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ORIGINAL ARTICLE

Gene expression profiling of breast cancer patients treated with docetaxel, doxorubicin, and cyclophosphamide within the GEPARTRIO trial: HER-2, but not topoisomerase II alpha and microtubule-associated protein tau, is highly predictive of tumor response

A. Rody[a,*], T. Karn[a], R. Gätje[a], A. Ahr[a], C. Solbach[a], K. Kourtis[a], M. Munnes[b], S. Loibl[a], S. Kissler[a], E. Ruckhäberle[a], U. Holtrich[a], G. von Minckwitz[a,c], M. Kaufmann[a]

[a]*Department of Obstetrics and Gynecology, J.W. Goethe-University, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany*
[b]*Diagnostics Research, Bayer Healthcare AG, Leverkusen, Germany*
[c]*German Breast Group, Neu-Isenburg, Germany*

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Summary Gene expression analysis in breast cancer patients undergoing neoadjuvant chemotherapy is an interesting tool for identification of gene signatures and new markers to predict tumor response. However, the detection of predictive markers strongly depends on the drugs used in the specific therapeutic setting. There is growing evidence that topoisomerase II-alpha (TOPO IIα) is a marker for anthracycline-, and microtubule-associated protein tau (MAPT) for taxane sensitivity. HER-2 has been described as a marker of both anthracycline and taxane sensitivity. We performed gene expression profiling of 50 patients within the GEPARTRIO study, an anthracycline and taxane neoadjuvant chemotherapy trial. Here we investigate the predictive value of TOPO IIα, MAPT and HER-2 mRNA expression for pathological complete response (pCR) in this setting. Interestingly, HER-2 gene expression was strongly predictive of pCR ($P = 0.017$) as well as overall

*Corresponding author. Tel.: +49 -69 -63014117; fax: +49 -69 -630183469.
E-mail address: achim.rody@em.uni-frankfurt.de (A. Rody).

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response ($P = 0.037$) and clinical complete response (cCR, $P = 0.050$). In contrast, for both TOPO IIα and MAPT no correlation with pCR was observed in our sample group.

Introduction

Neoadjuvant or primary systemic therapy (PST) is the standard care for inflammatory and inoperable mammary carcinomas. Large randomized trials comparing PST and adjuvant therapy revealed no difference in clinical outcome. However, PST offers the following advantages: the rate of breast conservation surgery can be increased by 10–20% depending on the drugs used, and the observed therapeutic effect of PST as response of the tumor to treatment can be directly monitored and exactly assessed by both clinician and patient, resulting in an in vivo assay for the chemosensibility of the tumor. In particular, the rate of pathological complete remissions (pCR) correlates very well with disease-free survival (DFS) and overall survival (OAS) and thus can be used as a surrogate marker for clinical outcome of the disease. In previous work, we were able to show that neoadjuvant chemotherapy with docetaxel (T), adriamycin (A) and cyclophosphamide (C) resulted in a rate of pCR of 17.9% in stage II–IV breast cancer disease.[1] To date, many efforts have been made to detect specific marker genes for predicting tumor response and disease prognosis. Global gene expression profiling by microarrays has been used as a valuable tool for the identification of prognostic marker genes. Perou et al.[2] reported that gene expression profiling by DNA microarray analysis of breast tumors is feasible and allows different tumor subtypes to be distinguished. Van't Veer et al.[3] were able to demonstrate that tumor clustering by gene expression profile is able to discriminate between breast cancers with a poor or good prognosis. Their prognostic gene signature was further validated in a larger cohort of 295 patients with primary breast carcinomas.[4] Sorlie et al.[5] classified breast carcinomas based on gene expression patterns in luminal A and B, basal-like, erbB2+, and normal breast-like subtypes and correlated these groups with OAS and DFS. Our group identified a 41-gene signature[6] that allowed the discrimination of patients with an unfavorable prognisis.[7]

The detection of predictive markers strongly depends on the drugs used in the specific therapeutic setting. There is growing evidence that topoisomerase II-alpha (TOPO IIα) is a predictive marker for anthracycline-based chemotherapy[8] and microtubule-associated protein tau (MAPT) for taxane sensitivity.[9] However, only a few reports investigating these genes as predictive markers in anthracycline- and taxane-based neoadjuvant chemotherapy are available. Chang et al.[10] demonstrated that a 92-gene signature is able to predict tumor response to docetaxel with 90% specificity and 85% sensitivity and recently Hannemann et al.[11] demonstrated a classifier encompassing 71 genes for neoadjuvant chemotherapy with doxorubicin and cyclophosphamide and a classifier with 17 genes for doxorubicin and docetaxel. Ayers et al.[22] revealed a signature containing 74 genes, which predicted tumor response after neoadjuvant chemotherapy with paclitaxel followed by 5-fluorouracil, adriamycin, and cyclophosphamide. However, neither MAPT, TOPO IIα, nor HER-2 was part of the signatures described. In contrast, Rouzier et al.[9] were able to demonstrate that a decrease in MAPT mRNA on microarray is a highly significant predictor of tumor response to paclitaxel-containing neoadjuvant chemotherapy. Furthermore, Slamon et al.[12] presented a subgroup analysis of the Breast Cancer International Research Group (BCIRG) 006 study, demonstrating TOPO IIα co-amplification as a marker for better response to anthracycline-containing chemotherapy in HER-2-positive patients.

The goal of our study presented here was to examine the predictive value of MAPT, TOPO IIα, and HER-2 mRNA expression in breast cancer patients who received neoadjuvant chemotherapy with docetaxel (T), doxorubicin (A), and cyclophosphamide (C) within the GEPARTRIO trial. Microarray analysis was used to obtain all measurements on the same platform and to compare these data with the results previously obtained from other groups using this approach.[9] Furthermore, this technique may allow the development of new diagnostic tools to predict tumor response in neoadjuvant chemotherapy of breast cancer patients.

Materials and methods

Selection of patients

Patients with previously untreated, unilateral or bilateral primary breast cancer were enrolled in

the study after informed consent was obtained. Inclusion criteria encompassed a measurable tumor lesion by palpation in two dimensions with one diameter of at least 2 cm, age 18 years or older, and a good performance status. Assessment of diagnosis by core-cut biopsy under ultrasound guidance was mandatory in all patients after determination of tumor dimensions by clinical examination, ultrasound, bilateral mammography, and/or magnetic resonance imaging (MRI). When breast cancer was diagnosed, patients were screened for metastatic disease by chest X-ray, abdominal ultrasound and/or computed tomography (CT) scans, and bone scans. One tissue sample from the core cut procedure was stored in liquid nitrogen for gene expression analysis after patients' informed consent was obtained. Pretherapeutic core biopsies were obtained from 70 patients. Samples were snap frozen in liquid nitrogen and part of the removed tumor tissue was used for diagnostic purposes. One 5-m tissue section (usually after 15, 30-min sections) of each biopsy and the first and the last section of each remaining tumor were stained with hematoxylin and eosin to monitor the percentage of tumor cells in the tissue. Only specimens with $>80\%$ of tumor cells were analyzed further. Samples were characterized according to standard pathology including immunohistochemistry of estrogen receptor, progesterone receptor, and amount of cancer cells. Patients were subsequently treated with two cycles of TAC.

Study design

All patients received two cycles of TAC (doxorubicin 50 mg/m^2, cyclophosphamide 500 mg/m^2, and docetaxel 75 mg/m^2 all on day 1, every 3 weeks). Tumor response was determined by palpation during the third week of the second cycle. Patients who demonstrated a tumor response defined as a tumor shrinkage of more than 50% were randomized for four or six further cycles of TAC. In the case of no tumor response, patients received either four further cycles of TAC or four cycles of NX (vinorelbine 25 mg/m^2 on days 1 and 8 plus capecitabine 1000 mg/m^2 orally twice a day on days 1–14 every 3 weeks) as a non-cross-resistant schedule after randomization. The primary aim of the trial was to determine the pCR rate of six or eight cycles of the combination of TAC and four cycles of NX in patients who did not respond after the first two cycles of TAC. Response was determined by clinical examination, ultrasound, and/or mammography or MRI. Surgery including axillary lymph node dissection was mandatory irrespective of tumor response. The primary goal was to achieve breast-conserving surgery. Patients with multicentric disease or large tumors underwent mastectomy. Secondary aims were determination of tumor response after two cycles of TAC and assessment of drug toxicity.

Evaluation of response

Clinical assessment of tumor response was evaluated by palpation, breast ultrasound, and/or mammography, and/or MRI. Clinical response was determined at every cycle by palpation and ultrasound. The clinical tumor response after the initial two cycles of TAC was assessed for further randomization. Tissue removed at surgery was investigated by pathologic examination. A pCR was defined as no microscopic evidence of a residual invasive or non-invasive tumor including single tumor cells in both all breast specimens and in lymph nodes. Patients who showed a tumor response of at least 50% were classified as having a partial response (PR) and less than 50% as non-responders (NR).

Gene expression and data analyses

Isolation of RNA was with Qiagen RNeasy reagents and expression profiling performed using Affymetrix Hg U133 Arrays (22 500 genes) according to the protocols of the manufacturer. More than two-thirds of the biopsies yielded sufficient amounts ($>5\,\mu$g) of RNA for expression profiling and high quality chip data. Microarray data were analyzed by EXPRESSIONIST software from GeneData (Basel, Switzerland) using the PM-MM model to obtain raw expression levels of MAPT, TOPO IIα, and HER-2. We previously demonstrated that for HER-2 as well as for the standard parameters ER and PR the expression data obtained from these microarrays correlated very well with immunohistochemical scoring by the pathologist.[13] Confirmation of Affymetrix microarray data on MAPT by protein expression has already been described.[9] The non-parametric Mann–Whitney test was applied to study the association between gene expression and response to therapy. For categorical variables Fisher's exact test was used. Spearman rank correlation was performed to evaluate a correlation of HER-2 and TOPO IIα expression. All statistical analyses were carried out using SPSS11.0 (SPSS, Chicago, IL, USA). Patients with missing values were excluded from the analyses and all reported *P*-values were two-sided. *P*-values of less

Table 1 Patient characteristics.

Age	Median 53	Range 30–69		
Menopausal status	Pre 25	Post 25		
Tumor stage	IIA 22	IIB 16	IIIA 6	IIIB 6
Tumor size	Median 3.5 cm	Range 2.5–12 cm		
Lymph node status	Positive 22	Negative 28		
Histological type	Ductal 42	Lobular 6	Mixed 2	
Grading	G1 4	G2 34	G3 11	GX 1
Receptor status	ER+ 33	ER− 17	PR+ 24	PR− 26
Study arm	TAC 40	TAC-NX 10		
Response after initial two cycles	Yes 27	No 23		
Clinical response	cCR 23	cPR 16	cNC 11	
OP	pCR 8	Residual disease 40	Not completed 2	

than 0.05 were considered to indicate a significant result.

Results

We enrolled 70 patients from the GEPARTRIO trial in our study. In 50 high-quality microarray expression data were obtained. Characteristics of these 50 patients are summarized in Table 1. The median age was 53 (range 30–69), half of the patients were premenopausal and half postmenopausal. Clinically 22 (44.0%) patients presented with stage IIA tumors at diagnosis, 16 (32.0%) stage IIB, 6 (12.0%) stage IIIA, and 6 (12.0%) stage IIIB. The median tumor size was 3.5 cm (range 2.5–12 cm). The histological type was invasive ductal in 84.0% and invasive lobular in 12.0%; in two cases, a mixed type was diagnosed. Lymph node involvement was clinically detectable in 44%. Four patients (8.0%) had grade I, 34 (68.0%) grade II, and 11 (22.0%) grade III tumors. Histological grading was not available for one case. Hormone receptor expression was positive for ER in 66.0% and for PR in 48.0% of the patients. All patients underwent surgical therapy after completion of chemotherapy. In 68.0% of all cases breast-conserving therapy was possible, whereas 32.0% had to undergo mastectomy. Seven patients (15.5%) developed a relapse after a median follow-up of 17 months. After two cycles of TAC 54.0% showed a tumor response, whereas 46.0% of the patients were classified as NR. A clinical complete response (cCR) was seen in 46.0% and clinical partial response (cPR) in 32.0% according to an overall response rate of 76.0% (cCR+cPR). Clinical tumor response after the initial two cycles of TAC was also a strong predictor in achieving a pCR ($\chi^2 = 4.303$, $P = 0.038$), overall response ($\chi^2 = 8.860$, $P = 0.003$), and cCR ($\chi^2 = 6.799$, $P = 0.009$). A pathological complete response (pCR) after neoadjuvant chemotherapy was achieved in eight patients (16.0%) by TAC chemotherapy, which is in accordance with data of the primary trial analysis (17.9%).[1] One patient demonstrated pCR after treatment with NX (2.2%).

When clinical variables were analyzed for their ability to predict tumor response, parameters such

Table 2 Correlation of clinical parameters and pCR to neoadjuvant chemotherapy.

	pCR	No pCR	*P*-value
Tumor stage			
IIA	3 (6%)	19 (38%)	
IIB	3 (6%)	13 (26%)	
III A	2 (4%)	4 (8%)	
III B	0 (0%)	6 (12%)	
Total	8 (16%)	42 (84%)	$P = 0.446$
Histology			
Ductal carcinoma	7 (14%)	35 (70%)	
Lobular carcinoma	1 (2%)	5 (10%)	
Mixed type	0 (0%)	2 (4%)	$P = 0.820$
*Grading**			
Low grade	0 (0%)	4 (8.2%)	
Intermediate grade	5 (10.2%)	29 (59.2%)	
High grade	2 (4.1%)	9 (18.3%)	$P = 0.107$
ER-status			
ER negative	6 (12%)	11 (22%)	
ER positive	2 (4%)	31 (62%)	$P = 0.013$
PR-status			
PR negative	7 (14%)	19 (38%)	
PR positive	1 (2%)	23 (46%)	$P = 0.050$

*Grading was not available for $n = 1$

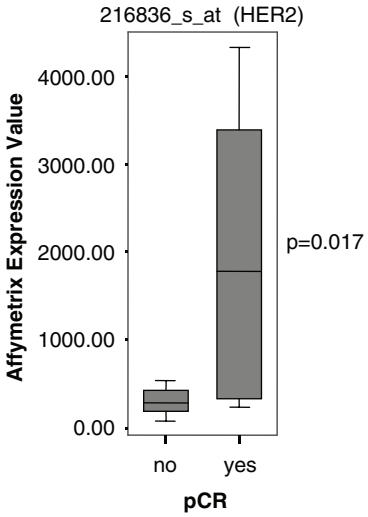

Figure 1 Correlation of HER-2 microarray expression and pathological complete response. Tumors were categorized according to pathological complete response and box plots of Affymetrix raw values for HER-2 mRNA expression (Affy probe set 216836_s_at) are shown.

as tumor stage, histological type, grading, and menopausal status showed no power in predicting a pCR after neoadjuvant chemotherapy (Table 2). On the other hand, ER and PR negativity were predictive of pCR, which is in accordance with most neoadjuvant trials. We previously demonstrated that mRNA expression data as determined by microarray analysis in this setting correlated well with the expression of routine parameters (ER and PR) determined by immunohistochemistry.[13] Thus, we assessed several further markers from microarrays for their ability to predict a pCR. Interestingly, HER-2 gene expression revealed to be strongly predictive of pCR ($P = 0.017$, Mann–Whitney test), clinical overall response (OR; $P = 0.037$, Mann–Whitney test), and cCR ($P = 0.050$, Mann–Whitney Test). Figure 1 demonstrates the difference in expression of HER-2 in patients who achieved a pCR and those with residual disease at the time of surgery. As presented in Fig. 2, TOPO IIα was not significant in predicting a pCR. However, there was a trend toward higher expression in responders, which was significant when clinical response was evaluated ($P = 0.049$ for OR and $P = 0.042$ for cCR respectively, Mann–Whitney test). Since TOPO IIα was demonstrated to be commonly co-expressed with HER-2, we tested for a possible correlation between those two genes, but did not achieve a significant result when Spearman rank correlation was used. MAPT, a proposed marker of taxane sensitivity, is represented by several probe sets on the Affymetrix U133A microarray. However, none of these probe sets revealed a correlation with pCR (see Fig. 3) or clinical response in our sample group.

Discussion

Interestingly, high levels HER-2 mRNA as evaluated by microarray analysis were predictive of achieving pCR, clinical OR, and cCR in our study. Consistent with these results, Konecny et al.[14] was able to find improved response rates in patients with advanced, HER-2 positive breast cancer receiving a combination of epirubicin and paclitaxel. In addition, Roche et al.[15] were able to demonstrate in a retrospective analysis of the adjuvant PACS01 trial an improved DFS and OS in patients with HER-2 overexpression or amplification by treatment with the combination of 5-fluorouracil, epirubicin, and cyclophosphamide (FEC) followed by docetaxel, compared with patients receiving FEC alone. The adjuvant BCIRG 001 study comparing six cycles of TAC with FAC chemotherapy could demonstrate a superiority of the taxane-containing regimen irrespective of HER-2 status (HER-2 positive: RR 0.60 [0.41–0.88], HER-2 negative: RR 0.76 [0.59–1.00], interaction test $P = 0.41$).[16] However, in our study we investigated

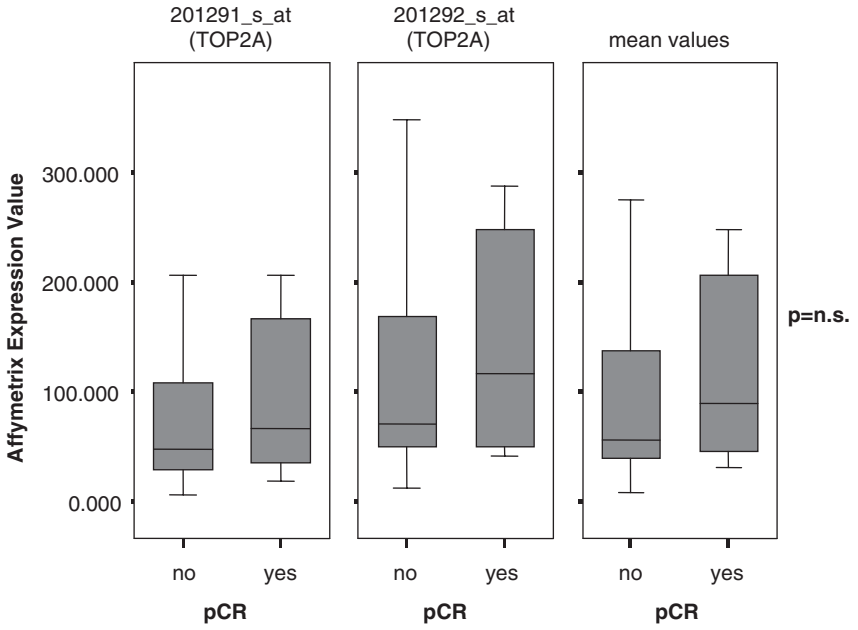

Figure 2 Correlation of Topo II-α microarray expression and pathological complete response. Tumors were categorized according to pathological complete response. Box plots of raw values for two different Affymetrix probe sets for Topoisomerase IIα (201291_s_at and 201292_s_at, respectively) as well as their mean values are given.

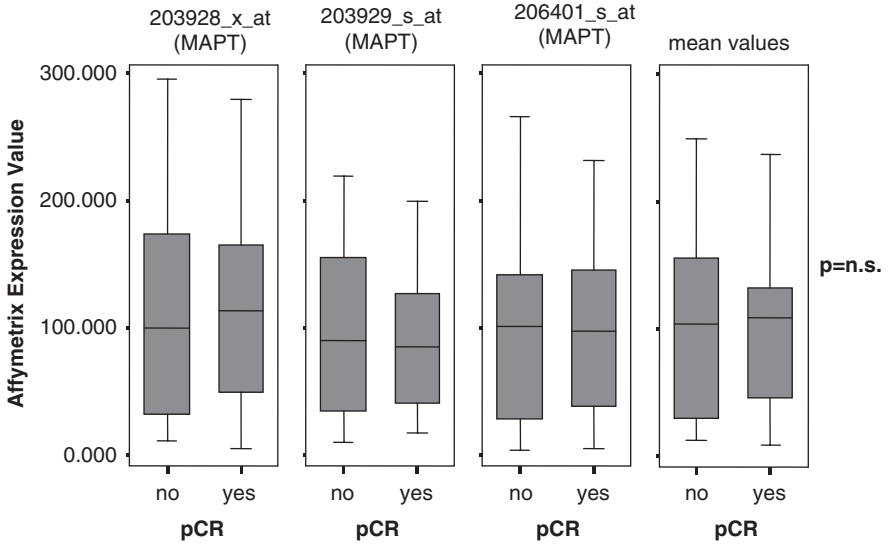

Figure 3 Correlation of MAPT microarray expression and pathological complete response. Tumors were categorized according to pathological complete response. Box plots of raw values for three different Affymetrix probe sets for microtubule-associated protein tau (203928_x_at, 203929_s_at and 206401_s_at, respectively) as well as the mean value of all three probe sets are given.

the predictive value of HER-2 exclusively and did not look for patients' prognoses stratified to different treatment arms as performed in both adjuvant trials.

High levels of TOPO IIα were also predictive of cCR and cPR in our study. These data are in accordance with the publication by Durbecq et al.[17] who demonstrated that overexpression of TOPO IIα is associated with a higher probability of tumor response in patients with advanced breast cancer receiving doxorubicin as a single agent compared with docetaxel. Also, Martin-Richard et al.[18] showed that TOPO IIα was overexpressed in 16 out of 41 (31%) tumors before treatment, and this overexpression was significantly associated with clinical response ($P = 0.03$). Notably, TOPO IIα overexpression was lost in specimens after chemotherapy ($P = 0.01$). In contrast, Petit et al.[19]

found no correlation of TOPO IIα and HER-2 overexpression with tumor response in 119 patients treated with neoadjuvant FEC. Coon et al.[20] found a strong correlation of TOPO IIα and HER-2 amplification in locally advanced breast cancer, which is explained by the close localization of the two genes on chromosome 17. Slamon et al.[12] also observed a high rate of co-amplification of TOPO IIα and HER-2 (35%) in HER-2 positive breast cancer and was able to demonstrate that the anthracycline sensitivity of these tumors was significant higher compared with those tumors that showed no co-amplification. Our data, however, did not reveal a correlation of TOPO IIα overexpression and HER-2 amplification/expression, which may be an explanation for the lack of predictive significance of TOPO IIα for a pCR. Notably, in contrast to HER-2, in which gene amplification correlates well with RNA expression as well as immunohistochemical data, this seems not to be the case for TOPO IIα. Mueller et al.[21] revealed that amplification of TOPO IIα did not correlate with protein expression levels in breast tumors, which may be one reason for discrepancies in different studies.

Rouzier et al.[9] described that decreased MAPT levels are highly predictive of achieving pCR in 82 patients receiving 12 cycles of weekly paclitaxel followed by a combination of four cycles of FAC as PST. However, we were not able to confirm these results in our study even though we used an identical microarray. Possible reasons for this discrepancy might arise from the different methods of obtaining tumor tissue (Rouzier: fine needle aspiration, our study: core cut biopsy), and the use of different taxanes (Rouzier: paclitaxel, our study: docetaxel). Moreover, the number of samples in both studies was rather small. Thus, differences in tumor characteristics between the two sample groups analyzed cannot be ruled out.

In conclusion, our data revealed that HER-2, but not TOPO IIα and MAPT, is highly predictive of pCR to neoadjuvant TAC chemotherapy. The conflicting results in terms of TOPO IIα and MAPT expression could be attributed to the low number of samples analyzed in the studies. Hence, an increase in the number of tumor samples investigated by gene expression profiling is necessary to clarify this controversy.

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References

1. Von Minckwitz G, Blohmer JU, Raab G, Lohr A, Gerber B, Heinrich G, et al. In vivo chemosensitivity-adapted preoperative chemotherapy in patients with early-stage breast cancer: the GEPARTRIO pilot study. *Ann Oncol* 2005;**16**:56–63.
2. Perou CM, Jeffrey SS, van de Rijn M, Rees CA, Eisen MB, Ross DT, et al. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. *Proc Natl Acad Sci USA* 1999;**96**:9212–7.
3. Van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002;**415**:530–6.
4. Van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002;**347**:1999–2009.
5. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001;**98**:10869–74.
6. Ahr A, Holtrich U, Solbach C, Scharl A, Strebhardt K, Karn T, et al. Molecular classification of breast cancer patients by gene expression profiling. *J Pathol* 2001;**195**:312–20.
7. Ahr A, Karn T, Solbach C, Seiter T, Strebhardt K, Holtrich U, et al. Identification of high risk breast-cancer patients by gene expression profiling. *Lancet* 2002;**359**:131–2.
8. Liu LF. DNA topoisomerase poisons as anti-tumor drugs. *Ann Rev Biochem* 1989;**58**:351–75.
9. Rouzier R, Rajan R, Wagner P, Hess KR, Gold DL, Stec J, et al. Microtubule-associated protein tau: a marker of paclitaxel sensitivity in breast cancer. *Proc Natl Acad Sci USA* 2005;**102**:8315–20.
10. Chang JC, Wooten EC, Tsimelzon A, Hilsenbeck SG, Gutierrez MC, Elledge R, et al. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. *Lancet* 2003;**362**:362–9.
11. Hannemann J, Oosterkamp HM, Bosch CA, Velds A, Wessels LF, Loo C, et al. Changes in gene expression associated with response to neoadjuvant chemotherapy in breast cancer. *J Clin Oncol* 2005;**23**:3331–42.
12. Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Pawlicki M, et al. on behalf of the BCIRG 006 Investigators. Phase III randomized trial comparing doxorubicin and cyclophosphamide followed by docetaxel (AC→T) with doxorubicin and cyclophosphamide followed by docetaxel and trastuzumab (AC→TH) with docetaxel, carboplatin and trastuzumab (TCH) in HER2 positive early breast cancer patients: BCIRG 006 study. *Breast Cancer Res Treat* 2005;**94**(Suppl.1) (abstract 1).
13. Rody A, Karn T, Gatje R, Kourtis K, Minckwitz G, Loibl S, et al. Gene expression profiles of breast cancer obtained from core cut biopsies before neoadjuvant docetaxel, adriamycin, and cyclophosphamide chemotherapy correlate with routine prognostic markers and could be used to identify predictive signatures. *Zbl Gynakol* 2006;**128**(2):76–81.
14. Konecny GE, Thomssen C, Luck HJ, Untch M, Wang HJ, Kuhn W, et al. HER-2/neu gene amplification and response to paclitaxel in patients with metastatic breast cancer. *J Natl Cancer Inst* 2004;**96**:1141–51.
15. Roche HH, Penault-llorca FM, Sagan C, Lacroix-triki M, Denoux Y, Verriele V, et al. Prognostic and predictive value of HER2, Pr, Er, and KI67 in the PACS01 trial comparing epirubicin-based

chemotherapy to sequential epirubicin followed by docetaxel. *J Clin Oncol* 2005;**23**(16s) (Abstract 605).
16. Martin M, Pienkowski T, Mackey J, Pawlicki M, Guastalla JP, Weaver C, et al. Adjuvant docetaxel for node-positive breast cancer. *N Engl J Med* 2005;**352**(22):2302–13.
17. Durbecq V, Paesmans M, Cardoso F, Desmedt C, Di Leo A, Chan S, et al. Topoisomerase-II alpha 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**:1207–14.
18. Martin-Richard M, Munoz M, Albanell J, Colomo L, Bellet M, Rey MJ, et al. Serial topoisomerase II expression in primary breast cancer and response to neoadjuvant anthracycline-based chemotherapy. *Oncology* 2004;**66**:388–94.
19. Petit T, Wilt M, Velten M, Millon R, Rodier JF, Borel C, et al. Comparative value of tumour grade, hormonal receptors, Ki-67, HER-2 and topoisomerase II alpha status as predictive markers in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy. *Eur J Cancer* 2004;**40**: 205–11.
20. Coon JS, Marcus E, Gupta-Burt S, Seelig S, Jacobson K, Chen S, et al. Amplification and overexpression of topoisomerase IIalpha predict response to anthracycline-based therapy in locally advanced breast cancer. *Clin Cancer Res* 2002;**8**: 1061–7.
21. Mueller RE, Parkes RK, Andrulis I, O'Malley FP. Amplification of the TOP2A gene does not predict high levels of topoisomerase II alpha protein in human breast tumor samples. *Gene Chromosome Cancer* 2004;**39**(4):288–97.
22. Ayers M, Symmans WF, Stec J, Damokosh AI, Clark E, Hess K, et al. Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer. *J Clin Oncol* 2004;**22**:2284–93.