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Editorial Comment

MDR1 inhibition: less resistance or less relevance?

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The P-glycoprotein (P-gp) family of proteins promotes resistance to cytotoxic agents by increased efflux from within the cell [1]. Numerous clinical studies have correlated expression of MDR1, a P-gp homologue found in human tissues, with increased drug resistance and decreased clinical response in selected haematological malignancies and solid tumours [2,3]. Whether MDR1 is indeed functionally relevant to tumour drug resistance instead of a tantalising epiphenomenon remains uncertain; it is but one of many proteins implicated in the cellular response to chemotherapy. None the less, this protein has been targeted intensely in recent therapeutic strategies aimed at reversing the drug resistance phenotype [4,5].

With this in mind, Davidson and colleagues (this issue) have attempted to sensitise refractory solid tumours to cytotoxic chemotherapy through pharmacological modulation of MDR1 activity. These authors administered a modified EVE regimen (etoposide, vincristine, and epirubicin—each is a MDR1 substrate) along with high-dose cyclosporin, a well-known MDR1 inhibitor, to 16 paediatric patients whose tumours had progressed on therapy or shortly thereafter. The results were modest: two partial responses and 7 patients with short-lived disease stabilisation. Davidson and colleagues add to a growing literature examining MDR1 blockade, including several large clinical trials. Together with previous observations, these data highlight lingering questions regarding the efficacy and clinical relevance of this mechanism, while illustrating several principles and pitfalls of drug resistance modulation and target-based strategies in general.

Before considering the clinical data on MDR1 modulation, it is useful to review some key concepts of the target validation process. At minimum, a 'valid' protein target must be: (1) expressed or induced in the cancer cells of interest; (2) physiologically active in the appropriate setting; and (3) relevant or limiting with respect to the mechanism being targeted. In clinical trials seeking to validate MDR1 as a drug resistance target, it would therefore seem necessary to confirm its presence and activity within tumours of the study population (this was not done in the Davidson study). To be sure, MDR1 clears such hurdles easily in the preclinical setting; indeed, it represents the principal multidrug-resistance factor in many *in vitro* systems and animal models [6,7]. At the same time, it is one member of a large family of potential drug resistance modulators ([8,9] Table 1). Other well-known processes (e.g. detoxifying enzymes, apoptosis factors, tumour microenvironment) may also play dominant roles in both intrinsic and acquired resistance [10–14]. Therefore, the validation of MDR1 as a clinical target is not an easy task.

None the less, several clinical studies performed during the last decade provided strong evidence linking MDR1 expression and function to a poor response to chemotherapy, particularly in patients with acute myelogenous leukaemia (AML). For example, the Southwest Oncology Group (SWOG) performed both protein expression and functional drug efflux analysis on leukaemic blasts from 211 elderly patients, and found a striking inverse correlation between MDR1 activity and the complete response (CR) rate [2]. A similar study in younger AML patients found that only MDR1 activity, and not other candidate resistance mechanisms, correlated significantly with the CR rate [15]. These data seemed to validate criteria 1 and 2 above for MDR1 as a target for drug resistance reversal.

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Table 1
The ABC transporter family and chemotherapy [9]

Name	Chemotherapy substrates	Tissue expression
MDR1 (P-glycoprotein)	Anthracyclines, vinca alkaloids, taxanes, lipophilic drugs	Liver, kidney, intestine, blood–brain barrier
MRP1	Heavy metals, antifolates, anthracyclines, oestrogen derivatives, vinca alkaloids (with glutathione)	Broad
MRP2, cMOAT	Methotrexate, oestrogen derivatives, platinum, vinca alkaloids (with glutathione)	Liver, kidney, intestine
MRP3, MOAT-D	Epipodophyllotoxins, oestrogen derivatives, methotrexate, vinca alkaloids (with glutathione)	Liver, kidney, intestine, pancreas, adrenal
MRP4-MOAT-B	Purine analogues, oestrogen derivatives	Intestine, prostate, testis, ovary, lung, pancreas
MRP5-MOAT-C	Thiopurines, cyclic nucleotides	Broad
MRP6-MOAT-E	May cooperate with MDR1	Liver, kidney
BRCP, MXR	Anthracyclines, camptothecins	Liver, intestine, breast, placenta

Accordingly, many investigators have attempted pharmacological inhibition of MDR1 concurrently with chemotherapy, in hopes of potentiating anticancer effects by reversing or preventing drug resistance. Most studies have employed cyclosporin or verapamil as MDR1 inhibitors; more recent and ongoing trials tested more potent agents, such as the cyclosporin D analogue PSC833 ([16] Table 2). Some of the clinical data appears tentatively promising, at least in haematological malignancies. A recent SWOG study of 226 patients with relapsed or high-risk AML found that addition of cyclosporin A to a daunarubicin/AraC regimen reduced the frequency of resistance to induction chemotherapy and improved both relapse-free and overall survival [17]. In this study, the addition of cyclosporin was associated with an improved response and survival in the subset of patients who had higher serum concentrations of daunorubicin and its major active metabolite daunorubicinol. The authors concluded that the addition of cyclosporin to anthracycline-containing leukaemic regimens decreases resistance in AML.

Table 2
MDR1 inhibitors in clinical development [16]

First generation	Second generation
Verapamil	PSC833
Quinine	LY335,979
Cyclosporin A	VX710, VX853
	MS-209
	XR-9576
	R10193

However, a closer look at this and other evidence tempers such an optimistic interpretation. One reason for uncertainty pertains to the complex pharmacokinetic effects of the MDR1 modulator with respect to the cytotoxic drug and its metabolites. In the SWOG study, for example, the median serum concentration of daunorubicin in patients also given cyclosporin was more than twice that of those given conventional therapy; that of daunorubicinol was 3–4 times higher, even as long as 10 days after treatment. Thus, much of the clinical benefit seen with cyclosporin in the SWOG trial may derive from effects on anthracycline pharmacokinetics, as opposed to leukaemic MDR1 inhibition.

Similar effects of MDR1 modulators; e.g., increased 'area under the curve' and decreased clearance of chemotherapeutic drugs, have been reported in trials of other cytotoxic MDR1 substrates, including etoposide and mitoxantrone [4,18,19]. The toxicity of such profound pharmacokinetic alterations can be substantial, and may reflect inhibition of drug or metabolite excretion into the bile by MRP-like transporters [4]. Frequently, this interaction requires dose reduction of coadministered cytotoxic agents, sometimes by 60% or more [20]. No pharmacokinetic data was reported in the Davidson study; however, its design reflects this uncertainty regarding cyclosporin's effects on drug clearance: the EVE regimen was administered at a 50% dose reduction in the presence of cyclosporin, and epirubicin was further reduced to 25% of standard paediatric doses to avoid cardiac toxicity. Together, these data suggest that augmented drug exposure in the presence of MDR1 modulators may contribute substantially, if not decisively, to the clinical responses observed. This pos-

sibility confounds interpretation of the Davidson study and other clinical data, as it pertains to the targeted mechanism of MDR1-mediated drug efflux within tumour cells.

Perhaps the most important reason for pessimism regarding MDR1 targeting stems from the substantial evidence that MDR1 function, though critical for cellular efflux in drug-selected experimental tumours, may not be particularly relevant to drug resistance in human cancers. In fact, most randomised studies interrogating MDR1 modulation over the past several years have failed to show an increased response to co-administered chemotherapy [21–23]. The outcomes in solid tumours have been particularly disappointing. Only one positive randomised trial has been reported in epithelial cancers: a small study of 99 patients with anthracycline-resistant breast cancer using verapamil as the MDR1 modulator [24]. The treatment regimen also included vindesine (a MDR substrate) and 5-fluorouracil (5-FU). Higher response rates and overall survival were noted in the study arm. However, neither this trial nor most other solid tumour studies address the possibility of altered pharmocokinetics described above. Studies of D-verapamil (better tolerated than the commercially-available racemic mixture) have shown marked pharmacokinetic interactions and increased anthracycline concentrations [25]. In addition, few trials included expression or functional studies to correlate efficacy with MDR1 expression or activity. These aspects bespeak a more general confounding issue within epithelial malignancies: although the majority are refractory to chemotherapy at the outset, it is not at all clear that this intrinsic tumour resistance involves MDR1. Thus, at least for solid tumours, MDR1 remains poorly validated as a target for modulation by any of the criteria outlined above.

Even in AML, where strong correlative data link MDR1 to refractory disease, MDR1 expression also correlates with more primitive myeloid precursor types [2]. Thus, it is possible that MDR1 actually constitutes a confounding factor marking refractory AML, but is not causally associated with chemotherapy resistance. Deciphering critical drug resistance pathways remains an enormous challenge that will continue to plague clinical and translational investigators. To derive effective rational therapeutics, we must circumvent this obstacle in part by firm adherence to rigorous criteria for validating target proteins and pathways. This goal will be greatly enhanced by the development of more accurate experimental models for clinically relevant tumour resistance mechanisms. At the same time, we must develop technologies that facilitate measurement of target expression and activity within individual patients; the available data show that this may simplify interpretation of clinical effects seen with resistance modulators [18]. Modalities that allow real-time, *in vivo* analysis of transporter function, such as imaging with 99Tcm-sestamibi, a MDR1 substrate, may prove useful in determining *in situ* activity of the resistance mechanism in question [26]. Finally, the advent of proteomics and other genome-scale approaches for the global analysis of cancer biology may accelerate the identification and characterisation of previously unrealised mechanisms responsible for tumour drug resistance [27].

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