Analytical Cellular Pathology 24 (2002) 17–23
IOS Press

Expression of multidrug resistance-associated markers, their relation to quantitative pathologic tumour characteristics and prognosis in advanced ovarian cancer [1]

Mariël Brinkhuis [a], Miguel A. Izquierdo [a], Jan P.A. Baak [a,*], Paul J. van Diest [a], Peter Kenemans [b], George L. Scheffer [a] and Rik J. Scheper [a]

[a] *Department of Pathology, Free University Medical Center, Amsterdam, The Netherlands*
[b] *Department of Obstetrics and Gynaecology, Free University Medical Center, Amsterdam, The Netherlands*

Received June 2001
Accepted April 2002

Abstract. Mean nuclear area has been consistently shown by different researchers to be a strong and independent prognostic factor in advanced ovarian carcinoma. However, the biological background of the prognostic value of nuclear area remains unclear. Others have found that the multidrug-resistance (MDR) related protein LRP has strong prognostic value. In the present study we have analysed whether the mean nuclear area and LRP are related in tumour tissue of the ovary obtained at the debulking operation before the administration of chemotherapy in 40 patients. The mitotic activity index, volume percentage epithelium, standard deviation of nuclear area and the other MDR-related proteins P-glycoprotein (JSB-1, MRK-16) and MRP have been investigated additionally for correlations and prognostic value.

No correlations were found between the morphometrical features and MDR-related proteins. Mean nuclear area tended to be larger in LRP positive tumours, but the correlation was not significant. In multivariate analysis LRP-protein expression and mean nuclear area had independent prognostic value.

Further studies are required to elucidate the biological background of the strong prognostic value of mean nuclear area in advanced ovarian cancer.

Keywords: Ovary, carcinoma, multi drug resistance, prognosis, morphometry

1. Introduction

Ovarian cancer remains the cancer of the female genital tract which deserves our greatest attention, since it forms the leading cause of death among all gynaecological cancers and is responsible for 6.5% of cancer deaths among women [30]. In the majority of ovarian cancer patients, disease has already spread to the peritoneal cavity or retroperitoneal lymph nodes (according to the International Federation of Gynecology and Obstetrics = FIGO stage III) or has metastasized to the abdominal wall, liver or even beyond the abdominal cavity (FIGO IV), as confirmed by diagnostic procedures and staging laparotomy. Treatment for patients with advanced ovarian cancer usually consists of cytoreductive surgery followed by cis- or carboplatin combination chemotherapy, which has numerous serious toxic side effects. In spite of these intensive treatment strategies 70% of patients will die of their disease with a median survival time of two years, be-

[1] Supported by grant #28-843 of the Health Research and Development Council of the Netherlands (ZONMW).
[*] Corresponding author: Professor J.P.A. Baak, Department of Pathology, SIR Hospital, Armauer Hansen veg 20, 4068 Stavanger, Norway. E-mail: jpabaak@yahoo.com.

cause their tumours become unresponsive to combination chemotherapy [21]. Therefore, it is considered important to develop accurate prognostic factors to select those patients who are expected to obtain benefit from cis-platin based combination chemotherapy. Then, alternative treatments aimed either at palliation or improvement of survival could be offered to those patients who are not expected to benefit.

Several prognostic variables in patients with advanced ovarian cancer have been identified and the most important among these are FIGO stage and residual tumour status after cytoreductive surgery [10]. Evidence has accumulated that quantitative pathological variables like the mean nuclear area of tumour cells (MNA), mitotic indices (MI) and the volume percentage of epithelium (VPE) have important prognostic value in ovarian cancer, stronger than DNA-ploidy by flow cytometry [2,4,5,8,12–15,20,22,30]. An important advantage of these techniques is that objective and reproducible values are obtained. Obviously, they are correlated to biological processes taking place in neoplastic growth, like proliferation (MI), nuclear differentiation (MNA) and architectural differentiation (VPE).

The emergence of multidrug-resistance (MDR) in the tumour cell population plays an important role in the success of chemotherapeutical treatment in advanced ovarian cancer. *In vitro* resistance to platinum and alkylating agents is caused by a decrease in drug accumulation, enhanced detoxification and increased DNA repair capacity [18]. MDR to natural products is linked to the over-expression of the MDR1 gene product, P-glycoprotein (Pgp) [11] and alteration in topoisomerase II activity [6]. To date, most clinical studies in ovarian carcinoma have failed to demonstrate a role for any of these mechanisms as a major determinant of response to chemotherapy and survival [1,7, 34,35]. Recently, two new drug resistance related proteins have been described, the MDR related protein (MRP) and the lung resistance protein (LRP). MRP is localized in the plasma membrane and it may cause resistance by extrusion of drugs out of the cell [33]. The LRP-gene product is localized immunohistochemically in the cytoplasm, in a granular pattern suggestive of a storage in vesicles. The LRP-gene was recently localized to the 16p13.2–16p11.2 chromosomal segment in human metaphases proximal to MRP at band 16p11.2 [29]. The LRP gene was recently cloned and sequenced in our laboratory and its product LRP was identified as the major human vault protein [26]. Vaults are cellular organelles which are thought to mediate nucleocytoplasmic transport of a wide variety of substrates [19,23]. Independent amplification of either or both the MRP and the LRP genes may be associated with the expression of acquired drug resistance [29]. In a recent study it was shown that the expression of the novel MDR-related protein LRP is a strong and independent prognostic factor to predict response to cis-platin combination chemotherapy and survival in advanced ovarian cancer [17].

Therefore, the objectives of the current investigation were to analyse whether relations exist between the expression of MDR-related proteins and quantitative pathological tumour characteristics in advanced ovarian cancer. Additionally, their value in predicting survival after cis-platin combination chemotherapy was assessed.

2. Material and methods

2.1. Patients

Forty patients diagnosed between 1984 and 1993 at the Free University Hospital, Amsterdam with advanced ovarian cancer of the common epithelial types were selected from whom frozen material from the primary tumour was available before chemotherapy. Borderline tumours were excluded. The age at diagnosis ranged from 29 to 84 years (median 68 years).

Nine patients were not treated with either cytoreductive surgery or cis-platin combination chemotherapy and were excluded from survival analysis, leaving 31 patients. Follow-up information was available in all cases and updated. The clinico-pathological characteristics and correlation of these quantitative pathological features are summarized in Tables 1 and 2.

2.2. Tissue processing and histopathology

Fresh tumour material was cut in 0.5 centimetre thick slices and fixed in neutral buffered formaldehyde. Four micron thick haematoxylin and eosin (H&E) stained sections were cut from the paraffin blocks for diagnosis and quantitative measurements. Histological types were assessed according to WHO criteria [27]. Twenty-eight were of the serous type, seven were mucinous, one was endometrioid and four were undifferentiated adenocarcinomas. One carcinoma was classified as well (grade I), nine as moderately (grade II) and thirty as poorly differentiated (grade III) [3]. The investigations for the immunohistochemical detection of MDR expression were performed on snap frozen (liquid nitrogen) material from the primary ovarian tumour.

Table 1

Correlations between clinicopathologic patient characteristics and the expression of MDR-related proteins in 40 patients with advanced ovarian cancer (tested with Pearson Chi-square statistics with Yates' correction)

	Pgp expression[1]			MRP expression[2]			LRP expression[3]		
	Negative	Positive	p-value	Negative	Positive	p-value	Negative	Positive	p-value
FIGO stage									
III ($n = 24$)	18	6	0.94	8	15	0.69	8	16	0.52
IV ($n = 16$)	13	3		7	8		3	13	
Residual disease									
#2 cm ($n = 13$)	7	6	*0.04*	3	10	0.25	4	9	1.00
>2 cm ($n = 27$)	24	3		12	13		7	20	
Grade									
I & II ($n = 10$)	5	5	*0.05*	5	4	0.46	1	9	0.31
III ($n = 30$)	26	4		10	19		10	29	
Ascites									
absent ($n = 12$)	21	5	0.59	10	15	1.00	7	19	0.98
present ($n = 26$)	8	4		4	7		4	8	
Histology									
serous ($n = 28$)	21	7	0.87	10	18	0.68	9	19	0.54
other ($n = 12$)	10	2		5	5		2	10	
Performance status									
good ($n = 17$)	12	5	0.84	5	11	1.00	4	13	0.83
bad ($n = 15$)	12	3		5	9		5	10	
Age (years)									
#68 ($n = 22$)	15	7	0.24	8	13	1.00	3	19	0.07
>68 ($n = 18$)	16	2		7	10		8	10	

[1]Pgp is p-glycoprotein, [2] MRP is MDR related protein, [3]LRP is lung resistance protein.

2.3. Quantitative methods

In the most representative primary tumour section from the ovary, the subjectively most poorly differentiated and epithelium rich area was marked for measurements in each case. Morphometrical and stereological assessments were done as described before [5, 8]. Briefly, the mitotic activity index (MAI) was assessed by counting the total number of mitoses in 25 fields at $\times$400 magnification (field diameter, 450 μm). Volume percentage of epithelium (VPE) was assessed by stereological point counting, and mean (MNA) and standard deviation of the nuclear area (SDNA) of 100 systematically at random selected tumour cell nuclei were measured with an interactive morphometric and stereological video overlay system (QPRODIT, Leica, Cambridge, UK).

2.4. Monoclonal antibodies (MAbs) and Immunohistochemistry

For the study of Pgp expression two murine MAbs were used which are specific for the MDR-1 gene product, MRK-16 (IgG2) and JSB-1 (IgG1). MRK-16 recognizes an external epitope of Pgp and JSB-1 reacts against and internal epitope of Pgp [16,24,31].

For the immunohistochemical detection of MRP the rat MAb MRPr1 (IgG2a) that was developed in our laboratory was used [9]. MRPr1 has been raised after immunisation with a fusion protein containing a segment of 168 amino acids in the amino-proximal half of the MRP protein, and has been well characterized by protein blot analysis, immunocytochemical and immunohistochemical studies. MRPr1 does not cross-react with the human Pgp.

For the study of LRP expression the murine MAb LRP-56 (IgG2b) was used which has been developed in our laboratory after immunization with the non-Pgp MDR human lung cancer cell line SW-1573/2R120 [29]. LRP-56 specifically recognizes a 110 kD protein overexpressed in a number of non-Pgp MDR cancer cell lines of different histogenetic origin.

Immunostaining was performed on the acetone-fixed cryostat sections as described in detail elsewhere [17]. Immunohistochemical expression of the MAbs was agreed upon and scored by two observers

Table 2
Results of univariate survival analysis in 31 patients with advanced ovarian cancer (MC = Mantel–Cox statistic)

	n	Survival		Mantel–Cox	p-value
		(%)	Median time (mo)		
FIGO					
III	20	45	30	6.6	0.01
IV	11	27	8		
Residual disease					
#2 cm	12	50	30	1.5	0.23
>2 cm	19	32	15		
Grade					
I & II	7	14	8	5.1	0.02
III	24	46	25		
Ascites					
absent	10	60	42	4.1	0.04
present	20	30	15		
Pgp					
#10%	22	41	20	0.6	0.43
>10%	9	33	12		
MRP					
#10%	11	36	15	1.8	0.18
>10%	19	42	27		
LRP					
#10%	7	71	N.R.[a]	5.6	0.02
>10%	24	29	15		
Mean nuclear area					
#90 μm^2	26	42	27	4.5	0.03
>90 μm^2	5	20	9		
SD of nuclear area					
#14	10	60	N.R.[a]	6.2	0.01
>14	21	29	12		
Volume % epithelium					
#75	17	41	20	0.0	0.9
>75	14	35	20		
Mitotic activity index					
#42	16	44	20	0.0	0.9
>42	15	33	25		

Pgp is p-glycoprotein, MRP is MDR related protein, LRP is lung resistance protein.
[a]N.R., not reached.

without knowledge of the clinical information of the patients. Samples were scored as positive if more than 10% of the tumour cells stained (for Pgp with both MRK-16 and JSB-1). In selected cases with equivocal results, the staining was repeated until a conclusive staining pattern was arrived at.

2.5. Statistics

Descriptive statistics were calculated for all variables. Relations between MDR expression and clinicopathological variables were tested with Pearsons' chi-square statistics using the Yates' correction. Differences between MDR expression (categorical variables) and quantitative pathological variables (continuous variables) were analyzed using the Mann–Whitney test. Survival and progression-free survival analysis were performed with the Mantel–Cox test and illustrated by Kaplan–Meier plots. For survival time, the time from diagnosis to death of disease or last date of follow up was used. Variables were analyzed for their prognostic value using (1) the median value, (2) in

three groups of equal size, and (3) according to previously established thresholds. The thresholds resulting in the lowest p-values are shown. Additional prognostic value of features was assessed with the multivariate Cox regression model (enter and remove limits 0.1). Variables that yielded prognostic value in univariate analysis were used as covariates. Hypotheses were evaluated at a significance level of 0.05. All statistical analyses were conducted with the Biomedical statistical software package (BMDP Statistical Software, Inc., Los Angeles, CA, USA).

3. Results

The correlations between the expression of MDR related proteins and clinicopathological features are summarized in Table 1. Nine out of 40 patients (23%) were Pgp positive, 23/38 patients (61%) were MRP positive and 29/40 patients (73%) were LRP positive. Pgp expression was significantly correlated with residual disease status and grade, respectively. As to LRP expression and age, LRP positive tumours tended to be associated with age under 68 years.

The MAI tended to be lower in LRP negative tumours. An interesting finding was that none of the LRP negative tumours had a MNA larger than 90 μm^2. However, no significant differences were observed between expression of the MDR-related proteins and quantitative pathological variables.

Twelve (39%) of the 31 evaluable patients survived with a median survival time of 34 months (range, 7 to 111 months) and 19 patients died with a median survival time of 10 months (range, 2 to 30 months). FIGO stage, SDNA, LRP-expression, MNA and the presence or absence of ascites had prognostic value in order of decreasing sequence (Table 2).

In multivariate survival analysis, LRP expression and MNA had additional prognostic value. LRP-expression was selected first followed by the MNA. The resulting formula was: (1.0226 $\times$ LRP-expression) + (0.0273 $\times$ MNA) (LRP-negative $=$ 1, LRP-positive $=$ 2). When the median value of this score was used as the cut-off point (4.90) a favourable subgroup could be distinguished with 50% survival (median survival time, 42 months) and an unfavourable group in which 27% survived (median survival time, 12 months) (MC $=$ 6.1, $p = 0.01$).

4. Discussion

In this study our principle objectives were to investigate whether or not correlations exist between quantitative pathological variables and the expression of MDR-related proteins and to assess the additional prognostic value of these variables in advanced ovarian cancer.

No significant differences in the size of nuclei (MNA), proliferation (MAI) or the relative amount of tumour epithelium (VPE) could be found in tumours with and without Pgp, MRP and LRP expression. The lack of association between Pgp and proliferative activity is in agreement with a recent study in colon cancer in which the proliferative activity was assessed with DNA flow cytometry [28]. An interesting finding was that in the LRP negative tumours the MNA was never larger than 90 μm^2. MNA above 90 μm^2 is known to be a very poor prognostic sign in ovarian cancer [5,8,20]. However, the final clue as to why patients with large nuclei in advanced ovarian cancer fare significantly worse than those with smaller nuclei does not seem to be related to MDR protein expression, even more since LRP-expression and the MNA had independent prognostic value in survival analysis. Also for the VPE and MAI no relations with the MDR-related proteins were established in this investigation. Therefore, the biological background for the strong prognostic value of mean and standard deviation of the nuclear area still remains to be determined.

Contrary to Pgp, MRP and LRP were frequently expressed in the untreated advanced ovarian cancers, denoting their potential as markers of clinical drug-resistance in this tumour.

Patients presenting with FIGO stage IV, large tumour cell nuclei (MNA values), ascites present, expressing LRP and a high SDNA had a very poor prognosis. Histological grade was also prognostically significant but its meaning is controversial since patients with poorly differentiated tumour had a better survival than patients with well or moderately differentiated tumours. This may be explained by the fact that only one patient presented with a well differentiated tumour and the other six patients probably belonged to the poor prognostic side of the spectrum of moderate differentiation. In the present investigation and also in previous studies [5,8,20,30], the nuclear area associated features were after FIGO stage the most important prognostic factors for survival. These nuclear size and variation features may reflect intratumour heterogeneity in the size of tumour cell nuclei and may therefore be indica-

Table 3

Correlations between quantitative pathologic variables and expression of MDR-related proteins in 40 patients with advanced ovarian cancer (Mann–Whitney test)

MDR-expression	n	MNA		SDNA		VPE		MAI	
		median	p-value	median	p-value	median	p-value	median	p-value
Pgp									
#10%	31	78	0.76	17.1	0.58	79	0.19	64	0.31
>10%	9	77		17.4		75		73	
MRP									
#10%	15	77	0.74	17.5	0.63	78	0.94	40	0.79
>10%	23	77		16.5		75		55	
LRP									
#10%	11	72	0.35	13.8	0.15	73	0.87	73	0.15
>10%	29	78		17.5		75		42	

MNA is mean nuclear area, SDNA is standard deviation of nuclear area, VPE is volume percentage epithelium, MAI is mitotic activity index, Pgp is p-glycoprotein, MRP is MDR related protein, LRP is lung resistance protein.

tive for the existence of different subclones of tumour cells in a patient. These subclones may have different profiles concerning their vulnerability to cytotoxic agents, like cis-platin. It may therefore be hypothesized that besides multidrug resistance other processes play a role in advanced ovarian cancer which determine its rapidly fatal course. Further investigations remain necessary to elucidate this problem.

It is well known that prognosis is extremely poor for those patients presenting with a FIGO stage IV and that most patients with considerable amounts of residual disease are likely to die. Therefore, it may be interesting to investigate the importance of LRP expression and quantitative pathologic variables in a group of patients with FIGO stage III and residual disease smaller than two centimetres.

In conclusion, no significant relations were found in this investigation between quantitative pathological variables and MDR related proteins in advanced ovarian cancer. The biological background for the prognostic value of the quantitative variables therefore remains unclear. Nevertheless, LRP and MNA both have important independent prognostic value for overall survival after cis-platin treatment in advanced ovarian cancer. Therefore, both features should be routinely assessed in such tumours and future trials should be designed to assess its significance for chemotherapy planning.

References

[1] S. Arao, H. Suwa, M. Mandai et al., Expression of multidrug resistance gene and localization of P-glycoprotein in human primary ovarian cancer, *Cancer* **54** (1994), 1355–1359.

[2] J.P.A. Baak, E.C.M. Wisse-Brekelmans, F.A. Langley, A. Talerman and J.F.M. Delemarre, Morphometric data to FIGO stage and histological type and stage for prognosis in ovarian tumours, *J. Clin. Pathol.* **39** (1986), 1340–1346.

[3] J.P.A. Baak, K.K. Chan, J.G. Stolk and P. Kenemans, Prognostic factors in borderline and invasive ovarian tumours of the common epithelial type, *Path. Res. Pract.* **182** (1987), 755–774.

[4] J.P.A. Baak, Possibilities and progress of quantitative pathological examination of ovarian tumours, *Eur. J. Obstet. Gynecol. Reprod. Biol.* **29** (1988), 183–189.

[5] J.P.A. Baak, N.W. Schipper, E.C.M. Wisse-Brekelmans et al., The prognostic value of morphometrical features and cellular DNA content in cis-platin treated late ovarian cancer patients, *Br. J. Cancer* **57** (1988), 503–508.

[6] W.T. Beck and M.K. Danks, Mechanisms of resistance to drugs that inhibit DNA topoisomerases, *Sem. Cancer Biol.* **2** (1991), 235–244.

[7] D. Bisset, K. McLaughlin, L.R. Kelland et al., Cis-platin-DNA damage recognition proteins in human tumour extracts, *Br. J. Cancer* **67** (1993), 742–748.

[8] P.J. van Diest, J.P.A. Baak, J. Brugghe, M.E.L. van de Burg, A.T. van Oosterom and J.P. Neijt, Quantitative pathologic features as predictors of long term survival in patients with advanced ovarian cancer treated with cis-platin, *Int. J. Gynecol. Cancer* **4** (1994), 174–180.

[9] M.J. Flens, M.A. Izquierdo, G.L. Scheffer et al., Immunohistochemical detection of MRP in human multidrug-resistant tumour cells by monoclonal antibodies, *Cancer Res.* **54** (1994), 4557–4563.

[10] M.L. Friedlander and A.J. Dembo, Prognostic factors in ovarian cancer, *Sem. Oncol.* **18** (1991), 205–212.

[11] L.J. Goldstein, I. Pastan and M.M. Gottesman, Multidrug resistance in human cancer, *Crit. Rev. Oncol./Hematol.* **12** (1992), 243–253.

[12] H. Haapasolo, Y. Collan, N.B. Atkin, E. Pesonen and A. Seppä, Prognosis of ovarian carcinomas: Prediction by histoquantitative methods, *Histopathology* **15** (1989), 167–178.

[13] H. Haapasalo, Y. Collan, A. Seppä, A.-L. Gidlund, N.B. Atkin and E. Pesonen, Prognostic value of ovarian grading methods – a method comparison study, *Histopathology* **16** (1990), 1–7.

[14] H. Haapasalo, Y. Collan, R. Montironi, E. Pesonen and N.B. Atkin, Consistency of quantitative methods in ovarian tumour histopathology, *Int. J. Gynecol. Pathol.* **9** (1990), 208–216.

[15] H. Haapasalo, N.B. Atkin, Y. Collan, E. Pesonen and L. Paljärvi, Tumour ploidy, morphometry, histological and clinical features in ovarian carcinoma: mutual relations, *Analyt. Cell. Pathol.* **3** (1991), 261–271.

[16] H. Hamada and T. Tsuruo, Functional role for the 170- to 180-kDa glycoprotein specific to drug-resistant tumour cells revealed by monoclonal antibodies, *Proc. Natl. Acad. Sci. USA* **83** (1986), 7785–7789.

[17] M.A. Izquerdo, A.G. van der Zee, J.B. Vermorken, P. van der Valk, J.A. Belien, G. Giaccone, G.L. Scheffer, M.J. Flens, H.M. Pinedo, P. Kenemans et al., Drug resistance-associated marker Lrp for prediction of response to chemotherapy and prognosis in advanced ovarian carcinoma, *J. Natl. Cancer Inst.* **87** (1995), 1230–1237.

[18] S.W. Johnson, R.F. Ozols and T.C. Hamilton, Mechanisms of drug resistance in ovarian cancer, *Cancer* **71** (1993), 644–649.

[19] N.L. Kedersha and L.H. Rome, Isolation and characterization of a novel ribonucleoprotein particle: large structures contain a single species of small RNA, *J. Cell. Biol.* **103** (1986), 699–709.

[20] C. Ludescher, A.-R. Weger, J. Lindholm et al., Prognostic significance of tumour cell morphometry, histopathology and clinical parameters in advanced carcinoma, *Int. J. Gynecol. Pathol.* **9** (1990), 343–351.

[21] J.P. Neijt, W.W. ten Bokkel Huinink, M.E.L. van der Burg et al., Long-term survival in ovarian cancer, *Eur. J. Cancer* **27** (1991), 1367–1372.

[22] C.J. Rodenburg, C.J. Cornelisse, J. Hermans and G.J. Fleuren, DNA flow cytometry and morphometry as prognostic indicators in advanced ovarian cancer: A step forward in predicting the clinical outcome, *Gynecol. Oncol.* **29** (1988), 176–187.

[23] L. Rome, N. Kedersha and D. Chugani, Unlocking vaults: organelles in search of a function, *Trends Cell. Biol.* **1** (1991), 47–50.

[24] R.J. Scheper, J.W.M. Bulte, J.G.P. Brakkee et al., Monoclonal antibody JSB-1 detects a highly conserved epitope on the P-glycoprotein associated with multidrug resistance, *Int. J. Cancer* **42** (1988), 389–394.

[25] R.J. Scheper, H.J. Broxterman, G.L. Scheffer et al., Overexpression of a 110 kD vesicular protein in non-P-glycoprotein mediated multidrug resistance, *Cancer Res.* **53** (1993), 1475–1479.

[26] G.L. Scheffer, P.L. Wijngaard, M.J. Flens, M.A. Izquierdo, M.L. Slovak, H.M. Pinedo, C.J. Meijer, H.C. Clevers, R.J. Scheper, The drug resistance-related protein LRP is the human major vault protein, *Nat. Med.* **1** (1995), 578–582.

[27] S.F. Serov, L.H. Scully and L.H. Sobin, *Histologic Typing of Ovarian Tumours*, World Health Organization, Geneva, 1973, pp. 17–18.

[28] F.A. Sinicrope, J. Hart, T.A. Brasitus, F. Michelassi, J. Lee and A.R. Safa, Relationship of P-glycoprotein and carcinoembryonic antigen expression in human colon carcinoma to local invasion, DNA ploidy and disease relapse, *Cancer* **74** (1994), 2908–2917.

[29] M.L. Slovak, J.P. Ho, S.P. Cole, R.G. Deeley, L. Greenberger, E.G. de Vries, H.J. Broxterman, G.L. Scheffer and R.J. Scheper, The LRP gene encoding a major vault protein associated with drug resistance maps proximal to MRP on chromosome 16: evidence that chromosome breakage plays a key role in MRP or LRP gene amplification, *Cancer Res.* **55** (1995), 4214–4219.

[30] O. Visser, J.W.W. Coebergh and L.J. Schouten, eds, *Incidence of Cancer in the Netherlands 1990*, Second report of the Netherlands Cancer Registry, Netherlands Cancer Registry, Utrecht, 1993.

[31] A.R. Weger, C. Ludescher, G. Mikuz et al., The value of prognostic indicators in advanced ovarian cancer, *Path. Res. Pract.* **185** (1989), 676–679.

[32] R.S. Weinstein, J.R. Kuszak, L.F. Kluskens et al., P-glycoproteins in pathology: the multidrug resistance gene family in humans, *Hum. Pathol.* **21** (1990), 34–48.

[33] G.J.R. Zaman, M.J. Flens, M.R. van Leusden et al., The human multidrug resistance-associated protein (MRP) is a plasma membrane drug efflux pump, *Proc. Natl. Acad. Sci.* **91** (1994), 8822–8826.

[34] A.G.J. van der Zee, B. van Ommen, C. Meijer et al., Glutathione S-transferase activity and isoenzyme composition in benign ovarian tumours, untreated malignant ovarian tumours, and malignant ovarian tumours after platinum/cyclophosphamide chemotherapy, *Br. J. Cancer* **66** (1992), 930–936.

[35] A.G.J. van der Zee, H. Hollema, A.J.H. Suurmeijer et al., The value of P-glycoprotein, glutathione S-transferase pi, c-erb-2, and p53 as prognostic factors in ovarian carcinomas, *J. Clin. Oncol.* **13** (1995), 70–78.