Prognostic factors in ovarian cancer: current evidence and future prospects

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Summary

In ovarian cancer, translational research on the prognostic impact of molecular biological factors has until now not led to clinical implementation of any of these factors. This is partly due to the often conflicting results of different prognostic factor studies on the same molecular biological factor. We have performed meta-analyses on studies in ovarian cancer on four putative prognostic molecular biological factors, epidermal growth factor-receptor (EGFR), HER-2/neu, glutathione-*S*-transferase (GST)-pi and p53. Odds ratios were estimated for the increase in death at 1 and 5 years for patients with ovarian cancer, harbouring aberrant EGFR, HER-2/neu, GST-pi and p53, respectively. Patients with aberrant Her-2/neu or p53 in their tumours had significantly worse odds of surviving 1 and 5 years, respectively. Patients with aberrant EGFR in their tumours only had a significantly greater risk of mortality at 5 years, while there seemed to be a trend for a decreased probability of 5-year survival for patients with aberrant GST-pi in their tumours. Despite inevitable flaws (such as small individual study sizes, publication bias, etc.) our meta-analysis confirms that therapeutic drugs targeted at EGFR, HER-2/neu, GST-pi and p53 may have therapeutic potential. Since ovarian cancer is a relatively rare disease, international collaboration to increase the number of patients to be analysed is critical for progress in translational research on the prognostic impact of molecular biological factors and on innovative treatment in ovarian cancer. In addition it is important to reach a consensus about guidelines for the design, conduct and analysis of translational studies in ovarian cancer.

Introduction

Ovarian cancer is the leading cause of death from gynaecological cancers and the fifth most common cause of cancer death in women after lung, breast, colorectal and pancreas cancers. It is estimated that 25,400 new ovarian cancer cases will be diagnosed in the United States in the year 2003 and that an estimated 14,300 deaths from ovarian cancer will occur [1]. This high death rate is related to the difficulty of detecting ovarian cancer at an early stage, as well as to the lack of effective therapies for advanced disease.

Current clinical decision-making in ovarian cancer is based on so-called "classic" clinicopathological prognostic factors, such as stage (according to the International Federation of Gynaecology and Obstetrics [FIGO]), differentiation grade and histiotype. Critical questions for the clinicians who treat patients with ovarian cancer are (in part) different in early-stage patients when compared with late-stage patients. In early-stage patients, it is particularly important: first, to identify those patients who will recur after macroscopically complete resection of disease, and second, to identify those patients with a high chance of recurrence who will benefit from adjuvant chemotherapy [2]. In late-stage ovarian cancer, it would be extremely helpful to be able to predict which patients would respond to chemotherapy and, ideally, which type of chemotherapy should be given [3]. Based on current classic clinicopathological prognostic factors, it is, however, not possible to answer these questions.

Ovarian cancer, like all cancers, is thought to result from an accumulation of genetic changes. Ultimately, when the genetic changes that underlie ovarian carcinogenesis are better understood, it should be possible to classify early- and late-stage ovarian cancer patients in a more specific way than is currently possible. Based on this "molecular biological" classifica-

tion, it should also become possible to treat patients more efficiently and in a more individualised manner.

In breast cancer, several cell biological prognostic markers have already been identified and their assessment in combination with therapeutic consequences has been implemented in daily clinical practice. For example, it has been clearly demonstrated that expression of oestrogen receptors is required for optimal responses to hormonal therapy and, more recently, that breast cancers that overexpress the epidermal growth factor receptor (EGFR) type II (HER-2/neu) are less sensitive to chemotherapy regimens lacking anthracyclines [4]. Trastuzumab, a monoclonal antibody targeting HER-2/neu, potentiates the antitumour effect of chemotherapy in patients with metastatic breast cancer [5].

In ovarian cancer, translational research on the prognostic impact of molecular biological factors has until now not led to the clinical implementation of any of these factors. Among other reasons, this is due to the often inconsistent or even contradictory results of different prognostic factor studies in ovarian cancer on the same molecular biological factor. In this review, we will present meta-analyses which we performed on studies in ovarian cancer on four putative prognostic molecular biological factors, namely EGFR, HER-2/neu, glutathione-*S*-transferase (GST)-pi and p53. These four markers were selected because of the large number of studies that have been performed on each of them, their possible role in response to chemotherapy and the availability of clinical intervention studies that target their expression in tumours from ovarian cancer patients. Our aim was to identify the methodological differences between the different prognostic studies and to produce a more precise estimate of the prognostic significance of these factors. In addition, we will also summarise the available studies in ovarian cancer that have applied the currently rapidly emerging DNA microarray technology to identify gene expression profiles that may predict prognosis. Finally, we discuss why translational research in ovarian cancer has not yet made any difference with regard to clinical decision-making, and how future translational research on the prognostic impact of molecular biological factors could be performed.

Meta-analyses

Search for prognostic studies

A PubMed search for studies investigating the prognostic significance of EGFR, HER-2/neu, GST-pi, and p53 in ovarian cancer was performed listing one of the search terms "EGFR", "HER-2/neu", "GST-pi", or "p53" in combination with one or more of the keywords "ovary", "ovarian cancer", "ovarian carcinoma", "ovarian neoplasm", "prognosis", "prognostic factor", "survival", or "response to chemotherapy". The references of all publications were hand-searched in order to identify missing relevant publications.

Methodological variability and inclusion criteria

Performing meta-analyses of prognostic factor studies in ovarian cancer is rather problematic. There is considerable variability between prognostic studies with respect to type of study design, patient inclusion criteria, assays or methods used to determine the status of the specific factor, determinations of factor cut-off points, and definition of study end-points for survival and response to chemotherapy. Furthermore, frequently no quantitative information beyond a *P*-value or even just "not significant" is provided. Therefore, the minimum criteria for studies to be included in our meta-analyses were: presence of a clear definition of positivity or negativity of the specific factor; availability of quantitative information on overall survival rates at 1 and/or 5 years (reported, or depicted in a graph) in relation to the status of the specific factor. In the subgroup of studies in which both mutation analysis as well as expression analysis of the specific factor was performed, only data as determined by mutational analysis were used, unless more data as determined by expression analysis were available. Where a single study had been reported on multiple occasions, only the most recent report or the report with the most complete data was included in the analysis.

Statistics

The power of heterogeneity tests is relatively low. Because the number of studies included was limited and the studies overall showed heterogeneity regarding the effect estimates, we show the results of the meta-analyses using random effects models. Random effects (DerSimonian-Laird) meta-analysis computes the odds ratios of the individual studies, the summary, the random effects variance, and heterogeneity. Woolf's formula was used for the calculations of the within variance. Studies with zero or infinite odds ratio were omitted, as their variance cannot be calculated sensibly.

The "plot" method that is presented in the figures shows standard meta-analysis plots. The 95% con-

fidence interval (CI) for each study is given by a horizontal line, and the point estimate is given by a square whose height is inversely proportional to the standard error of the estimate. The summary odds ratio is drawn as a diamond with horizontal limits at the confidence limits and width inversely proportional to its standard error. Odds ratios lower than 1 indicate a decreased risk to survive for patients affected by ovarian tumours with aberrant EGFR, HER-2/neu, GST-pi or p53 (e.g. an increased risk of death). Meta-analyses were performed using the Rmeta package of the R Project for Statistical Computing (Build1.7.0).

The class I family of receptor tyrosine kinases

The class I family of receptor tyrosine kinases consists of four closely related receptors that use kinase activity as the signal transduction trigger: EGFR (erbB-1), HER-2/neu (erbB-2), HER-3 (erbB-3), and HER-4 (erbB-4). These receptors modulate signalling pathways which control cell proliferation and differentiation [6]. It has further been shown by studies in human ovarian cancer cell lines that EGFR and HER-2/neu overexpression may play a role in sensitivity to chemotherapy. In two ovarian cancer cell lines, it was demonstrated that blockage of EGFR expression by an antibody resulted in an enhanced response to platinum compounds [7–9]. However, in one of those cell lines, short exposure to EGF also led to an increased response to cisplatin [10,11]. In addition, the effect of reversal of HER-2/neu overexpression in the ovarian cancer cell line SK-OV-3 on sensitivity to paclitaxel, doxorubicin and cisplatin has been studied. The data regarding sensitivity to paclitaxel are conflicting; one study reported an increased and one a decreased sensitivity of the SK-OV-3 cells to paclitaxel upon reversal of Her-2/neu overexpression. The sensitivity of the ovarian cancer cells to doxorubicin and cisplatin was not altered [12,13]. However, it was shown *in vivo* that an anti-HER-2/neu antibody enhanced the cytotoxicity of cisplatin against the SK-OV-3 cell line [14]. In another ovarian cancer cell line, it was also found that exposure to an anti-HER-2/neu antibody increased *in vitro* sensitivity to cisplatin [15].

Epidermal growth factor receptor (EGFR)

Nineteen studies were identified that investigated whether EGFR status of ovarian cancers was related with prognosis (see Table 1). EGFR status was determined by three different methods: a radioligand-binding assay, immunohistochemistry and reverse transcriptase-polymerase chain reaction. EGFR positivity was detected in 12–82% of the ovarian cancers. EGFR positivity of ovarian tumours was associated in one study with a better and in 8 studies with worse overall survival. In 6 studies, no relationship between EGFR positivity of ovarian tumours and overall survival was found.

Thirteen studies were included in the meta-analysis [16–28]. The meta-analysis demonstrated a decrease in survival at 1 and 5 years for patients with EGFR-positive ovarian tumours with respective summary odds ratios of 0.94 (95% CI: 0.58–1.51, not significant) and 0.38 (0.24–0.59, significant) (see Figs. 1 and 2). The χ^2 with df (degrees of freedom) = 10 for survival at 1 and 5 years was 15.65 ($P = 0.1101$) and 21.08 ($P = 0.0206$), respectively.

Several agents that block the activation of the EGFR have been developed, including small-molecule tyrosine kinase inhibitors (TKIs), such as ZD1839 ("Iressa") and OSI-774. ZD1839 has been evaluated in three phase I trials, including 33 ovarian cancer patients. Of these 33 patients, three patients had stable disease during treatment with ZD1839 [29]. A phase II trial of OSI-774 in 34 patients with heavily pre-treated ovarian cancer showed three patients with partial responses, while 42% of the patients had stable disease [30,31]. No data are as yet available on a possible relationship between response to OSI-774 and EGFR expression levels in the individual tumours.

HER-2/neu (c-erbB2)

Twenty-five studies were identified that evaluated the prognostic value of the HER-2/neu status of ovarian cancers (see Table 2). Most studies used immunohistochemistry to determine the HER-2/neu status of the ovarian tumours. HER-2/neu overexpression and/or amplification was observed in 5–66% of ovarian cancers. Nineteen studies were eligible for the meta-analysis [19,21,22,27,32–46] The meta-analysis showed that ovarian cancer patients with HER-2/neu-positive tumours had a worse probability of survival at 1 and 5 years with respective significant summary odds ratios of 0.52 (0.36–0.76) and 0.61 (0.4–0.92) (see Figs. 3 and 4). The χ^2 for survival at 1 and 5 years was 30.6 ($P = 0.0151$) and 25.07 ($P = 0.0226$), respectively.

HER-2/neu overexpression can be inhibited in ovarian cancer cells by repressing the HER-2/neu promoter via the adenovirus type 5 *E1A* gene, which encodes a well-known transcription factor. In *in vitro* and in animal studies, this inhibition appeared to abolish the tumorigenicity and metastatic capability

Table 1
Prognostic significance of EGFR-positivity in ovarian cancer

Author [Ref. no.]	*N*, FIGO stage	% EGFR positive tumours	Technique	Correlation with survival	Correlation with response to chemotherapy
Bauknecht et al. [16]	98,I/II/III/IV	35	Binding assay ^{125}I-EGF	UA, better OS ($P = 0.0004$–0.011)	UA, better response ($P < 0.01$)
Kohler et al. [17]	140, I/II/III/IV	36	Binding assay ^{125}I-EGF	UA, no relationship with OS in stage III/IV	UA, better response ($P < 0.01$)
Berchuck et al. [18]	87, I/II/III/IV	77	IHC, mAb 528 (frozen)	UA, worse OS ($P < 0.05$)	UA, no relationship ($P = 0.1$)
Scambia et al. [116]	72, III/IV	54	Binding assay ^{125}I-EGF	UA, worse PFS ($P = 0.0005$); MA, ($P = 0.0005$)	UA, no relationship
van der Burg et al. [117]	50, I/II/III/IV	63 82 78	Binding assay ^{125}I-EGF; IHC, mAb 2E9 (frozen); mAb EGF-R1 (frozen)	UA, no relationship with PFS ($P > 0.3$)	
Janinis et al. [118]	40, I/II/III/IV	40	IHC, mAb 6080-1 (paraffin)	UA, no relationship with OS ($P = 0.559$)	
Dam et al. [19]	80, I/II/III/IV	12	IHC, mAb R1 (frozen)	MA, worse OS ($P = 0.03$)	UA, no relationship
Scambia et al. [20]	117, I/II/III/IV	54	Binding assay ^{125}I-EGF	UA, worse OS ($P = 0.0022$) and PFS ($P = 0.0033$); MA, ($P = 0.014$), ($P = 0.03$)	UA, worse response in stage II–IV ($P = 0.031$); MA, ($P = 0.052$)
Devitt et al. [21]	50, I/II/III/IV	50	IHC, mAb 31G7 (paraffin)	UA, no relationship with OS ($P = 0.31$)	UA, no relationship
Meden et al. [22]	266, I/II/III/IV	13	IHC, mAb (paraffin)	UA, no relationship with OS	
Bartlett et al. [23]	62, I/II/III/IV	73	RT-PCR	UA, worse OS ($P = 0.007$); MA, ($P = 0.1$)	
Khater et al. [119]	35, I/II/III/IV	60	IHC, pAb4 (paraffin)	UA, worse OS and PFS	
Fischer-Colbrie et al. [24]	108, I/II/III/IV	61	Binding assay ^{125}I-EGF	UA, worse OS ($P = 0.043$) and PFS ($P = 0.0533$)	UA, worse survival rate after chemotherapy, indicating resistance ($P = 0.03$)
Kamel [25]	104, I/II/III/IV	66	IHC, pAb4 (paraffin)	UA, worse OS ($P < 0.01$); MA, ($P = 0.007$)	
Goff et al. [120]	54, III/IV	55	IHC, 31G7 (paraffin)		UA, no relationship ($P = 0.44$)
Baekelandt et al. [26]	185, III	22	IHC, pAb (paraffin)	UA, no relationship with OS ($P = 0.0669$)	UA, no relationship ($P = 0.458$)
Skirnisdottir et al. [27]	106, I/II	35	IHC, mAb (paraffin)	UA, worse OS ($P = 0.025$); MA, ($P = 0.0183$)	
Nagai et al. [28]	39, I/II/III/IV	77	Binding assay ^{125}I-EGF	UA, no relationship with OS ($P = 0.3559$); MA, ($P = 0.6840$)	
Ferrandina et al. [121]	76, III/IV	57	IHC, mAb 108 (paraffin)		UA, no relationship

IHC, immunohistochemistry; mAb, monoclonal antibody; pAb, polyclonal antibody; RT-PCR, reverse transcriptase polymerase chain reaction; UA, univariate analysis; MA, multivariate analysis; OS, overall survival; PFS, progression free survival; EGFR, epidermal growth factor receptor; FIGO, International Federation of Gynecology and Obstetrics.

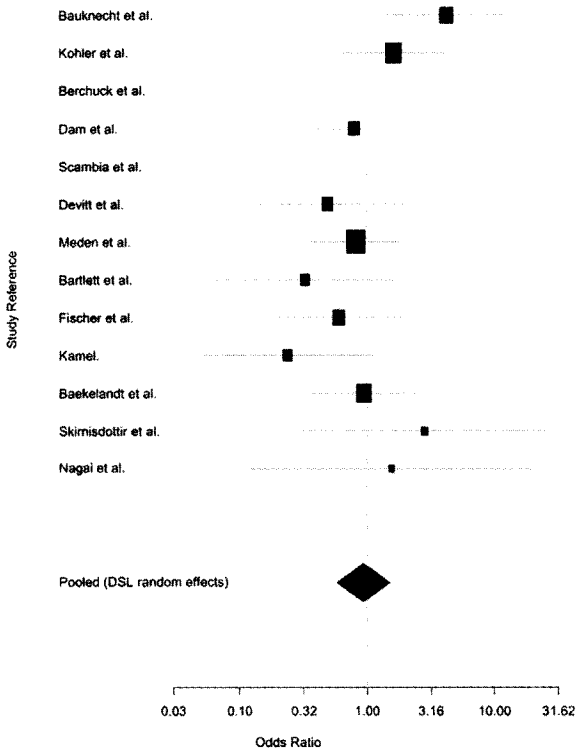

Fig. 1. Odds ratios and 95% CI of survival at 1 year for patients with EGFR-positive tumours. Individual odds ratios: squares whose height are inversely proportional to the standard error of the estimate, and their respective CIs; horizontal lines. Summary odds ratio: diamond with horizontal limits at the CIs and width inversely related to its standard error. Odds ratios lower than 1 indicate a decreased risk to survive for patients affected by EGFR-positive ovarian tumours.

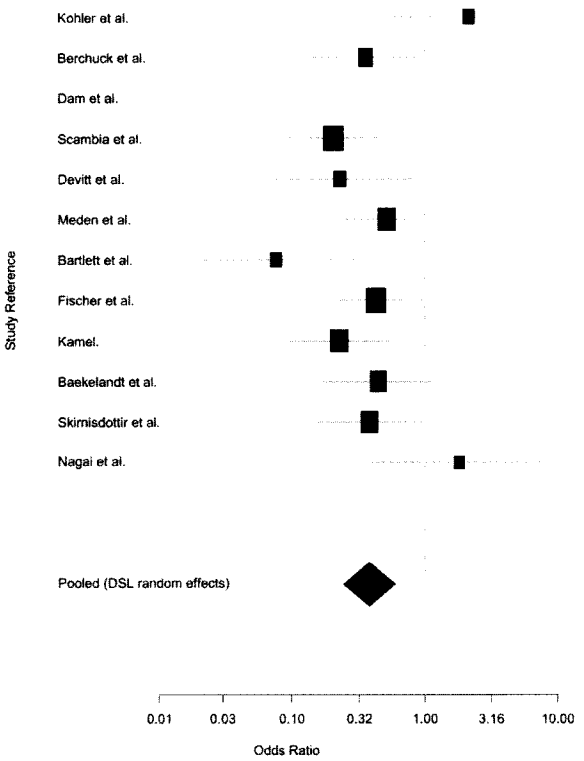

Fig. 2. Odds ratios and 95% CI of survival at 5 years for patients with EGFR-positive tumours (symbols as in Fig. 1).

induced by the HER-2/neu oncogene. In a phase I clinical trial, it has been demonstrated that cationic liposome-mediated *E1A* gene transfer in patients with HER-2/neu-overexpressing breast and ovarian cancers was feasible [47]. In addition, anti-HER-2/neu monoclonal antibodies (Mabs) have been designed to specifically antagonise the function of the HER-2/neu receptor in HER-2/neu-positive tumours (e.g. trastuzumab). A phase II trial with single agent trastuzumab in patients with recurrent or refractory ovarian or primary peritoneal cancer with overexpression of HER-2/neu showed a low frequency of HER-2/neu overexpression and low rate of objective response among patients with HER-2/neu overexpression [48]. Currently, two trials are ongoing, using trastuzumab in combination with weekly paclitaxel and carboplatin, one in patients with newly diagnosed, advanced stage ovarian cancer, and the other in patients with platinum-sensitive recurrent epithelial ovarian cancer [49].

Glutathione (GSH)/glutathione-*S*-transferase pi (GST-pi)

Glutathione (GSH) is a non-protein thiol, which is most abundantly present in the cytoplasm [50]. GSH synthesis depends on the formation of precursors by the enzyme gamma-glutamyl transpeptidase (GGT), and the successive actions of cytosolic enzymes such as gamma-glutamylcysteine synthetase (GCS) and glutathione synthetase [51,52]. GSH plays an important role in the cellular detoxification of various xenobiotics, such as platinum-drugs [50]. GSH can bind cisplatin (or alkylating agents) in a glutathione-cisplatin chelate complex. This complex is eliminated from tumour cells by a cell membrane-bound export pump, thereby preventing the formation of drug-DNA adducts [53,54].

Apart from a direct binding of platinum compounds to GSH, binding of platinum compounds to GSH may also occur by conjugation, mediated by gluthatione-*S*-transferases (GSTs). Currently, five isozymes of GST have been identified; alpha, mu, pi, theta and zeta, of which GST-pi appears to be the predominant GST

Table 2
Prognostic significance of HER-2/neu positivity in ovarian cancer

Author [Ref. no.]	*N*, FIGO stage	% amplification/ overexpression	Technique	Correlation with survival	Correlation with response to chemotherapy
Slamon et al. [32]	120	26	Southern analysis	UA, worse OS ($P < 0.0001$); MA, ($P < 0.0001$)	
Berchuck et al. [33]	73, III/IV	32	IHC, mAb TA1 (frozen)	UA, worse OS ($P < 0.001$)	UA, worse response ($P < 0.05$)
Kacinski et al. [34]	72, I/II/III/IV	43	IHC, mAb-1 (paraffin)	UA, no relationship with OS or RFS	
Scambia et al. [35]	94, III/IV	35	IHC, mAb (paraffin)	UA, no relationship with OS ($P = 0.86$) or PFS ($P = 0.63$)	
Rubin et al. [36]	105, III/IV	24	IHC, mAb 9G6 (frozen)	UA, no relationship with OS; MA, ($P = 0.09$)	No relationship
Singleton et al. [37]	56, III/IV	18	IHC, mAb-1 (paraffin)	UA, stainability was not related with OS ($P = 0.37$)	
Rubin et al. [38]	40, I/II	20	IHC, mAb 9G6 (frozen)	UA, no relationship with OS or RFS	
Dam et al. [19]	80, I/II/III/IV	24	IHC, mAb NEU3 (frozen)	UA; MA, no relationship with OS	UA, no relationship
Makar et al. [122]	74, I/II/III/IV	5	IHC, mAb NCL-CB11 (paraffin)	UA, no relationship with OS	
Devitt et al. [21]	61, I/II/III/IV	10	IHC, mAb 4D5 (paraffin)	UA, worse OS ($P < 0.05$)	
Meden et al. [22]	266, I/II/III/IV	18	IHC, mAb 9G6 (paraffin)	UA, worse OS ($P = 0.002$); MA ($P = 0.0118$)	
Felip et al. [39]	106, I/II/III/IV	22	IHC, mAb CB11 (paraffin)	UA, worse OS in stage III/IV; MA, ($P < 0.001$)	UA, worse response ($P = 0.0043$)
Fajac et al. [40]	65, I/II/III/IV	14	Southern analysis	UA, worse OS ($P = 0.04$); MA, ($P = 0.19$)	
van der Zee et al. [78]	89, I/II/III/IV	20	IHC, mAb CB11 (paraffin)	UA, no relationship with OS or PFS	No relationship
Wong et al. [123]	46, I/II/III/IV	48	Differential PCR	UA, no relationship	
Medl et al. [41]	196, I/II/III/IV	40	Quantitative PCR	UA, no relationship with OS ($P = 0.67$)	
Tanner et al. [42]	79, I/II/III/IV	20	S1 nuclease assay	UA, no relationship with OS in stage I–IV ($P = 0.7$), worse OS in stage III/IV ($P = 0.04$)	
Goff et al. [120]	54, III/IV	28	IHC, Ab-3 (paraffin)		UA, no relationship ($P = 0.19$)
Beckmann et al. [43]	79, I/II/III/IV	28	FdPCR	UA, worse OS and RFS ($P < 0.05$)	
Meden et al. [44]	208, I/II/III/IV	22	IHC, mAb 9G6 (paraffin)	UA, worse OS ($P = 0.0003$)	Significant dose-response effect in patients with HER-2/neu negative tumours ($P = 0.0341$)
Ross et al. [124]	61, I/II/III/IV	66	FISH (paraffin)	UA; MA, no relationship with OS	
Hengstler et al. [45]	77, I/II/III/IV	52	S1 nuclease assay	UA, worse OS ($P = 0.0001$); MA, ($P = 0.035$)	
Seki et al. [46]	48, I/III/IV	25	Differential PCR	UA, no relationship with OS	
Skirnisdottir et al. [27]	106, I/II	19	IHC, (paraffin)	UA, no relationship with OS ($P = 0.5872$)	
Ferrandina et al. [121]	76, III/IV	21	IHC, mAb 300G9 (paraffin)		UA, no relationship

IHC, immunohistochemistry; mAb, monoclonal antibody; pAb, polyclonal antibody; PCR, polymerase chain reaction; FdPCR, fluorescent differential PCR; FISH, fluorescence *in situ* hybridisation; UA, univariate analysis; MA, multivariate analysis; NS, not significant; OS, overall survival; PFS, progression-free survival; RFS, relapse-free survival.

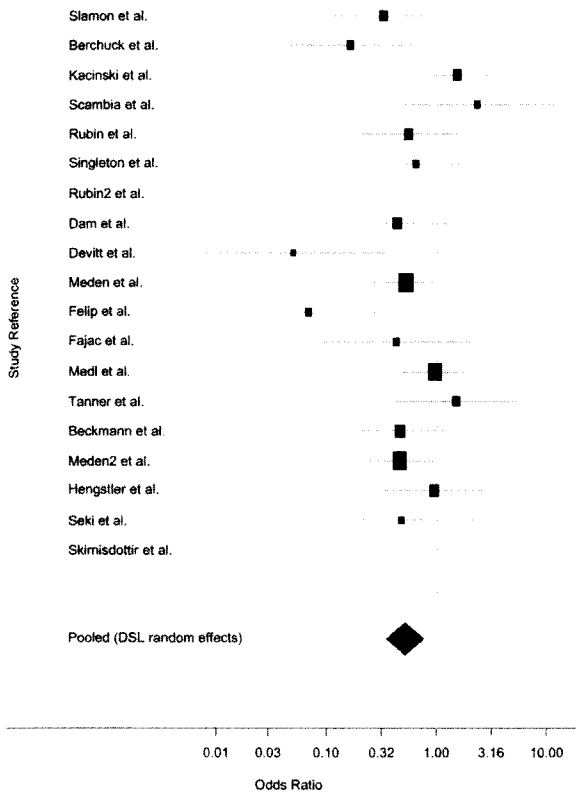

Fig. 3. Odds ratios and 95% CIs of survival at 1 year for patients with HER-2/neu-positive tumours (symbols as in Fig. 1).

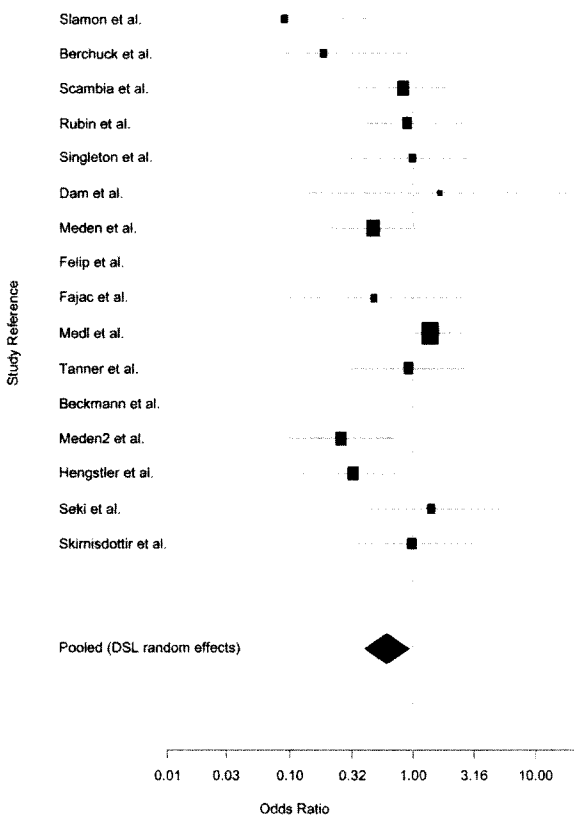

Fig. 4. Odds ratios and 95% CIs of survival at 5 years for patients with HER-2/neu-positive tumours (symbols as in Fig. 1).

isozyme in ovarian cancers [55–58]. However, in two different panels of ovarian cancer cell lines, no association was found between GST levels and resistance to platinum compounds [59,60].

Twenty-one studies were identified which have analysed the predictive value of GST-pi in ovarian cancer with respect to survival and/or response to chemotherapy (see Table 3). With immunohistochemistry, GST-pi was detected in 25–100% of ovarian cancers. Only eight studies could be included in our meta-analysis [61–68]. The meta-analysis illustrated that patients with GST-pi-positive tumours had an increase in mortality at 1 and 5 years with respective summary odds ratios of 0.56 (0.24–1.31, not significant) and 0.57 (0.3–1.07, not significant) (see Figs. 5 and 6).The χ^2 for survival at 1 and 5 years was 22.26 ($P = 0.0011$) and 26.3 ($P = 0.0004$), respectively.

Cellular GSH can be depleted by buthionine sulfoximine, a selective inhibitor of γ-glutamylcysteine synthetase. Phase I and II clinical trials have been performed in ovarian cancer with buthionine sulfoximine in conjunction with melphalan. Only results of the phase I study are available. A progressive decline of cellular GSH was found in peripheral mononuclear cells, while GSH was depleted in sequential tumour biopsies to a variable extent, but with a similar time course [69]. In addition, a phase II clinical trial of TLK286, a GST-pi activated glutathione analogue, has been performed in 21 patients with platinum-resistant ovarian cancer. Of the 15 patients that were evaluable for response to TLK286, 5 patients had stable disease and one patient had a partial response. No data are available on the GST-pi status of the ovarian tumours [70].

p53

*p*53 is a human tumour suppressor gene which in normal cells plays a key role in co-ordinating cell cycle arrest, DNA repair and apoptosis in response to DNA damage (caused by radiotherapy, DNA alkylating chemotherapy, or foreign DNA synthesis). In cancer cells, the loss of wild-type p53 function (via different mechanisms such as mutation, protein degradation or sequestration) can lead to more aggressive

Table 3
Prognostic significance of GST-pi positivity

Author [Ref. no.]	*N*, FIGO stage	% overexpression	Technique	Correlation with survival	Correlation with response to chemotherapy
Murphy et al. [58]	53, I/II/III/IV		GST activity assay		UA, no relationship ($P = 0.653$)
van der Zee et al. [56]	17, III		HPLC		UA, no relationship
Green et al. [61]	86, I/II/III/IV	99 (38, +; 53, ++; 8, +++)	IHC, (paraffin)	UA, stainability was related with worse OS ($P < 0.01$)	UA, stainability is related with worse response ($P = 0.003$)
Hamada et al. [62]	61, I/II/III/IV	54	IHC, (paraffin)	UA, worse OS ($P < 0.005$)	UA, worse response ($P < 0.005$)
van der Zee et al. [78]	89, I/II/III/IV	89	IHC, pAb (paraffin)	UA, no relationship with OS or PFS	UA, no relationship
Cheng et al. [125]	20, III/IV		Western blot analysis		UA, higher levels after chemotherapy are related with resistance ($P < 0.05$)
Hirazono et al. [63]	45, I/II/III/IV	57	IHC, pAb (paraffin)	UA, worse OS ($P < 0.05$–0.001)	
Ghazal-Aswad et al. [126]	39	87	IHC, pAb (paraffin)		UA, no relationship ($P > 0.05$)
Wrigley et al. [127]	97, I/II/III/IV	85	IHC, pAb (paraffin) ; Western blot analysis; GST activity assay	UA, no relationship with OS or PFS	UA, no relationship (overexpression is related to worse response to combination chemotherapy ($P = 0.025$))
Germain et al. [128]	86, I/II/III/IV	37	IHC, pAb (paraffin)		UA, no relationship ($P = 0.708$)
Tanner et al. [64]	121, I/II/III/IV		Western blot analysis	UA, no relationship with OS ($P = 0.14$)	
Codegoni et al. [129]	33, III/IV		Northern blot analysis	UA, no relationship with OS ($P = 0.085$)	UA, no relationship ($P = 0.276$)
Ferrandina et al. [65]	145, I/II/III/IV		GST activity assay	UA, better OS ($P = 0.043$) and PFS (0.037); MA, in stage III/IV ($P = 0.057$), ($P = 0.008$)	UA, better response in stage II/III/IV ($P = 0.014$); MA, ($P = 0.013$)
Silvestrini et al. [130]	168, III/IV	25	IHC, pAb NCL-GST-pi (paraffin)	Slightly higher chance of 3-year OS after cisplatin treatment	UA, no relationship
Kase et al. [131]	87, I/II/III/IV	63	IHC, mAb (paraffin)		UA, worse response ($P < 0.01$)
Joncourt et al. [132]	39, I/II/III/IV		GST-pi activity assay	UA, no relationship with OS	UA, no relationship
Kigawa et al. [133]	26, relapsed patients	65	IHC, mAb (paraffin); Western blot analysis		UA, no relationship with response to second line chemotherapy
Muso et al. [66]	58, I/II/III/IV	79	IHC, (paraffin)	UA, worse OS ($P = 0.0337$)	
Yokoyama et al. [134]	58, I/II/III/IV	59	IHC, pAb (paraffin)	UA, worse DFS ($P < 0.05$)	UA, worse response ($P < 0.05$); MA, ($P = 0.075$)
Mayr et al. [67]	213, I/II/III/IV	50	IHC, pAb (paraffin)	UA, worse OS in stage III ($P < 0.03$); MA, (0.093)	UA, worse response ($P < 0.027$)
Satoh et al. [68]	117, I/II/III/IV	66	IHC, mAb (paraffin)	UA, stainability was related with worse OS ($P < 0.01$–0.05)	

IHC, immunohistochemistry; mAb, monoclonal antibody; pAb, polyclonal antibody; HPLC, high performance liquid chromatography; UA, univariate analysis; MA, multivariate analysis; GST, glutathione-*S*-transferase.

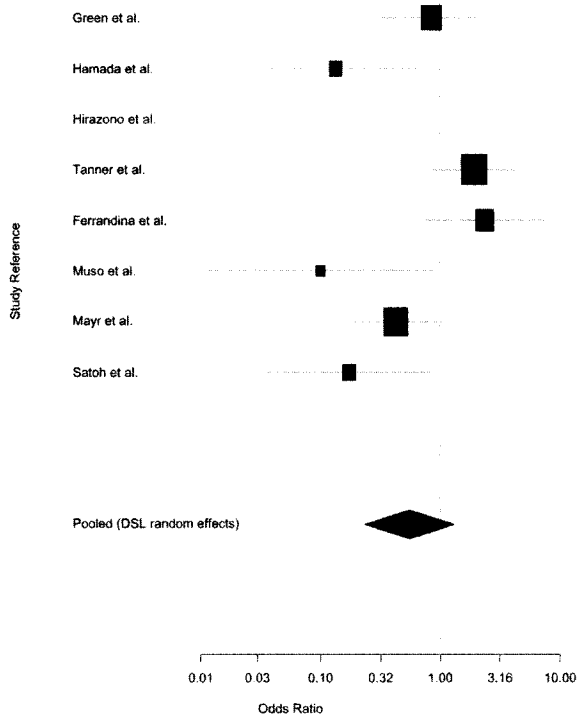

Fig. 5. Odds ratios and 95% CIs of survival at 1 year for patients with GST-pi-positive tumours (symbols as in Fig. 1).

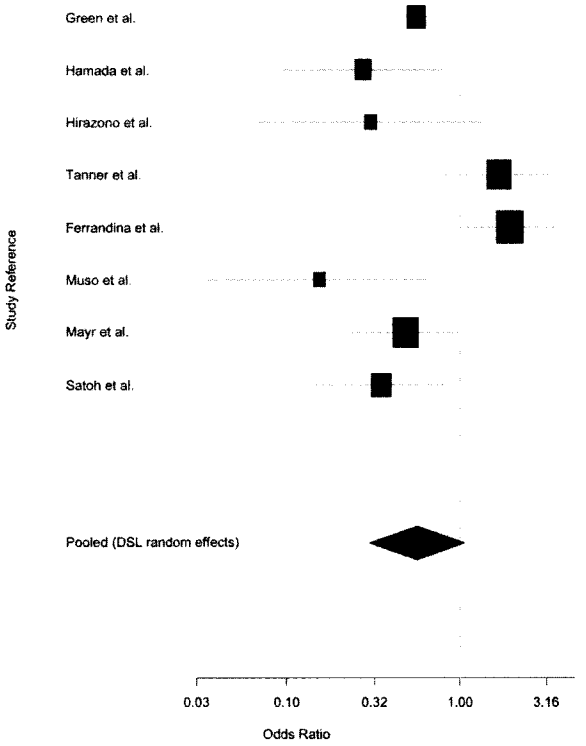

Fig. 6. Odds ratios and 95% CIs of survival at 5 years for patients with GST-pi-positive tumours (symbols as in Fig. 1).

tumour growth and worse response to chemotherapy. Fifty-three studies were identified that examined the prognostic impact of the p53 status in ovarian cancers (see Table 4). p53 overexpression was detected in 14–79% of the ovarian cancers. Thirty-two studies were eligible for the meta-analysis [67,71–101]. The meta-analysis demonstrated that patients with p53-overexpressing ovarian tumours had a decrease in survival at 1 and 5 years with respective summary odds ratios of 0.61 (0.5–0.75, significant) and 0.39 (0.3–0.5, significant) (see Figs. 7 and 8). The χ^2 for survival at 1 and 5 years was 19.1 ($P = 0.832$) and 51.33 ($P = 0.0006$), respectively.

The adenovirus dl1520 (ONYX-015) has been attenuated by deletion of its E1B 55-kd gene region, the protein product of which is known to bind and inactivate p53 and thereby allows continued DNA synthesis and viral replication. Mutants such as dl1520 lacking this early gene product are severely deficient in their ability to replicate in normal cells. Yet, ONYX-015 selectively replicates in and destroys cancer cells lacking p53 function e.g. by presence of a p53 mutation. Cells with non-functioning p53, which are therefore resistant to apoptosis, are permissive for replication, leading to virus spread and subsequent cytolysis of the cancer cell population [102,103]. In a phase I trial, no conclusive proof of viral replication in p53-mutant cancer cells or clear-cut antitumour activity could be demonstrated. Before proceeding with studies of intraperitoneal (i.p.) ONYX-015 in ovarian cancer, either as a single agent or in combination with chemotherapy, it will be important to provide evidence of either clear-cut clinical efficacy or at least a biological effect (e.g., cancer cell infection) [103].

SCH58500 (rAD/p53) is a replication-deficient adenovirus encoding human, recombinant, wild-type p53. A phase I/II clinical trial of SCH58500 gene replacement alone and sequentially in combination with platinum-based chemotherapy in heavily pre-treated recurrent ovarian cancer showed vector-specific transgenic expression in cancer by RT-PCR in cells from both ascitic fluid and tissue biopsies, while SCH58500 combined with platinum-based chemotherapy was associated with a significant reduction of serum CA125 [104]. In a subsequent study, the long-term follow-up was evaluated of the

patients who had been enrolled onto the phase I/II trial of SCH58500 gene transfer therapy and it appeared that the 12–13-month median survival compared favourably with the 16-month median survival for individuals treated with paclitaxel at the time of initial recurrence of this disease and is more than double the 5-month survival seen with palliative radiotherapy or paclitaxel failure [105]. Recently, a phase III trial comparing carboplatin/paclitaxel against carboplain/paclitaxel with SCH58500 i.p. was closed prematurely for as yet unspecified reasons.

Microarray

Recently established DNA microarray techniques allow for the simultaneous analysis of the expression profiles of thousands of genes in ovarian tumour samples. Technically it is now possible to analyse the expression of all known genes and expressed sequence tags (ESTs) as described by the Human Genome Project in one experiment [106]. However, as Simon et al. demonstrated, that there are several possible pitfalls in the use of microarray data for prognostic classification. Experience is needed in the analytical steps required to convert tens of thousands of noisy data points into reliable and interpretable biological information. In their discussion, Simon et al. point out that microarray studies trying to identify gene expression profiles that will affect clinical decision-making should be performed with statistical rigour and be reported clearly and with unbiased statistics [107]. Until now, there are only three studies in ovarian cancer available that examined the potential use of DNA microarrays to predict (treatment) outcome. Yet, studies using DNA microarrays that tried to identify the molecular determinants of the classic clinicopathological factors (stage, grade and histiotype) in ovarian cancer have also been presented. The sample size of all of those studies is small, which severely affects the statistical reliability of the conclusions. Shridhar et al. attempted to identify molecular differences between early and late-stage ovarian carcinomas by cDNA microarray and found that the gene expression profiles were very similar [108]. Jazaeri et al. investigated patterns of gene expression in well ($n = 8$) and poorly ($n = 4$) differentiated serous papillary ovarian carcinomas and identified 99 genes whose expression was significantly different. A disproportionate number of these differentially expressed genes were located on the chromosomal region 20q13 and all exhibited higher expression in grade III tumours [109]. Three studies used DNA microarrays for identifying gene expression patterns that may predict prognosis (e.g. survival or response to chemotherapy). Lancaster et al. compared differences in gene expression profiles between short- (dead of disease within two years, $n = 10$) and long-term (still alive after seven years, $n = 8$) survivors and detected a pattern of gene expression (predictor) that correctly classified ovarian cancers into either the short- or long-term survival group in 83% (15/18) of cases. However, it should be mentioned that these data are still preliminary [110]. Wei et al. profiled methylation alterations of CpG islands in advanced stage ovarian tumours ($n = 19$) to identify candidate markers for diagnosis and prognosis of the disease. A higher degree of CpG island methylation was associated with early disease recurrence after chemotherapy. Also a select group of CpG island loci was identified that may be used to predict treatment outcome in ovarian cancer patients [111]. Sakamoto et al. compared gene expression profiles between chemotherapy-sensitive ($n = 2$) and chemotherapy-resistant ($n = 2$) ovarian tumours and identified six genes that were overexpressed, and eight genes that were underexpressed in the chemotherapy-resistant tumours [112].

Discussion

During the course of our meta-analyses on studies aimed at determining the prognostic value of EGFR, HER-2/neu, GST-pi and p53, it became more and more clear that the methodological variability between the different prognostic studies is considerable. As illustrated in our review, many of the prognostic studies are especially affected by small sample size, which may lead to either over- or underestimation of the relevance of the factors under investigation. Only by accepting more or less flexible study inclusion criteria for the meta-analysis did it become possible to combine the results of the various prognostic studies regarding a specific factor. Apart from the methodological differences between the prognostic studies, another important factor affecting the reliability of our meta-analyses is the problem of publication bias. Negative (non-significant) findings are seldom written up and much less published. However, despite these limitations, the results of the meta-analyses should represent a more precise estimate of the prognostic significance of EGFR, HER-2/neu, GST-pi and p53, than the individual studies by themselves. As illustrated in our meta-analyses, patients with aberrant Her-2/neu or p53 in their tumours had significantly worse odds of surviving 1 and 5 years, respectively. Patients with aberrant EGFR in their

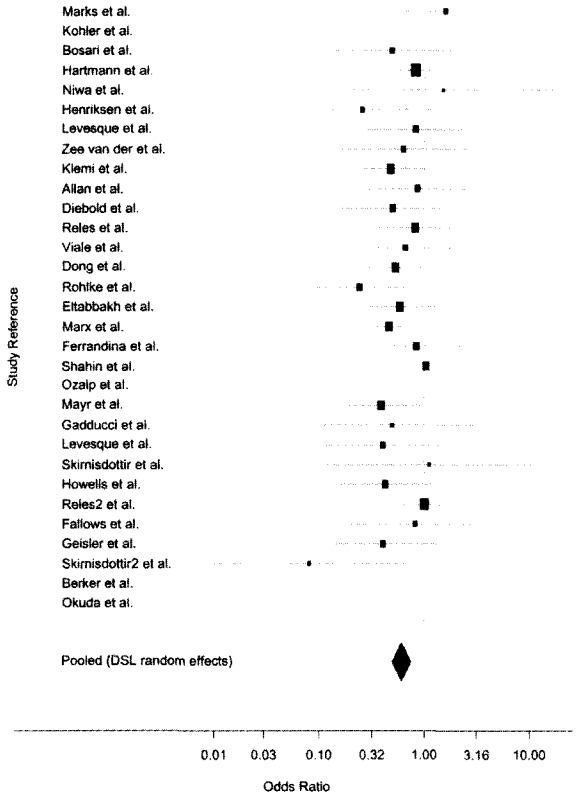

Fig. 7. Odds ratios and 95% CIs of survival at 1 year for patients with p53-positive tumours (symbols as in Fig. 1).

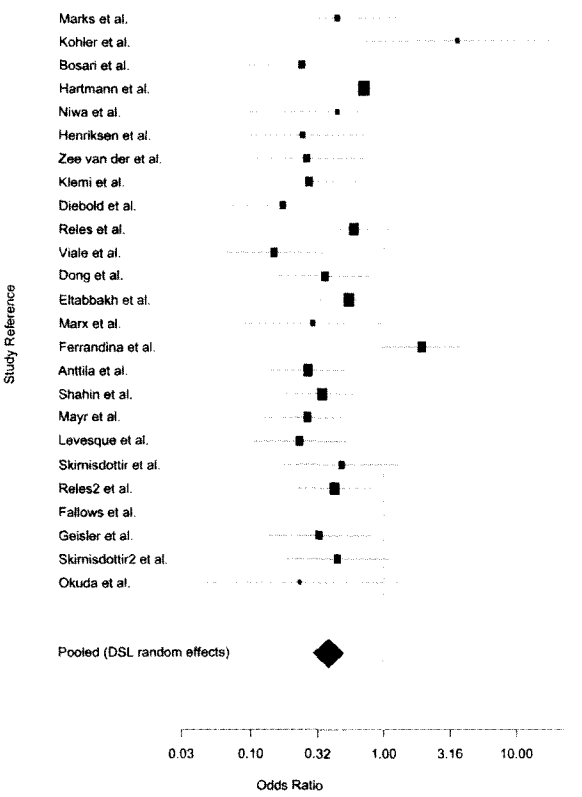

Fig. 8. Odds ratios and 95% CIs of survival at 5 years for patients with p53-positive tumours (symbols as in Fig. 1).

tumours only had a significantly greater risk of mortality at 5 years, while there seemed to be only a trend for a decreased probability of 5-year survival for patients with aberrant GST-pi in their tumours. Therapeutic agents targeting these molecular biological factors, therefore, may have therapeutic potential.

To evaluate the possible clinical relevance of molecular biological factors, large studies examining clinically relevant patient groups are needed, especially when the prevalence of the molecular biological factor under investigation is relatively low. To illustrate the number of patients that is needed for evaluation of the prognostic impact of the molecular biological factors, we have performed a Log Rank Survival Power Analysis (Simple) for early- and late-stage ovarian cancers separately to calculate the sample size needed for a study on the prognostic impact of mutant p53. It was assumed that the prevalence of mutant p53 is approximately 50%, the 5-year survival rate of early- and late-stage ovarian cancers is 70% and 20%, respectively, that a survival difference of 10% is clinically relevant, and no patients would be lost during follow-up. The statistical power achieved had to be 80% at a significance level of 5%. For a study on the prognostic impact of mutant p53 in early- and late-stage ovarian cancers an overall sample size of 711 patients and 295 patients, respectively, is required. None of the studies on the prognostic impact of p53 in ovarian cancer have included the number of early stage and/or late stage ovarian cancer patients needed according to our calculations to assure an adequate statistical power (see Table 4).

Due to the relative rarity of ovarian cancer, it is obvious that it will be difficult to include sufficient numbers of patients in future studies to obtain statistically reliable results. The incidence of ovarian cancer is about 8.4 times lower than the incidence of breast cancer [1]. The relative low incidence of ovarian cancer is particularly a problem in prognostic studies in early-stage ovarian cancer, since only about 30% of ovarian cancer patients have disease that is confined to the pelvis at diagnosis. The inclusion of an adequate number of patients is further complicated by the fact that approximately 36% of the patients with ovarian cancer are seventy years of age or older at the time of diagnosis [113]. Because of co-morbidity, older patients with ovarian cancer

Table 4
Prognostic significance of p53 positivity in ovarian cancer

Author [Ref. no.]	N, FIGO stage	% p53-positive tumours	Technique	Correlation with survival	Correlation with response to chemotherapy
Marks et al. [71]	107, I/II/III/IV	50	IHC, mAb PAb1801 (frozen)	UA, no relationship with OS in stage III/IV ($P > 0.2$)	
Kohler et al. [72]	52, I/II	29	IHC, mAb PAb1801 (paraffin)	UA, no relationship with OS or DFS	
Bosari et al. [73]	98, I/II/III/IV	45	IHC, mAb PAb1801 (paraffin)	UA, worse OS ($P = 0.0025$) and DFS ($P = 0.0011$)	
Hartmann et al. [74]	284, I/II/III/IV	62	IHC, mAb PAb1801 (paraffin)	UA, worse OS ($P = 0.04$); MA, (NS)	UA, no relationship ($P = 0.23$)
Sheridan et al. [135]	93, I/II/III/IV	47	IHC, mAb D07 (paraffin)	MA, no relationship with OS ($P = 0.286$)	
Niwa et al. [75]	31, I/II/III/IV	42	PCR-SSCP analysis + sequencing	UA, no relationship with OS ($P > 0.5$)	
Niwa et al. [75]	36	33	PCR-SSCP analysis + sequencing		UA, no relationship ($P > 0.4$)
Henriksen et al. [76]	55, I/II/III/IV	44	IHC, mAb PAb1801 (frozen)	UA, worse OS ($P = 0.002$)	
Renninson et al. [136]	50, I/II/III/IV	56	IHC, pAb CM1 (paraffin)		UA, no relationship ($P = 0.33$)
Levesque et al. [77]	90, I/II/III/IV	43	Immunofluorometric assay, mAb Pab240 + pAb CM1 (frozen)	UA, worse OS ($P = 0.06$) and DFS ($P = 0.03$); MA, ($P = 0.72$), ($P = 0.63$)	
van der Zee et al. [78]	89, I/II/III/IV	35	IHC, pAb CM1 (paraffin)	UA, worse OS ($P < 0.0001$) and PFS ($P < 0.001$)	UA, no relationship
Klemi et al. [79]	136, I/II/III/IV	44	IHC, mAb DAKO-p53 (paraffin)	UA, worse OS ($P = 0.002$); MA, ($P = 0.008$)	
Righetti et al. [137]	33, III/IV	61	IHC, mAb D07 (paraffin); PCR-SSCP analysis + sequencing		UA, p53 accumulation/ missense mutations are significantly related with resistance
Allan et al. [80]	61, I/II/III/IV	61	IHC, mAb PAb240, mAb PAb1801 (frozen), pAb CM1 (paraffin) + PCR-SSCP analysis + sequencing	UA, no relationship with OS ($P = 0.095$) or DFS ($P = 0.340$); MA, (NS)	
Diebold et al. [81]	148, I/II/III/IV	36	IHC, mAb D01 (paraffin)	UA, worse OS ($P = 0.0001$); MA, in stage I/II ($P < 0.005$), in stage III/IV ($P < 0.0001$)	
Reles et al. [82]	179, I/II/III/IV	44	IHC, mAb D01 (paraffin)	UA, no relationship with OS ($P = 0.12$) or DFS ($P = 0.26$)	
Herod et al. [138]	70, II/III/IV	61	IHC, mAb PAb1801 (paraffin)	UA, no relationship with OS ($P = 0.45$); MA, ($P = 0.05$)	UA, no relationship ($P = 0.69$)
McMenamin et al. [139]	30, I/II/III/IV	53	IHC, mAb D07 (paraffin)	UA, no relationship with OS	

Table 4 (continued)

Author [Ref. no.]	N, FIGO stage	% p53 positive tumours	Technique	Correlation with survival	Correlation with response to chemotherapy
Geisler et al. [140]	83, I/II/III/IV		IHC, mAb PAb1801 (frozen)	MA, worse OS ($P = 0.044$)	
Viale et al. [83]	112, I/II/III/IV	55	IHC, mAb PAb1801 (paraffin)	UA, worse OS ($P = 0.0004$); MA, (NS)	UA, no relationship
Dong et al. [84]	123, I/II/III/IV	46	IHC, mAb D07 (paraffin)	UA, stainability was related with worse OS ($P = 0.003$)	UA, worse response ($P < 0.05$)
Rohlke et al. [85]	83, T1/T2; T3/M1	35	IHC, mAb 6 (paraffin)	UA, worse OS ($P < 0.025$); MA, (0.023)	UA, no relationship
Eltabbakh et al. [86]	221, I/II/III/IV	48	IHC, mAb DAKO-p53 (paraffin)	UA, worse OS ($P = 0.049$); MA, ($P = 0.16$)	
Buttitta et al. [141]	53, I/II/III/IV	53	PCR-SSCP analysis + sequencing	UA, worse PFS ($P = 0.05$)	UA, worse response ($P = 0.009$)
Goff et al. [120]	54, III/IV	72	IHC, mAb D07 (paraffin)		UA, no relationship ($P = 0.058$)
Silvestrini et al. [130]	168, III/IV	65	IHC, mAb Pab1801	UA, no relationship with 3-year OS	UA, no relationship
Skomedal et al. [142]	347, I	50	IHC, pAb CM1 (paraffin)	UA, worse RFS ($P = 0.02$); MA, ($P = 0.56$)	
Marx et al. [87]	187, I/II/III/IV	14	IHC, mAb D07 (paraffin)	UA, worse OS ($P = 0.037$); MA, (NS)	UA, dose response effect in negative tumours ($P = 0.01$)
Smith-Sorensen et al. [143]	45, II/III	27	Mutation analyses		UA, better RFS in paclitaxel/cisplatin group compared to cyclophosphamide/cisplatin group ($P = 0.002$)
Shimizu et al. [144]	51, I/III/IV	35	IHC, mAb (paraffin)	MA, no relationship with OS	
Ferrandina et al. [88]	162, I/II/III/IV	52	IHC, mAb D07 (paraffin)	UA, no relationship with OS or PFS in stage III/IV	UA, related to poor response ($P = 0.012$); MA, ($P = 0.022$)
Anttila et al. [89]	316, I/II/III/IV	26	IHC, pAb CM1 (paraffin)	UA, worse OS ($P < 0.00005$) and RFS ($P = 0.0006$); MA, worse RFS ($P = 0.013$)	UA, worse response ($P = 0.001$);
Wen et al. [145]	105, I/II/III/IV	69 57	IHC, mAb D07 (frozen); PCR-SSCP + sequencing	UA, mutation and combination of overexpression and mutation were related with worse OS ($P = 0.049$), ($P = 0.02$); MA, (NS)	
Daponte et al. [146]	19, III	79	IHC, mAb D07 + pAb240 (paraffin)	UA, worse OS ($P = 0.029$)	
Shahin et al. [90]	171, I/II/III/IV	43	PCR-SSCP + sequencing	UA, worse OS ($P = 0.03$); MA, missense mutations ($P = 0.002$)	
Schmider et al. [147]	106, I/II/III/IV	48	IHC, mAb D01 + BP53-12-1 (paraffin)	IHC, no relationship with OS ($P = 0.41$)	
Ozalp et al. [91]	26, I/II/III/IV	46	IHC, mAb D07 (paraffin)	UA, worse OS ($P = 0.0053$); MA, ($P < 0.01$)	

Table 4 (continued)

Author [Ref. no.]	N, FIGO stage	% p53 positive tumours	Technique	Correlation with survival	Correlation with response to chemotherapy
Laframboise et al. [148]	43, II/III/IV	54	PCR-SSCP + sequencing	UA, no relationship with OS or DFS	UA, no relationship
Geisler et al. [149]	103, I/II/III/IV	68	IHC, mAb pAb1801 (frozen)	MA, worse OS ($P = 0.0032$)	
Lavarino et al. [150]	48, II/III/IV	60	PCR-SSCP analysis + sequencing		UA, better response to platinum/paclitaxel based therapy ($P = 0.0008$); MA, ($P = 0.024$)
Mayr et al. [67]	213, I/II/III/IV	46	IHC, mAb D07 (paraffin)	UA, worse OS in stage III ($P < 0.01$); MA, ($P = 0.012$)	UA, no relationship
Gadducci et al. [92]	38, III/IV	63	IHC, mAb pAb1801 (frozen)	UA, no relationship with OS ($P = 0.1271$)	UA, no relationship ($P = 0.717$–0.956)
Levesque et al. [93]	120, I/II/III/IV	50	Immunofluorometric Assay, mAb Pab240 + pAb CM1 (frozen)	UA, worse OS ($P < 0.01$) and DFS ($P = 0.04$); MA, ($P = 0.03$), ($P = 0.05$)	UA, worse response ($P = 0.03$)
Skirnisdottir et al. [94]	106, I/II	22	IHC, (paraffin)	UA, worse OS ($P = 0.046$); MA, ($P = 0.37$)	
Howells et al. [95]	81, I/II/III/IV	42	IHC, mAb D07 (paraffin)	UA, no relationship with OS ($P = 0.45$)	UA, no relationship
Schuyer et al. [151]	102, I/II/III/IV	44	IHC, mAb D01 (frozen); PCR-SSCP + sequencing	UA, overexpression and combination of overexpression and mutation were related with worse OS ($P = 0.03$), ($P = 0.008$); MA, (NS)	UA, no relationship
Reles et al. [96]	178, I/II/III/IV	57	PCR-SSCP analysis	UA, worse OS ($P = 0.015$) and PFS ($P = 0.029$); MA, worse OS ($P = 0.49$)	UA, worse response
Fallows et al. [97]	73, I/II/III/IV	44	PCR-SSCP analysis + sequencing	UA, no relationship with OS or DFS	UA, no relationship
Geisler et al. [98]	103, I/II/III/IV	71	IHC, mAb pAb1801 (frozen)	MA, worse OS ($P = 0.015$)	
Skirnisdottir et al. [99]	109, I/II	36	IHC, Ab (paraffin)	UA, worse OS ($P = 0.007$); MA, ($P = 0.02$)	
Berker et al. [100]	50, I/II/III/IV	66	IHC, mAb PAb1801 (frozen)	UA, no relationship ($P > 0.05$); MA, (NS)	
Sagarra et al. [152]	90, I/II/III/IV	47	IHC, mAb D07 (paraffin)	UA; MA, no relationship with OS, DFS or PFS	
Kupryjanczyk et al. [153]	229, II/III/IV	59	IHC, mAb pAb1801 (paraffin)	UA, no relationship with OS	UA, no relationship
Okuda et al. [101]	27, I/II/III	63	PCR-SSCP analysis + sequencing	UA, worse OS ($P = 0.09$); MA, ($P = 0.008$)	

IHC, immunohistochemistry; mAb, monoclonal antibody; pAb, polyclonal antibody; PCR–SSCP, polymerase chain reaction – single-strand conformational polymorphism; UA, univariate analysis; MA, multivariate analysis; NS, not significant; OS, overall survival, PFS, progression-free survival; DFS, disease-free survival; RFS, relapse-free survival.

are often not treated according to protocol, whereas (as much as possible) uniformity of treatment is important in prognostic studies in ovarian cancer.

Based on these considerations it is clear that to reach sufficient number of ovarian cancer patients in studies on prognostic factors, international collaboration is critical to ensure progress in translational research. Our power calculations are based on a minimal difference in prognosis to be detected of 10 percent. In this respect, it is encouraging to note that in a recent study by van de Vijver et al., microarray analysis on 295 breast tumours allowed the identification of gene expression signatures in breast cancer associated with differences in prognosis much larger than 10 percent [114,115]. Using DNA microarrays, it may therefore also be possible to identify gene expression profiles/signatures that can better predict prognosis in ovarian cancer than the current classic clinicopathological factors. However, it remains of the utmost importance to reach a consensus about guidelines for the design, conduct and analysis of such studies in ovarian cancer. Ideally, future prognostic studies should include ovarian tumour samples derived from patients treated in clinical trials with identical regimens, follow-up and salvage strategies. Hopefully, in that way, molecular biological factors will be identified that will make a difference in clinical decision-making in ovarian cancer, ultimately resulting in effective, individualised targeted therapy.

Acknowledgement

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