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Original Paper

Expression of the *p53* Tumour Suppressor Gene as a Prognostic Marker in Platinum-treated Patients with Ovarian Cancer

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Drug resistance is one of the most important clinical problems in the treatment of ovarian cancer. This study was designed to determine whether expression of p53 could be used as a marker for predicting the response to chemotherapy of ovarian cancer. Tissue blocks were obtained from 187 patients with diagnosed untreated ovarian cancer. Paraffin sections from the primaries were immunohistochemically analysed for p53 expression. All patients underwent platinum-based chemotherapy after surgery. We analysed whether the number of chemotherapy cycles was related to survival in women with p53 positive and p53 negative ovarian cancer. 27/187 cases were p53 positive. Expression of p53 was associated with other factors of unfavourable prognosis. Patients with p53 positive tumours had a significantly worse prognosis compared with patients with p53 negative tumours ($P = 0.037$). There was a statistically significant dose–response effect of platinum-based chemotherapy in patients with p53 negative tumours, which could not be seen in patients with p53 positive tumours ($P = 0.01$ versus $P = 0.553$). This could also be observed in patients with residual tumour after surgery ($P = 0.0001$ versus $P = 0.8866$). Expression of p53 may be an additional useful marker in predicting response to chemotherapy. Thus, it is possible to identify a subgroup of patients who may benefit from alternative therapy regimens. © **1998 Elsevier Science Ltd. All rights reserved**

Key words: ovarian cancer, tumour suppressor gene, p53, chemotherapy, prognosis
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INTRODUCTION

One of the most important clinical problems in the treatment of ovarian cancer is the development of resistance to platinum-based chemotherapy. To date, the prognostic value of various molecular markers which adequately account for treatment outcome of ovarian cancer patients is still unclear. Recently, we showed that there is no significant dose–response effect of platinum-based chemotherapy in ovarian cancer patients with c-*erb-B2* positive tumours [1]. However, the mechanism of drug resistance in ovarian cancer cells is less well understood and multiple resistance factors are described [2]. Since it is supposed that cisplatin as a DNA damaging agent may induce apoptosis in treated cells [3], various studies have focused on the role of apoptosis-associated genes in predicting the prognosis and response to chemotherapy of ovarian cancer. The wild-type nuclear phosphoprotein p53 acts as an important transcriptional activator of cell cycle regulating proteins leading to either growth arrest or apoptosis of cells with damaged DNA [4–6]. Perego and coworkers [7] showed that mutant p53 can be detected in cisplatin resistant ovarian cancer cell lines. Moreover, those cells showed reduced induction of apoptosis after drug treatment [7]. Thus, loss of p53 function may contribute to drug resistance of ovarian cancer. Nevertheless, there are diverging results concerning p53-associated drug resistance [8–17].

Owing to its prolonged half life, it is thought that mutant p53 accumulates in transformed cells and can thereby be detected by immunohistochemical techniques [4, 18]. Comparing different numbers of chemotherapy cycles in 187 ovarian cancer patients, we studied whether the number of

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chemotherapy cycles was related to survival in women with immunohistochemically p53 positive and p53 negative ovarian cancer.

PATIENTS AND METHODS

Patients

The investigations were based on tumour tissue of the primaries from 187 patients with ovarian cancer, treated at the Department of Obstetrics and Gynaecology of the University of Göttingen between 1982 and 1992, who had been given neither cytostatic nor radiological pretreatment. All patients underwent platinum-based chemotherapy after surgery. Until 1987, the standardised chemotherapy protocol included cisplatin/cyclophosphamide (80 mg/1000 mg/m^2 of body surface area every 4 weeks). In 1988, the standard chemotherapy protocol was changed to carboplatin/cyclophosphamide (350 mg/600 mg/m^2 of body surface area every 4 weeks) in order to reduce side-effects and to improve the patient quality of life. First-line chemotherapy was planned for six cycles. For different reasons (e.g. non-compliance, toxicity, tumour progression, multimorbidity, old patients, platinum resistant cases), platinum-containing polychemotherapy could not be administered for six cycles in all patients. Thus, it was possible to compare patients with different numbers of chemotherapy cycles.

The archived formalin-fixed, paraffin-embedded tissue of the primaries was reviewed by the same gynaecological pathologist (A.S.) for the determination of histological cell type and grade according to criteria of the World Health Organization (WHO) classification of ovarian tumours [19]. Patients' conditions were staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO).

Table 1. *Clinicopathological characteristics of patients* (n = 187) *with different cycles of platinum and cyclophosphamide (PC)-based chemotherapy (< 4×PC; ≥ 4×PC)*

	< 4×PC, *n* = 84 *n* (%)	≥ 4×PC, *n* = 103 *n* (%)
Stage (FIGO)		
I	13 (15)	5 (5)
II	8 (10)	14 (14)
III	40 (48)	67 (65)
IV	23 (27)	17 (17)
Grade		
G1	10 (12)	7 (7)
G2	21 (25)	27 (26)
G3	33 (39)	44 (43)
G4	20 (24)	25 (24)
Histology		
Serous	33 (39)	49 (48)
Mucinous	13 (15)	14 (14)
Endometrioid	15 (18)	16 (16)
Clear cell	2 (2)	8 (8)
Undifferentiated	21 (25)	16 (16)
Residual tumour after primary surgery (*n* = 119)		
No evidence of disease	2 (4)	3 (4)
Residual tumour	50 (96)	64 (96)
Age (years)		
< 60	21 (25)	52 (50)
≥ 60	63 (75)	51 (50)
p53		
Positive	14 (17)	13 (13)
Negative	70 (83)	90 (87)

FIGO, International Federation of Gynecology and Obstetrics.

Immunohistochemical analysis

For immunohistochemical studies, 1–2 μm sections from formalin-fixed, paraffin-embedded tumour tissue were air dried overnight at 37°C. Expression of p53 was determined using the monoclonal antibody DO-7, diluted 1:50, which recognizes mutant and wild-type p53 (Dako, Hamburg), and the APAAP (alkaline phosphatase–anti-alkaline phosphatase) technique. Prior to immunohistochemistry, slides were

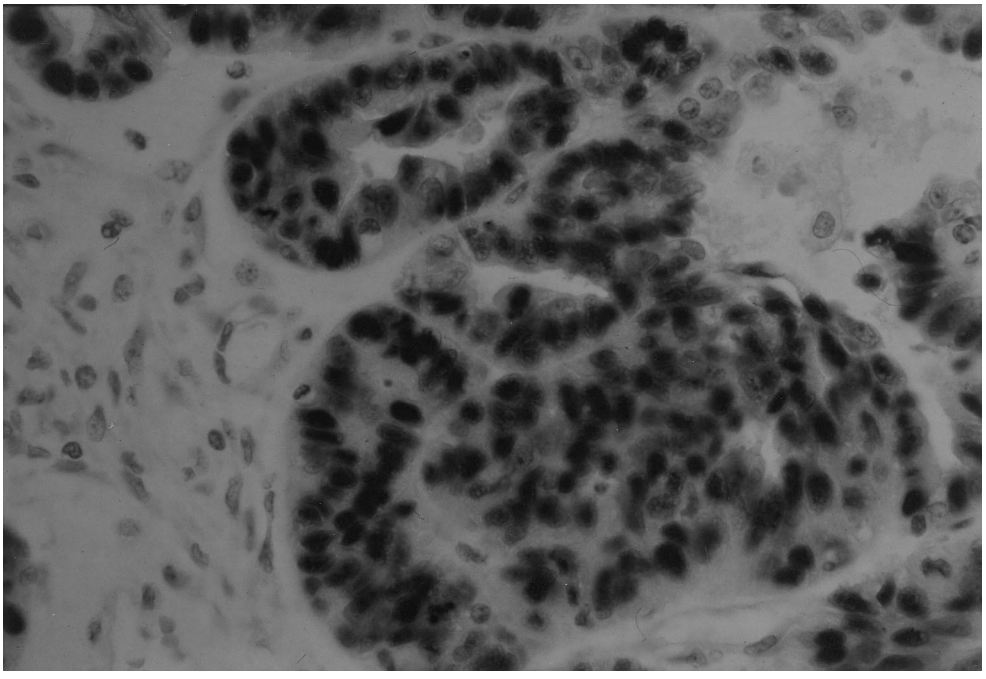

Figure 1. Immunohistochemical nuclear staining of a p53 positive endometrioid ovarian carcinoma.

heated in a microwave oven (720 W, 3×5 min). All slides were evaluated for p53 expression by two investigators without knowledge of patient information. Tissue was considered to be positive for p53 expression if immunoreactivity was found in more than 20% of the tumour cells.

Proliferative activity was measured according to Marx and associates [20] using the monoclonal antibody MIB-1 (Dianova, Hamburg). Immunohistochemistry for c-erb-B1 and c-erb-B2 expression was performed according to the protocol of Meden and colleagues [21]. According to the same protocol, c-erb-B3 expression was detected using the monoclonal antibody RtJ1, kindly provided by W.J. Gullick (London, U.K.).

Statistical analyses

The clinical data were taken from patient records. Follow-up data were obtained from the clinical registries and in co-operation with the doctors responsible for tumour follow-up. Regarding chemotherapy, patients with ≥ 4 cycles platinum/cyclophosphamide were compared with patients with < 4 cycles platinum/cyclophosphamide. Survival time was calculated from the date of diagnosis to the date of death.

Table 2. Clinicopathological characteristics of patients with p53 positive and p53 negative tumours (n = 187)

	p53 positive, $n = 27$ n (%)	p53 negative, $n = 160$ n(%)	P value (chi-square)
Stage (FIGO)			
I	1 (6)	17 (94)	
II	2 (9)	20 (91)	
III	18 (17)	89 (83)	
IV	6 (15)	34 (85)	0.159
Grade			
G1	1 (6)	16 (94)	
G2	1 (2)	47 (98)	
G3	21 (27)	56 (73)	
G4	4 (9)	41 (91)	0.001
Histology			
Serous	12 (15)	70 (85)	
Mucinous	1 (4)	26 (96)	0.086
Endometrioid	4 (13)	27 (87)	
Clear cell	4 (40)	6 (60)	0.018
Undifferentiated	6 (16)	31 (84)	
Residual tumour after primary surgery ($n = 119$)			
No evidence of disease	1 (20)	4 (80)	
Residual tumour	17 (15)	97 (85)	

FIGO, International Federation of Gynecology and Obstetrics.

Table 3. Expression of the c-erb-B *oncogene family and proliferative activity (measured by MIB-1 positive tumour cells) of p53 positive and p53 negative tumours*

	p53 positive n (%)	p53 negative n(%)	P value (chi-square)
c-erb-B1 positive	7 (27)	19 (73)	
c-erb-B1 negative	20 (12)	141 (82)	0.05
c-erb-B2 positive	9 (22)	32 (78)	
c-erb-B2 negative	18 (12)	128 (82)	0.121
c-erb-B3 positive	6 (11)	47 (89)	
c-erb-B3 negative	10 (12)	86 (88)	0.956
MIB-1 index < 17%	7 (8)	82 (92)	
MIB-1 index ≥ 17%	20 (20)	78 (80)	0.015

The medians and life tables were computed using the product–limit estimate of Kaplan and Meier [22] and the curves were examined by the log-rank test.

RESULTS

Patients and clinicopathological characteristics

Table 1 provides the clinical data for the 187 patients in both treatment groups. Patients in both treatment groups had similar clinicopathological features.

p53 Expression and clinicopathological characteristics

p53 Positive tumour cells have red staining of the nucleus (Figure 1). Overall, 14% (27/187) of the cancers were positive for p53. Within the various histological subtypes, the lowest rate of p53 expression was found in mucinous carcinomas, whereas clear cell carcinomas showed the highest rate of p53 expression (Table 2). There was no significant association between tumour stage and p53 expression. Nevertheless, the p53 expression rate was clearly decreased in stage I (6%) and stage II (9%) tumours as compared with stage III (17%) and stage IV (15%) tumours (Table 2). A significant association ($P = 0.001$) was found between tumour grade and p53 expression (Table 2). As seen in Table 3, an inverse relationship between p53 expression and either c-erb-B1 ($P = 0.05$) or c-erb-B2 expression (not significant) could be found, whereas c-erb-B3 expression seemed not to be connected with p53 expression. Concerning the proliferative activity, the 187 cases were segregated into two groups according to Marx and associates [20]: (1) MIB-1 index $< 17\%$ ($n = 89$) and (2) $\geq 17\%$ ($n = 98$). p53 Expression rate was significantly ($P = 0.015$) increased in tumours with higher proliferative activity (Table 3).

Survival

The median follow-up for all 187 patients was 22 months with a range of 1–162 months. Univariate survival analysis

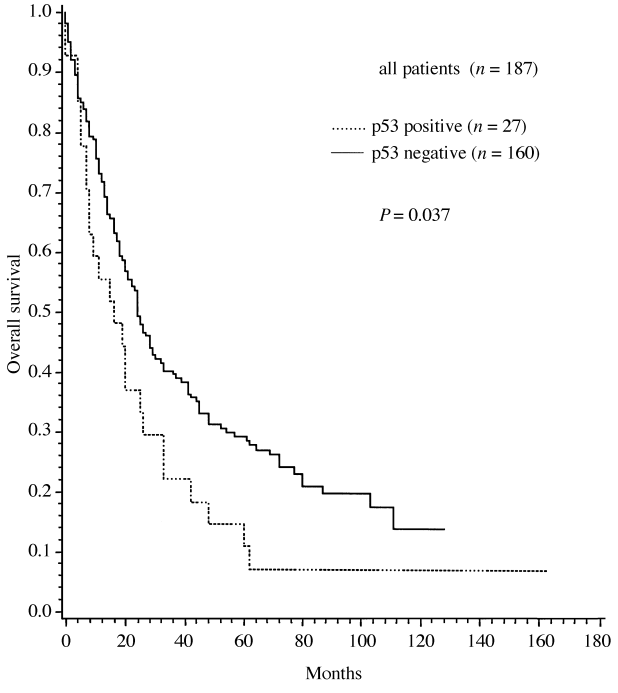

Figure 2. Overall survival for patients with p53 positive tumours versus those with p53 negative tumours ($n = 187$).

showed a shorter survival for patients with p53 positive tumours as compared with those patients with p53 negative tumours (median survival 16 months versus 24 months, $P = 0.037$). The 5-year survival rate was 11% for patients with p53 positive tumours and 30% for those with p53 negative tumours (Figure 2). However, multiple analyses including age, stage, degree of malignancy, histological subtype, residual tumour after primary surgery, proliferative activity and expression of c-erb-B2 could not confirm the results of the univariate analyses (data not shown).

Postsurgical chemotherapy

A significant dose–response effect (median survival 14 months versus 32 months, $P = 0.01$) was seen in the group of patients with p53 negative tumours ($n = 160$) with an advantage for patients with ≥ 4 cycles of chemotherapy (Figure 3a). In contrast, in patients with p53 positive tumours ($n = 27$), there was no statistically significant survival difference ($P = 0.553$) comparing patients with ≥ 4 cycles of chemotherapy with those with < 4 cycles (Figure 3b). Nevertheless, the median survival time for patients with < 4 cycles of

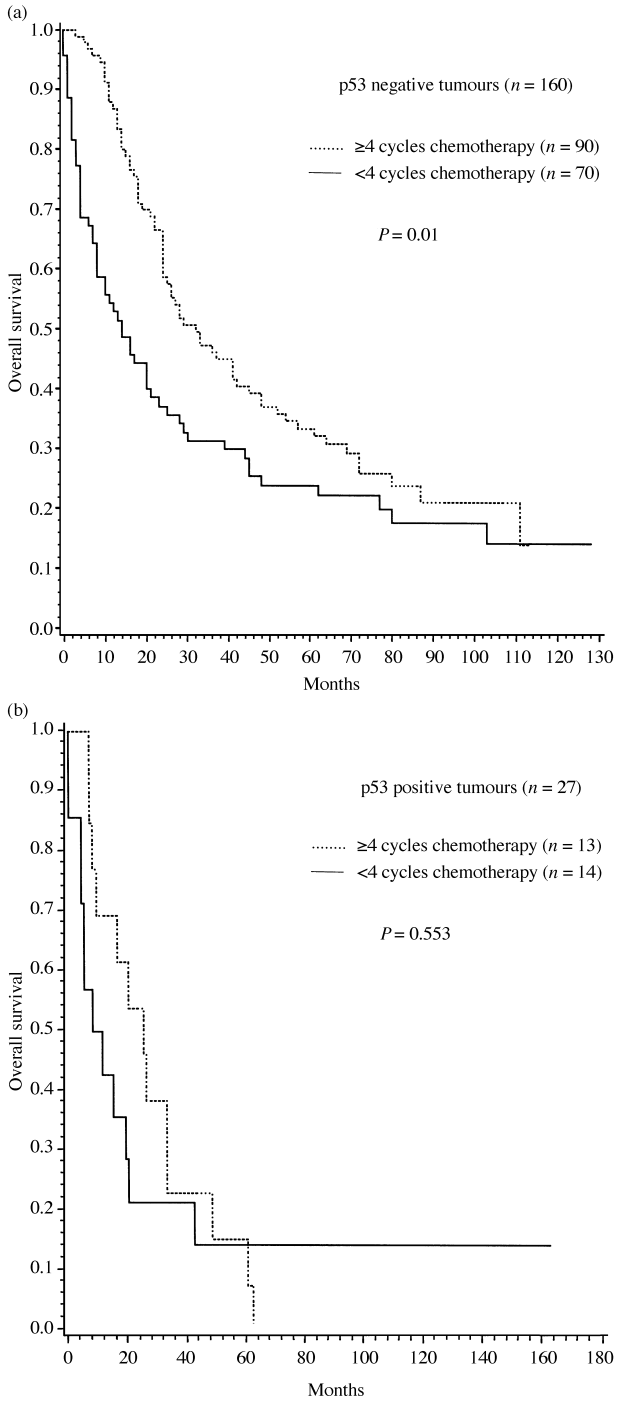

Figure 3. Overall survival in patients with (a) p53 negative tumours ($n = 160$) or (b) p53 positive tumours ($n = 27$) according to treatment group.

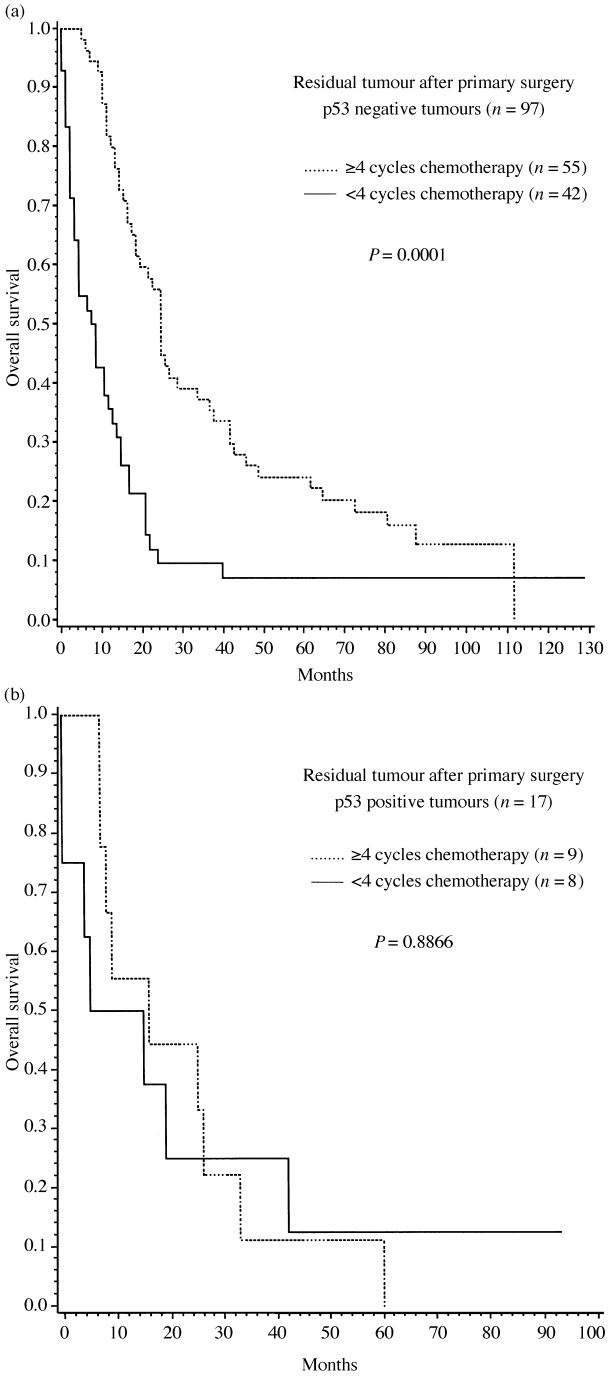

Figure 4. Overall survival in patients with (a) p53 negative tumours and residual tumour after primary surgery ($n = 97$) or (b) p53 positive tumours and residual tumour after primary surgery ($n = 17$) according to treatment group.

Table 4. Overall survival and median survival times according to treatment group and p53 and c-erb-B2 expression

	Median survival (months)		
	$<4\times$PC *n* (%)	$\geq 4\times$PC *n* (%)	Overall survival (*P* value)
All patients			
p53 and/or c-erb-B2 positive	15.5 ($n=28$)	19.5 ($n=36$)	0.7705
p53 and c-erb-B2 negative	12 ($n=57$)	36 ($n=71$)	0.0057
Patients with residual tumour after primary surgery			
p53 and/or c-erb-B2 positive	15 ($n=17$)	16 ($n=29$)	0.305
p53 and c-erb-B2 negative	4 ($n=33$)	24 ($n=39$)	0.0001

PC, platinum and cyclophosphamide-based chemotherapy.

chemotherapy was clearly decreased (10 months versus 25 months). These results could also be demonstrated for patients with residual tumour after primary surgery (Figure 4).

Recently, we demonstrated that there is no evidence of a dose–response effect in patients with c-erb-B2 positive tumours [1]. Considering patients with p53 and/or c-erb-B2 positive tumours, there was neither a statistically significant dose–response effect nor a difference in median survival times, either for the total group or for patients with residual tumour after primary surgery (Table 4). In contrast, a significant dose–response effect was observed in the subgroup of patients with tumours negative for both proteins (Table 4).

DISCUSSION

Identification of variables correlating with tumour aggressiveness and response to therapy would contribute to the selection of treatment for individual patients. During the past few years, the prognostic significance of p53 expression in ovarian cancer has been reported by many authors. Consistent with the present data, most of the other investigators demonstrated a statistically significant correlation between p53 expression and other factors of unfavourable prognosis, such as higher degree of malignancy [11, 14, 22–25] or higher proliferative activity [23, 26] and reduced survival [11, 22–25]. This suggests a possible association of p53 expression mainly with poorly differentiated tumours. There was no statistically significant association of p53 expression with tumour stage. Nevertheless, concordant with Levesque and colleagues [23] and Henriksen and associates [24] in stage I and stage II disease p53 expression rates were clearly decreased compared with stage III and stage IV tumours. However, multiple analyses, including age, stage, degree of malignancy, histological subtype, residual tumour after primary surgery, proliferative activity and expression of c-erb-B2 could not confirm the results of the univariate analyses. This may be due to the high statistically significant association between p53 expression and tumour grade, where tumour grade remains dominant in a multiple analysis. As the clinical relevance of the above findings with regard to resistance to chemotherapy is unclear and recent results are conflicting [11–14], we undertook the investigations presented here.

p53 Expression was significantly associated with treatment outcome in this study. The longer period of chemotherapy resulted in a significantly longer survival in patients whose tumours did not express p53 as compared with those with p53 expression. These data are in general consistent with results from Buttitta and colleagues [14], who demonstrated a significant association either of p53 expression or *p53* mutations, with less sensitivity to cisplatin-based chemotherapy. Confirming results were also reported by Righetti and associates [13]. Based upon 33 patients with advanced disease they showed that resistance to high-dose cisplatin therapy is significantly associated with immunohistochemically detectable p53 expression but, in contrast to Buttitta and associates [14], not with the overall frequency of mutations. Furthermore, they found a portion of immunohistochemically p53 positive non-responsive cases without detectable mutations. This is consistent with results from Brown and colleagues [27] who found an increased accumulation of wild-type p53 in cisplatin resistant ovarian cell lines. Resistance may therefore also be a result of increased repair of damaged DNA induced by wild-type p53 [28]. In contrast, van-der-Zee and associates [11] and Renninson and colleagues [12] failed to reveal any statistically significant association between p53 expression and altered response rate. Of particular interest are the results for patients with residual tumour after primary surgery, demonstrating for this subgroup the most obvious statistically significant evidence of a dose–response effect in patients with p53 negative tumours, which could not be seen in patients with p53 positive tumours. As one of the most important factors in predicting the prognosis of ovarian cancer is residual tumour after primary surgery, these results suggest that the observed p53-dependent altered response rate seems to be independent of surgical aspects.

It could be assumed that patients who receive <4 cycles of chemotherapy tend to have resistant primary tumours and a worse prognosis. However, patients with p53 positive tumours had a poor clinical outcome as compared with patients with p53 negative tumours with either <4 or ≥ 4 cycles of chemotherapy. Furthermore, patients in both treatment groups had nearly similar clinicopathological features. This suggests that p53 expression, along with other possible mechanisms, is involved in the development of chemoresistance in patients with primary ovarian cancer.

In a previous study, we showed that there is statistically significant evidence of a dose–response effect in patients with c-erb-B2 negative tumours, which could not be seen in patients with c-erb-B2 positive tumours [1]. Considering c-erb-B2 and p53 immunoreactivity (p53 and c-erb-B2 negative, p53 and/or c-erb-B2 positive), these results were clearly confirmed.

With 14%, the number of p53 positive cases in the present study is much smaller than in other investigations on ovarian cancer [11–14, 23–26]. The differences in the frequency and prognostic value of p53 expression may be partly due to methodical aspects (e.g. thinner sections and archived paraffin-embedded material used in this study), to different compositions of the investigated cases or to the type of *p53* mutations. For example, Righetti and colleagues [13] showed

that only missense mutations are significantly associated with protein accumulation and a portion of cases showed immunohistochemically detectable wild-type p53. Moreover, the staining results may vary using different antibodies and staining procedures [29].

In conclusion, patients whose tumours express p53 may benefit from alternative therapy. Trials are underway to determine whether, for example, high-dose chemotherapy with autologous bone marrow support improves the outcome for women with ovarian cancer. Such treatment involves substantial toxicity as well as a high cost. Therefore, analyses that help to identify those patients most likely to benefit from intensive treatment will be important, especially with regard to the quality of life. Beside c-erb-B2 [1], p53 expression in patients with primary ovarian cancer may be an additional marker of relative resistance to standard dose chemotherapy. However, more studies are needed to confirm our observations and to determine whether p53 and c-erb-B2 expression or other biological markers can identify tumours that are resistant or responsive to specific chemotherapeutic agents or to escalated doses of such agents.

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