Title: Development of taxane resistance in a panel of human lung cancer cell lines

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Summary

Using a selection process designed to reflect clinically relevant conditions, a panel of taxane-selected variants were developed to study further the mechanisms of resistance in lung cancer. Unlike continuous or pulse exposure to high concentrations of chemotherapeutic drugs which yield high resistance and often cross resistance, most variants developed here displayed low level resistance to the selecting drug with slight cross-resistance. Pulsing with taxol resulted in more highly resistant clones (up to 51.4-fold). Analysis of taxol and taxotere in the four major lung cancer cell types showed the taxanes to be more effective against NSCLC (with the exception of SKMES-taxane selected variants) than against the SCLC. Comparison of taxol and taxotere shows that taxol induces higher levels of resistance than taxotere. Further, in taxotere-selected cell lines, the cells are more resistant to taxol than taxotere, suggesting that taxotere may be a superior taxane from a clinical view. Taxol treatment resulted in increased cross-resistance to 5-FU in all classes of lung cancer except DMS-53. The high levels of Pgp in the DMS-53 and selected variant suggests this mechanism is not related to Pgp expression. Analysis of the Pgp and MRP-1 status by combination inhibitory assays and Western blotting showed no consistent relationship between expression of the membrane pumps Pgp or MRP-1 and resistance. However, where high level resistance was seen, the parent cell line expressed Pgp or MRP-1 and was accompanied by increased levels in the variants.

Abbreviations: SCLC = Small Cell Lung Cancer; LCLC = Large Cell Lung Cancer; NSCLC = Non-small Cell Lung Cancer; H1299 = NCI-H1299; H460 = NCI-H460; FCS= foetal calf serum; Pgp = P-glycoprotein; MRP = MDR related protein; 5-FU = 5-fluorouracil; VP-16 = etoposide.

Introduction

Lung cancer is the most lethal cancer worldwide with 1.5 million cases diagnosed in 2003 [1] exceeding those from breast, colon and prostate combined [2].   Causes of lung cancer include smoking (about 87%), exposure to chemicals such as asbestos and arsenic, as well as the naturally occurring radioactive gas radon [3].   Despite being one of the easiest cancers to prevent, it remains one of the most difficult to cure, due to the late stage of presentation, the presence of metastasis and the development of chemotherapy resistance.

As surgery is only curative in early stage lung cancer, radiation and chemotherapy are the alternative treatments. The chemotherapeutic drugs used depend upon which classification the cancer belongs to, namely small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC). NSCLC patients are traditionally treated with cisplatin, carboplatin, VP-16, vinblastine, vindesine and more recently taxol, taxotere, irinotecan, vinorelbine and gemcitabine [2].   In the treatment of SCLC, primarily cisplatin, carboplatin VP-16, cyclophosphamide, adriamycin, vincristine, and ifosfamide are used [4]. The most commonly used combination in the treatment of SCLC is etoposide and cisplatin.

The development of resistance to a particular or a variety of unrelated chemotherapeutic drugs (multiple drug resistance, MDR) is a major obstacle in the treatment of cancer. Drug resistance is caused by a variety of changes in the cancer cells, for example the increased activity of drug pumps (Pgp and MRP family), up or down regulation of topoisomerase or glutathion activities and changes in the apoptotic pathways [5,6].

SCLC representing 20% of all lung cancers have the poorest prognosis, being very aggressive and generally present with distant metastases at diagnosis [4]. SCLC initially responds to chemotherapy but acquires resistance. NSCLC includes squamous cell carcinoma (30%), adenocarcinoma (40%) and large cell carcinoma (10%) often shows intrinsic multidrug resistance [7]. The emergence of taxanes as a treatment for lung cancer represents significant progress showing substantial activity and a unique mechanism of action. Taxanes are versatile as they are active as a single agent or in combination (especially platinum derivatives) and have shown to have radiosensitising activity [8].

While many studies on lung cancer and resistance have been carried out, resistant variants developed from these previous studies were derived using drug levels not pharmacologically achievable and the drug administration regime did not reflect the clinical setting [9]. In this study, we focused on the development of drug resistance in lung cancer across a panel of chemotherapy naïve cell lines with taxol or taxotere. Pulse selection with low levels of chemotherapeutic drugs was used to develop resistant variants that would reflect the clinical setting.

Materials and Methods

All chemicals (unless otherwise stated), FBS, glutamine and Sulindac were obtained from Sigma (Poole, UK). Cell culture media was supplied by Gibco BRL (Paisley, UK). Adriamycin and carboplatin were obtained from EBEWE arzneimittel Ges. m.b.h. (Austria). Vincristine and cisplatin were obtained from Mayne Pharma Plc, (Warwickshire, UK). VP-16 and taxol were obtained from Bristol-Myers Squibb (Dublin, Ireland). Taxotere was obtained from Aventis Pharmaceuticals (Surrey, UK) and 5-FU was obtained from Faulding Pharmaceuticals (Warwickshire, UK). GF120918 was obtained from GlaxoSmithKline (Middlesex, UK). Primary and secondary antibodies ß-actin and anti-mouse were obtained from Sigma (Poole, UK). Anti-Pgp and anti-MRP-1 antibodies were obtained from Santa Cruz (California, USA).

Cell lines

The adenocarcinomas (A549 and SKLU1), large cell lung cancer (NCI-H1299 and NCI-H460) and the squamous cell lung carcinoma SKMES1, were obtained from the ATCC (Bethseda MD). The small cell lung cancer DMS-53 was obtained from the European collection of cell culture. The poorly differentiated squamous cell lung carcinoma DLRP, was developed in our centre [10]. DMS-53 and variants were grown in RPMI 1640 with 10% FBS, 1% NEAA, sodium pyruvate and L-glutamine while H460, H1299 and variants were supplemented with 5% FBS. DLRP, A549 and variants were grown in ATCC with 5% FBS and L-glutamine. SKLU1, SKMES1 and variants were grown in MEM with 1% NEAA, sodium

pyruvate and L-glutamine and 5% or 10% FBS respectively. All taxol-selected variants were referred to as –txl e.g. A549-txl and taxotere-selected variants were referred to as –txt e.g. SKMES1-txt.

Toxicity testing

Toxicity was assessed in 96-well plates with acid phosphatase activity as the end point [11]. Cells were set up in growth medium at 1×10^3 per well and allowed to incubate overnight at 5% CO_2 and $37^{o}C$. Cytotoxic drug dilutions were prepared freshly at 2X final concentration and an equivolume added to each well. The plates were incubated for a further 6 or 7 days until confluency was being approached in the control cells before assessment of cell survival. The concentration of drug causing 50% kill (IC_{50} of the drug) was determined from a plot of % survival versus cytotoxic drug concentration.

Combination assays

Cells were set up as for Toxicity assays. After overnight incubation, cytotoxic drug dilutions and inhibitors were prepared at 4X final concentration. Volumes of 50μl drug dilution and 50μl inhibitor were added to cells to a final 1X concentration and allowed to incubate for 6 – 7 days at 5% CO_2 and $37^{o}C$ until confluency was being approached in the control cells. Toxicity was assessed in 96-well plates with acid phosphatase activity as the end point [11].

Pulse selection of parent cell lines

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Parent cell lines were pulsed with taxol or taxxotere. SKMES1 and DMS-53 were pulsed with taxol (140nM and 70nM respectively) or taxotere (74.4nM and 49.6nM respectively). DLRP was pulsed with taxotere (6.2nM). A549, SKLU1, H1299 and H460 were pulsed taxol (175nM, 11.7nM, 175nM and 58.3nM respectively). The cells were pulsed with the selecting drug for four hours once a week for a period of 10 weeks.

Western blotting

Western blotting for detection of Pgp and MRP-1 was performed on cell lysates that were centrifuged at 1000rpm to remove nuclear material. Protein determination was made using the Biorad method. Samples were separated on a 10% SDS gel [12] with 20μg loaded per well. After Western blotting [13], blots with primary antibodies (Santa Cruz Biotechnology) were incubated overnight at 4°C. Secondary antibody conjugated to horse-radish peroxidase (Sigma) were detected by enhanced chemiluminesence (ECL, Amersham). Positive controls were used for each antibody.

Results

Toxicity profile of lung cancer cells to selecting drugs

In order to study trends in lung cancer resistance, a panel of cell lines encompassing the 4 main types (adenocarcinoma; large cell lung carcinoma; squamous lung carcinoma and small cell lung carcinoma) were chosen on the basis of no previous exposure to chemotherapeutic drugs, and were exposed to taxol or taxotere.

Toxicity profiles to taxol and taxotere (Figure 1) show considerable differences in the sensitivity of the parent cell lines. Adenocarcinomas (A549 and SKLU1) exhibit a lower IC_{50} to taxol than LCLC (H1299 and H460), 1.3nM as compared to 4.8nM respectively on average. Sensitivity to taxol in the squamous cell lines (SKMES1 and DLRP) was not as consistent with DLRP (10.45nM IC_{50}) being 7.5-fold more resistant to taxol than SKMES1 (1.4nM IC_{50}). The SCLC, DMS-53 was most resistant to taxol. Taxotere, the other taxane used in these studies, did not show the same profile as taxol. In NSCLC, the IC_{50} for taxotere was consistently low (about 1nM) whereas the SCLC cell line DMS-53 has a high innate resistance to taxotere with the IC_{50} being 10-fold higher than the other cell lines in the panel. DLRP and both LCLC are very sensitive to taxotere but not taxol.

The resistance of the parental cell lines to a variety of other chemotherapeutic drugs was also assessed (Table I). Across all the parent cell lines, the taxanes (shown in Figure 1) are the most effective on a molar basis with the next being vincristine and then adriamycin, with IC_{50}s

ranging 18nM – 71nM and 2nM – 28nM respectively, while the platinum drugs (carboplatin and cisplatin) and 5-FU are active at micromolar concentrations. For adriamycin, the high IC_{50} in DLRP correlates to the high innate resistance to taxol but no such correlation is seen with DMS-53. For vincristine, the pattern of resistance follows a similar trend to that of taxotere with all the other cell lines having similar IC_{50} values of 11nM-13nM (except SKLU1 at 2.4nM), while DMS-53 is twice as resistant. The panel was least resistant to cisplatin and 5-FU. Cisplatin, like carboplatin was more effective against DMS-53 (IC_{50} 0.4μM – 0.5μM) than NSCLC (0.7μM – 2.2μM) but was more toxic to the cells than carboplatin. The effect of 5-FU was quite variable in all the subtypes except LCLC (1.3μM). SKLU1, DLRP and to a lesser extent DMS-53, were less sensitive to 5-FU. For VP-16, DLRP and DMS-53 displayed greater than 2-fold higher resistance than the other cell lines, with IC_{50}s of 0.74μM and 0.48μM respectively.

The panel of cell lines was pulsed with a taxane (taxol or taxotere) to generate a number of drug-resistant variants to investigate what happens to the innate resistance of these cell lines. The cells were pulsed with or near to pharmacologically achievable levels for 4 hour intervals over a 10 week period to closely reflect the clinical scenario [8]. The changes in IC_{50} after pulsing are shown in Figure 2. All the cell lines except DLRP were pulsed with taxol. Taxol-selected variants resulted in a moderate increase in resistance for the adenocarcinomas and LCLC with IC_{50}s ranging from 7nM to 20nM, corresponding to 2.5-fold to 5.5-fold increases relevant to the

parent. SKMES1-txl displayed high resistance to taxol with an IC_{50} of 57.5nM, corresponding to a 24.7-fold increase compared to parent. DMS-53 variants selected with taxol displayed the highest IC_{50} values of all the variants developed for this work at 94.5nM, corresponding to 6.3-fold relative to the parent. The DMS-53 parent displayed the highest intrinsic resistance to taxol of the panel.

Three of the cell lines were selected with taxotere. The greatest change in resistance was seen in SKMES1, with the IC_{50} increasing from 0.9nM to 25nM, but this was unstable and started to decrease with time. At the highest IC_{50}, this cell line displayed a 29.1-fold increase compared to parent. The increased resistance in DLRP was a modest 4.1-fold (IC_{50} of 3.8nM) while for DMS-53 with high innate resistance, only a 1.8-fold increase in resistance is observed.

Cross resistance profile to chemotherapeutic drugs in selected variants

Each of the pulse-selected drug variants were screened with a panel of chemotherapeutic drugs to look at the cross-resistance profile (Figure 3). Modest cross-resistance was observed to adriamycin in most of the cell lines ranging from 1.3 to 7.5-fold. The LCLC variants showed no change in response to adriamycin compared to parental cells. The SKMES1 taxol- and taxotere- selected variants showed a dramatic cross-resistance to 5-FU (10-fold and 13.2-fold) which was not observed in any of the other cell lines. No major cross-resistance developed to the platinum agents cisplatin and carboplatin in any of the variants with the largest changes observed in H1299-txl of 1.5-fold increase in resistance to cisplatin and 1.7-fold

increase in resistance to carboplatin. Some of the variants, however, developed increase sensitivity to the platinum agents, especially to carboplatin (H460-txl, SKMES1-txl and SKMES1-txt). Cross-resistance to VP-16 was observed in A549-txl (4.2-fold), SKMES1-txl (9.2-fold), SKMES1-txt (3.9-fold) and DLRP-txt (3.6-fold). Across all the chemotherapeutic agents tested the most cross-resistance was displayed against vincristine with SKMES1-txl and SKMES1-txt showing 24.4-fold and 19.6-fold resistance respectively. The other variants showed slight changes in the range of 1-3.5-fold.

Detection and inhibition of Pgp and MRP-1

Combination assays with MRP-1 inhibitor sulindac or Pgp inhibitor GF120918 were conducted to investigate the involvement of these membrane pumps in drug resistance in the panel of cell lines. Toxicity assays were carried with a combination of the non-steroidal anti-inflammatory drug sulindac with adriamycin or a combination of GF120918 with taxol. For each cell line or variant, the percentage cell survival was calculated for the drug, the inhibitor and the combination. Increased cell kill in the combination indicates the contribution of either pump to cellular resistance. For instance, in Figure 4a, taxotere at 7.5ng/ml and GF120918 at 75μg/ml were not inhibitory individually, while the combination causes approximately 75% cell kill in SKMES1-txt. This is slightly more than the kill observed in the parent with taxotere at the same concentration, suggesting that alterations in the expression of Pgp could almost wholly

account for the increased resistance in SKMES1-txt. Consistent with this, is the expression of Pgp in the taxotere-selected variant (Figure 4b).

A summary of the results observed in all combination assays can be seen in Table II.

Sulindac combination with adriamycin show mixed results across the panel of cell lines. For the adenocarcinomas (A549, SKLU-1), selection with taxol did not increase the combination effect from that of the parent (effect or none for A549 and SKLU-1 respectively). For A549 as mentioned previously, this may reflect the high basal level of MRP-1 detected in the parent. Western blotting for MRP-1 correlated for A549 and variants with no change, however for SKLU-1, both selected variants expressed MRP-1 while the parent did not. The combination of GF120918 and taxol showed no enhanced effect in any of the adenocarcinomas, correlating with the lack of expression of Pgp detected by Western blotting. In LCLC, no significant effect on combination was seen. The MRP-1 status of these cell lines was not consistent. The H460 parent and selected variants were positive for MRP-1, while H1299 and H1299-tax were negative. The combination of GF120918 and taxol only showed an enhanced effect in the parent H1299 and not in the selected variants or any of the H460 cell lines. However, Pgp expression was detected in H1299 and H1299-txl.

For the squamous lung cancer cell lines, the GF120918/taxotere combination showed an effect for SKMES1 but not DLRP parents. DLRP showed no effect by combination assay. For SKMES1, the taxotere-selected variant was negative for MRP-1 and the carboplatin-selected variant was positive. SKMES1-txt was more effective in this combination

and the carboplatin less effective. Western blots for Pgp similarly show increased expression of Pgp in the taxane-selected variants of SKMES1. For the SCLC, only the DMS-53 and variants were tested. While the parent showed activity for the sulindac/adriamycin combination, none of the variants did. The GF120918/taxol combination showed increased effect in the variants with the taxane- selected variants.   Western blot analysis confirmed increased expression of Pgp in the taxane-selected variants.

Changes in in vitro invasiveness

The invasive abilities of the selected cell lines were compared to parental cells (Figure 5).   In three of the cell lines (A549, H1299 and SKMES1) taxol selection led to a slight increase in invasiveness. Two of the taxol-selected variants (SKLU1 and H460) displayed a decrease in invasion compared to parent. The two taxotere-selected cell lines tested displayed increased invasion. The SCLC cell line DMS53 and its variants were non-invasive so were not included in these experiments.

Discussion

The introduction of taxanes in the treatment of lung cancer represented a significant progress, being effective alone or in combination (with a platinum agent or VP-16) to treat NSCLC and SCLC respectively [14]. A downside is the development of drug resistance [15]. Most in vitro studies of drug resistance are based on continuous exposure of cells to non-pharmacologically achievable levels of chemotherapeutic drugs. These in vitro models usually display very high resistance up to 1000-fold, which is

unlikely to represent resistant tumour cells <u>in situ</u>. Hence the factors causing resistance in highly resistant variants may not reflect those involved in clinical resistance.

In the clinical setting, patients receive chemotherapy treatment once every 1-3 weeks allowing for recovery in between [8]. By pulsing chemotherapeutic naïve cells once weekly for four hours and allowing the cells time to recover, resistant variants were developed exhibiting modest changes in resistance with some cross resistance. These variants were selected at sustainable pharmacological levels [16]. These variants thus provide a suitable model for <u>in vitro</u> investigations into drug resistance.

Comparison of taxol and taxotere shows differences in resistance despite having a similar mechanism of action. In the present study, resistance to taxol was higher than taxotere regardless of whether the selecting drug had been taxol or taxotere. In similar studies in our lab, a taxotere-selected squamous lung cancer cell line (DLKP) had IC_{50}s of 166.2nM and 536.7nM for taxotere and taxol respectively (unpublished data). A similar study, [17] found taxol-selected ovarian cancer cells KF-tx to be more resistant to taxol (IC_{50} 705.3nM) than taxotere (IC_{50} 351nM). These studies indicate cross-resistance is present, but the cell lines are not fully cross-resistant as suggested by Adroulakis *et al*[18]. That the fold difference for taxotere in taxol-selected variants is greater than taxol suggests that taxol and taxotere share some mechanisms of action but also that taxotere through increased affinity for microtubules [19] or additional mechanisms exerts greater toxicity. Interestingly, at clinical doses, in KF-tx cells, taxotere induced Bcl-2 phosphorylation while taxol did not. There was also a greater degree

of tubulin polymerisation activity on treatment with taxotere than taxol with no changes in cell cycle distribution as determined by flow cytometry [17]. Haldar and colleagues have shown that taxotere is 10- to 100-fold more potent at Bcl-2 phosphorylation than taxol [20].

The cross-resistance profiles show taxol selection increased adriamycin resistance in squamous and adenocarcinomas and SCLC but not in LCLC. For the adenocarcinomas, the increased resistance in adriamycin and less so to vincristine correlate with the presence of MRP-1 found in A549 and variants and SKLU1-txl (Figure 3). Interestingly, there is a 4-fold increase in cross-resistance to VP-16 in A549-txl. That the cross resistance is greater than that seen with adriamycin suggests mechanisms other than increased MRP-1 may also be active including inhibition of microtubule formation or decreased topoisomerase II activity [14]. Taxol-resistant variants of A549 with 17 and 9-fold resistance to taxol [21] both showed a mutation in the α-tubulin iso-type Kα1 and low level expression of P-gp in A549-T24 and not in A549-T12.

Resistance to vincristine was high in squamous cell carcinomas, modest in LCLC and SCLC and low in adenocarcinomas. VP-16 cross resistance was sporadic. Cross-resistance to 5-FU was low in all except squamous cell carcinomas, although taxol treatment resulted in increased cross-resistance to 5-FU in all classes of lung cancer. The exception was DMS-53, having high basal levels of Pgp in the parent and selected variants. Previous studies have shown that Pgp expression dose not correlate with 5-FU cross-resistance [22]. As resistance to taxol and vincristine decreases, the resistance to 5-FU decreases only slightly. A previous study in our lab on

the nasal septum carcinoma cell line RPMI-2650 found that taxol-selected variants displayed an increase in cross-resistance to 5-FU that was not observered in melphalan-selected variants [23].

The increased resistance in taxane-selected variants observed in the present study may be due to a number of mechanisms including increased drug efflux, alterations in microtubule dynamics, mutations/alterations in tubulin [24] and changes in expression of apoptosis-related proteins e.g. caspases and the bcl-2 family [25]. While alterations in Pgp expression have been linked to clinical resistance in breast cancer cell lines and tumour samples [22] and NSCLC [26], the results from Western blotting and combination assays on the variants in this work, suggest that other mechanisms are also important. Changes in the ß-tubulin isotypes have been noted in taxol-resistant variants with low level exposure where no change in MDR1 was observed [27].

From the combination assays here, it can be seen that if a combination of GF120918 and taxol show an effect in the parent, it is increased in the taxane-resistant variants. Further, no significant effect with GF120918 and taxol was observed in Adenocarcinomas or LCLC except for H1299 parent. Interestingly, no increase in H1299 resistant variants is seen. In H460 and variants, MRP-1 but not Pgp expression is detected whereas in H1299 and H1299-txl, the reverse is seen (Table II). The combination assays however show no strong effect on taxol-selected variants in either of the LCLC cell lines.

Pulse-selection with a chemotherapeutic agent has been known to affect other functions of the cell, such as proliferation and invasion. Taxol

treatment has been linked to decreased invasion and motility. A study on the effect of taxol on the adhesive and motility properties of human ovarian carcinoma cell lines, OVCAR 5, SK-OV-3, and HOC-1OTC [28] found that taxol significantly inhibited the motility of these cell lines but it did not affect their adhesion to the subendothelial matrix. In the present study, mixed effects on in vitro invasion were observed with taxol selection. H460-txl showed a dramatic decrease in invasion in agreement with the above study. However taxotere exposure led to a consistent increase in invasion. Previous studies in our laboratories have shown that selection with some chemotherapeutic agents can lead to increased in vitro invasiveness [23]. Another study on the invasive breast cancer cell line MDA-MB-435S-F found that selection with the agents taxol and adriamycin led to a more aggressive invasive phenotype termed "superinvasive" [29].

Conclusion

In this study, we have found that development of drug-resistant variants selected to reflect the clinical scenario, can produce variants with mechanisms of resistance different to those seen at high doses and/or continuous exposure. These variants thus provide a suitable in vitro model to study clinically relevant resistant mechanisms.

Conflict of interest

None declared

Acknowledgements

Figure legend:

Figure 1: IC_{50} values of parent cell lines to taxol and taxotere.

Cells were exposed to increasing drug concentrations and growth measured using the acid phosphatase assay. The IC_{50} value for each cell line was obtained from a plot of drug concentration versus percentage cell survival. Results are expressed as the average of three separate repeats.

Figure 2: Fold resistance of variants to taxol and taxotere.

The value for each IC_{50} was obtained from a plot of drug concentration versus percentage cell survival. Results are expressed as the average of three separate repeats. (SKLU1 IC_{50} values for taxotere were inconsistent and were not included).

Figure 3: Cross resistance profile of selected variants compared to the parental cell lines.

IC_{50} values (n=3) for six chemotherapeutic drugs (adriamycin, 5-fluorouracil, cisplatin, carboplatin, VP-16 and vincristine) were compared in the resistant cell lines.

Figure 4: Combination of taxotere and GF120918 in SKMES1-txt and expression of Pgp protein in SKMES1 and SKMES1-txt.

Graph (a) shows the percentage survival of SKMES1-txt treated with low-toxicity quantities of taxotere and the Pgp inhibitor GF120918 and the effect of the combination of these agents.

(b) Western blot analysis of Pgp protein expression in SKMES1 and SKMES1-txt. B-actin is included as an endogenous control.

Figure 5: Changes in <u>in vitro</u> invasiveness of taxol- and taxotere-selected variants compared to parental cell lines.

This invasion was quantified by counting the number of invading cells and results are expressed as the average of three separate repeats. (n=3).

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	Adr (nM)	Cis (uM)	Carbo(μM)	5-FU (uM)	VP-16 (uM)	Vcr (nM)
A549	29.41 ± 0.34	1.93 ± 0.11	15.78 ± 0.54	1.16 ± 0.11	0.20 ± 0.03	12.58 ± 1.7
SKLU1	46.07 ± 0.68	1.34 ± 0.07	7.53 ± 0.64	9.40 ± 0.57	0.20 ± 0.01	2.38 ± 1.02
H1299	51.51 ± 1.53	1.44 ± 0.09	9.95 ± 1.61	1.17 ± 0.12	0.19 ± 0.02	8.16 ± 0.34
H460	18.87 ±2.72	0.83 ± 0.10	6.46 ± 0.54	1.45 ± 0.13	0.13 ± 0.01	12.58 ± 2.04
SKMES1	12.41 ± 0.68	2.22 ± 0.26	12.37 ± 0.54	0.69 ± 0.05	0.10 ± 0.00	12.92 ± 1.02
DLRP	69.19 ± 5.27	0.74 ± 0.07	9.42 ± 0.97	9.61 ± 1.02	0.74 ± 0.07	11.12 ± 0.71
DMS-53	25.50 ± 2.89	0.50 ± 0.04	3.23 ± 0.27	5.63 ± 0.44	0.50 ± 0.04	27.78 ± 3.26

Table I: IC$_{50}$ values of parent cell lines to adriamycin, cisplatin, carboplatin, 5-FU, VP-16 or vincristine (Vcr).

Results are the average of a least three separate experiments.

Cell line	*Sulindac and adriamycin*	*MRP-1 expression*	*GF120918 and taxol**	*Pgp expression*	
A549	+	Y	-	N	AC
A549-tax	+	Y	-	N	
SKLU1	-	N	-	N	
SKLU1-tax	-	Y	-	N	LCC
H1299	-	N	+	Y	
H1299-tax	-	N	-	Y	
H460	-	Y	-	N	SCC
H460-tax	-	Y	-	N	
DLRP	-		-		
DLRP-txt	-		-		
SKMES1	-		++	Y	SCLC
SKMES1-tax	-		Nd	Y	
SKMES1-txt	-		+++	Y	
DMS-53	+		+	Y	
DMS-53-tax	-		+++	Y	
DMS-53-txt	-		+++	Y	

Table II: Summary of effects observed in combination assays.

AC=adenocarcinoma; LCC=large cell carcinoma; SCC=squamous cell carcinoma and SCLC=small cell lung carcinoma. + indicates at >20% enhanced effect; ++ indicates at >40% enhanced effect; +++ indicates at >60% enhanced effect; - indicates no effect; nd=not done. * Indicates that taxotere was used in combination assays for SKMES1, DLRP, DMS-53 and corresponding variants with GF120918.
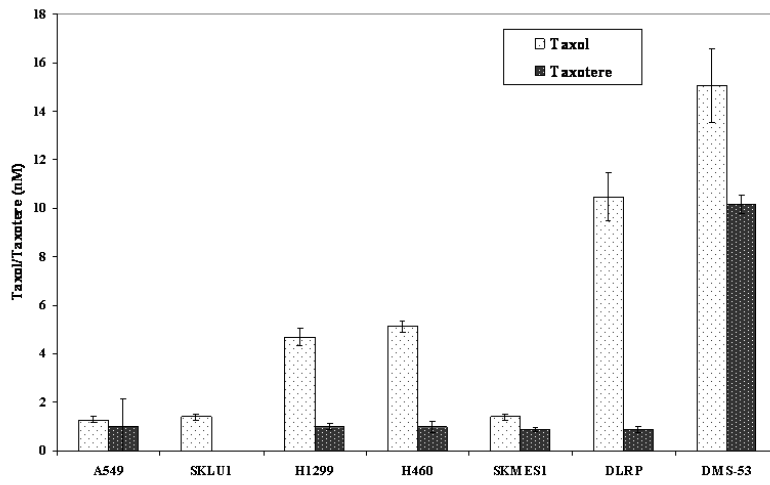
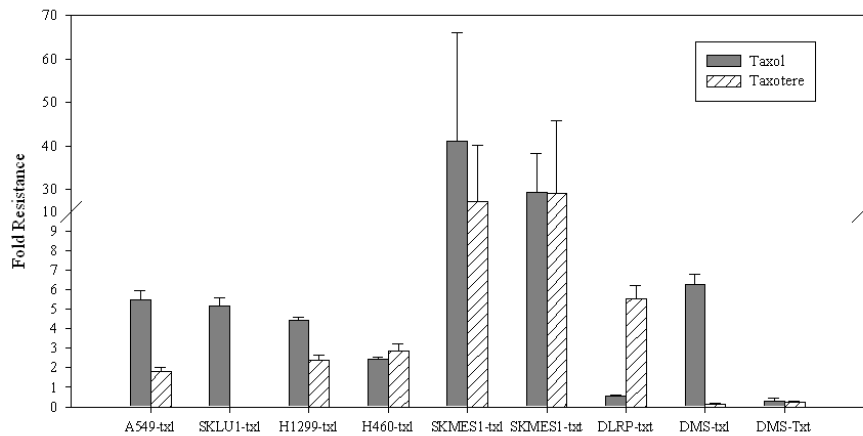**Figure 1: IC$_{50}$ values of parent cell lines to taxol and taxotere.**

Figure 2: Fold resistance of variants to taxol and taxotere.
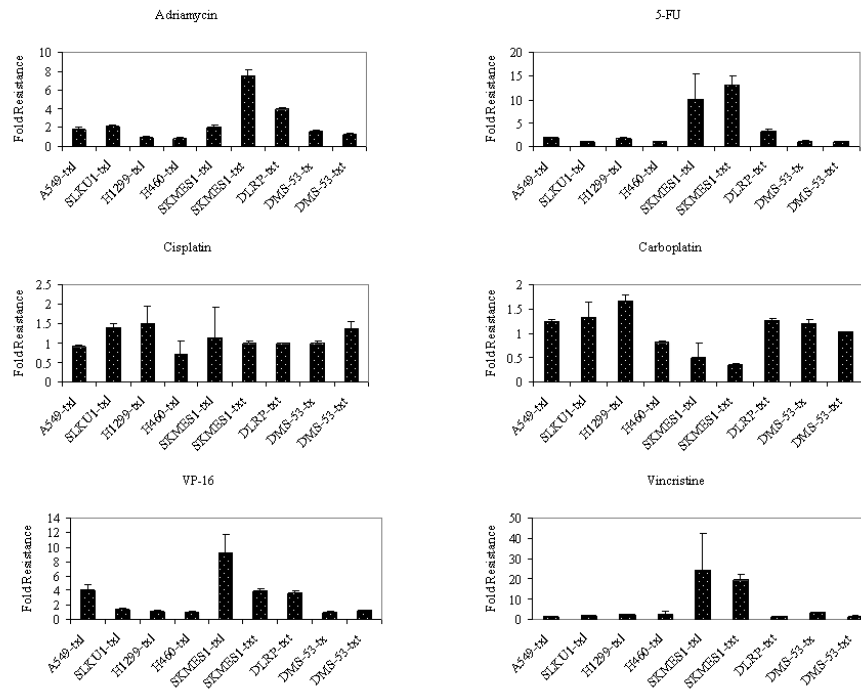
Figure 3: Cross resistance profile of selected variants compared to the parental cell lines.
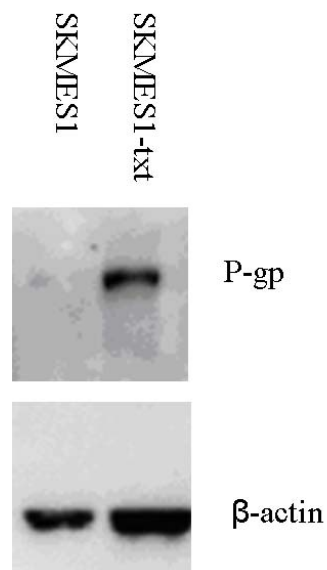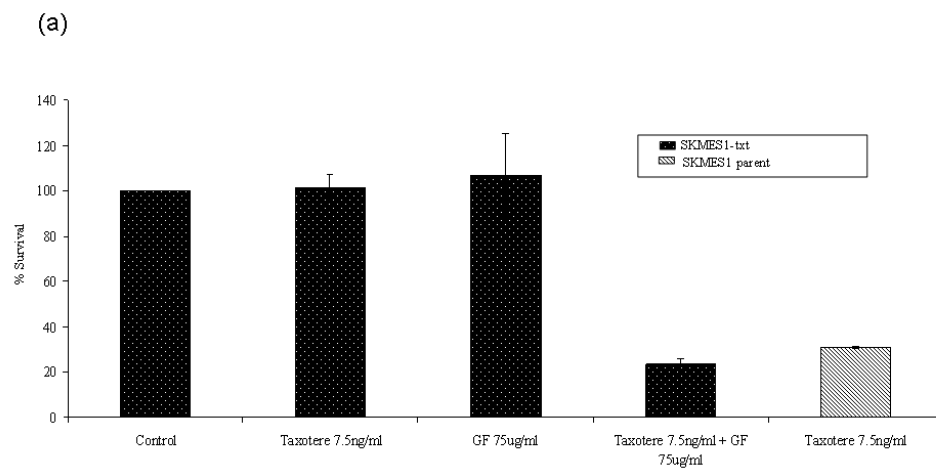
(a)

Figure 4: Combination of taxotere and GF120918 in SKMES1-txt and expression of Pgp protein in SKMES1 and SKMES1-txt.
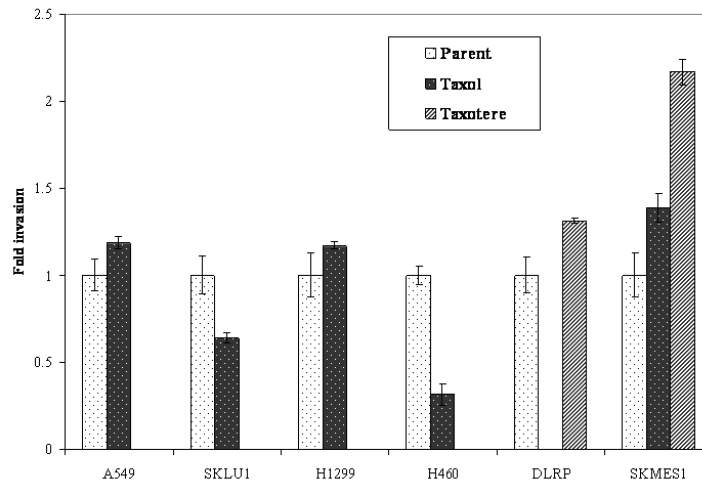
Figure 5: Changes in <u>in vitro</u> invasiveness of taxol- and taxotere-selected variants compared to parental cell lines.