HER-2 overexpression/amplification and its interaction with taxane-based therapy in breast cancer

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Received 8 March 2007; revised 7 June 2007; accepted 8 June 2007

Breast cancer (BC) is the most common cancer in women and it is incurable when metastases are diagnosed. Taxanes, namely docetaxel and paclitaxel, are effective chemotherapeutic agents in the metastatic, neoadjuvant and adjuvant settings. HER-2 overexpression/amplification is detected in 25–30% of BCs and confers aggressive tumor behavior as well as resistance to some systemic treatments; nevertheless, its association with response to taxane-based chemotherapy is still unclear, with conflicting results in both in vitro and in vivo preclinical studies. This review will address the impact of HER-2 overexpression/amplification in BC patients treated with taxanes. Prospective, randomized trials incorporating important biological hypotheses are either ongoing or just closed, and their results will hopefully help to shed more light on this issue.

Key words: breast cancer, ECD, HER-2 overexpression, taxane resistance, trastuzumab

introduction

Taxanes are agents that disrupt microtubule dynamics by stabilizing microtubules and preventing their depolymerization. Paclitaxel, the first taxane used in clinical trials, was initially identified as the active extract from the bark of the Pacific yew Taxus brevifolia [1]. Docetaxel was subsequently generated from the needles of the European yew Taxus baccata via a semi-synthetic approach [2, 3]. These two drugs are among the most active agents in the treatment of metastatic breast cancer (BC), with response rates ranging from 30% to 60% when used as a single agent in patients with minimum or no prior therapy, and 20% to 45% in patients with extensive prior chemotherapy (CT) [4]. Their role in the adjuvant setting, particularly for node-negative disease, is still controversial since the magnitude of benefit on average is likely to be small and most probably confined to a subgroup of patients yet to be identified. Cost is also an issue, particularly for docetaxel which can not yet be replaced by generic drugs.

Amplification/overexpression of HER-2, also known as c-erbB2 or neu, is observed in 25–30% of human BCs, and has been associated with poor outcome, in both node-negative and node-positive early BC [5–8]. In vitro, HER-2 overexpression confers increased resistance to paclitaxel in BC cells, while HER-2 degradation increases docetaxel-induced apoptosis [9–11]. This is further supported by data from a phase III clinical trial showing that paclitaxel response rate was significantly improved in BC patients when HER-2 was downregulated by the humanized anti-HER-2 antibody, trastuzumab [12].

The aim of this review is to identify the possible mechanisms of resistance to taxanes in HER-2-overexpressing BC patients. Preclinical and clinical data as well as the role of trastuzumab in overcoming taxane resistance are discussed.

materials and methods

A computer-based literature search using PubMed was performed. Articles were selected applying the key words ‘HER-2/neu’ and ‘paclitaxel resistance’ or ‘docetaxel resistance’, and ‘trastuzumab plus taxanes’. Only papers published in English and before 1 August 2006 were reviewed. Published abstracts or abstracts presented at International Meetings were also included.

HER-2 overexpression and resistance to taxanes: in vitro studies

Human BC cell lines with high HER-2 receptor levels had shown increased resistance to paclitaxel in the order of at least 100-fold as well as high levels of the mdr-1-encoded P-glycoprotein (p170abk−1). This protein functions as a drug-efflux pump (to reduce the cellular accumulation of specific drugs), the expression of which has been highly correlated with taxane resistance [13]. The addition of emodin, a HER-2 tyrosine kinase inhibitor, to paclitaxel-induced synergistic inhibition of MDA-MB-361 BC cells proliferation by 70%, which was higher than the effect seen with emodin or paclitaxel alone (28% and 16%, respectively). Similar results were also observed in BT-474 cells and suggest that HER-2 receptor tyrosine kinase activity contributes to paclitaxel resistance in HER-2-overexpressing BC cells [14].
Cell lines expressing both HER-2 and HER-3 receptors present high levels of phosphorylated-activated Akt, which may suppress apoptosis due to interaction with and phosphorylation of several key downstream effectors, and may therefore contribute to several anticancer drugs including taxanes. In MCF-7/Her-2-transfected cells, Akt activation may be mediated by the PI-3K pathway, leading to resistance to different drugs, including paclitaxel [15].

Pegram et al. [16] reported that Her-2 transgene was not sufficient to induce drug resistance and that response to paclitaxel was not statistically different between HER-2-overexpressing and control cancer xenografts. In contrast, Yu et al. [9] reported that Her-2-transfected MDA-MB-435 cells were more resistant to paclitaxel than parental cells (P < 0.00012), independently of cell cycle characteristics, oncogenic transformation or mdr-1. Other possible mechanisms of resistance are inhibition of both apoptosis and p34Cdc2 kinase activation through p21Cip1 upregulation [17] or Cdc2 phosphorylation of tyrosine Y15, which leads to resistance to paclitaxel-induced apoptosis [18].

MCF-10A Ha-ras cells show increased sensitivity to taxanes, while MCF-10A-Her-2 and MCF-10A-HE cells exhibit relative resistance to paclitaxel and docetaxel, with a 3.5- to 6.5-fold higher IC50 as compared with the parental cells. Furthermore, inhibition of type I cAMP-dependent protein kinase (PKAI) with antisense oligonucleotides overcomes the effect of HER-2 overexpression in MCF-10A cells [19].

In constitutively active EGFR- and Her-2-transfected cell lines, a 2- to 3-fold paclitaxel resistance and suppression of tubulin polymerization were reported when compared with the parental cell lines. Inhibition of constitutively active EGFR with an inactivating mutation in the kinase domain partially reversed paclitaxel resistance and showed 50% decrease in paclitaxel-resistant class IVA β-tubulin. These results suggest that modulation of tubulin isoform expression by oncogenic growth factor receptors may modulate resistance to tubulin-binding agents [20].

Activation of mitogen-activated protein kinases (ERK and p38 MAPK) and Raf-1 kinase by paclitaxel may explain the enhanced cytotoxic effects of this drug in HER-2-overexpressing cells [21]. Several studies have clearly demonstrated the involvement of the ERK cascade in paclitaxel-induced apoptosis [22–29], as well as the JNK cascade [30], p38 MAPK or even a PKA-induced pathway [29]. Daly et al. [31] demonstrated that apoptosis and G1/M transition induced by NDF/heregulin is dependent on p38 activation in HER-2-overexpressing BC cell lines, and that the addition of paclitaxel has an additive effect on p38 activation [31, 32]. Thus, the MAPK pathway plays an essential role in paclitaxel-induced cellular death and might be an interesting treatment target to be tested in combination studies [21]. Table 1 summarizes the main results from these selected articles and the ErbB signal transduction cascade is shown in Figure 1.

**HER-2 as a predictive marker of response to taxanes: clinical data**

In 2001, Yamauchi et al. [33] conducted an extensive review of HER-2 as a predictive marker of response to BC therapies concluding that it should not be used to determine whether a woman should receive adjuvant CT or endocrine therapy. Since then, many other articles have been published with controversial results regarding the relationship between HER-2 and taxane benefits in BC. These studies are summarized in Tables 2, 3 and 4 and are discussed below.

In vitro hypothesis for taxane resistance in HER-2-overexpressing breast cancer cells

<table>
<thead>
<tr>
<th>BC cells</th>
<th>Drug</th>
<th>Probable mechanism</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB-435.eB (Her-2-transfected)</td>
<td>P</td>
<td>Not mdr-1 mechanisms</td>
<td>[9]</td>
</tr>
<tr>
<td>MDA-MB-435.eB (Her-2-transfected)</td>
<td>P</td>
<td>Akt kinase activity</td>
<td>[17]</td>
</tr>
<tr>
<td>MDA-MB-435.eB versus MDA-MB-435</td>
<td>P</td>
<td>Inhibition p34&lt;sup&gt;Cdc2&lt;/sup&gt; activation</td>
<td>[18]</td>
</tr>
<tr>
<td>MCF-10A-Her-2, MCF-10A Ha-ras, MCF-10 HE (Her-2-ras) versus MCF-10A</td>
<td>P and D</td>
<td>Activation of PKA I</td>
<td>[19]</td>
</tr>
<tr>
<td>WiEGFR-NIH3T3 (CO12), EGFRvIII-NIH3T3 (HC2), HER-2-Ras-NIH3T3 (Val12)</td>
<td>P</td>
<td>Reduced β-tubulin polymerization</td>
<td>[20]</td>
</tr>
<tr>
<td>HER-2 mutant-HC2H (T691)</td>
<td>P</td>
<td>↑ Class IVα tubulin</td>
<td>[21]</td>
</tr>
<tr>
<td>MCF-7/Her-2 versus MCF-7</td>
<td>P</td>
<td>ERK and p38 MAPK pathways</td>
<td>[21]</td>
</tr>
</tbody>
</table>

**Abbreviations:** PKA I, type I c-AMP-dependent protein kinase; D, docetaxel; P, paclitaxel; ↑, upregulation.
In another study, Di Leo et al. [35] retrospectively analyzed patients with MBC enrolled into a phase III trial and randomly assigned to receive either doxorubicin (A) or docetaxel (D) (TAX 303 trial). FISH amplification was determined as a ratio of the number of Her-2 signals/centromeric 17 signals > 2. ORR was significantly higher with D than with A (161 patients: 47.8% compared with 165 patients: 33.3%, respectively; \( P = 0.008 \)) in all patients and in the Her-2-positive subgroup (67% compared with 27%, respectively; \( P = 0.04 \)), although this difference did not translate into a higher time to progression (TTP) or improved OS. In the D arm, the ORR was significantly better in patients with amplified Her-2 than in non-amplified cases. Interestingly, the interaction between Her-2 status and treatment was the only significant factor associated with increased probability of response to D in the multivariate analysis (OR = 3.64, 95% CI 1.39–9.54; \( P = 0.01 \)).

The European Organization for Research and Treatment of Cancer (EORTC) 10923 trial was a phase III study designed to compare single-agent doxorubicin with paclitaxel, with cross-over at the time of progression, in MBC patients. In the paclitaxel arm (56 patients treated first line and 31 second line), ORR was seen in 21 patients (24%), and no correlation between HER-2 status and response to paclitaxel was found [36]. Similarly, HER-2 expression was not predictive of response to single-agent paclitaxel either in univariate or in multivariate analysis (\( P = 0.511 \)) in a population of 144 MBC patients enrolled in nine different clinical trials utilizing different paclitaxel doses (ORR 42%) [4].

Figure 1. ErbB signal transduction cascade. Republished with permission of AlphaMed Press, from Burris HA 3rd, Dual kinase inhibition in the treatment of breast cancer: initial experience with the EGFR/ErbB2 inhibitor lapatinib. Oncologist 2004: 9 Suppl 3: 10–15; permission conveyed through Copyright Clearance Center, Inc.

Table 2. Published studies evaluating the predictive value of HER-2 to taxane-based therapy (single agent)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Patient [number in TAX arm (C/T)]</th>
<th>Trial phase</th>
<th>Disease stage</th>
<th>Method and antibody</th>
<th>ORR in HER-2+ (%)</th>
<th>ORR in HER-2− (%)</th>
<th>P value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P or D</td>
<td>202/122</td>
<td>II</td>
<td>IV</td>
<td>IHC (mAB 4D5)</td>
<td>65.2</td>
<td>35.5</td>
<td>0.002</td>
<td>[1]</td>
</tr>
<tr>
<td>D</td>
<td>131/66</td>
<td>III</td>
<td>IV</td>
<td>IHC (HercepTest)</td>
<td>53</td>
<td>53</td>
<td>0.50</td>
<td>[34]</td>
</tr>
<tr>
<td>D</td>
<td>161/85</td>
<td>III</td>
<td>IV</td>
<td>FISH (PathVysion)</td>
<td>67</td>
<td>40</td>
<td>0.03</td>
<td>[35]</td>
</tr>
<tr>
<td>P</td>
<td>166/87</td>
<td>III</td>
<td>IV</td>
<td>IHC (Biogenex)</td>
<td>71a</td>
<td>28.5a</td>
<td>&lt;0.05</td>
<td>[37]</td>
</tr>
<tr>
<td>P</td>
<td>21/15</td>
<td>II</td>
<td>T2-3</td>
<td>IHC (Biogenex)</td>
<td>38</td>
<td>28</td>
<td>0.067</td>
<td>[38]</td>
</tr>
<tr>
<td>P or D</td>
<td>71</td>
<td>II</td>
<td>II</td>
<td>FISH (Vysis)</td>
<td>16b</td>
<td>10b</td>
<td>0.68</td>
<td>[39]</td>
</tr>
<tr>
<td>P</td>
<td>29/29</td>
<td>II</td>
<td>T3-4</td>
<td>IHC (Biogenex)</td>
<td>38</td>
<td>28</td>
<td>0.667</td>
<td>[38]</td>
</tr>
<tr>
<td>P or D</td>
<td>71</td>
<td>II</td>
<td>II</td>
<td>IHC (HercepTest)</td>
<td>7b</td>
<td>18b</td>
<td>0.355</td>
<td>[40]</td>
</tr>
</tbody>
</table>

Abbreviations: C/T, clinical/translational study; D, docetaxel; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NR, not reported; ORR, overall response rate; P, paclitaxel; TAX, taxane.

aClinical or pathological complete response.
bPathological complete response.
In neoadjuvant trials, HER-2 was associated with an improved response to dose-dense paclitaxel in 15 of 21 stage T2–3 BC patients; clinical response (CR) was more than double in patients with HER2-positive tumors treated with paclitaxel ($P < 0.05$). Although this trial was prospective, it was not randomized, had a small number of patients and has been published only as an abstract [37]. Another small prospective study failed to demonstrate any correlation between HER-2 positivity and pathological complete response (pCR) in 29 patients with locally advanced breast cancer (LABC), T3 or T4, treated with doxorubicin followed by paclitaxel or paclitaxel followed by doxorubicin in a dose-dense regimen [38]. These negative results were also confirmed using FISH (Her-2/CEP17 > 2) in 71 patients treated with neoadjuvant paclitaxel or docetaxel given every 3 weeks [39]. Estevez et al. [40] also failed to demonstrate correlation between pathological or clinical response and HER-2 expression ($P = 0.355$ and $P = 0.942$, respectively) in 56 stage II–III BC patients treated with weekly neoadjuvant docetaxel (ORR 68% and pCR 16%). However, all these three trials were non-randomized, had few patients and in two studies, HER-2 status was not confirmed by FISH.

Interesting findings from Modi et al. [41] showed that phosphorylated-activated HER-2 is associated with clinical resistance to taxanes in 126 patients enrolled in different trials with single-agent taxanes for MBC and, perhaps, functional assessment of HER-2 status may provide unique predictive information not seen with conventional assessment.

The role of HER-2 was also analyzed in combination CT regimens and the main studies are summarized in Table 3.

### Table 3. Studies evaluating the predictive value of HER-2 to taxane-based therapy (combination chemotherapy)

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Patient [number in TAX arm (C/T)]</th>
<th>Trial phase</th>
<th>Disease stage</th>
<th>Method and antibody</th>
<th>ORR in HER2+ (%)</th>
<th>ORR in HER2− (%)</th>
<th>$P$ value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED</td>
<td>97/97</td>
<td>II</td>
<td>I–III</td>
<td>FISH (PathVysion)</td>
<td>NR</td>
<td>NR</td>
<td>0.876</td>
<td>[42]</td>
</tr>
<tr>
<td>AD</td>
<td>19/19</td>
<td>II</td>
<td>IIb, IIa, IIb</td>
<td>IHC (HercepTest)</td>
<td>56</td>
<td>10</td>
<td>0.05</td>
<td>[43]</td>
</tr>
<tr>
<td>PET</td>
<td>200/100</td>
<td>III</td>
<td>IIb</td>
<td>IHC (NR)</td>
<td>31</td>
<td>5</td>
<td>0.037</td>
<td>[44]</td>
</tr>
<tr>
<td>TAC</td>
<td>745/648</td>
<td>III</td>
<td>&gt;2 cm</td>
<td>FISH (NR)</td>
<td>24.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17</td>
<td>[45]</td>
</tr>
<tr>
<td>ACD</td>
<td>45/45</td>
<td>III</td>
<td>I–IIIA</td>
<td>IHC (Zymed)</td>
<td>78</td>
<td>81</td>
<td>0.99</td>
<td>[46]</td>
</tr>
<tr>
<td>P + RT</td>
<td>36</td>
<td>II</td>
<td>IIb, IIa, IIb</td>
<td>IHC (BioGenex)</td>
<td>NR</td>
<td>NR</td>
<td>0.11</td>
<td>[47]</td>
</tr>
<tr>
<td>EP</td>
<td>516/138</td>
<td>III</td>
<td>IV</td>
<td>FISH (Vysis)</td>
<td>76</td>
<td>50</td>
<td>0.005</td>
<td>[52]</td>
</tr>
<tr>
<td>AP</td>
<td>49/49</td>
<td>II</td>
<td>IV</td>
<td>FISH (NR)</td>
<td>50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17</td>
<td>&lt;0.02</td>
<td>[53]</td>
</tr>
</tbody>
</table>

Abbreviations: AC, doxorubicin + cyclophosphamide; ACD, doxorubicin + cyclophosphamide + docetaxel; AP, doxorubicin + paclitaxel; CT, clinical/ translational study; EC, epirubicin + cyclophosphamide; ED, epirubicin + docetaxel; EP, epirubicin + paclitaxel; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NR, not reported; ORR, overall response rate; PET, cisplatin, epirubicin, paclitaxel; RT, radiotherapy; RT–PCR: real time quantitative polymerase chain reaction; TAX, taxane.

<sup>a</sup>Complete response.

<sup>b</sup>Pathological complete response.

<sup>c</sup>Total number in both arms.

### Table 4. Main results of the in vitro studies with trastuzumab and taxanes

<table>
<thead>
<tr>
<th>Drug</th>
<th>BC cells</th>
<th>Effects/mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>P or D (IC 50)</td>
<td>MCF-7, SKBR-3, MDA-MB-453</td>
<td>Synergism</td>
<td>[60]</td>
</tr>
<tr>
<td>D</td>
<td>SKBR-3, MDA-MB-453</td>
<td>Synergism</td>
<td>[60]</td>
</tr>
<tr>
<td>P (IC 30–70)</td>
<td>SKBR-3</td>
<td>Synergism</td>
<td>[61]</td>
</tr>
<tr>
<td>D + carbo</td>
<td>BT-474</td>
<td>Additive</td>
<td>[62]</td>
</tr>
<tr>
<td>P</td>
<td>Transfected MCF-7</td>
<td>Additive</td>
<td>[63]</td>
</tr>
<tr>
<td>P</td>
<td>SKBR-3</td>
<td>Additive</td>
<td>[64]</td>
</tr>
<tr>
<td>BT-474 and mice bearing BT-474 xenografts</td>
<td></td>
<td>1. Two drugs acting in two different targets?</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Upregulation of HER-2 receptor by P?</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>MDA-MB-435, SKBR3</td>
<td>Additive effect on p34 Cdc2 activation</td>
<td>Cdc2-Tyr-15 dephosphorylation p21Cip1 downregulation</td>
</tr>
</tbody>
</table>

Abbreviations: BC, breast cancer; carbo, carboplatin; D, docetaxel; P, paclitaxel.
enrolled in the GeparTrio trial and treated with docetaxel, doxorubicin and cyclophosphamide (TAC) or vinorelbine plus capcitabine. pCR was obtained in 51 patients (17.9%), with no difference seen between Her-2-positive and Her-2-negative patients [45]. In a retrospective study of 121 patients enrolled in a randomized trial comparing preoperative doxorubicin and cyclophosphamide (AC) with or without docetaxel, no difference in clinical response was seen between the two arms (55% and 82%, respectively), although a higher, but not significant, ORR was reported regardless of Her-2 status in the docetaxel arm [46].

Pathological response was seen in 12 of 36 LARC patients (33%) treated with weekly paclitaxel combined with radiotherapy and higher pathological responses (complete and partial) were reported in patients whose tumors had low Her-2 gene expression by RT-PCR (P = 0.009); however, this significance disappeared when IHC was performed (P = 0.11). Whether Her-2-overexpressing tumors confer resistance either to paclitaxel or to radiation, or to both, is still not known [47].

In the adjuvant setting, the BCIRG 001 trial, which compared TAC versus 5-fluorouracil, doxorubicin and cyclophosphamide (FAC) showed, at a median follow-up of 53 months, a 39% relative reduction in the risk of cancer recurrence in a subgroup of patients with one to three positive nodes treated with TAC compared with those patients treated with FAC (P < 0.001). In patients with Her-2-overexpressing tumors, a 40% relative reduction in the risk of cancer recurrence was reported in patients treated with TAC compared with FAC with a 24% relative reduction in those treated with FAC (P = 0.046) [48, 49]. Data from the CALGB 9344 (adjuvant paclitaxel followed by AC in node-positive BC) trial, presented at the ASCO 2006 meeting, showed that treatment with paclitaxel resulted in a greater benefit in OS (P = 0.0056) and DFS (P = 0.0093) in Her-2-positive patients. The subset of ER-negative and HER-2-positive tumors benefitted the most from paclitaxel [50].

In the metastatic setting, Koncny et al. [51] reported a retrospective analysis from the AGO trial that compared epirubicin plus cyclophosphamide (EC) with epirubicin plus paclitaxel (ET) in 516 MBC patients. Similar ORR was seen in both arms in the clinical trial (46% and 41%, respectively). Her-2 status was evaluated by FISH (ratio < 2) in 275 patients from whom tumor blocks were available (137 patients assigned to EC and 138 assigned to ET). Patients with Her-2-positive tumors had a significantly greater objective response rate than those with Her-2-negative tumors following treatment with ET but not with EC; a trend for improved progression-free survival (PFS) and OS was reported in patients with HER-2-positive tumors receiving taxane treatment [52]. Doxorubicin combined with paclitaxel (DT) as first-line treatment in 49 MBCs resulted in 31% complete response (15 patients) that increased to 50% in those with HER-2-positive tumors, a statistically significant improvement in comparison with the 17% ORR seen in those with HER-2-negative tumors [53].

In a recent meta-analysis of taxanes alone or in combination with anthracyclines as first-line CT for MBC, single-agent anthracycline was significantly better than single-agent taxane in terms of PFS, but not in terms of OS. Moreover, taxane-based combinations resulted in a significantly better ORR than anthracycline-based combinations, but were only marginally better in terms of PFS and not in terms of OS [54], taxane in combination with trastuzumab. (i) In vitro data. Trastuzumab, a recombinant humanized anti-p185HER2/neu monoclonal antibody with high affinity for the HER-2 receptor was engineered from a cloned human IgG framework and the antigen-binding residues of the murine monoclonal antibody 4D5 [55]. Trastuzumab has been routinely used in HER-2-overexpressing MBC, and has recently shown striking benefit in both DFS and OS in early BC when given concomitantly or sequentially with adjuvant CT [56–58]. It is hypothesized that trastuzumab may sensitize HER-2-overexpressing BC cells by repressing HER-2-mediated upregulation of p21\(^{Cip1}\), allowing the activation of p34\(^{cdk2}\) kinase by paclitaxel, thus resulting in apoptosis [59].

At the IC\(_{50}\), the combination of trastuzumab with either paclitaxel or docetaxel led to synergism in MCF7 (HER-2+), MDA-MB453 (HER-2+), and SKBR3 (HER-2+/-) cell lines. However, in the IC\(_{50}\)–IC\(_{70}\) concentration range, trastuzumab interacted additively with docetaxel in SKBR3 and MDA-MB453 cell lines, while additive and synergistic interactions were achieved with paclitaxel in SKBR3 and MDA-MB453, respectively [60]. Synergistic interactions have also been reported for trastuzumab in combination with paclitaxel/carboplatin (combination index, CI: 0.64, P < 0.0001) and docetaxel/carboplatin (CI: 0.34, P < 0.00004) [61]; a CI of <1 indicates synergy and =1 indicates additive effects [62]. Additive cytotoxic effects were observed with trastuzumab in combination with paclitaxel (CI = 0.91, P = 0.21) in vitro as well as in vivo, resulting in a significant reduction in Her-2-transfected MCF7 xenograft volume compared with CT alone (P < 0.05) [62]. These results were in agreement with the data reported by Baselga et al. [63], who observed that trastuzumab enhanced the cytotoxic effect of paclitaxel in HER-2-overexpressing BT-474 and SKBR3 cells; an effect confirmed in vivo with 93% growth tumor inhibition observed in mice bearing BT-474 xenografts and treated with trastuzumab plus paclitaxel. Growth tumor inhibition at 5 weeks was superior in the combined group when compared with paclitaxel alone (P = 0.016), but not to trastuzumab alone (P = 0.4). Moreover, the highest tumor eradication rate at 5 weeks was observed in animals treated with trastuzumab plus paclitaxel when compared with either paclitaxel or trastuzumab alone (P = 0.004 and P = 0.04, respectively). In this study, two mechanisms are proposed to explain the capacity of trastuzumab to enhance the tumoricidal effects of paclitaxel: (i) the sum of effects of two anticancer drugs that act on different targets; and (ii) the exposure to paclitaxel may produce a functional upregulation of the HER-2 receptor, rendering the cells more sensitive to the antiproliferative effects of trastuzumab [63].

Downregulation of HER-2 expression leading to inhibition of HER-2-mediated p21\(^{Cip1}\) upregulation and Cdc2-Tyr15 phosphorylation was observed in HER-2-overexpressing BC cells (MDA-MB-435, MDA-MB-453 and SKBR3) treated with trastuzumab, but not in control or in paclitaxel-treated cells. Trastuzumab plus paclitaxel treatment led to an additive effect on p34\(^{cdk2}\) activation, which is required for paclitaxel-induced apoptosis. Cdc2-Tyr-15 dephosphorylation and p21\(^{Cip1}\) downregulation occur at least 24 h after trastuzumab treatment, and trastuzumab pretreatment increases paclitaxel-induced apoptosis and cytotoxicity in vitro and more effectively inhibits the growth of tumor xenografts with enhanced in vivo apoptosis [64].

(ii) Clinical data. Transporting the previous hypothesis into the clinic, some randomized trials with taxanes in combination with trastuzumab have been conducted in HER-2-positive MBC and their results are summarized in Table 5. The M77001 trial, which enrolled patients with HER-2 positivity (IHC 3+ or FISH+), compared docetaxel alone with docetaxel plus trastuzumab as first-line therapy for MBC. The addition of trastuzumab to docetaxel resulted in significantly improved ORR, TTP and OS with the effect being greater if the combination was given from the start as compared with adding trastuzumab at the time of progression under docetaxel [65].

Two trials have evaluated paclitaxel given either every 3 weeks [12] or weekly [66], alone or in combination with trastuzumab. Both trials resulted in significant improvement in ORR and TTP for the trastuzumab arm in the subset of HER-2-positive patients. A triple combination using paclitaxel/carboplatin/trastuzumab was given to 188 MBC patients with HER-2-overexpressing tumors (IHC 2+/3+). Improvements in ORR and in TTP were reported with the triple combination in patients who had HER-2 IHC 3+ tumors [67]. Paclitaxel given weekly in combination with carboplatin and trastuzumab in HER-2-overexpressing MBC (IHC 3+ or...
HER-2 extracellular domain as predictive factor of response to taxanes. The extracellular domain of the HER-2 receptor (HER-2 ECD), a protein of ~105 kDa, is cleaved and shed from the receptor [72]. It can be detected in the serum of women with MBC by enzyme linked immunosorbent assay (ELISA). The average percentage of patients with MBC having an elevated circulating HER-2 ECD level is 45.6% (range, 23–80%) [73]. Its presence has been correlated with higher tumor burden and a good correlation exists between HER-2 ECD and HER-2 overexpression using IHC with an optimized cut-off at 15 ng/ml [72]. Some studies have addressed the role of HER-2 ECD in patients treated with taxanes with conflicting results, as shown in Table 6.

In a prospective study, the levels of HER-2 ECD were predictive of response, with a significant inverse relationship in MBC patients treated with doxorubicin and paclitaxel. Additionally, a shorter response duration was found in patients with high levels of HER-2 ECD in multivariate analysis (P = 0.04) [74]. These results were further confirmed in two other studies: (i) worse response rate and shorter response duration (7.9 months compared with 14.4 months; P = 0.04) were seen in patients with high levels of HER-2 ECD treated with a biweekly combination of gemcitabine and paclitaxel [75]; (ii) elevated ECD levels were found to be associated with a better response rate (67% versus 25%; respectively, P = 0.02). Although this was a small randomized phase II trial, the results were provocative and the trial was prematurely stopped. A recent update showed that the efficacy remained unchanged after inclusion of results of 22 additional patients [70]. Another neoadjuvant trial tested the efficacy of docetaxel/carboplatin with or without trastuzumab in stage III BC patients. Of 37 patients with complete pathology verification, 11 (29%) showed pCR of the primary tumor with five cases being HER-2 positive. Among the 22 HER-2-positive patients who completed neoadjuvant treatment, pCR was noted in 36.4% in the trastuzumab group and 9% in the docetaxel/carboplatin group [71].

Among these seven trials, five were randomized phase II trials [66–69], and only two did not enrol patients with MBC [69, 71]. Therefore, the interpretation of their results is difficult and does not allow the conclusion that HER-2 is involved in taxane resistance in these patients.

Table 5. Randomized phase II and III trials with trastuzumab plus taxanes in HER-2-overexpressing metastatic breast cancer patients

<table>
<thead>
<tr>
<th>Study phase</th>
<th>Patients</th>
<th>Treatment arm</th>
<th>ORR (%)</th>
<th>TTP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>96</td>
<td>P</td>
<td>17</td>
<td>3.0 months</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td>92</td>
<td>P + H</td>
<td>42</td>
<td>6.9 months</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>186</td>
<td>D</td>
<td>34</td>
<td>6.1 months</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D + H</td>
<td>61</td>
<td>11.7 months</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>124</td>
<td>Weekly P</td>
<td>47.5</td>
<td>272 days</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weekly P + H</td>
<td>84.5</td>
<td>369 days</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>98</td>
<td>P + carbo q3w + w H</td>
<td>57</td>
<td>13.8 months</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>P + carbo + H q3w</td>
<td>36</td>
<td>7.6 months</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>43</td>
<td>w P + carbo + H</td>
<td>65</td>
<td>9.9 months</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>w P + carbo + H</td>
<td>81</td>
<td>13.8 months</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Carbo, carboplatin; D, docetaxel; H, herceptin; HD, herceptin + docetaxel; HP, herceptin + paclitaxel; NR, not reported; ORR, overall response rate; P, paclitaxel; P +, paclitaxel; P + carbo, paclitaxel + carboplatin; TTP, time to progression; q3w, every 3 weeks; w, weekly.

Table 6. Clinical trials using ECD to evaluate taxane resistance in metastatic breast cancer

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Patient number</th>
<th>Trial phase</th>
<th>Disease stage</th>
<th>ORR in HER-2 ECD+ (%)</th>
<th>ORR in HER-2 ECD- (%)</th>
<th>P value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>54</td>
<td>III</td>
<td>IV</td>
<td>29.4</td>
<td>41.9</td>
<td>NR</td>
<td>[72]</td>
</tr>
<tr>
<td>EP</td>
<td>47</td>
<td></td>
<td></td>
<td>50</td>
<td>46.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP</td>
<td>58</td>
<td>II</td>
<td>IV</td>
<td>62</td>
<td>78</td>
<td>0.021</td>
<td>[74]</td>
</tr>
<tr>
<td>GP</td>
<td>42</td>
<td>II</td>
<td>IV</td>
<td>42</td>
<td>83</td>
<td>0.02</td>
<td>[75]</td>
</tr>
<tr>
<td>Paclitaxel-based</td>
<td>280</td>
<td>III</td>
<td>IV</td>
<td>40.9</td>
<td>38.5</td>
<td>0.4</td>
<td>[79]</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>35</td>
<td>II</td>
<td>IV</td>
<td>42</td>
<td>83</td>
<td>0.02</td>
<td>[75]</td>
</tr>
<tr>
<td>D+AC</td>
<td>17</td>
<td>II</td>
<td>IV</td>
<td>42</td>
<td>83</td>
<td>0.02</td>
<td>[75]</td>
</tr>
<tr>
<td>AC+D</td>
<td>16</td>
<td></td>
<td></td>
<td>42</td>
<td>83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P ± T</td>
<td>275</td>
<td>II</td>
<td>IV</td>
<td>59</td>
<td>50</td>
<td>0.35</td>
<td>[81]</td>
</tr>
<tr>
<td>P + T</td>
<td>30</td>
<td>II</td>
<td>IV</td>
<td>76</td>
<td>33</td>
<td>0.04</td>
<td>[82]</td>
</tr>
</tbody>
</table>

Abbreviations: AC, doxorubicin + cyclophosphamide; D, docetaxel; EC, epirubicin + cyclophosphamide; DP, doxorubicin + paclitaxel; EP, epirubicin + paclitaxel; GP, gemcitabine + paclitaxel; NR, not reported; ORR, overall response rate; P, paclitaxel; RT, radiotherapy; T, trastuzumab.
...a shorter response duration ($P < 0.005$) and shorter TTP ($P < 0.005$) in 38 MBCs treated with docetaxel and epirubicin as first line CT [76]. Conversely, a lack of correlation between HER-2 ECD and response to ET was reported in patients treated as first-line CT, although a longer progression-free interval was seen in patients with increased HER-2 ECD when treated with ET instead of EC ($P = 0.0341$) [72].

Aiming to analyse the correlation between HER-2 ECD and response to taxanes, data on 280 of 739 patients enrolled in the ECOG 1193 study were reviewed. One hundred and nine patients received single-agent paclitaxel, 107 patients received doxorubicin and 64 initially treated with doxorubicin crossed over to paclitaxel at progression. No association between HER-2 ECD positivity and objective response was found ($P = 0.51$) [77]. This clinical study was published recently; however, this specific analysis was not reported [78]. In patients treated with weekly dose-intensified paclitaxel (90 mg/m$^2$ for 6 weeks every 9 weeks), no difference in RR was found between patients with HER-2 ECD negative and positive (cut-off 15 ng/ml) [79]. HER-2 ECD concentration was tested before and after three cycles of docetaxel and no significant difference was observed regarding HER-2 ECD status in the docetaxel arm [80].

Paclitaxel given either weekly or every 3 weeks with or without trastuzumab resulted in similar RR between patients with high and low levels of HER-2 ECD. However, the proportional decline in HER-2 ECD in 34 responders was 61% compared with 38% in 15 non-responders ($P = 0.067$) [81]. ORR was higher in patients with elevated HER-2 ECD at baseline and treated with weekly paclitaxel and trastuzumab as first- or second-line therapy [82]. Additionally, in patients with an elevated HER-2 ECD at baseline, a reduction to $<15$ ng/ml at week 12 was associated with a greater likelihood of treatment response to weekly trastuzumab and paclitaxel ($P = 0.005$) [83].

In a combined analysis of four randomized trials in MBC and metastatic lung cancer involving 375 patients, Leyand-Jones et al. [84] did not find a clinical utility for HER-2 ECD using two different cut-offs (15 and 100 ng/ml).

For all these studies, different types of ELISA test were used: Oncogene in four studies [72, 75, 76, 79], Calbiochem in one [74], Bayer Diagnostics in two [82, 83], and Bender Medsystems in one [80]. Only three studies did not use a cut-off of 15 ng/ml; one used 30 ng/ml [75] and the other two 450 fmol/ml [74] or serum 9ErB2 concentrations level [80]. All studies analysed MBC patients and two of them were phase III clinical trials [72, 77].

discussion

CT with docetaxel or paclitaxel alone or in combination plays an important role in BC treatment (metastatic, adjuvant or neoadjuvant settings) and, currently, there are no clinically useful predictive markers to identify patients who are likely to respond to taxanes. In a considerable proportion of BC patients, treatment with taxanes does not result in response, but can induce significant side-effects including neuropathy. The ability to identify predictors of response or resistance to these agents should allow for improved tailoring of treatment to the individual patient.

HER-2-positive BC tumors have been associated with poor prognosis and poor response to systemic treatments (CT and/or endocrine therapy). Agents that may present drug resistance in HER-2-overexpressing tumors are taxanes, cisplatin [17], 5-fluorouracil [85] and tamoxifen [86–89]. Different mechanisms are possibly involved in taxane resistance such as apoptosis inhibition or activation of the tyrosine kinase pathway, as shown in various in vitro studies. Understanding these mechanisms may help to overcome resistance and better target available therapies.

The ‘2000 update of tumor markers guidelines’ released by the American Society of Clinical Oncology does not recommend the use of HER-2 data to determine the prescription of taxane-based CT in either the adjuvant or metastatic setting due to the scant and contradictory results [90]. The American Society of Clinical Oncology Technology Assessment also does not recommend, outside a clinical trial setting, the use of CT sensitivity and resistance assays to select chemotherapeutic agents for individual patients. Oncologists are hence forced to take CT-related decisions based on published reports of clinical trials, patient’s health status and treatment preferences. Therefore, participation in clinical trials evaluating these technologies remains a priority [91].

Preclinical and clinical studies have provided contradictory results regarding the potential predictive value of HER-2 for response to taxanes, suggesting that it may correlate with either resistance or sensitivity to these agents. This is, in part, due to the great heterogeneity in patient selection, treatments, methods and scoring systems used to define HER-2 positivity. In terms of study design and patient selection, most of the studies are retrospective analyses of archival tissue, and only a few are randomized clinical trials; there is a lack of distinction between potential prognostic versus predictive roles of HER-2; included patients differ in TNM stages and may have received one of many types of therapy. Furthermore, not all patients enrolled in the initial trial were included in the translational research study, potentially resulting in bias. In terms of methods of detection, there were different assays, different reagents between assays, different positive/negative cut-off levels, tissue versus circulating HER-2 and different specimen preparations.

The predictive value of HER-2 in single-agent taxane studies is controversial, although three studies showed higher ORR to taxanes in patients with HER-2-positive MBC [1, 35, 37]. When taxanes were used in combination with other chemotherapeutic drugs in MBC, ORR was, most of the time, higher in patients with HER-2-overexpressing tumors than in non-overexpressing ones. Unfortunately, the mechanisms for this better response have not been properly elucidated and many questions remain unanswered.

Trastuzumab may sensitize HER-2-overexpressing BC cells to taxanes resulting in an additive and/or synergistic interaction. Whether this response is caused by trastuzumab itself or by the HER-2 receptor alteration is still uncertain and further research is needed. The HER-2 ECD has been evaluated in MBC patients and the reduction of its levels during treatment seems to be related to a higher ORR, although only three studies generated statistically significant results [74, 75, 82]. Therefore, HER-2 status and serum ECD are not yet ready to be used routinely as predictive markers for resistance to taxane-based CT.

More recently, gene expression profiling techniques have been used for the development of a prediction model for response to docetaxel and paclitaxel. Chang et al. [92] have reported 92 genes that correlated with docetaxel response ($P = 0.001$) using microarray technology. Sensitive tumors had higher expression of genes involved in cell cycle, cytoskeleton, adhesion, protein transport, protein modification, transcription and stress or apoptosis; whereas resistant tumors showed...
increased expression of some transcriptional and signal transduction genes. However, this study was not designed to discover specific genes for docetaxel response or resistance, but rather to identify patterns of many genes that could be used as a predictive test in patients with BC. Due to difficulties in performing microarray studies, this same group studied gene expression profiling using adapter-tagged competitive transcriptase–polymerase chain reaction (ATAC-PCR) in 44 docetaxel-treated patients. Diagnostic profiles in non-responders were characterized by elevated expression of genes controlling the cellular redox environment (i.e. redox genes, such as thioredoxin, glutathione-S-transferase and peroxiredoxin). Overexpression of these genes protected cultured mammary tumor cells from docetaxel-induced cell death, suggesting that enhancement of the redox system plays a major role in docetaxel resistance [93].

Ayers et al. [94] have examined the feasibility of developing a multigene predictor of pCR to sequential weekly paclitaxel and FAC (T/FAC) neoadjuvant CT for BC. pCR was achieved in 13 patients (31%) out of 42 patients: 24 patients were used in the training set and 18 patients in the validation set. The authors could not identify any single marker that was sufficiently associated with pCR to be used as an individual predictor. A multigene model with 74 markers (P ≤ 0.09) was built using data from the training samples and tested on the validation samples. Overall, a 78% predictive accuracy was observed, with a 100% positive predictive value for pCR, a 73% negative predictive value, a sensitivity of 43% and a specificity of 100%. Recently, this study has increased the number of patients to 133, with a pCR rate of 26% (34 patients). A nominally best 30-probe set Diagonal Linear Discriminant Analysis classifier was selected for independent validation. A significantly higher sensitivity (92%) compared with 61% was observed, with a 100% positive predictive value for pCR, a 73% negative predictive value, a specificity of 100% and a sensitivity of 43% and a specificity of 100%. The weak described above and based on the high-throughput technologies suggests that resistance/sensitivity to taxanes will be better predicted by multigene signatures rather than by a single molecular discrimination.

In conclusion, despite more than a decade of research, the predictive value of HER-2 regarding taxane-based CT is not yet validated and hence not ready to use in current clinical practice. This is, unfortunately, the same situation for all other predictive markers of response to CT. To obtain the desired level 1 evidence, a well-conducted patient-based meta-analysis and/or prospective biologically based trials are needed. Some of these trials are ongoing, such as the BIG-EORTC-p53 trial, and these results are eagerly awaited.

acknowledgements

E. Azambuja is a PhD student in the Programa de Pós-graduação em Medicina: Ciências Médicas, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. Dr D. D. Rose has received financial support from CAPES, Brazil.

Conflict of interest

F.C. and M.L.P. have declared conflict of interest.

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