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Alterations in the p53 pathway and prognosis in advanced ovarian cancer: A multi-factorial analysis of the EORTC Gynaecological Cancer group (study 55865)

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ABSTRACT

Purpose: The study was designed to determine independent prognostic variables in suboptimally debulked advanced ovarian cancer patients entered in the randomised phase III study EORTC 55865.

Experimental design: Retrospectively collected paraffin blocks from 169 patients with stages IIb–IV epithelial ovarian cancer, taken at primary debulking surgery, were analysed. All patients were treated with cyclophosphamide and cisplatin (CP), and followed up for a median of 10 years. Expression of p53, bcl-2, P21, Ki-67 and HER-2 status was assessed by immunohistochemistry (IHC).

Results: Expression of p21, a downstream effector of the p53 gene, was found to be a favourable prognostic factor for survival (HR 0.58, CI 0.36–0.94, $p = 0.025$) in addition to FIGO stage (HR 1.54, CI 1.08–2.21, $p = < 0.02$). For progression free survival (PFS), both p21 (HR 0.52) and Ki-67 (HR 0.6) were significant factors.

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MIB-1
Morphometry
DNA ploidy
Immunohistochemistry

Conclusion: P21 overexpression is a positive prognostic factor for survival and PFS in advanced ovarian carcinoma with residual lesions of more than 1 cm.

1. Introduction

The 5-year survival of all ovarian carcinoma patients remains low at 35% despite advances in surgery and chemotherapy. This study was designed to determine independent prognostic variables in suboptimally debulked advanced ovarian cancer patients entered in the randomised phase III study (EORTC 55865) of interval debulking surgery following induction chemotherapy.[1] Previous studies have identified performance status, degree of differentiation, residual tumour after primary debulking surgery, FIGO stage and response to chemotherapy as independent clinical prognostic factors in advanced disease.[2–4] DNA ploidy has been shown to be of value in predicting prognosis in early disease, while morphometric assessment of mean nuclear area has been shown to be of prognostic value in multivariate analysis in stages III and IV disease.[5,6] Many other molecular parameters have been studied, often in small series, but not as yet demonstrated to be independently related to the outcome.[7]

The epidermal growth factor receptor is a family of four related transmembrane receptors, of which HER2/neu (erbB2) has been the most extensively studied. Prognosis has been associated with expression of both EGFR (erbB1) and HER2 and advanced ovarian cancer.[8–10] The MIB-1 antibody recognises the proliferation dependent antigen Ki-67, for which there is preliminary evidence as a prognostic factor in ovarian cancer.[11,12]

The tumour suppressor gene p53 has been extensively studied in many epithelial tumours as a prognostic marker, and it is of particular interest in ovarian cancer in view of the association of platinum sensitivity and p53 pathway alterations. There are over 200 published studies of p53 expression assessed by immunohistochemistry (IHC) in ovarian cancer, and two meta-analyses have shown an adverse prognosis for p53 overexpression.[13,14] The majority of studies, however, were of inadequate quality and size.[15] Studies in non-small cell lung cancer, head and neck cancer as well as breast cancer showed a similarly small but significant adverse effect for p53 alterations as demonstrated by IHC.[16,17]

Sequence alteration or overexpression may not be fully informative for the biological function of TP53.[18,20–22] Additional information on apoptotic capacity may be provided by studying the downstream genes of TP53, including the cell cycle inhibitor p21 and the apoptosis antagonist Bcl-2.[23,24] Three studies have investigated the association between p53 and apoptosis.[25–27] Studies on the prediction of response to platinum and taxanes have not been conclusive,[19,28–30] although they suggested that wild type p53 was important for platinum induced apoptosis, and is a less critical requirement for the cytotoxicity of taxanes.

In this study, we investigated the role of the prognostic markers p53, p21, bcl-2, c-erbB2, Ki-67, morphometry and DNA ploidy to identify independent prognostic variables for survival and progression free survival in advanced ovarian cancer patients with residual lesions of more than 1 cm, entered in the EORTC 55865 phase III study of interval debulking surgery following induction chemotherapy.[1,3]

2. Patients and methods

2.1. Tissue and patient characteristics

Tissue samples were available from 169 of the 425 patients who entered in the randomised EORTC GCG study 55865, which investigated the value of interval debulking surgery in patients with a suboptimal primary debulking surgery. This compliance rate of 39.8% increases to 59.7% overall when those countries not taking part in the translational study are excluded. Approval for the translational study was given by the Wirral Ethical Committee, United Kingdom. This EORTC 55865 study was opened in March 1987 and closed in May 1993, and the median follow up is in excess of 9 years. Eligible patients had to have epithelial ovarian carcinoma with an International Federation of Gynaecology and Obstetrics (FIGO) stage of IIb–IV and residual lesions > 1 cm in diameter after primary debulking surgery, WHO performance status of 0–2, age less than 75 years, and adequate bone marrow and renal function. Primary surgery was required less than 6 weeks before the start of the treatment.

All patients received 3 cycles of CP, cyclophosphamide (750 mg/m^2) and cisplatinum (75 mg/m^2) every 3 weeks. After the third cycle, patients with a clinical response or stable disease were randomised between interval debulking surgery or no surgery, and all patients continued treatment with at least three additional CP cycles. In the clinical study, in which patients with progression or a contraindication to surgery went off the study, all patients with available tissue blocks, both the randomised and not randomised patients, were included in the prognostic factor analysis. WHO performance status, FIGO stage, histological subtype, grade, ascites, number of residual lesions and largest residual tumour size following primary surgery were included in the multivariate model.[3] Patient and tumour characteristics were well balanced between the 169 patients of the study group and the 425 patients of the EORTC GCG 55865 study, except for both non-serous cell type and number of lesions >10, which were overrepresented in the study group (Table 1).

2.2. Immunohistochemistry

The methodology for staining with microwave pretreatment and the scoring-system for immunohistochemical staining have been previously reported.[31] In short, first a representative tumour area was selected in the slides and a qualitative

Table 1 – Characteristics of included patients in prognostic factor analysis and total entered in clinical trial (EORTC 55865)

Variable	Included in translational study				Total (clinical trial)
	Surgery (N = 63), N (%)	No Surgery (N = 62), N (%)	Not rand (N = 44), N (%)	Included total (N = 169), N (%)	(N = 425), N (%)
Age					
Median	58.3	61.4	57.5	59.6	59.2
Range	31.6–75.2	45.2–83.1	33.3–75.4	31.6–83.1	21.6–83.1
N obs	63	62	44	169	425
Performance status					
0	18 (28.6)	17 (27.4)	6 (13.6)	41 (24.3)	143 (33.6)
1	29 (46.0)	32 (51.6)	26 (59.1)	87 (51.5)	194 (45.6)
2	16 (25.4)	12 (19.4)	12 (27.3)	40 (23.7)	81 (19.1)
3	0 (0.0)	1 (1.6)	0 (0.0)	1 (0.6)	2 (0.5)
Missing				0 (0.0)	5 (1.2)
Figo stage					
IIB, IIc, III	48 (76.2)	47 (75.8)	27 (61.4)	122 (72.2)	313 (73.6)
IV	15 (23.8)	15 (24.2)	17 (38.6)	47 (27.8)	107 (25.2)
Missing				0 (0.0)	5 (1.2)
*Cell type**					
Serous	27 (42.9)	28 (45.2)	15 (34.1)	70 (41.4)	233 (54.8)
Non-serous	36 (57.1)	34 (54.8)	29 (65.9)	99 (58.6)	187 (44.0)
Missing				0 (0.0)	5 (1.2)
Tumour grade					
1–2	25 (39.7)	28 (45.2)	19 (43.2)	72 (42.6)	161 (37.9)
3	36 (57.1)	31 (50.0)	21 (47.7)	88 (52.1)	240 (56.5)
Missing	2 (3.2)	3 (4.8)	4 (9.1)	9 (5.3)	24 (5.6)
Tumour size (cm)[a]					
0–5	18 (28.6)	11 (17.7)	6 (13.6)	35 (20.7)	100 (23.5)
5–10	18 (28.6)	19 (30.6)	11 (25.0)	48 (28.4)	106 (24.9)
>10	25 (39.7)	30 (48.4)	11 (25.0)	66 (39.1)	133 (31.3)
Missing	2 (3.2)	2 (3.2)	16 (36.4)	20 (11.8)	86 (20.2)
Number of lesion					
1–2	15 (23.8)	14 (2.6)	6 (13.6)	35 (20.7)	126 (29.6)
3–9	10 (15.9)	5 (8.1)	6 (13.6)	21 (12.4)	78 (18.4)
>10	37 (58.7)	42 (67.7)	32 (67.7)	111 (65.7)	208 (48.9)
Missing	1 (1.6)	1 (1.6)	0 (0.0)	2 (1.2)	13 (3.1)
Ascites					
No	11 (17.5)	12 (19.4)	5 (11.4)	28 (16.6)	89 (20.9)
Yes	52 (82.5)	50 (80.6)	39 (88.6)	141 (83.4)	331 (77.9)
Missing				0 (0.0)	5 (1.2)
Overall survival (years)	2.44 (1.96–3.28)	1.57 (1.25–2.07)	0.57 (0.44–1.08)	1.53 (1.26–1.92)	1.52 (1.28–1.67)
Median (95% CI)	53	60	43	156	371
N events					

a Size assessment mainly described as large bulk.

* Both cell type ($p = 0.0001$) and number of lesions ($p = 0.0001$) were found to be unbalanced between the two groups.

assessment of the immunohistochemical staining was performed to estimate visually whether any positivity of staining was present in the cells. The nuclear staining pattern was used for p53, Ki-67, p21 and membrane staining HER2, and cytoplasmic staining for bcl2. In the defined area of the lesion, fields of vision systematically spread over the whole area of interest were chosen. The first field was chosen at random and cells were selected using a point grid and the positivity of these cells was scored. In practice, 200 cells were sampled from 50 to 100 fields of vision. The cutoff values were for p53, bcl-2, p21, Ki-67 and HER-2 were 10%, 35%, 3%, 20% and 1%, respectively, based on earlier publications.[24,31,32]

The mouse monoclonal antibodies were as follows: for TP53 clone DO-1, Santa Cruz Biotechnology, Santa Cruz, CA, USA for Bcl-2 (clone 124, Dako, Glostrup Denmark) for MIB-1 antibody to Ki-67 (Dako), for p21 clone 2G12 (Pharmingen, San Diego, USA) and for HER2 antibody.[33] The slides were then incubated with biotinylated rabbit anti-mouse immunoglobulin for 30 min at room temperature. They were subsequently incubated with avidin–biotin peroxidase complex and counterstained with haematoxylin. Omission of the primary

Table 2 – Prognostic variable expression at predetermined cut-off points by randomised group

Variable	Treatment			
	Surgery (N = 63)	No surgery (N = 62)	Not randomised (N = 44)	Total (N = 169)
p53 d01				
<10%	23 (36.5)	19 (30.6)	16 (36.4)	58 (34.3)
⩾10%	32 (50.8)	35 (56.5)	25 (56.8)	92 (54.4)
Missing	8 (12.7)	8 (12.9)	3 (6.8)	19 (11.2)
bcl-2				
<35%	42 (66.7)	46 (74.2)	34 (77.3)	122 (72.2)
⩾35%	11 (17.5)	8 (12.9)	6 (13.6)	25 (14.8)
Missing	10 (15.9)	8 (12.9)	4 (9.1)	22 (13.0)
p21				
<3%	46 (73.0)	47 (75.8)	30 (68.2)	123 (72.8)
⩾3%	9 (14.3)	7 (11.3)	11 (25.0)	27 (16.0)
Missing	8 (12.7)	8 (12.9)	3 (6.8)	19 (11.2)
Ki-67				
<20%	15 (23.8)	10 (16.1)	15 (34.1)	40 (23.7)
⩾20%	25 (39.7)	27 (43.5)	16 (36.4)	68 (40.2)
Missing[a]	23 (36.5)	25 (40.3)	13 (29.5)	61 (36.1)
HER2				
<1%	50 (79.4)	50 (80.6)	38 (86.4)	138 (81.7)
⩾1%	5 (7.9)	4 (6.5)	3 (6.8)	12 (7.1)
Missing	8 (12.7)	8 (12.9)	3 (6.8)	19 (11.2)
s-phase fraction				
<6%	10 (15.9)	7 (11.3)	6 (13.6)	23 (13.6)
⩾6%	32 (50.8)	35 (56.5)	24 (54.5)	91 (53.8)
Missing	21 (33.3)	20 (32.3)	14 (31.8)	55 (32.5)
DNA-ploidy (image)				
Diploid	28 (44.4)	26 (41.9)	20 (45.5)	74 (43.8)
Non-diploid	16 (25.4)	21 (33.9)	12 (27.3)	49 (29.0)
Missing	19 (30.2)	15 (24.2)	12 (27.3)	46 (27.2)
Vol epithelium				
<65%	25 (39.7)	(37.1)	15 (34.1)	63 (37.3)
⩾65%	34 (54.0)	29 (46.8)	20 (45.5)	83 (49.1)
Missing	4 (6.3)	10 (16.1)	9 (20.5)	23 (13.6)
Mitotic index				
<30	21 (33.3)	22 (35.5)	19 (43.2)	62 (36.7)
⩾30	38 (60.3)	30 (48.4)	16 (36.4)	84 (49.7)
Missing	4 (6.3)	10 (16.1)	9 (20.5)	23 (13.6)
Mean nuclear index				
<80	52 (82.5)	44 (71.0)	34 (77.3)	130 (76.9)
⩾80	7 (11.1)	8 (12.9)	1 (2.3)	16 (9.5)
Missing	4 (6.3)	10 (16.1)	9 (20.5)	23 (13.6)
Performance status				
0	18 (28.6)	17 (27.4)	6 (13.6)	41 (24.3)
1	29 (46.0)	32 (51.6)	26 (59.1)	87 (51.5)
2	16 (25.4)	12 (19.4)	12 (27.3)	40 (23.7)
3	0 (0.0)	1 (1.6)	0 (0.0)	1 (0.6)
Figo stage				
IIb, IIc, III,	48 (76.2)	47 (75.8)	27 (61.4)	122 (72.2)
IV	15 (23.8)	15 (24.2)	17 (38.6)	47 (27.8)
Cell type				
Serous	27 (42.9)	28 (45.2)	15 (34.1)	70 (41.4)
Non-serous	36 (57.1)	34 (54.8)	29 (65.9)	99 (58.6)
Tumour grade				
1–2	(39.7)	28 (45.2)	19 (43.2)	72 (42.6)
3	36 (57.1)	31 (50.0)	21 (47.7)	88 (52.1)
Missing	2 (3.2)	3 (4.8)	4 (9.1)	9 (5.3)

Table 2 – *continued*

Variable	Treatment			
	Surgery (*N* = 63)	No surgery (*N* = 62)	Not randomised (*N* = 44)	Total (*N* = 169)
Tumour size (cm)				
0–5	18 (28.6)	11 (17.7)	6 (13.6)	35 (20.7)
5–10	18 (28.6)	19 (3.6)	11 (25.0)	48 (28.4)
>10	25 (39.7)	30 (48.4)	11 (25.0)	66 (39.1)
Missing	2 (3.2)	2 (3.2)	16 (36.4)[b]	20 (11.8)
Ascites				
No	11 (17.5)	12 (19.4)	5 (11.4)	28 (16.6)
Yes	52 (82.5)	50 (80.6)	39 (88.6)	141 (83.4)

a 61 (36%) missing or incomplete size measurement mainly described as large tumour bulk.
b Size measurement mainly described as large bulk.

antibody was performed as a negative control. All slides were evaluated for immunostaining without any knowledge of the clinical outcome or other clinicopathological data.

2.2.1. *DNA-ploidy*

DNA-ploidy was performed according to previously defined methods. In short, preparation, staining and analysis of paraffin-embedded tumours for flow cytometry were modified from Hedley *et al.*[34] and have been described previously.[35,36] Classification of the histograms was performed according to the recommendations of The Society of Analytical Cytology[36] without knowledge of the final outcome or other clinical information.

2.3. *Morphometry*

The morphometry was performed according to methods described in detail in earlier papers[5,37] and assesses mitotic index and the volume percentage epithelium.

2.4. *Statistical methods*

Overall and disease-free survival time was defined as the period that elapsed between primary surgery and death or relapse, respectively. Kaplan and Meier analyses[38] and the log-rank test[39] were used to estimate and compare overall and disease-free survival curves. The independent effects of prognostic factors and other covariates on survival function were determined by the Cox proportional-hazards regression model,[40] stratified for the assigned treatment group. Correlations between various factors were assessed by Spearman rank correlations. The criterion for inclusion of a variable was $p < 0.05$ in the univariate analysis and for removing a variable $p > 0.05$ in the multivariate analysis. The proportionality assumptions of the method were tested graphically by looking at the log minus log survival function plots. The stability of the variables entered in the final model was examined by repeating the analysis excluding each factor in turn.

Table 3 – Univariate survival analysis of prognostic factors for the 169 advanced ovarian cancer patients described in Table 2

Variable	Survival				Progression free survival		
	n patients	*p*-Value	HR	CI (95%)	HR	*p*-Value	CI (95%)
P53 d01	58/92	0.80	0.96	(0.67–1.36)	0.81	0.24	(0.57–1.15)
bcl-2	122/25	0.86	1.04	(0.66–1.65)	1.11	0.66	(0.70–1.76)
P21	**123/27**	**0.03**	**0.58**	**(0.36–0.94)**	**0.52**	**0.01**	**(0.33–0.83)**
Ki-67	40/68	1.00	1.00	(0.66–0.94)	**0.60**	**0.02**	**(0.40–0.91)**
DNA-ploidy (flow)	55/92	0.85	1.03	(0.73–1.47)	0.97	0.84	(0.68–1.38)
s-Phase fraction	23/91	0.59	0.88	(0.54–1.42)	0.74	0.22	(0.46–1.20)
Vol% epithelium	63/83	0.71	1.07	(0.76–1.51)	1.32	0.12	(0.93–1.87)
Mitotic index	62/84	0.88	0.97	(0.69–1.38)	0.95	0.76	(0.67–1.34)
Ps (0 *versus* 1)	41/87	0.77	1.06	(0.71–1.58)	1.09	0.68	(0.73–1.61)
Ps (0 *versus* 2)	41/40	0.91	0.97	(0.61–1.55)	1.14	0.58	(0.72–1.80)
FIGO stage	**122/47**	**0.02**	**1.54**	**(1.08–2.21)**	1.16	0.42	(0.81–1.65)
Cell type	70/99	0.07	1.35	(0.98–1.87)	1.12	0.48	(0.81–1.54)
Tumour grade	72/88	0.97	1.01	(0.73–1.40)	0.74	0.06	(0.54–1.02)
Tumour size (0–5 *versus* 5–10)	35/48	0.81	1.06	(0.66–1.70)	1.38	0.17	(0.87–2.18)
Tumour size (0–5 *versus* >10)	35/66	0.22	1.32	(0.85–2.04)	1.10	0.68	(0.71–1.69)
Lesions (1, 2 *versus* 3)	35/21	0.50	1.22	(0.69–2.17)	0.91	0.74	(0.52–1.59)
Lesions (1, 2 *versus* 4)	35/111	0.46	1.17	(0.78–1.77)	0.87	0.49	(0.59–1.30)
Ascites	28/141	0.23	1.31	(0.84–2.04)	1.11	0.62	(0.73–1.69)

3. Results

3.1. *Distribution of patient characteristics within subgroups*

Patient and tumour characteristics were well balanced between the two randomised subgroups comprising the surgery group (63 patients) and the no surgery group (62 patients). Closer examination of the non-randomised subgroup shows that adverse factors including FIGO stage IV, non-serous histology and presence of ascites were over-represented in this subgroup, which also had a worse prognosis than either of the other subgroups or the total patients entered (Table 1). The characteristics of the total patients entered on the translational study with those entered on the clinical trial were balanced except for non-serous cell type and for number of lesions >10 (Table 1).

3.2. *Morphologically assessed variables*

No association was found between PFS or overall survival and DNA ploidy, assessed either by flow cytometry or image analysis, S phase fraction, volume percentage epithelium or mitotic index (Table 3).

3.3. *Immunohistochemistry*

Table 2 shows the frequencies of prognostic factors based on their pre-determined cutoff levels. The correlation between p21 and p53 was low (<0.03 Spearman rank), while that between the other variables was less than 0.3, and it is therefore reasonable to consider all parameters as separate variables in the subsequent analyses.

3.4. *Survival and progression free survival*

In the univariate analysis, p21 and FIGO stage (IIb, IIc, III *versus* IV) were found to be significant for survival, p21 HR 0.58 CI 0.36–0.93 ($p = 0.025$), and FIGO stage HR 1.54 CI 1.08–2.2 ($p = 0.018$) as shown in Table 3. The benefit in survival at two years is 8% (48% *cf.* 40%) for p21 and 21% (47% *cf.* 26%) for FIGO stage. Patients with p21 values ⩾3% had a median survival of 23 months compared to 18 months for patients with a p21 value <3%. In all three treatment groups (surgery,

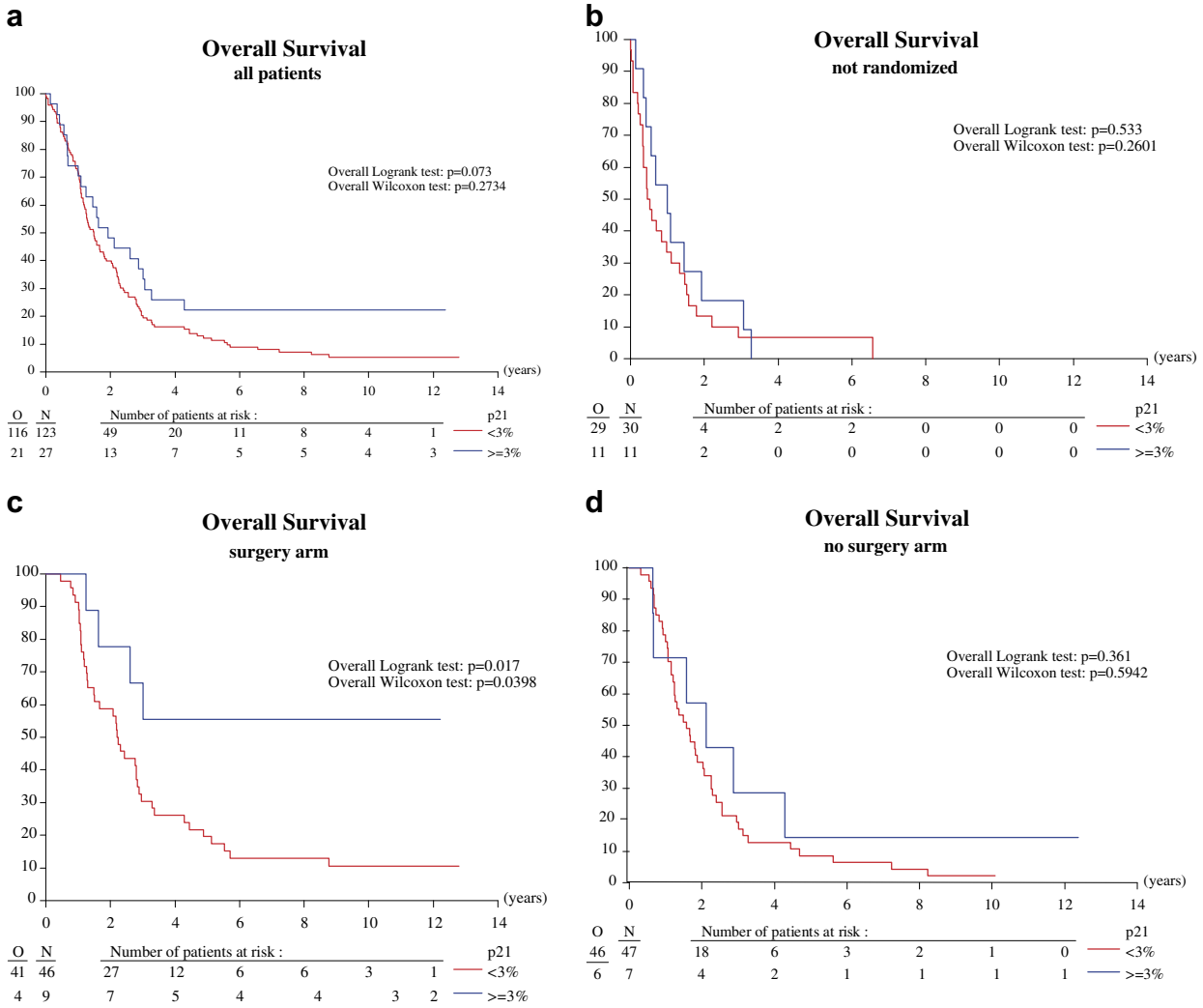

Fig. 1 – Overall survival curves for p21 expression (IHC)j for all 150 patients analysed (a), debulking surgery alone arm $n = 55$ (b), no surgery arm $n = 54$ (c), not randomised – progressed on chemotherapy or declined surgery $n = 41$ (d).

Table 4 – Response by FIGO stage (n = 169 cases) and Ki-67 (n = 108 cases)

Variable	Responses				
	CR	PR	NC/progression	NE/missing	Total
Figo stage[a] IIb, IIc, III	47 (38.5)	23 (18.9)	27 (22.1)	25 (20.5)	122
IV	14 (29.8)	11 (23.4)	8 (17.0)	14 (29.8)	47
Ki-67[b] < 20%	15 (37.5)	4 (10.0)	9 (22.5)	12 (30)	40
>20%	22 (32.4)	14 (20.6)	14 (20.5)	18 (26.4)	68

a CR *versus* rest $p = 0.37$.
b CR + PR *versus* rest $p = 0.69$.

Table 5 – Comparison of p21 and p53 staining by IHC in 169 cases

Variable	P21	<3% (N = 123), N (%)	⩾3% (N = 27), N (%)	Missing (N = 19), N (%)	Total (N = 169), N (%)
P53d01					
	<10%	47 (38.2)	11 (40.7)	0 (0.0)	58 (34.3)
	⩾10%	75 (61.0)	16 (59.3)	1 (5.3)	92 (54.4)
	Missing	1 (0.8)	0 (0.0)	18 (94.7)	19 (11.2)
P53d07					
	<10%	47 (38.2)	11 (40.7)	0 (0.0)	58 (34.3)
	⩾10%	74 (60.2)	16 (59.3)	1 (5.3)	91 (53.8)
	Missing	2 (1.6)	0 (0.0)	18 (94.7)	20 (11.8)

The correlation in staining between D01 and D07 was 0.886, while that with p21 was low (Spearman rank 0.028 and 0.022, respectively).

no surgery and not randomised), the patients with p21 < 3% have the worst prognosis. The corresponding Kaplan–Meier curve for p21 is shown in Fig. 1. For progression-free survival, p21 and Ki-67 were important prognostic factors: p21 HR 0.52 CI 0.33–0.83 ($p = 0.006$), Ki-67 HR 0.6 CI 0.40–0.91 ($p = 0.02$) as shown in Table 4. FIGO stage was no longer a prognostic factor for progression free survival (HR 1.16), and tumour grade was of borderline significance HR 0.74 CI 0.54–1.02 ($p = 0.06$). The benefit in progression free survival at 1 year is 17% (55% *cf*. 38%) for p21 and 16% (27% *cf*. 11%) for Ki-67. These values for Ki67 should be interpreted with caution in view of the 36% missing values on account of lack of material (Table 2).

In multivariate analysis, these differences in survival were retained for p21 with a HR of 0.54 CI 0.33–0.88 ($p = 0.014$) and for FIGO stage (HR 1.58 CI 1.0–2.4, $p = 0.03$). The difference for progression free survival was for p21 HR 0.52 CI 0.3–0.89 ($p = 0.02$) and for Ki-67 HR 0.57 CI 0.37–0.86 ($p = 0.008$). Expression of c-erbB2 was only found in 12% of patients, hence there were only a very few events, and significance was not achieved. The HR values were 1.23 for overall survival and 1.26 (CI 0.69–2.30) for progression free survival (see Tables 4 and 5).

A sensitivity analysis was carried out with the two populations of biopsies, those from the ovarian tumours and those where a metastatic site was selected at the time of surgery. However, all samples analysed in this study were taken at primary surgery, and samples from interval or second procedures are not included. When the 89 primary ovarian tumours were analysed (34 surgery, 30 no surgery, 25 not randomised), p21 and FIGO stages were confirmed as prognostic factors for OS. No variable was found to be significant in the analysis of the metastatic site subgroup ($n = 80$).

When the tumour biopsies taken at initial surgery were analysed by treatment allocation group, p21 retained significance only in the surgery group alone ($n = 55$ analysed out of 63 randomised) HR 0.31 CI 0.11–0.86 ($p = 0.03$). In the no surgery arm ($n = 54$ analysed out of 62 randomised) and in the non-randomised patients who developed progressive disease after first line chemotherapy ($n = 44$), no factor emerged as significant. The survival curves for p21 expression in these subgroups are shown in Fig. 1.

4. Discussion

The most consistent finding in this study was overexpression of p21 being a favourable prognostic factor in advanced stage ovarian cancer. The significance of alterations in p53, HER2 (erbB2), bcl2, and Ki-67 is uncertain.

This is one of the largest series of prognostic factor analysis in ovarian cancer. By using archival material from the original diagnostic tissue block (formalin fixed paraffin embedded FFPE) from patients entered into a multinational clinical trial, variation in management, in particular selection of chemotherapy, frequency and quality of follow-up are all kept to a minimum. All the patients who entered had suboptimal debulking surgery and received platinum based chemotherapy, consistent with the standard of care at that time, and median follow-up is now approaching 10 years. The distribution of prognostic factors is consistent with the inclusion of

the non-randomised patients with predominantly progressive disease in the analysis. These patients as expected have a poor prognosis as they were either chemoresistant or otherwise unsuitable for surgery. Clinicopathological stage emerges as a significant prognostic factor, as expected, and FIGO stage IV showed a 50% greater risk of death compared with FIGO stages IIb–III. The response rate was slightly higher in stage IIb–III compared to stage IV, but this did not achieve significance. The ploidy and morphometry assessments are consistent with previously published data in stages III and IV ovarian cancer,[5,37] but none of these variables was of prognostic value in this analysis.

The proportion of patients positive for the variable HER2 is lower than in many of the published data in the literature. However, the strictly defined cutoffs based on prior experience in each participating laboratory, and the use of a previously published consensus document by this group for IHC assessment,[31] indicate that the data presented here may be true reflections of the prevalence of abnormalities of expression of this variable. HER2 (c-erbB2) might be a promising marker with potential significance but the data available were insufficient to draw further conclusions. The overexpression found in 12% of the cases is at the lower end of the published series, in keeping with the more recent studies,[9,10] the majority of which have found that overexpression is associated with poor prognosis The overall proportion of patients who tested positive for p53 by IHC (54%) is at the upper range of the published values,[13,14] which may be related to the selection of an advanced stage and bulky tumours. The proportion of tumours with grade 3 histology is also high (52%). However, much of the published literature on IHC is of poor quality with low specificity, either as a result of methodological or assessment variation.[15]

P21 was found to be the best prognostic factor for survival and progression-free survival in the investigated population of 169 patients. The hazard ratio of 0.5 implies that patients whose p21 value are >3% are at much smaller risk and will have a longer expected progression-free and overall survival compared to those with lower expression. This protein is one of the several downstream effectors of p53, and is part of a pathway which has been linked to prediction of response to a number of agents, in particular platinum compounds which were used as the basis for chemotherapy in this study, and represent the most active agents in the treatment of ovarian cancer. Earlier series have in general also found an association between p21 expression and prolonged survival,[41,42] but others have found contrasting results with low p21 expression associated with survival.[44] In part, these differences may relate to differences in the cutoff values used in the different studies. The largest series of 267 cases used a cutoff of 2%, similar to ourselves. These authors found p21 status overall to be a positive prognostic factor, but a negative one in the small subgroup of p53 null tumours, an observation which they could not explain.[45] We postulate that the more pronounced beneficial effect of p21 expression in the surgical resection subgroup may in some way be related to selection of cases based on their response to chemotherapy. Some authors have reported correlation of p21 and p53 expression,[42–44,48] but one other study did not demonstrate any association,[42] as in the present study. This discrepancy could be explained by activation of a p53 independent pathway.

Ki-67 was significant for progression-free survival in these patients, and those with Ki 67 <20% had almost double the risk of progression or death compared with patients with Ki-67 >20%. This is at variance with the majority of the published literature,[11,12,43,47] but consistent with the conclusion of the series by Hartmann *et al.*[45] One explanation may be that the study population is selected from a group of patients with bulky tumours, and differ in biology from 60% to 65% of advanced ovarian cancer patients in whom initial optimal debulking is feasible and who are subsequently treated with platinum based chemotherapy. The literature on this variable in ovarian cancer is not sufficiently extensive to make further conclusions or recommendations.

This study suggests that larger studies of the extended p53 pathway are required, and these should take account of the standardised assay and assessment criteria used in this study. The prognostic factors grade and ploidy, which may broadly reflect genomic instability, apply in early stage but not in advanced disease[8] as confirmed in this study, and there are other less well validated molecular differences which may include the p53 gene status.[49,46]

There has, however, been further progress in identifying the relevant molecular pathways in ovarian cancer progression, and the development of targeted therapies at the PI-3 kinase and EGF receptor pathways. The current EORTC study 55041 evaluates the role of maintenance erlotinib, an EGF receptor inhibitor, in advanced ovarian cancer after primary chemotherapy There is a need to combine candidate gene/pathway analyses and non-specific proliferation markers with gene expression and gene copy number assessment to develop molecular prognostic factors in advanced disease, where heterogeneity may dictate that multiple factors may need to be considered.

Conflict of interest statement

None declared.

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