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Improving cancer chemotherapy with modulators of ABC drug transporters

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Abstract

ATP-binding cassette (ABC) transporters, P-glycoprotein (P-gp, ABCB1) and ABCG2, are membrane proteins that couple the energy derived from ATP hydrolysis to efflux many chemically diverse compounds across the plasma membrane, thereby playing a critical and important physiological role in protecting cells from xenobiotics. These transporters are also implicated in the development of multidrug resistance (MDR) in cancer cells that have been treated with chemotherapeutics. One approach to blocking the efflux capability of an ABC transporter in a cell or tissue is inhibiting the activity of the transporters with a modulator. Since ABC transporter modulators can be used in combination with chemotherapeutics to increase the effective intracellular concentration of anticancer drugs, the possible impact of modulators of ABC drug transporters is of great clinical interest. Another possible clinical use of modulators that has recently attracted attention is their ability to increase oral bioavailability or increase tissue penetration of drugs transported by the transporters. Several preclinical and clinical studies have been performed to evaluate the feasibility and the safety of this approach. The primary focus of this review is to discuss progress made in recent years in the identification and applicability of compounds that may serve as ABC transporter modulators and the possible role of these compounds in altering the pharmacokinetics and pharmacodynamics of therapeutic drugs used in the clinic.

Keywords

ABC transporters; ABCG2; blood-brain barrier; chemotherapy; modulators; multidrug resistance; oral bioavailability; P-glycoprotein

Multidrug resistance and ABC drug transporters

Chemotherapy is usually the most effective treatment for cancer patients with advanced/metastatic tumors or hematological malignancies. However, cancer cells often develop simultaneous resistance to many functionally and structurally unrelated anti-cancer drugs, a phenomenon known as multidrug resistance (MDR), which is a major problem in the treatment of cancer (1). Cancer cells with the MDR phenotype may have either inherent resistance to anti-cancer drugs or resistance acquired after cycles of chemotherapy. Several mechanisms of anti-cancer drug resistance, such as reduced cellular drug uptake, increased or decreased expression of metabolic enzymes, mutation of the target, alterations of the

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apoptotic pathway, changes in cellular repair mechanisms and increased expression/activity of drug efflux pumps have been characterized (2).

One of the most important MDR mechanisms is an increased efflux rate of the anti-cancer drug from cancer cells by the members of the superfamily of ATP-binding cassette (ABC) transporters, which is one of the largest protein families in living organisms (3, 4). There are 48 genes in the human genome that encode ABC transporters, which are divided into seven subfamilies (ABCA-ABCG) based on the amino acid sequence identity of ATP-binding domains (4). ATP-binding domains, also known as nucleotide binding domains (NBDs), and trans-membrane domains (TMDs), each with six alpha helices, are the essential units of an ABC transporter. The NBD has the highly conserved Walker A, B, and signature C motifs and are well conserved in all the organisms. On the other hand, the TMD confers transport substrate specificity, and is not well conserved (5, 6). The members of this family play a crucial role in physiology, and mutations in ABC transporters result in several human diseases including progressive familial intrahepatic cholestasis (ABCB11), Dubin-Johnson syndrome (ABCC2), cystic fibrosis (ABCC7), and adrenoleukodystrophy (ABCD1) (3, 4). So far, at least 15 transporters have been shown to export anti-cancer drugs *in vitro* (7). Among them, P-glycoprotein (P-gp; MDR1, ABCB1), multidrug resistance-associated protein 1 (MRP1, ABCC1), and ABCG2 (breast cancer resistance protein; BCRP, mitoxantrone resistance protein; MXR) are considered major players in the development of MDR in cancer cells.

P-gp, discovered in 1976, is one of the best characterized ABC transporters (8). It is composed of two homologous halves, each containing a NBD and a TMD, and transports exogenous and endogenous amphipathic substrates out of cells using energy from ATP (9). It is localized at the apical surface of the cells and is highly expressed in capillary endothelial cells of the blood-brain barrier, placental trophoblasts, the testes, intestines, the liver, kidneys and the adrenal gland (3). These tissues function as barriers, suggesting the physiological role of P-gp is to protect the body from xenobiotics and toxins. P-gp pumps out many structurally unrelated anti-cancer drugs, such as vinca alkaloids (vinblastine, vincristine, vindesine, vinorelbine), anthracyclines (doxorubicin, daunorubicin) and taxanes (paclitaxel, docetaxel), suggesting the flexible nature of the substrate binding site of P-gp (10, 11). P-gp is highly expressed in leukemia, breast, ovarian, colon, kidney, adrenocortical, and hepatocellular cancers and its overexpression is inversely correlated with poor clinical prognosis (12–14).

ABCG2 is a half transporter which contains one TMD and one NBD, and is therefore thought to homodimerize or heterodimerize to form the functional unit (15–17). Interestingly, similar to the MDR family of transporters in yeast, the location of the TMD and NBD is reversed in ABCG2 compared to P-gp (18). Similar to P-gp, ABCG2 is localized to the apical membrane in epithelial cells and normally expressed in organs such as the placenta, brain, liver, prostate, and intestine (16). ABCG2 is also detected in hematopoietic and other stem cells, suggesting that it may play an important role in the protective function of pluripotent stem cells (19). Overexpression of ABCG2 renders cancer cells resistant to many anti-cancer drugs including mitoxantrone, topotecan and methotrexate and it is associated with poor response to chemotherapy in leukemia and breast cancer patients (20, 21).

MRP1 (ABCC1) was the first member of the MRP family to be identified (in 1992) and has been linked to the development of MDR (22). The structure of MRP1 is similar to that of P-gp, except five additional transmembrane helices are present at the amino-terminal end of the transporter. It is highly expressed in the adrenal gland, bladder, choroid plexus, colon, in erythrocytes, bone marrow, the kidneys, lungs, placenta, spleen, stomach, testes, in helper T

cells and in muscle cells (23). MRP1 transports some substrates conjugated with glucuronide, sulfate or glutathione, vinca alkaloids, anthracyclines, methotrexate and also leukotriene C4, which is an endogenous substrate for the transporter (24, 25). The localization of MRP1 is different from that of P-gp, as it is expressed in the basolateral membrane in polarized epithelial cells and transports substrates in to the bloodstream (26). Overexpression of MRP1 has also been shown in lung, breast, prostate, and ovarian cancer, gastrointestinal carcinoma, melanoma, and leukemia (27). While some studies have reported MRP1 expression levels to be of prognostic significance (28, 29), others have found no correlation between clinical outcome and its expression (30, 31). A comprehensive role of MRP1 in clinical drug resistance is still debatable; therefore the present review will mainly focus on two major ABC drug transporters, P-gp and ABCG2.

Approaches to improving chemotherapy

A combination of two or multiple drugs is often used in chemotherapy, as each drug inhibits a specific target and the combination therefore could maximize the killing effect on cancer cells, additively and synergistically (32). The combination of drugs targets several cellular pathways simultaneously, which not only augments the tumoricidal effect of anti-cancer drugs, but also lessens the occurrence of drug resistance, thereby providing an optimal therapeutic outcome (2).

In addition to conventional anti-cancer drugs, many novel drugs have been developed based on an understanding of molecular mechanisms, which revealed a number of new potential targets for drugs. These novel agents such as the monoclonal antibody cetuximab, which targets the extracellular domain of EGFR by blocking ligand binding, and the small-molecule inhibitor erlotinib, that blocks EGFR intracellular tyrosine kinase activity, have demonstrated clinical advantages over the established drug regimen (33). Several newer approaches such as cancer vaccines, antisense oligonucleotides, and small-interfering RNAs (siRNAs) are also promising approaches for targeting cancer cells and deserve further clinical investigation (33). These approaches show a great promise for improving the efficiency of chemotherapy, but are still not sufficient to alter the outcome for many cancer patients.

ABC drug transporters: Targets for improving chemotherapy

MDR mediated by ABC transporters in cancer cells can be overcome by methods such as specific inhibition of transporters at the cell surface level, blocking the signaling pathways that control amplification and overexpression of these transporters, and targeting the transcription factors that regulate the expression of the pumps (34). Historically, research on the reversal of ABC transporter-mediated MDR has been directed towards inhibiting the activity of the transporter at the cell surface by drugs known as modulators or inhibitors and due to the scope of this review, we will focus on this strategy as a possible way to improve chemotherapy and/or oral bioavailability. A majority of modulator drugs used for this purpose are basically transport substrates of ABC drug transporters that inhibit efflux of anti-cancer agents in both *in vitro* and *in vivo* systems.

Almost three decades ago, Tsuruo *et al.* discovered that verapamil (a calcium channel blocker used as a coronary vasodilator) enhanced the cytotoxicity of vincristine and vinblastine in a P-gp-expressing vincristine-resistant cell line (35). Slater *et al.* later reported that cyclosporine A (CsA), an immunosuppressant drug, completely reversed primary resistance to vincristine and daunorubicin in a drug-resistant cell line of human T cell acute lymphatic leukemia (36). Additional studies showed that both verapamil and CsA reversed the MDR phenotype by interacting directly at the drug-substrate site on P-gp (37–39). Several other ‘off the shelf’ drugs which were already in clinical use such as nifedipine and

nifedipine were shown to reverse Pgp-mediated MDR (reviewed in (34)) but many of these agents were toxic, as higher concentrations of these drugs were required for inhibiting the transporters. These drugs have been described as the first generation modulators of P-gp. In 1991, an analog of CsA PSC833 (valspodar) was synthesized which was not only 10-fold more potent than CsA in inhibiting Pgp activity but also showed no immunosuppressive side effect (40). Discovery of this molecule marked the beginning of the second generation of ABC transporter modulators. Many other modulators were developed based on the quantitative structural activity relationship approach. A number of clinical studies were carried out using these modulators. These trials demonstrated some advantage over the previous agents, but patients still suffered from side effects associated with the therapy (41–44). Since then, third generation modulators such as tariquidar (XR9576) (45), elacridar (GF120918) (46), zosuquidar (LY335979) (47) and dofequidar (48) have been developed which have shown improved selectivity and inhibitory activity towards ABC transporters. Martin *et al.* reported that tariquidar, unlike verapamil and CsA, was not a transport substrate of P-gp, and that it inhibits P-gp function by binding at a site distinct from the vinblastine and paclitaxel site (49). Tariquidar potentiated the cytotoxicity of several drugs including doxorubicin, paclitaxel, etoposide, and vincristine at very low concentrations (25–80 nM). Co-administration of tariquidar potentiated the antitumor activity of doxorubicin without a significant increase in toxicity in mice (50). Two Phase III trials of tariquidar in combination with paclitaxel/carboplatin, or vinorelbine were initiated in patients with non-small-cell lung cancer (NSCLC) but these studies were terminated later owing to chemotherapy-related toxicity in the tariquidar arm (51). Recent results of another phase I study with vinorelbine have indicated shown that tariquidar is a potent P-gp antagonist, without significant side effects and much less pharmacokinetic interaction than previous P-gp antagonists (52). This and other ongoing clinical studies at the National Cancer Institute (NIH, Bethesda, USA) with tariquidar suggest that there is still optimism that therapeutic modulation of MDR mediated by ABC transporters may be beneficial to patients.

The leukotriene LTD₄ receptor antagonist MK571 and a fungal toxin, fumitremorgin C (FTC) were identified as specific modulators of MRP1 and ABCG2, respectively (53, 54). Since then MK571 and FTC have been used as standard modulators for inhibiting MRP1- and ABCG2-mediated transport, respectively, in laboratory assays. However, unlike P-gp, clinically useful modulators of MRP1 and ABCG2 have not been extensively investigated in clinical trials. Some modulators of P-gp such as biricodar (VX-710) dofequidar (MS-209), elacridar (GF120918) or CBT-1 have also been shown to inhibit MRP-1 and/or ABCG2-mediated drug transport/resistance in *in vitro* and in clinical studies (48, 55–61). Although the effect of these modulators on each transporter is not well established in clinical trials, these drugs might be useful against cancer cells which express a combination of transporters linked to MDR. The efficacy and safety of these modulators in clinical studies is still under evaluation.

Newer approaches have been developed in the laboratory to overcome ABC transporter-mediated MDR, including a monoclonal antibody that binds to human P-gp and inhibits drug transport (62), siRNA technology that specifically decreases the expression of *ABCB1* (63), and regulation of transcription factors that inhibit P-gp activation (64). Although these approaches might help to overcome transporter-mediated MDR, clinical studies using these alternative approaches are still lacking to establish their efficacy in cancer patients.

Modulators of ABC drug transporters and oral bioavailability of chemotherapeutics

Although modulators of ABC drug transporters were initially envisioned as drugs that can be used to overcome ABC transporter-mediated MDR in cancer cells or tumor tissue, these

drugs are recapturing attention for another possible use: increasing oral bioavailability of drugs which were earlier ruled out as oral chemotherapeutic agents due to poor bioavailability (65). Pgp and ABCG2 have now been confirmed to play significant physiological roles including preventing the uptake of many drugs and food components from the gut into the body. Both P-gp and ABCG2 are expressed in the brush-border membranes of enterocytes in the intestine, which results in the excretion of their drug-substrates into the lumen, resulting in a potentially limiting net absorption (Figure 1). The association of drug transporter levels in the intestine and oral drug bioavailability was initially demonstrated by Spareboom *et al.*, who showed that P-gp in the intestine limits the uptake of orally administered paclitaxel (66). Later Greiner *et al.* also showed that the plasma concentration of digoxin, another P-gp substrate, was inversely correlated with intestinal P-gp expression in patients (67). These observations led researchers to evaluate if modulators of ABC transporters could possibly be used to temporarily inhibit the efflux activities of the transporters, thereby increasing the oral bioavailability and plasma concentrations of some poorly absorbed drugs. Several preclinical *in vitro* and *in vivo* studies have since then reported the use of specific modulators of ABC drug transporters to increase the oral bioavailability or efficacy of drugs such as docetaxel, vinblastine, etoposide, digoxin, indinavir, saquinavir, tacrolimus, nelfinavir, talinolol, topotecan, methotrexate, irinotecan, SN-38, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and erlotinib, which are either P-gp or ABCG2 substrates (reviewed in (65)). This is consistent with biochemical studies demonstrating that there is significant overlap in substrate specificity of Pgp, ABCG2 and MRP1 (11). Recently, we showed that inhibition of intestinal abcg2 in mice by curcumin results in increased oral bioavailability of its substrate, sulfasalazine, which suggested that curcumin, a non-toxic natural product modulator of P-gp and ABCG2, may be used to enhance drug exposure (68). A very recent study reported a newly discovered modulator of Pgp, HM30181 increased oral bioavailability of paclitaxel in rats and inhibition of growth of tumor xenografts in mice which was better than intravenous paclitaxel (69). These results identify HM30181, as a highly selective and potent inhibitor of MDR1, which in combination with paclitaxel may provide an orally effective anti-tumor regimen. Table 1 summarizes recent studies which reported the use of P-gp or ABCG2 modulators to increase the oral bioavailability or plasma concentrations of drugs. These studies suggest a pharmacological advantage of using chemotherapeutic drugs in combination with modulators of ABC drug transporters.

Although using modulators in combination with other drugs is an appealing strategy, it does have the risk of increased toxicities associated with the combination therapy because both P-gp and ABCG2 are also expressed in epithelial cells of the colon, adrenal cortex, kidney, liver and bile canalicular membranes and gall bladder. These organs influence the excretion of drugs. Moreover, as P-gp and ABCG2 also protect vital organs such as the brain, the testes, and the fetus, against toxins that enter the body, inhibition of the activity of these transporters may lead to toxicity associated with these organs. In addition, some modulators also inhibit metabolic enzymes such as cytochrome P450-3A4, which may significantly delay drug clearance from the body (70). Considering these factors, the use of modulators in a combination regimen for improving oral bioavailability is an approach which should be carefully monitored for minimal toxicity associated with improved therapeutic outcome.

Modulators of ABC drug transporters increase brain accumulation of chemotherapeutics

P-gp and ABCG2 expression in the brain has been found in numerous species, including humans, primates, rats, mice, and pigs (71). These transporters are principally expressed at the luminal membrane of the endothelial cells in brain capillaries (blood-brain barrier; BBB) and pump substrates back into the circulation, which is a critical determinant for the

efficacy/toxicity of chemotherapeutics for the brain (Figure 1). The importance of P-gp in the brain was first demonstrated by Schinkel *et al.*, who showed that brain uptake of drugs was higher in P-gp knockout mice, suggesting that Pgp protects the brain from toxic effects under physiological conditions (72). Later, several other studies reported that P-gp drug-substrate levels were significantly higher in the brains of *mdr1a* knockout mice than wild-type mice (73–76). Brain accumulation of topotecan, a camptothecin analog and a substrate of both P-gp and ABCG2 that is used for treating ovarian, cervical and small cell lung cancer, was also found to be 3.7-fold higher in *mdr1a/b/Abcg2*-knockout mice compared to wild-type mice (77). Recently, Lagas *et al.* also showed P-gp and ABCG2 knockout mice have drastically increased dasatinib brain concentrations, both after oral and i.p. administration (78). Collectively, these studies provide strong evidence for the notion that inhibition of ABC transporters at the BBB can also be used to enhance the efficacy of drugs which are targeted for brain disorders and do not penetrate the BBB by virtue of being substrates of the transporters. This has been demonstrated in several *in vivo* and *in vitro* studies in which modulators of ABC transporters such as elacridar, pantoprazole, valsopodar and zosuquidar were used to increase accessibility of several clinically important drugs into the brain (78–80). Table 2 summarizes the list of modulators that have been evaluated for the purpose of increasing brain accumulation of clinically important drugs. Taken together, modulators of ABC drug transporters can possibly be used to augment brain accumulation of drugs which cannot cross the BBB due to the action of transporters, thereby increasing their therapeutic potential.

Modulators as adjuvants in clinical studies

As stated above, inhibition of ABC drug transporters by modulators may be used to achieve two independent clinical objectives: (1) reversal of MDR in drug resistant tumors during chemotherapy (2) increasing a drug's exposure in body {tissues} for purposes such as increasing oral bioavailability or enhanced brain accumulation of chemotherapeutics. Inhibiting ABC transporters as a way to reverse drug resistance in tumors has been reported several times, but most studies failed to show a dramatic improvement in clinical outcome. We have earlier reviewed the recent progress made in clinical studies by third generation ABC transporter modulators (34). Here, we will describe recent clinical studies reporting promising results. In a Phase I/II clinical trial, Zosuquidar (LY335979) in combination with CHOP (cyclophosphamide, hydroxydaunorubicin, oncovin (vincristine), and prednisone) was shown to have little effect on the pharmacokinetics of anti-cancer drugs in patients with non-Hodgkin's lymphoma, which suggests that this combination may be pursued further in phase III studies (81). Another phase I dose escalation study showed that a combination of oral topotecan and tariquidar was well tolerated in patients and warranted a further phase II evaluation (82). Saeki *et al.* also reported that a dual inhibitor of Pgp and MRP1, dofequidar (MS-209) in combination with cyclophosphamide, doxorubicin, and fluorouracil (CAF) displayed a significantly increased efficacy in breast cancer patients who did not receive prior therapy. Although the patient numbers in these analyses were small, the results are important within these clinically significant patient populations (83). In addition, studies from Oostendorp *et al.* also suggest that co-administration of ritonavir with docetaxel could also be evaluated for its efficacy in patients with solid tumors (84). Although more positive outcomes are expected now because of the increasing knowledge about modulators with drug transporters, studies such as those reported by Lhomme *et al.* compared the safety and efficacy of paclitaxel administered with or without PSC833, in patients with advanced ovarian or primary peritoneal cancer, showed that the addition of PSC833 to paclitaxel did not lead to an arrest of the disease progression or overall survival and the combination was more toxic compared with paclitaxel in untreated patients with advanced ovarian or primary peritoneal cancer (85).

The apparent lack of response to modulators to reverse MDR in clinical studies can be attributed to multiple factors. One of the most challenging issues in using modulators with chemotherapeutic agents is alteration of the pharmacokinetics of the chemotherapeutic agent, which has a toxic effect. In addition, inhibition of ABC drug transporters adds to non-specific dose limiting toxic effects and it may also result in drug-drug interaction related side effects. As a result, many of these studies had to be discontinued because of toxicities in patients (51). Genetic variability (SNPs) in ABC transporters (discussed below) and the variability in the expression levels of transporters among individuals selected for the clinical studies may also be one of the factors responsible for the poor outcome of the use of modulators. Another important aspect is patient selection in clinical studies. As development of MDR is a multifactorial phenomenon, reversal of MDR may have been studied in patients in whom drug resistance was not due to the overexpression of ABC drug transporters. Considering this, patients who show higher levels of Pgp or ABCG2 in tumor tissue should be the ones selected to evaluate the modulators in clinical studies. Moreover, the presence of other ABC transporters in tumors can complicate the use of specific modulators of ABC transporters. Taking the above factors into consideration, the use of modulators to inhibit ABC drug transporter mediated-MDR in patients is a strategy that is still a viable approach for treating drug resistant cancer. However, it is critical to find the right inhibitor for the right transporter with the right dosing, which would allow minimal pharmacokinetic interaction of the chemotherapeutic drug and maximal specific inhibition of the transporters in the target tumor.

Single-nucleotide polymorphisms in ABC drug transporters and its role in modulator efficiency

ABC drug transporters directly influence drug efficacy and toxicity and the expression of these transporters determines the degree of resistance of cancer cells to chemotherapy. Several single nucleotide polymorphisms (SNPs) in the coding region of both P-gp and ABCG2 have been reported that influence their substrate specificity and interaction with the transporters [reviewed in (86, 87)]. Such genetic variability in transporters often explains the inter-individual variability in interactions with substrate or modulators and drug disposition, ultimately resulting in differences in clinical endpoints including toxicity and response to the modulators. Kimchi-Sarfaty et al. reported that a synonymous mutation (C3435T) in a particular MDR1 haplotype causes a change in the conformation of substrate/modulator binding sites (88). Another example of a common non-synonymous SNP (421C>A) in ABCG2 has been linked to increased oral bioavailability of topotecan in patients, decreased survival of prostate cancer patients and increased exposure to atorvastatin and rosuvastatin, statins commonly used in the treatment of high cholesterol (89–91). In addition, SNPs in metabolic enzymes such as cytochrome P450s (CYPs) are often found to be associated with altered pharmacokinetics of transporter substrate drugs (92). This is especially important when the metabolism of the drug/modulator by the CYPs may be the rate-limiting step in drug clearance. Since the efficacy and toxicity of drugs is ultimately determined by plasma pharmacokinetic parameters, studies investigating polymorphisms in ABC drug transporters as they relate to drug administration are becoming increasingly important in the clinical setting. In conclusion, polymorphisms in ABC drug transporters in various ethnic populations may significantly affect the pharmacokinetics of substrates or modulators, suggesting that they may play a more important role than expected in the efficiency of therapeutic treatment of cancer patients.

Conclusions and therapeutic perspective

In the last decade, a huge amount of effort has been invested in the field of ABC drug transporters to identify, develop, and clinically evaluate a variety of agents known to

antagonize the function of these transporters as a means of overcoming tumor resistance. The application of this approach—using modulators as adjuvants in chemotherapy especially for drug-resistant tumors is still debatable due to very little success in the clinical studies that have been performed. The major reasons for the failure of this strategy could be explained in retrospect by multiple factors and variable components that are involved in the development of drug resistance in patients. It still seems that it will take some time to develop an ideal modulator that can be used to overcome drug resistance that develops as a result of cancer treatment or one that can be used in combination with other treatments because of complex substrate recognition factors and the physiological relevance of ABC drug transporters. Moreover, these transporters are only one part of a multi-component system that works to develop the drug resistance phenotype in a cancer cell. Physiologically, transporters play a vital role in protecting the cells from xenobiotics. Altering the function of any of these transporters may lead to a severe physiological imbalance resulting in high levels of toxicity. Therefore it is essential to evaluate the following factors critically to expect a successful outcome from the use of modulators of ABC drug transporters in the clinic: (a) Toxicity is one of the significant issues reported in several clinical studies, as most of these modulators showed non-specific toxic effects which are not considered acceptable during chemotherapy and prevents their safe usage during treatment; (b) Overlapping substrate specificity and redundancy in the expression of ABC transporters for the same substrate makes it very difficult to target one modulator for a specific drug transporter. Therefore, an ideal modulator should be able to specifically recognize and inhibit the ABC drug transporter responsible for drug resistance in a specific tumor; (c) Patient selection for clinical studies is a factor which seems to have been completely ignored in previous clinical studies and may well be a major reason for the failure of those trials. Patients whose tumors express high levels of transporters will obviously receive the most benefit from modulators. Therefore, drug-resistance reversal trials should ideally be performed in individuals with tumors that initially are chemosensitive but develop drug resistance following initial therapy, which is marked by an increase in the expression of ABC drug transporters; (d) In addition, SNPs in an ABC drug transporter are also linked to sensitivity to drugs. Therefore it is imperative to determine polymorphisms in these transporters in patients before making decisions about combination chemotherapy; (e) Improved *in vivo* imaging studies with probes specific for ABC transporters and high throughput genotype analysis can provide a quantitative assessment of the functioning of the transporters and would help in identifying patients for whom modulators of ABC drug transporter may be used as adjuvants to improve the clinical outcome of chemotherapy. It would also be important to evaluate whether the use of modulators of ABC drug transporters would prevent the selection of resistant cells with characteristics of cancer stem cells with increased expression of ABC transporters such as P-gp or ABCG2; (f) It may be worthwhile to test whether short-term treatment with modulators to block P-gp or ABCG2 just prior to chemotherapy would be effective. This type of short-term treatment could minimize not only the toxic side effects associated with the use of modulators but also help to lower the required doses of chemotherapeutic drugs.

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(a) Intestine **(b) Blood-brain barrier**

Drug-substrate of ABC transporters (P-gp and ABCG2)

Figure 1.

Schematic showing the localization of P-gp and ABCG2 at the apical surface in the (a) intestinal enterocytes and b) brain capillary endothelial cells. The drug substrates of the transporters are pumped inside the lumen of the intestine and brain capillaries by these transporters, resulting in reduced oral bioavailability and decreased brain accumulation (see text for details).

Table 1

Preclinical/clinical studies demonstrating the use of modulators of ABC drug transporters to improve oral bioavailability

Transporter	Drug	Inhibitor	Reference
P-gp	Paclitaxel	HM30181	(69)
P-gp	Paclitaxel	Valspodar (PSC833)	(93)
P-gp	Docetaxel	Ritonavir	(84)
P-gp	Talinolol	Verapamil	(94)
P-gp	Paclitaxel, Digoxin, Fexofenadine	Biochanin	(95)
P-gp	Paclitaxel	Elacridar (GF120918)	(96)
P-gp	Paclitaxel	Cyclosporin A	(97)
P-gp	Docetaxel	Ritonavir	(98)
P-gp	Paclitaxel	Cyclosporin A	(99)
P-gp	Digoxin	Quinidine	(100)
P-gp	Docetaxel	Cyclosporin A	(101)
P-gp	Digoxin	Talinolol	(102)
P-gp	Paclitaxel	Elacridar (GF120918)	(103)
P-gp	Paclitaxel	HM30181	(69)
P-gp, ABCG2	Topotecan	Elacridar (GF120918)	(82, 104)
P-gp, ABCG2	Etoposide	Elacridar (GF120918)	(105)
P-gp, ABCG2	Topotecan	Elacridar (GF120918)	(106)
ABCG2	Sulfasalazine	Curcumin	(68)
ABCG2	Methotrexate	Pantoprazole	(107)
ABCG2	Irinotecan	Gefitinib	(108)
ABCG2	Methotrexate	Omeprazole/lansoprazole;	(109)

Oral bioavailability of Erlotinib (110), Gefitinib (111) and Paclitaxel (112) has been shown to be increased in *mdr1a*, *mdr1b*, *Abcg2* triple knock-out and *mdr1a*, *mdr1b* knock-out mice, respectively.

Table 2

Preclinical/clinical studies demonstrating use of modulators of ABC drug transporters to improve brain accumulation of drugs.

Transporter	Drug	Inhibitor	Reference
P-gp	Vincristine	Quercetin	(113)
P-gp	Colchicine, Vinblastine	Valsopodar (PSC833), Elacridar (GF120918)	(114)
P-gp	Colchicine, Vinblastine	Verapamil, Valsopodar (PSC-833)	(115, 116)
P-gp	Colchicine	Valsopodar (PSC 833)	(117)
P-gp	Paclitaxel	Cyclosporin A, Valsopodar (PSC833), Elacridar (GF120918)	(118)
P-gp	Paclitaxel	Zosuquidar (LY335979)	(119)
P-gp	Paclitaxel	PSC833 (Valsopodar)	(120)
P-gp, ABCG2	Dasatinib	Elacridar (GF120918), Zosuquidar (LY335979) (only for P-gp)	(121)
P-gp, ABCG2	Dasatinib	Elacridar (GF120918)	(78)
P-gp	Imatinib	Valsopodar (PSC 833), Zosuquidar (LY335979)	(80, 122)
P-gp, ABCG2	Imatinib	Elacridar (GF120918), Pantoprazole	(79)
ABCG2	Imatinib	Elacridar (GF120918)	(80, 122)
ABCG2	Mitoxantrone	Elacridar (GF120918)	(123)
ABCG2	Mitoxantrone	Elacridar (GF120918)	(124)

Brain accumulation of Topotecan (77) and Dasatinib (121) has been shown to be increased in *mdr1a*, *mdr1b*, *Abcg2* triple knock-out mice.