence alterations (Hainaut et al. 1997). and the recent focus on *TP53* status as a possible determinant of cancer therapy response. raised the question of whether or not the *TP53* gene has an influence on paclitaxel therapy. Several groups have used various cell systems to investigate the topic (Delia et al. 1996: Hawkins et al. 1996: Wahl et al. 1996: Wu and El-Diery. 1996). Some authors could demonstrate an increased paclitaxel sensitivity for cells harbouring inactivated p53 protein (Hawkins et al. 1996: Wahl et al. 1996). whereas

Table 1   Relapse-free survival of patients with advanced ovarian cancer in relation to *TP53* status in their tumours

Patient number	Relapse	Relapse-free survival in months	TP53 status[a]
Paclitaxel/cisplatin therapy			
OV1	R	6.6	wt
OV2		23.6 rf	wt
OV3		23.2 rf	wt
OV11		18.1 rf	wt
OV12		17.5 rf	wt
OV15	R	11.1	wt
OV50	R	22.6	wt
OV42	R	13.7	Ex 5. T→C. 141. TGC→CGC. Cys→Arg
OV40	R	6.1	Ex 5. G ins. 141. TGC→TGGC. Cys→frame shift
OV10		19.6 rf	Ex 5. A ins. 166. TCA→TCAA. Ser→ frame shift
OV5		22.3 rf	Ex 5. G→A. 175. CGC→CAC. Arg→His
OV38	R	11.7	Ex 5. C→T. 179. CAT→TAT. His→Tyr
OV39	R	11.1	Ex 5. A→G. 179. CAT→CGT. His→Arg
OV46	R	9.9	Ex 6. T→G. 218. GTG→GGG. Val→Gly
OV14		16.3 rf	Ex 7. G→T. 238. TGT→TTT. Cys→Phe
OV44		18.5 rf	Ex 7. G→A. 245. GGC→AGC. Gly→Ser
OV35	R	7.1	Ex 8. G→A. 266. GGA→GAA. Gly→Glu
OV7		20.6 rf	Ex 8. C→T. 282. CGG→TGG. Arg→Trp
OV45		18.0 rf	Ex 10. 16-bp ins between 339 and 340→ frame shift
OV13		17.5 rf	Ex 5. splice mutation 5'. ag→aa
OV49	R	8.9	Ex 9. splice mutation 5'. ag→gg
OV47		17.5 rf	Ex 8. mutation detected with TTGE
OV6		22.0 rf	Ex 9. mutation detected with TTGE
Cyclophosphamide/cisplatin therapy			
OV26		20.3 rf	wt
OV29	R	9.7	wt
OV31	R	13.5	wt
OV32	R	4.3	wt
OV33		17.0 rf	wt
OV34	R	10.0	Ex 4. T→G. 113. TTC→TGC. Phe→Cys
OV16	R	5.5	Ex 5. G→A. 175. CGC→CAC. Arg→His
OV22	R	2.1	Ex 5. G→A. 175. CGC→CAC. Arg→His
OV23	R	5.1	Ex 5. G→T. 176. TGC→TTC. Cys→Phe
OV25	R	9.9	Ex 5. C→T. 178. CAC→TAC. His→Tyr
OV24	R	8.2	Ex 8. G→A. 272. GTG→ATG. Val→Met
OV18	R	14.9	Ex 8. G→A. 273. CGT→CAT. Arg→His
OV19	R	4.9	Ex 8. G→A. 273. CGT→CAT. Arg→His
OV28	R	4.6	Ex 8. G→A. 273. CGT→CAT. Arg→His
OV30	R	8.3	Ex 8. G→A. 273. CGT→CAT. Arg→His
OV41	R	7.5	Ex 8. C→G. 278. CCT→GCT. Pro→Ala
OV37	R	8.4	Ex 8. C→G. 278. CCT→CGT. Pro→Arg
OV48	R	13.1	Ex 10. A del. 345. AAT→AT. Asn→frame shift
OV43		11.8	Ex 8. 3-bp ins before start of ex 8
OV21		23.0 rf	Ex 9. splice mutation 3'. gt→at
OV27	R	2.5	Ex 5. mutation detected with TTGE
OV20	R	19.7	Ex 6. mutation detected with TTGE

[a]wt, Wild type. Specific mutations are indicated as follows: Ex (exon). base change. affected codon. codon change and amino acid change. TTGE. temporal temperature gradient electrophoresis: rf. relapse-free at last contact: bp. base pair: ins. insertion: del. deletion.

others demonstrated that cells became more resistant to treatment (Wu and El-Diery. 1996). The contradictory findings can be explained by the use of different cell systems. normal primary cells vs cancer cells. and the fact that. in some experimental settings. cells with inactivation of one *TP53* allele by point mutation were used. whereas others used expression of tumour virus proteins known to bind to the p53 protein. These in vitro studies have pointed out the need to investigate *TP53* status in tumours from patients treated with paclitaxel. Our first aim was to investigate in a clinical material whether or not the *TP53* status in tumours influenced the outcome of paclitaxel/cisplatin chemotherapy. In the present study. tumours from 45 patients randomized

to treatment with paclitaxel and cisplatin or cyclophosphamide and cisplatin were analysed for *TP53* mutations. The *TP53* status was related to the response of therapy and relapse-free survival.

MATERIAL AND METHODS

Patients and tumour specimens

Forty-five patients with advanced epithelial ovarian cancer (FIGO stage III for all patients except one patient with IIC) initially treated with cytoreductive surgery were randomized to two different chemotherapy regimens. either paclitaxel (175 mg m^{-2}) and cisplatin (75 mg m^{-2}) (23 patients) or cyclophosphamide (750 mg m^{-2}) and

cisplatin (75 mg m⁻²) (22 patients), given every 3 weeks for six cycles. None of the patients had received any preoperative treatment. Median age at diagnosis was 53 years, range 29–73 years. Response evaluation was performed according to WHO criteria: imaging (i.e. radiography, computerized tomography scan, magnetic resonance imaging and ultrasound) was performed if deemed appropriate at baseline and after every third cycle. Clinical evaluation was performed after the third and sixth cycle. Clinical response was documented with the same investigation technique on two occasions not less than 28 days apart. In cases of progressive disease and relapse, the patients were crossed over to either paclitaxel or doxorubicin regimens. Second-look laparotomy was performed in 17 patients in the paclitaxel/cisplatin group, and in seven patients who received cyclophosphamide/cisplatin.

Tumour specimens from 16 patients were obtained at initial surgical resection and immediately stored at – 70°C. Total mRNA was extracted and successfully analysed from 11 of the samples, whereas genomic DNA was isolated from the remaining five. Tumour material from the remaining 29 patients was only available as formalin-fixed paraffin-embedded tissue. Haematoxylin and eosin-stained sections were used to evaluate the approximate percentage of tumour tissue. Samples with less than 20% tumour tissue were not used in further analyses.

Mutation analyses of TP53 using cDNA

Frozen tissue samples stored at – 70°C were used for extraction of mRNA using the QuickPrep Micro mRNA Purification Kit (Pharmacia Biotech). The final RNA pellet was dissolved in 20 µl of elution buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) and stored at – 70°C. Reverse transcription (RT) was performed using the first-strand cDNA synthesis kit GeneAmp RNA polymerase chain reaction (PCR) (Perkin Elmer). The reaction contained 5 mM magnesium chloride, 10 mM Tris-HCl, pH 8.3, 50 mM potassium chloride, 1 mM of each dNTP, 20 U of RNase inhibitor, 2.5 µM random hexamers primer, 50 U of MuLV reverse transcriptase and 3 µl of RNA sample solution in a final volume of 20 µl. The cDNA reaction mixture was incubated at 42°C for 30 min, followed by 5 min at 95°C before storing at –20°C.

The TP53 cDNA was amplified using the following primers (sense) 5'-GTGACACGCTTCCCTGGATTG-3' and (antisense) 5'-AGTGGGGAACAAGAAGTGGAG-3', as described previously (Frebourg et al. 1992). The PCR contained 1.25 mM magnesium chloride, 60 mM Tris-HCl, pH 8.5, 15 mM ammonium sulphate, 0.2 ng µl⁻¹ bovine serum albumin (BSA), 0.01% 2-mercaptoethanol, 160 µM of each dNTP, 60 pmol of each primer, 3.8 U of Pfu DNA polymerase (Stratagene) and 2 µl of RT reaction in a final volume of 40 µl. The PCR reaction mixture was heated for 3 min at 95°C followed by 35 cycles of 94°C for 1 min.

65°C for 1 min 10 s and 78°C for 2 min, followed by 2 min at 78°C using a GeneAmp PCR System 2400 (Perkin Elmer). The PCR products were analysed for purity by running 7.5% PAGE, followed by staining with ethidium bromide. After purification using MicroSpin Columns (Pharmacia Biotech), TP53 cDNAs were submitted to direct sequencing using four internal primers, covering the open reading frame, and the Dye Terminator Cycle Sequencing Ready Reaction DNA Sequencing Kit (Perkin Elmer). The sequence analysis was performed in an automated 373 DNA Sequencer (Applied Biosystems). All sequence alterations reported in Table 1 were confirmed by independent PCR reactions.

Mutation analyses of TP53 using genomic DNA

Genomic DNA was isolated using standard procedures (phenol–chloroform extraction and ethanol precipitation) in cases for which frozen specimens were available. Extraction of genomic DNA from paraffin-embedded tissues was performed using ten 5-µm sections from each block. The samples were deparaffinized using two rinses with xylene, followed by one with absolute ethanol, in a microcentrifuge tube. After the final centrifugation, all ethanol was removed and the tissue pellet was briefly dried (55°C for 30 min). DNA was extracted using the Puregene DNA Isolation Kit (Gentra Systems). After dissolving the final DNA pellet in 100 µl (DNA Hydration Solution), the sample was stored at 4°C.

PCR primer sequences for analyses of exons 2, 5–9 and 11 of the TP53 gene using temporal temperature gradient electrophoresis (TTGE) were as previously described for constant denaturant gel electrophoresis (CDGE) analyses (Borresen et al. 1991; Smith-Sørensen et al. 1993), see Andersen and Borresen 1995 for modification of primers for exon 2 and 7 (Andersen and Borresen, 1995). PCR using DNA isolated from paraffin-embedded tissue as template contained 2.5 U of AmpliTaq Gold (Perkin Elmer) in a total volume of 50 µl. When template DNA was from frozen tissue extracted with phenol–chloroform, the PCR contained 2.5 U of AmpliTaq DNA Polymerase (Perkin Elmer). The PCR products were analysed for purity by running 7.5% PAGE followed by staining with ethidium bromide.

TTGE is a modification of the concept of denaturing gradient gel electrophoresis (DGGE) described by Fischer and Lerman (1983) and was performed using 10% polyacrylamide gels (acrylamide bis ratio 37.5:1) in 1.25 × TAE buffer (50 mM Tris-acetate, 1.25 mM EDTA, pH 8.0). The gels contained 31% denaturant (100% denaturant corresponds to 7 M urea and 40% (v/v) formamide) for analysing exons 6, 9 and 11 fragments, and 37% denaturant for analysing exons 2, 5, 7 and 8 fragments. Electrophoresis was perfomed in 1.25 × TAE running buffer using the DCode Universal Mutation Detection System (Bio-Rad). The temperature was programmed to increase from 63°C to 68°C

Table 2 Therapy response according to treatment group and TP53 tumour status in 38 clinically evaluable patients

Clinical response	Paclitaxel/cisplatin		Cyclophosphamide/cisplatin	
	TP53 wild type	TP53 mutation	TP53 wild type	TP53 mutation
Complete	4	10	3	3
Partial	1	2	0	5
Stationary disease	0	1	0	0
Progressive disease	1	1	1	6

during 3 h run at 130 V. After electrophoresis, the gels were stained for 4 min using 2 μl of SYBR Green I nucleic acid gel stain (FMC BioProducts) in 100 ml of 1 × TAE and photographed using a UV transilluminator. New PCR products were made using flanking primers from samples showing abnormal migrating bands after the TTGE analyses. These PCR products were purified and subjected to direct DNA sequencing as described above.

Statistical method

Relapse-free survival (RFS) was defined as the time interval between start of treatment and date of relapse or 1 January 1997. Survival probabilities for RFS were estimated using the Kaplan–Meier method, and the difference in survival curves for different subgroups was evaluated using the log-rank method. A *P*-value less than 0.05 was considered to have statistical significance.

RESULTS

Response to therapy

Out of the 45 patients included in the study, 38 had clinically evaluable disease (Table 2). Among patients who received paclitaxel/cisplatin therapy, clinical response was considered for 20 patients, whereas, in the group of patients treated with cyclophosphamide/cisplatin, 18 patients were evaluable. Of 20 patients with positive clinical response (complete or partial) second-look laparotomy confirmed the response evaluation by pathological review in 17 cases and by macroscopic review in three cases. The positive response rate to the paclitaxel/cisplatin treatment was 85% (17 out of 20) 61% (11 out of 18) for the cyclophosphamide/cisplatin regimen. The median relapse-free period for paclitaxel/cisplatin-treated patients was 17.5 months (range 6.1–23.6), while patients who received cyclophosphamide/cisplatin had a median relapse-free period of 9.9 months (range 5.1–23). RFS was compared for the two treatment groups, and a significant difference in favour of paclitaxel/cisplatin chemotherapy was found (*P* = 0.001).

TP53 gene mutations

In the 45 cases included in this study, the entire open reading frame was examined for mutations by direct sequencing of *TP53* cDNA in 11 specimens. For the other 34 specimens, codons of exons 2, 5–9 and 11 were screened for *TP53* mutations by TTGE after amplification of genomic DNA. DNA from samples showing bands with abnormal migration were further analysed by direct sequencing to describe the sequence alteration in detail. A total of 33 samples (73%) had detectable sequence alterations in the *TP53* gene after TTGE analyses (Table 1). Four of the observed sequence alterations after TTGE analyses were not detected by DNA sequencing (samples OV6, OV20, OV27 and OV47). In three of these cases, the amount of mutated DNA vs wild-type was less than 20%, which is below the detection level of direct DNA sequencing methods (Andersen and Børresen, 1995). When analysing the first half of exon 5 of sample OV27, an abnormal migrating band appeared with the same intensity as that of the wild-type band. However, the low quality of the DNA extracted from this paraffin-embedded tissue did not allow amplification of the exon 5 fragment used for direct sequencing. The larger size of the sequencing fragment (288 base pairs) compared with the size
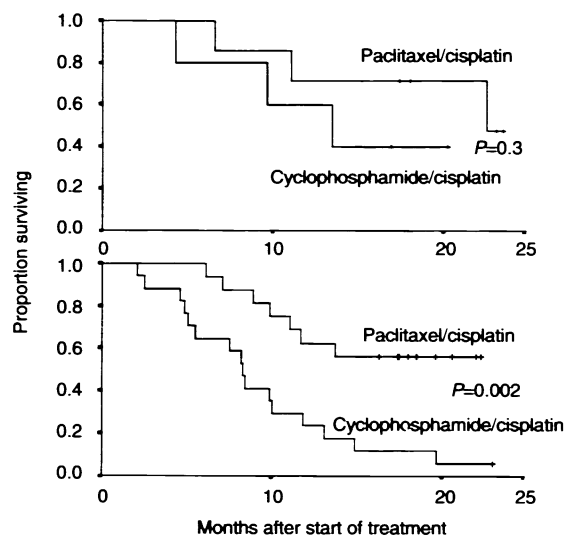

Figure 1 Relapse-free survival according to treatment group in 33 patients with *TP53* alterations in their tumours (lower part), and for 12 patients with tumours with wild-type *TP53* (upper part). The differences between patients with and without *TP53* tumour alteration within each treatment group had a *P*-value of 0.55 for paclitaxel/cisplatin and 0.15 for cyclophosphamide/cisplatin (not included in the figure)

of the fragment analysed in the TTGE screen (150 base pairs) explains the unsuccessful attempt to get this sequence alteration in detail.

Among the 29 described sequence alterations, missense mutations resulting in amino acid substitutions were detected in 21 cases (Table 1). Insertion and deletion mutations resulting in frame shift and premature stop codons were observed in four cases. Splice site mutations were observed in four cases, and these alterations are expected to lead to altered transcripts, resulting in abnormal proteins. A majority of the described sequence alterations occurred in highly conserved regions encoding motifs important for the p53 protein structure (Vogelstein and Kinzler, 1994). Two of these regions (codon 163–195 and codon 236–251) contribute to the binding of a zinc atom, which is important for stabilizing the protein structure, and 12 of the described sequence changes affect the zinc binding regions. The *TP53* mutation spectrum of the present study does not diverge from other reports of ovarian carcinomas (Hainaut et al, 1997).

TP53 status in relation to outcome of treatment

Out of the 17 patients with complete or partial response to paclitaxel/cisplatin therapy, six relapsed during the observation period. Four of these tumours harboured *TP53* alterations. Among the 11 patients without relapse during the observation time, *TP53* mutations in tumours were found in eight cases. In the cyclophosphamide/cisplatin treatment group, relapse occurred in 8 of the 11 cases with complete or partial response. Seven of these tumours had *TP53* alterations. In the tumours from the three relapse-free patients, one was found to contain a *TP53* mutation.

After cytotoxic therapy, nine patients had progressive disease (two in the paclitaxel/cisplatin group and seven in the cyclophosphamide/cisplatin group). Tumours with *TP53* alterations were found in seven of these cases, one in the paclitaxel/cisplatin group and six in the cyclophosphamide/cisplatin group (Table 2).

Within the group of patients treated with cyclophosphamide and cisplatin, relapse had taken place more often in patients with TP53 tumour alterations (16 out of 17, 94%) compared with patients with wild-type TP53 in their tumours (three out of five, 60%). For the group of patients treated with paclitaxel in combination with cisplatin, relapse had occurred in 7 out of 16 patients (44%) with TP53 mutations in their tumours compared with three out of seven patients (43%) having tumours with wild-type TP53.

When RFS was estimated for patients with TP53 tumour alterations, the paclitaxel/cisplatin group had a significantly better outcome than the cyclophosphamide/cisplatin group ($P = 0.002$), as shown in Figure 1. Specific regions in the TP53 gene, encoding domains important for binding the zinc atom, have previously been found to have influence on doxorubicin treatment of breast carcinomas (Aas et al. 1996). RFS for patients with TP53 alterations affecting the two zinc binding domains L2 and L3 (eight patients in the paclitaxel/cisplatin group and four patients in the cyclophosphamide/cisplatin group) showed a significantly better outcome for the paclitaxel/cisplatin-treated patient group than for the cyclophosphamide/cisplatin-treated group ($P = 0.001$).

DISCUSSION

Despite the introduction of combined use of cytoreductive surgery and chemotherapy in advanced epithelial ovarian cancer patients, long-term survival has not improved significantly over the last few years. Prognosis is currently based on clinical and histopathological parameters, and identification of new prognostic markers would be of great importance in identifying individuals who may benefit from new and more effective therapy. So far, the gene most frequently reported to be mutated in ovarian cancer is TP53 (Marks et al. 1991; Mazars et al. 1991; Okamoto et al. 1991). The high frequency of mutations may indicate an important role for this gene in ovarian carcinogenesis, and TP53 aberrations have been reported to be associated with advanced stages of the disease (Buttitta et al. 1997). It has also been observed that patients with p53 mutations have a significantly shorter RFS than patients with p53-negative tumours (Levesque et al. 1995; Buttitta et al. 1997). The treatment in these studies included platinum-based chemotherapy.

In the present study, TP53 alterations were observed in 73% of the tumours. All TP53 missense mutations detected affected codons previously reported to contain sequence alterations in other ovarian cancer studies. The most frequently mutated TP53 codons in ovarian carcinomas are 273, 175 and 248. We detected sequence alterations of codon 273 and 175 in four and three cases, respectively, but none in codon 248. When considering the patients treated with cyclophosphamide and cisplatin, a tendency toward a shorter RFS was observed for patients with TP53 mutations compared with the patients with wild-type TP53 ($P = 0.15$). However, with the rather small number of cases studied, this difference was not statistically significant. Whether or not the TP53 status influences the outcome of paclitaxel treatment has previously been investigated in different cell systems (Delia et al. 1996; Hawkins et al. 1996; Wahl et al. 1996; Wu and El-Diery, 1996). Primary embryo fibroblasts from p53-null mice have shown a ninefold increased cytotoxicity to paclitaxel compared with corresponding control cells (Wahl et al. 1996). Sensitivity to paclitaxel was less increased in fibroblasts isolated from heterozygous TP53 (+/−) mouse embryos compared with TP53 (−/−) mouse embryo cells. A sevenfold increased cytotoxicity to paclitaxel in normal human fibroblasts after targeted degradation of wild-type p53 through expression of HPV E6 has also been reported (Hawkins et al. 1996; Wahl et al. 1996). On the other hand, Wu and El-Deiry (1996) reported that ovarian carcinoma cells became more than 100 times as resistant to paclitaxel after depletion of functional p53 by E6 expression. Based on these apparently contradictory findings, it has been suggested that the relation between TP53 status and chemoresistance may be tissue specific and that the outcome of loss of wild-type p53 function in normal and cancer cells may be different. p53 independent response to paclitaxel in vitro has been reported for EBV-immortalized lymphoblastoid cells carrying heterozygous mutations of TP53 (Delia et al. 1996). The in vitro studies have clearly pointed out the need to investigate the TP53 status in tumours from ovarian cancer patients treated with paclitaxel. Results from a study of 13 patients (treated with carboplatin and paclitaxel) indicated that paclitaxel-based therapy has a value in overcoming resistance associated with p53 tumour inactivation in patients with ovarian cancer (Lavarino et al. 1997). In the present study, no association was seen between TP53 tumour status and prognosis within the patient group treated with paclitaxel and cisplatin, while within the group treated with cyclophosphamide and cisplatin there seems to be a better prognosis and less relapse for patients with tumours with wild-type TP53.

In an attempt to answer the central question of whether ovarian cancer patients with TP53 mutations in their tumours benefit from paclitaxel-inclusive chemotherapy, we compared the two different treatment groups in which TP53 alterations were observed. A significantly better outcome for the group treated with paclitaxel and cisplatin was found. The present study is, however, too small to evaluate whether or not specific TP53 mutations influence treatment response, as recently reported for doxorubicin treatment of breast carcinomas (Aas et al. 1996).

Several studies have demonstrated that the product of the TP53 tumour-suppressor gene is responsible for the G_1 checkpoint (Kastan et al. 1991; Kuerbitz et al. 1992). In response to genotoxic stress, the level of p53 protein increases and a transient arrest of cell cycle progression in the G_1 phase gives the cells time to repair critical DNA damage (Kastan et al. 1991; Kuerbitz et al. 1992), if apoptosis is not induced (Clarke et al. 1993; Lowe et al. 1993b). The main mechanism of cytotoxicity of the anti-cancer drug cisplatin is thought to be through induction of DNA damage, primarily in the form of intrastrand cross-links at the N-7 positions of adjacent guanine bases (Sherman and Lippard, 1987). The active from of cyclophosphamide is also known to directly bind to DNA. Several reports indicate that the TP53 status has influence on the effect of these DNA-damaging drugs, and many cisplatin-sensitive tumour types have been found to express wild-type p53. The results from the patients treated with cyclophosphamide and cisplatin in the present study are in agreement with these observations.

Recently, it has been suggested that p53 also plays a role in regulating the G_2/M transition (Agarwal et al. 1995; Guillouf et al. 1995; Stewart et al. 1995). However, it has also been documented that the G_2/M transition is regulated independently of p53, as cells that are p53 null or with mutated p53 show DNA damage-induced G_2 arrest (Kastan et al. 1991; Kuerbitz et al. 1992). The anti-cancer drug paclitaxel promotes assembly of tubulin dimers to form excessively stable microtubules by preventing depolymerization (Schiff et al. 1979). Cells incubated with paclitaxel accumulate in the G_2/M phase (Horwitz, 1992). Induction of apoptosis after paclitaxel treatment both in vitro (Bhalla et al. 1993; Gangemi et al. 1995) and in vivo (Milas et al. 1995; Milross et al. 1995) has

been observed. Our results do not reveal any differences in the outcome of treatment with paclitaxel in combination with cisplatin between patients with wild-type or mutant *TP53* in their tumours.

Paclitaxel has been reported to induce both wild-type p53 and Waf-1 protein (Blagosklonny et al, 1995). Induction of Waf-1 has also been demonstrated in cells lacking p53 expression and therefore is assumed to be independent of functional p53. Interestingly, Blagosklonny et al (1995) demonstrated that depletion of c-raf-1, an upstream regulator of MAP kinase, significantly abrogated the ability of paclitaxel to induce both wild-type p53 as well as Waf-1. Based on these recent results, we studied Waf-1 protein expression in tumours from patients treated with the paclitaxel combination (data not shown). As for the *TP53* status, we could not observe any association between prognosis and Waf-1 status, but Waf-1 expression was more common in wild-type *TP53* tumours than in tumours with *TP53* gene alterations.

The results of the study by Haldar et al (1996) have indicated that paclitaxel may execute some of its anti-cancer action through phosphorylation of Bcl-2. Bcl-2 is an intracellular integral membrane protein that resides in the outer mitochondrial membrane, nuclear envelope and parts of the endoplasmic reticulum. It has been observed that Bcl-2 by dimerizing with the bax protein protects cells from the apoptotic effect of bax homodimers (Sato et al, 1994; Yin et al, 1994; Yang et al, 1995). Experiments have further indicated that phosphorylated Bcl-2 is unable to form heterodimers with bax, leading to more bax homodimers and cell death (Haldar et al, 1996). It appears that Bcl-2 phosphorylation is not always a necessary component of the apoptotic response to paclitaxel in all cell types. Paclitaxel-induced apoptosis in some tumour cell lines has been suggested to act through Bcl-2-independent pathways (Bhalla et al, 1994). Considering these reports, we also included Bcl-2 expression analysis of the tumours from patients treated with paclitaxel in combination with cisplatin (data not shown). We could not observe any association between Bcl-2 status and prognosis, but expression was less common in wild-type *TP53* tumours than in tumours harbouring *TP53* mutations.

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