

## Review

### Recent advances in systemic therapy

# New diagnostics and biological predictors of outcome in early breast cancer

Catherine Oakman<sup>1</sup>, Silvia Bessi<sup>2</sup>, Elena Zafarana<sup>1</sup>, Francesca Galardi<sup>2</sup>, Laura Biganzoli<sup>1</sup> and Angelo Di Leo<sup>1</sup>

<sup>1</sup>Sandro Pitigliani<sup>1</sup> Medical Oncology Unit, Hospital of Prato, Istituto Toscano Tumori, 59100 Prato, Italy

<sup>2</sup>Translational Research Unit, Hospital of Prato, Istituto Toscano Tumori, 59100 Prato, Italy

Corresponding author: Angelo Di Leo, [adileo@usl4.toscana.it](mailto:adileo@usl4.toscana.it)

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## Abstract

The key to optimising our approach in early breast cancer is to individualise care. Each patient has a tumour with innate features that dictate their chance of relapse and their responsiveness to treatment. Often patients with similar clinical and pathological tumours will have markedly different outcomes and responses to adjuvant intervention. These differences are encoded in the tumour genetic profile. Effective biomarkers may replace or complement traditional clinical and histopathological markers in assessing tumour behaviour and risk. Development of high-throughput genomic technologies is enabling the study of gene expression profiles of tumours. Genomic fingerprints may refine prediction of the course of disease and response to adjuvant interventions. This review will focus on the role of multiparameter gene expression analyses in early breast cancer, with regards to prognosis and prediction. The prognostic role of genomic signatures, particularly the Mammaprint and Rotterdam signatures, is evolving. With regard to prediction of outcome, the Oncotype Dx multigene assay is in clinical use in tamoxifen treated patients. Extensive research continues on predictive gene identification for specific chemotherapeutic agents, particularly the anthracyclines, taxanes and alkylating agents.

## Introduction

Over the past decade there have been exciting developments in gene expression analysis [1]. Assessment of the genetic profiles of tumours furthers our understanding of their composition and behaviour. These signatures are enabling improved diagnosis, prognostic classification and more accurate prediction of benefit from chemotherapy for individual patients. Genetic profiles also assist pharmacogenomic development by providing potential new targets for therapies.

Breast cancer is a prevalent disease and a leading cause of cancer death in women. Adjuvant systemic therapy improves disease-free survival and overall survival (OS) in some women [2,3]. Patients with poor prognostic features benefit the most from adjuvant therapy and identification of these high risk women is an ongoing challenge. Individualised systemic treatment for these women should improve outcomes. Conversely, identification of women with a good prognosis, or low risk of recurrent disease, may be spared the rigours and potential complications associated with adjuvant therapy.

Traditionally, patients have been stratified according to risk of recurrence by clinical and histopathological features. These features have not proven adequate to identify patients who will most benefit from adjuvant therapy. For patients and clinicians there is a fear of under-treating in the adjuvant setting, potentially resulting in recurrent, incurable metastatic disease. Consequently, over-treatment in the adjuvant setting is not uncommon.

## Prognosis

Molecular identification and classification of tumours enables important distinctions to be made between tumours that may appear similar based on traditional clinical and histopathological systems [4]. Traditional prognostic factors include age, tumour size, lymph node status, histological type, grade, human epidermal growth factor receptor-2 (Her-2) status and hormone receptor status. [5-7]. A devastating feature of any tumour is its capacity to metastasize. It is possible that the ability to metastasize is not a late acquisition of a cancer as

CI = confidence interval; CAF = cyclophosphamide, adriamycin and 5-fluorouracil; CMF = cyclophosphamide, methotrexate and 5-fluorouracil; CTC = circulating tumour cell; DFS = distant recurrence-free survival; ER = oestrogen receptor; Her-2 = human epidermal growth factor receptor-2; HR = hazard ratio; NSABP = National Surgical Adjuvant Breast and Bowel Project; OS = overall survival; PgR = progesterone receptor; qRT-PCR = quantitative reverse transcriptase PCR; RS = Recurrence Score; TAILORx = Trial Assigning Individualised Options for Treatment; Topolla = topoisomerase IIa.

previously thought, but an early and inherent genetic property of breast cancer that may be detected at diagnosis of the primary tumour.

Gene expression profiles are powerful tools. Development and validation of these profiles are providing greater understanding of tumour behaviour. The clinical role for these tools is potentially great but their specific role is still being explored and refined.

### **MammaPrint**

MammaPrint is a 70 gene expression profile marketed by Agendia. The MammaPrint assay was developed based on research initially conducted at the Netherlands Cancer Institute, Amsterdam, and collaborating institutions.

To identify a genetic signature strongly predictive of short time to distant metastases van't Veer and colleagues [8] undertook DNA microarray analysis on primary breast tumours of 117 young women (<55 years old) with lymph node-negative disease. Snap frozen tissue was used to derive RNA. Unsupervised analysis with 25,000 genes revealed clustering of approximately 5,000 significant genes. Supervised analysis of 78 patients with sporadic, node-negative breast cancer revealed a 70 gene set to identify early relapse. The poor prognosis group included 34 of the 78 patients who developed distant metastases within 5 years of diagnosis. The poor prognosis signature comprised genes regulating cell cycle, invasion, metastasis, signal transduction and angiogenesis. Interestingly, there was omission of previously identified individual genes associated with outcome, for example, those encoding the oestrogen receptor (ER), Her-2, and cyclin D1. This supports the power of a collective genetic signature over individual genes. A small validation was performed on 19 young, lymph node-negative women, 12 of whom had developed metastases within 5 years of original diagnosis. Of these 19, 17 were correctly classified. Results indicate that prognosis can be derived from primary tumour gene expression.

The same research group further validated this 70-gene profile in 295 young women (<53 years old) with lymph node-negative or -positive disease [9]. Sixty one of the lymph node-negative patients were also used in the original study; 130 patients received chemotherapy and/or hormonal therapy. The genetic signature based on the 70-gene profile predicted metastasis-free survival and OS. With multivariable Cox analysis the signature was independent of more traditionally recognised prognostic markers. The hazard ratio for distant metastasis (HR = 5.1, 95% confidence interval (CI) 2.9 to 9.0,  $p < 0.001$ ) remained significant, even when analysed according to lymph node status. This independence from lymph node status was surprising as lymph node status is traditionally recognised as one of the strongest histopathological markers for prognosis.

Espinosa and colleagues [10] sought to reproduce the results of the 70-gene profile with quantitative reverse transcriptase PCR (qRT-PCR) rather than by microarray analysis. They divided 96 patients with node-positive or -negative disease and a median age of 57 years into good and poor prognosis groups. qRT-PCR reproduced the microarray results for the 70-gene profile. Relapse-free survival and OS differed significantly between the two groups. For good and poor prognosis groups at 70 months, relapse-free survival was 85% versus 62% and OS was 97% versus 72%, respectively. From multivariate analysis only lymph node status and gene profile were significant for OS.

Buyse and colleagues [11], through the TRANSBIG research network [12], undertook independent validation of the 70-gene prognostic signature for women with lymph node-negative breast cancer. This multinational, retrospective trial analysed 307 women, aged less than 61 years, with lymph node-negative disease who did not receive adjuvant therapy. Median follow-up was 13.6 years. The patients were divided into high and low risk groups based on gene signature and clinical risk factors. Clinical risk was assessed using Adjuvant! Online software [13]. As predicted by the gene signature, time to distant metastases had a HR of 2.32 (95% CI 1.35 to 4.0) and OS had a HR of 2.79 (95% CI 1.60 to 4.87). The 70-gene microarray signature was superior to clinicopathological risk assessment in predicting all endpoints.

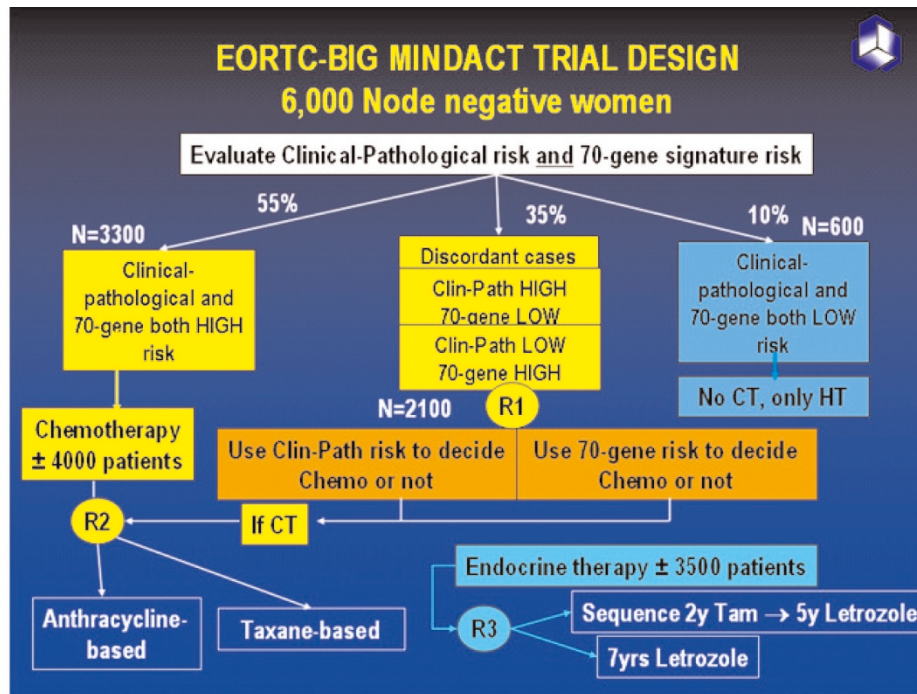
### **Rotterdam**

Another multiparameter gene expression tool, the 'Rotterdam Signature', was created at the Erasmus MC/Danail den Hoed Cancer Centre, Rotterdam. Of interest, this 76-gene set shares only three genes with the aforementioned MammaPrint.

Analysis was undertaken on stored tissue of 286 patients with lymph node-negative primary breast cancer who did not receive any adjuvant systemic therapy [14]; median age was 52 years. From an original training set of 115 tumours, a 76-gene signature was identified for good and poor prognosis. This was validated in a second set of 171 patients with 93% sensitivity and 48% specificity. The HR for distant recurrence within 5 years was 5.67 (95% CI 2.59 to 12.4) and only slightly less in a multivariate analysis (HR 5.55, 95% CI 2.46 to 12.5). After 5 years, the absolute differences in distant metastases-free survival and OS between good and poor signatures was 40% and 27%, respectively.

Independent multicentre validation of this tool was undertaken by Foekens and colleagues [15]. The previously identified 76-gene signature was applied to 180 lymph node-negative, untreated patients. Results of this study confirmed the signature as a strong predictive marker. The gene set identified patients at high risk of distant metastases within 5 years of original diagnosis with a HR of 7.41 (95% CI 2.63 to 20.9), which was maintained in multivariate analysis (HR

Figure 1



Outline of the TRANSBIG MINDACT trial. Clin-path, clinical-pathological; CT, chemotherapy; HT, hormone therapy; y, year.

11.36, 95% CI 2.67 to 48.8). An interesting comparison of the gene set with the 2003 St Gallen [16] and the 2001 National Institute of Health guidelines [17] was described. Approximately 40% of patients identified as high risk by these traditional clinicopathological risk assessments would have been spared their adjuvant treatment using the gene signature.

TRANSBIG assessed the 76-gene assay in the same population of patients it used to validate the 70-gene set [18]. The results showed that the two signatures performed similarly and were both superior to the traditional risk assessment tools.

#### Current clinical application of microarray analysis

The results from the Mammprint and Rotterdam signature are encouraging. However, several criticisms and concerns about the studies were highlighted [19]. Patient numbers in the training and validation sets were small. The Mammprint study also had 61 patients overlap between their two groups. Patient selection varied between the trials in terms of age inclusion, lymph node status and adjuvant therapy. The trials were retrospective and performed on frozen, stored tissue. Only three genes were shared between the two microarray signatures. This lack of gene overlap in the two signatures, which were designed to assess the same risk, may reflect different microarray platforms, different techniques and different experimental conditions.

A large, multicentre, prospective, randomised trial is necessary to test these microarray genomic profiles. TRANSBIG is now undertaking such a trial using the MammaPrint profile. This trial, MINDACT ('Microarray for Node Negative and 1 to 3 Positive Node Disease may Avoid Chemotherapy'), aims to recruit 6,000 women with node-negative early breast cancer in which patient decisions will be made based on a random assignment to use the MammaPrint assay or not [20] (Figure 1).

TRANSBIG has opted for the 70-gene signature for this study. Earlier concerns about concordance between different laboratories have been addressed, with TRANSBIG satisfactorily showing concordance between laboratory results if adherence to protocols is maintained [21]. The focus of this trial will be on patients who have discordance between risk assessment by the 70-gene set and traditional clinicopathological risk assessment using Adjuvant!. Prospective validation of the gene set as a prognostic tool is imperative, but the trial design also allows for potential predictive power of the tool for specific response to anthracycline- or docetaxel-based chemotherapy.

Clearly, the results of this trial will be eagerly awaited to guide the clinical use of these genomic profiles. Until these results are available there is currently not strong enough data to implement the gene arrays in daily practice for prognosis of patients.

Prognosis determination, regardless of the means of assessment, provides information about the natural history of a patient with early breast cancer. Prognostic tools have been valuable in identifying patients with aggressive disease and, as we have been deficient in clinically reliable predictive tools, they have been used to guide adjuvant therapies. However, the critical issue in early breast cancer is not actual prognosis, but whether a specific intervention will significantly improve prognosis. A patient at low risk of disease relapse - that is, with a good prognosis - may still consider adjuvant intervention if the relative risk reduction is significant and the risk of treatment is low. Conversely, a patient at high risk of relapse may have appropriate reservations about aggressive therapy with only minimal expected relative benefit. The critical issue is to find predictive tools to specify therapy for a specific patient, whether it be endocrine manipulation, chemotherapy and/or immunotherapy. As reliable predictive clinical tools evolve, the need for prognostic markers will diminish.

### Prediction

A significant number of women are diagnosed with lymph node-negative and hormone receptor-positive disease and many of these women currently receive adjuvant chemotherapy. Only few will receive much additional benefit from the chemotherapy over the benefit from endocrine therapy. Also, a small group of women may not be offered chemotherapy who will derive benefit. Ideally, molecular signatures would be able to not only identify the women at highest risk of recurrence, but also predict their benefit from therapy. Molecular signatures have been examined from women with recurrent disease and women without recurrent disease to compare their disease profiles and benefit from therapy. Patients with similar tumours clinically may respond differently to treatment, in terms of response and toxicities, likely attributable to genetic heterogeneity despite similar phenotypes.

### Oncotype Dx

The 21-gene assay Oncotype Dx is the first clinically validated multigene assay that quantifies the likelihood of breast cancer recurrence. It was developed specifically for women with hormone receptor (ER)-positive and lymph node-negative disease [22]. A real-time RT-PCR assay was developed for RNA extracted from routine paraffin embedded tissue; 250 candidate genes were identified from published literature and genomic databases. Three studies involving 447 patients were used to identify any link between the 250 genes and risk of breast cancer recurrence. Twenty-one genes associated with recurrence were identified: 16 cancer related genes and 5 reference genes (Figure 2). This gene panel is used to calculate a Recurrence Score (RS), a number between 0 and 100 that correlates to a specific likelihood of breast cancer recurrence within 10 years of original diagnosis. Patients are then assigned as having a low, intermediate or high risk of distant recurrence.

**Figure 2**

#### PROLIFERATION

*Ki67*  
*STK15*  
*Survivin*  
*CCNB1 (Cyclin B1)*  
*MYBL2*

#### *GSTM1*

#### *BAG1*

#### *CD68*

#### ESTROGEN

*ER*  
*PR*  
*BCL2*  
*SCUBE2*

#### INVASION

*MMP11 (Stromolysin 3)*  
*CTSL2 (Cathepsin L2)*

#### *HER-2*

*HER-2*  
*GRB7*

#### REFERENCE

*ACTB ( $\beta$ -actin)*  
*GAPDH*  
*RPLPO*  
*GUS*  
*TFRC*

The 21 genes identified for Oncotype Dx: 16 cancer related genes and 5 reference genes. These are used to calculate a Recurrence Score [22].

Once the 21-gene RT-PCR assay was defined it was prospectively validated using a cohort from the National Surgical Adjuvant Breast and Bowel Project (NSABP) trial B-14 [22]. This phase III trial compared adjuvant tamoxifen and placebo in patients with lymph node-negative and hormone receptor-positive primary breast cancer. Tamoxifen was superior for reducing risk of recurrence and death [23]. Low, intermediate and high risk groups were pre-specified as having a RS <18, RS 18 to 31, and RS >31, respectively. Of 668 tamoxifen-treated patients, 51% were categorised as having a low RS, 22% as having an intermediate RS and 27% as having a high RS. Ten-year Kaplan Meier estimates for distant recurrence were 6.8%, 14.3% and 30.5%, respectively. The RS was also predictive of overall survival ( $p < 0.001$ ).

Paik and colleagues [24] also applied the 21-gene assay in the placebo arm of the NSABP B-14 trial to assess its prognostic ability; 645 patients (355 placebo and 290

tamoxifen treated) were analysed. The RS was significantly associated with distant recurrence-free survival (DFS) in the placebo arm ( $p < 0.05$ ).

A second prospective validation was performed by Habel and colleagues, who evaluated the performance of the 21-gene assay among lymph node-negative patients who did not receive adjuvant chemotherapy [25]. From the Northern California Kaiser Permanente Tumour Registry of 4,964 patients, 220 cases (patients identified with death from breast cancer as their first event) were matched to 570 controls. The 10-year risk of breast cancer death in tamoxifen treated patients was 2.8%, 10.7% and 15.5% for those with a low, intermediate and high RS, respectively. In patients not treated with tamoxifen, the risks were 6.2%, 17.8% and 19.9%, respectively. This further validated use of the 21-gene assay with RS being strongly associated with breast cancer death.

Use of Oncotype Dx to predict benefit from chemotherapy was shown using a cohort of patients from the NSABP B-20 trial. This phase III trial in 2,306 ER-positive, lymph node-negative women compared adjuvant tamoxifen alone or with chemotherapy - methotrexate and 5-fluorouracil (MF) or cyclophosphamide, methotrexate and 5-fluorouracil (CMF). This trial showed a 4% absolute decrease in 10-year risk of recurrence in the CMF and tamoxifen group [26]. Analysis of 651 patients from this trial using the 21-gene assay showed that the benefit from chemotherapy in patients with a high RS was dramatic, a 27.6% absolute reduction in 10-year distant recurrent rate. Conversely, there was no clear benefit from chemotherapy in patients with a low RS. There was uncertainty in the intermediate group [27].

Oncotype Dx has also been analysed in lymph node-positive patients. Albain and colleagues [28] assessed the 21-gene assay in a cohort of postmenopausal, node-positive, ER-positive breast cancer patients. The original phase III trial randomized 1,158 women to adjuvant therapy with tamoxifen alone versus cyclophosphamide, adriamycin and 5-fluorouracil (CAF) with concurrent tamoxifen versus CAF with delayed tamoxifen. CAF with delayed tamoxifen was the superior arm for DFS and OS at 10 years [29]. A cohort of 367 women had 21-gene analysis of archived tissue, 148 from the tamoxifen alone arm and 219 from the CAF plus tamoxifen group. The RS distribution was 40% low, 28% intermediate and 32% high. RS was prognostic for DFS in tamoxifen-treated patients with positive nodes ( $p = 0.006$ ). CAF with tamoxifen added no apparent benefit to tamoxifen alone in the low RS patients, whereas there was a large benefit for CAF in the high RS group. The study also identified a group of patients with node-positive disease with low RS who did not seem to benefit from the chemotherapy.

Goldstein and colleagues [30] assessed whether Oncotype Dx could more reliably predict outcome at 5 years compared with standard clinicopathological risk assessment (based on

an algorithm based on Adjuvant!) in a cohort from Intergroup E2197. This phase III trial randomised 2,952 women with node-positive (one to three nodes positive) and high risk node-negative breast cancer to adjuvant doxorubicin/docetaxel versus doxorubicin/cyclophosphamide. There was no significant difference in DFS or OS at 76-month follow-up [31]. A group of 465 patients with ER-positive disease had 21-gene analysis; 99 patients had recurrent disease. RS predicted recurrence in node-positive and node-negative patients ( $p < 0.001$  for both). This prediction was more accurate than with using traditional risk factors.

In summary, the 21-gene Oncotype Dx assay is prognostic for hormone receptor-positive, lymph node-negative patients. A low RS is predictive of tamoxifen benefit in hormone-positive, node-negative cases. A high RS is predictive of chemotherapy benefit over hormonal therapy in hormone receptor-positive patients, regardless of lymph node status. These trials were performed with tamoxifen. It remains to be seen whether the tool may be predictive for other endocrine therapy, particularly the aromatase inhibitors.

An unanswered question regarding the Oncotype Dx assay is whether it adds more in predicting benefit than a combination of the histopathological markers ER, progesterone receptor (PgR), Her-2 and ki-67. Whilst Oncotype Dx has been compared with Adjuvant!, the latter is known to assess ER and PgR, but not Her-2 or ki-67. These histopathological markers are limited themselves in reproducibility with current standard methods, both within and between laboratories. However, a combination of these markers at a high quality laboratory, in combination with other clinical and pathological features, may still be as good as the evolving genomic signatures. These evolving technologies are certainly not without extra effort and expense. The genomic signatures have greater reproducibility and this may be their strength. A large, prospective, randomised trial comparing a combination of ER, PgR, Her-2 and ki67 with Oncotype Dx might be useful to better define in which clinical situations the use of Oncotype Dx can be recommended.

#### *Current clinical application of 21-gene recurrence score*

A potential clinical role for Oncotype Dx is in patients with hormone receptor-positive disease with uncertain levels of hormone sensitivity. Oncotype Dx is already commercially available in some centres for use in newly diagnosed patients with lymph node-negative, ER-positive primary breast cancer.

A large prospective, multicentre trial, TAILORx (Trial Assigning Individualised Options for Treatment) is underway for 10,000 patients with lymph node-negative, hormone receptor-positive breast cancer [32]. Patients with an intermediate RS (defined in this trial as RS 11 to 25) will be randomised to chemotherapy and endocrine therapy or endocrine therapy alone. Whilst the retrospective trials reviewed tamoxifen-, CMF- and anthracycline-based therapies,

TAILORx leaves the choice of specific endocrine agent and chemotherapy regimen up to the treating physician.

This trial does not challenge Oncotype Dx. It presumes that low and high RS are correct. These two groups would likely have been identified using standard biomarkers (that is, ER, PgR, Her-2, ki-67) and had their treatment directed accordingly. However, the trial does focus on patients with intermediate risk and results may clarify adjuvant intervention in this group, specifically whether endocrine therapy alone is as good as chemotherapy followed by endocrine therapy.

### Prediction of specific chemotherapy benefit

Whilst gene expression signatures are providing great advances, identification of single genes in a tumour can also provide essential data about the tumour and its innate behaviour. Important features of single genes include their presence, and alterations and interactions of them with other encoded genes. Specific genes may potentially provide specific new targets for pharmaceutical interventions and may also function as predictive biomarkers for response to systemic treatment. Empirical application of chemotherapy may be replaced by drugs specifically identified by molecular markers as beneficial in a particular patient.

### Anthracyclines

Anthracycline-based chemotherapy is commonly used in the adjuvant setting of early breast cancer. Anthracyclines have a survival benefit [33] but identification of the subgroup of women who will benefit from them is a challenge. Traditional markers have not satisfactorily identified this subgroup and predictive biomarkers, particularly Her-2 and topoisomerase IIa (Topolla), are under ongoing intensive investigation.

#### *Her-2, topoisomerase IIa and anthracyclines*

Her-2 overexpression occurs in about 30% of patients with breast cancer and is a recognised poor prognostic marker. Many trials have assessed Her-2 in predicting response to anthracycline versus non-anthracycline regimens. Results have been inconsistent. Whilst some trials have concluded a predictive role of Her-2 overexpression for improved efficacy of anthracycline-based adjuvant therapy [34-37], other studies have not. Two recent meta-analyses suggest greater benefit from the anthracycline-based therapy in women with Her-2 overexpression for disease-free survival and overall survival [38,39]. The mechanism underlying the interaction between Her-2 and anthracycline therapy is not fully understood. Her-2 may be serving as a surrogate marker for another drug target.

A principle mechanism of action of anthracyclines is inhibition of the Topolla enzyme, which is a key enzyme in DNA replication. *In vitro* and *in vivo* studies suggest greater anthracycline sensitivity in Topolla overexpression [40,41]. Conversely, suppression of Topolla produces resistance to anthracyclines [41]. Amplification of Topolla results in over-

production of the Topolla protein and this increase in drug target may account for improved drug efficacy. Contrasting data confuse this issue, however, as Topolla deletions have also been associated with benefit from anthracycline-based therapy [42].

Her-2 and Topolla co-inhabit chromosome 17. Topolla amplification occurs in 40% of Her-2 amplified breast cancers, whilst Topolla gene aberration is rarely detected in Her-2 non-amplified breast cancers [40]. Co-amplification of Her-2 and Topolla may predict anthracycline benefit [36,37].

Current data suggest a benefit for Her-2-overexpressing patients from an anthracycline versus non-anthracycline regimen. Further results are awaited specifically addressing the issues of the predictive role of Her-2 and Topolla co-amplification, Topolla amplification as an independent predictive marker, the effect of co-administration of anthracyclines with trastuzumab in Her-2 amplified patients, and clinically feasible Topolla measurements. Topolla measurement is an interesting evolving area of research. Regulation of the Topolla protein is complex and multifactorial (Figure 3). It may be that identification, quantification and intracellular localisation of the Topolla protein may be more clinically relevant than quantification of the Topolla gene [43].

### Taxanes

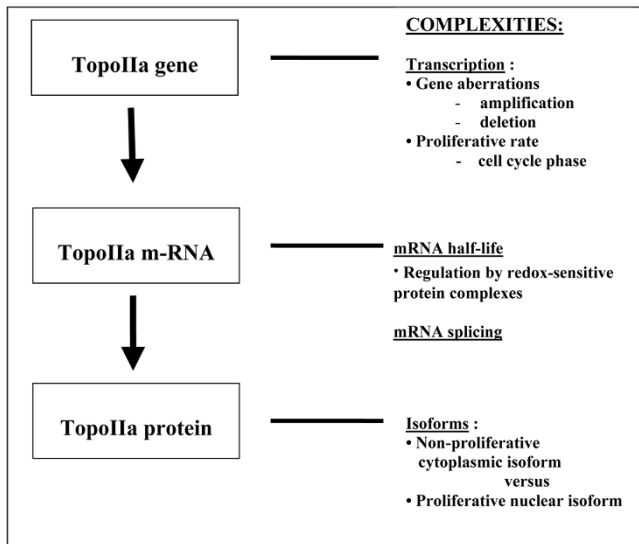
Taxanes cause apoptosis by binding to the interior surface of the beta-tubulin subunit of microtubules and disrupting cell architecture.

#### *Her-2 and taxanes*

Four trials in early breast cancer have retrospectively assessed Her-2 as a predictive biomarker for response to taxane therapy, one of these in the neoadjuvant setting [44-47]. The CALGB 9344 trial compared the addition of paclitaxel to anthracycline-based chemotherapy with anthracycline-based chemotherapy alone [44]. It showed a statistically significant improvement in DFS and OS with addition of paclitaxel only in Her-2-overexpressing disease. There was no taxane benefit seen in the Her-2-negative group. A meta-analysis of the three adjuvant trials suggested that both Her-2 amplified and Her-2 non-amplified patients benefit from the addition of the taxane, with greater benefit in the Her-2 amplified group [38].

#### *Protein tau and taxanes*

Microtubule-associated protein tau promotes microtubule assembly and stabilises microtubules. Gene expression analysis in the neoadjuvant setting with paclitaxel has identified low expression of tau with increased chemosensitivity and increased pathological complete response [48]. This negative correlation was validated using immunohistochemistry on tissue arrays to assess tau. Subsequent elegant *in vitro* work by the same group revealed that pre-incubation of tubulin with tau resulted in reduced taxane

**Figure 3**

Multifactorial regulation of topoisomerase IIa (TopoIIa). TopoIIa gene transcription is under control of gene signals and proliferative signals. Redox-sensitive protein complexes regulate mRNA half-life. Variable mRNA splicing produces protein isoforms: the cytoplasmic form is inactive, the nuclear form is active. Nuclear receptors regulate transport of these isoforms depending on the cell proliferative phase [43].

binding. Tau may compete with taxanes for microtubule binding. Low tau expression may become a predictive clinical biomarker for taxane sensitivity.

Tau expression also closely correlates with ER expression and may predict endocrine sensitivity in ER-positive breast cancers [49,50]. Potentially high tau levels may predict ER-positive tumours with increased sensitivity to endocrine therapy, whereas low tau levels may distinguish ER-positive tumours more chemosensitive to taxane therapy [50].

#### *p53 and taxanes*

p53 is a critical tumour suppressor playing an integral role in cellular apoptosis and regulation of normal cell growth. Wild-type p53 may undergo amplification, deletion or mutation. A meta-analysis reviewing the link between p53 and breast cancer revealed a poorer outcome for DFS and OS with a p53 alteration [51]. A large study using gene sequencing and specific mutation detection reported p53 as an independent negative prognostic marker in breast cancer [52].

The role of p53 as a predictive marker is not clear. Some studies have not shown any clear correlation between p53 alterations and clinical or pathological clinical response with taxanes [53,54]. Other *in vitro* and *in vivo* studies have confirmed taxane response in the presence of p53 mutation, supporting the hypothesis of p53-independent mechanisms of action for the taxanes [55,56]. This is in contrast to

anthracyclines, whose DNA damaging effects mediate apoptosis via p53-dependent pathways and, as such, are dependent on normal p53.

The complexity of p53 renders the detection of clinically important p53 alterations an ongoing challenge. Immunohistochemistry is associated with misclassification, detecting both wild-type and mutated p53. Identification of specific genetic mutations is expensive and not widely available. 'Functional' inactivation, which is the key concern with p53, may occur at many levels and with cross-talk between many pathways. Inactivation may be best assessed not by single gene analysis, but by a p53 multigene signature. Interestingly, some p53 wild-type tumours have been shown to express the mutant p53 signature and behave aggressively [57]. Genetic polymorphisms may also impact on p53 activity and may need to be incorporated into clinical predictive tools [58].

#### **DNA damaging agents**

Alkylating agents, namely cyclophosphamide, and platinum derivatives, carboplatin and cisplatin, inhibit cell growth and induce cell death by damaging DNA, particularly by intra- and inter-DNA strand binding. Cyclophosphamide is frequently used in the adjuvant treatment of breast cancer, whilst the role of platinum derivatives is not yet clearly defined.

Hereditary breast cancer accounts for 5% to 10% of all breast cancer cases. *BRCA* mutations account for 24% to 40% of hereditary breast cancers. Women with a *BRCA1* mutation have a 56% to 85% lifetime risk of developing breast cancer [59]. The normal *BRCA1* gene, on chromosome 17, encodes DNA repair proteins required for maintenance of normal DNA genomic integrity. *BRCA1* mutation prevents DNA repair. In the absence of DNA repair, DNA damaging agents exert greater effect. Certainly, preclinical and clinical studies reveal hypersensitivity of *BRCA1*-associated breast cancer to DNA damaging agents [60-62]. The clinical neoadjuvant studies were small but confirmed increased complete clinical response in *BRCA1* mutated patients, compared with *BRCA2* and sporadic cases.

Recent molecular classification of breast cancer has defined luminal-like and basal-like tumours [63], and there is evolving evidence of a strong link between *BRCA1* deficiency and the basal phenotype. The basal 'triple negative' tumours are ER-negative, PgR-negative and Her-2-negative, and also positive for epidermal growth factor receptor and basal cytokeratins. They are typically high grade, aggressive tumours with a poor prognosis. Analyses have shown that basal tumours are similar to *BRCA1* germline mutated tumours in clinical course, immunohistochemistry and genetic signature [64]. Whilst somatic *BRCA1* mutations are uncommon in sporadic breast cancer, there are non-mutational mechanisms causing *BRCA1* dysfunction. Down-regulation of *BRCA1* mRNA and protein expression may be mediated by acquired methylation of the *BRCA1* promoter or upstream pathway regulation

malfunction [59]. Like the *BRCA1* tumours, there are small, retrospective reviews of basal tumours being hypersensitive to DNA damaging chemotherapy [65,66].

BRCA1 and the basal phenotype may be valuable predictive biomarkers of response to DNA damaging agents, but further exploration in larger prospective clinical trials is required.

### Future directions

Traditional and genetic markers offer prognosis and possibly predict treatment response based on the characteristics or signatures of the primary tumour. Another approach is to identify and quantify micrometastatic disease postoperatively and correlate residual disease with outcome and benefit from treatment. In the neoadjuvant and metastatic settings, the bulk of disease and response to therapy may be gauged by measurable disease, clinically or radiologically, or with the aid of surrogate tumour markers or reported symptoms. In the adjuvant setting this is not possible. Recognition of patients with aggressive primary disease and measurable micrometastatic disease may guide future adjuvant interventions. Tools to assess residual disease include identification of micrometastases in bone marrow and blood, and possibly proteomic or metabolomic profiles

### Circulating tumour cells

Circulating tumour cells (CTCs) may be detected with high sensitivity and specificity using immunomagnetic separation [67] or microchip technology [68]. The ability to isolate, quantify and molecularly categorise CTCs is a tremendous challenge. The significance of these cells is yet to be clearly defined. The presence of these cells may not necessarily correlate with future relapse.

CTC profiles have been compared with the profiles of their primary tumour. Using the gene expression of the primary tumour to determine adjuvant therapy assumes concordance between the primary tumour and micrometastatic deposits. However, there is evidence that genetic alteration between the primary tumour and CTCs may alter both prognosis and therapy options; for example, a Her-2-negative primary breast cancer based on immunohistochemistry and fluorescence *in situ* hybridisation may in fact release CTCs that are positive for Her-2 [69]. This may worsen the prognosis but opens the therapeutic window for Her-2 directed therapy

In metastatic breast cancer the number of circulating tumour cells has been shown to be an independent predictor of progression-free survival and OS [70]. In the adjuvant setting the role of CTCs is undergoing intensive research. The significance of the presence, quantity and rate of post-operative clearance of CTCs, as well as response of CTCs during adjuvant therapy and appropriate reactions to these responses, are being explored and will hopefully be defined to incorporate CTCs into optimising clinical practice.

### Proteomics and metabolomics

A multitude of complex variables impact on the interaction between a tumour and its host: there are alterations that may occur at the RNA, protein and/or metabolite level; there is the host immune response to the tumour; and host handling of particular chemotherapeutics may dictate dosing, scheduling and predictive benefit for that individual. These issues are not addressed by analyses of the genome, but are being incorporated into analyses in proteomics and metabolomics. These rapidly developing fields seek to further personalise our approach in management of early breast cancer by analysing both tumour and host.

Proteomics identifies proteins expressed in the body, particularly defining sets of proteins expressed at a certain time under specified conditions [71]. Metabolomics is a new field of research aiming to analyse complete biological systems. This high throughput study of vast quantities of small molecules/metabolites in simple biofluids aims to provide a 'fingerprint' of metabolic processes and metabolic responses to pathophysiological and pharmaceutical intervention [72]. Proteomic and metabolomic analyses may enhance screening, diagnosis and targeted drug therapy discovery, and may also define surrogate markers for prognosis, response and treatment toxicity [73].

### Conclusion

Identification of specific single genes and gene expression signatures is refining our approach in early breast cancer. Such analyses are leading the way in individualisation of prognosis and prediction. This shift in approach from traditional clinical and histopathological risk assessment, which provides estimates of outcomes from disease and interventions for a similar population of patients, to specific tumour assessment in a specific patient with a genetic basis for prognosis and treatment decisions is exciting. Whilst some of the early data are encouraging, much of the research to date is inconclusive or conflicting, and often from small retrospective trials using archived tissue. Large prospectively designed and well powered clinical trials will help to specify definitive roles for these new diagnostic tools in daily clinical practice.

### Competing interests

The authors declare that they have no competing interests.

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## References

- Hanash S: **Integrated global profiling of cancer.** *Nat Rev Cancer* 2004, **4**:638-644.
- Early Breast Cancer Trialists' Collaborative Group: **Polychemotherapy for early breast cancer: an overview of the randomised trials.** *Lancet* 1998, **352**:930-942.
- Early Breast Cancer Trialists' Collaborative Group: **Tamoxifen for early breast cancer: an overview of the randomised trials.** *Lancet* 1998, **351**:1451-1467.
- Workman P, Johnston PG: **Genomic profiling of cancer: what next?** *J Clin Oncol* 2005, **29**:7253-7256.
- Carter CL, Allen C, Henson DE: **Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases.** *Cancer* 1989, **63**:181-187.
- Galea MH, Blamey RW, Elston CE, Ellis IO: **The Nottingham Prognostic Index in primary breast cancer.** *Breast Cancer Res Treat* 1992, **22**:207-219.
- Press MF, Bernstein L, Thomas PA, Meisner LF, Zhou JY, Ma Y, Hung G, Robinson RA, Harris C, El-Naggar A, Slamon DJ, Phillips RN, Ross JS, Wolman SR, Flom KJ: **HER-2/neu gene amplification characterized by fluorescence *in situ* hybridization: poor prognosis in node-negative breast carcinomas.** *J Clin Oncol* 1997, **15**:2894-2904.
- van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH: **Gene expression profiling predicts clinical outcome of breast cancer.** *Nature* 2002, **415**:530-536.
- van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ, Parrish M, Atsma D, Witteveen A, Glas A, Delahaye L, van der Velde T, Bartelink H, Rodenhuis S, Rutgers ET, Friend SH, Bernards R: **A gene expression signature as a predictor of survival in breast cancer.** *N Engl J Med* 2002, **347**:1999-2009.
- Isidoro A, Casado E, Redondo A, Acebo P, Espinosa E, Alonso AM, Cejas P, Hardisson D, Fresno Vara JA, Belda-Iniesta C, González-Barón M, Cuezva JM: **Breast cancer prognosis determined by gene expression profiling: a quantitative RT-PCR study.** *J Clin Oncol* 2005, **23**:7278-7285.
- Buyse M, Loi S, van't Veer L, Viale G, Delorenzi M, Glas AM, d'Assignies MS, Bergh J, Lidereau R, Ellis P, Harris A, Bogaerts J, Therasse P, Floore A, Amakrane M, Piette F, Rutgers E, Sotiriou C, Cardoso F, Piccart MJ; TRANSBIG Consortium: **Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer.** *J Natl Cancer Inst* 2006, **98**:1183-1192.
- Breast International Group: **Breast International Group and TRANSBIG** [<http://www.breastinternationalgroup.org>]
- Ravdin PM, Siminoff LA, Davis GJ, Mercer MB, Hewlett J, Gerson N, Parker HL: **Computer program to assist in making decisions about adjuvant therapy for women with early breast cancer.** *J Clin Oncol* 2001, **19**:980-991.
- Wang Y, Klijn JG, Zhang Y, Sieuwerts AM, Look MP, Yang F, Talantov D, Timmermans M, Meijer-van Gelder ME, Yu J, Jatkoe T, Berns EM, Atkins D, Foekens JA: **Gene expression profiles to predict distant metastasis of lymph node negative primary breast cancer.** *Lancet* 2005, **365**:671-679.
- Foekens JA, Atkins D, Zhang Y, Sweep FC, Harbeck N, Paradiso A, Cufer T, Sieuwerts AM, Talantov D, Span PN, Tjan-Heijnen VC, Zito AF, Specht K, Hoefler H, Golouh R, Schittulli F, Schmitt M, Beex LV, Klijn JG, Wang Y: **Multicenter validation of a gene expression-based prognostic signature in lymph node-negative primary breast cancer.** *J Clin Oncol* 2006, **24**:1665-1671.
- Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thürlimann B, Senn HJ: **Updated international expert consensus on the primary therapy of early breast cancer.** *J Clin Oncol* 2003, **21**:3357-3365.
- Eifel P, Axelson JA, Costa J, Crowley J, Curran WJ Jr, Deshler A, Fulton S, Hendricks CB, Kemeny M, Kornblith AB, Louis TA, Markman M, Mayer R, Roter D: **National Institutes of Health Consensus Development Conference Statement: Adjuvant therapy for breast cancer, November 1-3, 2000.** *J Natl Cancer Inst* 2001, **93**:979-989.
- Desmedt C, Piette F, Loi S, Wang Y, Lallemand F, Haibe-Kains B, Viale G, Delorenzi M, Zhang Y, d'Assignies MS, Bergh J, Lidereau R, Ellis P, Harris AL, Klijn JG, Foekens JA, Cardoso F, Piccart MJ, Buyse M, Sotiriou C; TRANSBIG Consortium: **Strong time-dependency of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multi-centre independent validation series.** *Clin Cancer Res* 2007, **13**:3207-3214.
- Simon R, Radmacher MD, Dobbin K, McShane LM: **Pitfalls in the use of DNA microarray data for diagnostic and prognostic classification.** *J Natl Cancer Inst* 2003, **95**:14-18.
- Bogaerts J, Cardoso F, Buyse M, Braga S, Loi S, Harrison JA, Bines J, Mook S, Decker N, Ravdin P, Therasse P, Rutgers E, van't Veer LJ, Piccart M; TRANSBIG consortium: **Clinical application of the 70-Gene profile: The MINDACT Trial.** *J Clin Oncol* 2008, **26**:729-735.
- Ach RA, Floore A, Curry B, Lazar V, Glas AM, Pover R, Tsalenko A, Ripoché H, Cardoso F, d'Assignies MS, Bruhn L, Van't Veer LJ: **Robust interlaboratory reproducibility of a gene expression signature measurement consistent with the needs of a new generation of diagnostic tools.** *BMC Genomics* 2007, **8**:148.
- Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N: **A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer.** *N Engl J Med* 2004, **351**:2817-2826.
- Fisher B, Costantino J, Redmond C, Poisson R, Bowman D, Couture J, Dimitrov NV, Wolmark N, Wickerham DL, Fisher ER, et al.: **A randomized clinical trial evaluating tamoxifen in the treatment of patients with node-negative breast cancer who have estrogen-receptor-positive tumors.** *N Engl J Med* 1989, **320**:479-484.
- Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Watson D, Bryant J, Costantino J, Wolmark N: **Expression of the 21 genes in the Recurrence Score assay and tamoxifen clinical benefit in the NSABP study B-14 of node negative, estrogen receptor positive breast cancer [abstract].** *J Clin Oncol* 2005, **23**:s510.
- Habel LA, Shak S, Jacobs MK, Capra A, Alexander C, Pho M, Baker J, Walker M, Watson D, Hackett J, Blick NT, Greenberg D, Fehrenbacher L, Langholz B, Quesenberry CP: **A population-based study of tumor gene expression and risk of breast cancer death among lymph node-negative patients.** *Breast Cancer Res* 2006 **8**:R25.
- Fisher B, Jeong JH, Dignam J, Anderson S, Mamounas E, Wickerham DL, Wolmark N: **Findings from recent National Surgical Adjuvant Breast and Bowel Project adjuvant studies in stage I breast cancer.** *J Natl Cancer Inst Monogr* 2001:62-66.
- Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, Cronin M, Baehner FL, Watson D, Bryant J, Costantino JP, Geyer CE Jr, Wickerham DL, Wolmark N: **Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer.** *J Clin Oncol* 2006, **24**:3726-3734.
- Albain K, Barlow W, Shak S, Hortobagyi G, Livingston R, Yeh I, Ravdin P, Yoshizawa C, Baehner F, Davidson N, Sledge G, Winer E, Hudis C, Ingle J, Perez E, Pritchard K, Shepherd L, Allred C, Osborne K, Hayes D. Southwest Oncology Group and The Breast Cancer Intergroup of NA, San Antonio, TX: **Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal, node-positive, ER-positive breast cancer (S8814, INT0100) [abstract].** *Breast Cancer Res Treat* 2007, **106**:A-10.
- Albain K, Barlow W, O'Malley F, et al.: **Concurrent (CAFT) versus sequential (CAF-T) chemohormonal therapy (cyclophosphamide, doxorubicin, 5-fluorouracil, tamoxifen) versus T alone for postmenopausal, node-positive, estrogen (ER) and/or progesterone (PgR) receptor-positive breast cancer: Mature outcomes and new biologic correlates on phase III Intergroup trial 0100 (SWOG-8814) [abstract].** *Breast Cancer Res Treat* 2004, **88**:37.
- Goldstein LJ, Gray R, Badve S, Childs BH, Yoshizawa C, Rowley S, Shak S, Baehner FL, Ravdin PM, Davidson NE, Sledge GW Jr, Perez EA, Shulman LN, Martino S, Sparano JA: **Prognostic utility of the 21-Gene assay in hormone receptor-positive operable**

- breast cancer compared with classical clinicopathologic features. *J Clin Oncol* 2008, **26**:4063-4071.
31. Sparano J, Paik S: Development of the 21-Gene assay and its application in clinical practice and clinical trials. *J Clin Oncol* 2008, **26**:721-728.
  32. North American Breast Cancer Intergroup [http://www.cancer.gov/clinicaltrials/digestpage/TAILORx]
  33. Early Breast Cancer Trialists' Collaborative Group: Multi-agent chemotherapy for early breast cancer. *Cochrane Database Syst Rev* 2002, **1**:CD000487.
  34. Paik S, Bryant J, Park C, Fisher B, Tan-Chiu E, Hyams D, Fisher ER, Lippman ME, Wickerham DL, Wolmark N: **ErbB-2 and response to doxorubicin in patients with axillary lymph node-positive, hormone receptor-negative breast cancer.** *J Natl Cancer Inst* 1998, **90**:1361-1370.
  35. Thor AD, Berry DA, Budman DR, Muss HB, Kute T, Henderson IC, Barcos M, Cirincione C, Edgerton S, Allred C, Norton L, Liu ET: **ErbB-2, p53, and efficacy of adjuvant therapy in lymph node-positive breast cancer.** *J Natl Cancer Inst* 1998, **90**:1346-1360.
  36. Di Leo A, Gancberg D, Larsimont D, Tanner M, Jarvinen T, Rouas G, Dolci S, Leroy JY, Paesmans M, Isola J, Piccart MJ: **HER-2 amplification and topoisomerase IIalpha 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**:1107-1116.
  37. Scandinavian Breast Group Trial 9401, Tanner M, Isola J, Wiklund T, Erikstein B, Kellokumpu-Lehtinen P, Malmström P, Wilking N, Nilsson J, Bergh J: **Topoisomerase IIa gene amplification predicts favorable treatment response to tailored and dose-escalated anthracycline-based adjuvant chemotherapy in HER-2/neu-amplified breast cancer: Scandinavian Breast Cancer Group Trial 9401.** *J Clin Oncol* 2006, **24**:2428-2436.
  38. Dhesy-Thind B, Pritchard KI, Messersmith H, O'Malley F, Elavathil L, Trudeau M: **Her-2/neu in systemic therapy for women with breast cancer: a systemic review.** *Breast Cancer Res Treat* 2008, **109**:209-229.
  39. Gennari A, Sormani MP, Pronzato P, Puntoni M, Colozza M, Pfeffer U, Bruzzi P: **HER-2 status and efficacy of adjuvant anthracyclines in early breast cancer: a pooled analysis of randomized trials.** *J Natl Cancer Inst* 2008, **100**:14-20.
  40. Jarvinen TA, Tanner M, Rantanen V, Bärlund M, Borg A, Grénman S, Isola J: **Amplification and deletion of topoisomerase II alpha associate with ErbB-2 amplification and affect sensitivity to the topoisomerase II inhibitor doxorubicin in breast cancer.** *Am J Pathol* 2000, **156**:839-847.
  41. Burgess DJ, Doles J, Zender L, Xue W, Ma B, McCombie WR, Hannon GJ, Lowe SW, Hemann MT: **Topoisomerase levels determine chemotherapy response in vitro and in vivo.** *Proc Natl Acad Sci USA* 2008, **105**:9053-9058.
  42. Knoop AS, Knudsen H, Balslev E, Rasmussen BB, Overgaard J, Nielsen KV, Schonau A, Gunnarsdóttir K, Olsen KE, Mouridsen H, Ejlersen B; Danish Breast Cancer Cooperative Group: **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**:7483-7490.
  43. Di Leo A, Moretti E: **Anthracyclines: the first generation of cytotoxic targeted agents? A possible dream.** *J Clin Oncol* 2008, **26**:5011-5013.
  44. Hayes DF, Thor AD, Dressler LG, Weaver D, Edgerton S, Cowan D, Broadwater G, Goldstein LJ, Martino S, Ingle JN, Henderson IC, Norton L, Winer EP, Hudis CA, Ellis MJ, Berry DA; Cancer and Leukemia Group B (CALGB) Investigators: **HER2 and response to paclitaxel in node-positive breast cancer.** *N Engl J Med* 2007, **357**:1496-1506.
  45. Martin M, Pienkowski T, Mackey J, Pawlicki M, Guastalla JP, Weaver C, Tomiak E, Al-Tweigeri T, Chap L, Juhos E, Guevin R, Howell A, Fornander T, Hainsworth J, Coleman R, Vinholes J, Modiano M, Pinter T, Tang SC, Colwell B, Prady C, Provencher L, Walde D, Rodriguez-Lescure A, Hugh J, Loret C, Rupin M, Blitz S, Jacobs P, Murawsky M, et al.: **Adjuvant docetaxel for node-positive breast cancer.** *N Engl J Med* 2005, **352**:2302-2313.
  46. Tzaida O, Gogas H, Dafni U, Kyroudi A, Papaspyrou I, Kyriakou V, Malamou-Mitsi V, Alamani M, Skopa C, Kostopoulos I, Kastiris E, Pectasides D, Briasoulis E, Kalofonos HP, Aravantinos G, Fountzilas G, Arapantoni-Dadioti P: **Evaluation of the prognostic value of HER-2 and VEGF in breast cancer patients participating in a randomized study with dose-dense sequential adjuvant chemotherapy.** *Breast Cancer Res Treat* 2006, **96**:251-261.
  47. Learn PA, Yeh IT, McNutt M, Chisholm GB, Pollock BH, Rousseau DL Jr, Sharkey FE, Cruz AB, Kahlenberg MS: **Her/2 neu expression as a predictor of response to neoadjuvant docetaxel in patients with operable breast cancer.** *Cancer* 2005, **103**:2252-2260.
  48. Rouzier R, Rajan R, Wagner P, Hess KR, Gold DL, Stec J, Ayers M, Ross JS, Zhang P, Buchholz TA, Kuerer H, Green M, Arun B, Hortobagyi GN, Symmans WF, Pusztai L: **Microtubule-associated protein tau: a marker of paclitaxel sensitivity in breast cancer.** *Proc Natl Acad Sci USA* 2005, **102**:8315-8320.
  49. Pusztai L: **Markers predicting clinical benefit in breast cancer from microtubule-targeting agents.** *Ann Oncol* 2007, **18**(Suppl 12):xii15-xii20.
  50. Andre F, Hatzis C, Anderson K, Sotiriou C, Mazouni C, Mejia J, Wang B, Hortobagyi GN, Symmans WF, Pusztai L: **Microtubule-associated protein-tau is a bifunctional predictor of endocrine sensitivity and chemotherapy resistance in estrogen receptor-positive breast cancer.** *Clin Cancer Res* 2007, **13**:2061-2067.
  51. Pharoah PD, Day NE, Caldas C: **Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis.** *Br J Cancer* 1999, **80**:1968-1973.
  52. Olivier M, Langerød A, Carrieri P, Bergh J, Klaar S, Eyfjord J, Theillet C, Rodriguez C, Lidereau R, Bièche I, Varley J, Bignon Y, Uhrhammer N, Winqvist R, Jukkola-Vuorinen A, Niederacher D, Kato S, Ishioka C, Hainaut P, Børresen-Dale AL: **The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer.** *Clin Cancer Res* 2006, **12**:1157-1167.
  53. Zhou B, Yang D, Xie F: **Biological markers as predictive factors of response to neoadjuvant taxanes and anthracycline chemotherapy in breast carcinoma.** *Chinese Med J* 2008, **121**:387-391.
  54. Noguchi S: **Predictive factors for response to docetaxel in human breast cancers.** *Cancer Sci* 2006, **97**:813-820.
  55. Wahl AF, Donaldson KL, Fairchild C, Lee FY, Foster SA, Demers GW, Galloway DA: **Loss of normal p53 function confers sensitization to Taxol by increasing G<sub>2</sub>/M arrest and apoptosis.** *Nat Med* 1996, **2**:72-79.
  56. Kandioler-Eckersberger D, Ludwig C, Rudas M, Kappel S, Janschek E, Wenzel C, Schlagbauer-Wadl H, Mittlböck M, Gnani M, Steger G, Jakesz R: **TP53 mutation and p53 overexpression for prediction of response to neoadjuvant treatment in breast cancer patients.** *Clin Cancer Res* 2000, **6**:50-56.
  57. Miller LD, Smeds J, George J, Vega VB, Vergara L, Ploner A, Pawitan Y, Hall P, Klaar S, Liu ET, Bergh J: **An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival.** *Proc Natl Acad Sci USA* 2005, **102**:13550-13555.
  58. Di Leo A, Tanner M, Desmedt C, Paesmans M, Cardoso F, Durbeq V, Chan S, Perren T, Aapro M, Sotiriou C, Piccart MJ, Larsimont D, Isola J; TAX 303 Translational Study Team: **p-53 gene mutations as a predictive marker in a population of advanced breast cancer patients randomly treated with doxorubicin or docetaxel in the context of a phase III trial.** *Ann Oncol* 2007, **18**:997-1003.
  59. James CR, Quinn JE, Mullan PB, Johnston PG, Harkin DP: **BRCA1, a potential predictive biomarker in the treatment of breast cancer.** *Oncologist* 2007, **12**:142-150.
  60. Moynahan ME, Cui TY, Jasin M: **Homology-directed DNA repair, mitomycin-c resistance, and chromosome stability is restored with correction of a Brca1 mutation.** *Cancer Res* 2001, **61**:4842-4850.
  61. Kloos I, Delaloge S, Pautier P, Di Palma M, Goupil A, Duvalard P, Cailleux PE, Lhomme C: **BRCA1-linked breast cancer (BC) is highly more chemosensitive than its BRCA2-linked or sporadic counterparts [abstract].** *Ann Oncol* 2002, **13**(Suppl 5):34.
  62. Chappuis PO, Goffin J, Wong N, Perret C, Ghadirian P, Tonin PN, Foukkes WD: **A significant response to neoadjuvant chemotherapy in BRCA1/2 related breast cancer.** *J Med Genet* 2002, **39**:608-610.
  63. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslén LA, Fluge O, Perga-

- menschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D: **Molecular portraits of human breast tumours.** *Nature* 2000, **406**:747-752.
64. Rakha E, Reis-Filho J, Ellis I: **Basal-like breast cancer: a critical review.** *J Clin Oncol* 2008, **26**:2568-2581.
  65. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, Ollila DW, Sartor CI, Graham ML, Perou CM: **The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes.** *Clin Cancer Res* 2007, **13**:2329-2334.
  66. Sirohi B, Arnedos M, Popat S, Ashley S, Nerurkar A, Walsh G, Johnston S, Smith IE: **Platinum-based chemotherapy in triple-negative breast cancer.** *Ann Oncol* 2008, **19**:1847-1852.
  67. Racila E, Euhus D, Weiss AJ, Rao C, McConnell J, Terstappen LW, Uhr J: **Detection and characterization of carcinoma cells in the blood.** *Proc Natl Acad Sci USA* 1998, **95**:4589-4594.
  68. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, Smith MR, Kwak EL, Digumarthy S, Muzikansky A, Ryan P, Balis UJ, Tompkins RG, Haber DA, Toner M: **Isolation of rare circulating tumour cells in cancer patients by microchip technology.** *Nature* 2007, **450**:1235-1239.
  69. Wülfing P, Borchard J, Buerger H, Heidl S, Zänker KS, Kiesel L, Brandt: **HER2-positive circulating tumor cells indicate poor clinical outcome in stage I to III breast cancer patients.** *Clin Cancer Res* 2006, **12**:1715-1720.
  70. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, Reuben JM, Doyle GV, Allard WJ, Terstappen LW, Hayes DF: **Circulating tumor cells, disease progression, and survival in metastatic breast cancer.** *N Engl J Med* 2004, **351**:781-791.
  71. Tyers M, Mann M: **From genomics to proteomics.** *Nature* 2003, **422**:193-197.
  72. Assfalg M, Bertini I, Colangiuli D, Luchinat C, Schäfer H, Schütz B, Spraul M: **Evidence of different metabolic phenotypes in humans.** *Proc Natl Acad Sci USA* 2008, **105**:1420-1424.
  73. Claudino WM, Quattrone A, Biganzoli L, Pestrin M, Bertini I, Di Leo A: **Metabolomics: available results, current research projects in breast cancer, and future applications.** *J Clin Oncol* 2007, **19**:2840-2846.