VX-710 (Biricodar) Increases Drug Retention and Enhances Chemosensitivity in Resistant Cells Overexpressing P-Glycoprotein, Multidrug Resistance Protein, and Breast Cancer Resistance Protein

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ABSTRACT

Purpose: The pipecolinate derivative VX-710 (biricodar; Incel) is a clinically applicable modulator of P-glycoprotein (Pgp) and multidrug resistance protein (MRP-1); we studied its activity against the third multidrug resistance (MDR)-associated drug efflux protein, breast cancer resistance protein (BCRP).

Experimental Design: VX-710 modulation of uptake, retention, and cytotoxicity of mitoxantrone, daunorubicin, doxorubicin, topotecan, and SN38 was studied in cell lines overexpressing Pgp, MRP-1 and wild-type (BCRP482) and mutant (BCRP482T) BCRP.

Results: In 8226/Dox6 cells (Pgp), VX-710 increased mitoxantrone and daunorubicin uptake by 55 and 100%, respectively, increased their retention by 100 and 60%, respectively, and increased their cytotoxicity 3.1- and 6.9-fold, respectively. In HL60/Adr cells (MRP-1), VX-710 increased mitoxantrone and daunorubicin uptake by 43 and 130%, increased their retention by 90 and 60%, and increased their cytotoxicity 2.4- and 3.3-fold. In 8226/MR20 cells (BCRP482), VX-710 increased mitoxantrone uptake and retention by 60 and 40%, respectively, and increased cytotoxicity 2.4-fold. VX-710 increased daunorubicin uptake and retention by only 10% in 8226/MR20 cells, consistent with the fact that daunorubicin is not a substrate for BCRP482, but, nevertheless, it increased daunorubicin cytotoxicity 3.6-fold, and this increase was not associated with intracellular drug redistribution. VX-710 had little effect on uptake, retention, or cytotoxicity of mitoxantrone, daunorubicin, doxorubicin, topotecan, or SN38 in MCF7 AdVP3000 cells (BCRP482T).

Conclusions: VX-710 modulates Pgp, MRP-1, and BCRP482, and has potential as a clinical broad-spectrum MDR modulator in malignancies such as the acute leukemias in which these proteins are expressed.

INTRODUCTION

Resistance to multiple structurally unrelated cytotoxic agents, termed multidrug resistance (MDR), is present in many cancers and represents a major obstacle to the success of cancer chemotherapy. MDR is frequently associated with overexpression of cell membrane proteins that function as energy-dependent drug efflux pumps, preventing substrate drugs from reaching intracellular targets (1). Drug efflux mediated by these proteins may be blocked by noncytotoxic competitive inhibitors, termed MDR modulators.

Cell membrane proteins that are overexpressed in multidrug-resistant cells include P-glycoprotein (Pgp; Ref. 2), multidrug resistance protein (MRP-1; Ref. 3), and breast cancer resistance protein (BCRP; Ref. 4). These proteins are members of the ATP-binding cassette superfamily of transport proteins (1), but they differ in structure and in substrate specificity. Pgp is a 170,000 kDa protein that confers cross-resistance to many natural product antitumor agents, including anthracyclines, mitoxantrone, epipodophyllotoxins, and taxanes (2). MRP-1 is structurally similar to Pgp but shares only 15% amino acid sequence identity (3); it also confers resistance to anthracyclines, mitoxantrone, and epipodophyllotoxins but differs from Pgp in the level of resistance to taxanes (5, 6). BCRP also confers resistance to mitoxantrone (4), but transport of anthracyclines was recently found to depend on mutations that alter amino acid 482 of the molecule (7). Anthracyclines are not transported by wild-type BCRP, with arginine at the amino acid 482 position (BCRP482), which is present in 8226/MR20 myeloma cells and in acute leukemia patient samples (8, 9). In contrast, they are transported by a mutant BCRP with threonine instead of arginine at the amino acid 482 position (BCRP482T), which is present in MCF7 AdVP3000 breast cancer cells. In addition, unlike Pgp and MRP-1, BCRP confers resistance to camptothecin analogues, including topotecan and SN38 (10, 11). A fourth MDR protein, lung resistance protein, is not an ATP-binding cassette transport protein, but rather a so-called vault protein; it mediates nuclear-cytoplasmic drug distribution rather than cellular drug efflux (12).

Although most MDR modulators used in the laboratory and...
in the clinic are specific for individual drug efflux proteins, the clinically applicable picleline derivative VX-710 (biricodar; (S)-N-[2-Oxo-2-(3,4,5-trimethoxyphenyl)-acetyl]piperidine-2-carboxylic acid 1,7-bis(3-pyridyl)-4-heptyl ester; Incel, Vertex Pharmaceuticals Incorporated, Cambridge, MA) is known to have activity against both Pgp and MRP-1 (13, 14). Here we demonstrate that VX-710 also has activity in increasing drug uptake and retention and reversing drug resistance mediated by wild-type BCRP (BCRP<sup>WT</sup>). These data provide support for further clinical development of VX-710 and testing in malignancies such as the acute leukemias in which these MDR proteins are expressed (8, 9, 15).

**MATERIALS AND METHODS**

**Cell Lines.** Well-characterized cell lines overexpressing Pgp (8226/Dox6, A2780/DX5b), MRP-1 (HL60/Adr), BCRP<sup>WT</sup> (8226/MR20; Refs. 16–18) and BCRP<sup>Pd842T</sup> (MCF7/AdVp3000; Ref. 7) were selected for study. Wild-type HL60 cells served as the negative control for expression and function of all three MDR proteins because the parental 8226 and MCF7 cell lines express low levels of BCRP (19, 20). Cell lines were maintained in exponential growth at 37°C in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies, Inc.), 2 mM L-glutamine, 20 units/ml penicillin, and 20 μg/ml streptomycin (Life Technologies, Inc.) buffered in 5% CO<sub>2</sub> in air. Selection and characterization of drug-resistant cell lines and culture conditions have been described previously (7, 16–18).

**MDR Protein Expression.** To detect Pgp, 0.5 to 1 x 10<sup>6</sup> cells were washed in PBS and incubated for 20 min on ice with MRK16 antibody (Kamiya Biomedical Company, Tukwila, WA) or mouse IgG2a isotype control matched according to immunoglobulin protein content, as described previously (21). Cells were then washed in PBS, incubated with phycoerythrin-conjugated goat-antimouse antibody for 20 min on ice, washed again in PBS, and stored on ice until analysis.

To detect MRP-1, 0.5 to 1 x 10<sup>6</sup> cells were fixed in 3.7% (w/v) formaldehyde for 10 min at room temperature, pelleted, resuspended in ice-cold methanol for 10 min, and washed three times in cold PBS with 1% BSA. Cells were then incubated for 40 min with the MRPM<sub>6</sub> monoclonal antibody (Kamiya) or IgG1 isotype control, washed in PBS with 0.01% Tween, incubated with fluorescein-conjugated goat-antimouse antibody for 20 min on ice, washed again in PBS with 0.01% Tween, and then stored on ice until analysis.

BCRP staining was described previously (19). Briefly, 0.5 to 1 x 10<sup>6</sup> cells were washed in PBS, fixed in 3.7% (w/v) formaldehyde for 10 min at room temperature, pelleted, resuspended in ice-cold methanol for 10 min, washed in PBS with 0.01% Tween and blocked with pooled human serum. The cells were then incubated for 60 min on ice with BXP-21 or BXP34 monoclonal antibody (gifts from Dr. R. J. Schepel, Free University Hospital, Amsterdam, the Netherlands; Ref. 22) in 2% BSA at a final concentration 2.5 μg/ml or with mouse IgG1 (BXP-34) or IgG2a (BXP-21) isotype control, matched according to immunoglobulin protein content. Cells were then washed in PBS with 0.01% Tween, incubated with fluorescein-conjugated goat-antimouse antibody for 20 min on ice, washed in PBS with 0.01% Tween, and stored on ice.

**Drugs.** Drugs studied included mitoxantrone (Sigma, St. Louis, MO), daunorubicin (Sigma), doxorubicin (Sigma), topotecan (LKT Laboratories, St Paul, MN) and SN38 (Pharmacia, Kalamazoo, MI). In studies of drug uptake and/or retention, mitoxantrone, daunorubicin, and doxorubicin were used at a concentration of 3 μM, topotecan at 100 μM, and SN38 at 2.5 μM. We have previously demonstrated that mitoxantrone at a concentration of 3 μM can be used to study modulation of drug retention in cell lines overexpressing Pgp, MRP-1, and BCRP with a standard flow cytometry setup (19). For cytotoxicity studies, cells were exposed to a 5-log range of drug doses with half-log increments. Drugs were prepared from frozen 5 mM stock solutions.

**Modulators.** VX-710 was compared with PSC-833 (Novartis Pharmaceutical Corporation, East Hanover, NJ), p-[diethylsulfamoyl]benzoic acid (Probenecid; Sigma), and fumitremorgin C (FTC; gift from Dr. Susan Bates, National Cancer Institute, NIH, Bethesda, MD), which are modulators specific for Pgp, MRP-1, and both wild-type and mutant BCRP, respectively. VX-710 and PSC-833 were studied at 2.5 μM, probenecid at 1 mM, and FTC at 10 μM; these concentrations were based on previous reports (13, 14, 21, 23–25).

**Modulation of Drug Uptake and Drug Retention.** To evaluate modulation of drug uptake, cells were incubated at a density of 1 x 10<sup>6</sup>/ml for 30 min at 37°C in RPMI 1640 with 10% fetal bovine serum and drug, with and without modulator. After 30-minute drug uptake, cells were washed once in cold PBS, resuspended in cold PBS, and kept on ice until analysis.

To study modulation of drug retention, after 30-minute drug uptake without modulator, cells were resuspended in RPMI 1640 with 10% fetal bovine serum with and without modulator, and incubated at 37°C for 90 min. Cells were then pelleted, resuspended in ice cold PBS, and kept on ice until analysis.

**Flow Cytometry.** Cellular content of the fluorescent drugs mitoxantrone, daunorubicin, doxorubicin, and topotecan was studied by flow cytometry. All of the samples were analyzed on a FacScan flow cytometer (Becton Dickinson, San Jose, CA) with an Argon laser for 488 nm excitation and 530/30 nm band-pass (FL1), 585/42 band-pass (FL2), and 670 long-pass (FL3) filters for emission collection. Flow cytometry data were analyzed using WinList software (Verity Software House, Topsham, ME).

**Flow Cytometry Data Analysis.** Staining intensity was evaluated by comparing binding of each antibody with that of its matched isotype control with the Kolmogorov-Smirnov statistic, expressed as a D-value ranging from 0 (identical distribution histograms) to 1.0 (no overlap in distribution histograms; Ref. 26). A D-value of ≥0.2 is considered positive for staining with MRPM<sub>6</sub>, BXP-21, and BXP-34, because cell fixation is required before staining, increasing the background signal (19). A D-value of ≥0.1 is considered positive for staining with MRK16, which stains unfixed cells (21).

Modulation of drug uptake was measured by comparing cellular fluorescence after drug uptake with and without modulator both by the Kolmogorov-Smirnov statistic and by calculating the relative increase in mean fluorescence intensity (ΔMFI), as [(MFI with modulator − MFI without modulator)/
MFI without modulator| $\times 100\%$. Modulation of drug retention was measured by comparing cellular fluorescence after 90-min efflux in the presence and absence of modulator (21) both by the Kolmogorov-Smirnov statistic and by calculating $\Delta$ MFI.

Although the $\Delta$ MFI calculation provides a more immediately understood comparison of distribution histograms, the Kolmogorov-Smirnov statistic is a more sensitive measurement and, as such, has become a standard method of analysis of modulation of drug retention in clinical samples (21, 25).

HPLC. Cellar content of SN38 was studied by high-performance liquid chromatography (HPLC). SN38 was measured by a validated reverse phase HPLC method with fluorescence detection, using a modification of the method of Warner and Burke (27). SN38 was extracted from 2 $\times$ 10$^6$ cells in 400 $\mu$l of cold acidified methanol by brief sonication. After centrifugation of the cell debris, the supernatant was evaporated and the residue reconstituted in 200 $\mu$l of a 1:1 solution of acidified methanol and 3% triethylamine. Eighty $\mu$l of the supernatant were injected into the HPLC system, which consisted of a Waters Associates M600 pump, M717 autosampler, and M474 fluorescence detector operated by a computer with Waters Millennium software. The separation was carried out on a Waters Nova-Pak C18 column equipped with a MicroPak guard column, with a mobile phase consisting of 20% acetonitrile and 80% triethylamine buffer (pH 5.5). The detection is by fluorescence, with excitation at 370 nm and emission at 510 nm. The standard curve range was 2.5–1000 ng/ml, with a limit of quantitation of 2.5 ng/ml.

Cytotoxicity Assays. To study cytotoxicity in suspension cell lines, cells were plated in 96-well tissue culture plates at a density of 10,000 cells/well in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 20 units/ml penicillin, and 20 $\mu$g/ml streptomycin. Drug was added to the culture medium to achieve final concentrations of 0.3 nM to 10 $\mu$M, with half-log increments, with and without VX-710 at a final concentration of 2.5 $\mu$M. The final volume of medium per well was 100 $\mu$l. Cells were incubated for 96 h at 37°C in a fully humidified atmosphere of 5% CO$_2$ in air. Cell growth was assessed by the sphere of 5% CO$_2$ in air. Cell growth was assessed by the

### RESULTS

M474. Cell lines were analyzed to confirm expression of Pgp, MRP-1, and BCRP (Table 1). Wildtype HL60 cells did not stain with antibodies to any of the three proteins. 8226/Dox6 and A2780/Dx5b cells expressed Pgp, HL60/Adr cells expressed MRP-1, and BCRP expression was confirmed in 8226/MR20 and MCF7 AdVp3000 cells. Wildtype 8226, MCF7, and 8226/Dox6 cells also expressed BCRP, as reported previously (19, 20). Thus, 8226/Dox6 cells coexpressed Pgp and BCRP, whereas A2780/Dx5b cells expressed only Pgp. Of note, BCRP antibody staining does not distinguish between wild-type and mutant forms of the protein.

Modulation of Mitoxantrone and Daunorubicin Transport in Resistant Cells Overexpressing Pgp, MRP-1, and BCRP$^{R482}$. Mitoxantrone, a fluorescent drug that is effluxed by cells that overexpress Pgp, MRP-1, or either BCRP$^{R482}$ or BCRP$^{R482T}$ and is retained in the absence of expression of these proteins (19), was used to assess modulation of drug transport by VX-710, compared with transport protein-specific modulators.

First, to demonstrate that VX-710 itself is not fluorescent, HL60 cells were incubated in medium with increasing VX-710 concentrations, up to 10 $\mu$M. With the FacScan instrument settings used to detect mitoxantrone, VX-710-associated fluorescence was not detected (data not shown).
In addition to modulating mitoxantrone uptake, VX-710 also effectively modulated mitoxantrone retention in cell lines expressing Pgp, MRP-1, and BCRP<sup>R482</sup> (Fig. 2). In A2780/Dox5b (Pgp), 8226/Dox6 (Pgp and BCRP<sup>R482</sup>), HL60/Adr (MRP-1), and 8226/MR20 (BCRP<sup>R482</sup>) cells, the presence of VX-710 increased mitoxantrone retention by 60% (D = 0.5), 100% (D = 0.53), 90% (D = 0.55) and 40% (D = 0.34). In comparison with transport protein-specific modulators, VX-710 modulated mitoxantrone retention similarly to PSC-833 (D = 0.5 and Δ MFI 60% in A2780/Dx5b cells) but less effectively than probenecid (D = 0.79 and Δ MFI 200% in HL60/ADR cells) and FTC (D = 0.71 and Δ MFI 140% in 8226/MR20 cells). In 8226/Dox6 cells (Pgp and BCRP<sup>R482</sup>), VX-710 modulated mitoxantrone retention more effectively than both PSC-833 (D = 0.33 and Δ MFI 20%) and FTC (D = 0.38 and Δ MFI 60%).

VX-710 did not modulate mitoxantrone uptake in HL60 cells (Fig. 1), but modulated mitoxantrone retention (Fig. 2), consistent with the previously described chemosensitizing effects of VX-710 at 2.5 μM in several drug-sensitive parental cell lines (13). In contrast, PSC-833, probenecid and FTC had no effect on mitoxantrone retention in HL60 cells (Fig. 2).

Because daunorubicin is a mainstay of therapy for acute myeloid leukemia (AML) and has been the substrate drug in MDR modulation clinical trials in AML (21, 30) and because AML cells express Pgp, MRP-1 and BCRP<sup>R482</sup> (8), VX-710 modulation of daunorubicin transport was also studied in cell lines overexpressing these proteins. VX-710 effectively modulated daunorubicin uptake and retention (Table 2) in cell lines overexpressing Pgp (8226/Dox6) and MRP-1 (HL60/ADR). In contrast, VX-710 only slightly increased daunorubicin uptake (D = 0.17 and Δ MFI 10%) and retention (D = 0.13 and Δ MFI 10%) in HL60 cells (Fig. 2). VX-710 did not modulate mitoxantrone uptake in HL60 cells (Fig. 1), but modulated mitoxantrone retention (Fig. 2), consistent with the previously described chemosensitizing effects of VX-710 at 2.5 μM in several drug-sensitive parental cell lines (13).

**Modulation of Mitoxantrone and Daunorubicin Cytotoxicity in Resistant Cells Overexpressing Pgp, MRP-1, and BCRP<sup>R482</sup>.** To assess whether VX-710-induced changes in drug uptake and retention correlated with drug sensitivity, modulation of mitoxantrone and daunorubicin cytotoxicity by VX-710 was evaluated in cell lines overexpressing Pgp, MRP-1, and BCRP<sup>R482</sup> (Table 3). IC<sub>50</sub>s were determined in the absence and presence of 2.5 μM VX-710, and the RMF was calculated as the ratio of the IC<sub>50</sub> with drug alone to that with drug and modulator.

We first demonstrated that VX-710 itself is not cytotoxic. Cells from each cell line were cultured for 96 h in 96-well plates in medium without modulator and with 2.5 μM VX-710. For all cell lines, less than 10% difference was observed between growth without and with modulator (data not shown).

As shown in Fig. 3, in concordance with the drug uptake and retention data, VX-710 sensitized cell lines expressing Pgp (8226/Dox6) and MRP-1 (HL60/ADR) to mitoxantrone, with RMFs of 3.1 and 2.4, respectively, and also sensitized these cell
lines to daunorubicin, with RMFs of 6.9 and 3.3, respectively. VX-710 also sensitized 8226/MR20 cells, which overexpress BCRP<sup>R482</sup>, to mitoxantrone, with a RMF of 2.4. Moreover, VX-710 sensitized 8226/MR20 cells to daunorubicin, with a RMF of 3.6, although only minimally increased daunorubicin retention in these cells. Finally, whereas VX-710 increased retention of mitoxantrone and daunorubicin in HL60 cells, it had no effect on cytotoxicity.

The effect of VX-710 on daunorubicin cytotoxicity in 8226/MR20 cells was unexpected, because it had little effect on daunorubicin retention. Because 8226/MR20 cells are known to also express lung resistance protein (25, 31), we hypothesized that VX-710 sensitization of 8226/MR20 cells to daunorubicin in the absence of an increase in retention might be attributable to modulation of intracellular distribution of daunorubicin. We used confocal microscopy to study intracellular daunorubicin distribution in 8226/MR20 cells after incubation with and without VX-710. In these experiments, 8226/MR20 cells were incubated for 2 h with 5 μM daunorubicin or for 24 h with 0.5 μM daunorubicin in the presence and absence of 2.5 μM VX-710.

VX-710 modulation of 8226/MR20 cells was not associated with changes in the nuclear/cytoplasmic distribution of daunorubicin under either of these conditions and, thus, could not be attributed to modulation of lung resistance protein. Fig. 4 shows representative examples of cellular daunorubicin distribution in 8226/MR20 cells after 2-h drug exposure to daunorubicin with and without VX-710.

**Table 2** Modulation of daunorubicin uptake and retention by VX-710 in resistant cell lines overexpressing P-glycoprotein (Pgp), multidrug resistance protein (MRP-1), and breast cancer resistance protein (BCRP<sup>R482</sup>), and in wild-type HL60 cells.

<table>
<thead>
<tr>
<th>Source</th>
<th>Modulation of uptake</th>
<th>Modulation of retention</th>
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<tbody>
<tr>
<td></td>
<td>D-value</td>
<td>Δ MFI (%)</td>
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<tr>
<td>HL60</td>
<td>0.35</td>
<td>20</td>
</tr>
<tr>
<td>HL60/Adr</td>
<td>0.82</td>
<td>130</td>
</tr>
<tr>
<td>8226/Dox6</td>
<td>0.71</td>
<td>100</td>
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<tr>
<td>8226/MR20</td>
<td>0.17</td>
<td>10</td>
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Fig. 2 Modulation of mitoxantrone retention (3 μM) by 2.5 μM VX-710, 2.5 μM PSC-833, 1 mM probenecid, and 10 μM fumitremorgin C in resistant cell lines overexpressing different multidrug resistance (MDR)-associated transport proteins and in wild-type HL60 cells. Modulation was evaluated by the Kolmogorov-Smirnov statistic, measured as a D-value as well as by determining the relative increase in mean fluorescence intensity (Δ MFI).
rubrirucin, topotecan, or SN38 cytotoxicity in MCF7 AdVp3000 cells, whereas FTC markedly increased cytotoxicity of all five drugs. Thus, in contrast to FTC, VX-710 does not modulate retention or cytotoxicity of substrate drugs in MCF7 AdVp3000 cells, which overexpress BCRP<sup>R482</sup>. In MCF7 parental cells, VX-710, like PSC-833, probenecid and FTC, had no effect on mitoxantrone uptake, retention (Δ MFI = 0 for all modulators), or cytotoxicity.

**DISCUSSION**

We have demonstrated that the pipecolate derivative VX-710 is an effective modulator of all three of the MDR-associated transport proteins Pgp, MRP-1, and BCRP<sup>R482</sup>, enhancing drug retention and chemosensitivity. The effects on Pgp (13) and MRP-1 (14) were previously known, and VX-710 was found to interact directly with Pgp and stimulate Pgp ATPase activity (13), and also to interact directly with MRP-1 (14). The effect of VX-710 on BCRP<sup>R482</sup> has not been previously described, and it is not known whether, as with Pgp and MRP-1, there is also a direct interaction with BCRP. On the basis of the data presented here, VX-710 has the potential to reverse clinical drug resistance mediated by all three of the known MDR-associated drug efflux pumps.

Although effective against BCRP<sup>R482</sup>, VX-710 was ineffective against BCRP<sup>R482T</sup>. The latter is expressed in MCF7 AdVp3000 breast cancer cells, in which BCRP was initially described (4) but, thus far, has not been found in clinical samples. Specifically, BCRP<sup>R482</sup> has been found in all cases of both AML (8) and acute lymphoblastic leukemia (9) studied to date. Thus, based on knowledge to date, the lack of effect on BCRP<sup>R482T</sup> should not compromise the efficacy of VX-710 as a clinical broad-spectrum modulator. The amino acid at position 482 of the BCRP protein is known to determine its substrate specificity (7); based on the data presented here, the amino acid at position 482 is also critical in determining modulator specificity.

In direct comparisons with MDR protein-specific modulators in the present study, VX-710 was as potent as PSC-833 in modulating Pgp, slightly less potent than probenecid in modulating MRP-1, and less potent than FTC in modulating BCRP<sup>R482</sup>. In this regard, it should be noted that probenecid and FTC are not suitable for development as clinical MDR modulators because of toxicities associated with their use at the concentrations required for modulation. VX-710 had been previously demonstrated to be as effective as cyclosporine A (CsA) in modulating Pgp, and ~2-fold more effective than CsA in modulating MRP-1 (14). VX-710 had also been found to be a more effective modulator than PSC-833 and CsA in neuroblastoma cell lines expressing Pgp, MRP-1, and lung resistance protein (32). Although less potent than specific MRP-1 and BCRP modulators, VX-710 has the advantages of efficacy against all three MDR-associated drug efflux pumps and of clinical applicability.

Both specific and broad-spectrum modulators have theoretical advantages for clinical MDR modulation. Clinical MDR appears to be multifactorial, and effective modulation may require targeting of multiple transport proteins. The use of a single broad-spectrum modulator may be preferable to the use of combinations of specific modulators to prevent drug interactions and cumulative toxicities. On the other hand, the use of specific modulators might minimize unwanted modulation of nontargeted transporters and minimize effects on nontumor tissues such as normal hematopoietic stem cells, bile canaliculi, and the blood–brain barrier. Clinical application of broad-spectrum...
modulation warrants testing in clinical trials with correlative laboratory studies.

Despite the theoretical concerns about potential toxicities of broad-spectrum modulation, VX-710 has been very well tolerated in clinical experience to date. Doses of VX-710 that produce plasma concentrations sufficient for complete MDR reversal in vitro have not been associated with toxicity (33).

In addition to being well tolerated, another advantage of VX-710 as a clinical modulator is its relative lack of pharmacokinetic interactions. VX-710 has been found to have minimal or no effects on the pharmacokinetics of doxorubicin (34, 35) and mitoxantrone (36), although it does alter paclitaxel pharmacokinetics (33, 37, 38). Thus, anthracycline dose reductions should not be required when these drugs are administered in combination with VX-710. The lack of substantial pharmacokinetic interaction of VX-710 with doxorubicin makes it an attractive agent for clinical MDR modulation in AML and other malignancies in which anthracyclines are mainstays of therapy.

Approaches to reversal of clinical MDR in AML have thus far yielded variable and generally disappointing results. Limitations of clinical trials have included modulator-associated toxicities and toxicities caused by modulator-associated pharmacokinetic interactions with chemotherapy drugs (21). The most successful clinical trial in leukemia to date has been the Southwest Oncology Group phase III CsA trial, in which remission duration and survival were prolonged significantly in patients with refractory and relapsed AML who received chemotherapy with CsA, compared with chemotherapy without CsA (30). In this trial, daunorubicin was administered by continuous infusion, which may have avoided high peak levels and, thus, averted enhanced toxicity associated with pharmacokinetic interaction with CsA. Additionally, CsA may also be more effective than PSC-833 by virtue of partial efficacy against MRP-1 (14).

Finally, based on the data presented here, VX-710 may have other chemosensitizing effects in addition to those resulting from modulation of drug retention mediated by Pgp, MRP-1, or BCRP<sup>482</sup>. In addition to increasing drug retention in cell
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