TP53 status determines clinical significance of ERBB2 expression in ovarian cancer

J Kupryjańczyk,1,2 R Madry,3 J Plisiecka-Hałasa,1 J Bar,4 E Kraszewska,5 I Ziółkowska,6 A Timorek,7 J Stełmachów,7 J Emerich,7 M Jędryka,7 A Płużańska,10 I Rzepka-Górska,11 K Urbaniński12 and J Markowska8

1Department of Molecular Pathology, The Maria Skłodowska-Curie Memorial Cancer Center, Institute of Oncology, ul. Roentgena 5, Warsaw 02-781, Poland; 2Department of Pathology, Medical Academy and Brodowski Hospital, ul. Kondratowicza 8, Warsaw 03-242, Poland; 3Chair of Gynecologic Oncology, Medical Academy, ul. Lakowa 12, Poznan 61-878, Poland; 4Department of Immunology, Medical Academy, ul. Dyrekcyjna 577, Wroclaw 50-528, Poland; 5Department of Biostatistics, The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, ul. Roentgena 5, Warsaw 02-781, Poland; 6Department of Gynecologic Oncology, The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, ul. Roentgena 5, Warsaw 02-781, Poland; 7Department of Obstetrics and Gynecology, Medical Academy and Brodowski Hospital, ul. Kondratowicza 8, Warsaw 03-242, Poland; 8Department of Gynecologic Oncology, Medical Academy, ul. Dyrekcyjna 577, Wroclaw 50-528, Poland; 9Department of Gynecologic Oncology, Medical Academy, ul. Paderewskiego 4, Lodz 93-509, Poland; 10Department of Gynecologic Oncology, Medical Academy, ul. Powstancow Wielkopolskich 72, Szczecin 70-111, Poland; 11Department of Gynecologic Oncology, Institute of Oncology, ul. Gamczarska 11, Krakow 31-115, Poland

ERBB2 expression has been found in 19 to 44% of ovarian carcinomas; however, its predictive value has not been demonstrated, and trastuzumab has not found clinical application in ovarian cancer patients. We evaluated clinical significance of ERBB2 expression in relation to TP53 accumulation in ovarian carcinoma patients treated with platinum-based regimens. Immunohistochemical analysis with CB11 and a novel NCL-CBE356 antibody (against the internal and external domains of ERBB2, respectively) was performed on 233 tumours (FIGO stage IB—IV); the US Food and Drug Administration-approved grading system with 0 to 3+ scale was used for evaluation, and the results were analysed by the Cox and logistic regression models. In all, 42% of the tumours expressed (category 1+, 2+ or 3+) either CB11 or CBE356 or both (CB11/CBE356 parameter). Associations between ERBB2 expression and clinical factors were observed only if tumours with staining category 1+ were grouped together with tumours showing staining categories 2+ and 3+. CB11/CBE356 parameter had a better predictive value than CB11 alone. CB11/CBE356 expression was negatively associated with platinum sensitivity (PS) in the TP53(+) group (P=0.022) and with disease-free survival (DFS) in the TP53(+) group (P=0.009). Our results may suggest that trastuzumab should be given postoperatively to patients with TP53(−)/ERBB2(+) ovarian carcinomas to enhance PS, and after completion of chemotherapy to patients with complete remission and TP53(+) or ERBB2(+) carcinomas to extend DFS time (in total to 30.4% of all patients analysed). Thus, novel criteria for ovarian cancer patient inclusion for clinical trials with trastuzumab should be considered and tested.

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ERBB2 (HER-2) is a transmembrane tyrosine kinase receptor protein that belongs to the epidermal growth factor receptor family (HER-1, HER-2, HER-3, HER-4) (Slamon et al, 1989; Baselga and Albanell, 2001; Ross et al, 2003). The HER family participates primarily in transduction of proliferation signals from various ligands, and this process involves dimer formation between different HER receptors. The presence of HER-2 in a heterodimer results in more efficient stability and signalling (Baselga and Albanell, 2001; Ross et al, 2003). Overexpression of HER-2 results also in the formation of homodimers, which may be constitutively active (Baselga and Albanell, 2001).

The expression of HER-2 is an established prognostic factor in breast cancer. A monoclonal antibody against the external epitope of HER-2, that is, trastuzumab, is used for therapy of breast cancer patients whose tumours express high levels of HER-2, that is, 2+ and 3+ US Food and Drug Administration-approved category (Press et al, 2002; Ross et al, 2003).

Some data from cell lines overexpressing ERBB2 showed that therapy with trastuzumab and cisplatin had a synergistic effect (Hancock et al, 1991). This could create a possibility of more efficient therapy in ovarian cancer patients. However, despite relatively frequent ERBB2 overexpression or gene amplification in
ovarian carcinomas (range 19–44%, Table 1), its clinical importance has been barely demonstrated (Berchuck et al, 1990; Kacinski et al, 1992; Rubin et al, 1993; Scambia et al, 1993; Meden et al, 1994; Fajac et al, 1995; Felip et al, 1995; Van der Zee et al, 1995; Tanner et al, 1996; Meden et al, 1998; Hengstler et al, 1999; Ferrandina et al, 2002). Only Berchuck et al (1990) and Felip et al (1998) observed lower frequency of complete remission (CR) in ERBB2-overexpressing tumours; however, they have not confirmed this by multivariate analysis. To date, trastuzumab has not found clinical application in ovarian cancer patients, neither combined with chemotherapy nor as monotherapy (Bookman et al, 2003).

We think that lack of clinical associations of ERBB2 overexpression in ovarian cancer studies may be due to (1) small group sizes (Table 1); (2) no regard to TP53 status and (3) overly restrictive criteria of ERBB2 overexpression.

We have recently shown that TP53 status may influence clinical importance of some molecular and clinical factors in ovarian carcinoma patients (Kupryjanczyk et al, 2003, 2004). The same may be true with regard to ERBB2. Some experimental studies have shown that wild-type TP53 protein limits or abrogates biological effects of ERBB2 stimulation. Both the growth inhibition and enhanced apoptosis were observed in wild-type TP53 cell lines after ERBB2 transfection. In the same conditions, TP53 mutant cells demonstrated enhanced growth (Casalini et al, 2001; Huang et al, 2002). Huang et al (2002) concluded that TP53 defects 'played a permissive role in ERBB2 upregulation', and 'ERBB2 overexpression phenotype might in turn select for the survival of cells with p53 mutations'.

The common criteria of ERBB2 positivity (2+ and 3+ in tumour tissues were established with reference to ERBB2 expression in normal tissues. It has been shown that ERBB2 is normally expressed (1+) on epithelial cell membranes including ovarian epithelium and this is not due to gene amplification (Berchuck et al, 1990; Press et al, 1990; Rubin et al, 1993). On the other hand, Press et al (2002) evaluated CB11 expression (anti-ERBB2 antibody approved by FDA for clinical testing) (Press et al, 1994; Felip et al, 1995; Van der Zee et al, 1995; Bookman et al, 2003) in tumours with determined ERBB2 gene amplification and mRNA expression, and found better CB11 sensitivity and accuracy for staining categories 1+ to 3+ than for 2+ and 3+ alone; the specificity was similar (98.6 vs 100%, respectively).

In the light of these findings, we aimed to evaluate the clinical significance of ERBB2 expression in a large group of advanced stage ovarian carcinomas, with the application of less stringent criteria of ERBB2 overexpression, and with respect to TP53 status. Another aim of the study was to test a novel CBE356 antibody (against external epitope of ERBB2) and to find out whether the evaluation of the internal (CB11) and external epitope of ERBB2 would be more clinically relevant than the evaluation of internal epitope only.

### MATERIALS AND METHODS

#### Patients and tumours

The study was performed on archival material from 233 ovarian carcinoma patients operated on in the years 1987–1999. Medical records were critically reviewed by at least two clinicians. The material was carefully selected out of 548 cases submitted to meet the following criteria: no chemotherapy before staging laparotomy, adequate staging procedure, International Federation of Gynecologists and Obstetricians stage IIB to IV disease (Creasman, 1989), standard CP (cisplatin–cyclophosphamide or carboplatin–cyclophosphamide) or CAP chemotherapy (CP with the addition of doxorubicin), tumour tissue from the first laparotomy available,
Outcome
Recurrence rate in a CR group 98/123 (80%)
Platinum resistant 132 (57%)
Platinum sensitive 101 (43%)

Response to chemotherapy
FIGO stage
IIIB+IIIC 17 (7%)
IIIA+IIIB 54 (23%)
IIIC 132 (57%)
IV 30 (13%)
Residual tumour size
0 52 (22%)
> 0 ≤ 2 cm 60 (26%)
> 2 cm 121 (52%)
Chemotherapy
CP 167 (72%)
CAP 66 (28%)

Response to chemotherapy
CR 123 (53%)
PR 36 (15%)
No change 8 (3%)
Progression 66 (28%)
Platinum sensitive 101 (43%)
Platinum resistant 132 (57%)

Recurrence rate in a CR group
CP 167 (72%)
CAP 66 (28%)
CR 123 (53%)
PR 36 (15%)
No change 8 (3%)
Progression 66 (28%)
Platinum sensitive 101 (43%)
Platinum resistant 132 (57%)

Evaluation of immunohistochemical stainings

The semiquantitative evaluation of immunohistochemical stainings was performed independently by two pathologists (JK, JB) without the knowledge of clinical data, and a consensus was reached in controversial cases. For the evaluation of ERBB2 expression, we applied the criteria approved by the US Food and Drug Administration, that is, lack of membranous staining or staining not exceeding 10% of cells was evaluated as negative; weak, barely perceptible incomplete membranous staining in at least 10% of cells was evaluated as 1+; weak or moderate staining in at least 10% of cells was evaluated as 2+; and strong staining in at least 10% of cells was evaluated as 3+ (Graziano, 1998; Jacobs et al, 1999). Five tumours representative of each ERBB2 staining category (in total 20) were stained once again for CBE356 expression 1 year after initial staining; the slides were evaluated without knowledge of initial results and the results were reproducible.

We combined CB11 expression with CBE356 expression (CB11/CBE356 parameter) by taking the highest result obtained with either antibody. For example, if a tumour was negative for CB11 and 3+ positive for CBE356, CB11/CBE356 parameter was scored 3+.

TP53 protein accumulation was described as present (more than 10% of positive cells) or absent (Kupryjanczyk et al, 2003).

Statistical analysis

Associations between ERBB2 expression and clinicopathological variables were analysed by χ² test. Probability of survival and DFS were calculated using the Kaplan–Meier method (Kaplan and Meier, 1958). Overall and DFS time analyses were performed with multivariate Cox’s proportional hazards models (Cox, 1972). Sensitivity to chemotherapy was evaluated with the multivariate
logistic regression model. Significant parameters were selected using the backward selection technique, where factors not significant at 0.1 were drawn one by one out of the model. All tests were two-sided and the level of significance was set at 5%.

The statistical analysis included the following independent variables: age of patients, FIGO stage, RT size (0 vs ≤ 2 cm, 0 vs > 2 cm), histological type and histological grade. We evaluated clinical significance of CB11 expression, and that of CB11/CBE356 expression separately. The analysis was performed separately in the TP53(+) and TP53(−) subgroup, as well as in the whole group. The latter analysis was performed to check whether the lack of some ERBB2 associations in the TP53 subgroups was due to lower group sizes.

RESULTS

CB11 and CBE356 expressions and their associations

Both antibodies gave membrane-bound (which is specific for ERBB2 receptor) and cytoplasmic staining and the latter was not taken into evaluation. Cytoplasmic staining for ERBB2 was usually observed also by other authors (Felip et al., 1995; Van der Zee et al., 1995; Bookman et al., 2003). SK-BR-3 cell line (3+) showed strong complete membranous staining in the majority of cells with both antibodies, and a variable cytoplasmic staining: MDA-175 cell line (1+) showed incomplete, barely perceptible membranous staining in a small percentage of the cells with both antibodies, as well as weak cytoplasmic staining in the Golgi region; MDA-231 cell line (negative for ERBB2) did not show specific staining with either antibody (Figure 1).

CB11 and CBE356 expressions were highly associated with each other (P<0.00001); however, they did not completely overlap (Table 3). CB11 expression was found in 54 tumors (23%), rates of tumours with category 1+, 2+ and 3+ were 7, 11 and 5%, respectively; CBE356 expression was found in 79 tumours (34%), a rate of tumours with category 1+, 2+ and 3+ was 13, 15.5 and 5.5%, respectively (Figure 2). In all, 42% of the carcinomas expressed either CB11 or CBE356 or both, and this is represented by CB11/CBE356 parameter; for this parameter, the rates of tumours with category 1+, 2+ and 3+ were 15, 20 and 7%, respectively. Thus, evaluation of both epitopes resulted in a much higher percentage of positive tumours.

To determine biological and clinical significance of category 1+ staining, first we compared tumours with categories 0, 1+ with those showing categories 2+, 3+, and then tumours with category 0 with those showing categories 1+ to 3+. Associations between ERBB2 expressions and biological or clinical factors were observed only if tumours with staining category 1+ were grouped together with tumours showing staining categories 2+ and 3+. Thus, the term ‘ERBB2 or CB11 or CB11/CBE356 expression’ will be further used in the meaning of staining categories 1+ to 3+.

CB11 expression was more frequent in the TP53(−) than in the TP53(+) carcinomas (30 vs 18%, P = 0.04), as well as in FIGO IV tumours than in other clinical stages (43% expressors in stage IV, 19% in stage IIIC, 20% in IIIB and 29% in IIIB and C, P = 0.032). CB11/CBE356 expression did not show such associations. CB11/CBE356 expression was significantly less frequent in the serous (37%) than in other types of carcinoma (58.5%) (P = 0.02). The rate of strong CB11/CBE356 expression (category 2+ and 3+) was particularly low in the serous type (23 vs 43.5%, P = 0.01). CB11 and CB11/CBE356 expressions were neither associated with tumour differentiation nor with RT size.

Associations of ERBB2 expression with clinical end points

CB11/CBE356 parameter (1+ to 3+) showed associations with DFS and platinum sensitivity (PS), while CB11 expression alone (1+ to 3+) was associated with PS at the border of significance only (Table 4). CB11/CBE356 expression enhanced 2.15 times the risk of recurrence in the TP53(+) group and not in the TP53(−) group (Figure 3). In the whole tumour group, the association and the relative risk of recurrence were weaker than in the TP53(+) group (Table 4).
Table 3  Comparison of immunohistochemical CB11 expression with CBE356 (clone 10A7) expression in 233 ovarian carcinomas

| No. of tumours | |
|----------------|-----------------|
| Both stainings positive | 35 (15%) |
| CB11 equal to 10A7 | 21 |
| CB11 stronger than 10A7 | 8 |
| CB11 weaker than 10A7 | 6 |
| Both stainings negative | 135 (58%) |
| One staining negative | |
| CB11 positive/10A7 negative | 19 |
| CB11 negative/10A7 positive | 44 |

Figure 2  Strong membranous expression of ERBB2 in an ovarian carcinoma (NCL-CBE356 antibody, clone 10A7, streptavidin–biotin–peroxidase method, haematoxylin counterstain).

Table 4  Associations of ERBB2 expression with DFS (i.e. risk of recurrence, Cox’s proportional hazards model) and probability of PS (logistic regression model) in the whole group of ovarian carcinomas, and in the TP53(+) and TP53(−) group

|                | All patients, N = 233, 189 deaths | TP53(−) group, N = 97, 80 deaths | TP53(+) group, N = 136, 109 deaths |
|----------------|-----------------------------------|----------------------------------|-----------------------------------|
| **DFS**        |                                   |                                  |                                   |
| CB11/CBE356*   | 0.012                             | CB11/CBE356                      | 0.009                            |
| RR = 1.71      |                                   | RR = 2.15                       |                                   |
| 95% CI (1.1, 2.6) |                                 | 95% CI (1.2, 3.82) |                               |
| FIGO IIIA, B   | 0.036                             | CB11                             | 0.07                             |
| FIGO IIIC      | <0.001                            | FIGO IIIA, B                     | 0.43                             |
| FIGO IV        | <0.001                            | FIGO IIIC                        | 0.001                            |
| FIGO IV        | <0.001                            | FIGO IV                          | 0.002                            |
| **PS**         |                                   |                                  |                                   |
| CB11/CBE356    | 0.06                              | CB11/CBE356                      | 0.022                            |
| OR = 0.33      |                                   | OR = 0.35                        |                                   |
| 95% CI (0.13, 0.85) |                   | 95% CI (0.12, 1.0) |                               |
| RT ≤ 2 cm vs 0 | 0.014                             | RT ≤ 2 cm vs 0                   | 0.03                             |
| <0.001         |                                   | <0.001                           |                                   |

*CB11/CBE356 and CB11 categories 1+, 2+ and 3+ were compared with category 0. When CB11/CBE356 and CB11 categories 2+ and 3+ were compared with category 0 and 1+, no associations have been found. FIGO stages depicted were compared with FIGO IIB and C; RR = relative risk; OR = odds ratio; DFS = disease-free survival; PS = platinum sensitivity; CI = confidence interval. Detailed statistical results of RT and FIGO stage analysis have been given in previous publications (Kupryjanycz et al, 2003, 2004).
On the other hand, CB11/CBE356 expression diminished (0.33 times) the probability of PS in the TP53(−) group, and not in the larger TP53(+) group. In the whole tumour group, CB11/CBE356 expression showed only a borderline association with PS. CB11 expression alone showed up as a factor influencing PS in the TP53(−) group, while it was not associated with this end point in the whole group (Table 4).

CB11/CBE356 expression showed weaker associations with DFS or PS than clinical parameters. DFS was also associated with FIGO stage in the TP53(+) and in the whole group (lack of association in the TP53(−) group may be due to lower group size, Table 4). Residual tumour size (RT) was the only clinical parameter associated with PS in this analysis. Interestingly, complete debulking with PS at RT ≤ 2 cm enhanced the probability of platinum-sensitive response in the TP53(−) group, while this has not been observed in the TP53(+) group (Table 4). Other factors evaluated did not show associations with DFS or PS. Overall survival and CR were not associated with ERBB2 expression. For full and detailed analysis of clinical factors in this patient group, the reader is referred to other publications (Kupryjanczyk et al, 2003, 2004).

DISCUSSION

We have demonstrated the clinical significance of ERBB2 expression in ovarian carcinoma patients, that is, associations with PS and DFS, using a novel approach to the analysis that included: (1) evaluation in subgroups determined by TP53 status, (2) broader criteria for ERBB2 positivity and (3) a combined end-result from two monoclonal antibodies that identified the external and internal domains of ERBB2.

In our study, TP53 protein accumulation determined the clinical significance of ERBB2 expression. Platinum resistance related to ERBB2 expression was observed in the TP53(−) group only. Pegram et al (1997) reported that ERBB2 CDNA transfection into breast and ovarian cancer cell lines differentially influenced their sensitivity to cisplatin. We have confronted their findings with the known TP53 status in some of those cell lines and the result is in accord with our observations. MCF-7/HER-2 and 2008/HER-2 cells that have wild-type TP53 showed resistance to cisplatin, while MDA-MB-435/HER-2, MDA-MB-231/HER-2 and Caov3-HER-2, which carry TP53 gene mutation, did not differ in this respect from their progenitors without HER-2 expression; an exception is the antibody-mediated proliferation stimulation was not observed either (Xu et al, 1999). It is possible that the biological and clinical effect of anti-ERBB2 therapy in ovarian carcinoma cases with weak or higher ERBB2 expression will become apparent, if this is evaluated with respect to TP53 status.

Three immunohistochemical assays approved by FDA for clinical testing, that is, HercepTest assay, CB11 and 4D5 ('Clinical Trials Assay') and the Ventana Pathway CB11, as well as many other anti-ERBB2 antibodies (about 30), have different sensitivity and specificity when confronted with more objective methods of evaluation of gene amplification or expression (Press et al 1994, 2002; Jacobs et al, 1999; Roche and Ingle, 1999). We have chosen the known CB11 antibody against the internal domain, because it is approved for clinical testing (Press et al, 1994; Pelip et al, 1995; Van der Zee et al, 1995; Bookman et al, 2003). We also wanted to test the novel 10A7 (NCL-CBE356) antibody against the external domain of ERBB2.

CB11 expression alone showed less clinical importance than the end-result from the two antibodies. During revision of this paper, for the purpose of discussion, we also checked whether CB356/10A7 expression alone associates with PS or DFS; however, it did not show any clinical significance. Thus, it appears that concomitant evaluation of expression of the internal and external ERBB2 domains may be more clinically useful; possibly, similar results could be obtained with other anti-ERBB2 antibodies.

With the methods applied, we have possibly identified subgroups of ovarian cancer patients who may benefit from anti-ERBB2 therapy, and timing of such a therapy. Our results could suggest that trastuzumab should be given postoperatively to patients with TP53(−)/ERBB2(+) ovarian carcinomas (in our study 18%) to enhance PS; as shown above, postoperative trastuzumab might be useless in patients with TP53(+) or ERBB2(+) carcinomas because in the TP53(+) group, ERBB2 expression does not diminish PS. However, trastuzumab should be given to patients with TP53(−)/ERBB2(+) ovarian carcinomas who reached CR, that is, after completion of chemotherapy (in our study 12.4% of all patients), because in this group it might extend DFS time. According to our results (lack of associations with overall survival), the group of patients with TP53(−)/ERBB2(+) tumours who did not reach CR would not qualify for trastuzumab treatment. Thus, novel criteria for ovarian cancer patient inclusion for clinical trials with trastuzumab should be considered and further tested. If confirmed, our results could create a perspective of the additional treatment for 30% of patients with advanced stage ovarian carcinomas.

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