Ex vivo reversal of chemoresistance by tariquidar (XR9576)

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The expression of P-glycoprotein (P-gp) has been demonstrated to confer resistance to several anticancer drugs, including anthracyclines, taxanes and vinca alkaloids. Tariquidar is a novel inhibitor of P-gp that has been shown to reverse resistance to cytotoxic drugs in tumor cell lines and mouse xenografts. We have used an ATP-based chemosensitivity assay (ATP-TCA) to compare the activity of cytotoxic drugs in combination with tariquidar against a variety of solid tumors ($n=37$). The expression of P-gp was determined in a subset of solid tumor samples by immunohistochemistry ($n=16$). Resistance was seen in 20 of 37 (54%) tumors tested with doxorubicin, in 27 of 34 (79%) samples tested with paclitaxel and 17 of 31 (55%) with vinorelbine. Tariquidar alone showed no activity over a wide range of concentrations up to 2 μM ($n=14$). The median IC_{90}s for doxorubicin, paclitaxel and vinorelbine, alone were 2.57, 27.4 and 15.5 μM. These decreased to 1.67 ($p<0.0005$), 20.6 ($p<0.05$) and 9.5 μM ($p<0.001$), respectively, in combination with tariquidar. Tariquidar also significantly decreased resistance in 14 of 20 (70%), six of 27 (22%) and six of 17 (35%) samples tested with doxorubicin, paclitaxel and vinorelbine, respectively. Immunohistochemical staining for P-gp was positive in nine of 16 (56%) samples and in all of these cases addition of tariquidar improved the activity of the cytotoxic. The results show that tariquidar is able to decrease resistance in a number of solid tumors resistant to cytotoxic drugs known to be P-gp substrates. These data support the introduction of tariquidar in combination with chemotherapy to clinical trials of patients expressing P-gp. *Anti-Cancer Drugs* 15:861–869 © 2004 Lippincott Williams & Wilkins.

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Introduction

The success of chemotherapy is often severely compromised by the development of resistance. Some tumors display resistance not only to the original agents used in treatment, but also to agents to which they have not previously been exposed, many of which are dissimilar in structure or mode of action. This phenomenon is known as classical multidrug resistance (MDR). MDR is strongly associated with overexpression of ATP-dependent pump molecules such as P-glycoprotein (P-gp). Many different chemotherapeutic agents have been shown to be susceptible to P-gp, including taxanes, vinca alkaloids and anthracyclines [1].

P-gp, the product of the multidrug resistance gene (MDR1), is a transmembrane efflux pump responsible for detoxifying normal cells as well as rendering tumor cells resistant to chemotherapy. Its role as a cellular efflux pump controlling intracellular concentrations of harmful substances is reflected by its cell- and organ-specific distribution in kidney, bile canaliculi, gut epithelium and capillary endothelium, as well as its ability to recognize and transport a wide range of compounds. In human tumors, P-gp expression is most commonly detected in colon, hepatic, renal and adrenal carcinomas. It has also been observed in human hematological malignancies, breast, ovarian, lung and gastric carcinomas, skin cancers, and certain germ cell tumors and sarcomas [1,2].

Several studies have shown an inverse correlation between P-gp expression and chemosensitivity or survival in a variety of tumor types, including leukemia [3–5], lymphomas [6], osteogenic sarcoma [7,8], small cell lung cancer [9,10], breast cancer [11–13], advanced ovarian cancer [14,15] and pediatric solid tumors [16–18]. However, it should be noted that associations with chemoresistance have also been reported for other transmembrane efflux pump molecules, including MRP, LRP/MVP and BCRP [19–21]. There is considerable controversy around which of these molecules are of greatest clinical importance to chemosensitivity or resistance, but reversal of such resistance could be of

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considerable benefit, and inhibitors of P-gp have therefore been developed.

A broad range of compounds that interact with P-gp and block drug efflux have been reported to reverse the MDR phenotype. The first generation modulators consisted of calcium channel blockers, calmodulin inhibitors, hormonal/steroidal derivatives, antibiotics, cardiovascular drugs and the cyclosporins [22]. These compounds were developed for pharmacological uses other than the reversal of MDR, and were relatively non-specific and weak inhibitors. The requirement for more selective and potent agents as resistance modifiers has led to the development of several 'second-generation' modulators such as the non-immunosuppressive cyclosporin D analog, PSC 833 (valdospar) and VX-710 (biricodar); however, these compounds have shown significant enhancement of pharmacokinetics and toxicity of various cytotoxics [23]. This effect has required the reduction of the cytotoxic drug dose when administered with these modulators. Further studies led to the development of third-generation molecules such as XR9576 (tariquidar; Fig. 1), LY335979 (zosuquidar), R101933 (laniquidar) and ONT-093 [24]. These agents have high potency and specificity for P-gp.

Tariquidar is a highly potent anthranilic acid derivative that has been demonstrated to reverse P-gp dependent multidrug resistance in tumor cell lines and animal models [25,26]. In healthy volunteers administration of tariquidar demonstrated P-gp inhibition in $CD56^+$ lymphocytes [27]. Tariquidar selectively binds to P-gp with high affinity and its long duration of action helps in the restoration of sensitivity of MDR^+ tumors to a range of chemotherapeutic agents. A series of phase IIa trials have been carried out to study the pharmacokinetic behavior of tariquidar when given with certain cytotoxic agents known to be affected by P-gp, i.e. vinorelbine, doxorubicin and paclitaxel. The results of these three studies demonstrated that tariquidar is a potent P-gp inhibitor, without significant side-effects and with less pharmacokinetic interaction than other inhibitors used previously [28]. MDR1 mechanisms may reduce the toxicity of some drugs such as anthracyclines and their inhibition may therefore change the side-effect profile of some drugs [23].

The ATP-Tumor Chemosensitivity Assay (ATP-TCA) is capable of determining drug sensitivity and drug resistance in individual tumor samples by measuring cell viability. This assay has a high evaluability rate (93% in ovarian cancer) [29] and measures the effect of multiple drugs or drug combinations at different concentrations.

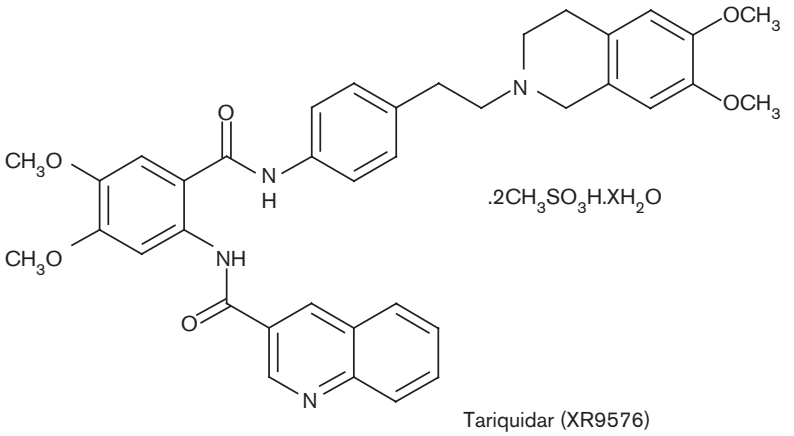
The aim of this study was to determine the ability of tariquidar to alter the chemosensitivity of various solid tumors to commonly used chemotherapeutic agents. We chose to study doxorubicin, paclitaxel and vinorelbine as examples of anthracyclines, taxanes and vinca alkaloids.

Materials and methods

Tissue samples

Of the 37 solid tumor samples studied, 21 were ovarian carcinomas, five were unknown primaries, five were skin melanomas, two were breast carcinomas, two were esophageal, one colon and one lung (non-small cell lung cancer) carcinomas. Patients consisted of 32 females and five males, having a median age of 58 (range 36–76). Several patients received one or more chemotherapy regimens, while 10 of 37 had no previous treatment (Table 1). All tumor samples were removed as part of patient treatment, with consent for tissue donation and Local Research Ethics Committee approval for use of the

Fig. 1



Tariquidar (XR9576)

Chemical structure of tariquidar.

Table 1 Patient and sample characteristics

Tumor type	No. samples	Patient age (median)	Sex	Sample type	Previous treatment[a]
Ovarian carcinoma	21	58 (38–76)	21F	5 solid, 2 pleural fluid, 14 ascites	carboplatin ($n=13$), platinum + taxane ($n=10$), liposomal doxorubicin ($n=3$), treosulfan + gemcitabine ($n=2$), carboplatin + gemcitabine ($n=1$), mitoxantrone + paclitaxel ($n=1$), topotecan ($n=1$), etoposide ($n=1$)
Skin melanoma	5	48 (36–66)	3M:2F	5 solid	melphalan ($n=1$), cyclophosphamide ($n=1$)
Unknown primary	5	61 (45–68)	5F	3 solid, 2 pleural fluid	carboplatin ($n=1$)
Breast carcinoma	2	49 (39–59)	2F	1 solid, 1 pleural fluid	anastrozole ($n=1$)
Esophageal carcinoma	2	62 (52–72)	2M	2 solid	epirubicin + cisplatin + 5-fluorouracil ($n=1$)
Colon carcinoma	1	39	1F	solid	irinotecan ($n=1$)
Lung (non-small cell lung cancer)	1	58	1F	pleural fluid	cisplatin + vinorelbine ($n=1$)
Total	37	58 (36–76)	5M:32F	17 solid, 20 fluid	

[a]The numbers in brackets indicate the number of patients who received the previously listed treatment. In the ovarian carcinoma subset several patients received more than one chemotherapeutic treatment.

tissue surplus to diagnostic requirements for chemosensitivity testing and further molecular studies.

Drugs

Tariquidar was provided by Xenova. A stock solution of tariquidar (XR9576 mesylate salt) was prepared in DMSO at a concentration of 1 mg/ml and stored as aliquots at −20°C. For cell treatments the stock solution was further diluted in culture medium with the final DMSO concentration less than 0.1%. The other drugs used in the assay were obtained as vials for injection from the pharmacy at Queen Alexandra Hospital. Doxorubicin was stored in aliquots at −20°C, vinorelbine was stored in the refrigerator at 4°C, while paclitaxel was kept at room temperature. The 100% test drug concentrations (TDC) used were derived from pharmacokinetic data, adjusted for protein binding to approximate the concentration clinically achievable in the patient and to provide good discrimination between tumors. The TDC for drugs included in the study were as follows, doxorubicin 2.5 μM, vinorelbine 11 μM, paclitaxel 15.9 μM and tariquidar 1 μM. Combinations were made up by adding both drugs at their 200% TDC to the wells at the beginning of the assay: sequential studies were not performed. Not all drugs or combinations were tested in every case.

Chemosensitivity assay

The ATP-TCA was performed as previously described [30,31]. Samples were transported to the laboratory in transport medium consisting of Dulbecco's modified Eagle's medium (DMEM; Sigma, Poole, UK; cat. no. D5671). Briefly, the tumor samples were first subjected to gentle enzymatic dissociation by collagenase (0.75 mg/ml; Sigma; cat. no. C8051) to produce a single-cell suspension. The cells were then cultured in serum-free media (Complete Assay Medium; DCS Innovative Diagnostik Systeme, Hamburg, Germany) in 96-well polypropylene plates (Corning-Costar, High Wycombe, UK) with or without the test drugs at six dilutions, allowing four drugs to be tested with triplicate wells for each data point. Combinations were made by direct addition of the two drugs at the same concentration used for the single agents. Two controls were included in 12 wells each: a maximum inhibitor (MI) which killed all the cells present giving a zero ATP count and a no drug control (MO) consisting of medium alone. The cells were cultured for 6 days at 37°C with 5% CO_2. Following incubation, a commercial detergent-based extraction reagent was used to lyse the cells and inhibit the ATPases contained within the cells (DCS Innovative Diagnostik Systeme). ATP quantification took place by adding the luciferin–luciferase counting reagent to the cell lysate. The amount of light produced was measured in a microplate luminometer (MPLX; Berthold, Hamburg, Germany). The results are expressed as the percent inhibition achieved at each concentration tested, calculated as: $\%\ \text{inhibition} = 1 - [(\text{Test} - \text{MI})/(\text{MO} - \text{MI})] \times 100$.

Immunohistochemistry

MDR1 (P-glycoprotein) monoclonal antibody (NCL-JSB1) from Novacastra (supplied by Vector, Peterborough, UK) was visualized using the Vectastain Universal Alkaline Phosphatase kit (Vector). Sections were cut on to positively charged slides (Surgipath Europe, Peterborough, UK) and dried at 60°C for 40 min. Prior to staining sections were de-waxed via immersion in solvent and alcohol before being rinsed in water. Antigen-presenting sites blocked by formalin fixation [32] were revealed by high-temperature antigen retrieval (pressure cooking) using sodium citrate buffer, pH 6.0, for 2 min. After cooling in water sections were incubated in normal horse serum for 20 min to block non-specific staining. Endogenous avidin-binding activity was blocked by a further 20 min of incubation in avidin and 20 min with biotin. Following this samples were incubated overnight in the primary antibody in a humid incubation chamber at 4°C. Concentration of antibody was determined by titration on positive control tissue (renal proximal tubules). The

antibody was diluted in Tris-buffered saline (TBS), pH 7.6, to its optimum dilution. Positive and negative controls were included with each batch of staining. The negative control consisted of a duplicate of the test section with the primary antibody omitted.

The following day, slides were allowed to acclimatize to room temperature before rinsing in TBS. These were then incubated for 30 min with diluted biotinylated universal secondary solution, rinsed and incubated for a further 30 min with Vectastain ABC-AP reagent. In order to visualize the reaction, slides were incubated for 20 min in Vector Red Alkaline Phosphate Substrate Kit (plus levamisole to inhibit endogenous alkaline phosphatase activity). The slides were counterstained with Gill's Hematoxylin, dehydrated and cleared using the Leica XL slide staining machine. The sections were thus mounted in Vector Mount producing a permanently mounted section with an optimal refractive index to retain the color intensity of Vector Red substrate reaction product.

Immunohistochemistry could only be performed in a proportion of samples, as some cytological specimens did not provide sufficient material for staining before treatment began.

Data analysis

Luminometer readings were entered into an Excel spreadsheet (Microsoft Office 2000), which automatically calculated IC_{90} and IC_{50} values for each drug and combination. The IC_{90} was used to correlate drug efficacy with immunostaining. Values of $IC_{90} \geq 100\%$ TDC were considered to suggest resistance, while values $IC_{90} < 100\%$ TDC were interpreted to suggest probable clinical sensitivity to the agent tested. When the addition of tariquidar decreased the cytotoxic IC_{90} to $< 50\%$ TDC, this was interpreted as a complete reversal of resistance. Assessment of slides was done using the *H*-score. Staining intensity (none = 0 points; weak = 1 point; moderate = 2 points; strong = 3 points) and percentage of positive tumor cells were multiplied to achieve a score between 0 and 300. A *H*-score of 100 or more was regarded as positive. The calculated and descriptive data were entered into an Access 2000 database (Microsoft) and analyzed using a Wilcoxon two-tailed paired rank sum test, the Mann–Whitney *U*-test for unpaired data or linear regression as appropriate (Statsdirect: Statsdirect, Sale, UK).

Results

Each of the agents tested produced a wide range of tumor inhibition consistent with considerable heterogeneity of chemosensitivity, as previously published [33–35]. As expected, tariquidar alone, tested in a subset of 14 tumors, showed no activity over a wide range of concentrations up to and including 2 μM (Fig. 2).

Doxorubicin

The median doxorubicin IC_{90} and IC_{50} values were 2.57 and 1.61 μM, respectively. The IC_{90} of doxorubicin was greater than 100% TDC (2.5 μM) in 20 of 37 (54%) samples. For the ovarian cancer subset, resistance according to IC_{90} was seen in 12 of 21 (57%) samples (Fig. 3a).

Immunostaining for P-gp was positive in nine of 15 (60%) samples tested with doxorubicin. The median doxorubicin IC_{90} values for P-gp-negative and -positive samples were 2.44 and 4.40 μM, respectively, but this difference did not reach statistical significance (NS, Mann–Whitney *U*-test). It is notable that five of six P-gp-negative samples were obtained from chemotherapy naive patients, while eight of nine P-gp-positive samples were from patients who had received previous treatment. No correlation was observed by linear regression analysis between the IC_{90} of doxorubicin and P-gp, but there was a correlation between the IC_{50} and P-gp expression ($p < 0.005$, Fig. 4). It should also be noted that 57% (four of seven) of the P-gp-negative samples showed sensitivity, while only 22% (two of nine) of the P-gp-positive samples showed sensitivity to doxorubicin on the basis of IC_{90}.

The addition of tariquidar to doxorubicin decreased the median IC_{90} of doxorubicin from 2.57 to 1.67 μM ($p < 0.0001$) and the IC_{50} from 1.61 to 0.92 μM ($p < 0.002$, Wilcoxon).

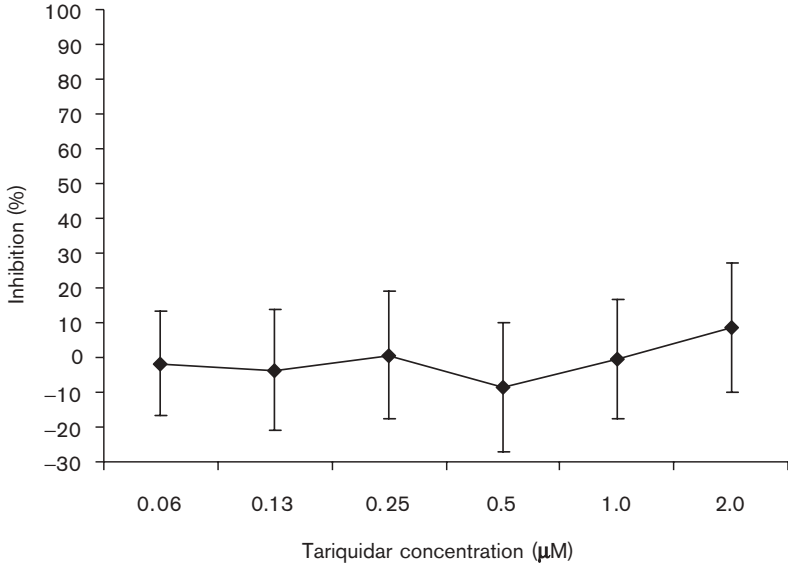
Tariquidar showed some reversal of resistance to doxorubicin in 14 of 20 (70%) samples classified as resistant and in two cases (10%) converted the sensitivity to an IC_{90} below 1.25 μM, which represents 50% TDC. Both these patients had recurrent ovarian carcinoma and none had previous anthracycline exposure. In the ovarian cancer subset, tariquidar showed some reversal of resistance to doxorubicin in 10 of 12 (83%) samples classified as resistant.

In all nine P-gp-positive cases the addition of tariquidar improved the activity of doxorubicin in the ATP-TCA. In four of nine cases (44%), the IC_{90} of doxorubicin decreased below 2.5 μM and in one of nine below 1.25 μM.

Vinorelbine

The median IC_{90} and IC_{50} values for vinorelbine were 15.5 and 2.3 μM. The IC_{90} of vinorelbine was greater than 100% TDC (11 μM) in 17 of 31 samples, indicating probable resistance in 55% of the tumors tested. For the ovarian cancer subset, resistance according to IC_{90} was seen in eight of 17 (47%) tumors (Fig. 3b).

Fig. 2



There is no activity of tariquidar as a single agent on tumor cell growth inhibition ($n = 14$). The data were normally distributed and are expressed as mean ± SD.

Immunohistochemical staining for P-gp was positive in seven of 12 (58%) samples tested with vinorelbine. The median IC_{90} values for P-gp-negative and -positive samples were 9.7 and 20.8 μM, respectively, but this difference did not reach statistical significance (NS, Mann–Whitney *U*-test). Semiquantitative visual assessment of the degree of staining in this relatively small number of cases showed no correlation with sensitivity to vinorelbine (IC_{90} and IC_{50}) by linear regression analysis (data not shown). However, it should be noted that only one of seven (14%) of the P-gp-positive samples showed sensitivity to vinorelbine on the basis of IC_{90}, while three of five (60%) of the P-gp-negative samples showed sensitivity to vinorelbine.

The addition of tariquidar decreased the median IC_{90} of vinorelbine from 15.5 to 9.5 μM ($p < 0.0003$, Wilcoxon). Tariquidar showed some reversal of resistance to vinorelbine in six of 17 (35%) samples classified as resistant and in one case (6%) converted the sensitivity to an IC_{90} below 5.5 μM, which represents 50% TDC (Fig. 3b). In the ovarian cancer subset, tariquidar showed some reversal of resistance to vinorelbine in three of eight (37%) samples classified as resistant. Tariquidar produced little or no effect on vinorelbine activity in P-gp-negative samples (Fig. 5). In all seven P-gp-positive cases the addition of tariquidar improved the activity of the vinca alkaloid in the ATP-TCA. In four of seven P-gp-positive cases (57%) the IC_{90} of vinorelbine decreased below 11 μM and in one of seven below 5.5 μM.

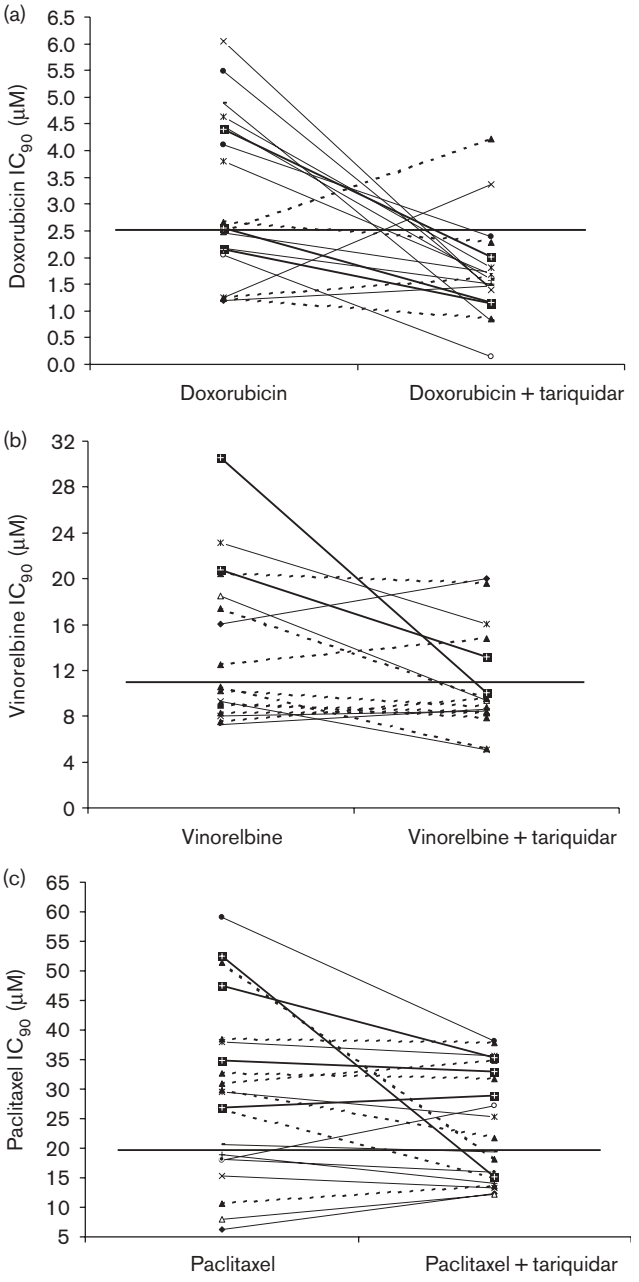
Paclitaxel

The median IC_{90} and IC_{50} values for paclitaxel were 27.4 and 9.7 μM, respectively. Resistance to paclitaxel according to IC_{90} was seen in 27 of 34 (79%) samples. In nine of 27 resistant cases, the patients had been previously exposed to a taxane-based regimen. In this particular subset the median IC_{90} for paclitaxel was 38.4 μM, while in the remaining 18 of 27 resistant cases the median IC_{90} decreased to 29.0 μM ($p < 0.05$, Mann–Whitney *U*-test). For the ovarian cancer subset, resistance according to IC_{90} was seen in 17 of 21 (81%) samples to paclitaxel (Fig. 3c).

Immunohistochemical staining for P-gp was positive in nine of 14 (64%) samples. Semiquantitative visual assessment of the degree of staining in this relatively small number of cases showed no correlation with sensitivity (IC_{90} and IC_{50}) by linear regression analysis (data not shown). It should be noted that only one of nine (11%) of the P-gp-positive samples showed sensitivity to paclitaxel on the basis of IC_{90}, while one of five (20%) of the P-gp-negative samples showed sensitivity to paclitaxel. Of those patients with P-gp-positive samples, eight of nine had received previous chemotherapy and three had been treated with a paclitaxel-based regimen. Of those patients with P-gp-negative, three of five were chemotherapy naive and one of five had previously received a taxane-based regimen.

The addition of tariquidar decreased the median IC_{90} of paclitaxel from 27.4 to 20.6 μM ($p < 0.05$, Wilcoxon).

Fig. 3



Effect of the addition of tariquidar on the activity of doxorubicin (a), vinorelbine (b) and paclitaxel (c) expressed as IC_{90} (μM). The samples labeled with a 'plus' in a black square are P-gp-positive tumors, while the samples labeled with a triangle and a dashed line are P-gp-negative tumors. A line has been drawn in each graph to indicate 100% TDC of each cytotoxic.

Tariquidar showed some reversal of resistance to paclitaxel in six of 27 (*22%*) samples classified as resistant.

Discussion

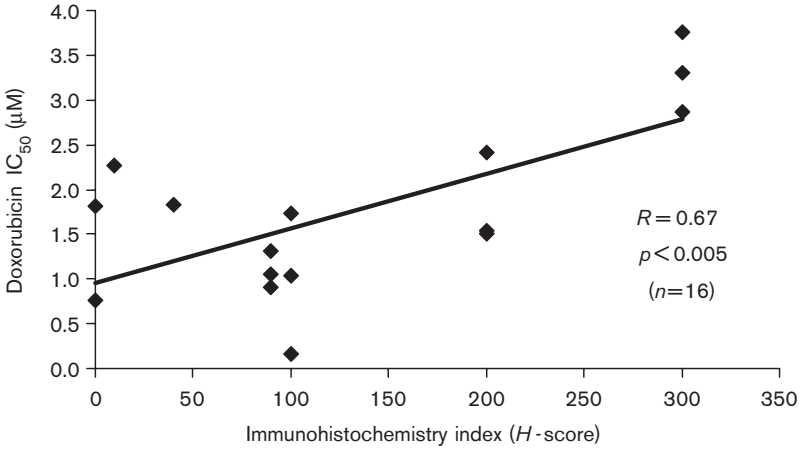
The results of this study show that in the ATP-TCA, tariquidar is able to reverse resistance to cytotoxic drugs known to be P-gp substrates. These data support the potential clinical benefit of tariquidar in combination with chemotherapy. However, not all tumors showed such potentially beneficial effects nor all drugs and it is clear from these results that patients must be carefully selected.

Almost all of those tumors that showed some increased sensitivity were P-gp-positive by immunohistochemistry, though there was a distinct lack of correlation with estimates of the degree of expression present. This may simply reflect the subjective and at best semi-quantitative nature of immunohistochemistry. Further, expression of P-gp may be rapidly up-regulated in response to chemotherapeutic agents both *in vitro* and *in vivo* [36–40]. These observations may explain the lack of correlation between P-gp expression at the time of diagnosis (before treatment) and eventual outcome in a number of studies [41–43].

Our data suggest that while P-gp-expressing tumors are nearly always resistant to pumped drugs, those that do not express P-gp are not always sensitive. Although the MDR phenotype mediated by P-gp appears to be an important mechanism of resistance to anthracyclines, taxanes and vinca alkaloids, other mechanisms may be of greater importance in many patients. It should be noted that many of our patients had been previously exposed to paclitaxel and there may have been induction of non-MDR mechanisms of resistance in these cases. Other ATP-dependent pumps such as MRP1 and BCRP have been shown to confer resistance to doxorubicin [44,45], although it should be noted that the role of MRP in paclitaxel and vinorelbine resistance is controversial and probably marginal [24,46–48]. Non-classical mechanisms of resistance are also likely to be involved. For microtubules interfering agents, altered microtubule dynamics, alterations in α- and β-tubulins, and/or altered binding sites may confer resistance. Altered metabolism and/or subcellular distribution, altered interaction of anti-tubulin agents with microtubules and inadequate induction of apoptotic signals are among the possible non-MDR mechanisms of resistance to tubulin-binding agents [49]. For anthracyclines such as doxorubicin, decreased levels of topoisomerase IIα have been associated with resistance and changes in DNA repair may also be important [50].

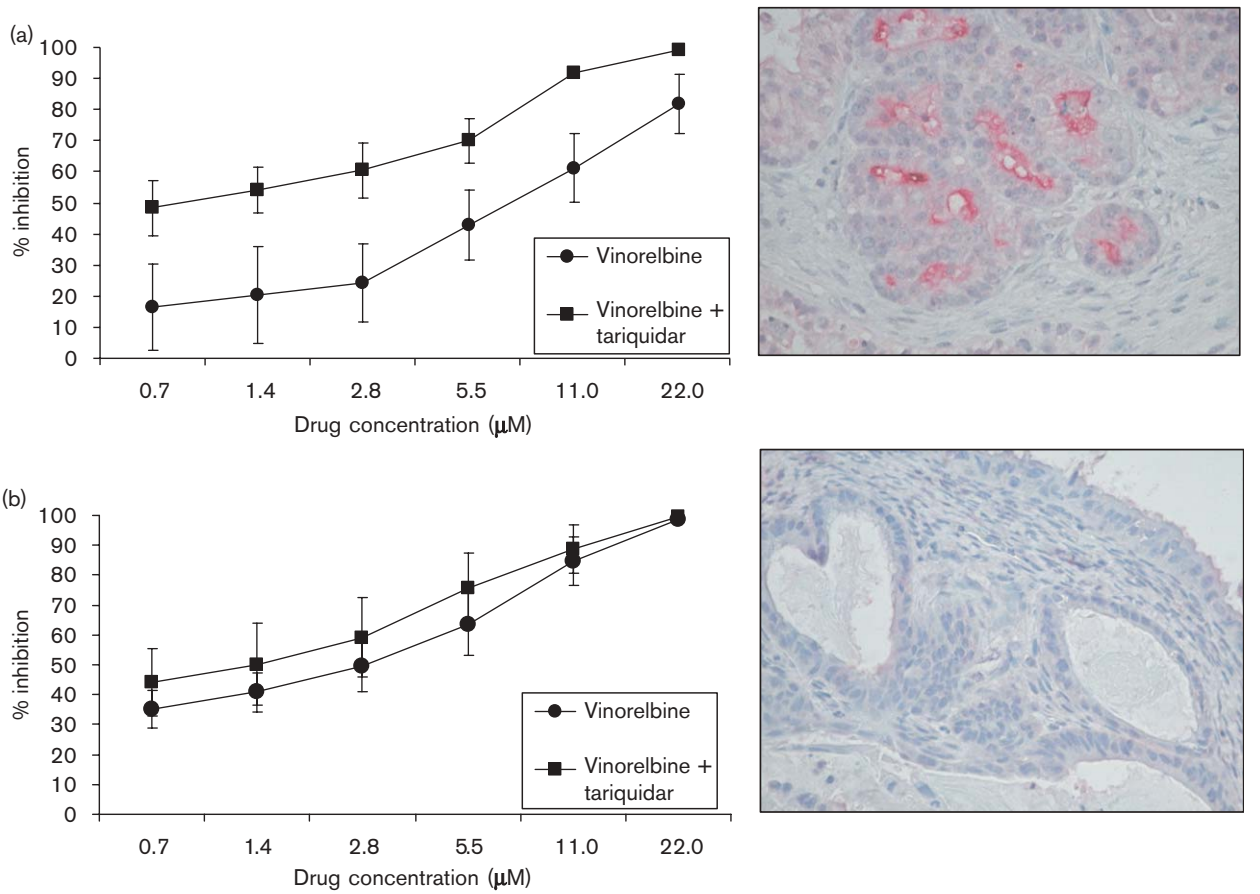
The ATP-TCA is a well-standardized assay capable of determining the activity of cytotoxic drugs *ex vivo* in tumor-derived cells, and shows excellent correlation with outcome in breast and ovarian cancer [51–53]. It could be used to select patients for tariquidar therapy and has the distinct advantage that it measures the effect of P-gp blocking agents in the context of other resistance mechanisms that may be present, but it is only applicable

Fig. 4



Linear regression analyses showing a correlation between P-gp immunostaining and sensitivity to doxorubicin.

Fig. 5



Effect of tariquidar on the vinorelbine activity in P-gp-positive (a) (*n*=7) and P-gp-negative (b) (*n*=5) tumors. Examples of a P-gp-positive and -negative ovarian cancer are shown in the right panels. The data are given as means ± SD.

to those from whom sufficient tumor tissue can be obtained for testing. Other functional assays of P-gp have been widely employed to study cell lines and hematological malignancies, but only occasionally in cells derived from solid tumors [54]. For these, *in vivo* radioimaging techniques have been developed [55,56]. The use of [^{99m}Tc]MIBI and analogous ^{99m}Tc-labeled agents (which have been shown to be P-gp substrates) allows the clinical assessment of P-gp function in cancer patients [57], but so far no large prospective clinical trials have been performed to investigate their potential as predictive tests [58].

Most studies of P-gp have used immunohistochemistry. A comparison of several studies reveals that there are inter-laboratory differences due to differing tissue fixation, processing and staining technique, experience of the observer in selecting hot spots, and the technique of counting positive cells [59].

In summary, the results of this study show that tariquidar is able to significantly reduce the median IC_{90}s for doxorubicin, paclitaxel and vinorelbine in a panel of human tumors. Furthermore, tariquidar is able to reverse resistance in a small percentage of solid tumors resistant to doxorubicin, paclitaxel and vinorelbine. The ideal patient for tariquidar treatment would express functional P-gp, but few if any of the other possible resistance mechanisms. Considerable translational research will be required to determine which are the most cost-effective predictive methods to employ, but it seems unlikely that immunohistochemistry alone will be sufficient and the use of functional assays showing *ex vivo* efficacy of tariquidar should be considered.

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References

1 Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer* 2002; **2**:48–58.
2 Cordon-Cardo C, O'Brien JP, Boccia J, Casals D, Bertino JR, Melamed MR. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. *J Histochem Cytochem* 1990; **38**: 1277–1287.
3 Marie JP, Zittoun R, Sikic BI. Multidrug resistance (*mdr1*) gene expression in adult acute leukemias: correlations with treatment outcome and *in vitro* drug sensitivity. *Blood* 1991; **78**:586–592.
4 Campos L, Guyotat D, Archimbaud E, Calmard-Oriol P, Tsuruo T, Troncy J, *et al.* Clinical significance of multidrug resistance P-glycoprotein expression on acute nonlymphoblastic leukemia cells at diagnosis. *Blood* 1992; **79**:473–476.
5 Damiani D, Michieli M, Ermacora A, Candoni A, Raspadori D, Geromin A, *et al.* P-glycoprotein (P-GP), and not lung resistance-related protein (LRP), is a negative prognostic factor in secondary leukemias. *Haematologica* 1998; **83**:290–297.
6 Yuen AR, Sikic BI. Multidrug resistance in lymphomas. *J Clin Oncol* 1994; **12**:2453–2459.
7 Baldini N, Scotlandi K, Barbanti-Brodano G, Manara MC, Maurici D, Bacci G, *et al.* Expression of P-glycoprotein in high-grade osteosarcomas in relation to clinical outcome. *N Engl J Med* 1995; **1333**:1380–1385.
8 Serra M, Scotlandi K, Reverter-Branchat G, Ferrari S, Manara MC, Benini S, *et al.* Value of P-glycoprotein and clinicopathologic factors as the basis for new treatment strategies in high-grade osteosarcoma of the extremities. *J Clin Oncol* 2003; **21**:536–542.
9 Savaraj N, Wu CJ, Xu R, Lampidis T, Lai S, Donnelly E, *et al.* Multidrug-resistant gene expression in small-cell lung cancer. *Am J Clin Oncol* 1997; **20**:398–403.
10 Hsia TC, Lin CC, Wang JJ, Ho ST, Kao A. Relationship between chemotherapy response of small cell lung cancer and P-glycoprotein or multidrug resistance-related protein expression. *Lung* 2002; **180**:173–179.
11 Linn SC, Giaccone G, van Diest PJ, Blokhuis WM, van der Valk P, van Kalken CK, *et al.* Prognostic relevance of P-glycoprotein expression in breast cancer. *Ann Oncol* 1995; **6**:679–685.
12 Gregorcyk S, Kang Y, Brandt D, Kolm P, Singer G, Perry RR. P-glycoprotein expression as a predictor of breast cancer recurrence. *Ann Surg Oncol* 1996; **3**:8–14.
13 Burger H, Foekens JA, Look MP, Meijer-van Gelder ME, Klijn JG, Wiemer EA, *et al.* RNA expression of breast cancer resistance protein, lung resistance-related protein, multidrug resistance-associated proteins 1 and 2, and multidrug resistance gene 1 in breast cancer: correlation with chemotherapeutic response. *Clin Cancer Res* 2003; **9**:827–836.
14 Baeklandt MM, Holm R, Nesland JM, Trope CG, Kristensen GB. P-glycoprotein expression is a marker for chemotherapy resistance and prognosis in advanced ovarian cancer. *Anticancer Res* 2000; **20**: 1061–1067.
15 Raspollini MR, Amunni G, Villanucci A, Baroni G, Boddi V, Taddei GL. Multidrug resistance in ovarian cancer: comparing an immunocytochemical study and ATP-tumor chemosensitivity assay. *J Chemother* 2002; **14**: 518–525.
16 Chan HS, Thorner PS, Haddad G, Ling V. Immunohistochemical detection of P-glycoprotein: prognostic correlation in soft tissue sarcoma of childhood. *J Clin Oncol* 1990; **8**:689–704.
17 Chan HS, Haddad G, Thorner PS, DeBoer G, Lin YP, Ondrusek N, *et al.* P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. *N Engl J Med* 1991; **325**:1608–1614.
18 Chan HS, Thorner PS, Haddad G, Gallie BL. Multidrug-resistant phenotype in retinoblastoma correlates with P-glycoprotein expression. *Ophthalmology* 1991; **98**:1425–1431.
19 Koshiyama M, Fujii H, Kinezaki M, Morita Y, Nanno H, Yoshida M. Immunohistochemical expression of topoisomerase IIalpha (Topo IIalpha) and multidrug resistance-associated protein (MRP), plus chemosensitivity testing, as chemotherapeutic indices of ovarian and endometrial carcinomas. *Anticancer Res* 2001; **21**:2925–2932.
20 Izquierdo MA, van der Zee AG, Vermorken JB, van der Valk P, Belien JA, Giaccone G, *et al.* Drug resistance-associated marker Lrp for prediction of response to chemotherapy and prognoses in advanced ovarian carcinoma. *J Natl Cancer Inst* 1995; **87**:1230–1237.
21 Kawabata S, Oka M, Soda H, Shiozawa K, Nakatomi K, Tsurutani J, *et al.* Expression and functional analyses of breast cancer resistance protein in lung cancer. *Clin Cancer Res* 2003; **9**:3052–3057.
22 Ford JM. Experimental reversal of P-glycoprotein-mediated multidrug resistance by pharmacological chemosensitisers. *Eur J Cancer* 1996; **32A**:991–1001.
23 Sparreboom A, Nooter K. Does P-glycoprotein play a role in anticancer drug pharmacokinetics? *Drug Resist Update* 2000; **3**:357–363.
24 Thomas H, Coley HM. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. *Cancer Control* 2003; **10**:159–165.
25 Roe M, Folkes A, Ashworth P, Brumwell J, Chima L, Hunjan S, *et al.* Reversal of P-glycoprotein mediated multidrug resistance by novel anthranilamide derivatives. *Bioorg Med Chem Lett* 1999; **9**:595–600.
26 Mistry P, Stewart AJ, Dangerfield W, Okiji S, Liddle L, Bootle D, *et al. In vitro* and *in vivo* reversal of P-glycoprotein-mediated multidrug resistance by a novel potent modulator, XR9576. *Cancer Res* 2001; **61**:749–758.
27 Stewart A, Steiner J, Mellows G, Laguda B, Norris D, Bevan P. Phase I trial of XR9576 in healthy volunteers demonstrates modulation of P-glycoprotein in $CD56^+$ lymphocytes after oral and intravenous administration. *Clin Cancer Res* 2000; **6**:4186–4191.
28 Boniface GR, Ferry D, Atsmon J, Inbar M, van Tellingen O, Abraham J, *et al.* XR9576 (tariquidar), a potent and specific P-glycoprotein inhibitor, has minimal effects on the pharmacokinetics of paclitaxel, doxorubicin and vinorelbine and can be administered with full-dose chemotherapy in patients with cancer. *Proc Am Soc Clin Oncol* 2002; **38**: abstr 2173.

29 Kurbacher CM, Cree IA, Bruckner HW, Brenne U, Kurbacher JA, Möller K, *et al.* Use of an *ex vivo* ATP luminescence assay to direct chemotherapy for recurrent ovarian cancer. *Anticancer Drugs* 1998; **9**:51–57.

30 Andreotti PE, Cree IA, Kurbacher CM, Hartmann DM, Linder D, Harel G, *et al.* Chemosensitivity testing of human tumors using a microplate adenosine triphosphate luminescence assay: clinical correlation for cisplatin resistance of ovarian carcinoma. *Cancer Res* 1995; **55**:5276–5282.

31 Cree IA. Luminescence-based cell viability testing. *Methods Mol Biol* 1998; **102**:169–177.

32 Norton AJ, Jordan S, Yeomans P. Brief, high temperature heat denaturation (pressure cooking): a simple and effective method of antigen retrieval for routinely processed tissues. *J Pathol* 1994; **173**:371–379.

33 Cree IA, Neale MH, Myatt NE, de Takats PG, Hall P, Grant J, *et al.* Heterogeneity of chemosensitivity of metastatic cutaneous melanoma. *Anticancer Drugs* 1999; **10**:437–444.

34 Whitehouse PA, Knight LA, Di Nicolantonio F, Mercer SJ, Sharma S, Cree IA. Heterogeneity of chemosensitivity of colorectal adenocarcinoma determined by a modified *ex vivo* ATP-tumor chemosensitivity assay (ATP-TCA). *Anticancer Drugs* 2003; **14**:369–375.

35 Mercer SJ, Somers SS, Knight LA, Whitehouse PA, Sharma S, Di Nicolantonio F, *et al.* Heterogeneity of chemosensitivity of esophageal and gastric carcinoma. *Anticancer Drugs* 2003; **14**:397–403.

36 Chaudhary PM, Roninson IB. Induction of multidrug resistance in human cells by transient exposure to different chemotherapeutic drugs. *J Natl Cancer Inst* 1993; **85**:632–639.

37 Abolhoda A, Wilson AE, Ross H, Danenberg PV, Burt M, Scotto KW. Rapid activation of MDR1 gene expression in human metastatic sarcoma after *in vivo* exposure to doxorubicin. *Clin Cancer Res* 1999; **5**:3352–3356.

38 Hu XF, Slater A, Kantharidis P, Rischin D, Juneja S, Rossi R, *et al.* Altered multidrug resistance phenotype caused by anthracycline analogues and cytosine arabinoside in myeloid leukemia. *Blood* 1999; **93**:4086–4095.

39 Stein U, Jürchott K, Schläfke M, Hohenberger P. Expression of multidrug resistance genes MVP, MDR1, and MRP1 determined sequentially before, during, and after hyperthermic isolated limb perfusion of soft tissue sarcoma and melanoma patients. *J Clin Oncol* 2002; **20**:3282–3292.

40 Tada Y, Wada M, Migita T, Nagayama J, Hinoshita E, Mochida Y, *et al.* Increased expression of multidrug resistance-associated proteins in bladder cancer during clinical course and drug resistance to doxorubicin. *Int J Cancer* 2002; **98**:630–635.

41 Arts HJG, Katsaros D, de Vries EGE, Massobrio M, Genta F, Danese S, *et al.* Drug resistance-associated markers P-glycoprotein, multidrug resistance-associated protein 1, multidrug resistance-associated protein 2, and lung resistance protein as prognostic factors in ovarian carcinoma. *Clin Cancer Res* 1999; **5**:2798–2805.

42 Yokoyama Y, Sato S, Fukushi Y, Sakamoto T, Futagami M, Saito Y. Significance of multi-drug-resistant proteins in predicting chemotherapy response and prognosis in epithelial ovarian cancer. *J Obstet Gynaecol Res* 1999; **25**:387–394.

43 Nakayama K, Kanzaki A, Ogawa K, Miyazaki K, Ne'amati N, Takebayashi Y. Copper-transporting P-type adenosine triphosphatase (ATP7B) as a cisplatin based chemoresistance marker in ovarian carcinoma: comparative analysis with expression of MDR1, MRP1, MRP2, LRP and BCRP. *Int J Cancer* 2002; **101**:488–495.

44 Grant CE, Valdimarsson G, Hipfner DR, Almquist KC, Cole SP, Deeley RG. Overexpression of multidrug resistance-associated protein (MRP) increases resistance to natural product drugs. *Cancer Res* 1994; **54**:357–361.

45 Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, Ross DD. A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci USA* 1998; **95**:15665–15670.

46 Shepard RL, Cao J, Starling JJ, Dantzig AH. Modulation of P-glycoprotein but not MRP1- or BCRP-mediated drug resistance by LY335979. *Int J Cancer* 2003; **103**:121–125.

47 Vanhoefer U, Cao S, Harstrick A, Seeber S, Rustum YM. Comparative antitumor efficacy of docetaxel and paclitaxel in nude mice bearing human tumor xenografts that overexpress the multidrug resistance protein (MRP). *Ann Oncol* 1997; **8**:1221–1228.

48 Dantzig AH, Tabas LB, Law KL. *In vitro* characterization of selective and highly potent tricyclic isoxazole inhibitors of the multidrug resistant protein MRP1. *Proc Am Ass Cancer Res* 2002; **43**:951.

49 Dumontet C, Sikic BI. Mechanisms of action and resistance to antitubulin agents: microtubule dynamics, drug transport and cell death. *J Clin Oncol* 1999; **17**:1061–1070.

50 Fedier A, Schwarz VA, Walt H, Carpini RD, Haller U, Fink D. Resistance to topoisomerase poisons due to loss of DNA mismatch repair. *Int J Cancer* 2001; **93**:571–576.

51 Cree IA, Kurbacher CM, Untch M, Sutherland LA, Hunter EM, Subedi AMC, *et al.* Correlation of the clinical response to chemotherapy in breast cancer with *ex vivo* chemosensitivity. *Anticancer Drugs* 1996; **7**:630–635.

52 Konecny G, Crohns C, Pegram M, Felber M, Lude S, Kurbacher C, *et al.* Correlation of drug response with the ATP tumor chemosensitivity assay in primary FIGO stage III ovarian cancer. *Gynecol Oncol* 2000; **77**:258–263.

53 Sharma S, Neale MH, Di Nicolantonio F, Knight LA, Whitehouse PA, Mercer SJ, *et al.* Outcome of ATP-based tumor chemosensitivity assay directed chemotherapy in heavily pre-treated recurrent ovarian carcinoma. *BMC Cancer* 2003; **3**:19.

54 Mechetner E, Kyshtoobayeva A, Zonis S, Kim H, Stroup R, Garcia R, *et al.* Levels of multidrug resistance (MDR1) P-glycoprotein expression by human breast cancer correlate with *in vitro* resistance to taxol and doxorubicin. *Clin Cancer Res* 1998; **4**:389–398.

55 Del Vecchio S, Ciarmiello A, Potena MI, Carriero MV, Mainolfi C, Botti G, *et al. In vivo* detection of multidrug-resistant (MDR1) phenotype by technetium-99 m sestamibi scan in untreated breast cancer patients. *Eur J Nucl Med* 1997; **24**:150–159.

56 Ciarmiello A, Del Vecchio S, Silvestro P, Potena MI, Carriero MV, Thomas R, *et al.* Tumor clearance of technetium 99m-sestamibi as a predictor of response to neoadjuvant chemotherapy for locally advanced breast cancer. *J Clin Oncol* 1998; **16**:1677–1683.

57 Agrawal M, Abraham J, Balis FM, Edgerly M, Stein WD, Bates S, *et al.* Increased ^{99m}Tc-Sestamibi accumulation in normal liver and drug-resistant tumors after the administration of the glycoprotein inhibitor, XR9576. *Clin Cancer Res* 2003; **9**:650–656.

58 Palmedo H. What can we expect from MDR breast cancer imaging with Sestamibi? *J Nucl Med* 2002; **43**:526–530.

59 Beck WT, Grogan TM, William CL, Cordon-Cardo C, Parham DM, Kuttesch JF, *et al.* Methods to detect P-glycoprotein-associated multidrug resistance in patients' tumors: consensus recommendations. *Cancer Res* 1996; **56**:3010–3020.