Protease inhibitors atazanavir, lopinavir and ritonavir are potent blockers, but poor substrates, of ABC transporters in a broad panel of ABC transporter-overexpressing cell lines

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Objectives: A possible mechanism for HIV therapy failure is the efflux of HIV drugs from viral target cells or certain body compartments by ATP-binding cassette (ABC) transporters, allowing ongoing viral replication. Here, we investigated the interaction between protease inhibitors (PIs) and ABC transporters.

Methods: To explore the potential blocking capacity of PIs, we exposed cells overexpressing multidrug resistance 1 P-glycoprotein (MDR1 P-gp), multidrug resistance protein 1 (MRP1) and breast cancer resistance protein (BCRP) to established cytotoxic substrates with or without one of the PIs atazanavir, lopinavir or ritonavir. Furthermore, to assess whether PIs serve as substrates, cell growth-inhibitory effects of these PIs were evaluated on cells overexpressing 1 of 11 ABC transporters and their parental counterparts.

Results: Atazanavir, lopinavir and ritonavir were highly effective in reversing resistance against established substrates in cells overexpressing MDR1 P-gp and MRP1, and, to a lesser extent, BCRP. Concurrently, however, PIs appeared to be relatively poor substrates for ABC transporters. Only a moderate level of resistance to atazanavir was observed in cells overexpressing MRP6 and MRP9 [resistance factor (RF): 2.0–2.6]. Cells overexpressing MDR1 P-gp, MRP3, MRP4 and MRP5 displayed low levels of resistance to atazanavir (RF: 1.3–1.7); MRP7- and MRP9-overexpressing cells to lopinavir (RF: 1.4–1.5); and MRP9-overexpressing cells to ritonavir (RF: 1.4).

Conclusions: PIs can act as potent blockers of MDR1 P-gp, MRP1 and BCRP, but they are poor substrates for 11 ABC transporters. Consequently, ABC transporters are unlikely to play a major role in PI failure, but still may contribute to drug-specific adverse events and drug–drug interactions.

Keywords: HIV protease inhibitors, HIV/AIDS, multidrug resistance, efflux pumps

Introduction

Since the introduction of combination antiretroviral therapy (cART) for HIV infection, treatment failure has become increasingly uncommon. Nevertheless, therapy failure remains an important problem.

HIV therapy failure is often the result of viral resistance-associated mutations. The virus has had the ability to mutate due to insufficient drug concentrations often related to drug non-adherence (not taking medication as prescribed) or caused by (temporary) decreased bioavailability (e.g. in the case of drug interactions or diarrhoea). However, sometimes therapy failure occurs while the patient is adherent and has adequate plasma drug concentrations. Additionally, in most patients on first-line cART, based on protease inhibitors (PIs), therapy failure cannot be ascribed to PI resistance-associated viral mutations. Possible explanations for these cases of therapy failure may have both a virological and a pharmacological character. Novel mutations may lead to viral resistance not detected or recognized by the current methods of genotyping. Additionally, viral replication may be due to suboptimal drug concentrations in cells or compartments, as potentially caused by ATP-binding cassette (ABC) transporters.

ABC transporters represent a large superfamily of ATP-dependent drug efflux pumps, of which ~11 have been described to be associated with multidrug resistance (MDR). Most prominent members include MDR1 P-glycoprotein (P-gp, ABCB1), MDR protein 1 (MRP1, ABCC1) and breast cancer
resistance protein (BCRP, ABCG2) that have proven capacities to efflux various cytostatic and immunosuppressive drugs. ABC transporters are differentially expressed in many different cell types, such as cells in the gut mucosa, cells of the blood–brain and blood–testis barriers, endothelium cells, and lymphocytes and macrophages. ABC transporters may therefore each make a different contribution to the biodistribution and effectiveness of drugs. The presence of these transporters on cancer cell membranes, either since onset or enhanced by cytostatic drug treatment, renders these cells resistant to the used drugs, potentially leading to therapy failure. Recently it was shown that ABC transporters may also play a role in therapy failure of other diseases, e.g. rheumatoid arthritis and epilepsy.

A role for ABC transporters has also been suggested in therapy failure in HIV, especially in those cases with normal plasma drug concentrations and proper adherence, in the absence of viral resistance-associated mutations. Efflux of HIV drugs from the viral target cells, i.e. CD4-positive lymphocytes and macrophages, or from certain body compartments, such as the CSF, would lead to subtherapeutic intracellular or intracompartmen
tal drug concentrations, allowing unhampered viral replication. More detailed knowledge of the contribution of the various ABC transporters to the efflux of antiretroviral drugs is thus needed to address incidental HIV therapy failures.

In particular, it is still unclear whether PIs, representing a prominent class of antiretroviral drugs, are substrates of ABC transporters. Earlier studies on the transport of some of the PIs available nowadays by ABC transporters have predominantly focused on MDR1 P-gp, and to a lesser extent on MRP1 and BCRP. For MDR1 P-gp, it was reported that PIs can be transported, but only to a limited extent; a 1.1- to 3-fold higher transport was observed when a cell line with MDR1 P-gp overexpression was compared with its parental cell line. For MRP1, transport of PIs was not consistently shown, and for BCRP no transport of PIs was observed. However, in vitro studies have suggested that PIs, although being poor substrates, might still act as blockers of these three ABC transporters. This would imply that PIs could hamper the physiological function of these and potentially other ABC transporters. Additionally, by blocking ABC transporters, PIs could interact with drugs that are efficient ABC transporter substrates.

In this study we selected the three most commonly used PIs, atazanavir, lopinavir and ritonavir, to further investigate whether they are capable of blocking ABC transporters and/or can be substrates for a broad panel of ABC transporters. Whereas atazanavir and lopinavir are actually prescribed as active PIs, ritonavir nowadays is only added in a low dose as a pharmacokinetic enhancer of other PIs because of its ability to block cytochrome P450 3A4, thereby increasing the concentration of most of the PIs. As a readout system for blocking by and transport of the PIs, we used cell growth inhibition assays. First, we tested atazanavir, lopinavir and ritonavir in cell lines with overexpression of MDR1 P-gp, MRP1 and BCRP to verify their ability to block transport of cytotoxic substrates and compare them with well-known blockers of the respective ABC transporters. Secondly, we used a broad panel of cell lines, each overexpressing one of the ABC transporters MDR1 P-gp, MRP1–MRP9 (ABCC1–6 and ABCC10–12) or BCRP, to explore their actual ability to transport these three PIs.

### Materials and methods

#### Reagents

Atazanavir was extracted from atazanavir 200 mg capsules manufactured by Bristol-Myers Squibb (Princeton, USA). The contents of the capsules were dissolved in 20 mL of dichloromethane. The solution was filtered through filter paper, after which the dichloromethane was evaporated by blowing in a stream of nitrogen. The precipitate was dissolved in 5 mL of ethanol and stored at −20°C. The atazanavir stock solution had a concentration of 2.34 mM measured by HPLC. Lopinavir and ritonavir were donated by Abbott (Chicago, USA). Both compounds were dissolved in ethanol (stock concentration of 14 mM) and stored at −20°C.

All chemicals and drugs used were from Sigma Chemical Co. (St Louis, MO, USA), unless stated otherwise. Mitoxantrone was purchased from AHP Pharma BV (Hoofddorp, The Netherlands), doxorubicin from Farmitalia Carlo Erba (Brussels, Belgium), trichloroacetic acid from ICN Biomedicals Inc. (Aurora, USA) and MK571 from Alexis Biochemicals (Grüningen, Germany); Ko143 was obtained from Professor G. J. Koomeen (University of Amsterdam, The Netherlands).

#### Cell lines

The human MCF-7 breast cancer cell line and its sublines MCF-7/Dox40 with MDR1 P-gp overexpression, selected with 400 nM doxorubicin, and MCF-7/MR cells with BCRP overexpression, selected with 80 nM mitoxantrone, were obtained from Dr W. S. Dalton (University of South Florida, Tampa, USA). MCF-7/Dox40 and MCF-7/MR cells were cultured in the presence of doxorubicin and mitoxantrone, respectively, until 1 week prior to the experiments. The 2008 ovarian carcinoma cell line and MRP1-transfected 2008 M1-4 and MRP2/cMOAT-transfected 2008 CM-23 cells, the human embryonic kidney HEK293 cells and MRP3-transfected HEK/MRP3, MRP4-transfected HEK293/MRP4 and MRP5-transfected HEK293/MRP5 were kindly provided by Professor P. Borst (Netherlands Cancer Institute, Amsterdam, The Netherlands). Chinese hamster ovary (CHO) cells and MRP6-transfected CHO/MRP6 cells were kindly provided by Dr G. Kruh (Fox Chase Cancer Center, Philadelphia, USA). As described, for cell growth inhibition experiments, CHO/MRP6 and the parental CHO cells were grown in the presence of 2 mM sodium butyrate for 24 h in order to up-regulate MRP6 expression, and the next day trypsinized cells were washed and resuspended in medium lacking sodium butyrate. MRP7-transfected HEK/MPR7 cells were generated by Dr G. Szakacs (Hungarian Academy of Sciences, Budapest, Hungary) using an MRP7 expression vector, kindly provided by Dr G. Kruh. MRP8-transfected HEK/MPR8 and MRP9-transfected HEK/MPR9 cells were kindly provided by Professor P. Borst.

All cells were maintained at 37°C in a 5% CO2 incubator in either RPMI 1640 or DMEM supplemented with 10% fetal calf serum, 2 mM l-glutamine and 100 mg/mL penicillin and streptomycin. All cell lines were routinely checked for the absence of mycoplasma infection. (Over)expression of the ABC transporters was confirmed by immunohistochemistry using specific monoclonal antibodies.

#### Cell growth inhibition assays—PIs as blockers of ABC transport

Cell suspensions of the ABC transporter-overexpressing cell lines MCF-7/Dox40 (P-gp), 2008/MRP1 (MRP1) and MCF-7/MR (BCRP) and their respective parental cell lines were pre-incubated with or without the PIs atazanavir, lopinavir or ritonavir, all at 5 μM, representing their maximum non-toxic concentration. Additionally, for each of the ABC transporters established blockers were included at their respective maximum non-toxic concentrations, i.e. 10 μM verapamil (MDR1 P-gp), 30 μM MK571 and 500 μM probenecid (both MRP1 blockers) and
300 nM Ko143 (BCRP). After 1 h of pre-incubation, cells were plated in triplicate in a volume of 200 μL in 96-well plates at a density of 5000 cells per well in the presence of a concentration range of the MDR1 P-gp and MRP1 substrate doxorubicin (0–50 μM) or the BCRP substrate mitoxantrone (0–10 μM). After 96 h, cell survival was determined using the sulforhodamine B method. The cellular resistance factor (RF) was calculated as the ratio of the IC50 (concentration of the compound that inhibits cell growth by 50%) of the ABC transporter-overexpressing cells and the IC50 of the parental cells.

Cell growth inhibition assays—PIs as ABC transporter substrates

ABC transporter-overexpressing sublines and their parental cell lines were plated in triplicate in a volume of 200 μL in 96-well plates at a density of 5000 cells per well in the presence of a concentration range of atazanavir, lopinavir and ritonavir (range 0–75, 0–50 and 0–60 μM, respectively). As positive controls for transport function, established sublines were added in parallel experiments, i.e. doxorubicin for P-gp, MRP1 and MRP2, and mitoxantrone for BCRP. After 72–96 h, cell survival was determined using the sulphorhodamine B method for the adherent cells (MCF-7, CHO and 2008) and the XTT method for the semi-adherent HEK293 cells. In the latter, 25 μg of XTT mixed with 0.15 μg of phenazine methosulphate in 25 μL of cell medium was added per well for 3–4 h. For each ABC transporter, cell growth inhibition experiments were performed 2–5 times. The RF was calculated as described above. An RF from 1.3 to 1.9 was considered low-level resistance, an RF from 2.0 to 9.9 as moderate-level resistance and an RF ≥ 10.0 as high-level resistance. An RF < 0.8 was considered as increased sensitivity.

Results

PIs are efficient blockers of MDR1 P-gp, MRP1 and BCRP

The hypothesized ability of PIs to block substrate transport by major ABC transporters was investigated in cell growth inhibition assays. As shown in Figure 1, the resistance of cell lines overexpressing MDR1 P-gp (Figure 1a), MRPl (Figure 1b) and BCRP (Figure 1c) exposed to cytotoxic substrates was reversed not only by the respective ABC transporter blockers, but also by atazanavir, lopinavir and ritonavir. Of note, in this experiment PIs at the tested concentration (5 μM) did not influence cell growth of MDR cell lines or of the respective parental cell lines (not shown).

Calculated IC50 values and RFs for all tested combinations are shown in Table 1. The RF of MDR1 P-gp-overexpressing MCF-7/Dox40 cells for doxorubicin was decreased from 116 to 3.2 with addition of the MDR1 P-gp blocker verapamil. RFs were also markedly decreased in the presence of the PIs: 23 for atazanavir, 13 for lopinavir and 20 for ritonavir. Using the MRPl-overexpressing 2008/MRP1 cells, the RF for doxorubicin (54) dropped substantially with the established blockers MK571 (19) and probenecid (20). Interestingly, similar levels of chemosensitization were observed when atazanavir (18), lopinavir (20) and ritonavir (13) were added. Regarding BCRP, the RF of MCF-7/BCRP cells for mitoxantrone decreased from 1650 to 75 with Ko143, while distinct but lower reduction of cytotoxicity was observed with atazanavir (792), lopinavir (1125) and ritonavir (1125). These results show that the PIs atazanavir, lopinavir and ritonavir are potent inhibitors of both MDR1 P-gp and MRP1, and to a lesser extent of BCRP.

PIs are poor substrates for a panel of 11 ABC transporters

To determine whether MDR1 P-gp, MRPl and BCRP as well as other less commonly studied ABC transporters can efflux PIs, 72–96 h growth inhibition assays were performed with atazanavir, lopinavir and ritonavir in a panel of 11 different ABC transporter-overexpressing cell lines. For the PIs, the used concentration ranges showed sigmoid growth inhibitory curves, as expected, for all sublines tested. The calculated IC50 values and RFs are presented in Table 2. In general, four profiles of PI growth effects were found: increased sensitivity (RF: <0.8); no resistance (RF: 0.8–1.2); low-level resistance (RF: 1.3–1.9); and moderate-level resistance (RF: 2.0–9.9). Representative growth curves of these observed profiles are shown in Figure 2(a–d), respectively. Of note, the substrate vehicle ethanol did not influence cell growth in any of the cell lines in a volume equivalent to the highest concentration of PIs used.

The complete overview of observed RFs of the panel of ABC transporter-overexpressing cell lines for atazanavir, lopinavir and ritonavir is shown in Figure 3. In general, the highest RFs were observed in sublines tested for atazanavir (Figure 3a). Also for this PI, resistance was observed in the highest number of transporter-overexpressing cell lines; cells with overexpression of MDR1 P-gp, MRPl, MRP3, MRP4, MRP5, MRP6 and MRP9 showed low-to-moderate-level resistance (RF: range 1.3–2.6). The highest RFs were observed in MRP6- and MRP9-overexpressing cell lines (2.6 and 2.0, respectively). Other sublines (overexpressing MRPl, MRP2, MRP7, MRP8 and BCRP) did not display resistance to atazanavir, while MRP2-overexpressing cells were slightly more sensitive to atazanavir (RF: 0.7).

For lopinavir (Figure 3b), only cells with overexpression of MRP7 and MRP9 showed low-level resistance (RF: 1.5 and 1.4, respectively). Other ABC transporter-overexpressing cell lines were found not to be resistant to lopinavir. Interestingly, cells with overexpression of MRP6 were more sensitive to lopinavir (RF: 0.6).

Finally, for ritonavir (Figure 3c), only cells with overexpression of MRP9 showed low-level resistance (RF: 1.4). Other ABC transporter-overexpressing cell lines were not resistant to ritonavir. As observed for lopinavir, MRP6-overexpressing cells were more sensitive to ritonavir (RF: 0.6).

Taken together, these experiments show that the PIs atazanavir, lopinavir and ritonavir are poor efflux substrates in a broad panel of ABC transporters.

Discussion

In HIV treatment, therapy failure is often caused by viral resistance-associated mutations. In the case of good adherence and appropriate plasma drug concentrations, other mechanisms may be important for the therapy failure. Here, we investigated the possible contribution of ABC transporters, known to play a role in cellular resistance to anticancer drugs. Some investigators had obtained results suggesting that, paradoxically, PIs are blockers of several ABC transporters, but nevertheless cannot be efficiently effluxed by these transporters.

Here, we confirmed that three commonly used PIs (atazanavir, lopinavir and ritonavir) are indeed potent blockers of MDR1 P-gp and MRPl, notably to an extent comparable to common ABC transporter...
Figure 1. Reversal of resistance of ABC transporter-overexpressing cell lines by PIs atazanavir, lopinavir and ritonavir. Sulphorhodamine B-determined growth curves for ABC transporter-overexpressing cells (black line) exposed for 96 h to a cytotoxic substrate in the absence (filled squares) or presence of a common blocker or one of the PIs atazanavir (filled triangles), lopinavir (filled diamonds) or ritonavir (filled circles). As a reference, parental cells in the absence of a blocker are shown as a grey broken line. (a) Curves for MDR1 P-gp-overexpressing MCF-7/Dox40 cells and parental MCF-7 cells exposed to doxorubicin as a substrate and verapamil (open squares) or the PIs. (b) Curves for MRP1-overexpressing 2008/MRP1 cells and parental 2008 cells exposed to doxorubicin as a substrate and MK571 (open triangles), probenecid (open diamonds) or the PIs. (c) Curves for BCRP-overexpressing MCF-7/MR cells and parental MCF-7 exposed to mitoxantrone as a substrate and Ko143 (open circles) or the PIs. ATV, atazanavir; LPV, lopinavir; RTV, ritonavir; VPM, verapamil; PBC, probenecid.

Table 1. IC50 values and RFs of ABC transporter-overexpressing cells in the presence of a cytotoxic substrate and common blockers or PIs

<table>
<thead>
<tr>
<th>ABC transporter (cell lines)</th>
<th>IC50, μM (RF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no blocker</td>
</tr>
<tr>
<td>MDR1 P-gp (MCF-7/Dox40)</td>
<td>6.5 (116)</td>
</tr>
<tr>
<td>MRP1 (2008/MRP1)</td>
<td>0.16 (54)</td>
</tr>
<tr>
<td>BCRP (MCF-7/MR)</td>
<td>8.8 (1650)</td>
</tr>
</tbody>
</table>

ATV, atazanavir; LPV, lopinavir; RTV, ritonavir; VPM, verapamil; PBC, probenecid; ND, not determined.

IC50 values and RFs were calculated from growth curves generated using the sulphorhodamine B method after 96 h of exposure of the parental and ABC transporter-overexpressing cell lines to cytotoxic substrates (doxorubicin for MDR1 P-gp and MRP1, and mitoxantrone for BCRP) with or without common blockers or PIs. RF (in parentheses) is defined as the ratio of drug concentration resulting in 50% cell growth inhibition for ABC transporter-overexpressing cells over parental cells. Results are presented as the means of experiments performed in triplicate.
Table 2. Resistance profile for PIs in a panel of 11 ABC transporter-overexpressing cell lines

<table>
<thead>
<tr>
<th>ABC transporter</th>
<th>Cell line</th>
<th>IC\textsubscript{50}, \mu M (SD)</th>
<th>RF (SD)</th>
<th>IC\textsubscript{50}, \mu M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1 P-gp</td>
<td>MCF-7/Dox40</td>
<td>8.5 (3.0)</td>
<td>1.0 (0.2)</td>
<td>3.3 (1.3)</td>
</tr>
<tr>
<td>MRP1</td>
<td>2008/MRP1</td>
<td>42.2 (9.1)</td>
<td>1.1 (0.1)</td>
<td>3.5 (2.1)</td>
</tr>
<tr>
<td>MRP2</td>
<td>2008/MRP2</td>
<td>32.1 (5.7)</td>
<td>0.6 (0.08)</td>
<td>19.8 (3.2)</td>
</tr>
<tr>
<td>MRP3</td>
<td>2008/MRP3</td>
<td>30.4 (13.9)</td>
<td>1.1 (0.2)</td>
<td>15.5 (1.1)</td>
</tr>
<tr>
<td>MRP4</td>
<td>2008/MRP4</td>
<td>6.5 (0.7)</td>
<td>0.9 (0.6)</td>
<td>3.5 (0.0)</td>
</tr>
<tr>
<td>MRP5</td>
<td>2008/MRP5</td>
<td>36.1 (4.3)</td>
<td>1.0 (0.2)</td>
<td>25.4 (4.3)</td>
</tr>
<tr>
<td>MRP6</td>
<td>2008/MRP6</td>
<td>42.2 (7.1)</td>
<td>0.6 (0.1)</td>
<td>20.2 (3.7)</td>
</tr>
<tr>
<td>MRP7</td>
<td>2008/MRP7</td>
<td>25.6 (5.1)</td>
<td>1.1 (0.1)</td>
<td>14.6 (1.1)</td>
</tr>
<tr>
<td>MRP8</td>
<td>2008/MRP8</td>
<td>4.6 (0.6)</td>
<td>0.9 (0.3)</td>
<td>1.4 (1.1)</td>
</tr>
<tr>
<td>BCRP</td>
<td>MCF-7/790R</td>
<td>23.4 (1.3)</td>
<td>1.2 (0.3)</td>
<td>3.9 (1.7)</td>
</tr>
</tbody>
</table>

IC\textsubscript{50}s and RFs were calculated from sulphorhodamine B- or XTT-determined growth curves for ABC transporter-overexpressing cell lines and the respective parental cell lines grown for 72–96 h in the presence of a concentration range of an established substrate (doxorubicin for MDR1 P-gp-, MRP1- and MRP2-overexpressing cell lines and mitoxantrone for the BCRP-overexpressing cell line). RF is defined as the ratio of drug concentration resulting in 50% cell growth inhibition for ABC transporter-overexpressing cells over parental cell lines. The highest levels of cellular resistance were observed for atazanavir in the MRP6-overexpressing CHO subline and in the HEK/MPR9 cells. Still, even these cells showed only moderate RFs of 2.6 and 2.0, respectively. Furthermore, low RFs for atazanavir were found in MDR1 P-gp-overexpressing MCF-7/Dox40, HEK/MPR3, HEK/MPR4 and HEK/MPR5. For the other two PIs examined, only low RFs were observed in some ABC transporter-overexpressing cells: for lopinavir in HEK/MPR7 and HEK/MPR9 cells, and for ritonavir in HEK/MPR5 cells.

Of course, there are limitations to the translation of such in vitro study results to the clinical situation. In particular, the PI concentrations which had clear effects on cell growth are above those that occur in clinical situations unless transiently, while our cells were exposed to these concentrations for 72–96 h. Furthermore, cell lines were used with supraphysiological expression of ABC transporters. However, our screening models were deliberately chosen to increase the chances of detecting putative interactions between the tested PIs and the ABC transporters.

Usually, compounds that are blockers of an ABC transporter exert their effect by acting as competitive substrates, or compete with ATP-binding sites of this transporter. In this context, our finding that PIs are blockers but are not efficiently effluxed by MDR1 P-gp, MRP1 and BCRP indicates that PIs do not operate as classical blockers of these transporters. Gupta et al. were the first to report this property for PIs in a BCRP-overexpressing cell line. Interestingly, a recent study showed that introduction of lopinavir-based cART in HIV-infected patients was accompanied by a blockage of the intestinal MDR1 P-gp activity, resulting in a 2-fold increase in digoxin exposure as revealed by the area under the curve.34

The apparent lack of efficient efflux of atazanavir, lopinavir and ritonavir by the MDR1 P-gp-overexpressing subline MCF-7/Dox40 is noteworthy in light of earlier reports suggesting that lopinavir and ritonavir can be transported by MDR1 P-gp using growth inhibition and transwell transport assays, as well as using human peripheral blood mononuclear cells and murine models. This may relate to even higher sensitivity of the used radioactively labelled PI-based transport assays in some of these studies. Still, the reported level of transport of lopinavir and ritonavir by MDR1 P-gp varied and was, similarly to our findings, quite low (1.1-3-fold compared with parental cells).14-17

Undoubtedly, the sensitivity of the growth inhibition assay is dependent on the expression levels and functionality of the transporters in the cell lines used. For all sublines in our studies, including MCF-7/Dox40, ABC transporter expression was confirmed by immunohistochemistry and, for those sublines with an established ABC transporter substrate available, functionality was confirmed by a marked resistance to the control blockers. Still, the PIs did not block BCRP as much as the established BCRP blocker Ko143. Subsequently, investigating whether ABC transporters might also be capable of effluxing PIs, growth-inhibitory effects of these PIs were evaluated in an extended panel of cell lines overexpressing MDR1 P-gp, MRP1–MRP9 or BCRP. Remarkably, our results show that none of the three tested PIs is an efficient substrate for any of the 11 ABC transporters tested, given the absence of appreciable differences in growth curves of the ABC transporter-overexpressing cell lines and their parental cell lines. The highest levels of cellular resistance were observed for atazanavir in the CHO subline and in the HEK/MPR9 cells. Still, even these cells showed only moderate RFs of 2.6 and 2.0, respectively. Furthermore, low RFs for atazanavir were found in MDR1 P-gp-overexpressing MCF-7/Dox40, HEK/MPR3, HEK/MPR4 and HEK/MPR5. For the other two PIs examined, only low RFs were observed in some ABC transporter-overexpressing cells: for lopinavir in HEK/MPR7 and HEK/MPR9 cells, and for ritonavir in HEK/MPR5 cells.
substrates. Of note, we detected minor but distinct MDR1 P-gp-mediated transport of atazanavir, as revealed by an increased IC50 (see Table 2, RF 1.5). Actually, atazanavir was the PI showing modest efflux by a wide range of ABC transporters, in contrast to lopinavir and ritonavir, which appeared to be poor substrates for most ABC transporters tested. Whether small
differences in chemical structure account for these findings
remains to be established. Of note, atazanavir differs from other
peptidomimetic PIs by its C-2 symmetric chemical structure.
Importantly, the clinical pattern of side effects of atazanavir
differs significantly from that of lopinavir, ritonavir and most
other PIs.5 When looking from a virological perspective, atazana-
vir also has a resistance pattern that differs from the other PIs.2,4,6

As far as data are available for the other ABC transporters
tested, our findings are in line with earlier reports showing that
PIs are poor substrates for MRP1, MRP3, MRP5 and BCRP2,25–27
but do not confirm a putative role for MRP2 in transporting
PIs.25,26 MRP6 stands out since this transporter appeared to
mediate distinct cellular resistance to atazanavir but rather
increased sensitivity to the other PIs. Increased sensitivity to a
drug in ABC transporter-overexpressing cell lines seems unex-
pected, but has been reported before.47,48 A study by Bergman et al.47
showed that increased sensitivity of MDR1 P-gp- and
MRP1-overexpressing cell lines to the anticancer drug gemcita-
bine was due to increased activity of the enzyme that phosphor-
ylates gemcitabine (deoxycytidine kinase), thereby potentiating
its cytotoxicity. In a similar way, MRP6 could mediate the extru-
sion of an intracellular metabolite that protects against toxicity
by lopinavir and ritonavir. Thus, the toxicity of these PIs would
be enhanced in MRP6-overexpressing cells. The pharmacological
role of MRP6 in MDR and HIV treatment warrants further evalu-
ation. Recently, MRP6 was found to transport several anticancer
drugs, such as etoposide and doxorubicin.35

MRP7 can confer cellular resistance to some chemotherapeu-
tic agents, e.g. docetaxel (9- to 13-fold resistance) and to
nucleotide analogues.49,50 With respect to the latter class of
drugs, this might point to interactions between lopinavir and
nucleotide reverse transcriptase inhibitors (NRTIs), such as teno-
fovir, which is one of the preferred drug combinations in cART.

Immuno- and drug–drug interactions, and explore if the transporters are
involvement of ABC transporters in drug-specific side effects
and certainly warrants further investigations into possible inter-
actions with viral suppression of PIs using HIV-infected cell-
based assays. Additionally, further studies could focus on the
involvement of ABC transporters in drug-specific side effects
and drug–drug interactions, and explore if the transporters are
responsible for concomitant immunosuppressive effects.

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None to declare.

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