

Prognostic and Predictive Factors

Laura Biganzoli

Prognostic factors are key elements for medical oncologists to select, among the group of patients with early breast cancer, those who are candidates for an adjuvant treatment based on their risk of tumor relapse. Predictive factors drive the decision of which type(s) of treatment should be given.

If we perform a literature search on prognostic and predictive factors in breast cancer we immediately realize how redundant is the number of markers that have been/are being evaluated and a dedicated book would be needed to review all of them critically. So, for the purpose of writing this chapter, I have decided to start from markers whose roles have been established to be relevant for patient care by an international panel of breast cancer experts [1].

Prognostic Factors

Three risk categories have been defined for patients with operated breast cancer by the St. Gallen Consensus's Panel. These categories are defined by patient's age, tumor size, axillary nodes status, histologic and/or nuclear grade, HER2/neu status, and presence or absence of peri-tumoral vascular invasion.

Age

Despite controversial data available on the value of age as a prognostic factor, the prognosis of breast cancer in very young women is generally considered to be unfavorable. Park and colleagues retrospectively evaluated the 10-year outcome of 1,098 breast cancer patients divided into 2 age groups (<35, n = 183, and >35 years) [2]. Age was observed to be an independent prognostic factor in

L. Biganzoli (✉)

“Sandro Pitigliani” Medical Oncology Unit, Hospital of Prato,
Tuscany Cancer Institute, Prato, Italy

the multivariate analysis with women aged 35 years or younger presenting a shorter loco-regional recurrence-free distant relapse, and overall survival. When the data was matched for stage and lymph node status, patients ≤ 35 years continued to show a poorer 10-year distant relapse free survival. Similar results were produced by Aebi and colleagues who evaluated the outcome of adjuvant therapy in a population of young (<35 years) premenopausal patients treated in four randomized trials [3]. Ten-year disease free survival and overall survival were worse in younger than in older (≥ 35 years) patients. Of interest, younger patients with estrogen receptor (ER) positive tumors had a poorer disease free survival than patients with ER negative tumors. In contrast, among older patients the DFS was similar irrespective of ER status. These data have been recently confirmed. Saghir et al. showed that young age had a negative impact on the survival of patients with positive axillary lymph nodes and positive hormonal receptors [4]. According to Colleoni and colleagues, compared with less young, very young patients with endocrine responsive and node-negative breast cancer have a worse prognosis [5].

Tumor Size

Tumor size is one of the most important independent prognostic factors for overall and recurrence free survival in breast cancer. Mirza and colleagues reviewed the literature looking for the role of prognostic factors in patients with node negative tumors entered in studies with sample size >200 patients and a follow-up in excess of 5 years [6]. In multivariate analysis, tumor size was an independent prognostic parameter for overall survival, being the second strongest factor after axillary lymph node status. A positive correlation has been found in several studies between tumor size and the frequency of axillary nodes involvement [7]. According to the St. Gallen experts, tumors larger than 2 cm indicated intermediate- or high-risk allocation, even in the absence of other adverse prognostic features [1]. The risk allocation of tumors below 1 cm in size and negative nodes remained controversial.

Axillary Nodes

Nodal status is the most powerful independent prognostic factor in breast cancer. There is evidence that overall survival decreases as the number of positive nodes increases [8, 9]. According to the St. Gallen experts, involvement of four or more nodes in the axilla by itself indicated high-risk, but patients with one to three nodes involved required HER2/neu overexpression or amplification to be included in the high-risk group, with other patients with one to three nodes included in the intermediate-risk category [1]. Although nodal micro-metastases were prognostically relevant in several studies [10, 11], the panel considered that neither they nor isolated tumor cells in lymph nodes should influence risk allocation.

Tumor Grade

The histological grade of breast carcinomas has long provided clinically important prognostic information. Elston and colleagues showed that patients with grade 1 tumors have a significantly better survival than those with grade 2 and 3 tumors [12]. Gene expression grade index appeared to reclassify patients with histologic grade 2 tumors into two groups with high vs low risks of recurrence [13]. Debate exists about the real role of grade 2 in defining patients' prognosis, i.e., intermediate risk.

HER2/neu Status

Several studies, mainly retrospective, have evaluated the prognostic role of HER2 overexpression or gene amplification in early breast cancer [14]. Of the 81 studies considering 27,161 patients reviewed by Ross et al., 73 (90%) of the studies and 25,166 (92%) of the cases found that either HER-2/*neu* gene amplification or HER-2 protein overexpression predicted breast cancer outcome on either univariate or multivariate analysis. In 52 (71%) of the 73 studies that featured multivariate analyses of outcome data, the adverse prognostic significance of the HER-2 gene, message, or protein overexpression was independent of all other prognostic variables. Thirteen (16%) of the studies reported prognostic significance on univariate analysis only (in eight studies, multivariate analysis was not performed). Only 8 (10%) of the studies encompassing 1,995 (8%) of the patients, showed no correlation between HER-2/*neu* status and outcome. According to the St. Gallen panelists, HER2 status should be regarded as useful for patient care, with overexpression indicating a worse prognosis [1].

Peritumoral Vascular Invasion

The prognostic significance of involvement of lymphatic or microvascular spaces in the primary tumor has been variably described [15–18]. There are data coming from retrospective studies indicating this to be an independent prognostic factor in both node-positive and node-negative patients [19, 20]. The St. Gallen panelists agreed that the presence of peritumoral vascular invasion defined intermediate risk for patients with node-negative disease but its value for patients with positive axillary lymph nodes was considered uncertain [1].

Others

Among the many putative prognostic factors, the detection of bone marrow metastasis, the expression of UPA/PAI-1 by the primary cancer, and the

recognition of simultaneous multiple gene expression patterns, or “signature” appear to be particularly promising.

The presence of tumor cells in the bone marrow of primary breast cancer patients at surgery has been shown to be an independent prognostic indicator of relapse [21]. This prognostic factor was not considered adequate by the St. Gallen panelists because of the absence of a standardized examination for detecting these cells.

UPA/PAI-1 over-expression, as determined by a highly validated and accurate ELISA in relatively large cancer sections, appears to be strongly prognostic. As shown by Harbeck et al., high levels indicates dire prognosis [22]. In contrast, patients with low UPA/PAI-1 and ER showed a particularly good prognosis [23].

Data in breast cancer have demonstrated the ability of microarray-based expression profiling to predict disease-free survival and overall survival from profiles in breast cancer surgical specimens [24–29]. Different microarray platforms have been used. Recently Hu and colleagues utilized a microarray data set combining method to create a large validation test set of over 300 tumors, and used it to validate a newly derived gene list for breast cancer prognostication and prediction [30]. When the new intrinsic gene set was used to cluster hierarchically this combined test set, tumors were grouped into LumA, LumB, Basal-like, HER2+/ER–, and Normal Breast-like tumor subtypes demonstrated by Perou et al. in previous datasets. These subtypes were associated with significant differences in relapse-free and overall survival. Multivariate Cox analysis of the combined test set showed that the intrinsic subtype classifications added significant prognostic information that was independent of standard clinical predictors. From the combined test set, the authors developed an objective and unchanging classifier based upon five intrinsic subtype mean expression profiles (i.e., centroids), which is designed for single sample predictions (SSP). The SSP approach was applied to two additional independent data sets and consistently predicted survival in both systemically treated and untreated patient groups. According to the authors this study validates the “breast tumor intrinsic” subtype classification as an objective means of tumor classification that should be translated into a clinical assay for further retrospective and prospective validation.

Predictive Factors

In the following paragraphs, an overview of the different predictive markers already tested or under evaluation in patients with early breast cancer, for both hormonal therapy and chemotherapy, will be presented. For each marker, the level of evidence reached so far will be stated. Table 1 reports the type and grading of evidence for recommendations.

Table 1 Type and grading of evidence for recommendations

Level	Type of evidence
I	Evidence obtained from meta-analysis of multiple well-designed controlled studies; randomized trials with low false-positive and low false-negative errors (high power)
II	Evidence obtained from at least one well-designed experimental study; randomized trials with high false-positive and/or negative errors (low power)
III	Evidence obtained from well-designed quasi-experimental studies; such as nonrandomized controlled single-group pre-post, cohort, time, or matched case-control series
IV	Evidence from well-designed non-experimental studies, such as comparative and correlation descriptive and case studies
V	Evidence from case reports and clinical examples
Category	Grade of evidence
A	There is evidence of type I or consistent findings from multiple studies of types II, III, or IV
B	There is evidence of types II, III, or IV and findings are generally consistent
C	There is evidence of types II, III, or IV, but findings are inconsistent
D	There is little or no systemic evidence
NG	Grade not given

Predictive Markers for Adjuvant Hormonal Therapy

The last overview performed by the EBCTCG (R. Peto, 2006, unpublished) provides data regarding the predictive power of ER when the efficacy of adjuvant tamoxifen given for approximately 5 years is evaluated. From these data it may be concluded that the activity of tamoxifen is strictly dependent on the ER status, and that there is a correlation between the level of ER positivity and the efficacy of tamoxifen. To date, ER is the only firmly established factor known to predict the efficacy of adjuvant hormonotherapy with level I/category A evidence. Nevertheless, about one-third of ER and/or PgR-positive tumors do not respond to endocrine therapy, clearly indicating the need for additional predictive markers.

With the exception of ER and PgR, the proto-oncogene HER-2 and its encoded protein have been the most extensively evaluated markers. Preclinical data suggest that HER-2 overexpression may be associated with decreased efficacy of tamoxifen, and even with a potential detrimental effect [31]. Several clinical studies, both in the metastatic and the adjuvant settings, have addressed this issue and provided contradictory results (Table 2) [32–37]. De Placido et al. [33] published the results of a retrospective study in which the activity of adjuvant tamoxifen was correlated with the expression of HER-2. They concluded that tumors overexpressing the HER-2 protein, measured by IHC, are less responsive to tamoxifen. Updates of the Swedish Breast Cancer Group study [34] and a study from a Spanish group [35] have been published, supporting the association between HER-2 overexpression and resistance to tamoxifen.

Table 2 HER-2/neu and adjuvant

Group (reference)	Study arms	No. of patients (in clinical trial)	Percentage with HER-2		Methods of HER-2 evaluation	Results
			Measured (%)	HER-2		
GÜN (De Placido) [33]	TAM	433	57	IHC		HER-2 is a strong predictor of adjuvant TAM failure, independently of ER
	No TAM					
Swedish group (Stal) [34]	TAM 2 years	871	66	DNA amplification assay (slot blot) flow cytometry		HER-2 overexpression decreases the benefit of prolonged adjuvant TAM treatment
	TAM 5 years					
Spanish group (Climent) [35]	Radical mastectomy	283	88	IHC		Patients treated with adjuvant TAM had significantly longer DFS and OS when HER-2 was negative
	Breast-conserving surgery (TAM assignment not rando)					
CALGB 8541 (Berry) [36]	CAF 600/60/ 600 mg/m ²	999	65	IHC, FISH, differential PCR		In ER + /node-positive patients, the efficacy of adjuvant TAM does not depend on HER-2 status
	CAF 400/40/ 400 mg/m ²					
	CAF 300/30/ 300 mg/m ²					
	(TAM assignment not rando)					
Danish group (Knoop) [37]	TAM	1716	88	IHC		The study does not support the hypothesis that HER-2 status could predict benefit from adjuvant TAM, in ER + early stage BC
	No TAM					

Rando, Randomized; GÜN, Gruppo Universitario Napoletano; CALGB, Cancer and Leukemia Group B; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; ER, estrogen receptor; CAF, cyclophosphamide + doxorubicin + 5-fluorouracil; OS, overall survival; BC, breast cancer
From Di Leo A, Cardoso F, Durbecq V, Giuliani R, Mano M, Atalay G, Larsimont D, Sotiriou C, Biganzoli L, Piccart MJ. Predictive molecular markers in the adjuvant therapy of breast cancer: state of the art in the year 2002. *Int J Clin Oncol.* 2002;24:5–53. Table 4. With kind permission of Springer Science and Business Media

On the other hand, the Cancer and Leukemia Group B (CALGB) and the Danish Breast Cancer Cooperative Group reported two trials in which no such association was found [36, 37].

Several facts could account for these conflicting results: (1) all the studies are retrospective; (2) the actual number of HER-2-positive patients who received adjuvant tamoxifen is low in all the studies; and (3) there is lack of standardization of methods for assessing HER-2 overexpression across different laboratories. More recently, early results from the TransATAC study suggest that in the adjuvant setting HER-2 and hormone-receptor positive breast cancer tends to be less sensitive to tamoxifen and aromatase inhibitors than HER-2 negative and hormone-receptor positive disease. The magnitude of anastrozole superiority over tamoxifen seems to be independent of the primary tumor HER-2 status [38]. This recent finding contrasts the main conclusions from two previously reported neoadjuvant studies, suggesting an increased superiority of aromatase inhibitors over tamoxifen in the presence of HER-2 and hormone-receptor positive disease [39, 40]. Of note, the two neoadjuvant studies correlated HER-2 status with objective response rates to neoadjuvant hormonotherapy [39, 40], while in the TransATAC study, disease-free survival was the main clinical outcome correlated with the primary tumor HER-2 status [38]. This difference between TransATAC and the two neoadjuvant studies might explain the apparent discordance.

The level of evidence regarding HER-2 as a predictive marker for adjuvant tamoxifen is, therefore, level II, category B.

Overexpression of the anti-apoptotic molecule bcl-2 is usually associated with high ER concentration and, contrary to expectation, has been associated with a higher likelihood of response to tamoxifen. In a total of 205 tumor samples from ER-positive metastatic breast cancer patients, high bcl-2 expression correlated with a better clinical response to tamoxifen (62% vs 49%; $p = 0.07$) and longer survival [41]. In the adjuvant setting, in 81 patients treated with tamoxifen, a significantly better relapse-free survival was found among those with bcl-2-positive tumors than in those with bcl-2-negative disease ($p = 0.02$) [42]. In another retrospective study, the interaction between bcl-2 and response to tamoxifen was evaluated in 289 patients with ER- and/or PgR-positive early breast cancer. This is the only study in which a “control” group of patients who did not receive treatment with tamoxifen exists, although the assignment to each group was not randomized. Despite the relatively small number of patients in each subgroup, there was a trend towards a greater benefit of tamoxifen in ER+/bcl-2-positive patients, as opposed to ER+/bcl-2-negative patients [43].

The potential predictive role of other markers, such as the β isotype of ER, *p*-53 mutations, the proliferation marker Ki67, and intra-tumoral aromatase activity (for aromatase inhibitors) is still under evaluation.

The National Surgical Adjuvant Breast and Bowel Project (NSABP) group has recently reported the results of a retrospective study evaluating the prognostic value of a recurrence score for hormone-receptor positive early breast

cancer patients treated with tamoxifen in the adjuvant setting [44]. The level of expression of 16 cancer related genes was measured by reverse-transcriptase-polymerase-chain-reaction (RT-PCR) in 668 archival samples from patients treated with adjuvant tamoxifen 20 mg daily for 5 years in the context of the NSABP B-14 trial. A prospectively defined algorithm led to the calculation of a recurrence score and allowed for the segregation of the study population into three distinct cohorts with different clinical outcomes. The Kaplan-Meier estimates of the rates of distant recurrence at 10 years in the low-risk, intermediate-risk, and high-risk groups were 6.8%, 14.3%, and 30.5%, respectively [44]. In a multivariate Cox model, the recurrence score provided significant predictive power that was independent of age and tumor size. In addition, the recurrence score was also predictive of overall survival. Of note, the 16 genes allowing for the determination of the recurrence score are involved in proliferation, invasion, HER-2, or hormone-receptor pathways [44].

The same group has recently reported the results of a second retrospective study in which node-negative ER positive patients were treated with either adjuvant tamoxifen or same treatment combined with a CMF-like adjuvant chemotherapy. The results of this study show that the benefit deriving from chemotherapy seems to be confined to the group of patients with a high recurrence score [45]. An adjuvant therapy clinical trial is ongoing in the U.S. to test the predictive value of the 16-gene signature in a prospective setting.

Predictive Markers for Adjuvant Chemotherapy

HER-2 as a Predictive Marker

The 2006 EBCTCG overview has confirmed the superiority of an anthracycline-based regimen over CMF in the adjuvant treatment of early breast cancer patients. The benefit is, however, modest and is associated with a definite increase in toxicity. This is the typical clinical situation in which the use of a predictive marker might help in selecting those patients for whom the benefits of the more aggressive treatment might be substantial and justify the increased toxicity.

HER-2 has been investigated in this setting. Data suggesting that HER-2-positive tumors might be resistant to adjuvant treatment with CMF (with or without prednisone) comes from three retrospective studies (Table 3) [46–48]. In two of these trials, when patients were divided into two subgroups according to the expression of HER-2, as measured by IHC in primary tumor samples, it was observed that adjuvant CMF (plus prednisone) was more effective than no adjuvant treatment only in the subset of HER-2-negative patients [46, 47]. In the third trial, all patients benefited from adjuvant CMF, but the magnitude of the benefit was superior in HER-2-negative patients [48]. However, in a study reported by the Milan group, HER-2 failed to show any predictive activity in a population of node-positive breast cancer patients randomly allocated to

Table 3 HER- and adjuvant CMF/CMF-like chemotherapy

Group (reference)	Study arms	No. of patients (in clinical trial)	Percentage with HER- 2 Measured (%)	Methods of HER-2 evaluation	Results
Intergroup Group 0011 (Allred) [46]	Observation CMFP	677	100	IHC	After CMFP, only HER-2 negative patients had longer DFS and OS, showing clear benefit from CT; no benefit in HER-2 positive patients
IBCSG trial V– Ludwig (Gusterson) [47]	N–: PeCT vs. Not N+: CMFP	2504	60	IHC	Tumors that overexpress HER-2 overexpression decreases are less responsive to CMF-containing adjuvant CT
ICRF study (Miles) [48]	Follow-up CMF	391	70	IHC	All patients benefited from CMF, but benefit was greater in HER-2-negative (median OS, 7.3 [follow-up group] vs 12.7 years [CMF group]) than in HER- 2-positive (median OS, 4.4 [follow-up group] vs 6.1 years [CMF group] patients
Milan trial (Menard) [49]	Follow-up CMF	386	87	IHC	Clinical benefit of CMF in patients with HER-2-positive, as well as in those with HER-2-negative tumors

PeCT, Perioperative cyclophosphamide + methotrexate + 5-fluorouracil (CMF); CMFP, postoperative CMF + prednisone; IBCSG, International Breast Cancer Study Group; ICRF, Imperial Cancer Research Fund; CT, chemotherapy
From Di Leo A, Cardoso F, Durbecq V, Giuliani R, Mano M, Atalay G, Larsimont D, Sotiriou C, Biganzoli L, Piccart MJ. Predictive molecular markers in the adjuvant therapy of breast cancer: state of the art in the year 2002. *Int J Clin Oncol.* 2002;24:5–53. Table 5. With kind permission of Springer Science and Business Media

receive CMF or no treatment [49]. Albeit based on a limited number of patients, this study is in contradiction with the previous ones. Therefore, regarding the predictive value of HER-2 for CMF-like chemotherapy regimens, the evidence is level II, category C.

Three retrospective studies performed by the National Surgical Adjuvant Breast and Bowel Project (NSABP), the Belgian Adjuvant Study Group, and the Milan group [50–52], evaluated the predictive value of HER-2 in a population of node-positive breast cancer patients, randomly assigned to receive CMF or anthracycline-based chemotherapy. These reports suggest reduced CMF efficacy in patients overexpressing the HER-2 oncoprotein, and all three studies agree in defining the HER-2-positive subgroup as the most sensitive to anthracycline-based adjuvant chemotherapy. Two other retrospective studies [53, 54] generated similar results regarding HER-2 overexpression and responsiveness to anthracyclines. Additionally, an Intergroup Study, presented at the 1998 ASCO meeting, showed that, in tumors overexpressing HER-2, chemohormonal therapy with cyclophosphamide, doxorubicin, 5-fluorouracil (CAF), plus tamoxifen seemed to yield better results than tamoxifen alone [55].

More recently, the Canadian group (NCI-C) has reported the results of a retrospective study exploring the predictive value of HER-2 gene amplification in a population of node-positive pre-menopausal patients treated in the context of the MA-5 phase III trial with either CMF or CEF [56]. This study suggests that the superiority of CEF over CMF is seen only in the population of HER-2 positive patients [56]. Conversely, the Danish group (DBCG) has not found a similar interaction between HER-2 and anthracyclines in their study comparing CMF and CEF in the adjuvant treatment of breast cancer patients [57]. Taken together, these studies provide level II, category C evidence concerning clinical practice recommendations.

Topoisomerase II Alpha (Topo II α)

The topo II α gene is located next to the HER-2 gene on chromosome 17q12-q21, and its amplification may lead to overexpression of the topo II α protein. Because this enzyme is inhibited by anthracyclines and is the main target of these drugs, its overexpression may render the cells more sensitive to topo II α inhibitors [58, 59]. Studies have shown that topo II α amplification only occurs with concurrent HER-2 amplification, and it is possible that the predictive value of HER-2 regarding anthracycline-based chemotherapy is explained by the concomitant amplification of the *topo II α gene* [58, 60–62]. Preclinical data also indicate that intra-tumoral topo II α levels may explain some forms of resistance to anthracyclines observed in in vitro systems [63].

Table 4 shows the results of those studies that have explored the predictive value of topoisomerase II α gene aberrations in a population of early breast cancer patients treated in the context of phase III trials with an anthracycline or non-anthracycline-based adjuvant chemotherapy [57, 64–66]. Based on the results of these retrospective studies, we have to conclude that the level of evidence for topo II α gene amplification is II category B.

Table 4 Phase III trials in which topo II gene has been tested as a predictive marker

Study (year)	Design	No. topo II Evaluable pts.	HER- 2 Status	% topo II Gene amplification	% topo II Gene deletion	Results
Di Leo et al. [64] (2002)	↑ CMF	61	+	38	13	HEC + EC > CMF if topo II amplified
	↑ HEC					HEC + EC = CMF if topo II non-amplified
	↑ EC					
Knoop et al. [37] (2005)	↑ CMF	773	+/-	12	11	FEC > CMF if topo II amplified or deleted
	↑ FEC					FEC = CMF if topo II normal
O'Malley et al. [65] (2006)	↑ CMF	443	+/-	11	6	FEC > CMF if topo II amplified or deleted
						FEC = CMF if topo II normal
	↑ FEC					
Slamon et al. [66] (2006)	↑ AC → DT	2990	+	35	5	AC → DT = DPT = AC → D if topo II amplified
	↑ DPT					AC → DT = DPT > AC → D if topo II non-amplified
	↑ AC → D					

CMF = cyclophosphamide-methotrexate-fluorouracil; HEC = standard doses epirubicin-cyclophosphamide; EC = moderate doses epirubicin-cyclophosphamide; AC → DT = doxorubicin-cyclophosphamide → docetaxel-trastuzumab; DPT = docetaxel-carboplatin-trastuzumab; AC → D = doxorubicin-cyclophosphamide → docetaxel; FEC = fluorouracil-epirubicin-cyclophosphamide

Factors preventing the use of topo II α gene amplification as a marker predicting the activity of anthracyclines in the adjuvant setting are: (1) the fact that also topo II α gene deletion seems to predict response to anthracyclines and this is hard to explain biologically [57, 65]; (2) the lack of correlation between gene status and topo II α protein levels evaluated by IHC [67–69]; (3) the lack of reproducibility studies showing an acceptable level of inter-laboratory agreement when topo II α gene status is evaluated on the same samples in different laboratories.

Markers Predicting the Activity of Taxane-Based Regimens

Taxanes have been evaluated in the adjuvant setting only recently. Therefore, most of the available results regarding possible predictive factors were obtained in the context of metastatic or neoadjuvant breast cancer studies. Three randomized studies have suggested that tumors overexpressing HER-2 might be more sensitive to a taxane-based than to anthracycline-based regimens (Table 5) [70–72]. Based on these results, the level of evidence is II category B. More clinical evidence is needed to implement the results of these retrospective studies into clinical practice.

In a clinical study of neoadjuvant chemotherapy, *p*-53 mutated tumors showed a high response rate when treated with taxanes, but a low response rate when treated with anthracyclines [73]. This and other in vitro and in vivo

Table 5 HER-2/neu and taxanes

Group	Design	Setting	No. pts.	HER-2 technique	Results
TAX 303 ⁷⁰	→ A	Advanced	176	IHC/FISH	HER-2+ TxT > A HER-2- TxT = A
	→ TxT				
UCLA ⁷¹	→ EC	Advanced	297	FISH	HER-2+ ET > EC HER-2- ET = EC
	→ ET				
CALGB ⁷²	→ AC	Early	1,500	IHC/FISH	HER-2+ AC → T > AC HER-2- AC → T = AC
	→ AC→T				

EC = Epirubicin-Cyclophosphamide; ET = Epirubicin-Paclitaxel; A = Doxorubicin; TxT = Docetaxel;

AC = Doxorubicin-Cyclophosphamide; T = Paclitaxel; FISH = fluorescence in-situ hybridization;

IHC = immunohistochemistry

studies have raised the hypothesis that *p*-53-mutated tumors might be less sensitive to anthracyclines, while retaining sensitivity to taxanes [74]. To test this hypothesis, a large multicenter international prospective trial has been

opened under the auspices of B.I.G. (Breast International Group) and coordinated by the EORTC.

The most attractive markers as far as taxane treatment is concerned are probably the microtubule-associated parameters (MTAP). These are a specific target for taxanes because these drugs interact with microtubules. Preclinical data suggest that mammary and pancreatic tumors with exquisite responsiveness to docetaxel in in vitro models have the highest expression of the *Tau* gene (MTAP-2 family) and of the α -tubulin protein [75]. Assessment of MTAP-2 expression by IHC in paraffin-embedded samples is feasible, making possible retrospective studies correlating MTAP-2 levels and docetaxel activity in both the metastatic and adjuvant settings.

The M.D. Anderson group has evaluated the predictive value of TAU protein in the context of a neoadjuvant phase II trial in which breast cancer patients were treated with a paclitaxel-based chemotherapy. The results of this retrospective study seem to suggest that TAU protein down-regulation is associated with a 44% pathologic complete response (pCR) rate, while in the TAU overexpression group pCR rate is 17% [76]. The same group has produced similar results in a pre-clinical study where TAU gene has been down-regulated and this has produced increased sensitivity of breast cancer cells to paclitaxel but not to epirubicin [76].

Of note, proteins associated with the mitotic spindle regulation have been suggested as markers predicting sensitivity or resistance to taxanes also in two different phase II neoadjuvant studies in which response to single-agent docetaxel or to paclitaxel-based sequential chemotherapy has been correlated with gene expression profiles evaluated by gene microarray technology on pre-treatment primary tumor samples [77, 78].

Although data exist on p-53 gene mutation or MTAP expression and resistance to taxanes, current levels of evidence do not recommend the use of these tools in clinical practice (level II C for p-53, level III B for MTAP).

Markers Predicting the Activity of Anti-HER-2 Therapies

The identification of patient candidates for anti-HER-2 therapies either in the early or in the metastatic setting is by far the most relevant information provided by HER-2 testing of breast cancer samples. Large phase III trials have unequivocally proved the efficacy of anti-HER-2 agents such as trastuzumab and lapatinib in patients carrying HER-2 positive tumors [79–84].

The identification of molecular markers complementing HER-2 scores with the aim to define better the profile of anti-HER-2 compound sensitive tumors is certainly a relevant research area. Different events seem to play a role in the onset of clinical resistance to anti-HER-2 compounds. Among these, activation of the insulin-like growth factor 1 (IGF-1) pathway, PTEN deficiency, PI3K gene mutations, compensatory signalling from other HER family members, and polymorphism of the FC receptor, have been suggested as potential markers of resistance [85]. Ongoing clinical studies will likely clarify the role

of these markers in predicting the likelihood of response of HER-2 positive tumors to anti-HER-2 therapies.

Conclusions

Hormone receptor expression for adjuvant hormonotherapy and HER-2 over-expression for anti-HER-2 compounds are the only predictive markers for which level I category A evidence justifies use in routine clinical practice.

The significant translational research efforts carried out in the past decade in this field have led to the generation of some fascinating hypotheses. New techniques now exist to test a number of these hypotheses. In particular, the use of cDNA micro-arrays will permit a better biological characterization of breast cancer, and perhaps even a new classification of the disease, based on distinct molecular profiles, which may be of prognostic and/or predictive value. It is now time to test these hypotheses in a new generation of prospective predictive marker studies, some of which are already ongoing, and the results of which are eagerly awaited. Their outcome may radically change the therapeutic approach to early breast cancer in the future.

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