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Predictive value of HER-2 and Topoisomerase IIα in response to primary doxorubicin in breast cancer

Edurne Arriola[a,*], *Abelardo Moreno*[b], *Mar Varela*[c], *Jose M. Serra*[d], *Catalina Falo*[a], *Enrique Benito*[e], *Agustin P. Escobedo*[a]

[a]*Servicio de Oncologia Medica, Institut Catala d'Oncologia, Duran I Reynals, Hospitalet de Llobregat, Barcelona, Spain*
[b]*Servicio de Anatomia Patologica, Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain*
[c]*Unidad funcional de mama, Institut Catala d'Oncologia, Duran I Reynals, Hospitalet de Llobregat, Barcelona, Spain*
[d]*Servicio de Cirugia Plastica, Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain*
[e]*Servicio de Ginecologia, Hospital Universitario de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain*

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ABSTRACT

Aim: To study the predictive role of HER-2 and Topoisomerase IIα (TOP2A) in response to primary doxorubicin.

Methods: Two hundred and thirty-two patients with operable breast cancer were treated with doxorubicin prior to surgery. ER, PgR, grade, Ki-67 and HER-2 status were prospectively assessed. HER-2 overexpression was evaluated with immunohistochemistry; positive cases were then studied for gene copy number of HER-2, TOP2A and chromosome 17 centromere by chromogenic in situ hybridisation. Clinical response was assessed by mammography. Pathological response was evaluated as the percentage of tumour replaced by changes due to chemotherapy.

Results: HER-2 amplification was associated with clinical response ($p = 0.04$). ER and PgR negativity, high Ki-67 and HER-2 amplification significantly correlated to pathological response ($p < 0.05$). Tumours with coamplification of HER-2 and TOP2A showed a higher percentage of pathological changes ($p = 0.6$). However, in the multivariate analysis for complete pathological response, ER negativity and high Ki-67 index were the only parameters that maintained statistical significance.

Conclusion: HER2 and Topoisomerase IIα amplification failed to show an association with pathological response to doxorubicin, whereas ER negativity and a high proliferation rate were predictive of complete pathological response to this regime.

1. Introduction

Response to neoadjuvant chemotherapy is correlated with a long-term outcome.[1] It is therefore necessary to identify predictive factors to guide clinicians in selecting the most appropriate drug for each patient. Several molecular markers have been related to prognosis and prediction of response to chemotherapy in breast cancer.

HER-2 is a protooncogene located in chromosome 17q11.2-q12 that encodes a transmembrane glycoprotein of 185-kDa with tyrosine kynase activity. This protein belongs to the family of growth factor receptors and activates substrates

* *Corresponding author:* Address: Breast Unit, Royal Marsden Hospital, Fulham Road, London SW3 6JJ, United Kingdom. Tel.: +44 2078082885; fax: +44 2073763918.
E-mail address: edurne.arriola@icr.ac.uk (E. Arriola).
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involved in cellular transduction. It is overexpressed/amplified in 20–30% of breast invasive carcinomas.[2] The overexpression/amplification of HER-2 has been associated with poor prognosis and shorter survival.[3,4]

In patients with HER-2 positive tumours, several reports have found a correlation between the use of chemotherapy with anthracyclines in the adjuvant setting and a better outcome.[5–7] The predictive value of HER-2 amplification/overexpression in tumour response to neoadjuvant doxorubicin has also been studied, but remains unclear.[8–10] HER-2's function as a marker of chemosensitivity/resistance may be due to associated genetic changes in Topoisomerase IIα (TOP2A). TOP2A is located in chromosome 17 (17q21-q22) and frequently shows alterations (amplification or deletion) in HER-2 amplified tumours.[11] TOP2A encodes for a nuclear enzyme that is essential in cell division and it is a molecular target of anthracyclines (including doxorubicin). It has been suggested that its deletion is correlated with resistance to anthracyclines and overexpression/amplification with responsiveness but these results were not conclusive.[12] Finally, recent reports have highlighted the role of chromosome 17 copy number in the accurate assessment of HER-2 and TOP2A status as the aneusomy of chromosome 17 is a frequent genetic alteration in breast cancer.[13]

In the current study, we have evaluated if HER-2 and TOP2A are predictive factors of clinical and pathological responses to primary doxorubicin.

2. Patients and methods

Between January 2000 and November 2002, patients under 66 years with operable breast cancer in our institution, entered a protocol for primary chemotherapy. Tumours were staged clinically, and those that were T2-3, N0-1 (according to TNM classification of UICC) were included in the protocol. All patients gave full informed consent to the proposed strategy that had been approved by our local Research Ethics Committee.

2.1. Pretreatment exams

Before starting the treatment, all patients underwent a complete physical examination, chest X-ray, isotope bone scan, electrocardiogram, bilateral mammography, biochemical test and a differential blood count. Diagnosis was performed by a fine needle aspiration (FNA) or a core biopsy of the primary tumour. Patients had a skin tattoo on the area of the palpable tumour to help with tumour location.

2.2. Primary chemotherapy

Patients received 75 mg/m^2 of doxorubicin every 21 days for four cycles previous to surgery. Physical examination was performed every 3 weeks during treatment.

2.3. Clinical response

A pretreatment mammography was performed before starting chemotherapy and a second mammography was done at least 15 days after the fourth cycle of doxorubicin in order to assess the response.

Clinical response was defined as a reduction greater than 50% of the products of maximum diameters measured in mammography. Progression (PD) was defined as an increase in the product of the diameters superior to 25%. The rest of the situations were categorised as stable disease (SD).

2.4. Pathological response (PR)

Complete pathological response (pCR) was defined as the total absence of invasive tumour cells in the surgical specimen (tumour and axillary lymph nodes). Pathological changes related to chemotherapy included distinct loose fibrosis with capillary neoformation, periductal inflammatory infiltrate (ductilitis), stromal calcifications and cumulus of foamy macrophages.[14] Cases with an incomplete PR were reported as the percentage of tumour tissue replaced by these features, using a scale with 5% intervals for evaluation. All samples were assessed by the same pathologist (A.M).

2.5. Surgical treatment

Surgery consisted of lumpectomy (when possible taking into account radical and aesthetic criteria), conservative surgery with the aid of oncoplastic techniques (bilateral reduction, muscular flaps and remodeling) or modified radical mastectomy with immediate or delayed reconstruction. In all cases, three level axillary dissection was performed.

2.6. Adjuvant treatment

Patients received 3 additional cycles of cyclophosphamide, methotrexate and fluorouracil (CMF 1.8) after surgery. Adjuvant tamoxifen 20 mg/d was administered to all patients with positive hormone receptor tumours. Breast irradiation was performed in all patients with conservative surgery and after mastectomy when indicated.

2.7. Biological factors assessment

Histological grade was assessed according to Contesso's criteria.[15] Oestrogen receptor (ER) and progesterone receptor (PgR) status were determined by IHC and were reported as positive when a nuclear staining greater than 10% was found. Ki-67 was evaluated with the MIB-1 antibody. Nuclear positivity of 25% or greater was considered as high proliferative index.

3. CISH for HER-2, TOP2A and chromosome 17 centromere detection and interpretation

Initially, HER-2 status was determined by an algorithm (IHC with 2 antibodies and FISH in discordant cases) developed in our institution and already reported.[16] According to previously published data, that suggests a very low prevalence of TOP2A alterations in HER-2 negative tumours,[17–19] only those cases with positive HER-2 status in our first assessment were evaluated by CISH for HER-2, TOP2A and chromosome 17 status (centromeric probe: CEN17) using consecutive sections of the same paraffin block for each probe. CISH is a

well-validated technique and has shown a high concordance with FISH (>90%).[20,21]

Briefly, 3 μm sections of formalin-fixed paraffin-embedded tissue were mounted on microscope slides and baked overnight at 65 °C. Slides were deparaffinised in xylene and washed in 100% ethanol at room temperature. Then they were boiled for 5 min in citrate 1X buffer. After washing with PBS, digestion with pepsine was performed during 8 min at 37 °C. Dehydration in graded ethanol series was done and then, denaturation with the probe (Zymed®) was performed at 95 °C in a thermocycler. Hybridisation, stringent wash, and immunodetection procedure were performed with the Spot-Light® kits according to the manufacturer's instructions (Zymed®). Slides were scanned at 10× objective to identify areas with optimal tissue digestion and non-overlapping nuclei. For the classification of each sample, signals had to be identified in at least 50% of the cancer cells.

Criteria for defining a sample as amplified/non-amplified/deleted for HER-2 and TOP2A are described in Table 1. Based on previously published studies,[21] we selected a cut-off point of >5 for HER-2 amplification when considering the absolute copy numbers. We also studied the implication of the evaluation of chromosome 17 centromere copy number for all cases with HER-2 amplification as this question has been addressed in recently reported studies with interesting results.[22,23] Polysomy of chromosome 17 was defined as a mean number of signals greater than 2.5.

3.1. *Statistical analysis*

Demographic information including diagnosis details, tumour characteristics, chemotherapy scheme and toxicity, surgical data, and response were entered prospectively into a database.

Continuous data are expressed as mean, and the range between parentheses. Qualitative data are presented as absolute numbers or percentages.

Clinical response was considered as a discrete variable divided into responders and non-responders (SD + PD).

Complete pathological response is the only pathological result that has been consistently correlated with outcome and is widely accepted as a surrogate for survival.[1] Therefore, we performed the analysis with complete pathological response as a discrete variable. Nevertheless, using the percentage of pathological changes produced by chemotherapy in the tumour bed has also been clearly correlated with outcome.[14,24] As any chosen cut-off point would be arbitrary, we evaluated the percentage of changes as a continuous variable with intervals of 5%.

HER-2 status (positive or negative) was reclassified for the whole series by combining the first assessment results with CISH results of the available cases. The cases that were positive by IHC (3+) and did not have a CISH assessment due to unavailability of the sample remained as positive for the analysis.

We performed a univariate analysis to assess the relationship between each biological parameter and clinical and PR to doxorubicin. The χ^2 test was used to compare frequencies. The Mann–Whitney *U* test was used to study the relationship between changes due to chemotherapy as a continuous variable and the potential predictive factors.

A multivariate analysis, in which pCR was the dependent outcome variable was performed by forward stepwise logistic regression.

Statistical significance was indicated by a two-sided $p < 0.05$. Data were analysed with SPSS™ (version 10.0, Chicago, IL).

Table 1 – HER-2 and TOP2A status definition criteria

	HER-2	TOP2A
Amplification	HER-2 > 5 HER-2/CEN17 > 2	TOP2A > 5 TOP2A/CEN17 > 2
Non-amplification	HER-2 = 2–5 HER-2/CEN17 = 0.8–2	TOP2A = 2–5 TOP2A/CEN17 = 0.8–2
Monoallelic deletion	HER-2 < 2 HER-2/CEN17 < 0.8	TOP2A < 2 TOP2A/CEN17 < 0.8

4. Results

4.1. *Patients*

Data from 232 patients who received treatment inside this protocol were analysed. The mean age of the patients was 47 years.

4.2. *Tumours characteristics*

Tumours characteristics are shown in Table 2.

HER-2 status was negative in 180 (81%) tumours and 43 (19%) tumours showed overexpression/amplification according to our initial assessment. Positive cases with available tissue underwent CISH: 33 samples were assessed for HER-2, 36 for TOP2A and 38 for CEN17. Results of the subgroup evaluated by CISH are shown in Table 3. The reclassification of the whole series for HER-2 status (positive and negative) is shown in Table 4. Ten cases that were HER-2 positive by IHC (3+) and could not be studied by CISH were considered as positive for the final analysis. One case had HER-2 CISH (positive with signal characteristic cluster) assessment but no material was available for CEN17 determination. This case was also considered positive in the combined results (Table 4).

Polysomy was observed in 29/38 (76%) HER-2 positive tumours. HER-2 amplification was positively correlated with ER negativity ($p = 0.003$) and TOP2A amplification was correlated with a high Ki-67 index ($p = 0.03$).

4.3. *Treatment*

All patients completed treatment with a median dose intensity of doxorubicin of 1. The rate of conservative surgery was 93%.

4.4. *Response assessment*

CR was evaluated in 229 patients, 130 (57%) showed response (CR) and 99 (43%) showed no response (SD and

Table 2 – Tumours characteristics (N: 232)

		Total number (%)
T	2	75 (30%)
	3	157 (70%)
N	0	139 (60%)
	1	93 (40%)
Histology	Ductal	203 (88%)
	Lobular	21 (9%)
	Other	8 (3%)
ER	POS	157 (67%)
	NEG	67 (29%)
	Unknown	8 (4%)
PgR	POS	121 (52%)
	NEG	99 (43%)
	Unknown	12 (5%)
GRADE	1	3 (1%)
	2	114 (49%)
	3	86 (37%)
	Unknown	29 (13%)
HER-2 (algorithm)	POS	43 (19%)
	NEG	180 (77%)
	Unknown	9 (4%)
Ki-67	High	39 (17%)
	Low	58 (25%)
	Unknown	135 (42%)

T: tumour size; N: Nodal status; ER: oestrogen receptor; PgR: progesterone receptor; POS: positive; NEG: negative: High $\geqslant$ 25%; Low < 25%.

Table 3 – CISH results with the two assessment methods

		HER-2[a]	TOP2A[a]
Amplification	Copy number > 5	30	10
	Ratio > 2	21	5
Non-amplification	Copy number = 2–5	3	22
	Ratio = 0.8–2	9	18
Monoallelic deletion	Copy number < 2	0	4
	Ratio < 0.8	2	13

a Total numbers of each group vary because some determinations could not be performed in all samples of each group because of lack of tissue.

Table 4 – HER-2 status according to the different assessment methods

	First assessment	Her-2 Copy number > 5	HER-2/CEN17
Positive	43 (19%)	40 (18%)	32 (14%)
Negative	180 (81%)	183 (82%)	191(86%)

CEN17: Chromosome 17 centromeric signals.

PD). Twenty (9%) of the responders showed complete clinical response. Only 5 (2%) patients showed progressive disease.

Regarding PR, pCR was observed in 27 samples (12%), 13 of which had residual in situ component. Seventy tumours (31%) showed no histological changes.

4.5. *Correlation of response with biologic parameters*

4.5.1. *Clinical response*

HER-2 amplification evaluated by the ratio (HER-2/CEN17) showed a significant association with clinical response ($p = 0.04$). HER-2 amplification evaluated by the absolute copy number and hormone receptor negativity showed a trend to predict clinical response (both p-values = 0.07).

4.5.2. *Pathological response*

Considering changes due to chemotherapy as a continuous variable, HER-2 overexpression/amplification, ER and PgR negativity and high Ki67 were predictors of pathological changes (Table 5). Grade 3 tumours showed a trend to associate with higher percentage of response but did not reach statistical significance. Lobular carcinomas had a mean percentage of changes of 11% whereas ductal carcinomas showed 45% of changes as a group ($p = 6 \times 10^{-5}$).

When assessing pCR, ER ($p = 1.2 \times 10^{-5}$) and PgR ($p = 0.01$) negativity, high Ki-67 ($p = 0.005$) and HER-2 amplified tumours (ratio) ($p = 0.03$) were predictive markers of pCR. HER-2 amplification assessed by the absolute copy number and grade 3 tumours showed a trend towards correlation but results were not statistically significant ($p = 0.12$ and 0.3, respectively). Ductal compared to lobular histology also showed a trend to correlate with pCR ($p = 0.07$).

Neither TOP2A status (amplification/deletion) nor polysomy of chromosome 17 were predictive of PR in our analysis. Moreover, although tumours with coamplification of HER-2 and TOP2A, had a mean percentage of changes higher than

Table 5 – Relationship between pathological changes and biological parameters

Mean percentage of changes (range)			p-value[a]
ER	POS	36 (26–45)	1.8×10^{-7}
	NEG	55 (39–71)	
PgR	POS	39 (29–49)	0.003
	NEG	46 (30–61)	
Ki-67	High	56 (43–69)	0.001
	Low	32 (22–43)	
Grade	2	39 (29–49)	0.1
	3	48 (32–62)	
HER-2 (first assessment)	Pos	53 (33–72)	0.028
	NEG	39 (29–47)	
HER-2 copy number	AMPL	62 (42–82)	0.006
	NONAMPL	37 (28–46)	
HER-2 ratio (HER-2/CEN17)	AMPL	58 (45–72)	0.002
	NONAMPL	37 (32–42)	

ER: oestrogen receptor; PgR: progesterone receptor; POS: positive; NEG: negative; AMPL: amplification; NONAMPL: non-amplification; CEN17: number of centromeric signals in chromosome 17.
a Mann–Whitney U test.

those that did not show coamplification (56% versus 48%), these results did not reach statistical significance ($p = 0.5$).

We performed a multivariate analysis for pCR. Ninety-seven cases had both Ki67 and ER measurements. When a logistic model was used to assess the relationship between Ki67 (below or $\geqslant 25$) and pCR, a high Ki67 rate was associated with a greater probability of pCR (HR = 5.8; 95% CI, 1.47–23.6; $p = 0.008$). An identical approach examining ER indicated that ER negativity was associated with a greater probability of response (HR = 4.9; 95% CI, 1.72–14.2; $p = 0.001$). Comparison of the predictive value of these two parameters did not indicate that either was a better predictor than the other (p-value for comparison $p = 0.065$).

5. Discussion

When anthracyclines became essential drugs in the treatment of breast cancer, HER-2 status was extensively evaluated as a potential marker of response to this therapy.

In the adjuvant setting, some trials with large number of patients, showed a better outcome for HER-2 positive patients when treated with anthracyclines.[5–7]

A number of studies of anthracyclines in the neoadjuvant setting have addressed the potential relationship between HER-2 status and response. Several do not demonstrate a clear association.[25,26] Those that do show a trend or a significant relationship, are heterogeneous and have included small numbers of patients with different stages of disease, retrospective data, different definitions of response and mostly polychemotherapy regimes rather than single agent anthracyclines.[9,10,25,27] Globally, these studies have failed to show a clear correlation between HER-2 status and response to anthracyclines. And more importantly, the biological mechanism for this relationship was not elucidated.

Biological rationale and in vitro data suggest that HER-2 could be the marker while TOP2A aberrations could account for both chemosensitivity and resistance to anthracyclines.[17] Reported clinical data for testing this hypothesis are heterogeneous and thus, it has been difficult to draw clear conclusions.[18,19,28,29]

Conversely, recent adjuvant studies involving large numbers of patients seem to suggest that TOP2A status might be a predictive marker of the benefit of anthracyclines.[30,31]

Our current work investigates a series of 232 consecutive operable breast cancer patients, with prospectively determined biological parameters, treated with primary doxorubicin as a single agent. Drug interactions seen in polypharmacy are therefore eliminated. Doxorubicin was administered with a correct dose intensity. This regimen, helped by optimal surgical techniques, resulted in a high percentage of conservative surgery.

Regarding clinical response, the only parameter that showed a predictive value was HER-2 amplification evaluated as a ratio.

When assessing pathological response, in our univariate analysis, ER, PgR negativity, high Ki-67 and HER-2 positivity (overexpression and amplification) seemed to predict a higher percentage of changes induced by doxorubicin. ER, PgR negativity, high Ki-67 and HER-2 amplification also predicted pCR.

Regarding histological grade, grade 3 tumours in our series, showed a trend towards correlation with both clinical and pathological responses. Previous reports support this predictive value of grade 3 in response to primary chemotherapy.[10,25] Having used Contesso's grading system that does not include the mitotic index in its evaluation criteria, instead of Elston and Ellis[32] we may have underestimated the predictive value of histological grade.

In agreement with previous reports,[33] lobular histology appeared as a clear marker of resistance to doxorubicin probably related to its biological characteristics. Interestingly, in our series, all lobular carcinomas were HER-2 negative and ER positive.

Tumours with coamplification of HER-2 and TOP2A, showed a higher percentage of changes due to chemotherapy but this result was not statistically significant. Furthermore, TOP2A amplification alone did not show any significant association with response.

Deletion of TOP2A occurred in only a small number of samples (1–6% depending on the assessment method). Even though there is a rationale and data[17] to support the existence of deletion as a marker of resistance to doxorubicin, we were unable to demonstrate this correlation (data not shown). The lack of association between TOP2A and PR to doxorubicin may be due to the small number of alterations of the gene in the global population of breast cancer patients and consequently, the small number of cases in our series and others' previously reported[18,19] or to a real lack of association. Moreover, the evaluation of TOP2A gene status is a laborious and time-consuming test and more definitive results are needed to define its predictive value and its use in routine clinical practice.

In the multivariate analysis, the only factors that maintained statistically significant association with pCR were ER negativity and high Ki-67. HER-2 overexpression/amplification failed to maintain its predictive role as reported in previous publications.[25,26,34]

ER positive tumours generally exhibit a less aggressive phenotype, and have been associated with chemoresistance. Proliferation markers (including Ki-67) are known to be associated with a higher likelihood to respond to preoperative chemotherapy. Our results clearly show the predictive value of these two parameters confirming previously reported results.[25,33,35]

These tests are available in most pathology departments with ER status assessment obligatory. We believe Ki-67 is a powerful predictor of response to doxorubicin and should also be taken into account when making a decision on chemotherapy for breast cancer patients.

Finally, chromosome 17 polysomy rate was high (76%) in the HER-2 positive tumours and its assessment reclassified 8 cases differently. These cases became non-amplified after CEN17 evaluation. This made correlation with PR more statistically significant. The meaning of these findings and their impact in response to chemotherapy remains to be clarified and needs further evaluation.

From our data, we can conclude that ER negativity, and high Ki-67 rate predict pathological response to doxorubicin in the primary setting.

In conclusion, individual predictive markers may be helpful as guidance for the clinician to choose the most appropriate treatment, but more work needs to be done with the help of high throughput technology in order to achieve a more clear insight in breast cancer biology and targeted therapy.

Conflict of interest statement

The authors state no conflict of interest.

REFERENCES

1. Chollet P, Amat S, Cure H, et al. Prognostic significance of a complete pathological response after induction chemotherapy in operable breast cancer. *Br J Cancer* 2002;**86**(7):1041–6.
2. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;**235**(4785):177–82.
3. Press MF, Bernstein L, Thomas PA, et al. HER-2/neu gene amplification characterized by fluorescence in situ hybridization: poor prognosis in node-negative breast carcinomas. *J Clin Oncol* 1997;**15**(8):2894–904.
4. Tandon AK, Clark GM, Chamness GC, Ullrich A, McGuire WL. HER-2/neu oncogene protein and prognosis in breast cancer. *J Clin Oncol* 1989;**7**(8):1120–8.
5. Muss HB, Thor AD, Berry DA, et al. c-erbB-2 expression and response to adjuvant therapy in women with node-positive early breast cancer. *N Engl J Med* 1994;**330**(18):1260–6.
6. Paik S, Bryant J, Park C, et al. erbB-2 and response to doxorubicin in patients with axillary lymph node-positive, hormone receptor-negative breast cancer. *J Natl Cancer Inst* 1998;**90**(18):1361–70.
7. Thor AD, Berry DA, Budman DR, et al. erbB-2, p53, and efficacy of adjuvant therapy in lymph node-positive breast cancer. *J Natl Cancer Inst* 1998;**90**(18):1346–60.
8. Petit T, Borel C, Ghnassia JP, et al. Chemotherapy response of breast cancer depends on HER-2 status and anthracycline dose intensity in the neoadjuvant setting. *Clin Cancer Res* 2001;**7**(6):1577–81.
9. Vincent-Salomon A, Carton M, Freneaux P, et al. ERBB2 overexpression in breast carcinomas: no positive correlation with complete pathological response to preoperative high-dose anthracycline-based chemotherapy. *Eur J Cancer* 2000;**36**(5):586–91.
10. Wang J, Buchholz TA, Middleton LP, et al. Assessment of histologic features and expression of biomarkers in predicting pathologic response to anthracycline-based neoadjuvant chemotherapy in patients with breast carcinoma. *Cancer* 2002;**94**(12):3107–14.
11. Jarvinen TA, Tanner M, Barlund M, Borg A, Isola J. Characterization of topoisomerase IIα gene amplification and deletion in breast cancer. *Genes Chromosomes Cancer* 1999;**26**(2):142–50.
12. Di Leo A, Gancberg D, Larsimont D, et al. HER-2 amplification and topoisomerase IIα gene aberrations as predictive markers in node-positive breast cancer patients randomly treated either with an anthracycline-based therapy or with cyclophosphamide, methotrexate, and 5-fluorouracil. *Clin Cancer Res* 2002;**8**(5):1107–16.
13. Ma Y, Lespagnard L, Durbecq V, et al. Polysomy 17 in HER-2/neu status elaboration in breast cancer: effect on daily practice. *Clin Cancer Res* 2005;**11**(12):4393–9.
14. Moreno A, Escobedo A, Benito E, et al. Pathologic changes related to CMF primary chemotherapy in breast cancer. Pathological evaluation of response predicts clinical outcome. *Breast Cancer Res Treat* 2002;**75**(2):119–25.
15. Contesso G, Mouriesse H, Friedman S, et al. The importance of histologic grade in long-term prognosis of breast cancer: a study of 1,010 patients, uniformly treated at the Institut Gustave-Roussy. *J Clin Oncol* 1987;**5**(9):1378–86.
16. Falo C, Moreno A, Lloveras B, et al. Algorithm for the diagnosis of HER-2/neu status in breast-infiltrating carcinomas. *Am J Clin Oncol* 2003;**26**(5):465–70.
17. Jarvinen TA, Tanner M, Rantanen V, et al. Amplification and deletion of topoisomerase IIα associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. *Am J Pathol* 2000;**156**(3):839–47.
18. Park K, Kim J, Lim S, Han S. Topoisomerase II-α (topoII) and HER2 amplification in breast cancers and response to preoperative doxorubicin chemotherapy. *Eur J Cancer* 2003;**39**(5):631–4.
19. Coon JS, Marcus E, Gupta-Burt S, et al. Amplification and overexpression of topoisomerase IIα predict response to anthracycline-based therapy in locally advanced breast cancer. *Clin Cancer Res* 2002;**8**(4):1061–7.
20. Gong Y, Gilcrease M, Sneige N. Reliability of chromogenic in situ hybridization for detecting HER-2 gene status in breast cancer: comparison with fluorescence in situ hybridization and assessment of interobserver reproducibility. *Mod Pathol* 2005;**18**(8):1015–21.
21. Tanner M, Gancberg D, Di Leo A, et al. Chromogenic in situ hybridization: a practical alternative for fluorescence in situ hybridization to detect HER-2/neu oncogene amplification in archival breast cancer samples. *Am J Pathol* 2000;**157**(5):1467–72.
22. Bhargava R, Lal P, Chen B. Chromogenic in situ hybridization for the detection of HER-2/neu gene amplification in breast cancer with an emphasis on tumors with borderline and low-level amplification: does it measure up to fluorescence in situ hybridization? *Am J Clin Pathol* 2005;**123**(2):237–43.
23. Hicks DG, Yoder BJ, Pettay J, et al. The incidence of topoisomerase II-α genomic alterations in adenocarcinoma of the breast and their relationship to human epidermal growth factor receptor-2 gene amplification: a fluorescence in situ hybridization study. *Hum Pathol* 2005;**36**(4):348–56.
24. Ogston KN, Miller ID, Payne S, et al. A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. *Breast* 2003;**12**(5):320–7.
25. Petit T, Wilt M, Velten M, et al. Comparative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II α status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. *Eur J Cancer* 2004;**40**(2):205–11.
26. Zhang F, Yang Y, Smith T, et al. Correlation between HER-2 expression and response to neoadjuvant chemotherapy with 5-fluorouracil, doxorubicin, and cyclophosphamide in patients with breast carcinoma. *Cancer* 2003;**97**(7):1758–65.
27. Di Leo A, Chan S, Paesmans M, et al. HER-2/neu as a predictive marker in a population of advanced breast cancer patients randomly treated either with single-agent doxorubicin or single-agent docetaxel. *Breast Cancer Res Treat* 2004;**86**(3):197–206.
28. Depowski PL, Rosenthal SI, Brien TP, et al. Topoisomerase IIα expression in breast cancer: correlation with outcome variables. *Mod Pathol* 2000;**13**(5):542–7.
29. Durbecq V, Paesmans M, Cardoso F, et al. Topoisomerase-IIα expression as a predictive marker in a population of advanced breast cancer patients randomly treated either with single-

agent doxorubicin or single-agent docetaxel. *Mol Cancer Ther* 2004;**3**(10):1207–14.
30. Knoop AS, Knudsen H, Balslev E, et al. Retrospective analysis of topoisomerase IIa amplifications and deletions as predictive markers in primary breast cancer patients randomly assigned to cyclophosphamide, methotrexate, and fluorouracil or cyclophosphamide, epirubicin, and fluorouracil: Danish Breast Cancer Cooperative Group. *J Clin Oncol* 2005;**23**(30):7483–90.
31. Press MF BL, Sauter G, Zhou JY, et al. Topoisomerase II-α gene amplification as a predictor of responsiveness to anthracycline-containing chemotherapy in the Cancer International Research Group 006 clinical trial of trastuzumab (herceptin) in the adjuvant setting. In: Sant Antonio Breast Cancer Symposium. University of Southern California, Los Angeles, CA; University Medical Center Hamburg-Eppendorf, Hamburg, Germany; GBG, Munchen, Germany; Maria Sklodowska-Curie (MSC) Centre, Warsaw, Poland; ICORG, Dublin, Ireland; US Oncology, Dallas, TX; CIRG, Paris, France; AbbottVysis, Inc., Downers Grove, IL; U.C.L.A., Los Angeles, CA [1045] 2005[Abstract].
32. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 1991;**19**(5):403–10.
33. Mathieu MC, Rouzier R, Llombart-Cussac A, et al. The poor responsiveness of infiltrating lobular breast carcinomas to neoadjuvant chemotherapy can be explained by their biological profile. *Eur J Cancer* 2004;**40**(3):342–51.
34. Cardoso F, Durbecq V, Larsimont D, et al. Correlation between complete response to anthracycline-based chemotherapy and topoisomerase II-α gene amplification and protein overexpression in locally advanced/metastatic breast cancer. *Int J Oncol* 2004;**24**(1):201–9.
35. Rouzier R, Extra JM, Klijanienko J, et al. Incidence and prognostic significance of complete axillary downstaging after primary chemotherapy in breast cancer patients with T1 to T3 tumors and cytologically proven axillary metastatic lymph nodes. *J Clin Oncol* 2002;**20**(5):1304–10.