

## ABC Transporter Proteins and Cellular Drug Resistance

### *P-Glycoprotein and Analogous Transporters*

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yea, at that very moment  
Consideration, like an angel, came  
And whipp'd the offending Adam out of him,  
Leaving his body as a paradise.  
To envelop and contain celestial spirits...  
*King Henry V*

### 1. Introduction

Mammalian cells possess a natural battery of defense mechanisms against xenobiotic assault. A class of proteins actively transports an extensive array of structurally unrelated large lipophilic compounds from the cell, providing what is often known as multiple drug resistance (MDR) (1). MDR is characterized by active efflux or pumping of xenobiotics and pharmaceuticals via transmembrane proteins acting as hydrophobic "vacuum cleaners" (2,3). The MDR1 gene encodes a 170 kD integral plasma membrane phosphorylated glycoprotein, P-glycoprotein (P-gp), which is the best known and most extensively studied among these transporters, and which thus far has the largest substrate list. The gross structural features of P-gp are shared by a large family of membrane transporters known as adenosine 5'-triphosphate (ATP)-binding cassette (ABC) transporters, which evidently act as ATP-driven pumps that remove xenobiotics from the interior of cells. Expression of P-gp in normal human tissues—particularly within the cellular membranes of the gastrointestinal tract, liver, blood–brain barrier (BBB), adrenal glands, and kidneys—suggests that the protein plays a role in cellular protection as well as in secretion (1–4). Although the primary function of this protein is unknown, its ability to confer resistance to a wide variety of structurally and chemically unrelated compounds remains impressive. Indeed, the substrate list for this transporter reveals that P-gp shares a similar tolerance or acceptance for chemicals as cytochrome P450 3A4 (CYP3A4), the predominant intestinal and hepatic cytochrome P450 oxygenase enzyme, and may even prove to be more extensive in its substrate recognition and as an avenue of drug elimination (5).

It is becoming evident that drug interactions ostensibly mediated by the cytochrome P450 3A4 oxidative pathway are also the result of P-gp inhibition (6,7). Given the

enormous number of substrates now known to be recognized by P-gp, and a binding site that is evidently hydrophobic, the substrates for CYP3A4 and P-gp clearly overlap. Among the more grave examples of clinical drug interactions are those of the H<sub>1</sub>-receptor antagonist terfenadine (Seldane) with ketoconazole and erythromycin (8), as well as those of simvastatin with itraconazole and mibefradil (9); all are substrates/inhibitors of P-gp (4–6). Additionally, P-gp polymorphisms may cause compound-specific drug reactions to treatment with P-gp substrates. If a polymorphic gene product of MDR1 has inferior selectivity toward a therapy, increased systemic exposure to that erstwhile P-gp substrate could be expected (10–12).

### 1.1. Function and Mechanism

One of the many intractable aspects of investigating P-gp is an absence of chemical change to its substrates and hence no product formation or gross substrate depletion to monitor or analyze. Because extensive *in vitro* and *in vivo* studies indicate that MDR enzymes transport/move lipophiles across a lipid bilayer against a concentration gradient, P-gp has been described as a translocase, a flippase, or even a hydrophobic vacuum cleaner. The purpose of this enzyme ostensibly is: (1) protection against exogenous toxins ingested with food; (2) excretion of (endogenous) metabolites or toxins; (3) prevention of toxic materials from entering the brain, gonads, and fetus; (4) transportation of steroid hormones; and (5) extrusion of peptides (cytokines) not exported via signal/cleavage.

As a member of the ABC superfamily of transporters, P-gp possesses two ATP binding sites and uses ATP (via hydrolysis) as the source of energy for “translocating” substrates (4). The large transmembrane protein P-gp has two homologous domains, each containing a nucleotide binding site (ATP) and a substrate binding site near or within the inner leaflet of the membrane. With ATP hydrolysis providing the energy for function, experimental quantitation of the coupling of ATP hydrolysis to efflux events indicates a ratio of approx 1 or 2 ATP per substrate molecule transported (13–19). Hence, measurement of the rate of ATP hydrolysis serves as an indirect assay of enzymatic activity. The inactivation at only one of the two ATP binding sites is sufficient to abolish entirely the activity of the transporter (20). An allosteric linkage has been shown between one ATP binding site and catalysis at the other homologous site; these sites appear to drive substrate transport in tandem.

The substrates enter the active transport sites from the inner-leaflet, cell-membrane lipid bilayer (21) and can bind to two (or more) nonidentical sites (17). Kinetic data indicating noncompetitive inhibition of P-gp-mediated transport by substrates and inhibitors suggest differing substrate specificity between the binding sites (17). Many of these studies show  $V_{\max}$  changes that are consistent with nonexclusive binding of an inhibitor or alternative substrate, whereas photoaffinity labeling has indicated nonidentical substrate binding sites (22). Indeed, binding studies also show biphasic binding curves and hence two distinct  $K_d$ 's for many P-gp substrates, further indications of affinities that are unique to the two binding sites (23). Moreover, allosteric and perhaps synergistic effects have been observed for certain substrate combinations and conditions (24,25), with recent evidence even suggesting interaction between the two binding sites (26,27) and/or the nucleotide binding sites (20,28,29). Such an allosteric linkage between sites could affect the inhibition characteristics of marker-substrate transport out of the cell by particular inhibitors. Under these circumstances, the inhibition of transport might differ from that observed by simple Henri-Michaelis-Menten

hyperbolic saturation plot of inhibition. Moreover, in light of the substantive evidence for two binding sites, it is probable that some substrates are uniquely recognized and/or transported by P-gp. This responsiveness or sensitivity would, in turn, determine the extent of influence (including concentration dependency) a particular inhibitor will have on such substrates (30).

## **2. Clinical Impact**

Numerous examples illuminating the critical and potent physiological impact of P-gp have been described. Striking effects on bioavailability are shown by genetic knock-out (KO) animals as well as the therapeutic intervention of P-gp function. KO animals have no functional *mdr1a* (P-gp null) and are highly sensitive to the neurotoxin ivermectin and many other P-gp substrates. Dramatic effects on exposure have been observed in particular sanctuaries protected by the activity of P-gp. For example, brain-tissue levels of ivermectin were 87-fold higher, and those of the carcinostatic drug vinblastine were 22-fold higher, in the KO animal vs wild-type (31). Furthermore, paclitaxel and digoxin (cardio-toxin) oral uptake is markedly increased. Indeed, even systemic plasma concentrations are increased 2-, 3.5-, 5.7-, and 4.1-fold, respectively, for cyclosporin A, digoxin, erythromycin, and saquinavir in the P-gp null mouse (32). Co-administration of the potent P-gp inhibitor PSC833 with digoxin in wild-type mouse increased brain exposure to levels almost that of KO mice by abolishing intestinal P-gp function (33). An example of the dramatic impact of P-gp on physiological exposure to xenobiotics in humans is the remarkable decrease of the cyclosporin A plasma area under the curve (AUC)-dependent on the location of absorption in the rank order stomach>jejunum/ileum>colon (intubation) (34). The decrease in absorption exhibited a marked correlation ( $r = 0.994$ ) to expression of mRNA for P-gp over the gastrointestinal (GI) tract (stomach<jejunum<colon).

### **2.1. Tissue Distribution and Toxicity**

The evidence thus far shows that P-gp has been found virtually wherever investigators have searched for it. P-gp has been detected in: adrenal glands (endothelial cells); kidney (brush border of proximal renal tubule); liver (biliary canaliculi); intestine (columnar epithelium); jejunum, ileum, pancreas (epithelia); colon, central nervous system (CNS; endothelial cells); pregnant uterus (glandular epithelial cells of the endometrium); CD34<sup>+</sup> bone marrow cells, circulating lymphocytes, and haematopoietic stem cells (1–4).

### **2.2. Blood–Brain Barrier and Neurotoxicity**

P-gp is expressed at the apical surface of the capillary endothelial cells that form the BBB, where it seems to play a dramatic role in the exclusion of many drugs from the brain. Studies have shown that the sensitivity of homozygous P-gp KO mice to the neurotoxicity (and CNS concentration) of ivermectin and vinblastine is increased 100-fold compared with that of wild-type animals (31). In addition to MDR1, another isoenzyme of the drug/xenobiotic efflux family, multidrug resistance-associated protein (MRP1), has been detected in about 30% of head and neck squamous cell carcinomas by immunohistochemistry.

A comparative analysis of 18 physicochemical properties revealed that drugs for CNS indications had fewer hydrogen bond donors, fewer positive charges, greater lipophilicity, lower polar surface area, and reduced flexibility compared with the non-

CNS group (properties that enhance membrane permeability) (35). Because a CNS drug should ideally have high passive permeability and not be a substrate of P-gp, it is not surprising that there was a threefold lower incidence of P-gp-mediated efflux of CNS drugs ( $n = 7$  of 48, 14.6%) than of non-CNS drugs ( $n = 19$  of 45, 42%) (35).

The striking differences in brain concentrations of P-gp substrate drugs between wild-type and KO mice is impressive even compared with plasma differences, indicating P-gp is more critical to brain exposure than intestinal absorption (*see below*). Administration of ivermectin (oral), digoxin intravenous, or cyclosporin intravenous in wild-type and KO mice resulted in 87-, 27-, or 55-fold higher brain levels in the KO over wild-type (32), yet the increases were less than fourfold in liver, kidney, and plasma. These results indicate that P-gp inhibitors should be used with caution to avoid potential neurotoxicity.

### **2.3. Intestinal Absorption and Bioavailability**

It is noteworthy that poor pharmacokinetic (PK) properties, such as poor oral bioavailability or duration of action (clearance), account for nearly half of the failures in clinical development (36). MDR1 (P-gp) and MRP1 are constitutively expressed in epithelia throughout the GI tract and are often overexpressed in carcinomas originating from these tissues. Double MDR1 genetic KO mice have shown decreased elimination of drugs resulting from impaired excretion by liver, kidney, or gut (depending on which route is important in the P-gp-mediated excretion of the drug), and/or enhanced reabsorption of drug from bile, gut lumen, or urine prior to elimination from the body (32).

For drugs that are excreted unaltered or as a conjugate that can be hydrolyzed after secretion, P-gp in the epithelial surfaces of bile ducts, gut, and kidney proximal tubule may prevent reabsorption of the excreted drug. P-gp generally has greater impact on drug uptake than on drug excretion. Clinical results with the (relatively low potency) inhibitors tested so far demonstrate, for instance, that these agents interfere with the elimination of anthracyclines, a process that results in considerable increases in AUC and concomitant toxicity (37). Moreover, oral bioavailability of paclitaxel (MDR substrate) increased from 9.3% to 67% with either intravenous or oral co-administration of the MDR inhibitor cyclosporin A (38). Similarly, high levels of bioavailability were observed with the P-gp KO mice (32).

There are many instances where absorption from the small intestine may be complete but bioavailability is poor owing to enterocyte recycling via P-gp (34,39–41). Reabsorption after ejection increases the compound exposure to enterocyte drug-metabolising enzymes, and P-gp may enhance intestinal metabolism of drugs. Midazolam (42) and cyclosporin (43) endure extensive first-pass gut elimination owing to active transport and recycling. The efficient P-gp substrate verapamil has a low oral bioavailability of about 20% at doses of at least 120 mg, and propranolol has a low bioavailability of about 26%.

The poor bioavailability of HIV protease inhibitors (44), digoxin (to the brain [45]), and taxol (46) is apparently owing to efficient transport by P-gp, with paclitaxel oral bioavailability of less than 5% (47). The tacrolimus (bioavailability 18%) concentration/dose ratios in a recipient of a small-bowel transplant correlated well with the levels of MDR1 mRNA, but not with CYP3A4, indicating that P-gp determines intraindividual variability in tacrolimus pharmacokinetics (48). Moreover, the pharmacokinetics of the  $\beta$ -blocking agents celiprolol, pafenolol, and talinolol are also significantly affected by P-gp in the intestine (49–53); the H2 receptor antagonists

ranitidine and cimetidine are similarly affected (54), though 50% absorbed at the high therapeutic doses.

However, intestinal P-gp may be saturated when drug concentrations in the intestinal lumen exceed the  $K_m$  values after high oral doses. Whereas P-gp substrate drugs, such as digoxin, given at low doses result in low and variable absorption, many of these drugs (talinolol, indinavir, etc.) exhibit dose-dependent absorption owing to P-gp saturation. Chiou et al. (55) recently concluded that 13 P-gp substrate drugs are not significantly impeded by efflux transport in vivo. Yet the absorption of some drugs that are administered at high doses is still significantly affected by intestinal P-gp. Despite the high clinical oral dose of cyclosporin and paclitaxel (200–700 mg and 100–200 mg, respectively), P-gp significantly limits their oral absorption (34,46), perhaps owing to poor solubility. Therefore, drug absorption is unlikely to be quantitatively limited by active efflux transport unless a very small oral dose is given, or the dissolution and/or membrane diffusion rates of the drug are very slow. To highlight further the importance of oral absorption, poor absorption is asserted to be causing a new public health issue because it increases the chemical burden on municipal waste treatment facilities (56).

#### **2.4. Excipients Affect Permeability by Inhibiting Transporters**

Nonabsorbable pharmaceutical excipients such as Tween-20, Tween-80, pluronic P85, or TPGS have been shown to be potent modulating agents of membrane transporters (57,58). Other nonionic surfactants affecting transport pumps include Cremophors, pluronic block copolymers, Nonidet P-40, and Spans. Surfactants that are commonly used as vehicles for solubilizing certain drugs can inhibit MDR in resistant cells at clinically achievable concentrations (59). Addition of the surfactants Solutol HS-15, Tween-40, and Cremophor EL (10 mg/mL each) decreased lipid fluidity of isolated crude plasma membranes of resistant cells (60), whereas noninhibiting surfactants (octylglucoside, hecameg) did not affect membrane fluidity. Furthermore, Tween-80 and Cremophor EL fluidized cell-lipid bilayers, whereas vitamin E TPGS rigidized lipid bilayers reducing the BL-AP permeability of rhodamine 123, and the noninhibitor *N*-octyl gulcoside did not modulate membrane fluidity (61). PEG-300 (polyethylene glycol) inhibited efflux-transporter activity in Caco-2 cell monolayers, probably caused by changes in the microenvironment of the cell membranes, perturbing the ability of these transporters to efflux substrates such as taxol and doxorubicin (62). Inhibition is observed at concentrations below the critical micelle concentration (cmc) for the different surfactants, suggesting that the monomer is responsible and may be partitioning into the membrane and inhibiting P-gp through a membrane-fluidizing mechanism. Indeed, low concentrations of the nonionic surfactant Triton X-100 inhibited azidopine binding to P-gp in vinblastine-resistant human lymphoma (63).

#### **2.5. Liver, Kidney, and Excretion**

P-gp plays a significant role in the biliary excretion of digoxin, doxorubicin, vincristine, and vinblastine in mice (64). The biliary clearance of digoxin is substantially greater in wild-type mice (2.3 mL/min/kg) than in KO mice (0.84 mL/min/kg) (65). Approximately 45% of digoxin is excreted in the bile of wild-type mice.

Digoxin is also actively secreted in the isolated perfused rat kidney with the P-gp inhibitors quinidine and verapamil inhibiting tubular secretion (66). The renal clearance of digoxin in wild-type mice was three times greater than that in KO mice (65). Therefore, digoxin appears to be actively secreted into the renal tubular lumen by P-gp.

## 2.6. Placenta and Teratogenicity

MDR1 functions as a critical component of the maternal blood–placental barrier, protecting the fetus from exposure to various maternal blood-borne chemicals. Pregnant dams of a mouse CF-1 subpopulation, known to lack P-gp, exposed to an avermectin were highly sensitive to fetal cleft palate (67). The degree of chemical exposure of fetuses within each litter was inversely related to expression of placental P-gp, which was determined by the fetal genotype.

## 3. Substrates, Their Diversity, and Drug–Drug Interactions

It is, as yet, unclear how P-gp can recognize and transport such a structurally diverse spectrum of compounds ranging in size from less than 250 Da (cimetidine) to more than 1800 Da (Gramicidin D). The only structural common denominator identified so far is that all transported substrates are at least somewhat hydrophobic and/or amphipathic in nature, containing a hydrophobic and often a polar or even a (generally positively) charged domain (1–4).

The ability of transported substrates to insert into biological membranes may be an essential requirement for recognition of the compound by P-gp. Favored partitioning into the lipid membrane would increase the effective substrate concentration at the transport binding site. Distinct but overlapping specificities of the drug binding/transport sites may help explain the broad substrate tolerance or lack of specificity (30).

### 3.1. Substrate Recognition

The substrate recognition abilities of P-gp are broad and tolerant, even among most xenobiotic-defense enzymes. The purported xenobiotic-protection role of P-gp (1,2) mimics that of CYP3A4. Both enzymes provide a protective role to many of the same cells and defend against a generally shared list of xenobiotic substrates. The extensive overlap between these two enzymes is probably fortuitous, as opposed to concerted, because of their great tolerance for and acceptance of large lipophilic substrates. Both enzymes appear to have large accommodating hydrophobic binding sites that do not discriminate among many lipophilic compounds. However, substrate recognition and preference are not this simple, because both enzymes have shown cooperativity and a role for decisively oriented hydrogen bonding in the substrate binding sites (7,68).

Most P-gp pharmacophore models can only address very general properties such as lipophilicity and size owing to the multiple binding sites, different assays, binding tolerance, and other complications. General properties appear to converge around hydrophobicity, presence of rings, size, and in particular, tertiary amines (extensively reviewed in refs. 4,69,70). Indeed, it appears that P-gp recognizes its substrates directly from the lipid phase (21,71–74), where they are expected to be much more concentrated owing to partitioning of the lipophilic compounds (75). However, the lipophilicity factor logP (a partition coefficient phase preference) often is not correlated with P-gp binding affinity, and certainly not across compound classes or series (70,76). Structure activity relationships have shown direct correlation of MDR inhibition to logP only for compounds within a closely related series (4). Litman et al. (77) showed that 34 inhibitors from different pharmacological classes have no significant correlation with calculated partition coefficients and that the size of the molecule (van der Waals surface area) was a better corollary. In fact, a P-gp inhibitor has been defined as a compound containing at least two aromatic rings separated by a basic chain with a secondary or tertiary amine (78–80),

and even stereospecific interactions have often been observed for pairs of chiral compounds (81–85). Furthermore, the contribution of hydrogen bonding has been shown for P-gp substrates (86–88). Important features of molecular recognition of substrates include multiple hydrophobic and hydrogen-bond acceptor features (89). Indeed, Seelig has described a pharmacophore with two general patterns for substrate recognition: the “type I unit” of two electron-donor groups (hydrogen-bond acceptors) with a spatial separation of  $2.5 \pm 0.3 \text{ \AA}$  and the “type II unit” of three electron-donor groups with a spatial separation of  $4.6 \pm 0.6 \text{ \AA}$  (87). A recent computational ensemble pharmacophore model supports these recognition patterns (90), although the less restrictive van der Waals interactions, stacking interactions, and the hydrophobic effect may generally combine to provide affinity with multiple diverse compounds (91). Although P-gp does not possess acidic residues in their membrane domains, it transports cationic amphipathic compounds. Therefore, another physical quality must provide this selectivity and the face of the aromatic ring structures of tyrosine, phenylalanine, and tryptophan residues can bind to cations (92,93). Furthermore, binding interactions are modulated by the membrane-lipid environment (75,94,95).

### 3.2. P-gp Inhibition and Pharmacokinetic Drug Interactions

Because of the likelihood of co-administered drugs sharing recognition by the transport site, the inhibition of P-gp causes many PK interactions (vide supra), such as the increase of the oral bioavailability of paclitaxel from 9.3% to 67% with co-administration of cyclosporin A (38). Cyclosporin A also inhibits the renal secretion of vincristine and vinblastine, and other P-gp inhibitors reduce the active biliary excretion of colchicine, doxorubicin, and etoposide by the liver (96,97). Intravenous administration of potent P-gp inhibitors resulted in up to 37-fold increase in HIV-1 protease inhibitor concentrations in the brain of mice (98); and the P-gp inhibitor GF120918 raised the HIV drugs' brain–plasma ratio about 100-fold (99). These and many further examples of significant clinical drug–herb interactions mediated by P-gp indicate that this transporter should be routinely examined in drug development for binding and inhibition caused by proposed therapies.

## 4. P-gp Elevation

Increased P-gp expression and/or activity will naturally have the opposite effect of thwarting the P-gp activity described earlier. Dramatic examples of PK interactions mediated by P-gp induction have been reported. Significant elevation of intestinal P-gp quantity and the suppression of talinolol (100) or digoxin (101) exposure with co-administration of rifampin, a P-gp inducer, were remarkably well-correlated. P-gp and CYP3A regulation appear to respond similarly to PXR binding and share some molecular-regulation signals (102). Following chemotherapy, tumor cells may mutate to present supernormal quantities of P-gp, although many malignancies are already MDR-positive at diagnosis (chemotherapy naïve). For example, in a clinical study, P-gp levels increased 3- to 15-fold, showing that tumors adjust rapidly to anticancer drugs (103). A major obstacle for successful chemotherapy of cancer is the resistance of tumors to multiple anticancer drugs (MDR). Because P-gp can account for up to a 100-fold increase in drug resistance, overexpression or upregulation of this transporter can be applied as a prognostic marker in certain diseases, such as leukemia, breast cancer, neuroblastoma, pancreatic cancer, or ovarian cancer. Indeed, many compounds are in clinical trials to inhibit P-gp, with the goal of overcoming MDR (*vide infra*).

Many other cytotoxic (xenobiotic) compounds are also inducers of P-gp quantity; for example, verapamil, nifedipine, nicardipine, diltiazem, rifampicin, cyclosporin A, progesterone, estradiol, phenobarbital, insulin, clotrimazole, reserpine, isosafrole, St. John's Wort, hyperforin, dexamethasone, androstanol, troglitazone, ecteinascidin, digoxin, some PAHs, 2-acetylaminofluorene, and anthracyclins (104,105). Many results indicate a tissue-dependent inductive response of P-gp to inducer exposure (106).

## 5. Genetics and Variability

### 5.1. Polymorphisms

As with the cytochrome P450 superfamily, MDR1 genetic polymorphism might result in observed outcomes in unique subpopulations, with naturally occurring MDR1 single-nucleotide polymorphisms (SNPs) having clinical and pharmacological relevance. In the 28 exons of MDR1 genomic DNA of healthy Caucasians, 15 SNPs were detected, including six in the coding region (11). Three of these altered the primary amino acid sequence of the protein. Phenotypical consequences for C3435T in exon 26 correlated with intestinal P-gp expression and uptake of orally administered P-gp substrates (12,107). Individuals homozygous for this polymorphism (TT, ~25%,  $n = 188$ ) showed significantly lower duodenal P-gp expression, lower *in vivo* activity of P-gp (approximately twofold), and increased digoxin plasma levels. However, C3435T is located at a noncoding, nonpromoter position in the MDR1 gene and is unlikely to influence P-gp expression. It is more likely linked to other as-yet-unidentified changes in regions of the MDR1 gene that control expression, e.g., in the promoter or enhancer region, or in sequences that are important for mRNA processing. Serving as a surrogate for the estimation of other tissue levels, the concentration of P-gp in a subset of lymphoid cells (CD56<sup>+</sup> natural killer [NK] cells) is also substantially lower in the T/T genotypes.

The frequency of C/C genotype (higher activity or function) in West Africans and African Americans is 83% ( $n = 172$ ) and 61 % ( $n = 41$ ), respectively, whereas in Caucasians it is 26% ( $n = 537$ ) (108) (see Table 1).

Higher doses of tacrolimus or cyclosporine were required in African Americans than Caucasians to attain similar plasma levels. Conversely, the T/T genotype patients attain lower plasma concentrations of the P-gp substrates nelfinavir and efavirenz—anti-HIV drugs (despite low expression of the MDR1 transcript and P-gp) (110)—although the T/T genotype responded better and faster (greater rise in CD4-cell count) to therapy. The 3435C/T polymorphism is noncoding and could be in linkage disequilibrium with a polymorphism elsewhere in the genome that modifies MDR1 expression or function. In another study, the T/T genotype (in the context of a C1236T, G2677T haplotype) was associated with high P-gp expression *in vitro* and low plasma concentrations of fexofenadine (111). The reason for this discrepancy with the C3435T allele subject observations (12) described earlier is currently unclear. There could be an indirect effect of the 3435 genotype, i.e., low P-gp could be compensated for by induction of other transporters (or CYP3A4) and dietary/environmental differences could contribute. The allelic variant MDR1\*2 (haplotype) exhibits enhanced efflux of digoxin, is statistically associated with lower fexofenadine exposure, and includes C1236T, C3435T, and G2677T[Ala893Ser] (62% of European Americans, 13% of African Americans). A recent haplotype analysis has been able to reconcile conflicting results of studies whose analysis is based solely in individual SNPs (112). Haplotype 12 (2677G/3435T) codes

**Table 1**  
**Prevalence of C/C Genotype in Different Ethnic Groups**

| Ethnic Group      | C/C Genotype (%) |
|-------------------|------------------|
| Ghanaian          | 83               |
| Kenyan            | 83               |
| African-American  | 84               |
| Sudanese          | 73               |
| British Caucasian | 48               |
| Portuguese        | 43               |
| Southwest Asian   | 34               |
| Chinese           | 53               |
| Filipino          | 59               |
| Saudi             | 55               |

Adapted from ref. 109.

for elevated concentrations of digoxin after oral dose and the superior haplotype analysis results match data of other MDR1 studies (112).

However, 20+ million humans in Africa and South America have been treated with ivermectin (with no evidence of neurotoxicity), an antiparasitic P-gp substrate and potent neurotoxin in P-gp-null genotype KO mouse. This suggests that: (1) MDR1 P-gp expression is highly conserved in humans overall and (2) defining a subgroup of humans with complete absence of P-gp expression is unlikely. Indeed, 10 SNPs do not result in amino acid changes or are in noncoding regions, and three have an unknown effect on function.

## 5.2. Expression Variability

Humans exhibit wide variation in liver expression of MDR1 mRNA and P-gp protein. The variability of enterocyte P-gp concentration is about 10-fold in transplant patients and a bit less in normal nonmedicated adults (about fourfold, interpatient); males expressed twofold higher amounts of P-gp than females (113,114). There is even an indication of up to eightfold interindividual variability in P-gp content (115), with more than eightfold differences in the P-gp expression observed in a small population (25 patients [116]). This variability is roughly similar to, or perhaps more than, what is observed for CYP3A4, though these studies are from a limited sample population. Temporal variation of P-gp levels is expected (*vide supra*), such as a threefold inpatient variability that was observed in a transplant case study (48)!

## 6. Role of P-gp Polymorphisms and Mutation in Cancer

Many cancer types have provided examples of gene modifications associated with drug resistance and P-gp primary structure. Naturally occurring mutations in the MDR1 gene associated with colorectal cancers with high microsatellite instability (MSI-H) were found in both the coding and promoter regions (117). A mutation in the promoter of the MDR1 gene in human hematological malignancies may contribute to the pathogenesis of resistant disease (118). Similarly, point mutations in the MDR1 promoter have been found in osteogenic sarcoma and various types of leukemia (119) and are associated with diminished in vitro responsiveness to MDR relevant drugs. Moreover, DNA methylation and hypermethylation can affect transcription and gene-product levels (120).

## 7. In Vitro to In Vivo Correlation

Although observations must be carefully judged in the context of contrived elemental systems, the correlation of in vitro experiments to clinical observations generally is quite good owing to the dramatic role or influence of P-gp. The significant impact of P-gp inhibition has been illustrated earlier, including some corollary to in vitro experiments. There are many further examples, including the increased digoxin concentrations in the brain of PSC833 (very potent P-gp inhibitor in vitro) orally treated, wild-type mice near the levels found in *mdr1a/1b*(-/-) double KO mice (33). The relative P-gp inhibition potencies of many herbs and drugs such as the azole antifungals (itraconazole, etc.) and statins (HMG-CoA reductase inhibitors) are consistent with their observed PK effects (121–124). Additionally, correlation between in vitro transcellular molecule transport ratios from transfected (L-*mdr1a*) cells (efficiency as a P-gp substrate) and brain concentration ratios of *mdr1a*(-/-) to *mdr1a*(+/+) CF-1 mice is remarkable and predictive ( $r^2 = 0.93$ ; 125). The cellular accumulation ratio and transcellular transport ratio methods for substrate characterization/quantification also correlated well and consistently (125). Sandwich-Cultured (SC) rat hepatocytes have been shown to be an in vitro model to assess and predict the biliary excretion of xenobiotics with notable correlation (126).

To evaluate substrate-transport kinetics, ATP hydrolysis is a useful assay for P-gp substrates (17,127), although some substrates do not significantly alter P-gp-mediated ATP hydrolysis (from baseline activity ostensibly caused by co-purified endogenous substrate) in the presence of standard lipid constituents (128). However, Caco-2 cells apparently do not afford good general correlation with gastrointestinal (GI) absorption owing to variable expression of P-gp, other ABC transporters, plasma protein binding, rates of passive diffusion, luminal saturation of P-gp, and so forth (55). Moreover, physiological factors such as gastric emptying, GI motility, mucus dissolution, intestinal pH, and blood flow, and lymph flow can uniquely impact each transport mechanism. Yet Caco-2 cells can be a useful indicator of jejunal drug efflux, if the low expression of BCRP (ABC-G2) and CYP3A is accounted for (129). Caco-2, HT-29, MDCK, TC7 can reproducibly display some properties of differentiated intestinal cells (130) and, therefore, suitable for qualitative predictions and molecular-permeability screening studies. Indeed, the in vivo to in vitro Caco-2 drug transport permeability measurements correlate well for passively or highly absorbed drugs ( $r^2 = 85\%$ ) (131,132; also see below) or small molecules (133). However, even some studies of peptidomimetics have resulted in reasonable correlations for slowly and incompletely absorbed drugs (134,135).

### 7.1. Overall Efficiency of Transport

The implications of P-gp activity and function must account for the ratio of permeability/active-transport for the distinction of a substrate from an inhibitor (136). Although a substrate typically competes with alternate substrates for the active site of a xenobiotic-disposition enzyme, many P-gp substrates are not competitive inhibitors for overall efflux. We have shown that many P-gp substrates have no effect on the ultimate ability of P-gp to cause removal of the marker substrates from a viable cell (136), and others have indicated a potentially similar distinction for other compounds (137–139). It is very important to properly define a compound as a substrate, inhibitor, or both in the context of evaluating the potential for drug interactions and drug–herb interactions as well as exposure to toxins and drugs. This disconnect between a sub-

strate and its ability to inhibit P-gp can be explained by an element of the natural system: P-gp exports its substrates across a lipid bilayer that is intended to preclude permeation of adverse xenobiotics. A requirement for a P-gp substrate also to be an inhibitor is the rapid passive transbilayer movement across the membrane bilayer, a process that allows the substrate to re-enter the cell quickly and hence effectively occupy the P-gp active site.

Many studies indicate the critical role of membrane permeation or passive transmembrane movement rate of a substrate toward the inhibition of P-gp (140,141). Although P-gp-mediated active rate of compound transfer or efflux is slow ( $\sim 900 \text{ min}^{-1}$ ; 142), the "flip-flop" rate of its many substrates is even slower ( $t_{1/2} > 2 \text{ min}$ ) against those for which there is "resistance." In other words, to create a concentration gradient, the P-gp substrate must have a relatively slow transmembrane passive transport rate. Conversely, P-gp substrates with relatively rapid permeation will overcome the pace of P-gp to re-enter the binding site and competitively inhibit function. The rate of active efflux transport relative to the rate of passive permeation or influx determines the net movement of drug from inside to outside of the cell membrane; a substrate cycling rapidly back into cytosol will compete for P-gp binding site access. MDR-type drugs are amphipathic (hydrophobic and positively charged) and as such bind readily to negatively charged phospholipid head groups of the membrane. Therefore, transmembrane movement often requires a "flip-flop" through the membrane bilayer and is slow. Indeed, the passive transbilayer diffusion of phosphatidylcholine (PC), the most abundant membrane lipid, is very slow ( $t_{1/2} \sim \text{d}$ ) in both artificial and natural membranes (143). By measuring the transport rate and passive transbilayer permeation rate of five inhibitors and five substrates, it was shown that P-gp inhibitors cross the bilayer membrane faster than the egress rate of P-gp, thereby resulting in rapid equilibration rates (139). At an approximated turnover rate of  $900 \text{ min}^{-1}$ , P-gp can keep pace with a compound like Rho with a transbilayer movement lifetime of minutes. Conversely, P-gp is inefficient in protecting MDR cells against molecules rapidly permeating through lipid bilayer membranes; for example, potent P-gp inhibitors such as the carrier-type ionophore valinomycin, which traverse membranes within microseconds ( $k > 25 \times 10^4 \text{ s}^{-1}$ ). Conversely, gramicidin, effectively excluded by P-gp, has a transmembrane "flip-flop" rate with a lifetime of minutes. Additionally, the transmembrane movement rate is critical to the overall efficiency of P-gp removal of rhodamine dyes from MDR cells. Indeed, rhodamine B and tetramethylrhodamine exhibit high affinity for P-gp, but rhodamine B was the fastest membrane-traversing dye and the least efficiently excluded from the cell. There was a similar corollary for all of the related dyes tested (144). Another efficient marker of efflux, doxorubicin exhibits a "flip-flop" rate with a  $t_{1/2}$  of approx 1.7 min, and 30% cholesterol addition to the vesicles to reduce membrane fluidity decreased the rate sevenfold (145). The fluidizer benzyl alcohol accelerated the rate, consistent with the role of membrane bilayer permeability dictating the rate of equilibration. Consequently, of compounds ejected by P-gp, those that are relatively slow to cross the lipid bilayer are efficient "substrates," and those that rapidly permeate the membrane are (also) good "inhibitors." This effect has been modeled using the highly permeable compound nifedipine and further supports this conclusion (39,146), as does ranitidine, which exhibits very low passive permeability (147) and is not a P-gp inhibitor.

Lipophilicity could sometimes be used to discriminate between P-gp substrates and inhibitors. Among a series of anthracyclines, the less lipophilic derivatives were corre-

lated with slower passive diffusion and resulted in lower intracellular accumulation (140,141,148,149). Indeed, lipophilicity is inversely correlated with P-gp efflux efficiency of some vinca alkaloids (150), steroids (151), and peptides (152); and directly relates to P-gp inhibition for cyclosporins (153), steroids (151), and linear hydrophobic peptides (154). Although more lipophilic compounds appear to be better inhibitors generally (as opposed to ejected efficiently as substrates), this correlation does not apply outside of a particular set of analogs (155,156). This result is probably owing to the fact that lipophilicity parameters are inadequate for judging the interaction of structurally diverse compounds with the complexities of membranes (76).

We can now appreciate that the number of P-gp substrates that are not also inhibitors is striking, and many substrates could be expected to have no substantial effect on P-gp function in the viable cell. Despite this absence of effect on P-gp overall function by many substrates, these P-gp substrates can still be affected pharmacokinetically by the modulators of P-gp activity. It is therefore important to characterize both the efflux-rate parameters and those of inhibition.

## 8. Pharmacological Modulation

Of the types of cellular multidrug resistance (resistance to unrelated drugs), the ATP-dependent efflux pumps are the dominant factor (157). Early studies showed that P-gp was highly expressed in colon, kidney, adrenocortical, and hepatocellular cancers (158), and P-gp expression is correlated with a reduced complete remission rate of acute myelogenous leukemia (AML) and a higher incidence of refractory disease (159). High P-gp expression is also well-correlated with the poor clinical outcome in childhood acute lymphoblastic leukemia (ALL) (119). Additionally, breast tumors, and possibly ovarian cancers and lung cancers, that expressed increased levels of P-gp after therapy were associated with over a threefold greater likelihood of treatment failure (160). After chemotherapy, a significant proportion of breast cancer patients express increased tumor levels of P-gp (161). In ovarian cancer samples, 16–47% were found to express P-gp, as measured by immunohistochemistry (162). Studies using mRNA detection or immunohistochemistry methods, ex vivo functional assays, or in vivo tumor imaging all show a strong association between therapy with MDR drugs, intrinsic or acquired expression of P-gp, reduced tumor-cell drug retention, and a poor treatment response in breast cancer patients (163,164). Owing to significant resistance to anticancer drugs, the mitigation of P-gp has been ardently sought for more than 20 yr (165). As mentioned earlier, PSC833 (cyclosporin analog) is a potent P-gp inhibitor and has been investigated in the clinic as an adjuvant therapy toward overcoming chemotherapy resistance (166), although it also causes profound PK effects (33). Most of these compounds, however, emerged as weak inhibitors that were toxic at high doses. The poor activity of current P-gp-inhibition compounds in patients has also been attributed to the presence of resistance factors in addition to P-gp (e.g., other ABC transporters), inappropriate design of clinical trials, toxicity, bioavailability, and/or lack of specificity of anti-P-gp reagents. Yet a Phase I/II trial of cyclosporin A added to daunorubicin and cytarabine in “poor-risk” patients with AML resulted in 62% complete remissions and a 69% overall response rate (167). Combinations of suboptimal doses of P-gp inhibitors were shown to be effective at 15–100 times less than the optimal doses, suggesting the possibility of avoiding associated toxicities of these agents (168).

An intriguing possibility of a dual-acting chemotherapy is found in SCH66336 (lonafarnib), an orally active, potent, and selective inhibitor of the farnesyl protein transferase (FPT) enzyme (169). This novel therapeutic agent has activity against a wide variety of human tumor xenografts and also causes regression of tumors in wap-H-ras transgenic mice. Enhanced antitumor activity has been reported in preclinical cancer models when SCH66336 is combined with cyclophosphamide, 5-fluorouracil (5-FU), or vincristine (169,170), all substrates of P-gp. Furthermore, a synergistic effect on antitumor activity of SCH66336 and taxanes (also P-gp substrates) has recently been described (171). Moreover, a recent report shows a synergy with co-administration of SCH 66336 with paclitaxel or docetaxel (two known substrates of P-gp) in vitro and in vivo (171). SCH66336 significantly enhanced the effect of paclitaxel in the NCIH460 lung-cancer xenograft model and was able to sensitize wap-ras/F mammary tumors as well as tumor cell lines to paclitaxel (171). We have directly characterized and quantified a specific synergy on P-gp function between SCH66336 and either tamoxifen, paclitaxel, or vinblastine (124). Relatively small concentrations of SCH66336 can increase the affinity (potency) of these additional compounds as inhibitors of P-gp function. Treatment with SCH66336 would be predicted to be synergistic with co-administered cancer therapeutics that are substrates of P-gp. A further benefit of co-administration of SCH66336 could be reduced chemotherapy dosage, hence, lower exposure to normal cells, and therefore less undesired toxicity.

### **8.1. Classes of Modulators**

Many of the characterized MDR modulators (inhibitors) can be categorized in various compound classes and this has been very well-reviewed (4,172,173). The classes include: (1) calcium-channel blockers (dihydropyridine analogs, i.e., verapamil); (2) calmodulin antagonists (phenothiazines and thioxanthenes, i.e., trifluoperazine); (3) cyclic peptides (cyclosporin A, PSC833); (4) steroids and hormonal analogs (progesterone, tamoxifen); (5) dipyridamole; (6) anthracycline/vinca alkaloid analogs; and (7) miscellaneous other compounds.

### **8.2. Toxicity and Pharmacokinetic Interactions**

Dose-limiting toxicities of the MDR modulators have often precluded their further clinical development (174). Ventricular arrhythmia (verapamil), myelosuppression, cerebellar ataxia (PSC833, valspodar), and hypertension (cyclosporin A), have been observed, but may be owing to PK interactions caused by P-gp inhibition (6). The antidiarrheal agent loperamide is a P-gp substrate, hence brain exposure is very limited normally; yet in the presence of quinidine (potent P-gp inhibitor), the brain loperamide concentrations increase resulting in serious neurotoxicity (175). Moreover, PK interaction could be caused by inhibition of other xenobiotic-defense enzymes such as the cytochrome P450s, and could also enhance exposure of the chemotherapy to "sanctuary" sites such as CNS and testis, thus unveiling new toxicities not previously seen with the cytotoxin alone. This suggests that a significant fraction of patients have been under-dosed, thus making efficacy interpretations difficult.

### **8.3. Clinical Success or "Proof of Principle"**

Clinical trials with some of the second-generation modulators are in progress, and some studies show clinical benefit from the use of modulators such as Valspodar

(PSC833) (176), although Valspodar is saddled with enhancement of toxicity owing to PK interactions. Furthermore, the more potent and specific modulators (GF120918 [elacridar], LY335979 [zosuquidar]) do not show significant PK interaction with doxorubicin, etoposide, and paclitaxel in animal studies (177–179). In a human Phase II study, a subgroup of paclitaxel-refractory advanced breast-cancer patients was resensitized by biricodar (VX-710) with adequate safety margins and PK parameters (180). Trials in multiple myeloma, non-Hodgkin's lymphoma (NHL), and acute leukemia have shown that positive responses to P-gp modulators may occur in patients who are refractory to standard chemotherapy regimens (180a–182).

These studies clearly indicate that the development of potent and selective P-gp inhibitors is an important approach to reversing MDR in the clinic. MDR of cancer cells is a potentially surmountable obstacle to effective chemotherapy of cancer.

## 9. MDR Pharmacological Inhibitors and Development Phase

The extensive list of MDR modulators cataloged in Table 2 serves as a strong illustration of the pursuit of an adjuvant cancer therapy to mitigate the action of P-gp. This table includes the pharmacological product classifications, compound name, pharmacological activity with parameter value, the evaluation stage as development phase, and the reference. This catalog was generated from the MF-line database and data management software.

## 10. Other ABC Transporters and the MDR Protein Multi-Gene Family

Much like the genetic superfamilies for cytochromes P450 and glutathione S-transferases, the xenobiotic transport enzymes are significantly contributing members of an extensive family that affects the overall disposition of many compounds. MDR1 and MRP1 exhibit much overlapping substrate specificity, although MDR1 currently seems to be broader in scope. However, MRP1 can act as a GS-X pump, i.e., it can transport drugs conjugated with GSH and glucuronide. MRP2 (also known as cMOAT; canalicular multispecific organic anion transporter) almost exclusively pumps out conjugates. Rats deficient in MRP2 show a chronic conjugated hyperbilirubinemia (model for the human disorder Dubin-Johnson syndrome). MRP homologs recently identified are MRP3, MRP4, MRP5, MRP6, MRP7, MDR3, BSEP, and MXR (205–208). MDR2 apparently translocates only phospholipids, e.g., phosphatidylcholine. It is believed that humans possess 48 genes encoding ABC transporters.

The presence of other cellular drug-resistance mechanisms in addition to P-gp is most likely responsible for the apparent ineffectiveness of some P-gp modulators to date. The expression of MRP, P-gp, or both can account for diminished accumulation and retention of daunorubicin in blast cells from AML patients (209). MRP2 is capable of mediating drug efflux, and a recent study showed increased bioavailability of several drugs and carcinogens in MRP2-null rats (210).

### 10.1. MRP1, MRP2, and MRP3

The multi-drug resistance protein (MRP) transporters are a subfamily of ABC transporters (ABC-C) related further to P-gp as expellers of various xenobiotics such as chemotherapy (194,205,208,211,212). Extruding many anionic amphipathic compounds and conjugates from cells, the MRPs have become well-appreciated for their significant role in chemotherapy resistance (213–215). MRP1 (ABC-C1) and MRP2

Table 2

**P-Glycoprotein (P-gp) Interaction of Selected Multidrug-Resistance Modulators (MDRMs) and Other Selected Compounds With Significant Activity on P-gp**

| Compound                                                | P-gp inhibition <sup>a</sup><br>IC <sub>50</sub> [K <sub>i</sub> ]*(μM)/References |                   | P-gp affinity <sup>b</sup><br>IC <sub>50</sub> [K <sub>i</sub> ]*(μM)/References |               |
|---------------------------------------------------------|------------------------------------------------------------------------------------|-------------------|----------------------------------------------------------------------------------|---------------|
| <b>MDRMs</b>                                            |                                                                                    |                   |                                                                                  |               |
| Biricodar dicitrate<br>(VX-710)                         | 2.5                                                                                | (183)             | 0.75                                                                             | (183)         |
| B9109-012                                               |                                                                                    |                   | 0.0107*                                                                          | (184)         |
| CP-100356                                               |                                                                                    |                   | 3.50                                                                             | (186)         |
| Dexniguldipine(HCl)                                     | 0.45                                                                               | (184)             | 2.5                                                                              | (185)         |
|                                                         |                                                                                    |                   | 0.0112*                                                                          | (184)         |
| Elacridar, GF120918                                     | 0.044–0.51                                                                         | (187,191)         | 0.0014–0.0025*                                                                   | (187)         |
| Laniquidar                                              | 0.2                                                                                | (192)             |                                                                                  |               |
| MS-209                                                  | 0.4*                                                                               | (193)             |                                                                                  |               |
| ONT-093                                                 | 0.03                                                                               | (194,195)         |                                                                                  |               |
| OC144-093                                               |                                                                                    |                   |                                                                                  |               |
| Progesterone derivative                                 | 0.6–0.8                                                                            | (196)             |                                                                                  |               |
| S-9788                                                  | 0.3                                                                                | (197)             | 60                                                                               | (92)          |
| Tariquidar (XR9576)                                     | 0.025–0.49                                                                         | (187,191,198)     | 0.55                                                                             | (191)         |
|                                                         |                                                                                    |                   | 0.0026–0.0042*                                                                   | (187)         |
| Timcodar-2CH <sub>3</sub> SO <sub>3</sub> H             | 0.2–0.4                                                                            | (199)             |                                                                                  |               |
| Valspodar                                               | 0.29–1.06                                                                          | (200)             |                                                                                  |               |
| XR-9051                                                 | 0.70                                                                               | (188,189,190)     | 0.0018–0.0106*                                                                   | (190)         |
| XR-9576                                                 |                                                                                    |                   | 0.0018–0.0106*                                                                   | (190)         |
| Zosuquidar(3HCl<br>(I.Y-335979)                         | 0.059*                                                                             | (178)             | 0.03–0.06                                                                        | (179)         |
| <b>Oncolytic drugs</b>                                  |                                                                                    |                   |                                                                                  |               |
| Paclitaxel                                              |                                                                                    |                   | 0.335*                                                                           | (187)         |
| Vinblastine-H <sub>2</sub> SO <sub>4</sub>              |                                                                                    |                   | 0.02*                                                                            | (187)         |
| Mitotane                                                | 10–30                                                                              | (180a)            |                                                                                  |               |
| <b>Immunosupressants</b>                                |                                                                                    |                   |                                                                                  |               |
| Cyclosporine                                            | 0.44–5.10                                                                          | (200,201,203)     | 0.6–1.17                                                                         | (191)         |
| FR-901459                                               | 6.0                                                                                | (201)             |                                                                                  |               |
| <b>Antifungal agents</b>                                |                                                                                    |                   |                                                                                  |               |
| Itraconazole                                            | 1.7                                                                                | (202)             |                                                                                  |               |
| Ketoconazole                                            | 5.6                                                                                | (202)             |                                                                                  |               |
| <b>Calcium antagonists</b>                              |                                                                                    |                   |                                                                                  |               |
| Fantofarone (SR33557)                                   | 2.5–20                                                                             | (203)             | 0.0083                                                                           | (203)         |
| Nicardipine(HCl)                                        |                                                                                    |                   | 0.011–0.182*                                                                     | (187)         |
| Verapamil                                               | 0.58–15.3                                                                          | (124,196,198,203) | 1.27–110                                                                         | (124,179,203) |
| <b>Farnesyltransferase inhibitors</b>                   |                                                                                    |                   |                                                                                  |               |
| Lonafarnib<br>(SCH-66336)                               | 2.7                                                                                | (124)             |                                                                                  |               |
| <b>Calmodulin antagonists,<br/>Dopamine antagonists</b> |                                                                                    |                   |                                                                                  |               |
| Trifluoperazine(HCl)                                    | 7.2                                                                                | (124)             |                                                                                  |               |
| <b>Others</b>                                           |                                                                                    |                   |                                                                                  |               |
| Silybin                                                 |                                                                                    |                   | 6.8*                                                                             | (204)         |
| Taxifolin                                               |                                                                                    |                   | 37.4*                                                                            | (204)         |
| CBT-1 (NSC-77037)                                       |                                                                                    |                   |                                                                                  | (204a)        |

<sup>a</sup>Inhibitory activity measured in different cell lines expressing P-gp and/or exhibiting multidrug resistance by means of different assays.

<sup>b</sup>Affinity to P-gp evaluated by displacement of different radioligands in different cell lines expressing P-gp and/or exhibiting MDR.

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(ABC-C2) have been shown to be expressed in cancers such as lung (216,217), leukemia, bladder, neuroblastoma, breast, ovarian, hepatic, gastric, and prostate (211,212,218–220) and have conferred resistance against various chemotherapeutics. Accordingly, MRP1 is ubiquitously distributed among many organs predominantly in the basal and lateral plasma membranes, whereas MRP2 is in intestine, liver, kidney, placenta, BBB, pancreas, spleen, and choroid plexus and localized in the apical membrane of these cells (211,212). The role in these locations is exemplified by the significant contribution of MRP2 to BBB function that was recently reported (221). MRP1 and P-gp share only modest amino acid sequence identity (15%), whereas MRP1 and MRP2 share 50% amino acid identity, with MRP1 and MRP3 sharing even more (58%)

Because MRP1 secretes compounds into the system or body on the basolateral side, its role is more of a cellular defense than one of total organism defense such as P-gp and MRP2 (eliminated drugs from the body). Absence of MRP1 causes etoposide levels to increase 10-fold in the cerebrospinal fluid (CSF) after intravenous administration of the drug (222). The body must have a basolateral transporter to protect sperm or CSF, because P-gp or other apical side efflux transporters would extrude drug into the sanctuary.

Substrate recognition by MRP1 and MRP2 is generally similar, with known examples being glucuronosyl-etoposide, estrone-3-sulfate, estradiol-17 $\beta$ -glucuronide, etoposide, vincristine (vinca alkaloids), sulfinpyrazone, methotrexate, leukotriene C4 (perhaps as a GSH conjugate), and anthracyclines. MRP1 can, however, exhibit remarkable selectivity, which probably contributes to cancer-therapy resistance. Whereas estradiol 17 $\beta$ -glucuronide is a good substrate, the 3-isomer is not (223). Furthermore, MRP2 transports HIV protease inhibitors, whereas MRP1 does not (224).

Cisplatin is an actively transported substrate of MRP2 (214,225–232). The introduction of MRP2 antisense cDNA into human hepatic cancer HepG2 cells results in increased sensitivity to cisplatin, vincristine, doxorubicin, and the camptothecin derivatives (231), with re-inoculated rats confirming an *in vivo* MRP2-mediated resistance to cisplatin (229). MRP2 mRNA expression is significantly associated with the resistance of colorectal cancer to cisplatin (227). In transfected cells, the overexpression of MRP2 resulted in resistance to cisplatin (10-fold), etoposide, doxorubicin, epirubicin, and MTX (225). Moreover, MRP2 mRNA levels correlate with cisplatin resistance in a subset of resistant cell lines (214), whereas cellular accumulation and drug sensitivity to cisplatin in human MRP2 transfectants decreased (214).

5-FU is a possible substrate for the MRP1 transporter. HL-60 cells selected for overproduction of MRP1 (10 $\times$  the level of parent HL-60 cells) showed resistance to 5-FU (233), and MRP1 expression is correlated with 5-FU resistance in seven GI cancer-cell lines (234). Treatment of cisplatin-resistant cell line with 5-FU increased the cytotoxicity of cisplatin fourfold, indicating that 5-FU may interact with MRP2 as well (235).

Tyrosine kinase inhibitors, particularly STI571 (imatinib mesylate; Gleevec), have been shown to interact directly with MRP1 and P-gp (236). In transfected cell lines expressing high levels of either MRP1 or P-gp, several tyrosine kinase inhibitors can inhibit transport function as well as substrate-stimulated ATP hydrolysis (236). Moreover, P-gp has been detected in cell lines resistant to STI571 (237).

A substrate of a trans-membrane transporter may not also be an inhibitor; conversely, inhibitors may not also be transported. Therefore, the list of substrates may not equal that of substrates. The known MRP1 inhibitors are nonspecific and include

sulfinpyrazine, probenecid, benzbromarone, indomethacin (nonsteroidal anti-inflammatory drugs [NSAID]), some flavonoids, and even ritonavir (238).

MRP3 also transports amphipathic anions on the basolateral side, like MRP1 (219); is found largely in the liver, gut, and kidney, like MRP2; and is also present in the adrenal gland, pancreas, gallbladder, lung, and ovary (239). MRP3 may contribute to enterohepatic recycling of bile salts (e.g., glycocholate) and removal of toxic anions during cholestasis (240). Substrates include estradiol-17 $\beta$ -glucuronide, glucuronosyl-drug conjugates, vinca alkaloids, methotrexate, etoposide, and teniposide and may contribute to chemotherapy resistance. During cholestatic conditions the high MRP2 levels drop, whereas MRP3 is significantly elevated. Therefore, many amphipathic anions (including bile salts) secrete to the basolateral instead of the apical direction, preventing intrahepatic accumulation to toxic concentrations (240).

### **10.2. MRP4 and MRP5**

MRP4 and MRP5 are on the basolateral side of cells in many tissue types and are able to transport therapeutic nucleoside-based compounds (241,242). Indeed, HIV was less effectively inhibited by the modified nucleoside analogs in cells overexpressing MRP4 (241). MRP4 expression may also affect cancer chemotherapy. MRP4-overexpressing cells were resistant to cytotoxic effects of 6-mercaptopurine and 6-thioguanine (and AZT, MTX), important drugs in the treatment of childhood leukemias (242). A role for MRP4 in the transport of DHEA (dehydroepiandrosterone 3-sulphate) and other conjugated steroids has recently been suggested (243).

With MRP5 transporting not only PMEA (adenine nucleotide analog, acyclic nucleoside phosphonate) but also monophosphate diphospho-thiopurines (244), this transporter may have a role in cancer chemotherapy as well. It was recently demonstrated that MRP5 is a cGMP transporter, yet a comparatively poor cAMP transporter (245). Because sildenafil (i.e., Viagra, as well as trequinsin and zaprinast), a potent phosphodiesterase inhibitor, is also a very effective MRP5 inhibitor, speculation suggests that the vasodilatory effects could be owing to inhibition of MRP5-mediated cyclic nucleotide transport. Although MRP4 seems to prefer methylated thio-IMP, MRP5 prefers the unmethylated thioinucleotides. Moreover, MRP4 mediates transport of glucuronate conjugates and methotrexate, whereas MRP5 apparently does not (246).

Many of the ABC transporters have been shown to be temporally regulating, or induced by exposure to xenobiotics. MRP4 transcription regulation may be controlled by its natural substrates, cyclic nucleotides (247), and MRP4 is upregulated in response to elevated levels of hepatic bile acids (248).

### **10.3. MRP6**

MRP6, located in the basolateral membranes of cells in the liver and kidney, transports an anionic cyclopentapeptide BQ-123 and certain glutathione conjugates (249). Possibly a highly selective pump for amphipathic anions (250), MRP6 can cause low levels of resistance to some anticancer agents (etoposide, teniposide, doxorubicin) (249). The absence of MRP6 causes pseudoxanthoma elasticum, a heritable disorder characterized by calcification of elastic fibers in skin, arteries, and retina (251).

### **10.4. BCRP/MXR/ABCG2**

The ABC transporter breast cancer resistance protein (BCRP), also known as mitoxantrone-resistance protein (MXR) (252), is overproduced in MCF7 breast can-

cer cells (253). As part of the ABC-G subfamily, this transporter has been renamed ABCG2 and apparently functions as a homodimer. It is not known if ABC-G2 can also heterodimerize with other proteins, such as those of the AGC-G class. Though possibly more selective in substrate recognition than P-gp, ABCG2 ejects mitoxantrone, topotecan derivatives, anthracyclines, bisantrene, etoposide, prazosin, and flavopiridol (254), as well as HIV-1 nucleoside reverse-transcriptase inhibitor zidovudine (AZT) (255). The active transport of indolocarbazole compound A was inhibited by indolocarbazole analogs but not by mitoxantrone, suggesting unique binding sites (256) reminiscent of P-gp. Other likely substrates include the experimental indolocarbazole topoisomerase inhibitors NB-506 and J-107088 and the active metabolite of the camptothecin analogue irinotecan/CPT-11 (257). However, vincristine, vinblastine, paclitaxel, cisplatin, colchicine, verapamil, calcein-AM, rhodamine 123, and doxorubicin are not significantly transported by AGC-G2 (258). It appears that P-gp, MRP1, and ABCG2 can account for most of the known active MDR (259), with relatively high expression of BCRP mRNA observed in approx 30% of AML cases (260). ABC-G2 mRNA also increased significantly from diagnosis to relapse or refractory disease, indicating that ABC-G2 levels may correlate with clinical resistance in AML (261). ABCG2 has been detected in the apical membranes of placental syncytiotrophoblasts, hepatocytes, the epithelial lining of the small intestine and colon, brain microvessel endothelium (262), the ducts and lobules of the mammary gland (263), and hematopoietic progenitor cells (264). Drug-resistant cell lines overexpressing ABC-G2 are derived from parent cells of fibroblasts, breast, colon, gastric, lung, or ovarian carcinomas, fibrosarcomas, and myelomas, which suggests that ABC-G2 may contribute to drug resistance in tumors of various tissue types.

ABC-G2 inhibitors may be useful to improving chemotherapy response, analogous to P-gp inhibitors. The P-gp inhibitors reserpine, GF120918, are potent inhibitors of the ABC-G2. Other inhibitors include fumitremorgin C and the tyrosine kinase inhibitor CI1033, whereas verapamil, cyclosporin A, PSC833 and some other P-gp inhibitors have little effect on ABC-G2.

Similar to P-gp, ABC-G2 could limit oral bioavailability. Indeed, the mRNA level of BCRP is significantly higher than that of MDR1 in jejunum (125). Moreover, inhibition of ABC-G2 by oral dose of GF120918 has been shown to cause a drug interaction with oral topotecan, raising plasma concentrations approximately sixfold. Biliary ABC-G2 also seems to contribute bioavailability and drug interactions because the GF120918 dose decreased hepatobiliary excretion of intravenously administered topotecan by approximately twofold (265). Furthermore, brain exclusion of xenobiotics may be significantly dependent on AGC-G2, with its mRNA more highly expressed than P-gp and MRP1 in porcine brain (266).

Differences in ABC-G2 function or expression among the population may result in diverse (and possibly dangerous) clinical exposure to substrate drugs, analogous to other polymorphic xenobiotic-metabolism enzymes. Analysis of DNA from 11 different ethnic populations revealed that there are several common natural allelic variants of ABC-G2, but their effect on function is yet to be examined (267). A 78-fold variation in expression of BCRP mRNA and significant variation in protein expression in human intestine could not be accounted for by one of the common allelic variants (267) (see Table 3).

### **10.5. Sister-P-gp (SPGP)/Bile Salt Export Protein (BSEP)/ABC-B11**

Sharing 50% amino acid identity with P-gp, sister-P-gp (SPGP), also known as the Bile Salt Export Pump (BSEP, ABC-B11), is significantly more selective with respect to substrate recognition. Expressed exclusively in the liver (268), BSEP appears to have a role in efflux of endogenous compounds (bile acids) and exogenous compounds (xenobiotics) into the bile (269,270). Some examples of endogenous substrates thought to be exported by BSEP include taurocholate, estradiol-17 $\beta$ -glucuronide, cholic acid, muricholates, and other monoanionic bile salts. Canalicular secretion of bile acids from the liver in the form of bile facilitates the emulsification of dietary lipids and fat-soluble vitamins. Defective bile secretion results in cholestasis with accumulation of bile salts and other toxic bile constituents within hepatocytes and blood plasma. Mutations in the *BSEP* gene can result in the absence of BSEP expression and are the cause of certain forms of progressive familial intrahepatic cholestasis (PFIC-2) (271,272). PFIC manifestations are jaundice, fibrosis, cirrhosis (caused by <1% of normal biliary bile salts), hyperbilirubinemia, suppressed lipid and cholesterol metabolism, and intestinal malabsorption of fat and fat-soluble vitamins.

BSEP has recently been characterized as a transporter that interacts with drugs and xenobiotics, including vinblastine, ditekiren, troglitazone, troglitazone sulfate, cyclosporin, rifamycin, glibenclamide (273), sulindac, and taxol (274). Moreover, the administration of troglitazone (275,276), cyclosporin, rifampicin, and bosentan (277)—all inhibitors of BSEP—has been linked with cholestasis. Xenobiotic-induced cholestasis is a significant clinical problem, though drug interactions mediated by BSEP may also have dangerous consequences. Because P-gp and BSEP are both expressed in the liver, it is clear that the extent of overlap between P-gp and BSEP drug substrates and inhibitors needs to be established to estimate the BSEP role in drug disposition. The importance of BSEP interactions at the level of hepatobiliary export processes should thus be considered in the evaluation of drug interactions.

Several cholestatic drugs have already been shown to potentially inhibit BSEP: cyclosporine A, rifampicin, glibenclamide, estradiol-17 $\beta$ -glucuronide, bosentan, troglitazone, and sulindac all can cause increased bile-salt concentrations in serum and eventually cholestatic liver injury and are BSEP inhibitors (273,275,278) and probably substrates (279). Thus far, the most potent inhibitors seem to be cyclosporin, tamoxifen, and valinomycin (280). There are likely to be much more potent inhibitors, and other substrates also may be more sensitive to inhibition of transport.

MDR3 is also involved in bile transport and another form of progressive familial intrahepatic cholestasis (PFIC-3). Sharing 75% amino acid identity with P-gp, it is inhibited by some known P-gp inhibitors and can also transport some amphipathic drugs (281). However, it is generally considered selective for efflux of biliary phospholipids (phosphorylcholine).

## **11. Conclusion**

Because of the wide tolerance of substrate recognition, P-gp can often be the mechanism for significant pharmacokinetic drug interactions when two or more drugs are competing for the P-gp transport site. P-gp levels are also inducible and can be even further elevated in cancer cells, thus contributing to the confounding pleiotropic resistance to chemotherapy and poor treatment prognosis. Consequently, a broad scope of research over 20 years has led to the evaluation of co-therapies intended to augment

**Table 3**  
**ABC Transporters Relevant to Xenobiotic Disposition**

| Name     | AKA              | Tissue                                                                | Side        | Substrates                                                                           | Notes                                 |
|----------|------------------|-----------------------------------------------------------------------|-------------|--------------------------------------------------------------------------------------|---------------------------------------|
| MDR1     | P-gp,<br>ABC-B1  | Ubiquitous                                                            | Apical      | Hydrophobic                                                                          | Induced<br>cholestasis                |
| MDR2     | MDR3,<br>ABC-B4  | ?                                                                     | Apical      | PL                                                                                   | xenobiotics                           |
| BSEP     | SPGP,<br>ABC-B11 | Liver                                                                 | Apical      | Bile salts                                                                           | PFIC                                  |
| MRP1     | ABC-C1           | Ubiquitous (low<br>liver)                                             | Lateral     | Lipophilic anions,<br>leukotriene,<br>conjugates                                     | Induced<br>cholestasis                |
| MRP2     | CMOAT<br>ABC-C2  | Liver, kidney,<br>gut, placenta, brain                                | Apical      | Conjugates,<br>lipophilic anions,<br>Bilirubin                                       | Suppressed<br>cholestasis             |
| MRP3     | ABC-C3           | Liver, brain,<br>adrenals,<br>pancreas<br>kidney, gut,<br>ovary, lung | Basolateral | Etoposide,<br>vincristine,<br>methotrexate,<br>glycocholate,<br>conjugates (anionic) | Induced<br>cholestasis                |
| MRP4     | ABC-C4           | Ubiquitous                                                            | Basolateral | Anti HIV                                                                             | Conjugates<br>Nucleoside<br>antiviral |
| MRP5     | ABC-C5           | Ubiquitous                                                            | Basolateral | Anti HIV<br>conjugates                                                               | Nucleoside<br>antiviral               |
| MRP6     | ABC-C6           | Liver, kidney,<br>brain                                               | Lateral     | Amphipathic anions,<br>conjugates                                                    |                                       |
| CFTR     | ABC-C7           | Exocrine tissue                                                       |             | Anions bicarbonate                                                                   | Cystic<br>fibrosis                    |
| BCRP/MXR | ABC-G2           | Ubiquitous                                                            | Apical      | Amphipathic,<br>Chemotherapy                                                         | Chemotherapy<br>resistance            |

chemotherapy by inhibiting P-gp. This review includes a list of the currently known P-gp inhibiting adjuvant candidates described in the literature, with associated references and summary data. The summary catalog of P-gp modulators illustrates the ardent pursuit to overcome this form of therapy resistance and gives examples of clinical success and failure. Significant *in vivo* and *in vitro* experimental observations as well as the extensive catalog of P-gp inhibitors shown earlier illuminate the critical pursuit of impeding MDR by inhibiting P-gp. However, there remain many difficulties and hurdles to effective and safer therapies intended to block the active efflux provided by P-gp owing to its broad selectivity and tissue distribution as well as clinical liabilities of the compounds.

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