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MDR1 Gene Expression in Solid Tumours

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INTRODUCTION

ALTHOUGH COMBINATION chemotherapy has had a significant impact on survival for malignancies such as Hodgkin's disease, testicular cancer, and acute childhood leukaemias, the majority of cancers are initially either resistant to chemotherapy (renal, colon, etc.) or chemosensitive, but acquire resistance during treatment, such as lymphoma and breast cancer. Resistance to chemotherapy remains an obstacle to the successful treatment of human cancer and has been the subject of numerous investigations aimed at identifying the molecular mechanisms of resistance in cancer cells. An increasing understanding of the *MDR1* gene and its role in drug resistance has been provided by data from several major laboratories [1–4].

Tumour cells that develop resistance through the MDR mechanism simultaneously develop cross-resistance to several structurally unrelated natural products. Cell lines characterised by the MDR phenotype are resistant to anthracyclines, vinca alkaloids, epipodophyllotoxins, taxanes and actinomycin-D (Table 1). Resistance to these agents is the result of energy-dependent drug efflux, eventuating in decreased intracellular drug accumulation. The malignant cell lines that display this MDR phenotype usually contain an amplified gene, *MDR1*, which encodes a 4.5 kb mRNA. P-glycoprotein (Pgp or P170), the protein encoded by this gene, is a 170 kd transmembrane protein. Intracellular concentrations of these cytotoxic agents are decreased as a consequence of the transmembrane protein, Pgp, transporting drugs extracellularly in an energy-dependent manner. P-glycoprotein is one of a large superfamily of ATP-binding cassette transport proteins with a conserved amino acid sequence resembling those of bacterial and other eukaryotic transport proteins.

An important feature of the MDR phenotype is its susceptibility to reversal by inhibition of drug efflux. Substances capable of reversing resistance *in vitro* include: verapamil, quinidine, quinine, amiodarone, phenothiazines, reserpine, cephalosporins, cyclosporins, calmodulin inhibitors, dipyridamole, tamoxifen and dihydropyridine [5–7] (Table 2). Thus, MDR represents a drug resistance mechanism that can be reversed *in vitro* and is potentially reversible in patients.

Table 1. Cytotoxic substrates of MDR

Anthracyclines	Taxanes
Doxorubicin	Paclitaxel
Daunomycin	Docetaxel
Vinca alkaloids	Other
Vincristine	Actinomycin-D
Vinblastine	Mitoxantrone
Epipodophyllotoxins	
Etoposide	
Teniposide	

Table 2. Inhibitors of MDR

Verapamil	Nifedipine
Diltiazem	Quinidine
Quinine	Reserpine
Cefoperazone	Cloroquine
Trifluoperazine	Tamoxifen
Progesterone	Cyclosporin
Dipyridamole	

MOLECULAR DIAGNOSIS OF MULTIDRUG RESISTANCE

Several molecular methods are available to detect and measure gene expression including evaluating mRNA and protein expression. Levels of mRNA may be measured using RNA slot blots, RNA protection assays, *in situ* hybridisation, Northern blot analysis, and reverse transcription followed by the polymerisation chain reaction (RT-PCR). Protein may be detected by Western blot analysis and immunohistochemical techniques. Each method must be considered in terms of sensitivity, specificity, reproducibility, use as a quantitative assay, and its effectiveness in the detection of *MDR1* gene expression within a heterogeneous background of non-expressing cells. Each methodology therefore carries its own advantages and disadvantages. Different laboratories have used different techniques for detecting expression of the *MDR1* gene in both normal and malignant tissues. Functional assays on tumour cells would be the most convincing evidence that the *MDR1* gene mediates clinical drug resistance; however, the only such techniques currently available are fluorescent antibody cell sorting (FACS) analysis and flow cytometry. In cell lines, indirect fluorescent labelling, dye efflux studies, and flow cytometry can be used to study the effect of various modifiers of MDR on the efflux of fluorescent rhodamine 123 or doxorubicin and serve as sensitive function assays [8, 9]. Clinically, FACS analysis has been used predominantly in leukaemias and ascitic fluid, which easily lend themselves to such study. Although this technique has been reported in solid tumours, the technology to disaggregate a solid tumour to provide viable tumour cells on which such assays can be performed may alter the protein and interfere

with detection and assessment of protein function [10]. In human tumour xenografts that were enzymatically dissociated, Broxterman and associates demonstrated alterations in drug uptake, consistent with *in vivo* functional Pgp. However, determining function at lower levels of Pgp expression, such as found in the KB 8-5 cell line, which is the range of most human tumours, has been inconsistent, suggesting that this technique may have limitations in a heterogenous population [11]. Although it is beyond the scope of currently available techniques to assess the function of P-glycoprotein in solid tumours by assessing tumour cell drug accumulation, it is possible and necessary to perform pharmacokinetic and pharmacodynamic studies when performing clinical trials using *MDR1* modulators.

EXPRESSION AND FUNCTION OF THE *MDR1* GENE IN NORMAL TISSUES

Localisation studies have previously demonstrated that the *MDR1* gene is normally expressed in bile canaliculi of the liver, pancreatic ductules, the lumina of the small and large bowel, the proximal tubules of the kidney, and the adrenal gland [12, 13]. More recently, P-glycoprotein has been found in the endothelial cells of the central nervous system (CNS) and testes, where it may be implicated in both the blood–brain barrier and the putative sanctuary sites in malignancies such as acute lymphocytic leukaemia [13, 14]. In addition, the human placenta expresses Pgp; in this case it may play a role in the transport of endogenous substances [13]. It has also been reported that CD34+ haematopoietic stem cells express Pgp [8]. The localisation of the protein product of the *MDR1* gene is consistent with the role of P-glycoprotein as a transport protein that has possibly evolved teleologically to be involved in detoxification of normal tissues. The potential functions of P-glycoprotein include protecting against exogenous toxins, excretion of metabolites, transport of steroid hormones, extrusion of polypeptides (cytokines) not excreted from cells via classic signal cleavage pathways, ion transport and cell volume regulation. To elucidate the normal function of P-glycoprotein, Schinkel and coworkers [15] recently reported the results of their knockout experiments, in which mice were generated that were homozygous for a disruption of the *mdr*1a gene (see pages 985–990). Phenotypically, these mice were viable, fertile, and appeared normal, but they exhibited an enhanced sensitivity to the centrally neurotoxic pesticide, ivermectin, and to the cytotoxic vinblastine. In these mice, *mdr*1a was noted to be the major P-glycoprotein in the blood–brain barrier and its absence resulted in increased drug levels in many tissues, especially brain, and in decreased drug elimination [16]. In mice homozygous for the disruption of *mdr*2, liver disease developed, which appears to be related to their complete inability to secrete phospholipids into bile [17]. In addition, mice deficient in *mdr*1b and in both *mdr*1a and *mdr*1b have been produced [18]. The *mdr*1b-defective mice display normal phenotypes, lifespan and fertility. A compensatory alteration in *mdr*1a expression has not been observed, and drug sensitivity and pharmacokinetic studies are pending. Mice with the double knockout—*mdr*1a(−/−), *mdr*1b(−/−)—would be the equivalent of eliminating the human *MDR1* gene. The observations of these animals are too preliminary to draw any conclusions except that the phenotype is normal. From these early studies, one can conclude that there is no essential physiological function that mice cannot do without, which is hopeful news with respect to experiments in humans using inhibitors of P-glycoprotein. However, organs normally protected by these proteins may become highly vulnerable to drugs.

MDR1 GENE EXPRESSION IN HUMAN TUMOURS

After the *MDR1* gene was cloned and found to be expressed in specific normal tissues, several investigators analysed human tumours for *MDR1* gene expression. Fojo and associates [19] found that untreated adenocarcinomas from tissues that normally expressed the *MDR1* gene overexpressed *MDR1* RNA. Within this tumour group, the *MDR1* gene has been studied most extensively in renal cell carcinoma. Immunohistochemical and *in situ* hybridisation studies localised *MDR1* in the proximal tubules, the site of origin of most renal cell carcinomas. In addition, the highest levels of *MDR1* have been found in the most differentiated renal cell carcinomas [12]. This is not surprising, since P-glycoprotein expression appears to be a normal, differentiated function of proximal tubule cells [20]. Other untreated malignancies including colon cancer, renal cell carcinoma, hepatoma, adrenocortical carcinoma, phaeochromocytoma, islet cell tumours of the pancreas, and carcinoid tumours frequently express high levels of the *MDR1* transcript [19–21] (Table 3). Clinically, these tumours are resistant to chemotherapy and many are derived from tumours that normally express the *MDR1* gene. A plausible explanation for the intrinsic resistance of these tumours is that their tissues of origin are highly expressive of the *MDR1* gene, which is conserved in these tumours. In addition, these findings demonstrate that once cells undergo malignant transformation, they may continue to express the *MDR1* gene.

Other untreated carcinomas occasionally show high or intermediate levels of *MDR1* RNA. This group includes acute leukaemia, chronic leukaemia, non-Hodgkin's lymphoma, chronic myelocytic leukaemia in blast crisis, neuroblastoma, non-small cell lung cancer with neuroendocrine properties (NSCLC-NE) and astrocytoma. Haematological malignancies within this group are at least initially sensitive to chemotherapy [21–29] (Table 3). Unfortunately, a significant fraction of these malignancies are unresponsive to therapy. In addition, Chan and colleagues [30–31] have demonstrated that overexpression of P-glycoprotein, measured by immunohistochemistry, correlates with a decrease in disease-free and overall survival in both paediatric sarcoma and neuroblastoma.

In many untreated tumours, we found relatively low or undetectable levels of *MDR1* RNA. Included in this group are breast cancer, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), bladder cancer, chronic myelocytic leukaemia (CML), oesophageal carcinoma, head and neck cancer, melanoma, mesothelioma, ovarian cancer, prostate cancer, sarcoma, thymoma, thyroid cancer, Wilms' tumour, and gastric carcinoma [21–32] (Table 3). Some of these tumours, for example, Wilms' tumour, are sensitive to chemotherapy. Others, such as melanoma, are clinically resistant to multiple cytotoxic agents that are substrates of P-glycoprotein and thus mechanisms other than MDR may be important in these malignancies.

Tumours that are initially sensitive to chemotherapy may acquire resistance during or after exposure to therapy. Malignancies that have high levels of *MDR1* RNA after exposure to at least one drug affected by the MDR phenotype include non-Hodgkin's lymphoma, adult and childhood acute lymphoblastic leukaemia, adult acute myelocytic leukaemia

Table 3. Expression of the MDR1 *gene in human tumours*

A.	High expression of the *MDR1* gene in untreated tumours	
	Colon	
	Renal	
	Hepatoma	
	Adrenocortical carcinoma	
	Pheochromocytoma	
	Pancreatic carcinoma	
	NSCLC-NE	
	Carcinoid	
	Multiple myeloma	
	CML-Blast Crisis	
B.	Occasionally high expression of the *MDR1* gene in untreated tumours	
	ALL (adult)	
	AML (adult)	
	Non-Hodgkin's lymphoma	
	Neuroblastoma	
	Astrocytoma	
	CLL	
C.	Low or no expression of the *MDR1* gene in untreated tumours	
	Breast	Mesothelioma
	NSCLC	Ovarian
	Bladder	Prostate
	CML-Chronic Phase	Sarcoma
	Oesophageal	SCLC
	Gastric	Thymoma
	Head and neck	Thyroid
	Melanoma	Wilms'
D.	High *MDR1* gene expression in tumours relapsing after treatment	
	Non-Hodgkin's Lymphoma	Breast
	Neuroblastoma	ALL (childhood)
	CML-Blast Crisis	Phaeochromocytoma
	ALL (adult)	Ovarian
	ANLL (adult)	CLL
	Multiple myeloma	

CML, chronic myelocytic leukaemia; SCLC, small cell lung cancer; NSCLC-NE, non-small cell lung cancer with neuroendocrine properties; ALL, acute lymphoblastic leukaemia; ANLL, acute non-lymphocytic leukaemia; AML, acute myeloblastic leukaemia; CLL, chronic lymphocytic leukaemia.

(AML), neuroblastoma, phaeochromocytoma, breast cancer, ovarian cancer, rhabdomyosarcoma, paediatric sarcoma, and multiple myeloma [21, 33–35] (Table 3).

For most of these cancers, only a small number of specimens have been evaluated for *MDR1* gene expression. In addition, few studies have analysed samples from the same patient before and after therapy to understand the contribution of both intrinsic and acquired drug resistance to clinical drug resistance. However, the more extensive experience with leukaemia, lymphoma, breast cancer, ovarian cancer, neuroblastoma and sarcoma does indicate an association between *MDR1* gene expression and resistance to therapy with agents of the MDR class. Preliminary studies were based on very small sample sizes, but there has been interest in the haematological malignancies as well as several extremely common malignancies, such as lung cancer and breast cancer, for which overcoming drug resistance would have a tremendous impact on response rates and survival in a large number of patients.

Although such data suggest a potential role in *MDR1* expression in the drug resistance of certain malignancies, these studies do not unequivocally verify true causation between *MDR1* expression and clinical drug resistance associated with a poor outcome. Below, we present a disease-oriented review of patterns of *MDR1* gene expression and its clinical significance in solid tumours including breast cancer, lung cancer, ovarian cancer, gastrointestinal malignancies, tumours of the genitourinary tract, sarcomas and neuroblastomas.

Breast cancer

Breast cancer was predicted to affect 183,000 women in 1995 and over 46,000 will succumb to their disease [36]. Although there is a significant response rate to doxorubicin and taxanes in breast cancer, resistance and relapse are the usual outcomes in advanced disease. The role of MDR in breast cancer has been studied by several investigators for the following reasons: (1) the high lifetime incidence of breast cancer in women; (2) patients with advanced disease are often initially responsive to chemotherapy but ultimately develop progressive and resistant disease; and (3) doxorubicin, paclitaxel, and vinblastine all have significant activity in breast cancer and are substrates of P-glycoprotein. The results of these studies have been limited by (1) the size of the study; (2) the retrospective nature of most studies, resulting in possible selection bias; (3) lack of clinical correlation; and (4) absence of sequential tumour sampling before and after treatment with a cytotoxic agent, which is a substrate of Pgp. This last point would lead to an inability to discern between the relative contribution of intrinsic and acquired resistance. A detailed analysis of the published studies of *MDR1* gene expression in breast cancer is illustrative of these points as well as other technical factors involved in explaining discrepancies between studies (Table 4). Issues of method selection for the detection of *MDR1* gene expression (RNA versus protein; quantitative versus qualitative assays), selection of a definition of a positive control that would affect the sensitivity of a chosen method, and sensitivity and specificity of given cDNA probes and monoclonal antibodies may all contribute to the disparate results and are explained in detail.

Many early studies were limited by small sample size, which is illustrated by the investigations of Ro and associates [37], Sugawara and associates [14], and Gerlach and associates [38], whose studies demonstrated little to no *MDR1* gene expression; however, the denominators are too small to reach any conclusions about the significance of the results. In addition, most of the studies, except that of Wallner and colleagues [39] and Verrelle and colleagues [40], are retrospective analyses carried out on archival frozen tissue without any clinical correlation. This may result in possible selection bias. Verrelle and colleagues [40] used the anti-Pgp monoclonal antibody, C494, in an avidin–biotin-immunoperoxidase technique and detected Pgp in 17 of 20 breast cancer specimens. The authors used a semiquantitative method of analysis by grading both the number of positive cells and the specific staining intensity. Although the number of patients in the study was limited, strong Pgp-positive staining found in the majority of tumour cells significantly correlated with no initial response to chemotherapy ($P < 0.02$) and with a shorter progression-free survival ($P < 0.02$). Further follow-up is needed to confirm if the results of Wallner and colleagues [39] from primary breast cancer specimens have any prognostic significance.

Another major limitation of these studies is the absence of sequential tumour sampling before and after treatment with a

Table 4. Multidrug resistance in breast cancer

Study	Treatment	MDR expression	%	Methods
Wallner and associates [39]	None	27/59 +	46	RNA slot blot
Goldstein and associates [21]	None	9/57 + (16%)	16	RNA slot blot
	MDR substrate	6/8 + (75%)	75	
Keith and associates [41]	None	25/49 +	51	Dot blot Northern blot
Merkel and associates [42]	None	0/219 +	0	Southern blot
	Doxorubicin	0/29 +	0	Western blot Northern blot
Gerlach and associates [38]	Unknown	0/3 +	0	Western blots (C219)
Verrelle and associates [40]	None	17/20 +	85	Immunological (C494)
Schneider and associates [45]	None or non-MDR	0/16 +	0	Immunohistochemistry
	MDR substrate	3/7 +	43	C219
Wishart and associates [44]	None			Immunohistochemistry
		21/29 +	72	C219
		16/29 +	55	MRK16
Sugawara and associates [14]	None	1/9 +	11	Immunohistochemistry (MRK16)
Ro and associates [37]	None	0/8 +	0	Immunoperoxidase
	Vincristine/doxorubicin	20/40 +	50	(C219)
Sanfillipo and associates [43]	None	10/34 +	29	C219 immunoblots
	Treated (9 with Doxorubicin)	9/14 +*	64	

*Positive expression was not related to doxorubicin therapy.

cytotoxic agent, which is a substrate Pgp. Such sampling would enable us to determine the role of MDR-mediated intrinsic and acquired drug resistance in breast cancer.

The differences reported in untreated breast cancer are difficult to reconcile. Studies measuring *MDR1* RNA by either slot blot, Northern blot, or RNA protection assays report that 0–51% of untreated breast cancer specimens are positive. Both Keith and associates [41] and Merkel and associates [42] used positive control cell lines, which expressed high levels of *MDR* RNA with positive results as 0% and 51%, respectively. Using control cell lines with high levels of *MDR1* as a positive control may lead to interpretation of low levels of *MDR1* expression as insignificant. Similarly, using a cell line with low expression as a positive control may score as positive some tumours that have only background expression. Sufficient data must be provided by the authors to allow closer comparison of the results. If Merkel and colleagues [42] accepted any level of expression on slot blot as positive, three of their 108 (3%) specimens would be considered positive. The study of Wallner and associates [39] and our own study [21] used the KB8-5 cell line as a positive control (which expresses relatively low levels of *MDR1* RNA) and reported 46% and 16% as positive, respectively. In these studies, therefore, parameters other than selection of a positive control and the definition of a positive result must account for the variability in results. While differences in the cDNA probe used in hybridisation could contribute to the differences in these reports, all but Merkel and colleagues used the same probe. In addition, RNA slot blot analysis is considered quantitative, whereas Northern blot analysis is merely qualitative.

Definition and significance of a positive result with the use of immunohistochemistry may also contribute to the inflated results especially when compared with RNA studies because in some studies using immunohistochemistry the authors use any positive cells as a positive result. The major difference between immunohistochemical techniques and RNA analysis is the measurement of protein as opposed to RNA, but neither method assesses protein function. Immunohistochemistry also has the advantage of analysing individual cells such that tumour cell expression can be differentiated from adjacent normal cells and stroma, whereas isolating RNA from a solid tumour lacks such discrimination. Of importance is the recent report that P-glycoprotein expression has been observed in stromal cells in breast cancer but not in cells of normal breast tissue [44]. Although immunohistochemistry is capable of detecting low levels of expression in individual cells, at the lower limit it may be incapable of distinguishing true expression from background, and in that respect may not be as sensitive for detection of *MDR1* RNA as reverse transcriptase–polymerase chain reaction (RT–PCR). In addition, because of the heterogeneous staining, immunohistochemistry is not as quantitative as RNA slot blot.

Another major factor contributing to discrepancies among reports is the sensitivity and specificity of the specific monoclonal antibody used. As noted, Schneider and coworkers [45] found no expression of P-glycoprotein. Verrelle and colleagues [40] detected expression in 85% of untreated specimens and Wishart and coworkers [44] found different results with different antibodies. The most commonly used monoclonal antibodies are C219 and MRK16. Others include C494, JSB1 and HYB241. C494 and JSB1 recognise internal epitopes, whereas the others recognise external epitopes. Specificity is a significant issue in that C219 crossreacts with *MDR2*, which has not been demonstrated to confer drug resistance, and also crossreacts with myosin. MRK16 is specific for *MDR1*, but may have heterogeneous staining even in control cell lines. Differences in fixation techniques may also contribute to the variability of results, even when the same antibody is used.

By RNA slot blot and RNA protection assay, we found that 9 of 50 (18%) untreated breast cancer specimens had low to intermediate levels of *MDR1* RNA, whereas six of eight (75%) samples from patients treated with at least one MDR cytotoxic

agent had moderate to high levels of *MDR1* mRNA [21]. In general, one could conclude that *MDR1* gene expression is generally low in untreated breast cancer specimens but it may play a significant role in acquired resistance in treated patients. The overall impact of both intrinsic and acquired *MDR1* gene expression in the multidrug resistance of breast cancer has yet to be clearly demonstrated, since only a limited number of patients have been evaluated before and after therapy. In studies that have attempted to do a prospective analysis, further follow-up is needed in the patients to correlate relapse-free and overall survival with *MDR1* expression. Since many of the substrates of P-glycoprotein have activity in breast cancer including doxorubicin, vinblastine and paclitaxel, there is the potential to modulate resistance and improve treatment outcome in breast cancer. In addition, the use of quantitative RT–PCR (Q-RT–PCR) has made it possible to determine *MDR1* expression in extremely small specimens such as those obtained by fine needle aspiration. This method carries the advantages of enhanced sensitivity and being a quantitative method, but it is plagued with the issues of sampling error and the inability to distinguish tumour tissue from stroma.

What is the future of MDR in breast cancer? Uziely and colleagues [46] recently reported preliminary data in 36 patients who had baseline biopsies for detection of P-glycoprotein (immunohistochemistry with C219 and JSB1) and *MDR1* expression (PCR), followed by the administration of paclitaxel. Seventy-four per cent of the patients had progressive disease, and all of these had moderate to high levels of P-glycoprotein. There were two complete remissions and four partial responses, and these patients had low to absent P-glycoprotein expression. PCR has been performed in only 10 patients thus far, but the limited results correlate with the immunohistochemical results. Schneider and associates found a significant association between *MDR1* and Her-2 neu expression in inoperable mammary carcinomas suggesting that *MDR1* may be a marker of tumour aggressiveness as opposed to indicating tumour resistance to chemotherapy [47]. In another small retrospective study of archival paraffin-embedded specimens from operable breast tumours, this group showed a distribution of high levels of Pgp expression with other unfavourable prognostic factors such as p53, PCNA, c-erb-B2 and cathepsin D [48]. In neither of these reports was a correlation made between response to chemotherapy and disease-free or overall survival. A recent Eastern Cooperative Oncology Group (ECOG) protocol for advanced breast cancer randomised patients to doxorubicin, paclitaxel, or the combination of the two in a randomised crossover manner. An ancillary laboratory study is evaluating the role of MDR expression in resistance to these agents and its relationship to their sequencing. Patients had biopsies performed before treatment and at the time of disease progression to determine MDR expression and its potential to predict response to therapy, as well as the time to progression. Prospective evaluations of MDR in locally advanced breast cancer will determine if P-glycoprotein expression predicts failure to respond to induction chemotherapy. Other trials have been designed to evaluate chemomodulators administered to patients at the time of progression while these patients are receiving MDR substrates. The ultimate goal would be to demonstrate a role for MDR in advanced breast cancer and to then use MDR modulators in the adjuvant setting to prevent recurrent resistant disease.

Lung cancer

Lung cancer is the most common malignancy in the United States. The responsiveness of lung cancers to chemotherapy depends on the histology. Ultimately, all patients with metastatic disease will die from their disease, and some form of drug resistance would seem to be involved with these poor outcomes. Small cell lung cancer is a rapidly growing tumour that is initially responsive to either etoposide and a platinum analogue, or the combination of cyclophosphamide, doxorubicin and vincristine. The majority of patients will have a response to initial chemotherapy, but will ultimately relapse and do poorly with salvage regimens. This outcome would seem to invoke a role for both intrinsic and acquired drug resistance in that perhaps a resistant clone expands in the face of chemotherapy, cytoreducing the sensitive population of cells. Most patients with metastatic non-small cell lung cancer (NSCLC) do not respond to a regimen containing etoposide and cisplatin or carboplatin (which are not MDR substrates). This failure would suggest a role for alternative intrinsic drug resistance.

All data regarding MDR expression in lung cancer are retrospective, and both electrophoretic and immunohistochemical methods of evaluation have been utilised. Lai and associates [32] examined 24 lung tumours and 67 lung cancer cell lines by RNA slot blot analysis. Fourteen (58%) tumours, both small cell and NSCLC, expressed intermediate levels of *MDR1* mRNA, but the only one to express high levels was NSCLC with neuroendocrine features (NSCLC-NE). Forty-five (67%) of the cell lines expressed low or undetectable levels of *MDR1* mRNA, but five of six cell lines derived from the NSCLC-NE subgroup had intermediate to high levels of MDR expression. The levels of *MDR1* mRNA expression were compared to nine matched tumour cell line pairs, and there was no significant difference between the two groups ($P = 0.07$). Of interest, the cell line studies showed no correlation between MDR expression and subsequent response to chemotherapy, and no correlation between MDR expression and *in vitro* chemosensitivity, but the levels of MDR expression were generally low, and therapy did not contain MDR substrates. It was therefore postulated that in addition to MDR, other mechanisms must be involved with the poor response of advanced lung cancer to chemotherapy. As a consequence of these findings, the multidrug-resistant related protein (MRP) gene was cloned from a small cell lung cancer cell line that expressed a multidrug-resistant phenotype but did not express *MDR* [49].

Holzmayer and colleagues [50] evaluated 24 untreated lung cancers and four treated lung cancers using RT–PCR analysis. Fifty per cent (four of eight) of small cell cancers and 80% (16 of 20) of untreated adenocarcinomas expressed *MDR1* mRNA. All four patients (three NSCLC, one small cell) treated with MDR regimens expressed *MDR1* mRNA. 7 patients with small cell lung cancer were subsequently treated with MDR regimens. The three MDR-negative patients responded; the four MDR-positive patients were unresponsive to regimens that employed MDR substrates such as doxorubicin, vincristine and etoposide ($P < 0.029$). Cordon-Cardo and O'Brien [51] evaluated lung tumours using immunohistochemistry and found that none of six small cell and 2 of 27 (7%) NSCLC expressed P-glycoprotein. The data regarding MDR in lung cancer are retrospective and the numbers are small, but the majority of patients were evaluated by PCR or slot blot analysis, which are sensitive techniques.

At this time, MDR probably represents just one of several resistance mechanisms in advanced lung cancer, and prospective trials are needed to define more clearly its role in prognosis. Recently, paclitaxel has been demonstrated to have activity in both small cell and NSCLC, and therefore the evaluation of MDR in these tumours may in fact have a significant impact in the treatment design of these common diseases [52–55].

Gynaecological tumours (Table 5)

The role of MDR in ovarian cancer has evolved over time. Historically, cyclophosphamide, doxorubicin and cisplatin (CAP) was a common initial therapy for advanced ovarian cancer. Subsequent studies demonstrated that full-dose cyclophosphamide and cisplatin (CP) resulted in similar response rates and survival compared to CAP, and therefore CP became the standard therapy [56]. More recently, a multi-institutional randomised trial of advanced ovarian cancer demonstrated significantly increased response rates and a suggestion of improved progression-free survival for the regimen of paclitaxel and cisplatin compared to CP [57]. The use of paclitaxel for initial therapy of advanced ovarian carcinoma should again spark interest in the examination of MDR in ovarian malignancies.

All evaluations of MDR in ovarian cancer have been retrospective thus far. Small numbers of patients have been evaluated, and patients exposed to MDR substrates appear to have higher levels of MDR expression than untreated patients. Bell and associates [58] used Western blot analysis with the monoclonal antibody C219 to show that two of five (40%) pretreated patients expressed P-glycoprotein. In untreated patients, we showed that none of 16 patients expressed *MDR1* mRNA [21]. Bourhis and associates [33] evaluated 50 ovarian tumour specimens by Northern blot and slot blot analysis. Three of 10 specimens (30%) treated with doxorubicin or vincristine expressed *MDR1* mRNA, while none of 35 untreated and none of 5 patients treated with a non-MDR regimen expressed it. Holzmayer and colleagues [50] evaluated 60 ovarian cancer patients for MDR expression with RT–PCR analysis. Thirty of 46 (65%) untreated specimens were positive, as were two of five (40%) specimens from patients treated with non-MDR regimens. Most strikingly, 10 of 10 specimens from patients treated with MDR regimens were positive for MDR expression. Five of seven (72%) patients who were MDR-negative responded to an MDR regimen, whereas only one of 10 (10%) who were MDR-positive responded ($P < 0.035$). These results vary from others in that a higher percentage of both untreated and treated patients expressed MDR, which may be due to the increased sensitivity of PCR analysis, and suggests that low levels of expression may be sufficient to confer resistance. This was a well-controlled study, as each experiment was performed in triplicate and both MDR-positive KB cells and negative controls were used. This is the only study to detect *MDR1* mRNA in untreated patients.

Studying samples prospectively using RT–PCR and/or immunohistochemistry analysis may help determine the significance of the low levels of MDR expression. Although these are small numbers of patients, there appears to be a potential role for MDR as an acquired form of drug resistance in women with ovarian cancer. In the future, as more women receive paclitaxel as part of a first-line regimen for ovarian cancer, prospective studies can be carried out to understand better the true prognostic role of MDR, as well as to evaluate the effects of MDR modulators in ovarian cancer.

Two studies have evaluated MDR in cervical cancer. Riou and colleagues [59] examined 92 specimens by slot blot analysis, 77 of which were of squamous cell histology. Twenty-four of 69 (35%) untreated squamous cell cancers expressed *MDR1* mRNA, while it was expressed by seven of eight (88%) tumours treated with chemotherapy or radiation. Not all patients who received chemotherapy were treated with MDR substrates. One patient had a low level in the untreated primary and normal liver, while a much higher level was demonstrated in a liver metastasis after a vincristine-containing regimen. Schneider and associates [60] used immunohistochemical analysis with C219 to show that 10 of 11 (91%) cervical cancers, including both tumours pretreated with vincristine, and all 10 normal human cervical controls expressed P-glycoprotein. The role of P-glycoprotein, if any,

Table 5. MDR (multidrug resistance) in gynaecological tumours

Study	Site	Treatment	MDR expression	%	Methods
Goldstein and associates [21]	Ovary	None	0/16 +	0	RNA slot blot
Bourhis and associates [33]	Ovary	None or non-MDR regimen	0/40 +	0	RNA slot blot Northern blot
		MDR substrates (vincristine or doxorubicin)	3/10 +	30	
Holzmayer and associates [50]	Ovary	None or non-MDR regimens	32/51 +	63	RNA slot blot
		MDR substrates	10/10 +	100	
Bell and associates [58]	Ovary	Doxorubicin-based regimens	2/5 +	40	Western blot (C219)
Riou and associates [59]	Cervix, squamous cell	None	24/69 +	35	RNA slot blot
		MDR and non-MDR regimens	7/8 +	88	
Schneider and associates [60]	Cervix	None	8/9 +	89	Immunohistochemistry (C219)
		Vincristine	2/2 +	100	
Schneider and associates [61]	Endometrium	None	20/20 +	100	Immunohistochemistry (C219)
		Doxorubicin	3/3 +	100	

in normal cervical tissue needs to be investigated, but these data, while limited, may explain why advanced cervical cancer responds best to platinum-based therapy and poorly to MDR substrates.

Schneider and associates [61] also evaluated endometrial cancer with the C219 and JSB1 antibodies for P-glycoprotein expression. Evaluation of 23 endometrial cancers, including three that had received prior doxorubicin-based therapy, showed that all overexpressed P-glycoprotein. In contrast to Thiebaut and associates [12], who did not detect P-glycoprotein in the normal uterus with the monoclonal antibody MRK16, all 10 normal endometrial controls overexpressed P-glycoprotein [61]. If these results are confirmed in a larger study, it could be postulated that P-glycoprotein plays a role in steroid hormone transport in the normal uterus. Doxorubicin, which is the most active agent in this disease, exhibits only a 20–30% response rate. Prospective prognostic analyses are needed to determine if MDR expression is predictive for those patients who are not responsive to doxorubicin.

Gastrointestinal tumours (Table 6)

Because the normal colon, pancreas, and liver express P-glycoprotein and tumours from these organs are relatively chemoresistant, gastrointestinal (GI) tumours have been extensively examined for MDR expression. These data are summarised in Table 6. Evaluations of MDR in colon cancer have been retrospective, the only correlation with chemosensitivity has been *in vitro*, and, with one exception, immunohistochemical techniques have been used exclusively. We showed by slot blot analysis that 35 of 41 (85%) untreated colon cancer specimens expressed *MDR1* mRNA [21], while Cordon-Cardo and O'Brien [51], by immunohistochemistry, found 9 of 11 (82%) colon specimens expressed P-glycoprotein. Yaumachi and associates [62] showed that three of eight (38%) colon cancer specimens expressed P-glycoprotein, and using tumour cell suspensions, each MDR-positive specimen was shown to be resistant to doxorubicin *in vitro*. Peters and associates [63] showed that 23 of 24 (96%) colon cancer specimens expressed P-glycoprotein, but these tumours also had increased amounts of GST-Pi.

Weinstein and associates found a statistically significant association between Pgp immunostaining in a specific subpopulation of cancer cells and a high prevalence of vessel invasion and lymph node metastases suggesting that Pgp may be a marker of invasiveness or aggressiveness [64]. One small study ($n = 52$) reported that Pgp had prognostic significance in Dukes' B2 stage colon cancers [65], but this was of only marginal significance ($P = 0.04$) and was not demonstrated in two other studies [66, 67]. Furthermore, no association between mutant p53 and Pgp expression was found in 34 colorectal tumours suggesting that mutant p53 does not induce Pgp overexpression in colorectal cancers as indicated by *in vitro* studies [68].

These studies show that the MDR phenotype helps to explain the resistance of colon cancer to chemotherapy, especially MDR substrates, but since modulated 5-fluorouracil (5-FU), which is a non-MDR substrate, only has a 35% response rate in advanced disease, alternative mechanisms of drug resistance must also be involved.

Surgery is the mainstay of therapy in localised hepatocellular carcinoma, whereas chemotherapy has been generally ineffective in advanced disease. Doxorubicin is the most active agent in this disease, but offers only a 20% response rate. Since P-glycoprotein is overexpressed in normal liver tissue, it has been postulated that MDR may be responsible for the resistance of hepatomas to chemotherapy. The series evaluating MDR in hepatomas was retrospective, with some limited data correlating expression to chemotherapy responses *in vitro* and *in vivo*. In untreated hepatomas, we found that 7 of 12 (58%) expressed *MDR1* mRNA [21]. Huang and colleagues [69] used slot blot analysis to evaluate 6 treated and 10 untreated patients. In the untreated patients, *MDR1* mRNA levels were higher in the tumour tissue than in the normal tissue. 5 of 6 patients (83%) who were treated with mitomycin C, doxorubicin, or both had increased levels of *MDR1* mRNA, and all 6 patients were clinically resistant to chemotherapy. Isshiki and colleagues [70] showed that 4 of 7 (57%) hepatoma cell lines expressed P-glycoprotein by immunohistochemical analysis with C219 and Western blot. This correlated with *in vitro* sensitivity to doxorubicin but not to cisplatin. Less staining was observed in tumour samples than in normal samples, but the tumours and normal samples were from different patients. Itsubo and colleagues [71] analysed 43 hepatomas by immunohistochemistry with the JSB-1 monoclonal antibody. Twenty-nine of the 43 (67%) hepatomas expressed P-glycoprotein, but this was not related to prior chemotherapy. These studies show that P-glycoprotein expression is common in primary and pretreated hepatocellular carcinoma, but prospective data are needed to explain the

Table 6. Multidrug resistance in gastrointestinal tumours

Study	Site	Treatment	MDR expression	%	Methods
Goldstein and associates [21]	Colon	None	35/41 +	85	RNA slot blot
	Hepatoma	None	7/12 +	58	
Peters and associates [63]	Colorectal	None	23/24 +	96	Western blot (C219)
Cordon-Cardo and O'Brien [51]	Colon	None	9/11 +	82	Immunohistochemistry (panel)
Yamauchi and associates [62]	Colon	None	3/8 +	38	Immunohistochemistry (C219)
Huang and associates [69]	Hepatoma	None	10/10 +	100	Dot blot
		Mitomycin C or doxorubicin	5/6 +	83	
Itsubo and associates [71]	Hepatoma	None	12/16 +	75	Immunohistochemistry (JSB1)
		Doxorubicin, epirubicin, and/or mitomycin C	17/27 +	63	

relationship of P-glycoprotein expression to chemotherapy resistance in this disease.

Genito-urinary tumours (*Table* 7)

The treatment of advanced renal cell cancer has continued to challenge clinicians. The aggressiveness with which this tumour may grow and metastasise varies from patient to patient, and renal cell carcinoma remains one of the most clinically drug-resistant human malignancies. Chemotherapeutic agents classically described as members of the MDR phenotype—such as vincristine/vinblastine and doxorubin—have shown marginal activity, with average response rates of only 7–10%, consistent with intrinsic drug resistance to these as well as to non-MDR cytotoxic agents. The presence of intrinsic resistance as well as the development of acquired resistance has led to the extensive investigation of MDR in renal cell carcinoma. Extensive characterisations of the levels and distribution of *MDR1* mRNA and P-glycoprotein in the human kidney and human renal cell carcinoma specimens have been reported in addition to grade, histology, degree of differentiation, and degree of chemosensitivity or resistance, and will be reviewed below. Data regarding *MDR1* gene expression in genito-urinary (GU) tumours are summarised in Table 7. The studies to date in renal cell carcinomas are retrospective evaluations that use RNA slot blots, Northern blots, or immunohistochemistry. All data in terms of chemosensitivity or modulation are *in vitro* data, and as yet there are no prospective clinical data.

With the use of slot blot analysis, Kanamaru and colleagues [20] demonstrated that the mean level of *MDR1* mRNA expression was higher in well differentiated renal cell carcinomas than in poorly differentiated tumours. This is consistent with the data showing that the renal proximal tubular epithelium, from which most renal cell carcinomas arise, expresses high levels of P-glycoprotein [12]. Rochlitz and coworkers [72] reported similar results, finding that 31 of 40 (78%) untreated renal cell carcinomas expressed P-glycoprotein, detected by immunohistochemical analysis with the C219 monoclonal antibody, with a greater staining intensity in the more differentiated tumours. We showed that 40 of 50 (80%) untreated renal cell cancers expressed *MDR1* mRNA, as determined by slot blot analysis [21]. Kakehi and associates [22] evaluated 14 previously untreated renal cell carcinoma specimens with Northern blot and slot blot analysis. Twelve of 14 (86%) had elevated *MDR1* mRNA levels, and this percentage was higher than that of other organ-specific GU cancers. By the use of cell cultures, Kakehi also demonstrated that *in vitro*, vinblastine sensitivity inversely correlated with *MDR1* mRNA levels, and *in vitro*, doxorubicin sensitivity was decreased in the renal cell carcinomas compared with the other GU tumours [22]. By the use of immunohistochemistry and short-term cultures of renal cell carcinomas from untreated patients, Bak and associates [73] showed that 10 of 17 (59%) renal cell carcinomas that were doxorubicin-resistant *in vitro* expressed P-glycoprotein, while none of four sensitive tumours had expression. Nishiyama and colleagues [74] used immunoblots with the C219 antibody to show that 33 of 38 (87%) renal cell carcinomas (one with prior treatment) expressed P-glycoprotein, while only 3 of 17 (18%) transitional cell carcinomas had detectable levels.

Unfortunately, renal cell carcinoma is refractory to most chemotherapeutic agents, and Volm and associates [75] showed that at least two of three resistance mechanisms (MDR, increased GST-P levels, decreased topoisomerase II levels) exist in 88% of untreated renal cell carcinomas. *In vitro* studies have suggested that MDR in renal cell carcinoma can be circumvented with pharmacological agents [76, 77], but this has not been examined *in vivo*. In summary, P-glycoprotein may contribute to the MDR phenotype in renal cell carcinoma, but it is unlikely that it is the exclusive cause of drug resistance. It is expressed in a significant number of tumours; there is decreased *in vitro* sensitivity to MDR sub-

Table 7. Multidrug resistance in genitourinary tumours

Study	Site	Treatment	MDR expression	%	Methods
Kanamaru and associates [20]	Renal	None	38/38 +	100	RNA slot blot
Kakehi and associates [22]	Renal	None	14/14 +, 12 high	100	RNA slot blot
	Bladder		6/6 +, 0 high	100	Northern blot
	Ureteral		1/1 +, 0 high	100	
	Prostate		2/2 +, 0 high	100	
	Testicular		1/1 +, 0 high	100	
	Renal pelvis		1/1 +, 0 high	100	
Goldstein and associates [21]	Renal	None	40/50 +	80	RNA slot blot
	Bladder		1/6 +	17	
Bak and associates [73]	Renal	None	10/21 +	48	Immunohistochemistry (C219, 265/F4)
Nishiyama and associates [74]	Renal	One with doxorubicin	33/38 + (treated patient +)	87	C219 immunoblot
	Bladder	None	3/17 +	18	
Rochlitz and associates [72]	Renal	None	31/40 +	78	Immunohistochemistry (C219)
Volm and associates [75]	Renal	None	23/31 +	74	Immunohistochemistry (panel)
Benson and associates [10]	Bladder	None	17/23 +	74	Flow cytometry
		Three with MDR substrates	2/3 +	67	(C219)
Siegsmund and associates [81]	Prostate	None	8/11 +	73	RT-PCR
Goldstein and associates [21]	Prostate	None	0/3 +	0	RNA slot blot

strates, and resistance has been reversed *in vitro* with pharmacological modulators of MDR. The limitations to investigating MDR modulators in renal cell carcinoma are (1) the lower incidence of this disease compared with other malignancies, making patient accrual difficult; (2) a reluctance to use conventional chemotherapeutic agents, even with a modulator as initial therapy when vinblastine, the most active drug, has a response rate on the order of 10%; and (3) the likelihood of multiple mechanisms of drug resistance.

Unlike renal cell carcinomas, other GU malignancies are not known to commonly express MDR, but the data are very limited and all reported studies are retrospective. Benson and colleagues [10] demonstrated transitional cell carcinoma (TCC) of the bladder from most untreated patients expressed low or undetectable levels of Pgp, but significantly higher levels can be observed in tumours of patients with prior therapy. We showed that only one of six (17%) bladder cancers expressed *MDR1* mRNA by slot blot analysis [21]. Cordon-Cardo and O'Brien [51] showed that 4 of 10 (40%) bladder cancer patients had patchy immunoreactivity with anti-P-glycoprotein antibodies. Although MDR does not appear to be highly expressed in untreated bladder cancer, it may be clinically useful to evaluate it in patients who progress on chemotherapy, especially vinblastine, doxorubicin and paclitaxel, to determine its importance in acquired drug resistance.

Although prostate cancer is the most common malignancy in males, the use of hormonal therapies in metastatic disease has limited the evaluation of MDR in this setting. Because chemotherapy has generally been ineffective in prolonging survival in hormone-refractory patients, investigations of drug resistance in this common disease may be useful. This is especially true in light of recent trials using vinblastine or paclitaxel in combination with estraumustine in this disease [78, 79]. With slot blot analysis, we reported *MDR1* mRNA levels to be undetectable in three untreated prostate cancer specimens [21]. Theyer and colleagues [80] demonstrated P-glycoprotein expression with Western blot (C219) in the PC-3 and DU-145 prostate cancer cell lines. With the use of flow cytometry, drug efflux in these cell lines was reversed by verapamil. Siegsmund and associates [81] demonstrated that 8 of 11 untreated prostate cancer specimens expressed *MDR1* mRNA using RT–PCR. In the same analysis, Siegsmund and colleagues also demonstrated that ketoconazole, which is used as an inhibitor of androgen synthesis in hormone-refractory patients, was able to reverse *in vitro* doxorubicin and vinblastine resistance in KB-V1 cells. If larger studies are able to demonstrate the MDR phenotype in prostate cancer, clinical trials evaluating ketoconazole or other pharmacological MDR modulators in this malignancy may be worthwhile. Although cisplatin is the most active agent in testicular cancer, etoposide is used in combination as part of the initial treatment regimen for testicular cancer. There are few data on the presence of the MDR phenotype in testicular cancer due to the high response rates to initial chemotherapy, and because relapsed patients are often salvaged with second-line therapy. Evaluation of diagnostic specimens for MDR expression may potentially demonstrate it to be a prognostic factor for the few patients who relapse or fail to respond to therapy.

Sarcomas

As previously discussed, Chan and colleagues [30] reported that MDR expression is an adverse prognostic factor in paediatric sarcomas. Doxorubicin is the mainstay of chemotherapy in adult soft tissue sarcomas, but response rates with combination chemotherapy are approximately only 40%. In adult sarcomas, we [21] found *MDR1* mRNA levels to be undetectable in 11 untreated sarcoma patients; but the clinical outcomes are unknown. Gerlach and colleagues [38] evaluated 25 specimens by immunoblotting with C219. Six of 25 (24%) specimens expressed P-glycoprotein, three of which had been previously treated. In all MDR-positive patients who received chemotherapy, progressive disease developed, and all regimens contained at least one MDR substrate. In 7 of the 8 MDR-negative patients who could be evaluated, progressive disease developed while they were receiving chemotherapy, but the remaining patient had a complete response. These data and the chemoresistance of adult soft tissue sarcomas led to a prospective evaluation of MDR expression in these tumours. Baldini and associates recently reported that increased levels of Pgp in high grade stage II osteosarcoma treated with surgery and chemotherapy was significantly associated with a decreased probability of remaining event-free after diagnosis ($P = 0.002$) [82]. In the 92 patients that were studied prospectively, Pgp status, as defined by immunostaining with the C219 antibody, was determined to be an independent predictor of clinical outcome by multivariate analysis. Of interest in this study is that Pgp expression did not correlate with the extent of tumour necrosis after preoperative chemotherapy indicating that Pgp expression was unrelated to tumour response to chemotherapy and suggesting that Pgp may be a marker of poor prognosis or tumour aggressiveness as opposed to a predictor of chemoresponsiveness at least in this disease.

Neuroblastomas

The paediatric data, although retrospective, may serve as a model for the evaluation of the clinical significance of MDR in adults. Studying samples from children with neuroblastoma, we found significantly higher levels of *MDR1* expression in samples from patients treated compared with those from untreated patients [24]. We also noted no correlation between *MDR1* expression and *N-MYC* oncogene amplification [24]. Chan and associates [31] reported that expression of the *MDR1* gene, detected by immunohistochemical analysis using the monoclonal antibody C494, significantly correlated with poorer overall survival in neuroblastoma (see Chan and associates, pages 1051–1061). In addition, Bourhis and colleagues [25] measured *MDR1* transcripts from neuroblastomas at the time of surgery with Northern and slot blot analysis and found 15 of 15 patients with low or undetectable *MDR1* mRNA levels responded to chemotherapy, while only 6 of 11 who were MDR-positive responded ($P = 0.007$). If these data are verified in prospective trials, MDR expression in neuroblastoma may yield important prognostic information by identifying patients who would be candidates for clinical trials of new drugs or MDR modulators.

Miscellaneous solid tumours

Some less common malignancies have also been evaluated for MDR, including adrenocortical carcinomas, phaeochromocytomas, soft tissue sarcomas, and brain tumours. By the use of a panel of four monoclonal antibodies, Flynn [83] demonstrated that 11 of 11 adrenocortical carcinomas expressed P-glycoprotein, and the intensity of immunoreactivity was equal to or greater than that of the normal adrenal

cortex. We found overexpression in 7 of 9 patients with adrenocortical carcinomas and in 15 of 20 untreated phaeochromocytomas [21]. These results are not surprising, because the normal adrenal cortex and medulla have been shown to express P-glycoprotein [12].

The resistance of brain tumours to chemotherapy is a challenging problem. Some chemotherapeutic agents lack the ability to penetrate the blood–brain barrier, and P-glycoprotein present in the capillary endothelial cells may prevent vincristine or doxorubicin from entering tumour cells. Henson and colleagues [84] with the use of HYB241, the mouse monoclonal antibody against P-glycoprotein, reported that three of 49 brain tumours expressed P-glycoprotein, including metastases. Vascular endothelial staining within the tumour specimens was present in 37 of 49 (76%) cases. We showed that two of four (50%) untreated primary brain tumours expressed *MDR1* mRNA (unpublished data). Therefore, the resistance of brain tumour cells to MDR substrates may not only be due to their failure to cross the blood–brain barrier, which may be related to the presence of P-glycoprotein in capillary endothelial cells in the brain, but also to the presence of P-glycoprotein in the tumours themselves.

REASONS FOR LOW *MDR1* EXPRESSION

Several explanations for low levels of *MDR1* RNA in some drug-resistant tumours are possible. First, there is the heterogeneity of cell types within a tissue sample. For example, in preparing RNA from a tumour specimen, part of it may contain non-tumour stromal cells or fibrous tissue. Heterogeneity may also exist at the cellular level within a tumour. That is, within a given tumour there are populations of cells that may or may not express the gene in question. In such cases, many available techniques lack the sensitivity necessary to detect such expression. Single cell techniques in combination with PCR technology are currently being developed to address this issue in leukaemia and myeloma, but they are not yet available for reproducible assays in solid tumours. Immunohistochemical evaluation of P-glycoprotein expression in breast cancer has demonstrated staining of stromal and other non-malignant cells within or adjacent to tumour tissue. This staining may represent true Pgp expression or non-specific hybridisation with other proteins. Finally, mechanisms other than MDR may account for resistance to chemotherapy in highly clinically drug-resistant tumours that do not express *MDR1*.

CONSIDERATIONS IN THE DESIGN OF FUTURE STUDIES

Appropriate disease

It is clear from the above data that expression of the *MDR1* gene in a variety of malignancies, both at initial diagnosis and at time of relapse, indicates that *MDR1* may be responsible for at least one mechanism of clinical drug resistance. Modification of multidrug resistance with reversing agents offers a potential mechanism by which the activity of chemotherapy may be improved. Well-designed phase I, II and III prospective clinical trials, using reversing agents in conjunction with chemotherapy, in appropriate malignancies that express the *MDR1* gene, are necessary before agents such as verapamil and quinidine, which carry innate toxicities can be used routinely. Epithelial tumours, such as colon and renal cell carcinoma, express the *MDR1* gene and are clinically resistant to a variety of cytotoxic agents, many of which are not substrates of P-glycoprotein. In this situation, MDR may be one of a complex array of drug resistance mechanisms. Thus tumours such as lymphoma, leukaemia, breast cancer, and ovarian cancer would be more appropriate diseases on which to base further investigation. These are malignancies for which many active chemotherapeutic agents are handled by MDR, and for which an alteration in drug efflux may indeed have an impact on response.

Correlation of MDR1 *gene expression*

To draw any conclusions with regard to agents modifying drug resistance on the basis of MDR, it is necessary to have sequential biopsy specimens for analysis of MDR expression so that one can correlate tumour response or resistance to *MDR1* gene expression. As discussed above, there are many techniques currently available for analysis of expression, and the current recommendations offer at least two levels of analysis—for example, with immunohistochemistry and RT–PCR. However, lack of standardisation of techniques currently makes it difficult to compare results from different reports. In addition to expression, functional assays would be more definitive in demonstrating that an alteration in response in the presence of an MDR modulator was a consequence of an alteration in drug efflux. Although such analyses are difficult to do in solid tumours, such as breast cancer, as described above, *ex vivo* plasma assays from patients being studied to determine if an adequate plasma concentration of drug is available to reverse resistance *in vitro* would be a reasonable substitute.

CONCLUSION

Drug resistance is a major obstacle in the treatment of malignancies. Although MDR-mediated drug resistance has been well characterised in preclinical models, its role in clinical drug resistance is not as well characterised and requires further investigation. There appears to be an association between *MDR1* expression and prognosis in leukaemia and lymphoma; but prospective studies are necessary to establish the role of *MDR1* gene expression in the clinical resistance of solid tumours. Associations such as these may represent one of a cascade of poor prognostic biological markers as suggested in colon cancer and by the recent osteosarcoma data. In addition, other mechanisms of resistance are likely especially in tumours such as colorectal carcinoma. Several candidate genes involved with drug resistance are topoisomerase II and MRP. Additionally, mechanisms involved in the blockade of the apoptotoic pathway such as mutated p53 or overexpression of bcl-2 may play a significant role in drug resistance.

The ability to identify tumours with increased *MDR1* gene expression has several potential applications, such as the prediction of response to chemotherapy and the design of studies aimed at reversal of resistance with agents that inhibit MDR-mediated drug. The initial goal of such trials is to demonstrate the ability to reverse *MDR1* mediated drug resistance in the appropriate advanced refractory malignancies. Ultimately, it will be important to incorporate these reversal strategies in the treatment of early stage disease, at which time the tumour burden is smaller and fewer mechanisms of resistance may be present. Prospective phase I, II and III clinical trials, that use reversing agents in conjunction with chemotherapy in malignancies that express the *MDR1* gene, such as the haematological malignancies and breast cancer, are necessary before agents such as verapamil, quinidine and cyclosporine and their

analogues which carry innate toxicities can be used routinely, and result in pharmacokinetic alterations of the cytotoxic agent in question. Recommendations for advancement in the area of clinical investigation of MDR modification include:

—Standardisation of reproducible, sensitive, and quantitative measures of *MDR* gene expression and potential function. This would permit an appropriate comparison among studies.
—Decision network to screen potential reversing agents.
—The development of more active MDR modulators.
—Well-designed phase I, II and III studies in the appropriate malignancies.

MDR is a mechanism of drug resistance that provides the potential for an alteration in drug efflux, which may have a significant impact on response and may possibly improve survival for some cancer patients.

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