HER-2 overexpression is an independent marker of poor prognosis of advanced primary ovarian carcinoma: a multicenter study of the GINECO group

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Background: Despite numerous studies, no biological marker has been identified that accurately predicts prognosis of advanced ovarian cancer. Tumors from a homogeneous population of 117 patients with a stage III/IV ovarian cancer, enrolled in a multicenter prospective GINECO clinical trial were analyzed retrospectively.

Patients and methods: All patients received the same platinum-based combination therapy and were followed-up for a median of 68 months. Tumor expression of Ki67, BCL-2, BAX, P53 or c-erbB-2 proteins was evaluated immunohistochemically on paraffin-embedded tissues and their prognostic impact analyzed.

Results: The median rate of Ki67-positive nuclear area was 30%. BCL-2, BAX and P53 proteins were expressed in 52, 54 and 71% of the tumors, respectively, while HER-2 protein was overexpressed in 16%. Only HER-2 overexpression was significantly associated with shorter progression-free survival and overall survival. According to our multivariate analysis, the HER-2 prognostic impact was independent of classical clinical prognostic factors.

Conclusion: HER-2 appeared to influence the outcome of advanced ovarian cancer patients included in a clinical trial with prolonged follow-up, thereby suggesting that HER-2 is a potential target for treatment of this cancer.

Key words: HER-2, ovarian cancer, prognosis

Introduction

At the time of initial diagnosis, most patients with epithelial ovarian cancer already have advanced disease. Despite a high response rate to first-line chemotherapy, prognosis remains poor due to recurrences and development of resistance to chemotherapy. Current recognized prognostic factors in advanced ovarian cancers are primarily clinical, including patient performance status and characteristics of disease extension or tumor volume such as FIGO (International Federation of Gynecology and Obstetrics) stage, residual tumor size after initial surgery and presence of ascites.

However, most prognostic models proposed in the literature do not include biological factors [1]. Thus, other markers of ovarian cancer are needed that can accurately identify subpopulations of patients whose disease behaves differently from that of the majority of patients and may benefit from adapted therapeutic options. Among the biological markers described in the literature, BCL-2, BAX, HER-2, P53 and Ki67 have the potential to be of prognostic value in ovarian carcinoma.

The BCL-2 gene was identified in the t(14;18) translocation in follicular lymphomas, which juxtaposes the immunoglobulin heavy chain locus and the BCL-2 gene, leading to overexpression of the latter [2]. The overexpressed protein is mainly located on the outer mitochondrial membrane and inhibits apoptosis by preventing the drop of the mitochondrial potential. Thus, BCL-2 overexpression, by inhibiting apoptosis induced by anticancer drugs, should be correlated with resistance to chemotherapy. In a clinical setting, results have associated BCL-2 with either a good or a poor prognosis, depending on the tumor considered. The BCL-2 family includes members which, like BCL-2, have anti-apoptotic activity and others which are pro-apoptotic. Among the latter, BAX is a major protein, and the susceptibility of a cell to apoptotic signals has been linked to the BCL-2:BAX ratio.

P53 is a nuclear protein that detects genetic damage and triggers repair mechanisms. When DNA damage is irreparable, P53 triggers apoptosis. When the P53 gene is mutated, it may lose its ability to induce apoptosis following DNA damage and thus might be responsible for resistance to chemotherapy. Because wild-type P53 is quickly degraded, it can not be detected immunohistochemically in normal cells. Therefore, P53 positivity usually corresponds to a dysfunctional protein, with a mutation of the gene in most cases.
Ki67 is an antigen expressed during the cell cycle from G1 to mitosis. It is widely used in routine practice because the rate of nuclear staining reflects tumor proliferation.

HER-2 is a 185-kDa transmembrane tyrosine kinase receptor that belongs to the family of epidermal growth factor receptors. All members of this family seem to play critical roles in epithelial cell growth and differentiation. Although no specific ligand has been identified to date, several lines of evidence suggest that HER-2 might augment signal transduction of the other family members. Its overexpression, linked in most cases with gene amplification [3], is observed in ~20–30% of breast cancers, where it has been reported to have prognostic and predictive value [4].

Although several studies have explored the prognostic impact of BCL-2, BAX, HER-2, P53 and Ki67 status in ovarian cancer, the results were contradictory, but most of those studies had been conducted on small series of patients or populations heterogeneous with respect to disease stage, treatment and follow-up.

In this study, histology and Ki67, BCL-2, BAX, P53 and HER-2 expressions were retrospectively analyzed on tissue samples from 117 patients with advanced ovarian cancer included in a GINECO multicenter first-line, phase III clinical trial.

### Patients and methods

#### Patients

Between February 1994 and June 1997, 164 consecutive patients with advanced ovarian cancer were recruited from 40 study centers. Eligibility criteria were: age between 18 and 70 years, histologically proven epithelial ovarian cancer, FIGO stage III or IV, World Health Organization (WHO) performance status <3, no previous chemotherapy, no major organ failure.

Every 21 days, patients received a chemotherapy regimen, combining intravenous (i.v.) cisplatin (75 mg/m²), epirubicin (50 mg/m²) and cyclophosphamide (CEP) for a total of six cycles. The cyclophosphamide dose was randomized between the standard dose of 500 and 1800mg/m² with granulocyte colony-stimulating factor (G-CSF) support.

After the six scheduled chemotherapy cycles, patients were monitored at 3-month intervals during the first 3 years and every 6 months thereafter. All clinical data were gathered in the GINECO data center. Response to chemotherapy was defined as a 50% or greater reduction in the product obtained from measurement of each lesion and no appearance of new lesions. Response was evaluated either clinically or with a second-look laparotomy.

### Tumor specimens

Histological slides from 117 of 164 patients could be obtained retrospectively. For each patient, one representative sample of tumor tissue from the first surgery (before chemotherapy) was chosen by the initial pathologist. Two pathologists (S.C.B., O.L.), unaware of the initial diagnosis, used a double-headed microscope to review each slide. In the case of disagreement, the slide was examined by three pathologists (S.C.B., O.L., A.L.T.) and the histological diagnosis was definitively retained when a consensus was reached using the World Health Organization (WHO) classification of ovarian epithelial tumors. Ninety-six samples were primary ovarian tumors and 21 (18%) were peritoneal metastases.

When sufficient material was available, primary tumors were graded as follows: grade 1, well-differentiated tumors without nuclear atypia; grade 2, moderately differentiated tumors without nuclear atypia; grade 3, moderately differentiated tumors with nuclear atypia; grade 4, poorly differentiated or undifferentiated tumors with nuclear atypia [5].

### Immunohistochemistry

Immunohistochemical studies were performed on paraffin-embedded material with antibodies raised against the following antigens: Ki67, P53, BCL-2, BAX and HER-2 (Table 1). Thin sections 4-µm thick were deparaffinized in a routine manner. After microwave pretreatment the primary antibody was incubated for 2 h. Labeling was visualized using a commercially available standard streptavidin–biotin–peroxidase kit (ABCys Biospa, Milano) and nuclei were counterstained with hematoxylin. Immunohistochemical labeling was performed in a single laboratory and was interpreted without any knowledge of the clinical data.

Ki67 expression in the 80 samples tested was analyzed using a digitized image analysis system (SIS Soft Imaging Software Corp., Reutlingen, Germany). For all slides showing only positive mitotic figures, another slide was tested or the result was considered unreliable. A Leitz DMR (Leica) microscope was used with a ×40 objective and hooked up to a CCD color video camera (DUX, 930P, Sony). For each slide, we analyzed three high-power fields, choosing those with the highest rates of positive cells. Results are expressed as percentages of positively labeled nuclear area/total of nuclear area (defined as positive area + negative counterstained nuclear area). For each specimen, the observer manually adjusted the positive and negative counterstained boundaries, so as to take into account the individual labeling patterns of each sample to another. To avoid overestimating Ki67 labeling, we used median as the cut-off point, rather than the statistically defined ‘optimal’ cutpoints.

Nuclear P53 labeling was scored as follows: 0, <10% positive nuclei in tumor tissue; 1, 10–80% positive nuclei, 2, >80% positive nuclei. The same scoring method was used to assess BCL-2 and BAX staining in the cytoplasm.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>Source</th>
<th>Dilution</th>
<th>No. of patients tested</th>
<th>Positive cases</th>
<th>Strong labeling a</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ki67</td>
<td>MIB-1</td>
<td>Immunotech</td>
<td>1:50</td>
<td>80</td>
<td>– 3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BCL-2</td>
<td>124</td>
<td>Dakopatts</td>
<td>1:50</td>
<td>109</td>
<td>57</td>
<td>52</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>BAX</td>
<td>B-9</td>
<td>Tebu</td>
<td>1:500</td>
<td>92</td>
<td>50</td>
<td>54</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>P53</td>
<td>DO 7</td>
<td>Novostra</td>
<td>1:50</td>
<td>94</td>
<td>67</td>
<td>71</td>
<td>51</td>
<td>54</td>
</tr>
<tr>
<td>HER-2</td>
<td>CB 11</td>
<td>Novostra</td>
<td>1:800</td>
<td>95</td>
<td>15</td>
<td>16</td>
<td>–</td>
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</tr>
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</table>

aStrong positivity was defined as the positivity of >80% of the tumor cells for BCL-2, BAX and P53.

bUnlike other markers, Ki67 positivity is expressed as the percentage of the nuclear area labeled (see immunohistochemistry).
For BCL-2, reactive lymphocytes were used as the internal positive control, whereas kidney tissue was used as control for BAX staining. All slides were analyzed twice independently by the same observer. Usually, the score chosen led to clear-cut and reproducible results. Difficult cases were discussed by two pathologists using a double-headed microscope for reaching consensus.

HER-2 overexpression was assessed by using two antibody dilutions. First, according to the manufacturer’s instruction, a 1:50 dilution was applied, which yielded intense, non-specific labeling of the cytoplasm of stromal cells and some tumor cells, rendering interpretation of the results difficult and not reproducible. Next, we adapted our technique diluting the primary antibody 1:800, according to Couturier et al. [6], as this procedure for breast cancer was in complete concordance with the gene amplification assessed by the fluorescent in situ hybridization (FISH) method. Using this dilution, the background noise disappeared and specific membrane labeling was easy to read and reproducible. Labeling was considered positive when the plasma membranes of >10% of the cells were moderately or strongly labeled. To evaluate the reliability of our technique, a highly HER-2-positive breast cancer sample was used as positive control. Moreover, we conducted in-situ hybridization to confirm the amplification of the HER-2 gene in seven immunohistochemically positive cases and the absence of amplification in seven negative samples (data not shown).

### Statistical analysis

The characteristics of the patients and their immunohistochemical results were compared using the chi-square test. Overall survival (OS) time and progression-free survival (PFS) were calculated, respectively, from the date of the first surgery until death, progression or last follow-up examination. PFS and OS curves were derived from the Kaplan–Meier estimates [7]. Univariate Cox model analysis [8] was performed to estimate and test the influence of clinical variables and biological markers/immunohistochemical labeling data. Multivariate analysis applied the Cox proportional hazards regression model [8] to evaluate the influence of these variables on outcome adjusted for the other prognostic factors. Results are presented with relative risk (RR) and their 95% confidence interval (95% CI). Only P values <0.05 were considered significant.

### Results

#### Patients

Clinical characteristics of the 164 patients included in the GINECO clinical trial are detailed in Table 2. Median follow-up was 68 months. The response rates, median PFS and OS rates did not
differ significantly as a function of the cyclophosphamide dose (500 or 1800 mg/m²/cycle) administered [9]. At the time of our analysis (December 2002), 134 (87%) progressions and 117 (71%) deaths had been recorded for the entire population. Median PFS duration was 15 months (95% CI 13.1–16.5). Median OS was 32 months (95% CI 27.7–36.2).

No statistical difference has been found between this retrospective series including 117 patients and the whole population (Table 2). At the time of our analysis, 106 (90%) progressions and 88 (75%) deaths had been recorded for this subgroup of patients. Their median PFS was 14.4 months (95% CI 12.5–17.3) and the median OS was 32 months (95% CI 27.3–36.7).

**Histology and tumor grade**

Diagnoses of the 117 specimens that could be analyzed were 74 (63%) serous or mixed epithelial carcinomas, four (3%) clear cell carcinomas, nine (8%) endometrioid carcinomas and 30 (26%) undifferentiated carcinomas. Clear cell component was found in 17 non-clear cell carcinomas. Among the 96 patients with primary ovarian tumor samples, 15 (16%) were considered grade 2, 47 (49%) grade 3 and 34 (35%) grade 4. No grade 1 tumor was identified.

**Expression of Ki67, BCL-2, BAX, P53 and HER-2**

Examples of the labeling patterns observed are shown in Figure 1A–E. The median Ki67-positive nuclear area was 30% (95% CI 26.7–32.6), which was considered the cut-off threshold for statistical analyses. Immunohistochemical labeling results are listed in Table 1. HER-2 expression on the plasma membrane was moderate or intense in >10% of the tumor cells in 15 of 95 (16%) specimens. In some cases, this HER-2 expression varied from one part of the tumor to another.

**Association between biological markers and other prognostic variables**

No relationship was found between BCL-2 and BAX or P53 expression. No relationship was found between HER-2 overexpression by tumor cells and clinical parameters, histological type or grade, or other tested biological variables.

**Analysis of survival and response to chemotherapy**

Response to chemotherapy was observed in 92 of the 117 patients (79%). None of the clinical (age, FIGO stage, ascites, performance status), histopathological (tumor type and grade) or immunohistochemical (Ki67-positive nuclear area, BCL-2, BAX, P53 and HER-2) variables tested were statistically linked to response to chemotherapy.

**Univariate analysis**

Univariate analysis of the potential prognostic impact of clinical, histopathological and immunohistochemical parameters identified the presence of performance status ≥1, ascites and HER-2 overexpression by tumor cells as being significantly associated with shorter OS and PFS (Table 3). Size of the residual lesions after first laparotomy was also significantly associated with poorer PFS.

None of the biological markers other than HER-2 overexpression were of prognostic value in terms of OS and PFS. For patients with or without HER-2 overexpression, respectively, median PFS rates were 12 and 15 months and median overall survival was 25 and 35 months (Figure 2).

Because the molecular markers may vary significantly between primary tumor and metastatic lesions, we have analyzed their prognostic impact in a subgroup, including only those patients with primary tumor samples. Results were similar to the whole population. Ki67, BCL-2, BAX and P53 expression did not retain prognostic significance. In contrast, HER-2 overexpression was still associated with a poorer prognosis, considering either OS (P = 0.02, RR 2.07, 95% CI 1.03–4.17) or PFS (P = 0.02, RR 2.13, 95% CI 1.13–4.01).

**Multivariate analysis**

To evaluate the impact on PFS, performance status, presence or absence of ascites, size of residual lesions after the first laparotomy, and HER-2 overexpression were entered into the Cox proportional hazards model. Only presence of ascites (P <0.01, RR 1.85, 95% CI 1.18–2.89) and HER-2 overexpression (P = 0.01, RR 2.16, 95% CI 1.19–3.93) were retained as independent prognostic factors of shorter PFS.

Similar results have been obtained in the primary tumor sample subgroup, showing independent prognostic impact for ascites (P <0.01, RR 1.94, 95% CI 1.19–3.16) and HER-2 overexpression (P = 0.02, RR 2.08, 95% CI 1.11–3.91).

The multivariate analysis of parameters prognostic of OS included performance status, presence or absence of ascites and HER-2 overexpression. Presence of ascites (P = 0.03, RR 1.67, 95% CI 1.04–2.68) and HER-2 overexpression (P = 0.01, RR 2.24, 95% CI 1.19–4.22) were retained as independent factors associated with shorter survival.

Similar results have been obtained in the primary tumor sample subgroup, showing independent prognostic impact for ascites (P = 0.04, RR 1.75, 95% CI 1.03–2.07) and HER-2 overexpression (P = 0.04, RR 2.07, 95% CI 1.03–4.15).

**Discussion**

In this study, the potential impact of the rate of Ki67-positive tumor nuclei and the expression of BCL-2, BAX, P53 and HER-2 proteins on the outcomes of patients with advanced ovarian cancer was analyzed. Among these parameters evaluated by immunohistochemistry, only HER-2 overexpression predicted a shorter PFS and OS.

The patients included in this series had FIGO stage III/IV epithelial ovarian cancer and participated in a multicenter GINECO phase III trial of the first-line CEP chemotherapy regimen. The two arms of the trial differed only by the cyclophosphamide dose delivered. All patients were analyzed as a single population because no significant differences were detected between the two study arms, in terms of response to chemotherapy, PFS and OS.
Figure 1. Immunohistochemical labeling results. (A) Plasma membrane HER-2 overexpression in >10% of the tumor cells. (B) Nuclear Ki67 expression. (C) Heterogeneous cytoplasmic BCL-2 expression in tumor cells and small reactive lymphocytes. (D) Strong cytoplasmic BAX expression. (E) Anti-P53 antibody-labeled tumor nuclei.
In addition, prolonged follow-up, with a median of 68 months, was available for this cohort. We retrospectively obtained the slides from 117 (71%) of the 164 patients included. This subpopulation was representative of the whole population (Table 2). All specimens were reviewed by a panel of pathologists for histological type and grade. No prognostic impact was found for these histological parameters but we were limited by the fact that, for most patients, only one sample was available for re-evaluation.

The percentage of the nuclear area recognized by anti-Ki67 antibody was analyzed by image analysis. The 30% median rate of positivity found in our study is similar to that previously reported by Anttila et al. [10], who used a similar technique. In contrast, Ki67 positivity bore no prognostic value in our study. Anttila et al.’s population of 316 ovarian cancer patients was, however, heterogeneous regarding stage and treatment, with only half of the patients having received adjuvant chemotherapy. This difference raises the possibility that the three-drug, platinum-based regimen received by all our patients attenuated the potential prognostic impact of the proliferation rate.

In accordance with the literature [11–17], BCL-2 and BAX were expressed in 52% and 54% of the specimens, respectively. Two controversial points must be addressed separately. First, BCL-2 expression seemed to decline with increasing tumor aggressiveness. Indeed, most authors who evaluated the frequency of BCL-2 expression in ovarian disease found that it was less expressed in carcinomas than benign and borderline tumors [16, 18–20]. In the context of advanced ovarian carcinoma, the anti-apoptotic protein BCL-2 should logically be linked with chemoresistance and thus a poorer prognosis. Contradictory results reported in the literature concerning the prognostic value of BCL-2 expression, indicated either a positive [11, 12] or negative prognostic impact [13, 15]. In our study, which has the advantage of analyzing a homogeneous population of advanced ovarian cancer patients, no prognostic impact was found for BCL-2. We compared BCL-2 expression (≥10% of positive tumor cells) versus no expression in the survival analyses, because the two groups were of similar size. How-

| Table 3. Univariate analysis for overall survival and progression-free survival of clinical, histopathological and immunohistochemical parameters |
|---|---|---|---|---|---|
| Factor | Overall survival | | | Progression-free survival | | |
| | RR | 95% CI | P | RR | 95% CI | P |
| Age | | | | | | |
| ≥60 versus <60 years | 1.08 | 0.70–1.65 | 0.7 | 1.09 | 0.74–1.60 | 0.68 |
| Performance status | | | | | | |
| 0 versus 1/2 | 1.81 | 1.08–3.02 | 0.02 | 1.80 | 1.13–2.86 | 0.01 |
| FIGO stage | | | | | | |
| IIc/IV versus IIIa/IIIb | 1.22 | 0.75–1.98 | 0.4 | 1.09 | 0.70–1.68 | 0.72 |
| Ascites | | | | | | |
| Presence versus absence | 1.68 | 1.09–2.58 | 0.01 | 1.83 | 1.23–2.72 | 0.003 |
| Residual tumor after first laparotomy | | | | | | |
| <2 cm versus ≥2 cm | 1.22 | 0.89–1.63 | 0.2 | 1.31 | 1.00–1.72 | 0.05 |
| Tumor type | | | | | | |
| Clear cell component versus others | 1.03 | 0.92–1.16 | 0.6 | 1.02 | 0.92–1.14 | 0.69 |
| Tumor grade | | | | | | |
| 1/2 versus 3/4 | 0.95 | 0.69–1.28 | 0.7 | 0.86 | 0.64–1.16 | 0.32 |
| Ki67 expression (% of nuclear area) | | | | | | |
| ≥30 versus <30 | 1.22 | 0.73–2.05 | 0.45 | 0.94 | 0.59–1.50 | 0.80 |
| BCL-2 | | | | | | |
| ≥10% versus <10% of tumor cells | 1.20 | 0.78–1.84 | 0.41 | 1.25 | 0.84–1.85 | 0.27 |
| BAX | | | | | | |
| ≥10% versus <10% of tumor cells | 0.93 | 0.58–1.47 | 0.74 | 1.30 | 0.84–2.01 | 0.24 |
| P53 | | | | | | |
| ≥10% versus <10% of tumor cells | 1.09 | 0.69–1.75 | 0.72 | 1.13 | 0.73–1.73 | 0.58 |
| HER-2 | | | | | | |
| ≥10% versus <10% of tumor cells | 2.12 | 1.13–3.98 | 0.01 | 1.99 | 1.12–3.54 | 0.02 |

RR, relative risk, 95% CI, 95% confidence interval.
However, when tumors strongly expressing BCL-2 (>80% positive tumor cells) were considered, a trend towards a poorer prognosis could be observed (data not shown).

The in vitro expression of the pro-apoptotic protein BAX seems to sensitize ovarian cancer to paclitaxel [17]. Among the 45 patients receiving this agent described by Tai et al. [17], BAX expression was associated with a good prognosis. In our study, elevated BAX expression had no prognostic impact, but the chemotherapy regimen given to our patients did not include paclitaxel. Our findings suggest that high BAX expression carries no intrinsic influence on the prognosis of advanced ovarian cancer. However, further studies are needed to confirm the role of BAX expression in the chemoresponsiveness of ovarian cancer patients who receive paclitaxel.

Because the BCL-2:BAX ratio has been reported to play a role in the deregulation of apoptosis, it has been suggested that the subgroup defined by the expression BCL-2–BAX+ was correlated with a poor outcome [14]. Unlike that study, we observed no prognostic impact considering the following subgroups: BCL-2−BAX−, BCL-2−BAX+ or BCL-2+BAX− and BCL-2+BAX+ (data not shown).

P53 accumulation was detected in 71% of our patients’ specimens. In most published studies 45–65% of the patients were positive [11–13, 15]. Nonetheless, no prognostic value was discerned for P53 accumulation in our series. Because results from P53 immunohistochemistry are not always similar to that of TP53 gene mutation, we cannot exclude the possibility of underestimating in our study a potential predictive role of P53 gene alteration.

HER-2 was overexpressed in 16% of our patients. This rate has varied in the literature from 8 to 66% [21–31]. This wide variation in heterogeneity is due in part to the different detection methods used, which measure either gene amplification (Southern blotting or FISH) or protein expression (immunohistochemistry). Some methods, such as Southern blotting and RT–PCR (reverse transcription–polymerase chain reaction) in pathological materials are limited by artifacts resulting from the extraction of specimens containing a highly heterogeneous amount of tumor and normal cell populations. In a clinical setting, in situ methods have the advantage of specifically analyzing tumor cells, whose importance is obvious, especially when assessing quantitative rather than qualitative alterations.

According to our univariate and multivariate analyses, HER-2 overexpression bore significant prognostic impact on PFS and OS. Contradictory results have been published concerning the role of HER-2 overexpression in patient outcome. The lack of standard detection technique might explain some of the negative results. For example, van der Zee et al. [21] used the CB11 antibody diluted 1:25 (we used the same antibody diluted 1:800) and interpreted plasma membrane as well as cytoplasmic labeling. Ross et al. [22] and Medl et al. [23] reported high rates, 66% and 40% respectively, of HER-2 gene amplification, suggesting that the different methods applied gave results which necessitated different definitions of positivity.

Some other studies included <50 patients with advanced disease, which restricted the impact of prognostic results [22, 24–26].

Our findings, obtained in a multicenter prospective clinical trial conducted on a homogeneous cohort of advanced ovarian cancer patients, confirm previously reported data [27–33] such as the two large series published by Meden et al. [27] and Felip et al. [30], who found that HER-2 overexpression was associated with a poor outcome. Moreover, our multivariate analyses showed that the influence of HER-2 overexpression was independent.

Ideally, assessing a purely prognostic role of a biological marker would require assigning some patients to receive no adjuvant therapy, which is ethically impossible nowadays in the context of advanced ovarian cancer. However, because all our patients received the same CEP regimen, the poor prognostic impact of HER-2 overexpression on outcome may reflect a real influence of this overexpression or chemoresistance of HER-2 overexpressing tumors. Indeed, it has been shown in breast cancer that HER-2 overexpression was associated with chemoresistance to alkylating agents and chemosensitivity to anthracyclines. All our patients were given cyclophosphamide (500 or 1800 mg/m2) but we did not observe any trend in favor of a dose–response effect for the alkylating agent in the subgroup (15 patients) of HER-2-positive patients (data not shown). Furthermore, this absence of a significant difference was also observed in the whole population, which comprised a majority of HER-2-negative advanced ovarian carcinomas. We could have expected that the use of the anthracycline epirubicin might have abrogated the poor prognosis of HER-2-positive patients, as reported for breast cancer [33]. However, the
dose of epirubicin (50 mg/m²/cycle) administered was perhaps too low to counteract the negative impact of HER-2.

In this study we demonstrated the independent prognostic impact of HER-2 overexpression. To our knowledge, this is the first study to examine a homogenous population of stage III/IV ovarian cancers, included in a multicenter prospective, clinical trial. Further studies are needed to analyze the impact of HER-2 overexpression in patients treated with other chemotherapy regimens, including paclitaxel, with the goal of identifying those patients most likely to benefit from chemotherapy with a higher anthracycline dose or specific anti-HER-2 molecules.

Acknowledgements


References