Gene expression profiles as biomarkers for the prediction of chemotherapy drug response in human tumour cells

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Genome profiling approaches such as cDNA microarray analysis and quantitative reverse transcription polymerase chain reaction are playing ever-increasing roles in the classification of human cancers and in the discovery of biomarkers for the prediction of prognosis in cancer patients. Increasing research efforts are also being directed at identifying set of genes whose expression can be correlated with response to specific drugs or drug combinations. Such genes hold the prospect of tailoring chemotherapy regimens to the individual patient, based on tumour or host gene expression profiles. This review outlines recent advances and challenges in using genome profiling for the identification of tumour or host genes whose expression correlates with response to chemotherapy drugs both *in vitro* and in clinical studies. Genetic predictors of response to a variety of anticancer agents are discussed, including the anthracyclines, taxanes, topoisomerase I and II inhibitors, nucleoside analogs, alkylating agents, and vinca alkaloids. *Anti-Cancer Drugs* 18:499–523 © 2007 Lippincott Williams & Wilkins.

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Introduction

There can be little doubt that the field of genomics will have a tremendous impact in the field of oncology. This impact will likely be felt at numerous levels, enabling us to understand better the process of carcinogenesis, the genetic risk factors associated with the incidence of specific cancers, classification of various solid and haematological tumours, prediction of patient prognosis, and prediction of response or toxicity to various chemotherapy drugs. As evidence of this impact, hierarchical clustering of DNA microarray data with or without principal component analysis has been used successfully to identify subsets of various cancers, including breast cancer [1] and various leukemias [2]. Moreover, genome profiling can be useful in predicting prognosis in cancer patients. van't Veer and colleagues [3] in 2002 used DNA microarray analysis of primary breast tumours with supervised classification approaches to identify gene expression signatures strongly predictive of poor prognosis, even for lymph node-negative patients, who are generally considered to be of low risk for disease recurrence. Although for some cancers (colorectal, in particular), histological classifications (TNM/R0/R1/R2) remain the major determinants of clinical outcome, other studies suggest that genome profiling by DNA microarray analysis may be superior to standard clinical and histological approaches for prediction of prognosis in cancer patients [4–6]. A highly effective approach for prediction of clinical outcome may involve the combination of clinicopathological staging systems with prognostic gene signatures [7,8]. The ability of genome profiling to help classify tumours and predict prognosis will likely help triage patients, based on these classifications and predictions. It would, however, appear in the study by Van't Veer and colleagues [3] that the genes identified were predictive of prognosis regardless of the treatment regimen. Such prognostic biomarkers may thus be of limited value in helping to reduce mortality rates for certain cancers and in choosing treatment options for patients. Recently, considerable effort has been spent on identifying groups of genes whose expression can help predict a patient's response to specific chemotherapy drugs (predictive biomarkers), preferably in a drug-specific or regimen-specific manner. This would permit the tailoring of therapies to individual patients, on the basis of the expression profile of specific tumour or host genes.

Direct genome profiling of patient tissues to predict treatment response is highly problematic, because differences in response to chemotherapy drugs amongst patients can be attributed to a wide variety of host and tumour factors, including the natural history of the disease, drug metabolism by the host, drug interactions, age, sex, nutritional status and the function of specific organs involved in drug activation, detoxification or clearance. Although it is highly likely that direct assessment of tumour or host gene expression by

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microarray studies will identify genes whose expression correlates with treatment response (given the tens of thousands of genes surveyed), the false discovery rate is typically quite high. In addition, without an internal measure of drug response, the correlation between the expression of specific genes and 'response' to drugs may be largely related to prognosis. Genes associated with a good prognosis after chemotherapy may correlate well with patient survival, but may be completely unrelated to drug response. Clinical studies ethically do not include a nontreatment arm to enable one to differentiate between genes affecting prognosis and genes involved in drug response. Thus, it may be necessary to restrict clinical pharmacogenomic studies involving patients to subsets of genes whose expression has been shown to correlate with drug response in in-vitro or animal studies. In contrast to recent reviews in this area focusing on the role of host germline polymorphisms in determining chemotherapy efficacy and/or toxicity [9–11], this review will focus on recent advances in the use of tumour or host genome profiling to predict patient response to chemotherapy agents. A number of the major classes of chemotherapy agents used in the treatment of human cancers will be discussed, including their mechanisms of action, the genes that appear to affect drug cytotoxicity *in vitro* and the utility of various genes to serve as biomarkers for the prediction of response to the various agents in cancer patients. A considerably lesser emphasis will be placed on gene mutations and germline polymorphisms associated with chemotherapy drug response or genetic factors associated with drug toxicity in patients.

Genomic predictors of response to chemotherapy agents

Anthracyclines

Anthracyclines such as doxorubicin, epirubicin and daunorubicin are widely used in the treatment of a variety of human malignancies. Doxorubicin and epirubicin are most commonly employed to treat solid tumours, whereas haematological malignancies are often treated with daunorubicin [12–14]. At clinically relevant doses, the anthracyclines intercalate between DNA strands in cells, and inhibit both topoisomerase II activity and DNA synthesis [15–18]. The cellular effects of anthracyclines and their mechanisms of action have been debated over the years, with recent findings supporting the importance of DNA intercalation, topoisomerase II inhibition and chromatin structure rearrangements in anthracycline action [13,17–19]. Other effects of anthracyclines have been observed in cells, but many at concentrations that exceed those used clinically (reviewed in [17,19]). A number of mechanisms have been identified that induce resistance to anthracyclines *in vitro*, including the induction of the Abcb1 [12,20] and Abcc1 [21] drug-efflux pumps, the downregulation of topoisomerase II levels and activity [22,23], elevated expression of drug-conjugating enzymes such as the glutathione-*S*-transferases (GSTs) [24,25], alterations in p53 function [26], and a disruption in the ability of the drugs to induce apoptosis through reduced Fas receptor expression [27].

Despite significant progress in understanding resistance to anthracyclines in cultured tumour cells, there has been little progress in identifying biomarkers, which can reliably be used to predict response to this class of chemotherapy agents. Recently, a number of putative biomarkers were assessed for their ability to predict patient response to anthracyclines. These included Abcb1 [28–30], p53 [31–32], ErbB2 [31,33,34], Bcl2 [32,33], Mib1 [33] and Brca1 [35]. The expression levels of these proteins, however, appeared to be minimally effective at predicting response to anthracyclines, with the highest predictive rates (65–67%) occurring when the tumours of breast cancer patients were scored for expression of *BRCA1* [35], a gene involved in Rad51-dependent DNA repair [36,37], which when mutated, is linked to higher breast cancer incidence [38,39]. Although there are conflicting reports of the ability of *ABCB1* expression to predict resistance to anthracyclines in human tumours [28–30,40–42], *ABCB1* expression, by itself, is insufficient to predict resistance to anthracyclines in all patients. Moreover, there appears to be consensus that agents that inhibit Abcb1 activity do not appear to enhance significantly response rates to anthracycline-based chemotherapy in cancer patients [43–44]. Other predictive markers assessed in the past have included specific mutations in the gene for topoisomerase II [45] and a single nucleotide polymorphism (SNP) in the gene for interleukin (IL)-6 in which the nucleotide at position 174 (guanine) is changed to cytidine (174G→C) [46]. To date, however, robust biomarkers for the prediction of patient response to anthracyclines have yet to be identified. This may be, in part, owing to variability in tumour and/or host response to anthracyclines in cancer patients. Liem and colleagues in 2003 [47] showed that doxorubicin could cause an increase in tumour levels of p53, but only in patients whose tumours expressed the wild-type protein. Cardiotoxicity towards doxorubicin also varies significantly between patients [48], suggesting possible differences among patients in terms of drug metabolism and/or detoxification.

With the emergence of DNA microarray analysis, investigators have been able to identify thousands of genes whose expression changes in response to anthracyclines. Kudoh *et al.* [49] surveyed 5760 genes to identify changes in gene expression, which accompany selection of MCF-7 breast tumour cells for resistance to doxorubicin. Of the 300 gene-expression differences identified, 17 were transiently regulated by doxorubicin and played a role in cell cycle progression. Recently, Villeneuve *et al.* [50] examined, by cDNA microarray analysis, changes in gene expression that accompany the establishment of resistance to doxorubicin in MCF-7 cells. The approach

used was similar to that of Kudoh *et al.* [49], including the use of the MCF-7 cell line. Despite the use of an identical cell line, the genes changing expression upon selection for doxorubicin resistance differed significantly from those identified by Kudoh *et al.* [49] (see Table 1). The contrasting findings may be owing to differences in the number and nature of probes present on the microarray slides, and differences in both the number of replicate array experiments and the data analysis and normalization tools utilized [51]. Also, unlike the study by Kudoh *et al.* [49], a number of the findings by Villeneuve *et al.* [50] were confirmed by quantitative reverse transcription polymerase chain reaction (RT-PCR) [50,52].

Watts *et al.* [53] used a similar approach to identify 380 genes that were differentially expressed between wild-type and doxorubicin-resistant multiple myeloma cells. Of these genes, 29 were involved in apoptotic signalling (in particular cellular ceramide metabolism and mitochondrial permeability). Although many of the current standards in DNA microarray analysis were not employed in a number of these early studies, approximately 90% of the gene expression changes identified by Watts and colleagues [53] were confirmed in Northern blotting experiments. This study thus suggests that doxorubicin resistance may, in fact, stem from alterations in gene expression that result in a repression of doxorubicin's ability to induce apoptosis in cells.

In 2004, Kang *et al.* [54] used high-density oligonucleotide arrays to identify genes in drug-resistant gastric cancer cells whose expression correlated with resistance to various chemotherapy agents. Large 'genetic signatures' for resistance to 5-fluorouracil (5-FU), doxorubicin and cisplatin were established. The only significant gene commonly upregulated in drug-resistant cells coded for neurite growth-promoting factor 2 (midkine). This protein has been shown to be expressed in other drug-resistant cells, in which its growth-promoting properties act as a mediator for intercellular transfer of drug resistance *in vitro* [55]. Furthermore of interest is that four additional genes were identified in this study, whose expression correlated with drug resistance (*TOP2A*, *CLU*, *S100P* and *ABCC1*). These genes were also found to have altered expression upon selection for drug resistance to paclitaxel or doxorubicin in the previously cited study by Villeneuve *et al.* [50].

Short-term incubation of cells with doxorubicin is also known to affect gene expression. The expression of these genes may thus also serve as useful biomarkers for the prediction of response (rather than resistance) to anthracyclines. Chang and colleagues [56] treated colon carcinoma cells with doxorubicin and divided the cells into either a proliferative or a drug-induced senescent population. They then used DNA microarray analysis to identify changes in the expression of genes as cells acquired senescence in response to doxorubicin. The genes identified included a host of genes involved in DNA repair, replication, mitosis and DNA segregation, including topoisomerase II (one of the known targets of doxorubicin). A study in hepatoma cells also identified changes in topoisomerase II protein expression associated with anthracycline resistance (along with alterations in carbonic anhydrase expression) [57], giving further credence to the importance of topoisomerase levels as a measure of response to anthracyclines.

The acute changes in gene expression induced by the anthracyclines appear to be a direct effect of drug exposure. Recently, Bonilla and colleagues [58] used cDNA differential library screening to determine the gene expression differences that occur upon treatment of mouse fetal oocytes with doxorubicin at clinically relevant concentrations. These investigators were able to determine that doxorubicin induced changes in the expression of genes involved in the mitochondrial respiratory chain and in intracellular transport (metaxin and syntaxin 5). Moreover, the ability of 5 μmol/l doxorubicin to alter the expression of a transfected metaxin gene construct containing NF-κB and AP-2-binding sites suggests that the anthracyclines may directly affect gene expression at clinically appropriate doses. Doxorubicin also likely induces epigenetic changes in gene expression, given that the study by Bonilla *et al.* [58] found that the drug was able to induce changes in the expression of methionine adenosyltransferase, spermidine and spermine transferase.

Anthracycline-induced changes in gene expression also appear to be highly dependent upon cell type. In contrast to the above studies involving colon carcinoma cells, a similar approach with hepatoma cells indicated that doxorubicin induced the expression of topoisomerase II-β and carbonic anhydrase [57]. Niiya *et al.* [59] examined the changes that occurred in lung cancer cells treated with doxorubicin. These authors showed that there was an induction of tumour necrosis factor (TNF) α, urokinase, interleukin-8 (IL-8) and macrophage chemoattractant protein-1 (MCP-1) upon treatment with doxorubicin, even in the absence of any receptors for these ligands [59].

Even for a specific type of cancer, variations in cell type may affect sensitivity to the anthracyclines. Recently, Troester *et al.* [60] assessed this hypothesis using breast tumour cell lines derived from either luminal or basal cells. By hybridizing cDNA samples from each of the cell lines onto oligonucleotide arrays containing 18 861 human genes, the authors of this study were able to construct a 'genetic signature' for response to doxorubicin or 5-FU in each of the two breast cell types. Moreover, by combining the data into basal-derived and luminal-derived signatures, they were

able to identify smaller signatures that were unique to the cell types' response to either doxorubicin or 5-FU.

When one examines the above-described genes associated with response or resistance to the anthracyclines, few (by themselves) are predictive across a variety of cell lines. Nevertheless, one common theme appears to emerge in predicting response to the anthracyclines. Resistance or response to anthracyclines in cultured cells appears to involve changes in the expression of genes whose products are involved in stress response or apoptosis, including clusterin [50,54,61], syntaxin [50,58], GADD45 [61], S100P [54], carbonic anhydrase [57,61] and specific chemokine receptors [57,59]. These would be in addition to alterations in the expression of Abcb1 and topoisomerase II-α, both of which have been repeatedly shown to play a role in resistance to anthracyclines *in vitro*.

Two recent studies suggest that genome profiling may prove useful in predicting response to anthracycline-based chemotherapy in cancer patients. Using direct microarray profiling of tumour core biopsies, Cleator and colleagues [62] recently identified 92 genes whose expression in patient tumours correlated with sensitivity to doxorubicin/cyclophosphamide chemotherapy. The accuracy rate, however, was 67%, which is only somewhat greater than chance alone. In a similar fashion, Gyorffy *et al.* [63] identified 79 genes whose expression correlated with doxorubicin sensitivity or resistance in in-vitro experiments involving a variety of tumour cell lines. The ability of these genes sets to predict response to doxorubicin monotherapy in breast cancer patients was then assessed. The patient group having an expression pattern similar to that of doxorubicin-sensitive cells exhibited a longer survival (49.7 ± 26.1 months) than the resistant group (32.9 ± 18.7 months). Although these findings are encouraging, the error suggests that such differences may be of questionable significance. Even if significant, the high degree of error makes it unlikely that these genes could be used to accurately predict response to doxorubicin in all patients. Nevertheless, with some refinement of gene selection, it may be possible to use in-vitro studies to accurately predict clinical responsiveness to doxorubicin. A summary of the above-mentioned studies assessing possible genetic predictors of anthracycline response or resistance is depicted in Table 1.

Taxanes

The taxane family of chemotherapy agents binds to cellular microtubules and blocks their depolymerization [64], resulting in cell cycle arrest at mitosis and the subsequent induction of apoptosis [65,66]. Paclitaxel and most recently docetaxel are highly effective and widely used members of the taxane family. The mechanisms by which resistance to paclitaxel can occur in cultured tumour cells are varied, and include elevations in cellular levels of Abcb1 [67], p53 [68], Akt [69] or AURORA-A [70], downregulation of Bcl-2 expression [71], or mutations in genes coding for the α- or β-chains of tubulin [72,73]. Although the mechanism of action of the taxanes is fairly well understood, reliable biomarkers for the prediction of response to this class of chemotherapy agents in cancer patients have yet to be identified.

Genes that may play a role in clinical resistance to paclitaxel have been identified in a recent study through genome profiling of wild-type and paclitaxel-resistant ovarian and breast tumour cell lines [74]. Although a number of differentially expressed genes were identified by comparison of each cell line and its paclitaxel-resistant counterpart, only eight genes had higher expression and three genes lower expression in all of the paclitaxel-resistant cell lines studied (see Table 2). The most notable gene that changed expression in all three cell lines was *ABCB1*. Expression was elevated between 33-fold and 240-fold. Two other genes that were commonly upregulated included *MGC4175* and *MGC14772*. Together, the three genes reside on band 21 of the long arm of chromosome 7 (7q21), raising the prospect that paclitaxel resistance can result from a selective amplification of the 7q21 region of the human genome. It is unclear at this point whether the gene products for *MGC4175* and *MGC14772* also contribute to paclitaxel resistance. Three downregulated genes associated with paclitaxel resistance in all of the cell lines (cyclic nucleotide-gated channel-α 3, fibronectin and the opiod receptor) have not been previously reported to play a role in drug resistance and may represent novel biomarkers for the prediction of paclitaxel resistance in human tumours. In a similar approach [50], cDNA microarray analysis was conducted to identify differences in gene expression between the wild-type MCF-7 cell line and its isogenic, paclitaxel-resistant derivative cell line (MCF-7_{TAX}). Similar to the above study, dramatically higher *ABCB1* expression was observed in the MCF-7_{TAX} cell line compared with its MCF-7 parent. The study also identified an additional 20 genes upregulated upon selection for paclitaxel resistance. These genes coded for proteins involved in cell adhesion, cell growth promotion, apoptosis inhibition and drug transport. Twelve downregulated genes were also identified, which coded for promoters of apoptosis, growth inhibitors and lysosomal proteins that may play a role in the degradation of growth factors and/or Abcb1 [50].

Acute changes in gene expression associated with exposure of head and neck tumour cell lines (HN12 and HN30) to docetaxel for 48 h were identified recently by Yoo and colleagues [75]. In a survey of 1191 genes, 153 genes had altered expression upon treatment with docetaxel. The genes identified are known to play a role in cell cycle progression, apoptosis, oncogenesis, angiogenesis and tyrosine kinase signal transduction. Three

Table 1 Genes associated with response or resistance to the anthracyclines

Material description	Drugs tested	Methodology	Major findings	Reference
Wild-type and doxorubicin-resistant MCF-7 cells	Doxorubicin	cDNA microarray (5760 elements)	Determined gene expression differences in transient exposure to doxorubicin and in cells selected for resistance to doxorubicin. Untreated doxorubicin-resistant MCF-7 cells had changes in the expression of around 300 genes relative to wild-type MCF-7 cells. Doxorubicin exposure led to decreased expression of transcription factors and increased expression of zinc-finger proteins.	[49]
Wild-type and doxorubicin-resistant RPMI 8226 cell lines	Doxorubicin	cDNA microarray (5375 elements)	Doxorubicin-resistant cell lines exhibited 380 changes in gene expression relative to wild-type cells. Of these genes, 29 were involved in ceramide-dependent apoptosis and in changes in mitochondrial permeability. A verification rate of 90% was achieved when a subset of these genes were assessed for their expression level in Northern blotting experiments.	[53]
HCT116 human colon carcinoma cell lines	Doxorubicin	cDNA microarray (>9000 elements) (UniGEM v2.0 Incyte Genomics, Wilminton, Delaware, USA)	Cells were treated with doxorubicin to induce cellular senescence and the population sorted into senescent and proliferating phenotypes. Differences in gene expression between the two populations were then assessed by microarray analysis, with 63 of the 74 differentially expressed genes being confirmed by RT-PCR. Gene changing expression as cells acquired doxorubicin-induced senescence were involved in DNA repair, replication, mitosis and DNA segregation (including topoisomerase II-α).	[56]
Chronic myelogenous leukaemia patients	Doxorubicin	RT-PCR	The study demonstrated that resistance to doxorubicin in patients with chronic myelogenous leukaemia did not correlate with the expression level of the ABCB1 and ABCC1 drug-efflux pumps.	[40]
Tumour biopsies of breast cancer patients	Epirubicin, cyclophosphamide	Q-PCR; immuno-histochemistry	Using Q-PCR and immunohistochemistry, the study determined levels of BRCA1, BRCA2, ErbB2 and p53 expression and assessed their relationships to treatment response. BRCA1 expression level correlated with response to chemotherapy.	[35]
Primary mouse fetal oocytes	Doxorubicin	cDNA differential display	Using cDNA library differential screening, the study identified several genes whose expression changed in response to doxorubicin, including: MAT, COX II and III, MTX, STX5, RPS16, ND2 and 16CE.	[58]
Eight hepatoma cell lines	Doxorubicin, epirubicin and six other drugs	cDNA microarray (2300 elements)	In this study, the sensitivity of the hepatoma cell lines to each drug was assessed, after which pair-wise comparisons were made between the expression of each gene on the microarray and drug sensitivity using a relevance network. Forty-two genes had expression levels that correlated with chemosensitivity. Resistance to doxorubicin or epirubicin was linked to topoisomerase II-β expression levels. Carbonic anhydrase I mRNA expression also related to chemosensitivity to anthracyclines.	[57]
12 Lung carcinoma cell lines	Doxorubicin	Cancer chip (v2) (425 elements)	This study showed that incubation of lung carcinoma cells with 2–4 μmol/l doxorubicin maximally induced expression of TNF-α, uPA, IL-8 and MCP-1 even in the absence of TNF-α, uPA, and chemokine receptors.	[59]
10 Drug-resistant gastric cancer cell lines	Doxorubicin (5-fluorouracil) cisplatin	Affymetrix array (HG-U133A) (22 282 elements)	Array profiling uncovered sets of genes whose expression correlated with resistance to 5-fluorouracil, doxorubicin and cisplatin, with the expression of midkine being elevated in all resistant cell lines. Alterations in the expression of clusterin and S100P were specifically observed upon selection of cells for resistance to doxorubicin.	[54]
Two basal and two luminal breast cancer cell lines	Doxorubicin, 5-fluorouracil	cDNA microarray (18 861 elements)	Differences in gene expression were used to distinguish between basal and luminal cell lines and their sensitivity to doxorubicin or 5-fluorouracil; 920 gene expression differences were noted. The expression level of 100 of these genes was then successfully used to classify tumours as basal or luminal and to classify their sensitivity to doxorubicin or 5-fluorouracil.	[60]
Primary breast cancer core biopsies	Doxorubicin, cyclophosphamide	Affymetrix array (HG-U133A) (22 282 elements)	In this study, patients were divided into chemotherapy-resistant and chemotherapy-responsive populations. Array profiling then identified 253 differentially expressed genes between the drug-sensitive and drug-resistant populations. The expression levels of these genes could be used to predict response to chemotherapy in patients with an accuracy of between 61 and 67%.	[62]
13 Human cell lines	Doxorubicin, mitoxantrone	CDNA microarray (43 000 elements)	The expression of 79 and 70 genes were found to correlate with resistance to doxorubicin and mitoxantrone, respectively. The gene profiles were then successfully used to predict patient survival after chemotherapy.	[63]
Patients with acute myeloid leukemia	Daunorubicin, cytarabine	Integrated ABCB1 score	This study observed that higher ABCB1 expression and function was correlated with clinical response to daunorubicin/cytarabine chemotherapy. Inhibition of ABCB1 function by PSC-833, however, had no effect on the efficacy of chemotherapy.	[43]
Wild-type, doxorubicin-resistant and paclitaxel-resistant MCF-7 cell lines	Doxorubicin, Paclitaxel	University health network 1.7K arrays (3840 elements)	Using cDNA microarray analysis, this study identified 29 genes which had altered expression upon selection of MCF-7 cells for resistance to doxorubicin. Thirteen of these changes in gene expression were verified by Q-PCR or immunoblotting experiments. The genes identified included CAV1, ABCB1, SAT,	[50]

Table 1 (Continued)

Material description	Drugs tested	Methodology	Major findings	Reference
			LGALS3, MDH2, FDFT1, STX3A, ZFP36L2, TOMM20, CRYAB, caspase-9 and ABCG2. Of these, only ABCB1 and ABCG2 expression were also found to have altered expression (in the same direction) upon selection for resistance to paclitaxel.	
Tumour cells (47 various tumour samples)	Doxorubicin and other chemotherapy agents	Q-PCR	This study assessed the changes in gene expression that occurred as tumour cells adapted to the presence of various chemotherapy agents over 6 days in culture. Genes that showed altered expression included ABCB1, ABCC1, MVP1 (upregulated) and TOP2A (downregulated). ABCB1 or ABCG2 expression was unchanged after exposure to doxorubicin for 6 days.	[116]
30 Cell lines	11 Chemotherapy drugs	Affymetrix array (HG-U133A) (22 282 elements)	This study found 67 genes whose expression correlated with resistance to four or more chemotherapy drugs. These changes in gene expression were verified by Q-PCR.	[136]
Tumour biopsies from breast cancer patients	Epirubicin	Immunohistochemistry	These investigators found that tumour p53 expression (but not bcl-2 expression) correlated with patient response to epirubicin chemotherapy. The data also suggested that the response rate was lower for patients expressing the ABCB1 drug-efflux pump.	[32]

IL-8, interleukin-8; Q, quantitative; RT, reverse transcription; PCR, polymerase chain reaction; TNF-α, tumour necrosis factor-α.

recent studies have also looked at the response of the genome to docetaxel in PC3 and LNCaP prostate cancer cell lines (alone or in combination with other agents). In the first study [76], the cell lines were treated with docetaxel for 6, 36 and 72 h. Of the genes surveyed, 1785 and 964 genes changed expression after 72 h of treatment with docetaxel in PC3 and LNCaP cells, respectively. The genes identified coded for tubulin, microtubule receptor-related and androgen receptor-related genes, and genes associated with cell cycle progression, apoptosis, oncogenesis and gene transcription. The remaining studies examined the changes in gene expression associated with treatment of the same prostate cancer cell lines with either Furtulon [77] or Estramustine [78]. Interestingly, very similar findings were obtained, except that additional genes were identified as a result of the addition of another chemotherapy agent. The combination treatments also increased expression of specific cytochrome P450 enzymes and S100P, similar to observations in paclitaxel-resistant MCF-7 cells [50]. This suggests that the expression of cytochrome P450 or S100P may be associated with general resistance to the taxanes rather than specifically in docetaxel resistance.

It is also worth noting that some drugs appear to have the capacity to induce very strong cross-resistance to other drugs. For example, our laboratory has shown recently [79] that selection of MCF-7 cells for resistance to doxorubicin resulted in the generation of an MCF-7_{DOX} cell line having 52-fold and 124-fold resistance to doxorubicin and epirubicin, respectively. In contrast, the MCF-7_{DOX} cell line exhibited 4720-fold and 14 640-fold cross-resistance to paclitaxel and docetaxel, respectively. This phenomenon is likely related to the nature of the proteins involved in the establishment of doxorubicin resistance and their ability to affect sensitivity to other drugs. For example, long-term exposure to doxorubicin may select for elevated expression of specific drug transporters that result in a greater increase in efflux of paclitaxel compared with doxorubicin, as suggested by the above-described study. Moreover, the study may further suggest that genes changing expression in response to selection for doxorubicin resistance may be equal or better predictors of response to taxanes and possibly other drugs.

Continued exposure of tumour cells to chemotherapy agents can often result in selective amplification of specific genes whose products promote drug resistance. For example, comparative genomic hybridization was used recently to identify changes in gene copy number between drug-sensitive breast tumour cell lines (MCF-7 and MDA-MB-231) and progeny of these cell lines selected for resistance to docetaxel [80]. Similar to observations with paclitaxel-resistant cell lines, both docetaxel-resistant cell lines exhibited a 7q amplification (as well as a loss of 10q). The increased *ABCB1* expression associated with the 7q amplification also resulted in increased expression of its gene product, as seen through immunoblotting experiments. The doctaxel-resistant MCF-7 cells also gained an additional chromosome 18 and lost the short arms of chromosomes 6 and 12 (6p and 12p). In contrast, the doctaxel-resistant MDA-MB-231 cells showed a gain of chromosomes 5p and 9, and a loss of chromosomes 4, 8q, 11q, 12q, 14q and 18. The authors of this study suggested that the amplification of 7q21 and loss of 10q may represent a common mechanism of acquired docetaxel resistance in breast cancer cells.

Drug resistance-related genes identified through the study of wild-type and drug-resistant tumour cell lines in culture may or may not have relevance in cancer patients whose tumours have three-dimensional structure and various levels of vascularization. Genes that may prove useful for the prediction of taxane response in patients

Table 2 Genes associated with response or resistance to the taxanes

Material description	Drug(s) tested	Methodology	Major findings	Reference
Primary breast cancer (24 patients, pretreatment)	Neoadjuvant, docetaxel	HgU95-Av2 GeneChip (Affymetrix)	This study identified 92 tumour genes associated with docetaxel response, with 14 genes having higher expression in docetaxel-resistant patients and 78 genes having higher expression in docetaxel-sensitive patients. Increased β-tubulin expression was associated with resistance in some tumours. The expression of these genes showed significant ability to predict response to docetaxel.	[81]
Primary breast cancer (13 patients, posttreatment)	Neoadjuvant, docetaxel	HgU95-Av2 GeneChip (Affymetrix)	Using posttreatment tumours from 13 patients in the above study, *t*-tests did not identify any changes in gene expression upon treatment with docetaxel by cDNA microarray analysis. The genetic profile of tumours was highly similar between patients after 3 months of docetaxel treatment.	[82]
Wild-type and paclitaxel-resistant ovarian carcinoma (SKOV-3 and OVCAR8) and breast carcinoma (MCF-7) cell lines	Paclitaxel	HgU95-Av2 GeneChip (Affymetrix)	When gene expression was compared between the paclitaxel-resistant cell lines and their parental counterparts by cDNA microarray analysis, hundreds of genes had either increased or decreased expression in the paclitaxel-resistant cell lines. Eight genes had increased expression in all three paclitaxel resistant cell lines (GBP1, Toll-like receptor 6, CATP-III, human testis-specific basic protein, MGC4175, MGC14772, NF-κB-2 and ABCB1). Three genes had decreased expression in all three paclitaxel-resistant cell lines (cyclic nucleotide-gated channel α-3, fibronectin and the opiod receptor).	[74]
Patients with primary or locally recurrent breast cancer (70 patients, pretreatment)	Docetaxel	ATAC-PCR (2453 genes)	In this study, ATAC-PCR was performed on RNA from the tumours of 44 patients (22 responders, 22 nonresponders). In this learning set, 61 genes were found to have higher expression in nonresponders and 24 genes had increased expression in responders. Using tumours from 26 patients as a validation set, it was determined that the genes had an 80.7% accuracy in differentiating between responders and nonresponders. Expression of tubulin was elevated in nonresponders, but the most prominent characteristic was increased expression of genes controlling the cellular redox environment. The study then confirmed the role of redox genes in paclitaxel resistance using in-vitro transfection experiments.	[83]
Human breast cancer cell lines (MCF-7, MDA-MB-231) and derivatives of the cell lines resistant to docetaxel	Docetaxel	CGH arrays	This study compared gene copy numbers between wild-type and docetaxel-resistant cell lines by comparative genomic hybridization. Found a common region of amplification (7q) and a common region of loss (10q) in both docetaxel-resistant cell lines. The docetaxel-resistant MCF-7 cell line had a gain of chromosome 18, but there was a loss of this chromosome in the docetaxel-resistant MDA-MB-231 cell line. Chromosome gains and losses were confirmed by fluorescence in-situ hybridization. Increased expression of ABCB1 (found on chromosome 7q) was confirmed in immunoblotting experiments.	[80]
Prostate cancer cell lines (PC3 and LNCaP)	Docetaxel	Affymetrix array (UG113A) (22 282 elements)	In cDNA microarray experiments, 1785 and 964 genes changed expression after 72 h of treatment with docetaxel in the PC3 and LNCaP cell lines, respectively. Genes changing expression coded for the six major classes of proteins: tubulin and microtubule-related proteins, cell cycle regulators, apoptosis modulators, oncoproteins, transcriptional regulators, and androgen receptor-related proteins.	[76]
Head and neck squamous carcinoma cells (HN12 and HN30)	Docetaxel	cDNA microarrays (1191 elements)	In this study, docetaxel treatment caused 153 genes to change expression in both of the cell lines. Changes in gene expression were confirmed by PowerBlot analysis, imunoblot experiments, flow cytometry and ELISA assays. Genes changing expression were involved in either cell cycle progression, the regulation of apoptosis, angiogenesis, or tyrosine kinase signal transduction.	[75]
Xenografts of the human ovarian carcinoma cell line 1A9 and its paclitaxel-resistant cell line 1A9PTX22	Paclitaxel	cDNA microarrays	In the 1A9 cells, 26 and 61 genes changed expression 4 and 24 h posttreatment with paclitaxel, respectively. Early responsive genes were mostly signal transduction proteins and transcriptional regulators, whereas late responsive genes were involved in cell proliferation/differentiation, apoptosis, cell metabolism, and protein biosynthesis and trafficking. In paclitaxel-resistant 1A9PTX22 cells, 9 and 7 genes changes expression 4 and 24 h posttreatment with paclitaxel, respectively, suggesting paclitaxel resistance. The only genes changing expression in the paclitaxel-resistant cell lines were CRADD, TFAP4, PCTP, SDHD, SEC61G and TCL6.	[84]
Prostate cancer cell lines (PC3 and LNCaP)	Docetaxel and/or furtulon	Affymetrix array (UG113A) (22 282 elements)	This study observed alterations in gene expression occurring as early as 6 h after treatment with docetaxel, and these changes were more evident with longer treatment. Docetaxel treatment caused a decrease in the expression of genes involved in cell cycle progression, transcription and oncogenesis. In contrast, the drug induced increased expression of genes involved in the induction of apoptosis, cell cycle arrest and tumour suppression. Docetaxel/Furtulon treatment upregulated some genes responsible for	[170]

Table 2 (Continued)

Material description	Drug(s) tested	Methodology	Major findings	Reference
			chemotherapy resistance, suggesting the induction of cancer cell resistance genes upon prolonged exposure to the drug combination.	
Prostate cancer cell lines (PC3 and LNCaP)	Docetaxel, estramustine	Affymetrix array (UG113A) (22 282 elements)	Both mono and combination treatment caused a downregulation of some genes for cell proliferation and apoptosis inhibition, transcription factors and oncogenesis. These treatments also induced an upregulation of some genes related to the induction of apoptosis, inhibition of cell proliferation and tumour suppression. The combination of both drugs caused even more gene changes not seen in monotreatment that were related to cell proliferation, oncogenesis, angiogenesis, invasion and differentiation.	[78]
Wild-type, doxorubicin-resistant, and paclitaxel-resistant MCF-7 cell lines	Doxorubicin, paclitaxel	University Health Network 1.7K arrays (3840 elements)	Using cDNA microarray analysis, this study identified 32 genes which had altered expression upon selection of MCF-7 cell for resistance to paclitaxel. Twelve of these changes in gene expression were documented by Q-PCR or immunoblotting experiments. The genes identified included RDC1, IFI30, FURIN, S100P, ABCB1, FXYD3, MCP, BCL2, FTT1, TUBB4, FDFT1 and ABCG2. Of these, only ABCB1 and ABCG2 expression were also found to have altered expression (in the same direction) upon selection for resistance to doxorubicin.	[50]
Patients with ovarian cancer	Paclitaxel carboplatin	Pyrosequencing	This study observed that the incidence of a single nucleotide polymorphism (G2677T/A) in the gene coding for ABCB1 correlated with response to paclitaxel/carboplatin chemotherapy. There was a dose-dependent relationship between the number of mutated alleles and response to treatment.	[88]

ATAC, adapter-tagged competitive; PCR, polymerase chain reaction; Q, quantitative.

were identified in two recent studies comparing gene expression between breast cancer patients before treatment and after 3 months of treatment with docetaxel. Expression profiling of the pretreatment samples was then conducted to determine which genes showed differential expression between the tumours that responded to docetaxel and those that did not [81,82]. Fourteen genes had higher expression in pretreatment tumours that were resistant to therapy, whereas 78 genes had increased expression in pretreatment tumours that responded to docetaxel treatment. Tumour genes associated with resistance to therapy encoded for proteins involved in protein translation, cell cycle progression or RNA transcription. An increase in the expression of β-tubulin was also associated with docetaxel resistance in some but not all tumours. Tumours sensitive to docetaxel treatment had alterations in the expression of genes involved in the cellular stress response, apoptosis, cell adhesion, protein transport, cellular signal transduction or RNA splicing [81]. When the posttreatment tumour samples from 13 random patients were similarly profiled, no statistically significant differences in gene expression between sensitive and resistant tumours could be obtained that could not have been found by chance alone [82]. This suggests that docetaxel-resistant and docetaxel-sensitive tumours could no longer be differentiated by gene profiling experiments 3 months after treatment [82]. The authors suggested that there are probably several possible explanations for the loss of gene expression differences between docetaxel-responsive and docetaxel-resistant tumours. These include the selection of drug-resistant clones upon treatment with docetaxel or enhanced detection of normal breast tissue after treatment. In the 13 patients that were assessed after treatment, however, the gene expression pattern was populated by genes involved in cell cycle arrest at G_2/M, and survival pathways involving the mammalian target of rapamycin (mTOR). The authors concluded that they could determine a specific and consistent gene expression pattern in residual tumours after docetaxel treatment that could provide therapeutic targets that could lead to improved treatment [82].

Another study aimed at identifying genomic predictors of docetaxel response in tumours of breast cancer patients was published recently. In this study, the highly quantitative approach of adapter-tagged competitive PCR was used to profile gene expression in docetaxel-responsive and docetaxel-resistant tumours [83]. This study included a total of 70 patients with primary or locally recurrent breast cancer, and surveyed a total of 2453 genes from the tumours before treatment with docetaxel. Of the 70 tumours collected, 44 (22 from responders and 22 from nonresponders) were used as a learning set, and 26 were used as a validation set. A total of 85 different genes were identified as differentially regulated in the learning set. Sixty-one genes had higher expression in nonresponders, whereas 24 had higher expression in responders. Using this gene set, there was an 80.7% accuracy in predicting responders versus nonresponders. Of the genes identified, tubulin expression was elevated in nonresponders, but the most prominent class of genes exhibiting increased expression were cellular redox genes (GST-π, glutathione peroxidase

I, thioredoxin, peroxiredoxin 1). The possible role of the redox genes in docetaxel resistance was confirmed in cell transfection experiments, whereby transfection of MCF-7 cells with constructs coding for the overexpression of either GST-π, thioredoxin or peroxiredoxin 1–GST were found to protect MCF-7 cells from docetaxel-induced death. Whereas these observations are highly encouraging, the genes associated with docetaxel response or resistance were widely different in the two above studies. Moreover, the ability of redox gene expression to predict tumour resistance to killing by docetaxel remains to be assessed in independent confirmatory clinical trials.

An additional approach to identify genetic predictors of response to the taxanes involved xenografting the human ovarian carcinoma 1A9 cell line or a paclitaxel-resistant variant of this cell line (strain 1A9PTX22) in nude mice [84]. Upon establishment of 1A9 or 1A9TX22 tumours, all mice were treated with paclitaxel for 0, 4 or 24 h, after which tumours were removed and cDNA microarray analysis was performed. In the 1A9 tumours, a 4 or 24 h incubation with paclitaxel resulted in changes in the expression of 26 and 61 genes, respectively. Early response (4 h) genes coded mostly for proteins involved in signal transduction and transcriptional regulation, whereas late response genes (24 h) coded for proteins involved in cell proliferation/differentiation, apoptosis, cell metabolism and protein biosynthesis and trafficking. In the resistant 1A9PTX22 tumours, only nine and seven genes changed expression after 4 or 24 h, respectively. The genes that changed expression solely in paclitaxel-resistant tumours included *CRADD*, *TFAP4*, *PCTP*, *SDHD*, *SEC61G* and *TCL6*. These genes code for proteins involved in apoptosis, metabolism and protein biosynthesis and trafficking. One significant advantage to the xenograft model is that it allows for the study of a homogeneous population of cells in a tumour microenvironment that includes the effects of tumour vascularization. Pharmacodynamic studies and an assessment of the role of cell substratum/intercell adhesion in drug resistance are also possible using this approach. The relevance of the genes identified above in paclitaxel resistance in humans, however, has not been assessed.

The large number of studies examining changes in gene expression associated with response to paclitaxel or docetaxel have helped to identify thousands of putative genes, which may serve as biomarkers of taxane sensitivity in cancer patients. Although taxane response or resistance genes varied considerably from study to study (except for genes such as *ABCB1*), the functional roles that their gene products play within cells appear to be similar. In general, the products of genes associated with taxane resistance play a role in the regulation of apoptosis, cell cycle progression, microtubule function and gene transcription (see Table 2). Thus, the pathways to taxane response or resistance may be shared among cells, but the precise mechanisms by which taxane response or resistance is achieved may vary considerably. Thus, changes in the expression of sets of genes representing various biochemical pathways may be better predictors of response/resistance to taxanes than changes in the expression of individual genes. Although changes in ABCB1 expression have been observed in numerous studies of taxane-resistant cells [67,85], its expression did not appear to be associated with acute response to taxanes in drug-sensitive cells [75,78,84]. Moreover, no clear correlation between *ABCB1* gene expression and clinical response to taxanes has been observed for a variety of tumours [86,87] and an inhibitor of ABCB1 activity showed little efficacy in increasing response rates for breast cancer patients treated with a taxane-based chemotherapy regimen [44]. This suggests that Abcb1 may not play a significant or general role in taxane response/resistance in patients with breast cancer. It remains possible that increased *ABCB1* expression may predict clinical resistance to the taxanes in subsets of breast cancer patients, in particular those with tumours exhibiting particular gene amplifications or polymorphisms. For example, the G2655T/A polymorphism in the *ABCB1* promoter has recently been shown to correlate with response to paclitaxel/carboplatin chemotherapy in women with ovarian cancer [88]. A summary of the above-mentioned studies assessing possible genetic predictors of taxane response or resistance is depicted in Table 2.

Topoisomerase I inhibitors – camptothecin analogs

The camptothecin family of chemotherapy agents [which include irinotecan (CPT-11) and topotecan (TPT)] are typically used in the treatment of colorectal cancer. They have, however, shown promise in the treatment of a variety of neoplasms including the leukemias, lung, cervical, ovarian and stomach cancers [89–94]. The primary target of the camptothecins is the enzyme topoisomerase I. This enzyme functions to induce single-strand nicks in DNA to relax supercoiling structures during replication. In addition, topoisomerase I phosphorylates certain splicing factors and acts as a recombinase, facilitating illegitimate recombination. The enzyme also regulates gene expression through its ability to associate with other proteins in various transcription complexes [95]. Camptothecins induce their cytotoxic effects by stabilizing a reversible covalent intermediate between topoisomerase I and the nicked 3′-phosphate end of the DNA strand [96,97]. This complex prevents DNA religation and upon the replication forks encountering the topoisomerase I-DNA lesions in S-phase, the replication machinery induces irreversible double-strand breaks and subsequent cell death via apoptosis [97,98]. Interestingly, the camptothecins appear to be able to induce apoptosis independent of their effects on DNA replication, as the will also kill nondividing neuronal cells [99].

Camptothecin was the first topoisomerase I inhibitor to be investigated for its anticancer properties. Phase I and

II clinical trials were, however, terminated owing to low antitumour efficacy and severe unpredictable toxicity [100]. Identification of topoisomerase I as a primary drug target led to rejuvenated interest in camptothecin and the development of viable sister compounds. Recent studies have helped identify possible molecular markers of resistance to camptothecin. Ubiquitin-mediated proteolysis of topoisomerase I or downregulation of topoisomerase I gene expression appear to both play a role in the ability of human tumour cells to evade the cytotoxic effects of camptothecin and such mechanisms have been demonstrated in recent studies [101,102]. Microsatellite instability and subsequent loss of the DNA mismatch repair gene *MLH1* have also been shown to decrease sensitivity to camptothecin and its analogs *in vitro* [103]. Certain members of the ATP-binding cassette (ABC) family of drug transporters have also been implicated in resistance to camptothecins, including Abcc2/cMOAT [104,105] and Abcg2/BCRP [106,107]. Camptothecins and related compounds, however, are not typically Abcb1 substrates [105,108]. cDNA microarray studies of RNA isolated from untreated and camptothecin-treated cells or from camptothecin-sensitive and camptothecin-resistant cells have allowed for the development of possible molecular signatures indicative of cellular response to camptothecins. Similar to what has been observed for other chemotherapy agents, genes related to intracellular signaling, cellular adhesion, apoptosis, cell cycle and metabolism have all been identified as having altered expression upon treatment of cells with camptothecin or upon selection for camptothecin resistance. Specifically, genes coding for proteins related to the NF-κB (TRAF2, TNF-α, IL-1), MAPK (c-fos/c-jun), Bcl-2 (Bax, Bcl-2) and p53 (p21/Waf, c-Myc) pathways were identified as important in camptothecin response [102,109–112]. Mutations within the topoisomerase I coding sequence (such as R364H) have also been shown to play a role in cellular response to campothecin, in which the mutations render the enzyme resistant to inhibition by camptothecin, although having no effect on catalytic activity [113].

Irinotecan (CPT-11) is a carboxylate derivative of the parental camptothecin molecule, which displays increased cytotoxicity levels approximately 1000 times that of the base compound. Intracellular or extracellular conversion of CPT-11 into SN-38 is accomplished by the carboxylesterases CE1 or CE2, with CE2 having a higher K_m value than CE1 [114]. Multiple polymorphisms have been identified in the CE2 gene, yet none affects catalytic activity and hence CPT-11 activation [115]. The transporters Abcg2/BCRP and Abcc4/Mrp4 have been demonstrated to confer considerable resistance to CPT-11 both *in vitro* and *in vivo*, with some studies suggesting that resistance to CPT-11 by ABC transporters requires continued selective pressure and is cell type-specific [116–118]. Downregulation of the gene for topoisomerase II has been associated with CPT-11 treatment across multiple tumour types and may be a useful biomarker for predicting clinical response to CPT-11 [119–121]. Changes in gene expression induced by CPT-11 as determined by DNA microarray studies identified pathways similar to those described previously for its parent compound (camptothecin), which may be useful in predicting clinical respone to CPT-11 [119–122]. The expression of NF-κB has been shown to be particularly effective in predicting clinical outcomes to the camptothecins (including CPT-11) and other chemotherapy drugs [123].

TPT is a second analog of camptothecin. As a result of its relatively recent introduction, however, there are few studies that have identified genes involved in response/resistance to this agent. It appears that the ABC transporters Abcg2/BCRP and Abcc4/Mrp4 may play a role in TPT resistance [124,125]. In addition, similar to camptothecin, loss of the DNA mismatch repair gene MLH1 owing to microsatellite instability appears to confer resistance to TPT *in vitro* [103]. Moreover, the MAPK (c-fos/c-jun) and p53 pathways were identified as being affected by TPT treatment, and clinical response to the agent in patients was found to be highly dependent upon the p53 status of their tumours [126,127]. A summary of the above-mentioned studies assessing possible genetic predictors of response or resistance to the camptothecin family of topoisomerase I inhibitors is depicted in Table 3.

Topoisomerase II inhibitors

Epipodophyllotoxins (EPPT) comprise the major group of topoisomerase II inhibitors. The molecular targets of EPPTs are the topoisomerase II-α and -β isoforms, which induce double-strand breaks in DNA to relax supercoiling, with subsequent religation to repair the induced lesions [128,129]. Although the complete mechanism of EPPT-induced cytotoxicity is not fully understood, this class of topoisomerase II inhibitors is thought to act, at least in part, in the step preceding topoisomerase II-mediated DNA religation [130]. Topoisomerase II inhibition results in the induction of apoptosis and decreased expression of genes associated with cell survival [131].

The presence or expression of several gene products may help predict patient response to EPPTs. For example, transfection of caspase-3-deficient MCF-7 cells with an expression vector coding for wild-type caspase-3 restores the ability of etoposide, an EPPT analog, to kill the cell line [132], suggesting that the presence and/or level of expression of caspase-3 in tumour cells may correlate with patient responsiveness to treatment with etoposide. The increased expression of antiapoptotic members of the Bcl-2 family of proteins (namely Bax and Bcl-x_L) has been also shown to be associated with prosurvival signals and decreased cellular sensitivity to etoposide [133]. Moreover, the expression of Bax and Bcl-x_L appears to be

Table 3 Genes associated with response or resistance to topoisomerase I inhibitors

Materials tested	Drugs tested	Methodology used	Major findings	Reference
Cervical cancer cells (HeLa)	Camptothecin	Affymetrix array (22 283 elements), viability assays	The study identified 683 genes associated with CPT resistance, including NF-κB, EGFR, genes involved in chromatin remodeling and p53. Treatment with an EGFR inhibitor decreased CPT resistance.	[109]
U87-MG and resistant U87-CPTR astrocytoma cells		Agilent human microarray (13 508 elements), Q-PCR	The study identified 494 genes associated with CPT response, including genes involved in NF-κB signaling, angiogenesis, apoptosis, drug metabolism, drug resistance, and DNA repair (topoisomerase II-α).	[102]
30 colorectal cancer cell lines		cDNA microarray (9216 elements), Q-PCR	The study identified 143 genes whose expression correlated with CPT-induced apoptosis. MMR and p53 levels did not appear to affect response to CPT.	[110]
HCT116 colorectal cancer (CRC) cells		cDNA microarrays (1694 elements)	Thirty-three genes relating to CPT toxicity were identified in this investigation, including proteins involved in p53 signalling, cell cycle arrest in G_2, and NF-κB survival pathways.	[111]
Wild-type and camptothecin-resistant prostate cancer cell lines		DNA sequencing of RT-PCR products	This study characterized the activity and sensitivity of a mutant form of topoisomerase 1 where arginine 364 was replaced with histidine (R364H). The mutant enzyme was fully active but CPT-resistant.	[113]
Prostate (DU-145) and CPT-resistant DU-145 cells		'Oncochip' cDNA microarray (1648 elements)	This investigation identified 181 genes correlating with response to CPT, including genes coding for proteins involved in chromatin modification, apoptosis (Bcl2 family members), Akt/protein kinase B signaling, and transcriptional regulation. A small nucleotide polymorphism in the gene for topoisomerase I also appears to play a role in CPT resistance.	[112]
CRC cells (HCT116)		Giemsa cytotoxicity assays	This study found that loss of the MMR gene (MLH1) but not MSH2 was associated with resistance to CPT.	[103]
Leukemia cell line (HL60) and patient leukemias CML (2), AML (2).	Irinotecan (CPT-11, SN-38)	cDNA microarray (3011 elements); Q-PCR	Thirty-one genes whose expression correlated with CPT-11 response were identified in this investigation, including genes coding for survivin, NF-κB and cell cycle/cell adhesion proteins. CPT-11 treatment of HL60 cells also affected the expression NF-κB, Bcl-2, AP-1 and cell cycle related proteins.	[119]
47 Tumours of various cell origins		Q-PCR, immunohistochemistry	In this study, administration of CPT-11 to patients resulted in the downregulation of topoisomerase I and topoisomerase II-α expression in tumours, while ABCG2 expression was moderately up regulated.	[116]
CRC (HT29) cells transfected with hCE-1 and hCE-2		Immunoblotting, cytotoxicity assays	This study found that hCE2 hydrolyzes irinotecan to SN-38 faster than hCE-1, resulting in greater cellular sensitivity to killing by the drug.	[114]
Colon tumours from patients (12)		Affymetrix U95-Av2 arrays (12 000), RT-PCR, immunohistochemistry	Microarray profiling identified genes that predict response to four independent regimens (3 patients/group). Only two genes (NOT, c-fos) were found to have altered expression (upregulated) in all four regimens.	[120]
Wild-type and p53 null CRC cells (HCT116)		Cytotoxicity assays, immunoblotting, RT-PCR	ABCG2 expression was increased in both cell lines upon treatment with irinotecan, while topoisomerase I and carboxylesterase expression was decreased. The p53 status did not affect drug sensitivity.	[117]
CRC cell line (SW-620)		Affymetrix HGU95A array (22 284 elements), Q-PCR	Array profiling identified 244 genes changing expression upon induction of apoptosis by SN-38. The genes are involved in cell cycle progression, transcription, DNA replication, signal transduction and apoptosis.	[121]
Human liver and lymphocyte DNA		PCR and sequencing	Identified 11 polymorphisms in the carboxylesterase gene CE2, with none affecting activity.	[115]
52 colorectal tumours with matched normal colon controls		Q-PCR	Significant differences in gene expression were noted between tumour and normal samples; however, none of the differences noted could predict clinical outcome. ABCC1 was found to be differentially expressed.	[122]
Liver cancer cells (transfected HepG2 cells)		Cytotoxicity assays, drug uptake assays, HPLC	Transfection of liver cancer cells with ABCC4 conferred resistance to CPT CPT-11 and SN-38.	[118]
Tumours from 43 esophageal cancer patients		Immunohistochemistry studies	At 2-years posttreatment with irinotecan/docetaxel/5-fluorouracil chemotherapy, NF-κB-positive tumours significantly correlated with lower overall survival (52%) than NF-κB negative tumours (95%).	[123]
Wild-type and topotecan (TPT) resistant A2780 ovarian cancer cells	Topotecan	Cytotoxicity assay, immunoblotting, antisense RNA	This study found that ABCG2 could confer resistance to TPT, without concomitant upregulation of ABCC1 or ABCB1.	[124]
Uterine cancer (HEC59) and CRC (HCT116) cell lines		Cytotoxicity assay	This investigation suggested that loss of the microsatellite instability and DNA mismatch repair gene MLH1 reduces cellular death induced by TPT.	[103]
Tumours from patients with ovarian carcinoma (28)		PCR, DNA sequencing	This study found that patients with ovarian tumours expressing wild-type p53 had a greater response to TPT in second-line chemotherapy (CR=46%) than tumours with mutant p53 (CR=27%).	[126]

Table 3 (Continued)

Materials tested	Drugs tested	Methodology used	Major findings	Reference
CRC cells (HCT116 and p53 null HCT116 cells)		National Cancer Institute 'Oncochips' (6500 elements), RT-PCR, Immuno-blotting	This investigation revealed 167 genes whose expression changed in response to TPT treatment. Although the genes identified were related to the MAPK, p53 and antiapoptotic pathways, only the expression of c-jun/c-fos (and not p53 status) were found to correlate with response to TPT.	[127]
Osteoblasts (Saos-2)		MTT, Westerns Blots	Transfection of osteoblasts with ABCC4 confers resistance to the camptothecins, but not the epipodophyllotoxins.	[125]

CE, carboxylesterase; CPT, camptothecin; CRC, colorectal cancer; HPLC, high-pressure liquid chromatography; MTT, 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide; Q, quantitative; RT, reverse transcription; PCR, polymerase chain reaction; EGFR, epidermal growth factor receptor; CR, complete response; MAPK, mitogen-activated protein kinase.

controlled by epigenetic factors [133], suggesting that the environment may play an important role in response to EPPTs. As further evidence of the role of epigenetic factors in cellular sensitivity to etoposide, it has been shown that phosphorylation of Ser-1106 within the catalytic domain of the topoisomerase II enzyme dramatically reduces sensitivity of the HL-60 human leukemia cell line to etoposide [134]. Other possible candidates whose expression may help predict response to EPPTs were identified in recent DNA microarray studies. These studies showed that etoposide treatment of tumour cells resulted in altered expression of DNA mismatch repair genes (MLH1, MSH2), the apoptosis modulators Bcl-2 and Bax, transcription factors (NF-κB, c-fos/c-jun), p53-responsive genes such as c-Myc and c-Myb, and genes involved in cell signaling (AP-1, TRAF) [131,135–137].

Resistance to EPPTs such as etoposide can occur *in vitro* through the enhanced expression of both the Abcc1/Mrp1 drug transporter, in particular with coelevated levels of the detoxification enzymes GST-π and γ-GCS [138], which likely modify the drug to increase its affinity for the Abcc1/Mrp1 drug transporter. Furthermore, the expression of the ABC transporters Abcc1/Mrp1 and Abcc3/Mrp3 has been directly correlated with resistance to etoposide in nonsmall-cell lung carcinomas (NSCLC) but not small cell lung carcinomas (SCLC) [139]. The downregulation of one of the protein targets of the EPPTs (topoisomerase II-α) also appears to be associated with resistance to these agents [140]. A summary of the above-mentioned studies assessing possible genetic predictors of response or resistance to topoisomerase II inhibitors is depicted in Table 4.

Nucleoside analogs

The nucleoside analogs are structurally similar to endogenous cellular nucleosides (both purines and pyrimidines). Their entry into cells is facilitated by nucleoside transporters. Upon entry, the nucleoside analogs are phosphorylated in a manner similar to endogenous nucleosides, in which they become nucleotide derivatives that compete with endogenous nucleotides to inhibit a variety of cellular processes, including DNA replication [141]. Examples of purine analogs include cladribine and fludarabine, which are both used effectively to treat a number of low-grade haematological malignancies. Cytarabine and capecitabine are pyrimidine analogs used in the treatment of leukaemia, and colorectal and breast cancers, respectively [141].

5-Fluorouracil (5-FU) is another nucleoside analog, which binds to the enzyme thymidylate synthase and inhibits its activity. 5-FU is thus able to block the conversion of dUMP into dTMP in cells. This results in dramatically reduced levels of dTMP in cells and a concomitant reduction in both DNA replication and cell cycle progression (particularly in rapidly dividing tumour cells). In addition, 5-FU is incorporated into both RNA and DNA, where it affects a variety of RNA-directed and DNA-directed processes (reviewed in [142]). It has, however, been proposed that the antiproliferative effects of 5-FU may also involve the inhibition of pre-rRNA processing [143]. 5-FU is most commonly used to combat the growth of colorectal tumours.

Given that thymidylate synthase is the target for 5-FU, it would appear likely that tumour response to 5-FU may be dependent upon the expression level of thymidylate synthase. This hypothesis was addressed recently in a study by Hu *et al.* [144] in 2003. In this study, thymidylate synthase gene expression, overall survival and tumour response to 5-FU were determined in patients with pancreatic cancer. Interestingly, in this study, median survival for patients with low intratumoral thymidylate synthase expression (18 months) was considerably ($P = 0.02$) longer than for patients with high thymidylate synthase expression (12 months). Moreover, patients having high thymidylate synthase expression had a significantly reduced risk of death for any adjuvant therapy involving 5-FU. Taken together, the data suggest that thymidylate synthase expression could be a useful marker for predicting tumour response to 5-FU. Patients with high intratumoral thymidylate synthase expression would be expected to benefit significantly from adjuvant therapy with 5-FU. Another study [145] suggested that amplification of the 20q13 locus [including the decoy receptor 3 (DcR3) gene] may be associated with

Table 4 Genes associated with response or resistance to topoisomerase II inhibitors

Materials tested	Drugs tested	Methodology used	Major findings	Reference
30 Cell lines of various tissue origins	Etoposide (VP-16)	Affymetrix U133A arrays (22 282 elements), Q-PCR	In this investigation, array profiling was used to identify 76 genes whose expression was altered upon treatment with etoposide at concentrations used clinically, including Bax, Bcl-2, IFITM1, MLH1, MSH2 and SERPINA5. Tissue-specific resistances to etoposide were also noted.	[136]
MCF-7 breast tumour cell line		Cytotoxicity assay, Immunoblotting, Flow cytometry	Transfection of the caspase-3 gene resensitizes MCF-7 cells to etoposide-induced apoptosis, suggesting that the expression/activity of caspase-3 is important for etoposide action.	[132]
Leukemia cell line (HL-60)		Atlas cDNA nylon microarrays (2858 elements), immunoblotting	In this study, 40 genes related to cell signaling (AP-1, TRAF), the cell cycle (cyclin A/B1), transcription (c-Myb/Myc) and apoptosis (Bcl-2 family) changed expression upon etoposide treatment, resulting in the generation of proapoptotic signals.	[131]
Isolated $CD34^+$ cells from human cord blood		R&D cDNA microarrays, annexin V, Q-PCR	44 Genes related to the cell cycle (cyclin A2), apoptosis (Bcl-2 family, p53), signal transduction (NF-κB, TRAF1) and cytokine signaling (interleukin-1β) had altered expression in response to etoposide.	[135]
Wild-type and drug-resistant melanoma cells		cDNA microarrays (30 000 elements), Q-PCR	Fifty genes were identified as changing expression upon selection for resistance to one or more chemotherapy agents. Many of the proto-oncogenes (c-myb, c-fos) were not present on the array. Expression of Bcl-2 and Bax was seen to be upregulated in response to drug treatment.	[137]
Wild-type and drug-resistant HL-60 cells		Mass spectrometry, 2-D electrophoresis, immunoblotting, PCR	This investigation observed that the phosphorylation of Ser-1106 in the catalytic domain of topoisomerase II-α causes resistance to etoposide.	[134]
B-cell lymphoma cell line (JLP119)		Immunoblotting, immunoprecipitation	The apoptotic inducer Bcl-xL was shown to be upregulated in etoposide resistance. Inhibition of NF-κB by (E)-capsaicin restored sensitivity to etoposide.	[133]
29 NSCLC and SCLC cell lines		Immunoblotting, cytotoxicity assay	This study established that the ABC transporters ABCC1 and ABCC3 are upregulated in cells resistant to etoposide and appear to cause resistance to etoposide.	[139]
Fibroblast cell lines (NIH3T3 and NIH3T3-MRP)		Immunoblotting, cytotoxicity assay, GSH conjugation	In this investigation, transfection of ABCC1-expressing cells with vectors coding for GST-π and γ-GCS conferred considerable resistance to etoposide.	[138]

Q, quantitative; PCR, polymerase chain reaction, NSCLC, nonsmall-cell lung cancer; SCLC, small cell lung cancer; GSH, glutathione; GST, glutathione-*S*-transferase.

resistance to 5-FU-based chemotherapy in patients with colorectal cancer. Patients with a normal DcR3 copy number derived greater clinical benefit from 5-FU-based chemotherapy than patients with an amplification of this gene. There have yet to be additional studies corroborating such findings.

Specific polymorphisms in the thymidylate synthase and methylenetetrahydrofolate reductase genes have also been found to predict patient response or toxicity to 5-FU across a number of studies. For example, the thymidylate synthase gene promoter possesses tandem, repeated 28 bp regulatory sequences, which serve as enhancers to increase thymidylate synthase expression. The number of copies of these regulatory sequences has been shown to be associated with increased expression of thymidylate synthase, reduced response to 5-FU and increased patient toxicity to 5-FU. Prediction of 5-FU resistance and toxicity through assessment of thymidylate synthase expression was even greater when the 677C→T polymorphism in the gene for methylenetetrahydrofolate reductase was also identified in patients [146]. Another study [147] identified a novel polymorphism in the 5′-flanking region of the gene for dihydropyrimidine dehydrogenase (DPD), which appears to affect cellular expression of DPD. As the enzyme is involved in nucleoside metabolism, its expression may also affect clinical response to 5-FU.

With the advent of cDNA microarray technology, additional studies have identified promising biomarkers for the prediction of 5-FU response in patients. Wang and colleagues [148] used cDNA microarray profiling to identify 91 genes whose expression was altered as tumour cells acquired resistance to 5-FU. A number of these genes are involved in 5-FU activation and were downregulated in resistant cells from 2.3-fold to 3.6-fold. The downregulated genes included thymidine kinase, orotate phosphoribosyltransferase, uridine monophosphate kinase and pyrimidine nucleoside phosphorylase. Elevated expression of thymidylate synthase and its adjacent gene, c-Yes, was also detected in the resistant cell lines. Interestingly, both NF-κB mRNA and protein were overexpressed in resistant cells, and transfection of vectors coding for both the p50 and p65 subunits of NF-κB was able to induce 5-FU resistance. Thus, it is possible that NF-κB serves as the transcription factor responsible for one or more of the changes in gene expression observed as cells acquired 5-FU resistance. An additional study [149] suggested that the expression of spermine/spermidine acetyl transferase, annexin II, thymosin-β-10, chaperonin-10 and the chloride conductance inducer protein MAT-8 (FXYD3) is increased both upon exposure of MCF-7 cells to 5-FU and upon selection for 5-FU resistance. A similar study in gastric tumour cells [150] identified 11 genes commonly upregulated only in 5-FU-sensitive cells lines and two

genes that were oppositely regulated in both sensitive and resistant cell lines. The genes were involved in 5-FU metabolism (orotate phosphoribosyltransferase, thymidylate synthase, DPD), as well as genes involved in cell signalling, apoptosis and cell cycle progression (see Table 5).

Can the expression of sets of genes identified by microarray analysis prove superior to known biomarkers of 5-FU response? Mariadason *et al.* [110] recently addressed this question. The investigators conducted gene profiling and apoptosis induction measurements on 30 colorectal tumour cells lines before and after treatment with 5-FU. They identified 50 genes whose expression correlated with 5-FU-induced apoptosis. In support of this approach, the expression of a set of 50 genes proved superior for the prediction of 5-FU-induced apoptosis than well-known determinants of 5-FU response, namely the thymidylate synthase, thymidine phosphorylase, p53 and the DNA mismatch repair proteins. Although these findings are very promising, it will be important to assess the ability of this gene set to predict clinical response to 5-FU across in multiple sets of patients.

Another nucleoside analog used widely in chemotherapy regimens [particularly for chronic lymphocytic leukemia (CLL)] is fludarabine. An analog of adenosine, fludarabine is primarily administered in its monophosphate form. The enzymes serum phosphatase and ecto 5′-nucleotidase (CD73) are responsible for converting fludarabine into its nucleoside form, which enters the cell via the hENT1, hENT2 and hCNT3 nucleoside transporters [151]. Deoxycytidine kinase then converts fludarabine into its active cytotoxic form (fludarabine triphosphate) (reviewed in [141,152]).

Recently, Rosenwald and colleagues [153] examined the changes in gene expression that occur when patients with CLL were treated with fludarabine. Similar to findings with 5-FU, genes that changed expression in response to fludarabine were mostly p53 target genes or genes involved in DNA repair. Interestingly, very similar changes in gene expression were observed when CLL cells were treated with either fludarabine or ionizing radiation *in vitro*. This raises the prospect that treatment of CLL patients with fludarabine may result in selection of tumour cells for p53 null variants in the population and this may be a mechanism for the acquisition of fludarabine resistance. Indeed, mutations of the p53 gene have been correlated with reduced survival and resistance to fludarabine in CLL patients [154,155]. In addition, other genes involved in DNA repair may also predict clinical response/resistance to fludarabine. For example, the DNA repair enzyme deoxycytidine kinase is known to be phosphorylated and activated by DNA damaging agents [156], and patients whose tumours are resistant to fludarabine often have decreased expression of deoxycytidine kinase [151]. As fludarabine accumulation in CLL cells is mediated predominantly by ENT-type (rather than CNT-type) transporters [157], responsiveness of CLL patients to fludarabine is probably also highly dependent upon the expression of ENT nucleoside transporters. This hypothesis was recently examined in a study that assessed, by quantitative reverse-transcription PCR, the role of nucleoside transport and metabolism genes in response to fludarabine chemotherapy for patients with CLL [151]. Interestingly, patients whose peripheral blood cells had elevated expression of hCNT3 experienced a lower complete response rate, suggesting hCNT3 expression may be coupled with resistance to fludarabine chemotherapy. The hCNT3 protein was not localized to the plasma membrane in the CLL cells and did not contribute to the intracellular uptake of fludarabine. The mechanism by which hCNT3 overexpression contributes to fludarabine resistance is unknown.

In another study, Gottardi *et al.* [158] examined the changes in gene expression as patients with $CD5^+$ B cell chronic lymphoytic leukemia (B-CLL) and mantle cell lymphoma in the leukemia phase (MCL) were treated with fludarabine/methylprednisolone chemotherapy. This investigation found that Bcl-2 expression was significantly downregulated after treatment in a subset of patients. Moreover, a partial or complete pathologic response was generally (but not always) observed in patients that exhibited a downregulation in Bcl-2 expression. This suggests that Bcl-2 gene expression may be a useful marker for response to fludarabine/methylprednisolone chemotherapy in these patients. A summary of the above-mentioned studies assessing possible genetic predictors of response or resistance to nucleoside analogs is depicted in Table 5.

Alkylating agents

As competent electrophiles, alkylating agents can form covalent bonds with DNA via electron-rich groups. Their ability to interact with DNA in turn disrupts DNA synthesis and the replicative fidelity of DNA during DNA replication. This may also help explain some additional effects of alkylating agents, including their carcinogenic and teratogenic properties (reviewed in [159]). Among the major groups of alkylating agents used in medicine are nitrogen mustards (e.g. cyclophosphamide), ethylenimines, alkylsulfonates, nitrosoureas and triazenes. Cyclophosphamide is considered to be relatively nontoxic or pharmacologically inactive against cells *in vitro*. In humans, however, the prodrug is almost immediately converted into a highly cytotoxic metabolite (aldophosphamide) by specific cytochrome P450 enymes (namely CYP2B1) in the liver [160,161].

One of the most noted mechanisms of resistance to cyclophosphamide is the increased activity and/or

Table 5 Genes associated with response or resistance to nucleoside analogs

Materials tested	Drugs tested	Methodology used	Major findings	Reference
Colorectal tumours and normal colorectal tissues	5-Fluorouracil	PCR amplification of TYMS genotypes	This study found that specific polymorphisms within the promoter and 3′ untranslated regions of the thymidylate synthase gene affected the toxicity of 5-fluorouracil in patients with colorectal cancer.	[196]
Extracts from pancreatic tumours		Immunohistochemistry	Study observed that high cellular levels of thymidylate synthase are linked to poor prognosis for pancreatic cancer patients after 5-fluorouracil-based adjuvant chemotherapy.	[144]
Wild-type and 5-fluorouracil-resistant colorectal (H630) and breast (MCF-7) tumour cell lines		Microarray analysis (HG-U133A arrays)	cDNA microarray profiling was used to identify genes whose expression is associated with resistance to 5-fuorouracil. These included genes involved in 5-fluorouracil activation (thymidine kinase, orotate phosphoribosyltransferase, uridine monophosphate kinase, and pyrimidine nucleoside phosphorylase), thymidylate synthase and c-Yes. The changes in gene expression may be mediated through NF-κB mRNA, since transfection of vectors coding for both the p50 and p65 subunits of NF-κB was able to induce 5-fluorouracil resistance in the cell lines.	[148]
Colorectal cancer (tissue biopsies)		PCR and immunohistochemistry	Observed an amplification of the DcR3 (decoy receptor 3) gene at the 20q13 locus and an overexpression of the DcR3 protein in 63 and 73% of colorectal tumours, respectively. Adjuvant chemotherapy with 5-fluorouracil, however, was significantly more beneficial in patients with normal DcR3 gene copy number than in patients with amplification. Amplification of the 20q13 locus may thus be a predictive marker for adjuvant chemotherapy in colorectal cancer.	[145]
30 colorectal cancer cell lines		PCR, microarray analysis (9216 elements)	Identified 50 genes whose expression correlated with 5-fluorouracil-induced apoptosis. The expression of this set of genes served as a better predictor of 5-fluorouracil response than well known determinants of 5-fluorouracil response, namely thymidylate synthase and thymidine phosphorylase activity, and p53 or mismatch repair status.	[110]
Gastric cancer cell lines (SNU)		cDNA microarray analysis (KUGI) (14 000 elements), RT-PCR analysis	Identified in gastric tumour cells 11 genes commonly upregulated only in 5-fluorouracil-sensitive cells lines and two genes that were oppositely regulated in both sensitive and resistant cell lines. The genes were involved in 5-fluorouracil metabolism (orotate phosphoribosyltransferase, thymidylate synthase, dihydropyrimidine dehydrogenase), as well as genes involved in cell signalling, apoptosis, and cell cycle progression (ZFP100, 4F2hc, FLJ11021, CSTF3, PPP1R14A, DDB2, C6orf139, CDKN1A, HOXC11 and FLJ38860). Findings confirmed by semiquantitative-PCR.	[150]
Wild-type and 5-fluorouracil-resistant breast (MCF-7) and colon (H630) cancer cell lines		Micromax human cDNA microarray; Northern blotting	Study observed that the expression of spermine/spermidine acetyl transferase, annexin II, thymosin-β-10, chaperonin-10 and the ion transport regulatory MAT-8 is increased both upon exposure of MCF-7 cells to 5-fluorouracil and upon selection for 5-fluorouracil resistance.	[149]
A variety of tumour cell lines		PCR, DNA sequencing, cytotoxicity assay	Study identified a novel polymorphism in the 5′ flanking region of the gene for dihydropyrimidine dehydrogenase, which appears to affect cellular expression of DPD. As the enzyme is involved in nucleoside metabolism, its expression may affect clinical response to 5-fluorouracil.	[147]
Liver metastases from patients with colorectal cancer		Melting curve analyses and tritium-release assays	Methylenetetrahydrofolate reductase gene polymorphisms at positions 677 and 1298 were found to affect response to 5-fluorouracil. This study also showed that thymidylate synthase activity measured in metastases was a significant predictor of 5-fluorouracil responsiveness. Addition of the 677C→T genotype status for MTHFR improved the ability to predict tumour response to 5-fluorouracil chemotherapy.	[146]
Leukemic cells from patients with chronic lymphocytic leukemia	Fludarabine	cDNA microarray (lymphochip, 17 856 elements)	This study monitored changes in gene expression in leukemic cells from CLL patients in response to fludarabine treatment. Both in-vitro and in-vivo studies suggest that fludarabine induces the expression of known p53 target genes as well as genes involved in DNA repair.	[153]
Peripheral blood lymphocytes from B-CLL and MCL patients		Flow cytometry, Northern blot, RT-PCR	This study identified a dose-dependent relationship between apoptosis induction in peripheral blood lymphocytes of B-CLL and MCL patients treated with fludarabine/methylprednisolone and the downregulation of Bcl-2 expression in these cells. Patient response to treatment was typically but not always correlated with Bcl-2 downregulation.	[158]
Peripheral blood and bone marrow samples from CLL patients		Q-PCR, confocal microscopy	An association between the overexpression of the nucleoside transporter hCNT3 and resistance to fludarabine chemotherapy was identified in patients with CLL. The hCNT3 protein, however, was not localized to the plasma membrane in the CLL cells and did not contribute to the intracellular uptake of fludarabine.	[151]

DPD, dihydropyrimidine dehydrogenase; TYMS, thymidylate synthase; Q, quantitative; RT, reverse transcriptions; PCR, polymerase chain reaction; CLL chronic lymphocytic leukaemia.

expression of aldehyde dehydrogenase, which is known to detoxify the aldophosphamide metabolite [162,163]. As cyclophosphamide cytotoxicity is activated by the cytochrome P450 enzymes, recent studies have focused on the possible ability of cytochrome P450 expression or activity to predict tumour cell responsiveness to cyclo-

phosphamide. Consistent with this view, treatment of rats with cyclophosphamide resulted in the induced expression of a variety of cytochrome P450 enzymes, including CYP2B1, CYP2B2, CYP3A2 and CYP2C11 [164]. Induction was highest (220-fold) for CYP2B1 [164]. A study using human liver slices indicated that CYP2B6 and CYP3A4 expressions are also induced by cyclophosphamide [165], whereas similar studies in human HL-60 leukemia cells were unable to detect an induction of cytochrome P450 enzymes [166]. Taken together, these observations suggest that there are cell type-specific differences in terms of cyclophosphamide's ability to induce expression of cytochrome P450 enzymes. Alternatively, studies in cell lines may not accurately reflect gene induction in organs such as the liver.

Although resistance to cyclophosphamide could possibly occur by selection for tumour cells having reduced expression of CYP2B1 or other cytochrome P450 enzymes (thereby blocking prodrug activation), in-vitro studies in HL-60 or MCF-7 cells suggest that resistance to cyclophosphamide can occur through enhanced activity/overexpression of GST-π or aldehyde dehydrogenase [166,167]. Evidence exists clinically for isoform-specific resistance to cyclophosphamide chemotherapy by the GSTs. Vester and colleagues [168] found that patients with nephrotic syndrome that were *GST-M1* null experienced a better response to cyclophosphamide, whereas patients who were heterozygous or homozygous for *GST-P1* had a greater susceptibility to further relapses in treatment.

Cisplatin (another alkylating agent) is used to treat a very broad range of neoplasms, including cancers of the head and neck, esophagus, lung, ovary and bladder cancer. Similar to cyclophosphamide and other alkylating agents, cisplatin induces inter and intrastrand DNA crosslinks and blocks various DNA-dependent processes, including DNA replication [169]. Resistance mechanisms to cisplatin also appear to be similar to other alkylating agents and include decreased drug accumulation, increased level of cellular thiols (such as GST), increased nucleotide excision–repair activity and decreased mismatch-repair activity. In general, the molecules responsible for each resistance mechanism are upregulated in cisplatin-resistant cells [170]. Specifically, alterations in the expression of glutathione transferase M3 [171] and several DNA repair enzymes (uracil DNA glycosylase, DNA polymerase-γ and ERCC1) [172,173] appear to correlate with the onset of cisplatin resistance. Consequently, quantification of tumour expression levels of these enzymes may prove highly useful in predicting response to cisplatin.

The gene for glycosylphosphatidylinositol-anchored molecule-like protein (*GML*) is specifically induced by wild-type p53, which appear to be closely related to tumour cell sensitivity to cisplatin and other drugs. Supporting this hypothesis, a recent study [174] using a small number of tumour specimens from patients with NSCLC, showed that *GML* expression is commonly detected in p53-negative NSCLCs in close association with good sensitivity to cisplatin. *GML* expression may therefore serve as a useful predictor of cisplatin-based chemotherapy for patients with NSCLC.

Evidence exists to suggest that select members of the plastin family of actin-binding proteins [175,176] may play a role in resistance to cisplatin. Hisano and colleagues [177] observed in differential display experiments involving a variety of wild-type and cisplatin-resistant cell lines, that the expression of the T-plastin gene was substantially higher in cisplatin-resistant cell lines compared with cisplatin-sensitive cells. Moreover, transfection of cisplatin-resistant T24/DDP10 cells with a vector encoding full-length T-plastin antisense RNA reduced T-plastin expression and increased sensitivity to cisplatin. It will be interesting to assess whether these observations are supported in clinical studies involving patients being treated with cisplatin chemotherapy.

Evidence exists for an upregulation of genes coding for subunits of the V-ATPase class of proton-translocating enzymes in response to cisplatin and in cisplatin resistance. Through differential display [178], it has been shown that cisplatin exposure can induce expression of ATP6C, a subunit of one of the V-ATPases. Using Northern blot experiments, this study also found that other members of the V-ATPase subunits are upregulated by cisplatin. Pump gene expression was also found to be upregulated in cell lines exhibiting cisplatin resistance but not vincristine or etoposide resistance. Also noteworthy in this study are the observations that the DNA-binding activity of cisplatin is markedly increased under acidic conditions and that proton-pump inhibitors can synergistically potentiate the cytotoxicity of cisplatin but not of etoposide or camptothecin. Taken together, these findings suggest that cellular pH may play a role in cisplatin resistance and that this resistance may be predicted by the upregulation of V-ATPase subunits. Additional genome profiling experiments have identified a large number of other genes whose expression may affect the response of tumour cells to cisplatin, including the induction of the proapoptotic regulator Bax by cisplatin [179] and the increased expression of genes coding for growth factors (*AXL* and *TRAP1*) and a DNA repair protein (*FANCG*) in cells exhibiting cisplatin resistance [180]. A summary of possible genetic predictors of response or resistance to alkylating agents is depicted in Table 6.

Vinca alkaloids

Vinca alkaloids are a family of microtubule-targeting chemotherapy agents that include vinblastine, vincristine, vindesine, vinorelbine and a recently developed derivative compound vinflunine [181]. These agents all bind β-tubulin at the vinca domain [182]. In contrast to the taxanes, higher concentrations of vinca alkaloids are required to be growth inhibitory and to stimulate the depolymerization of microtubules. Nevertheless, both the taxanes and vinca alkaloids block the dynamics of microtubules [183] without significantly affecting their mass. These agents consequently block cell cycle progression in metaphase/anaphase, resulting in growth arrest and ultimately cell death by the induction of apoptosis (reviewed in [184]). Studies in cellular pharmacology demonstrate that the vinca alkaloids are taken up by tumour cells through multiple mechanisms, including passive diffusion and energy-dependent and temperature-dependent active transport systems. Pharmacokinetic parameters of vinca alkaloids are time-dependent and dose-dependent, and large inter- and intraindividual variabilities have been observed [185]. The vinca alkaloids are used to treat a variety of human cancers, often in combination with other chemotherapy agents. For example, vinblastine is used primarily in the treatment of advanced Hodgkin's disease and germ cell cancer of the testes [182]. It is also used in combination with other chemotherapeutic drugs such as methotrexate, doxorubicin, cisplatin or rituximab for the treatment of bladder cancer [186]. Vincristine is used in combination with other drugs for the treatment of acute lymphoblastic leukaemia, both Hodgkin's and non-Hodgkin lymphoma [182] or chemorefractory low-grade or follicular non-Hodgkin's lymphoma (NHL) [187]. Vinorelbine is a semisynthetic vinca alkaloid with a broader spectrum of antitumour activity *in vitro*, which appears to affect the rate and extent of microtubule polymerization (rather than depolymerization) [188]. Clinically, vinorelbine has mainly shown efficacy in the treatment of advanced NSCLC and the treatment of metastatic breast cancer [189]. Vindesine, a semisynthetic derivative of vinblastine, is ranked among the most effective drugs for the treatment of NSCLC (when used with cisplatin) [190].

To date, there is some evidence to support the existence of genes whose expression may help predict tumour cell sensitivity to vinca alkaloids both *in vitro* and in cancer patients. Fan *et al.* [191] found that treatment of KB3 lung carcinoma cells with vinblastine resulted in the downregulation of p53 (and its target p21) and an upregulation of TNF-α, Bak and other proteins. These vinblastine-mediated changes in gene expression were not observed in identical cells transfected with a dominant-negative inhibitor of c-Jun, suggesting a central role for the AP-1 pathway in mediating resistance to vinblastine *in vitro*.

With respect to putative genetic predictors of clinical response to vinca alkaloids, microarrays representing 23 040 genes were used to profile gene expression in 85 cancer xenografts derived from nine human organs. The xenografts were examined for their sensitivity to nine different anticancer drugs, including vincristine and vinblastine. In this study, 1578 genes were identified whose expression correlated significantly with chemosensitivity to one or more of the drugs under study [192]. Interestingly, a strong correlation was observed between the expression of *MAP2K3* and tumour sensitivity to vincristine, but only for xenografts of breast tumour cells. These findings suggested that *MAP2K3* may only be predictive of vinca alkaloid cytotoxicity for breast tumours and likely only for vincristine. In a recent translational research study, the expression of *MAP2K8* was shown to be useful in distinguishing between responders and nonresponders in patients with bladder cancer being treated with vinblastine, methotrexate, doxorubicin and cisplatin (M-VAC) [186]. The true ability of *MAP2K3* to predict tumour sensitivity to select vinca alkaloids and possibly other drugs in cancer patients will, nevertheless, only be determined upon completion of a series of independent confirmatory clinical studies.

Recently, Györffy *et al.* [136] conducted gene expression profiling of 30 cell lines to establish whether the expression of specific genes correlated directly or indirectly with resistance to a variety of chemotherapy agents including one vinca alkaloid (vinblastine). Thirty-five genes exhibited alterations in gene expression that correlated with the degree of resistance to vinblastine. The level of expression of these genes in the tumours of cancer patients may thus be useful to predict patient resistance to vinblastine. The genes identified were, however, also useful in predicting resistance in cell lines to between four and eight additional chemotherapy drugs (none of which were vinca alkaloids). This suggests that the genes identified may be biomarkers for prediction of general drug resistance and not specifically for resistance to vinblastine or vinca alkaloids. Similar findings [193] were observed when 45 human cancer cell lines were profiled for changes in gene expression associated with resistance to vinorelbine. Whereas 27 and 29 genes related to vinorelbine sensitivity and resistance were identified, respectively, a number of these genes also affected sensitivity to other chemotherapy agents. The ability of either of the above gene sets to predict resistance to vinca alkaloids or other classes of drugs in the tumours of cancer patients has yet to be assessed.

A few recent studies have begun to assess whether the expression of specific gene polymorphisms in cancer patients can effectively predict response to vinca alkaloids. Plasschaert *et al.* [194] examined the effect of SNPs in *ABCB1* on vincristine pharmacokinetics and toxicity in children being treated for acute lymphoblastic

Table 6 Genes associated with response or resistance to alkylating agents

Materials tested	Drugs tested	Methodology used	Major findings	Reference
Leukemia and hepatoma cell lines	Cyclophosphamide	RT-PCR, Southern blot hybridization	In cyclophosphamide-resistant leukemia cells, the transcript levels and catalytic activities for glyceraldehyde-6-phosphate dehydrogenase and aldehyde dehydrogenase 1 were found to have increased expression and activity, suggesting a role for these genes in cyclophosphamide metabolism and resistance.	[162]
Wistar/Fu male rats		Q-PCR	After treatment with upto 200 mg/kg cyclophosphamide, rats were examined for their expression of various cytochrome P450 enzymes. Cyclophosphamide treatment dramatically increased the expression of CYP2B1, CYP2B2, CYP3A2, and CYP2C11, in particular the CYP2B1 and CYP2B2 enzymes. Microsomal activities of these enzymes were also increased (albeit to a much lesser extent).	[164]
Wild-type (HL-60S) and multidrug-resistant (HL-60R) leukemia cell lines		RT-PCR, Q-PCR	This study observed that wild-type and multidrug-resistant HL-60 cells do not contain measurable levels of mRNA for CYP2B6, CYP3A4, CYP2C9 and CYP2C19 before or after treatment with cyclophosphamide. In contrast, the CYP1B1 mRNA was expressed in the HL-60 cell line and treatment with cyclophosphamide suppressed this expression in a concentration-dependent manner. β-Actin gene expression was also decreased. No resistance to cyclophosphamide was seen in HL-60R cells.	[166]
Patients with nephrotic syndrome		Multiplex PCR	This investigation found that patients with nephrotic syndrome having a GST-M1 null genotype experienced a better response to cyclophosphamide treatment, whereas patients who were heterozygous or homozygous for GST-P1 had a greater susceptibility to further relapses in treatment.	[168]
Surgical samples from nonsmall cell lung cancer patients	Cisplatin	RT-PCR	In this study, it was observed that the expression of the gene for glycosylphosphatidylinositol-anchored molecule-like protein was correlated with p53 expression and in-vitro chemosensitivity to cisplatin.	[174]
Wild-type and cisplatin-resistant cell lines from bladder, prostate, and head and neck cancers.		Differential display	T-plastin, which is part of the plastin family of actin-binding proteins, was shown in this investigation to be upregulated in cells exhibiting cisplatin resistance. Transfection of cisplatin-resistant T24/DDP10 cells with a vector encoding full-length T-plastin antisense RNA reduced T-plastin expression and increased sensitivity to cisplatin.	[177]
Epidermoid and prostate cancer cell lines		Differential display, Northern blotting	This study used differential display to demonstrate that cisplatin induces the expression of ATP6C, a subunit of the V-ATPase class of proton-translocating enzymes. Northern blot analysis revealed an induction of other members of the V-ATPase subunits. Pump induction also occurred in cell lines exhibiting cisplatin (but not vincristine or etoposide) resistance. DNA binding to cisplatin was markedly increased in acidic conditions, suggesting that the cisplatin cytotoxicity is dependent upon cellular pH.	[178]
Colon cancer cell lines		Affymetrix (HG-U95A) array, RT-PCR	The study identified 15 genes that play a role in survival of colon cancer cells in the presence of cisplatin. The list of genes (confirmed by RT-PCR) included the drug-conjugating enzyme GST-M3 and the DNA repair enzyme ATM.	[171]
Ovarian tumour cell lines		Northern blot, mobility shift assay	This study measured mRNA levels of the DNA excision repair enzyme ERCC-1 in response to cisplatin for various periods of time. ERCC-1 mRNA expression increased in a dose- and time-dependent manner. The study also observed increased levels of AP-1 and c-Jun phosphorylation, which may play a role in the activation of ERCC-1 transcription.	[173]
Gastric cancer patients		Affymetrix (HG-U133A) array	Gene expression profiling was conducted on tumours from gastric cancer patients exhibiting either sensitivity or resistance to cisplatin/5-fluorouracil chemotherapy. Eighty-six genes were identified whose expression correlated with response to therapy including genes coding for proteins involved in signal transduction, the immune response, apoptosis, the stress response, DNA repair and DNA/RNA metabolism.	[172]
Wild-type and cisplatin-resistant ovarian adeno-carcinoma cells		Microarray analysis, RT-PCR	This study demonstrated that cisplatin-resistant ovarian adenocarcinoma cells demonstrate a differential response to the ErbB ligand compared to wild-type cells. This was not due to varying expression of ErbB receptors. Genes associated with cisplatin resistance were identified, including the growth factors AXL and TRAP1 and the DNA repair enzyme FANCG.	[180]
Hepatocellular carcinoma cells		Atlas human apoptosis array, RT-PCR, and immunoblotting	Using cDNA microarray analysis, genes changing expression in Hep3B cells in response to cisplatin were identified. A series of genes coding for proteins involved in the regulation of cell-cycle progression, apoptosis and signal transduction by cisplatin were identified, including the proapoptotic mitochondrial protein Bax.	[179]

Q, quantitative; RT, reverse transcription; PCR, polymerase chain reaction.

leukemia. Unfortunately, whereas the Abcb1 transporter can induce resistance to the vinca alkaloids *in vitro*, no correlation could be established between the presence of specific *ABCB1* polymorphisms and the large variability in vincristine pharmacokinetics observed in patients [187]. On a more promising note, a pilot study has identified a

number of gene polymorphisms, which when present, appear to correlate with response to cisplatin/vinblastine/dacarbazine/IL-2/interferon-α chemotherapy in patients with advanced (stage IV) melanoma [195]. The interferon-γ (+874A→T) gene polymorphism was associated in a statistically significant manner with response to treatment, progression-free survival, and overall survival. Moreover, the further classification of patients based on the presence of additional polymorphisms in the genes for IL-10 (−1082G→A) and excision repair cross-complementing gene 1 (ERCC1; codon 118) resulted in the ability to stratify patients into four distinct groups with significantly different clinical outcomes. Whether the genes identified are simply prognostic biomarkers or predictive of a clinical response to vinblastine specifically remains to be assessed. A summary of the above-mentioned studies assessing possible genetic predictors of response or resistance to the vinca alkaloids is depicted in Table 7 [52,179,184–186,188].

Concluding remarks

The above sections document much of the recent advances in the identification of genetic biomarkers for the prediction of response or resistance to some of the major classes of chemotherapy agents used in the treatment of human cancers. Although the genes or 'genetic signatures' associated with drug response vary from study to study, some common themes emerge when one reviews the various summary tables presented in this review. For example, genes whose expression clearly affects drug response/resistance across a large number of well-conducted studies tend to be drug-specific. To illustrate this point, tumour cell sensitivity *in vitro* to nucleoside analogs has been reproducibly shown to be dependent upon the expression level of thymidylate synthase and a variety of enzymes involved in nucleoside metabolism. This has not been observed for the anthracyclines, where drug sensitivity appears often to be dependent upon the level of expression of specific ABC transporters or clusterin and on the functionality of specific pathways (ceramide and mitochondrial permeability) that play a role in the ability of anthracyclines to induce apoptosis in tumour cells. As chemotherapy agents block the growth of tumour cells via very specific and different mechanisms of action, it is not surprising that the genes identified to play a role in drug response or drug resistance would vary from tissue to tissue. Despite this, there also appear to be genes whose expression appears to correlate with drug response across a wide variety of classes of chemotherapy agents, despite their different structures and mechanisms of action. For example, the expression of the transcription factor NF-κB (known to play a role in the cellular stress response) appears to be associated with the acquisition of resistance *in vitro* to selected members of the taxane, nucleoside analog, topoisomerase I inhibitor and topoisomerase II inhibitor families. Similarly, the Bcl-2 family of apoptosis regulators appears to play a role in response to nucleoside analogs, alkylating agents, taxanes, topoisomerase I inhibitors and topoisomerase II inhibitor in in-vitro

Table 7 Genes associated with response or resistance to the vinca alkaloids

Material tested	Drug(s) tested	Methodology	Major findings	Reference
30 Cell lines	Eleven chemotherapy drugs	Affymetrix (HG-U133A) array (22 282 elements)	This investigation identified 67 genes whose expression correlated with resistance to four or more chemotherapy drugs. These changes in gene expression was verified by Q-PCR.	[136]
Mouse xenografted tumours (85 human cancer cell lines from nine organs)	Nine anticancer drugs, including vincristine and vinblastine	cDNA microarray (23 040 elements)	In this gene profiling study, 1578 genes showed significant correlation with sensitivity to one or more drugs. The expression of 333 and 32 genes correlated with sensitivity to more than two and more than six drugs, respectively. A strong correlation was observed between the expression of MAP2K3 in tumours and their sensitivity to killing by vincristine, but only for xenografts of breast tumour cells.	[192]
Forty-five human cancer cell lines (JFCR-45)	53 Anticancer drugs	Atlas human cDNA microarray (3537 elements)	This study identified 27 and 29 genes related to sensitivity and resistance to vinorelbine, respectively. The expression of a number of these genes also affected sensitivity to other chemotherapy agents.	[193]
Tumour biopsies from 27 bladder cancer patients	Methotrexate, vincristine, doxorubicin cisplatin	cDNA microarray (27 648 elements)	In this study, 14 genes were identified which were differentially expressed between chemotherapy responders and nonresponders. Although the genes may prove to be predictive of response to the combination of chemotherapy agents, genes associated specifically with response to the vinca alkaloids were not identified.	[186]
Peripheral blood mononuclear cells from 90 patients with stage IV melanoma	Vinblastine, cisplatin, cacarbazine, IL-2, IFN-α, ± tamoxifen	Amplification refractory mutation system, PCR, RFLP Analysis	This investigation demonstrated that the presence of the IFN-γ (+874A→T) polymorphism in patients was strongly associated with response to chemotherapy, but the role of this polymorphism specifically in response to vinblastine was not assessed.	[195]
KB3 lung carcinoma cell line	Vinblastine	Atlas apoptosis microarray	This study found that vinblastine activates the c-JUN NH_2-terminal protein kinase/AP1 pathway in apoptosis. Vinblastine treatment also resulted in the downregulation of p53 expression and an upregulation of p21 expression.	[191]

IL-2, interleukin; IfN-α, interferon-α; RFLP, restriction fragment length polymorphism; Q, quantitative; PCR, polymerase chain reaction.

studies. Another common theme appears to be that tumour cells use a variety of methods to resist the killing action of chemotherapy drugs, including the sequestration or efflux of drugs, conversion of drugs into less active metabolites, downregulation of apoptotic pathways that likely act downstream of drug targets and the stimulation of growth/survival pathways. Gene expression profiling studies suggest that a number of these mechanisms are operative in cells that have acquired resistance to chemotherapy drugs and the expression or activity of a number of gene products will be necessary to serve as useful predictors of drug response across cancer patients.

Interestingly, the genes identified as affecting response to a particular drug *in vitro* appear to be highly useful in some instances in predicting response to therapy in cancer patients. For example, the level of expression of wild-type p53, topoisomerase II-α, NF-κB and Bcl-2 have been shown across multiple studies to affect patient response to the topoisomerase II inhibitors (see Table 3). Similarly, patient response to nucleoside analogs has been shown repeatedly to be dependent upon the expression of thymidylate synthase (see Table 5). For most chemotherapy agents, however, the expression of individual genes or small sets of genes is insufficient to serve as useful biomarkers for the prediction of drug response on an individual patient basis. Given the large number of genes and proteins affecting patient response to chemotherapy drugs, it appears likely that large sets of drug response genes will be required to accurately predict response to specific chemotherapy regimens in cancer patients. In support of this approach, a 50-gene set proved superior for the prediction of 5-FU-induced apoptosis in colon tumour cells than well-known determinants of 5-FU response, namely the thymidylate synthase, thymidine phosphorylase, p53 and the DNA mismatch repair proteins [110]. Whether this will be borne out in future clinical studies remains to be seen.

The identification of set of genes able to predict patient response to specific chemotherapy drugs or regimens remains a challenging task. Some gene sets were identified based on studies of cell lines cultured within the laboratory, which do not accurately reflect tumours in patients, in which cell/substratum adhesion, intercell adhesion and variations in vascularization strongly affect gene expression and tumour properties. In addition, cell lines often acquire many genetic and epigenetic changes as they adapt in culture, necessitating the use of a control cell line in microarray experiments that accounts for changes in gene expression associated with cell culture. These controls are often absent in microarray experiments. Nevertheless, the direct study of tumours or host tissues is also problematic, owing to the significant genetic variability amongst patients, the presence of contaminating tissues in tumour samples (if they are not removed by laser capture microdissection) and significant variability in the degree of vascularity and anoxia associated with tumour tissues. Also problematic is the very high false discovery rate inherent in gene profiling studies, as the probability by chance alone of finding one or more genes whose expression correlates with response to a particular drug is very high when tens of thousands of genes are being surveyed. To illustrate this point, two recent studies used direct microarray profiling of tumours from breast cancer patients to identify genes whose expression appeared to be predictive of clinical response to docetaxel [81,83]. Interestingly, both studies identified genes correlating with patient response to docetaxel in training sets that were supported in a validation study using an independent subset of patients. Unfortunately, the lists of genes predictive of docetaxel response were highly different between the studies, suggesting that many of these genes may have been false positives. Consequently, to improve the success of finding useful genetic biomarkers for the prediction of drug response, it may be necessary to limit assessment to a subset of putative predictive genes (whose expression has been shown to correlate with drug response *in vitro*). Additional sources of variation in microarray studies relate to varying methods used in the normalization and statistical analysis of microarray data, the array methodology used and the definition of clinical response in patients.

It is also worth noting that cancer patients do not constitute a uniform population. The expression of a set of genes may only be predictive of response to treatment in certain subsets of patients based on host genetic factors or on the expression of specific tumour markers (such as the estrogen receptor, progesterone receptor, or HER-2/*neu* in breast cancer patients). To conduct such studies, significantly larger samples sizes will be required to obtain reliable findings. Thus, although there has been strong progress in the identification of genes whose expression correlates with response to chemotherapy drugs *in vitro*, there remains to be a robust set of genes identified whose expression clearly predicts response to therapy across multiple studies involving different sets of patients. This will be an important step in our ultimate quest of providing tailor-made chemotherapy regimens for individual cancer patients on the basis of their genetic background or the genetics of their tumours.

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