Markers predicting clinical benefit in breast cancer from microtubule-targeting agents

L. Pusztai*

Department of Breast Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, USA

Taxanes (e.g. paclitaxel, docetaxel) and epothilones (e.g. ixabepilone) are microtubule-targeting agents, which disrupt cellular processes and induce apoptosis. Although their mechanisms of action are similar, clinical data in breast cancer patients support at least partial non-cross resistance between the classes, and even between individual compounds. Several biomarkers might contribute to the identification of patient groups likely to derive benefit from one class of microtubule-targeting agent or even one agent. Overexpression of P-glycoprotein is associated with resistance to taxanes, but not ixabepilone, in vitro; its role in vivo remains unclear. Mutations in β-tubulin linked to resistance to taxanes but not epothilones are observed in vitro; somatic mutations of β-tubulin appear rare clinically. Overexpression of the βIII-tubulin isoform is associated with taxane resistance in cell lines; some clinical studies support a relationship between poor response to taxanes and overexpression of βIII-tubulin. βII-tubulin overexpression seems not to affect sensitivity to ixabepilone [1]. Estrogen receptor negativity, low expression of microtubule-associated protein tau, and perhaps HER2 amplification may define a subset of patients with higher than average sensitivity to paclitaxel. Large scale pharmacogenomic analysis has identified molecular markers potentially capable of distinguishing patients with differential sensitivity to paclitaxel and ixabepilone. These markers require validation in clinical trials.

Key words: breast cancer, epothilone, ixabepilone, taxane, microtubule-targeting agents, tubulin

Microtubules, filaments formed by the polymerisation of heterodimeric α/β-tubulin subunits, play a fundamental role in diverse cellular functions including mitotic cell division and endosomal transport. Agents that disrupt the processes of microtubule polymerisation and depolymerisation cause the cell cycle to arrest, resulting in the induction of apoptosis. The taxanes (e.g. paclitaxel, docetaxel) and vinca alkaloids (e.g. vinorelbine) are two families of chemotherapeutic agents that exert their activity via interaction with tubulin subunits. The taxanes are widely used in the treatment of breast cancer and have shown important clinical activity as adjuvant chemotherapy [2, 3] and in the treatment of metastatic disease [4]. However, primary and secondary (acquired) resistance is a problem in taxane therapy [5]. The utility and limitations of taxanes in breast cancer have stimulated searches for other types of cytotoxic agent that might target microtubules. The epothilones have emerged from this search as a new class of microtubule-targeting agent. Epothilones are macrocyclic, originally derived from the myxobacterium Sorangium cellosum. Several epothilone analogues are now in clinical development, with ixabepilone being the most advanced [5].

Like the taxanes, the epothilones induce microtubule bundling, formation of multipolar spindles and mitotic arrest [6]. Epothilones compete with paclitaxel for binding to microtubules and suppress microtubule dynamics in a manner similar to paclitaxel. However, they have a non-common pharmacophore for microtubule binding [7] and recent evidence indicates their binding, although overlapping, is not identical to those of the taxanes [5]. Although the taxanes and epothilones have a similar mechanism of action, there are clinical data, largely from Phase II studies, indicating there is at least partial non-cross resistance between these classes of microtubule-targeting agent. For example, in 29 women previously treated with anthracycline and taxane, epothilone D (KOS-862) produced a 14% response rate [8]. A large body of evidence indicative of non-cross resistance comes from studies of ixabepilone, which has shown activity not only in patients with anthracycline pretreated metastatic breast cancer (n = 65, response rate 42%, 17% of patients also pretreated with taxanes) [9] and taxane-naïve breast cancer (n = 23, response rate 57%) [10], but also in patients with metastatic disease who progressed during or within 4 months of taxane therapy in the metastatic setting or experienced recurrence within 6 months of adjuvant taxane therapy (n = 49, response rate 12%) [11]. Ixabepilone has also shown efficacy in an even more challenging 'triple-refractory' patient population who were resistant to anthracyclines, taxanes and capcitabine (n = 113, response rate (investigator) 19%) [12]. There is also evidence of differential sensitivity within the taxanes in
46 patients with paclitaxel-resistant disease, docetaxel therapy was associated with a response rate of 18% [13]. This evidence that different microtubule-targeting agents, although operating via essentially the same mechanism, can have differing clinical effects raises an important question: how can we know which type of microtubule-targeting agent, or even which individual agent, will be the most effective in a particular patient? Novel biomarkers might contribute to the identification of patient groups likely to derive significant clinical benefit from specific microtubule-targeting agents (Figure 1). Recent studies suggest several potential biomarkers of sensitivity to microtubule-targeting agents, and, perhaps more significantly, markers that may predict differential sensitivity to taxanes and epothilones (Table 1).

**P-glycoprotein-mediated drug resistance**

Efflux of drugs via the activity of the ATP-binding cassette protein P-glycoprotein has long been recognised as a mechanism of multidrug resistance in tumour cell lines [14]. P-glycoprotein, the product of the MDR1 gene, is a pump that, in normal tissues such as the gastrointestinal tract and brain, prevents accumulation of toxic substances [15]. Overexpression of P-glycoprotein is thought to be one of the most common mechanisms underlying resistance to taxanes in cancer models [16, 17]. In contrast, ixabepilone is a poor substrate for drug-transporting ATP-binding cassette proteins and has shown cytotoxicity in cell lines overexpressing P-glycoprotein [18, 19]. This makes overexpression of P-glycoprotein by tumour cells a potential marker of resistance to taxanes but not to ixabepilone.

Although there is substantial evidence from in vitro systems that P-glycoprotein mediates resistance to taxanes, demonstrating its role in vivo has been problematic. Accurate quantification of a marker is essential to evaluating its prognostic or predictive utility. This has not yet been achieved for P-glycoprotein, owing to a lack of universally accepted guidelines for analytical or clinical validation, differences in methods of tissue collection and preparation, different molecular assay targets (mRNA or protein), and low sensitivity and specificity of currently used immunohistochemistry methods [14, 15]. A meta-analysis of 31 breast cancer trials found that P-glycoprotein was expressed in 41% of tumours and that treatment with chemotherapy agents was associated with an increase in the proportion of tumours expressing P-glycoprotein, suggesting that chemotherapy induces expression of P-glycoprotein. Tumour expression of P-glycoprotein after treatment was associated with a three-fold reduction in response to chemotherapy [20]. There was, however, a wide range in P-glycoprotein expression rates in the individual studies, and the validity of these findings remains controversial [14]. A study investigating the relationship between P-glycoprotein expression and response to docetaxel in breast cancers found no significant correlation [21].

If P-glycoprotein mediates resistance to certain agents in vivo, inhibitors of its activity may be able to restore sensitivity. Translating this idea into clinical practice has been difficult, as a result of low efficacy of inhibition among candidate inhibitors, toxicity, pharmacokinetic interactions, and inadequate clinical trial design [14]. Newer agents, such as the third-generation inhibitor tariquidar, may overcome such limitations, but results to date have been inconclusive [14, 15]. In an interventional study, tariquidar showed limited ability to restore sensitivity to chemotherapy in 17 women with stage III-IV breast cancer who progressed or had stable disease on anthracycline or taxane therapy, and the trial was terminated due to lack of efficacy [22]. Of the 17 patients, five showed an increase of at least 10% in uptake of 99Tc-sestamibi, indicating that P-glycoprotein activity was being inhibited. The patient with the greatest increase in sestamibi uptake, who also showed inducible P-glycoprotein expression, did demonstrate a partial response to therapy. Careful selection of patients who could benefit from P-glycoprotein-inhibiting therapy will be needed to confirm the efficacy of P-glycoprotein inhibition in large-scale trials. Research is also required into whether certain drugs are not susceptible to P-glycoprotein mediated resistance. It may be, for example, that patients who overexpress P-glycoprotein are resistant to taxanes but respond to ixabepilone, but this has not yet been investigated in clinical studies.

**Table 1.** Cellular mechanisms of resistance to microtubule binding agents

<table>
<thead>
<tr>
<th>Molecular Mechanism</th>
<th>Implicated in paclitaxel resistance</th>
<th>Implicated in ixabepilone resistance</th>
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<tr>
<td>P-glycoprotein [17–19]</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Tubulin mutations and isoforms [24, 28, 1]</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Impaired apoptotic response (bcl-2, bax) [49, 50]</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Alterations in microtubule-binding proteins (stathmin, tau) [39, 42, 51]</td>
<td>yes</td>
<td>yes</td>
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More than one mechanism may be active in any particular cell and host characteristics such as pharmacokinetics can also influence drug response.
**β-tubulin mutations**

Alterations in the structure of β-tubulin have the potential to modify interactions with microtubule-targeting agents. Mutation in β-tubulin has been identified as a mechanism of resistance to paclitaxel in vitro. For example, resistance to paclitaxel in an ovarian cancer cell line has been linked to its expression of a modified β-tubulin containing an alanine-to-threonine mutation at residue 364. Interestingly, epothilone cytotoxicity is unaffected by this mutation [23, 24].

Again, results in vitro have not been replicated in vivo. Early findings linking mutations in β-tubulin with paclitaxel resistance in non-small cell lung cancer were misleading, owing to amplification of pseudogenes, and have since been contradicted [21]. Likewise, mutational analysis of class I β-tubulin in human breast cancers has indicated that somatic mutation of this gene is rare, occurring at a rate of only 1 in 62 (1.6%) [25]. The contribution of mutations in β-tubulin to clinical resistance to taxanes and their use as a prognostic marker remains unknown.

**Variation in isotypes of β-tubulin**

Eight different isoforms of β-tubulin have been identified [26]; of these, the βIII and βIV isoforms appear to have reduced stability, which could potentially counteract the stabilising effect of taxanes [21]. In normal and tumour breast tissue, class II and I/IV isoforms are the most abundant [27].

Overexpression of the βIII-tubulin isoform has been associated with resistance to paclitaxel in a number of human cancer cell lines [28]. The emergence of resistance to paclitaxel in breast cancer cell lines correlates with an increase in the abundance of the βIII isoform [29]. Preliminary data indicate that an increase in βIII-tubulin expression does not affect sensitivity to ixabepilone in a breast cancer cell line derived from a patient with primary resistance to paclitaxel [1]. This cell line has an abnormal β-tubulin isotype composition combining loss of the βII isoform and overexpression of the βIII isoform. Evaluation of efficacy in a mouse model demonstrated that whereas paclitaxel, docetaxel and vinorelbine (a vinca alkaloid) tested at their maximum tolerated doses produced log cell kill values of only 0.3, 0.2 and 0.1, respectively, ixabepilone produced 1.6 log cell kill at its maximum tolerated dose [1].

Although some clinical studies have failed to link response to taxanes with levels of βIII-tubulin expression [26], others have provided positive results. A relationship between overexpression of βIII-tubulin and resistance to paclitaxel has been demonstrated in a study of 70 patients with metastatic breast cancer treated with paclitaxel-based combination chemotherapy [30]. Expression of βIII-tubulin was predictive of no response to chemotherapy or rapid progression after chemotherapy, with only 2% of those with low βIII-tubulin expression progressing after chemotherapy in comparison with 38% of those with high βIII-tubulin expression. Similarly, in a series of 92 patients with advanced breast cancer treated with first-line paclitaxel-based chemotherapy, a significantly higher proportion of those with high βIII-tubulin expression showed no response and progression of disease (35%) compared to those with low expression of βIII-tubulin (7%, P <0.002) [29]. In addition, high βIII-tubulin expression is associated with poor response to docetaxel in breast cancer [21]. If standardised scoring methods for assessing expression can be derived, βIII-tubulin expression might be of use in the selection of patients for taxane therapy, and can also be investigated in clinical trials of ixabepilone.

**HER2 overexpression as a marker of paclitaxel sensitivity**

Although HER2 overexpression or forced expression of HER2 in xenografts does not affect sensitivity to paclitaxel [31], retrospective analysis of the CALGB9344 clinical trial, in which four cycles of doxorubicin-cyclophosphamide (AC) followed by four cycles of paclitaxel improved disease-free and overall survival in comparison with four cycles of AC alone, has suggested that HER2 amplification may be a marker of benefit from paclitaxel [32]. Patients with HER2-negative, estrogen receptor (ER)-positive tumours derived no significant benefit from paclitaxel therapy, whereas patients with HER2-positive disease, whether ER-positive or ER-negative, showed significant improvements in 5-year disease-free survival in comparison with patients not given the taxane. Patients with HER2-negative, ER-negative disease derived a lesser degree of benefit than those with HER2 amplification. Even if HER2 itself is not mechanistically involved in determining response to chemotherapy it may be a marker of a disease phenotype that is sensitive to taxanes and anthracyclines. Indeed, HER2 amplification is associated with co-amplification of topoisomerase IIα and lower expression of microtubule-associated protein tau that could mechanistically explain increased sensitivity to chemotherapy [33].

**Gene expression profiling to identify biomarkers**

The potential biomarkers discussed above have been investigated for mechanistic reasons. An alternative approach is to explore the expression of a large number of genes in tumour tissue, without a prior mechanistic hypothesis as to why these genes might be significant. The basis of such an approach is that powerful, previously unknown markers may be discovered and that combinations of genes may predict response to therapy more reliably than expression of any single gene [34].

Gene expression profiling has been used in a study of preoperative ixabepilone therapy in a group of 161 patients with stage II-III breast cancer, 18% of whom showed complete pathological response in the breast while 82% had residual cancer [35, 36]. Pretreatment core needle biopsies were used to identify genes expressed differentially in the responsive patient population. Genes encoding the ER and microtubule-associated protein tau had the greatest predictive value in this analysis (Table 2).

In another study, a multigene predictor of pathological complete response to preoperative paclitaxel-containing...
chemotherapy has been developed using pretreatment biopsies from 82 patients with stage I-III breast cancer who received preoperative weekly paclitaxel and fluorouracil-doxorubicin-cyclophosphamide therapy (T/FAC) [37]. The aim was to identify genes whose expression is associated with extreme sensitivity to this combination regimen. Pathological complete response was seen in 26% of the patients. Of 56 differentially expressed probes identified (at a false discovery rate of 1%), a set of the best 30 predicted complete pathological response to T/FAC therapy with high sensitivity (92%) and negative predictive value (96%). The most significant differentially expressed gene was that encoding microtubule-associated protein tau. Low tau expression was significantly more common among patients experiencing complete pathological response than among those with residual disease. This finding suggests tau expression might be a marker for sensitivity to paclitaxel-containing chemotherapy.

**Table 2.** Performance characteristics of single gene markers of complete pathological response to ixabepilone neoadjuvant chemotherapy in breast cancer [35]. Point estimates and 95% confidence intervals are shown.

<table>
<thead>
<tr>
<th></th>
<th>PPV</th>
<th>NPV</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Error</th>
</tr>
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<tbody>
<tr>
<td>ER IHC</td>
<td>0.29 (0.20–0.41)</td>
<td>0.90 (0.81–0.95)</td>
<td>0.72 (0.54–0.85)</td>
<td>0.59 (0.50–0.67)</td>
<td>0.345</td>
</tr>
<tr>
<td>ER mRNA</td>
<td>0.37 (0.19–0.59)</td>
<td>0.92 (0.80–0.97)</td>
<td>0.64 (0.35–0.85)</td>
<td>0.79 (0.66–0.87)</td>
<td>0.289</td>
</tr>
<tr>
<td>Tau mRNA</td>
<td>0.29 (0.14–0.50)</td>
<td>0.89 (0.77–0.95)</td>
<td>0.55 (0.28–0.79)</td>
<td>0.73 (0.60–0.83)</td>
<td>0.361</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; IHC, immunohistochemistry

**Figure 2.** Expression of microtubule binding protein Tau mRNA in cell lines with variable sensitivity to paclitaxel. The IC₅₀ concentration of paclitaxel was determined for 23 breast cancer cell lines. Cells with IC₅₀ less than the mean of the log IC₅₀ were defined as sensitive (light) and those above the mean as resistant (dark). Tau mRNA expression detected by Affymetrix U133A gene chips is shown for each cell line as bar graphs [38]. Reprinted from Wagner P. et al. Cell Cycle 2005.

**microtubule-associated protein tau as a marker of taxane sensitivity**

Several lines of evidence indicate that low tau mRNA expression correlates with sensitivity to paclitaxel *in vitro* (Figure 2). Tau expression examined in 23 different cell lines correlates with IC₅₀ for paclitaxel [38]. Down-regulation of tau expression in breast cancer cell lines (ZR75.1 and MCF7) increases sensitivity to paclitaxel [39]. Tau promotes microtubule assembly and stabilises microtubules, and it is possible that tau competes with taxanes for microtubule binding. Binding of fluorescent paclitaxel to tubulin is reduced by pretreatment with tau, and paclitaxel-induced tubulin polymerisation is also reduced by preincubation with tau in a concentration-dependent fashion [39].

Reduced tau protein expression also predicted response to single agent paclitaxel therapy in gastric cancer patients [40] and an inverse correlation between tau expression and sensitivity to a novel benzoylphenylurea sulfur analogue (SG410) that destabilises microtubules was reported in pancreatic cancer models as well [41].

Tau expression is positively correlated with ER expression in breast cancer [42]. The gene encoding tau contains an imperfect ER response element upstream of its promoter and tau is an estrogen-induced protein in several cell lines [43, 44]. Given that tau may be regulated by estrogen, is tau predictive of endocrine sensitivity? Comparison of tau expression data from ER-positive patients in the T/FAC study referred to above [37] with data from two different sets of ER-positive patients, one of whom received no systemic therapy [45] and the adjuvant tamoxifen only [46], indicates that low tau is predictive of resistance to endocrine therapy in this patient group: ER-positive patients with low tau expression showed higher rates of recurrence after five years of tamoxifen therapy than those with high tau expression [42].

Current knowledge of the role of tau in breast cancer therapy suggests that: tau and paclitaxel bind to the same pocket on the inner surface of microtubules [39, 47]; low tau expression renders microtubules more accessible and therefore more vulnerable to paclitaxel; and, in ER-positive patients, high tau expression is an indicator of endocrine activity and increased benefit from adjuvant tamoxifen [42]. The evidence suggests that tau may be a marker of sensitivity to all microtubule stabilising drugs, rather than to a specific compound or class.

**markers of differential sensitivity to individual microtubule-targeting agents**

In an attempt to identify biomarkers predictive of differential sensitivity to paclitaxel and ixabepilone, pharmacogenomic data from the two analyses described above have been combined [35–37, 48]. It is important to remember that there are several differences between these studies that need to be considered when interpreting the results: ixabepilone was given as four cycles of monotherapy whereas the other group of patients received six months of combination chemotherapy; core needle biopsy was used in the ixabepilone study whereas fine needle aspiration was employed in the T/FAC study. As expected with a single agent, there was a lower rate of complete
pathological response in the ixabepilone study. In addition, since these were two independent studies, patients were not randomised to ixabepilone versus paclitaxel, such that incidental differences in the patient populations included in the trials might have led to the identification of different markers.

From the combined pharmacogenomic analysis it was possible to identify individual molecules and combinations of molecules that were predictive of response to ixabepilone therapy but not of response to the paclitaxel-based chemotherapy regimen [48]. A study is planned to validate these findings. This study will involve 300 patients with ER-negative/HER2-negative disease, who, after receiving four cycles of intravenous doxorubicin and cyclophosphamide at 3-weekly intervals, will be assigned at random to receive either ixabepilone (four cycles at 3-weekly intervals) or paclitaxel (weekly for 12 weeks). Complete pathological response will be assessed after surgery to enable estimation of complete pathological response rates associated with the subsets of biomarkers identified as potentially predictive in the combined pharmacogenomic analysis.

conclusions

Different microtubule-targeting agents have been shown to have only partially overlapping resistance mechanisms in the clinical setting: docetaxel, ixabepilone and KOS-862 have all shown activity in paclitaxel-refractory or pre-treated cases. Various biomarkers associated with differential sensitivity to different microtubule-targeting agents have been identified in vitro, and there is evidence that some of these may have clinical utility. ER-negative status, low tau expression and perhaps HER2 amplification define a subset of patients with breast cancer who have higher than average sensitivity to paclitaxel. Pharmacogenomic analysis has identified sets of molecular markers that may distinguish sensitivity to paclitaxel and ixabepilone: these markers require validation in prospective clinical trials.

disclosures

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references


