ABC Transporters as Potential Targets for Modulation of Drug Resistance

L. Gatti, G.L. Beretta, G. Cossa, F. Zunino and P. Perego*

Department of Experimental Oncology and Laboratories, Fondazione IRCCS Istituto Nazionale Tumori, via Venezian 1, 20133 Milan, Italy

Abstract: ATP binding cassette transporters are implicated in multidrug resistant phenotypes of tumor cells and may be cancer stem cell markers. Inhibitors of drug efflux pumps represent an emerging group of potentially useful agents for the improvement of antitumor therapy. Here we provide an overview of drug transporter functions and modulation.

Key Words: ATP binding cassette transporters, antitumor drugs, modulators.

INTRODUCTION

Drug resistance represents a major obstacle towards the cure of cancer, being one of the main causes of failure of antitumor therapy. Preclinical studies clearly support that some tumors are already resistant to drugs when the therapy starts (intrinsic resistance), whereas others acquire resistance after some cycles of therapy in spite of the initial responsiveness (acquired resistance). Under both circumstances, tumor cells frequently exhibit simultaneous resistance to multiple structurally unrelated anticancer drugs (Table **1**), a phenotype known as multidrug resistance (MDR) [1]. A major cause of the MDR phenotype is the overexpression in tumor cells of members of a highly conserved family of transmembrane proteins characterized by an ATP-binding cassette (ABC), i.e., the ABC superfamily of transporters [1, 2]. An extraordinarily different array of structurally unrelated chemotherapeutic agents is substrate for such transporters which act as efflux pumps decreasing the intracellular amount of toxins. Overexpression of ABC transporters usually occurs at the level of the plasma membrane, the final goal being to reduce the intracellular amount of drug available for interaction with the drug target, thereby preventing cell death. A recent study indicating non-genetic transfer of the MDR phenotype suggests the occurrence of multiple mechanisms to increase the function of ABC transporters [3].

Whole genome approaches have documented the existence of a wide family of ABC type transporters in human cells [1]. Fifty different ABC transporters have been identified that can be divided into seven classes (A-G) based on sequence similarities. Members of the A, B, C, and G classes have been shown to confer drug resistance in cultured cells [4]. Although all share a highly conserved ABC, containing 3 core consensus motifs (Walker A, Walker B and Signature C) essential for ATP binding, their domain structures are organized differently, with diverse numbers and locations of trans-membrane domains [5]. With the exception of BCRP, these transporters contain two ABCs in a single molecule.

*Address correspondence to this author at the Fondazione IRCCS Istituto Nazionale Tumori, *via* Venezian 1, 20133 Milan, Italy; Tel: +39-0223902237; Fax: +39-0223902692;
E-mail: paola.perego@istitutotumori.mi.it

MDR efflux transporters have been initially shown to be localized mainly at the plasma membrane of malignant cells. However, transporters can be localized at subcellular sites including nucleus and Golgi apparatus [6]. Also, a mechanism of drug sequestration in extracellular vesicles localized at cell-cell attachment zones in breast cancer cells resistant to mitoxantrone has been reported [7].

Since it is reasonable that the success of antitumor therapy will depend on the specific features of the treated tumors and on the structural properties of the drugs used for the treatment, a precise knowledge of the functions of novel transporters is expected to contribute to optimization of antitumor therapies. In this context, the poor pharmacokinetics, the effect of inhibitors in other tissues and the lack of patient selection, rather than the lack of selectivity, could represent the major cause of therapy failure. Based on this background, the present review focuses on the pharmacological significance of selected ABC transporters, with particular reference to perspectives in the development of useful modulators of their function, as therapeutic agents. In particular, a characterization of the substrate specificity of the novel transporters is provided, together with a comprehensive description of the available modulators of efflux pumps with an indication of their efficacy in the clinical use.

ABC TRANSPORTERS

ABCB1/P-gp/MDR1

The best known member of the ABC transporter superfamily is the 170 kDa P-glycoprotein (P-gp), encoded by the MDR1 (ABCB1) gene that was identified over 30 years ago [8]. P-gp can transport a variety of molecules including cytostatic drugs and endogenous substrates such as steroid hormones and cytokines, and binds and transports drugs against a concentration gradient using ATP [2]. It has been proposed that the drug molecule binds to a specific site of P-gp within the lipid bilayer of the cell plasma membrane, and by means of the energy of ATP hydrolysis is transported out of the cell [9]. A negative correlation between P-gp expression levels and chemosensitivity has been documented in a range of human malignancies [10]. P-gp activity undergoes a complex regulation involving multiple genes (i.e, *p53, EGR1*, etc.) [9]. Evidence for multiple regulators of *MDR1*/

1389-5575/09 $55.00+.00

Table 1. Recognition of Widely Used Anticancer Drugs by ABC Transporters*

Compound	Target	P-gp	MRP1	MRP2	MRP3	MRP4	MRP5	MRP6	MRP7	MRP8	BCRP
Methotrexate	Dihydrofolate reductase		+	+	+	+	+			+	+
Vincristin, Vinblastin	Tubulin	+	+	+					+		
Taxol	Microtubules	+		+					+		
Mitoxantrone	Top II			+							+
Anthracyclines	Top II	+	+	+				+	+		+
Etoposide	Top II	+	+	+	+			+	+		+
Camptothecins	Top I	+	+	+		+			+		+
Cisplatin	DNA			+							
6-thioguanine	DNA			+		+	+				
Imatinib	Bcr-ABL c-kit PDGF-R	+	+								+
Erlotinib	EGF-R	+									+
Gefitinib	EGF-R	+									+

*Drug recognition may vary depending on the allelic status of genes coding for ABC transporters or on the drug exposure time used in cell sensitivity assays.

P-gp transcription/activity underlines the great importance of this defence system for cell protection against xenotoxins.

ABCG2/BCRP

The BCRP (breast cancer resistance-related protein, ABCG2), described as mitoxantrone-resistance associated protein (MXR) and as placenta-specific ABC protein (ABCP) [11-13], is a half-transporter consisting of only 1 nucleotide binding domain and 1 membrane-spanning domain [11,12]. BCRP, which may function as a homodimer or homotetramer, is a 72 kDa protein that localizes to the plasma membrane in cells which overexpress the protein through gene amplification or rearrangement [14]. In normal tissues, it is abundantly expressed in throphoblast cells, liver canalicular membrane, ducts and lobules of the breast, in the apical side of the epithelium of the small intestine and colon. Such a localization suggests a role in epithelial barriers, and in particular in the uptake of orally administered drugs. BCRP is also expressed by normal stem cells, in which it may play a protective function by preventing toxins from entering cells and by regulating differentiation [15]. The overexpression of BCRP was initially found in cells exhibiting resistance to mitoxantrone and anthracyclines [12,16], but not to vinca alkaloids and taxanes. The transporter was then shown to confer resistance to selected camptothecins, etoposide and methotrexate [17-20]. However, although methotrexate is clearly a substrate for BCRP the transport of the antifolate appears dependent on drug exposure time as well as on certin polymorphisms, in particular those affecting the 482 Arg (wt) residue (Arg→Gly, Arg→Thr) [21,22]. In fact, BCRP-mediated resistance to methotrexate can be evidenced in cell sensitivity assays only when using clinically relevant short term exposure [21,22]. In addition, wild-type BCRP but not mutated, is a methotrexate-polyglutamate transporter [23], since antifolate metabolites resulting from long-term exposure (i.e., long chain polyglutammates) can not be effluxed by mutated BCRP. The relevance of mutations in the amino acid 482 of BCRP have been observed also in resistance to doxorubicin and camptothecin [16,21,24,25]. Interestingly, the 7-modified camptothecin analogue gimatecan has been shown to overcome BCRP-associated resistance in a mitoxantrone-selected cell line exhibiting cross-resistance to topotecan and SN38 [26]. The multidrug-resistant phenotype due to BCRP expression is only in part overlapping with that of P-gp [12]. Indeed, unlike Pgp, which appears to transport unmodified drugs and xenobiotics, BCRP is an organic anion pump capable of transporting conjugates of sulfates, glutathione, and glucuronic acid [27]. It is most efficient in transporting GSH conjugates and thus the metabolism of the drug appears critical for determining possible transport by BCRP and therefore drug resistance.

A constitutive expression of BCRP in human endothelial cells of the blood-brain barrier suggests that together with P-gp, BCRP plays a role in limiting drug access to the brain [28]. The role of BCRP in clinical cancer drug resistance remains to be defined [29, 30].

The ABCC Family (MRPs)

The human multidrug resistance-associated proteins (MRPs) represent another subfamily of the ABC transporter

superfamily encoded by *MRP*-related genes. Some components of such a subfamily have an established role in transporting several drugs, particularly glutathione (GSH)-conjugated derivatives of toxic compounds so that some members have been defined "GS-X pumps" [31]. The best characterized member of such a subfamily is MRP1 [32].

The overexpression of MRPs plays an important role in the development of drug resistance, as the expression of the MRP transporters is induced by cytotoxic drugs [33,34]. There are currently 10 members of the MRP family, most of them implicated in resistance to clinically relevant antitumor drugs (Table **1**). MRPs are organic anion pumps that transport anionic drugs (e.g. methotrexate) and neutral drugs conjugated to acidic ligands, such as GSH, glucuronate, or sulfate, and in such features they differ from P-gp which has a low affinity for such negatively charged compounds [31]. However, MRP1, MRP2 and MRP3 can also cause resistance to neutral organic drugs that are not known to be conjugated to acidic ligands by transporting these drugs together with free GSH [35]. For example, MRP1 can confer resistance to arsenite, apparently by transporting it in complexes with GSH [36]. Mutational studies have provided insights into the significance of different aminoacidic residues in interaction with substrates [37].

Increased expression of MRP2 has been related to resistance to cisplatin as a result of increased efflux of the GSH-cisplatin conjugate. The pattern of drug resistance associated with increased levels of MRP2 (vincristine, vinblastin, doxorubicin, etoposide, mitoxantrone, CPT11, SN38 and paclitaxel), may eventually turn out as being similar to that shown for MRP1, except than for cisplatin resistance that has been related mainly to MRP2 overexpression [2,38,39]. The relationship between MRP2 expression and cisplatin resistance has been approached in model systems using hammerhead ribozymes and RNA interference [40,41] and also in patients [42], suggesting that MRP2 may be relevant for resistance to cisplatin treatment in colorectal cancer. In contrast to these results, there are reports in which an association between cisplatin resistance and MRP2 could not be demonstrated [43]. The reasons for this variability are unclear, but may be attributable to differences in cell culture condition/genetic background, modes of selection of cell systems, and to the choice of transfected host cell lines, all features that may affect both the expression level and the subcellular localization of MRP2 [43].

Among the MRP family members, MRP3 shares the highest amino acid sequence identity (58%) with MRP1, and both localize to the basolateral membranes of polarized cells [44]. Its only known substrates are epipodophyllotoxins (etoposide and teniposide) and metotrexate [44,45]. Novel substates of MRP3 are expected to be identified *in vivo* using approaches employing wild-type and Mrp3 -/- mice [46].

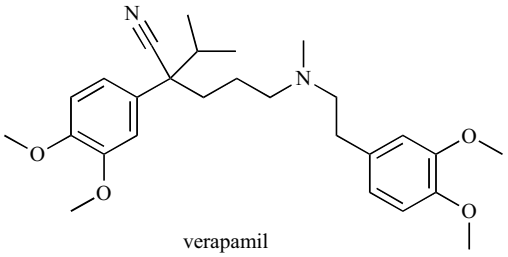
MRP4 is a cyclic nucleotide transporter implicated in the transport of different compounds including organic anions, antiviral and antiretroviral compounds and proinflammatory mediators (i.e, leukotriene B4 and C4) [47,48]. MRP4 overexpression is associated with high-level resistance to nucleoside analogues used as anti-human immunodeficiency virus drugs [2]. The analysis of the drug resistance profile has documented a role of the transporter in the cellular efflux of methotrexate, camptothecins, cisplatin and 6-mercaptopurine [49-53]. Expression of MRP4 may be a marker of poor prognosis in neuroblastomas in which preclinical studies have shown that the transporter confers resistance to irinotecan [54]. An analysis of the expression of MRPs in childhood and adult acute lymphoblastic leukaemia suggests that MRP4 is not involved in predicting prognosis in this malignancy, differently from what observed for other components of the MRP superfamily, including MRP1, MRP2, MRP3, MRP5 and MRP6 [55]. Overexpression of MRP5 has been shown to confer resistance to 6-mercaptopurine, 6-thioguanine and antifolate [56,57]. In addition to MRP1, MRP2, MRP3 and MRP4, also MRP6 has been shown to confer low levels of resistance to several commonly used anticancer agents, including etoposide, doxorubicin, daunorubicin and actinomycin D [58,59].

Among ABCC subfamily of transporters, MRP7 (ABCC10) and MRP8 (ABCC11) are at a relatively early stage of investigation. Those proteins are lipophilic anion pumps that are able to confer resistance to chemotherapeutic agents. In particular, MRP7 has been reported to confer resistance to vinca alkaloids, taxanes, gemcitabine, camptothecin, etoposide, daunorubicin and cytarabine, while MRP8 has been implicated in methotrexate and 5-FU efflux [60-62].

MODULATORS OF THE FUNCTION OF ABC TRANSPORTERS

In principle, on the basis of the mechanism of action, inhibitors of ABC transporters can be grouped in two main classes, i.e., compounds that are substrates for the transporters and then act as competitive antagonists, and inhibitors that are not transported. Molecular modelling and crystallographic approaches, together with homology studies support the concept that the modulators bind to a common recognition site that is large, flexible and rich in aromatic aminoacids producing hydrophobic interactions in addition to electrostatic bonds [63]. According to this model, interaction would occur with different binding modes. For some inhibitors, binding to different domains has been documented (i.e., flavonoids C terminal NDB, steroids hydrophobic region close to the ATP binding site). Structural activity studies have recognized that the features required to reach target inhibition include the presence of aromatic rings and of a protonable nitrogen atom as well as the amphipatic nature of the molecule [64].

Structurally dissimilar classes of modulators of ABC transporters have been described including terpenoids, flavonoids, steroids, phenols (e.g. curcumins) and polyphenols (e.g. catechins), quinolines, etc. In this context, the recent application of high-troughput technologies (i.e, libraries of synthetic and natural compounds) has allowed to identify molecularly targeted reagents including ABC transporters inhibitors [65]. After the discovery of the P-gp inhibitory activity of the calcium channel blocking verapamil (Fig. **1**), second and third-generation P-gp inhibitors have been reported which include the non-immunosuppressive Cyclosporin A derivative valspodar (PSC833) and tariquidar (XR9576) [66].



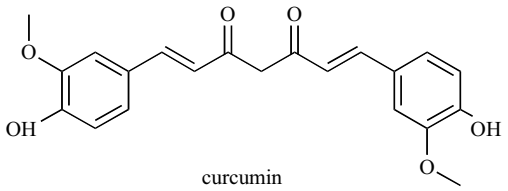


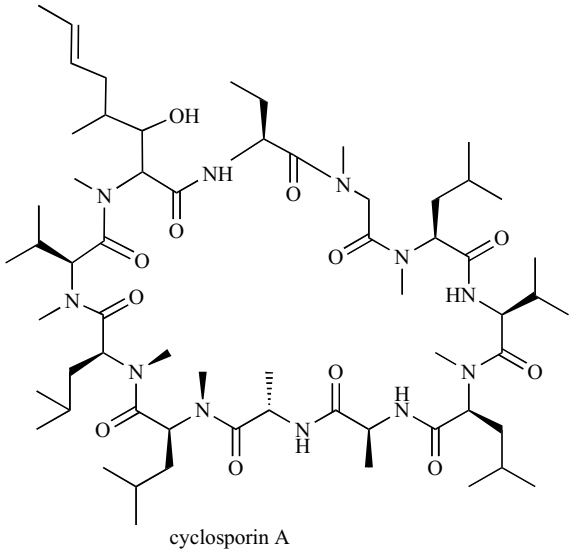
Fig. (1). Chemical structures of representative P-gp inhibitors.

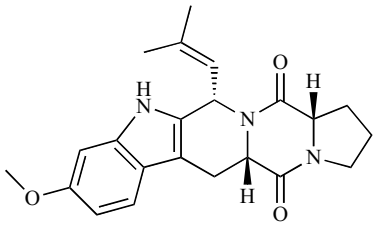
The clinical use of such modulators has been limited by the severe toxic side-effects observed at the concentrations required to inhibit P-gp *in vivo*. More recently, natural phenolic compounds known as curcuminoids (Fig. **1**) have been shown to be potential modulators of P-gp functions, thereby suggesting the interest of this class of compounds endowed with a range of pharmacological activities as MDR chemosensitizers [67]. The spectrum of activity of curcumin appears not limited to inhibition of ABCB1, because the *in vivo* efficacy of the modulator on ABCG2 has already been established in animal models [68].

The fact that several efflux pumps can transport a specific anticancer agent has relevant implications for reversal of drug resistance. The anthracycline doxorubicin, for example, is substrate for P-gp, MRPs and BCRP. Thus, inhibition of one transporter may be hampered by the presence of another. This observation may explain why drug reversal trials in the clinic have not achieved satisfactory results [12]. However, as mentioned above, P-gp modulators can exhibit inhibitory capability towards other ABC transporters. Thus, the difficulties encountered with several generations of these products in modulating the MDR phenotype may be counterbalanced by the recently acquired knowledge about novel transporters through the design of multi-specific inhibitors. Some preclinical studies have provided the proof of principle of the relevance of transporters as targets for modulation. Indeed, small interfering RNA-induced suppression of h*MDR1* gene restores sensitivity in MDR preclinical models [69]. Reversal of different drug-resistant phenotypes has been achieved in gastric and ovarian carcinoma cell lines overexpressing P-gp and MRP2 by using an autocatalytic multitarget multiribozyme [70].

Inhibitors of BCRP, which could be used to address its contribution to drug resistance, have not been readily made available until recently [19,71]. Several P-gp inhibitors have been tested for their capability to reverse BCRP-associated decrease in drug accumulation [72]. Some of them (i.e cyclosporin A, Fig. **2**) displayed capability to partially restore drug accumulation in BCRP-overexpressing cells [72]. Specific inhibitors have also been designed. Among them, fumitremorgin C (Fig. **2**) has been shown to modulate BCRP-mediated, but not P-gp-or MRP-mediated resistance through a mechanism that has not been clarified [72,73]. Since neurotoxic effects of fumitremorgin C preclude its use *in vivo*, another tetracyclic analog (Ko143) has been evaluated as specific inhibitor of BCRP [74].

Interestingly, it has been shown that tyrosine kinase inhibitors such as imatinib mesilate (Gleevec ™; Fig. **3**), a phenylamino-pyrimidine kinase inhibitor for Bcr-ABL, c-kit and PDGF-R, as well as gefitinib (Iressa ™) and erlotinib (Tarceva ™), both EGF-R tyrosine kinase inhibitors, are potent P-gp and BCRP inhibitors [75-81]. Imatinib mesilate, gefitinib and erlotinib have been reported as being both in-





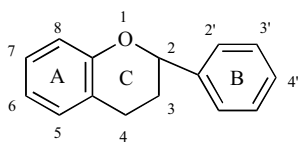


Fig. (2). Chemical structure of selected BCRP inhibitors.

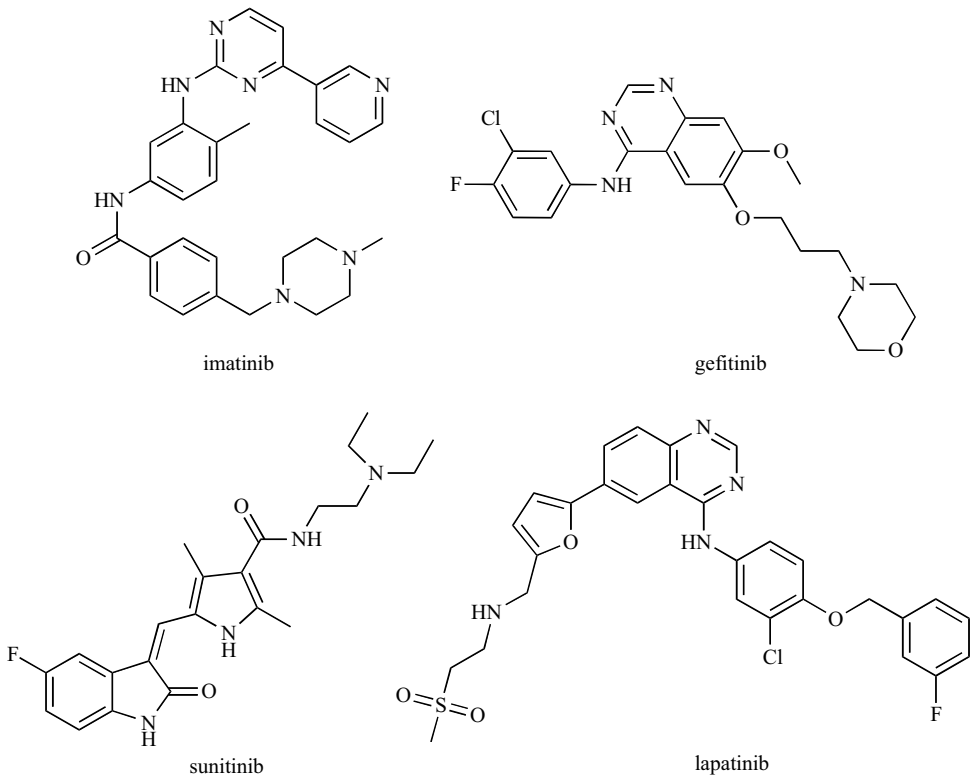


Fig. (3). Chemical structures of tyrosine kinase inhibitors. The shown compounds have been reported to inhibit ABCG2 and or ABCB1.

hibitors and substrates for BCRP, and the concentration of these drugs seems to determine whether they behave as substrates or inhibitors [82]. In particular, gefitinib and erlotinib appear to be BCRP substrates at clinically achievable plasma concentrations, whereas they inhibit BCRP at higher doses that are possibly achieved locally in tissues and tumors [82, 83]. Also sunitinib malate (Fig. **3**), that inhibits cellular signalling of multiple targets (ie, PDGF-R and VEGF-R) has been shown to block the function of ABCB1 and ABCG2 in *in vitro* cell systems [84]. In fact, a direct interaction of sunitinib with the substrate binding pocket of the two transporters has already been documented [84]. Evidence of the capability of lapatinib (Fig. **3**) to reverse the MDR phenotype by inhibition of ABCB1 and ABCG2 has been provided recently [85].

These findings are expected to have relevant clinical implications because the specific targeting of tyrosine kinases represents a promising therapeutic approach aimed at blocking cellular signalling pathways involved in tumor cell growth and metastasis. Indeed, gefitinib (Fig. **3**) has been documented to enhance the antitumor activity and oral bioavailability of irinotecan in preclinical models [86]. Also HIV protease inhibitors as well as flavonoids (Fig. **2**) have been reported to inhibit BCRP (Fig. **2**) [87,88]. Further studies are needed to clarify the precise mechanism of BCRP inhibition of several of the compounds as well as to define specificity of inhibition with particular reference to other ABC transporters.

Attempts to find inhibitors for MRPs have been mainly focused on MRP1 and MRP2. Substrates for MRP1 and MRP2 are organic anions with a substantial hydrophobic moiety and at least one, but preferably two, negative charge(s) [2]. Other inhibitors of MRP1 are organic acids originally developed to inhibit transport of uric acid, but as charged compounds do not easily enter cells, such inhibitors do not represent obvious lead compounds for drug development, but are excellent laboratory tools. Better compounds could be synthesized by making pro-drugs in which the charged moiety is shielded [2]. Among MRP1 inhibitors, the interest of indomethacin analogues has emerged from structure activity studies showing the possibility to generate compounds targeting only MRP1 in the absence of substantial inhibition of COX-1 or COX-2 and of glutathione-S-transferase [89]. In the absence of effective and specific pharmacological MRP inhibitors, it is not easy to analyze the contribution of MRPs to resistance by use of intervention studies in which anticancer drugs transported by MRPs are combined with inhibitors [2].

Several ABC transporters inhibitors have undergone clinical evaluation (www.clinicaltrial.gov; Fig. (**4**) and Table **2**). Among them valspodar has reached phase III in spite of the evidence of effects on the pharmacokinetics of the co-administered antitumor drug.

Other inhibitors are under evaluation due to the apparent lack of interaction with the combined chemotherapeutic agents at the pharmacokinetic level (i.e., LY335979, zosuquidar; XR9576, tariquidar; VX-710, biricodar; GF-120918, elacridar; R101933, laniquidar). In particular biricodar, an inhibitor of P-gp, MRP1 and BCRP has shown good tolerability but has failed to enhance antitumor activity of doxorubicin and vincristine as well as survival [90]. Some inhibitors (ie, tariquidar and zosuquidar) have shown in-

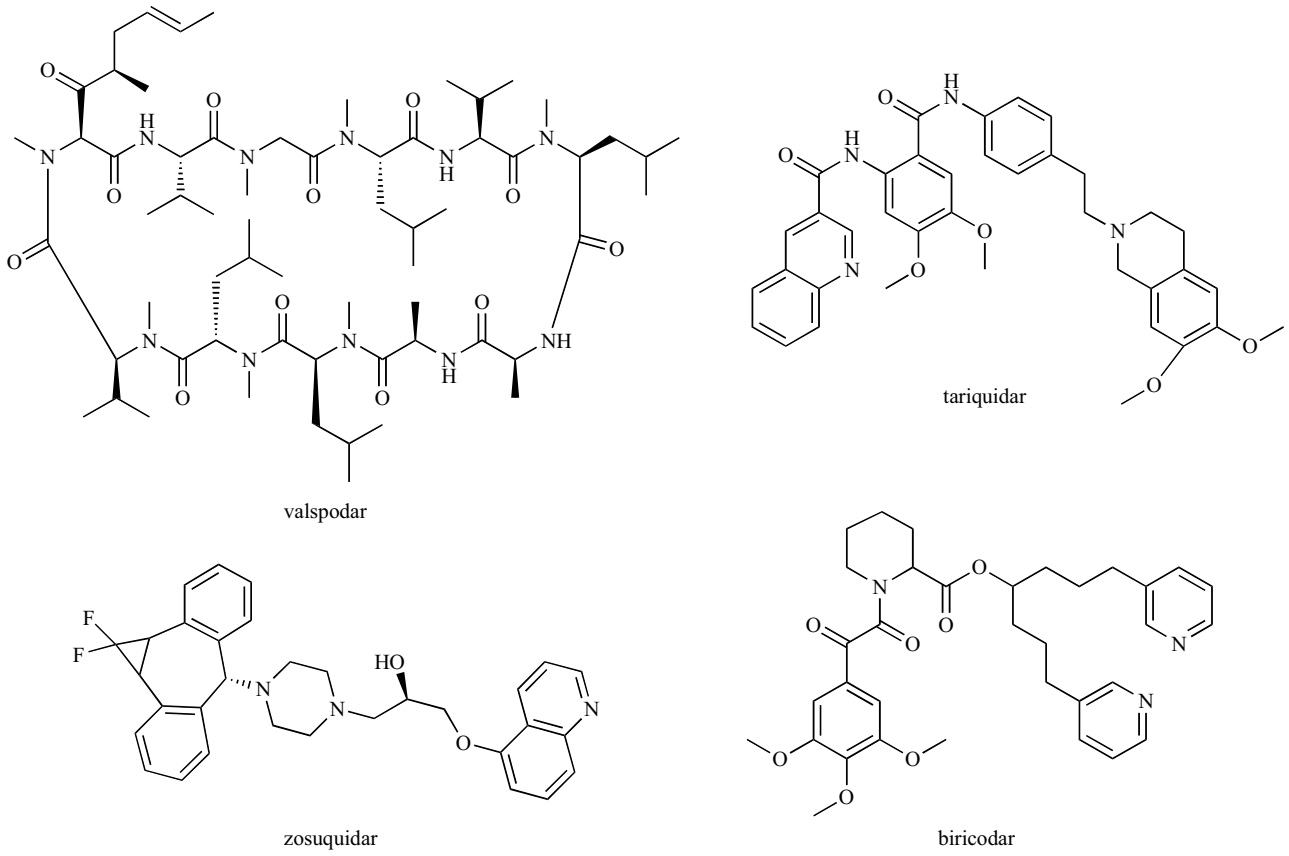


Fig. (4). Chemical structures of MDR modulators under clinical evaluation.

creased potency and specificity for Pgp at the preclinical levels [91]. Also orally administrable inhibitors are being developed. The promising features of CBT-1, a natural bisbenzylquinoline capable of inhibiting P-gp and MRP1 mediated transport [92] have led to the design of trials in which the compound is used in combination with paclitaxel.

ABC TRANSPORTERS AND CANCER STEM CELLS

Several lines of evidence support that in all differentiated mammalian normal tissues there is a small number of cells that can differentiate in response to environmental stimuli, because they maintain stem-cell like features [93]. Recent investigations have proposed the existence also in tumors of a cell fraction endowed with self-renewal, differentiating and tumor initiating properties designates as CSCs or tumor-initiating cells [94,95]. In this view, only one population of cancer cells initiates and sustains the growth of a tumor. CSCs have been identified in different tumor types, but the precise phenotypic/functional features of such cells still need to be defined [96]. Expression of proteins conferring drug-resistance, and in particular of ABC transporters, in putative CSCs versus differentiated cancer cells is being regarded as a major feature of CSCs that could be targeted to improve the efficacy of treatment [97].

The significance of ABC transporters in relation to stem cell physiology has been originally mainly addressed in normal haematopoietic stem cells (HSC). A small fraction of bone marrow cells that can be evidenced by flow cytometry for the ability to efflux the fluorescent dye Hoechst 33342 (known as "side population" and enriched for HSC), has been identified in the haematopoietic compartments of different organisms including humans and in non haematopoietic tissues [98,99]. Normal HSC express at least 2 ABC transporters, but the complexity of the ABC transporter family suggests that other members could be present [100]. Studies in mice have indicated that BCRP but not MDR1 determines the HSC phenotype [98,101]. Expression of the murine hortologue of ABCG2 appears a constant feature of murine stem cells from different sources such as bone marrow, skeletal muscle, and embryos. In murine HSC, Abcg2 is highly expressed and is down-regulated during differentiation. Gain of function and loss of function studies have shown that the side population phenotype is dependent on Abcg2 [102].

Since the expression of ABC transporters has been shown in different models of CSCs, these transporters are considered phenotypic markers of CSCs and are regarded as functional regulators. For example, putative prostate stem cells and prostate tumor stem cells in benign and malignant tumors have been defined by expression of BCRP and concomitant lack of adrogen receptor [103]. In this view, expression of BCRP by normal/tumor prostate stem cells may protect from androgen deprivation, hypoxia or chemotherapy, thus favouring recurrence of prostate cancer [103]. In keeping with a pro-survival role of the expression of BCRP in CSCs is the observation that the PI3K/Akt pathway regu-

Table 2. Clinical Trials Including Efflux Pump Inhibitors*

Efflux Pump	Inhibitor	Clinical Trial
P-gp	Verapamil	Phase II (ongoing)
	Valspodar (PSC833)	Phase III (ongoing)
	Tariquidar (XR9576)	Phase III (terminated)†
	Zosuquidar (LY335979)	Phase III (ongoing)
	Biricodar (VX-710)	Phase II (ongoing)
	Elacridar (GF-120918)	None‡
	Laniquidar (R101933)	Phase II (ongoing)
	CBT-1	Phase III (ongoing)
	Curcumin	Phase III (ongoing)
	Imatinib	Phase II (ongoing)
	Erlotinib	None
	Gefitinib	Phase III (ongoing)
	Lapatinib	Phase I (ongoing)
	Sunitinib	None
BCRP	Curcumin	Phase III (ongoing)
	Fumitremorgin C	None
	Ko143	None
	Biricodar (VX-710)	Phase II (ongoing)
	Imatinib	ongoing but not recruiting participants
	Erlotinib	None
	Gefitinib	None
	Lapatinib	Phase II (ongoing)
	Sunitinib	None
MRP1	Biricodar	None
	CBT-1	None

* The clinical data are from www.clinicaltrial.gov.

‡ None: tested only in preclinical models.

† Terminated: recruiting or enrolling participants has halted prematurely and will not resume.

lates the side population phenotype and BCRP activity in human glioma [104].

In addition to BCRP, the ABCB5 transporter has been implicated in CSC biology, with particular reference to malignant melanoma [105], in which it has been proposed as a marker of melanoma-initiating cells. Indeed, ABCB5 has been shown to mark CD133-expressing progenitor cells among human epidermal melanocytes and to positively regulate the propensity of this subpopulation to undergo cell fusion, a process contributing to culture growth and differentiation [106]. ABCB5 has also been involved in doxorubicin efflux transport and it has been already exploited as therapeutic target by development of a specific antibody [107].

This fast-moving field of research is still at an early stage and functional studies are required to establish a precise link between expression of BCRP or other ABC transporters and stem cell–like features/behaviour. The available evidence supports that protection of CSC against drugs and toxins is mediated by expression of several ABC transporters, thus providing therapeutic opportunities to overcome resistance [97].

CONCLUSION

The recent definition of the complexity of the ABC transporter family suggests that it will be a difficult task to clarify which ABC transporters contribute to resistance to specific drugs in the different tumor types and to define the best targets for modulation of antitumor therapy. Among the characterized transporters, the MRP family appears remarkable because of the range of anticancer drugs handled by its members. Whereas P-gp transports a wide range of neutral or slightly basic organic compounds, the members of the MRP family are even more versatile. Transport by MRPs also provides a link between drug efflux and the GSH system [2]. BCRP displays peculiar structural characteristics and particular physiological functions as it is expressed by normal stem cells and in selected models of CSCs. Hopefully, CSC-related research will provide knowledge useful for develop-

ment of novel therapeutic strategies involving targeting of ABC transporters.

Also pharmacogenomics approaches aimed at finding the genetic bases for variability among drug responses are expected to increase the available knowledge, thereby allowing to explain inter-individual differences [4, 108-111]. Genes for drug pumps are prominent in all of the genomes of several organisms recently sequenced, and the identification of the complete family of human ABC transporters underlines the complexity of the mechanisms developed also by human cells to extrude toxic compounds. In a context where the contribution of different transporters to drug resistance remains to be clarified, it is already evident that the mutational status of specific transporters can affect interaction with substrate. Thus, the reversal activity of all modulators may be influenced by the gene status of the transporters, a feature that have a major impact also on the efflux of the antitumor drug. Single nucleotide polymorphisms have been reported for some ABC transporters and they are expected to be clinically relevant considering that variability among individuals has been shown in terms of oral bioavailability and clearance of drugs that are substrates for ABC transporters [112,113].

Several studies have addressed the functional characterization of polymorphisms affecting ABCB1/MDR1 and alteration of protein expression/function has been documented [114]. For example, using a vaccinia virus expression system, it has been documented that the common MDR1 coding polymorphisms produce P-gps with cell surface distribution and transport properties similar to wild-type P-gp [115]. Moreover, an altered pattern of cross-resistance in cells overexpressing a mutated BCRP has been described. In particular, mutations affecting the amino acid 482 (R482G, R482T) of BCRP confer a higher resistance to selected camptothecins (i.e homocamptothecins) than the wild-type transporter as well as high levels of resistance to various hydrophilic antifolates [20,22]. For the ABCG2 gene, several different naturally occurring sequence variations have been described in human samples [112]. Details about possible functional implications of single nucleotide polymorphisms (i.e., Q141K) that could cause increased sensitivity of normal cells to camptothecins, including topotecan and SN38, have been reviewed [112].

Naturally occurring mutations in MRP and MRP-related drug transporters have also been reported [72]. Whereas the role of at least 5 MRPs in the elimination of drugs and other organic anions and in maintainence of blood tissues barriers has been established by knock out mice studies, *in vitro* site-directed mutagenesis approaches are expected to allow predicting the consequences of aminoacid changes [116].

A better definition of the transport properties of recently identified ABC transporters might contribute to provide insights in an attempt to overcome MDR and in the design of novel modulators. Studies on larger numbers of tumor specimens could generate more conclusive data about the role of different MRPs and their function. The clinical trials with the use of modulators of drug transporters (e.g. valspodar, tariquidar, CBT-1) are expected to provide a better understanding of the relevance of transporters in clinical resistance of various tumor types. Of particular interest are compounds (e.g. elacridar and tariquidar) that inhibit both P-gp and BCRP. A critical aspect of their use is the possible increase of treatment toxicity related to the expression of these transporters in normal tissues.

ACKNOWLEDGEMENTS

This work was partially supported by the Associazione Italiana per la Ricerca sul Cancro, Milan, by the Ministero della Salute, Rome, by the Fondazione Italo Monzino, Milan and by the EU grant CHEMORES.

REFERENCES

[1] Gottesman, M.M.; Ling, V. The molecular basis of multidrug resistance in cancer: the early years of P-glycoprotein research. *FEBS Lett*., **2006**, *580*, 998-1009.

[2] Borst, P.; Evers, R.; Kool, M.; Wijnhold, J.A. A family of drug transporters: the multidrug resistance-associated proteins. *J. Natl. Cancer Inst*., **2000**, *92*, 1295-302.

[3] Ambudkar, S.V.; Sauna, Z.E.; Gottesman, M.M.; Szakacs, G. A novel way to spread drug resistance in tumor cells: functional intercellular transfer of P-glycoprotein (ABCB1). *Trends Pharmacol. Sci.,* **2005**, *26*, 385-7.

[4] Gillet, J.P.; Efferth, T.; Remacle, J. Chemotherapy-induced resistance by ATP-binding cassette transporter genes. *Biochim. Biophys. Acta*, **2007**, *1775,* 237-62.

[5] Gottesman, M.M.; Fojo, T.; Bates, S.E. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat. Rev.Cancer*, **2002**, *2*, 48-58.

[6] Babakhanian, K; Bendayan, M.; Bendayan R. Localization of P-glycoprotein at the nuclear envelope of rat brain cells. *Biochem. Biophys. Res. Commun.*, **2007**, *361*, 301-6.

[7] Ifergan, I.; Scheffer, G.L.; Assaraf, Y.G. Novel extracellular vesicles mediate an ABCG2-dependent anticancer drug sequestration and resistance. *Cancer Res.*, **2005**, *65*, 10952-58.

[8] Juliano, R.L.; Ling, V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim. Biophys. Acta*, **1976**, *455*, 152-62.

[9] Stavrovskaya, A.A. Cellular mechanisms of multidrug resistance of tumor cells. *Biochemistry,* **2000**, *65*, 95-106.

[10] Chan, H.S.; Haddad, G.; Thorner, P.S.; DeBoer, G.; Lin, Y.P.; Ondrusek, N.; Yeger, H.; Ling, V. P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. *N. Engl. J. Med*., **1991**, *325*, 1608-14.

[11] Doyle, L.A.; Yang, W.; Abruzzo, L.E.; Krogmann, T.;. Gao, Y.; Rishi, A.K.; Ross, D.D. A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 15665-70.

[12] Litman, T.; Brangi, M.; Hudson, E.; Fetsch, P.; Abati, A.; Ross, D.D.; Miyake, K.; Resau, J.H. The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2). *J. Cell Sci*., **2000**, 113, 2011-21.

[13] Allikmets, R.; Schriml, L.M.; Hutchinson, A.; Romano-Spica, V.; Dean M. A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. *Cancer Res*., **1998**, *58,* 5337-9.

[14] Bates, S.E.; Robey, R.; Miyake, K.; Rao, K.; Ross, D.D.; Litman, T. The role of half-transporters in multidrug resistance. *J. Bioenerg. Biomembr*., **2001**, *33*, 503-11.

[15] Ejendal, K.F.; Hrycyna, C.A. Multidrug resistance and cancer: the role of the human ABC transporter ABCG2. *Curr. Protein Pept. Sci*., **2002**, *3*, 503-11.

[16] Ross, D.D.; Yang, W.; Abruzzo, L.V.; Dalton, W.S.; Schneider, E.; Lage, H.; Dietel, M.; Greenberger, L.; Cole, S.P.; Doyle, L.A. Atypical multidrug resistance: breast cancer resistance protein messenger RNA expression in mitoxantrone-selected cell lines. *J. Natl. Cancer Inst*., **1999**, *91*, 429-33.

[17] Allen, J.D.; Brinkhuis, R.F.; Wijnholds, J.; Schinkel, A.H. The mouse Bcrp1/Mxr/Abcp gene: amplification and overexpression in cell lines selected for resistance to topotecan, mitoxantrone, or doxorubicin. *Cancer Res*., **1999**, *59*, 4237-41.

[18] Volk, E.L.; Farley, K.M.; Wu, Y.; Li, F.; Robey, R.W.; Schneider, E. Overexpression of wild-type breast cancer resistance protein mediates methotrexate resistance. *Cancer Res*., **2002**, *62*, 5035-40.

[19] Allen, J.D.; van Dort, S.C.; Buitelaar, M.; van Tellingen, O.; Schinkel, A.H. Mouse breast cancer resistance protein (Bcrp1/Abcg2) mediates etoposide resistance and transport, but etoposide oral availability is limited primarily by P-glycoprotein. *Cancer Res.*, **2003**, *63*, 1339-44.

[20] Bates, S.E.; Medina-Perez, W.Y.; Kohlhagen, G.; Antony, S.; Nadjem, T.; Robey, R.W.; Pommier, Y. ABCG2 mediates differential resistance to SN-38 (7-ethyl-10-hydroxycamptothecin) and homocamptothecins. *J. Pharmacol. Exp. Ther.*, **2004**, *310*, 836-42.

[21] Chen, Z-S.; Robey, R.W.; Belinsky, M.G.; Shchaveleva, I.; Ren, X-Q.; Sugimoto, Y.; Ross, D.D.; Bates, S.E.; Kruh, G.D. Transport of methotrexate, methotrexate polyglutamates, and 17β-estradiol 17-(β-D-glucuronide) by ABCG2: effects of acquired mutations at R482 on methotrexate transport. *Cancer Res*., **2003**, *63*, 4048-54.

[22] Shafran, A.; Ifergan, I.; Bram, E.; Jansen, G.; Kathmann, I.; Peters, G.J.; Robey, R.W.; Bates, S.E.; Assaraf, Y.G. ABCG2 harboring the Gly482 mutation confers high-level resistance to various hydrophilic antifolates. *Cancer Res.,* **2005**, *65*, 8414-22.

[23] Volk, E.L.; Schneider, E. Wild-type breast cancer resistance protein (BCRP/*ABCG2*) is a methotrexate polyglutamate transporter. *Cancer Res*., **2003**, *63*, 5538-43.

[24] Honjo, Y.; Hrycyna, C.A.; Yan, Q.W.; Medina-Perez, W.Y.; Robey, R.W.; van de Laar, A.; Litman, T.; Dean, M.; Bates, S.E. Acquired mutations in the MXR/BCRP/ABCP gene alter substrate specificity in MXR/BCRP/ABCP-overexpressing cells. *Cancer Res.*, **2001**, *61*, 6635-9.

[25] Allen, J.D.; Jackson, S.C.; Schinkel, A.H. A mutation hot spot in the Bcrp1 (Abcg2) multidrug transporter in mouse cell lines selected for Doxorubicin resistance. *Cancer Res*., **2002**, *62*, 2294-9.

[26] Perego, P.; De Cesare, M.; De Isabella, P.; Carenini, N.; Beggiolin, G.; Pezzoni, G.; Palumbo, M.; Tartaglia, L.; Pratesi, G.; Pisano, C.; Carminati, P.; Scheffer, G.L.; Zunino, F. A novel 7-modified camptothecin analog overcomes breast cancer resistance protein-associated resistance in a mitoxantrone-selected colon carcinoma cell line. *Cancer Res*., **2001**, *61*, 6034-7.

[27] Muller, M.; Mijer, C.; Zaman, G.J.; Borst, P.; Scheper, R.J.; Mulder, N.H.; de Vries, E.G.; Jansen, P.L. Overexpression of the gene encoding the multidrug resistance-associated protein results in increased ATP-dependent glutathione S-conjugate transport. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 13033-7.

[28] Aronica, E.; Gorter, J.A.; Redeker, S.; van Vliet., E.A.; Ramkema, M.; Scheffer, G.L.; Scheper, R.J.; van der Valk, P.; Leenstra, S.; Baayen, J.C.; Spliet, W.G.; Troost, D. Localization of breast cancer resistance protein (BCRP) in microvessel endothelium of human control and epileptic brain. *Epilepsia*, **2005**, *46*, 849-57.

[29] Robey, R.W.; Polgar, O.; Deeken, J.; To, K.W.; Bates, S.E. ABCG2: determining its relevance in clinical drug resistance. *Cancer Metastasis Rev*., **2007**, *26,* 39-57.

[30] Lemos, C.; Jansen, G.; Peters, G.J. Drug transporters: recent advances concerning BCRP and tyrosine kinase inhibitors. *Br. J. Cancer*, **2008**, *98,* 857-62.

[31] Ishikawa, T.; Kuo, M.T.; Furuta, K.; Suzuki, M. The human multidrug resistance-associated protein (MRP) gene family: from biological function to drug molecular design. *Clin. Chem. Lab. Med.*, **2000**, *38*, 893-7.

[32] Cole, S.P.; Bhardwaj, G.; Gerlach, J.H.; Mackie, J.E.; Grant, C.E.; Almquist, K.C.; Stewart, A.J.; Kurz, E.U.; Duncan, A.M.; Deeley, R.G. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science*, **1992**, *258*, 1650-4.

[33] Wielandt, A.M.; Vollrath, V.; Manzano, M.; Miranda, S.; Accatino, L.; Chianale, J. Induction of the multispecific organic anion transporter (cMoat/mrp2) gene and biliary glutathione secretion by the herbicide 2,4,5-trichlorophenoxyacetic acid in the mouse liver. *Biochem. J*., **1999**, *341*, 105-11.

[34] Oguri, T.; Isobe, T.; Suzuki, T.; Nishio, K.; Fujiwara, Y.; Katoh, O.; Yamakido, M. Increased expression of the MRP5 gene is associated with exposure to platinum drugs in lung cancer. *Int. J. Cancer*, **2000**, *86*, 95-100.

[35] Hipfner, D.R.; Deeley, R.G.; Cole, S.P. Structural, mechanistic and clinical aspects of MRP1. *Biochim. Biophys. Acta*, **1999**, *1461*, 359-76.

[36] Konig, J.; Nies, A.T.; Cui, Y.; Leier, I.; Keppler, D. Conjugate export pumps of the multidrug resistance protein (MRP) family: localization, substrate specificity, and MRP2-mediated drug resistance. *Biochim. Biophys. Acta*, **1999**, *1461*, 377-94.

[37] Kimchi-Sarfaty, C.; Gribar, J.J.; Gottesman, M.M. Functional characterization of coding polymorphisms in the human MDR1 gene using a vaccinia virus expression system. *Mol. Pharmacol*., **2002**, *62*, 1-6.

[38] Cui, Y.; Konig, J.; Buchholz, J.K.; Spring, H.; Leier, I.; Keppler, D. Drug resistance and ATP-dependent conjugate transport mediated by the apical multidrug resistance protein, MRP2, permanently expressed in human and canine cells. *Mol. Pharmacol*., **1999**, *55*, 929-37.

[39] Huisman, M.T.; Chhatta, A.A.; van Tellingen, O.; Beijnen, J.H.; Schinkel, A.H. MRP2 (ABCC2) transports taxanes and confers paclitaxel resistance and both processes are stimulated by probenecid. *Int. J. Cancer*, **2005**, *116*, 824-9.

[40] Materna, V.; Holm, P.S.; Dietel, M.; Lage, H. Kinetic characterization of ribozymes directed against the cisplatin resistance-associated ABC transporter cMOAT/MRP2/ABCC2. *Cancer Gene Ther*., **2001**, *8*, 176-84.

[41] Materna, V.; Stege, A.; Surowiak, P.; Priebsch, A.; Lage, H. RNA interference-triggered reversal of ABCC2-dependent cisplatin resistance in human cancer cells. *Biochem. Biophys. Res. Commun*., **2006**, *348,* 153-7.

[42] Hinoshita, E.; Uchiumi, T.; Taguchi, K.; Kinukawa, N.; Tsuneyoshi, M.; Maehara, Y.; Sugimachi, K.; Kuwano, M. Increased expression of an ATP-binding cassette superfamily transporter, multidrug resistance protein 2, in human colorectal carcinomas. *Clin. Cancer Res.*, **2000**, *6*, 2401-7.

[43] Young, L.C.; Campling, B.G.; Cole, S.P.; Deeley, R.G.; Gerlach, J.H. Multidrug resistance proteins MRP3, MRP1, and MRP2 in lung cancer: correlation of protein levels with drug response and messenger RNA levels. *Clin. Cancer Res*., **2001**, *7*, 1798-804.

[44] Zelcer, N.; Saeki, T.; Reid, G.; Beijnen, J.H.; Borst, P. Characterization of drug transport by the human multidrug resistance protein 3 (ABCC3). *J. Biol. Chem.,* **2001**, *276*, 46400-7.

[45] Zeng, H.; Chen, Z.S.; Belinsky, M.G.; Rea, P.A.; Kruh, G.D. Transport of methotrexate (MTX) and folates by multidrug resistance protein (MRP) 3 and MRP1: effect of polyglutamylation on MTX transport. *Cancer Res.,* **2001**, *61*, 7225-32.

[46] Zelcer, N.; van de Wetering, K.; de Waart, R.; Scheffer, G.L.; Marschall, H.U.; Wielinga, P.R.; Kuil, A.; Kunne, C.; Smith, A.; van der Valk, M.; Wijnholds, J.; Elferink, R.O.; Borst, P. Mice lacking Mrp3 (Abcc3) have normal bile salt transport, but altered hepatic transport of endogenous glucuronides. *J. Hepatol*., **2006**, *44*, 768-75.

[47] Schuetz, J.D.; Connelly, M.C.; Sun, D.; Paibir, S.G.; Flynn, P.M.; Srinivas, R.V.; Kumar, A.; Fridland, A. MRP4: A previously unidentified factor in resistance to nucleoside-based antiviral drugs. *Nat. Med*., **1999**, *5*, 1048-51.

[48] Rius, M.; Hummel-Eisenbeiss, J.; Keppler, D. ATP-dependent transport of leukotrienes B4 and C4 by the multidrug resistance protein ABCC4 (MRP4). *J. Pharmacol. Exp. Ther.*, **2008**, *324*, 86-94.

[49] Lee, K.; Klein-Szanto, A.J.; Kruh, G.D. Analysis of the MRP4 drug resistance profile in transfected NIH3T3 cells. *J. Natl. Cancer Inst*., **2000**, *92*, 1934-40.

[50] Chen, Z.S.; Lee, K.; Walther, S.; Raftogianis, R.B.; Kuwano, M.; Zeng, H.; Kruh, G.D. Analysis of methotrexate and folate transport by multidrug resistance protein 4 (ABCC4): MRP4 is a component of the methotrexate efflux system. *Cancer Res*., **2002**, *62*, 3144-50.

[51] Tian, Q.; Zhang, J.; Tan, T.M.; Chan, E.; Duan, W.; Chan, S.Y.; Boelsterli, U.A.; Ho, P.C.; Yang, H.; Bian, J.S.; Huang, M.; Zhu, Y.Z.; Xiong, W.; Li, X.; Zhou, S. Human multidrug resistance associated protein 4 confers resistance to camptothecins. *Pharm. Res*., **2005**, *22*, 1837-53.

[52] Wakamatsu, T.; Nakahashi, Y.; Hachimine, D.; Seki, T.; Okazaki, K. The combination of glycyrrhizin and lamivudine can reverse the cisplatin resistance in hepatocellular carcinoma cells through inhibition of multidrug resistance-associated proteins. *Int. J. Oncol*., **2007**, *31*, 1465-72.

[53] Peng, X.X.; Shi, Z.; Damaraju, V.L.; Huang, X.C.; Kruh, G.D.; Wu, H.C.; Zhou, Y.; Tiwari, A.; Fu, L.; Cass, C.E.; Chen, Z.S. Up-regulation of MRP4 and down-regulation of influx transporters in human leukemic cells with acquired resistance to 6-mercaptopurine. *Leuk. Res.*, **2008**, *32*, 799-809.

[54] Norris, M.D.; Smith, J.; Tanabe, K.; Tobin, P.; Flemming, C.; Scheffer, G.L.; Wielinga, P.; Cohn, S.L.; London, W.B.; Marshall,

G.M.; Allen, J.D.; Haber, M. Expression of multidrug transporter MRP4/ABCC4 is a marker of poor prognosis in neuroblastoma and confers resistance to irinotecan *in vitro*. *Mol. Cancer Ther*., **2005**, *4*, 547-53.

[55] Plasschaert, S.L.; de Bont, E.S.; Boezen, M.; vandr Kolk, D.M.; Daenen, S.M.; Faber, K.N.; Kamps, W.A.; de Vries, E.G.; Vallenga, E. Expression of multidrug resistance-associated proteins predicts prognosis in childhood and adult acute lymphoblastic leukemia. *Clin. Cancer Res*., **2005**, *11*, 8661-8.

[56] Wielinga, P.R.; Reid, G.; Challa, E.E.; van der Heijden, I.; van Deemter, L.; de Haas, M.; Mol, C.; Kuil, A.J.; Groeneveld, E.; Schuetz, J.D.; Brouwer, C.; De Abreu, R.A.; Wijnholds, J.; Beijnen, J.H.; Borst, P. Thiopurine metabolism and identification of the thiopurine metabolites transported by MRP4 and MRP5 overexpressed in human embryonic kidney cells. *Mol. Pharmacol*., **2002**, *62*, 1321-31.

[57] Assaraf, Y.G. The role of multidrug resistance efflux transporters in antifolate resistance and folate homeostasis. *Drug Resist. Update*, **2006**, *9*, 227-46.

[58] Belinsky, M.G.; Chen, Z.S.; Shchaveleva, I.; Zeng, H.; Kruh, G.D. Characterization of the drug resistance and transport properties of multidrug resistance protein 6 (MRP6, ABCC6). *Cancer Res*., **2002**, *62*, 6172-7.

[59] Nomura, M.; Matsunami, T.; Kobayashi, K.; Uchibayashi, T.; Koshida, K.; Tanaka, M.; Namiki, M.; Mizuhara, Y.; Akiba, T.; Yokogawa, K.; Moritani, S.; Miyamoto, K. Involvement of ABC transporters in chemosensitivity of human renal cell carcinoma, and regulation of MRP2 expression by conjugated bilirubin. *Anticancer Res.,* **2005**, *25*, 2729-35.

[60] Hopper-Borge, E.; Xu, X.; Shen, T.; Shi, Z.; Chen, Z.S.; Kruh, G.D. Human multidrug resistance protein 7 (ABCC10) is a resistance factor for nucleoside analogues and epothilone B. *Cancer Res*., **2009**, *69*, 178-84.

[61] Hopper-Borge, E.; Chen, Z.S.; Shchaveleva, I.; Belinsky, M.G.; Kruh, G.D. Analysis of the drug resistance profile of multidrug resistance protein 7 (ABCC10): resistance to docetaxel. *Cancer Res*., **2004**, *64*, 4927-30.

[62] Krush, G.D.; Guo, Y.; Hopper-Borge, E.; Belinsky, M.G.; Chen, Z.S. ABCC10, ABCC11, and ABCC12. *Pflugers Arch*., **2007**, *453*, 675-84.

[63] Teodori, E.; Dei, S.; Martelli, C.; Scapecchi, S.; Gualtieri, F. The functions and structure of ABC transporters: implications for the design of new inhibitors of Pgp and MRP1 to control multidrug resistance (MDR). *Curr. Drug Targets*, **2006**, *7*, 893-909.

[64] Wiese, M.; Pajeva, I.K. Structure-activity relationships of multidrug resistance reversers. *Curr. Med. Chem*., **2001**, *8*, 685-713.

[65] Shoemaker, R.H. The NCI60 human tumour cell line anticancer drug screen. *Nat. Rev. Cancer*, **2006**, *6*, 813-23.

[66] Shukla, S.; Wu, C.P.; Ambudkar, S.V. Development of inhibitors of ATP-binding cassette drug transporters: present status and challenges. *Expert Opin. Drug Metab. Toxicol*., **2008**, *4*, 205-23.

[67] Chearwae, W.; Anuchapreeda, S.; Nandigama, K.; Ambudkar, S.V.; Limtrakul, P. Biochemical mechanism of modulation of human P-glycoprotein (ABCB1) by curcumin I, II, and III purified from Turmeric powder. *Biochem. Pharmacol*., **2004**, *68*, 2043-52.

[68] Shukla, S.; Zaher, H.; Hartz, A.; Bauer, B.; Ware, J.A.; Ambudkar, S.V. Curcumin inhibits the activity of ABCG2/BCRP1, a multidrug resistance-linked ABC drug transporter in mice. *Pharm. Res*., **2009**, *26*, 480-7.

[69] Wu, H.; Hait, W.N.; Yang, J.M. Small interfering RNA-induced suppression of MDR1 (P-glycoprotein) restores sensitivity to multidrug-resistant cancer cells. *Cancer Res*., **2003**, *63*, 1515-9.

[70] Kowalski, P.; Surowiak, P.; Lage, H. Reversal of different drug-resistant phenotypes by an autocatalytic multitarget multiribozyme directed against the transcripts of the ABC transporters MDR1/P-gp, MRP2, and BCRP. *Mol. Ther*., **2005**, *11*, 508-22.

[71] Henrich, C.J.; Bokesch, H.R.; Dean, M.; Bates, S.E.; Robey, R.W.; Goncharova, E.I.; Wilson, J.A.; McMahon, J. A high-throughput cell-based assay for inhibitors of ABCG2 activity. *J. Biomol. Screen*., **2006**, *11*, 176-83.

[72] Bates, S.E.; Robey, R.; Knutsen, T.; Honjo, Y.; Litman, T.; Dean, M. *Emerging Ther. Targets*, **2000**, *4*, 561-80.

[73] Mao, Q.; Unadkat, J.D. Role of the breast cancer resistance protein (ABCG2) in drug transport. *AAPS J*., **2005**, *7,* 118-33.

[74] Allen, J.D.; van Loevezijn, A.; Lakhai, J.M.; van der Valk, M.; van Tellingen, O.; Reid, G.; Schellens, J.H.M.; Koomen, G-J.; Schinkel, A.H. Potent and specific inhibition of the breast cancer resistance protein multidrug transporter *in vitro* and in mouse intestine by a novel analogue of fumitremorgin C. *Mol. Cancer Ther.,* **2002**, *1*, 417-25.

[75] Houghton, P.J.; Germain, G.S.; Harwood, F.C.; Schuetz, J.D.; Stewart, C.F.; Buchdunger, E.; Traxler, P. Imatinib mesylate is a potent inhibitor of the ABCG2 (BCRP) transporter and reverses resistance to topotecan and SN-38 *in vitro*. *Cancer Res*., **2004**, *64*, 2333-7.

[76] Ozvegy-Laczka, C.; Hegedus, T.; Varady, G.; Ujhelly, O.; Schuetz, J.D.; Varadi, A.; Keri, G.; Orfi, L.; Nemet, K.; Sarkadi, B. High-affinity interaction of tyrosine kinase inhibitors with the ABCG2 multidrug transporter. *Mol. Pharmacol*., **2004**, *65*, 1485-95.

[77] Hamada, A.; Miyano, H.; Watanabe, H.; Saito, H. Interaction of imatinib mesilate with human P-glycoprotein. *J. Pharmacol. Exp. Ther*., **2003**, *307*, 824-8.

[78] Kitazaki, T.; Oka, M.; Nakamura, Y.; Tsurutani, J.; Doi, S.; Yasunaga, M.; Takemura, M.; Yabuuchi, H.; Soda, H.; Kohno, S. Gefitinib, an EGFR tyrosine kinase inhibitor, directly inhibits the function of *P*-glycoprotein in multidrug resistant cancer cells. *Lung Cancer*, **2005**, *49*, 337-43.

[79] Kawamura, K.; Yamasaki, T.; Yui, J.; Hatori, A.; Konno, F.; Kumata, K.; Irie, T.; Fukumura, T.; Suzuki, K.; Kanno, I.; Zhang, M.R. *In vivo* evaluation of P-glycoprotein and breast cancer resistance protein modulation in the brain using [(11)C] gefitinib. *Nucl. Med. Biol.*, **2009**, *36*, 239-46.

[80] Shi, Z.; Peng, X-X.; Kim, I-W.; Shukla, S.; Si, Q-S.; Robey, R.W.; Bates, S.E.; Shen, T.; Ashby Jr., C.R.; Fu, L-W.; Ambudkar, S.V.; Chen, Z-S. Erlotinib (Tarceva, OSI-774) antagonizes ATP-binding cassette subfamily B member 1 and ATP-binding cassette subfamily G member 2-mediated drug resistance. *Cancer Res*., **2007**, *67*, 11012-20.

[81] Shi, Z.; Parmar, S.; Peng, Z-X.; Shen, T.; Robey, R.W.; Bates, S.E.; Fu, L-W.; Shao, Y.; Chen, Y-M.; Zang, F.; Chen, Z-S. The epidermal growth factor tyrosine kinase inhibitor AG1478 and erlotinib reverse ABCG2-mediated drug resistance. *Oncol. Rep*., **2009**, *21*, 483-9.

[82] Li, J.; Cusatis, G.; Brahmer, J.; Sparreboom, A.; Robey, R.W.; Bates, S.E.; Hidalgo, M.; Baker, S.D. Association of variant ABCG2 and the pharmacokinetics of epidermal growth factor receptor tyrosine kinase inhibitors in cancer patients. *Cancer Biol. Ther*., **2007**, *6*, 432-8.

[83] Lemos, C.; Kathmann, I.; Giovannetti, E.; Calhau, C.; Jansen, G.; Peters G.J. Impact of cellular folate status and epidermal growth factor receptor expression on BCRP/ABCG2-mediated resistance to gefitinib and erlotinib. *Br. J. Cancer*, **2009**, *100*, 1120-7.

[84] Shukla, S.; Robey, R.W.; Bates, S.E.; Ambudkar, S.V. Sunitinib (Sutent, SU11248), a small-molecule receptor tyrosine kinase inhibitor, blocks function of the ATP-binding cassette (ABC) transporters P-glycoprotein (ABCB1) and ABCG2. *Drug Metab. Dispos*., **2009**, *37*, 359-65.

[85] Dai, C.L.; Towari, A.K.; Wu, C.P.; Su, X.D.; Wang, S.R.; Liu, D.G.; Ashby, C.R. Jr.; Huang, Y.; Robey, R.W.; Liang, Y.J.; Chen, L.M.; Shi, C.J., Ambudkar, S.V.; Chen, Z.S.; Fu, L.W. Lapatinib (Tykerb, GW572016) reverses multidrug resistance in cancer cells by inhibiting the activity of ATP-binding cassette subfamily B member 1 and G member 2. *Cancer Res*., **2008**, *68*, 7905-14.

[86] Stewart, C.F.; Leggas, M.; Schuetz, J.D.; Panetta, J.C.; Cheshire, P.J.; Peterson, J.; Daw, N.; Jenkins, J.J. 3rd; Gilbertson, R.; Germain, G.S.; Harwood, F.C.; Houghton, P.J. Gefitinib enhances the antitumor activity and oral bioavailability of irinotecan in mice. *Cancer Res*., **2004**, *64*, 7491-9.

[87] Gupta, A.; Zhang, Y.; Unadkat, J.D.; Mao, Q. HIV protease inhibitors are inhibitors but not substrates of the human breast cancer resistance protein (BCRP/ABCG2). *J. Pharmacol. Exp. Ther.,* **2004**, *310*, 334-41.

[88] Cooray, H.C.; Janvilisri, T.; van Veen, H.W.; Hladky, S.B.; Barrand, M.A. Interaction of the breast cancer resistance protein with plant polyphenols**.** *Biochem. Biophys. Res. Commun*., **2004**, *317*, 269-75.

[89] Touhey, S.; O'Connor, R.; Plunkett, S.; Maguire, A.; Clynes, M. Structure-activity relationship of indomethacin analogues for MRP-1, COX-1 and COX-2 inhibition. Identification of novel che-

motherapeutic drug resistance modulators. *Eur. J. Cancer*, **2002**, *38*, 1661-1670.

[90] Gandhi, L.; Harding, M.W.; Neubauer, M.; Langer, C.J.; Moore, M.; Ross, H.J.; Johnson, B.E.; Lynch, T.J. A phase II study of the safety and efficacy of the multidrug resistance inhibitor VX-710 combined with doxorubicin and vincristine in patients with recurrent small cell lung cancer. *Cancer*, **2007**, *109*, 924-32.

[91] Marcelletti, J.F.; Multani, P.S.; Lancet, J.E.; Baer, M.R.; Sikic, B.I. Leukemic blast and natural killer cell P-glycoprotein function and inhibition in a clinical trial of zosuquidar infusion in acute myeloid leukemia. *Leuk. Res*., **2009**, in press.

[92] Robey, R.W.; Shukla, S.; Finley, E.M.; Oldham, R.K.; Barnett, D.; Ambudkar, S.V.; Fojo, T.; Bates, S.E. Inhibition of P-glycoprotein (ABCB1)- and multidrug resistance-associated protein 1 (ABCC1)-mediated transport by the orally administered inhibitor, CBT-1((R)). *Biochem. Pharmacol*., **2008**, *75*, 1302-12.

[93] McCulloch, E.A.; Till, J.E. Perspectives on the properties of stem cells. *Nat Med*., **2005**, 11, 1026-8.

[94] Clevers, H. Stem cells, asymmetric division and cancer**.** *Nat. Genet*., **2005**, *37*, 1027-7

[95] Bjerkvig, R.; Tysnes, B.B.; Aboody, K.S.; Najbauer, J.; Terzis, A.J.A. Opinion: the origin of the cancer stem cell: current controversies and new insights. *Nat. Rev. Cancer*, **2005**, *5*, 899-904.

[96] Dalerba, P.; Cho, R.W.; Clarke M.F. Cancer stem cells: models and concepts. *Annu. Rev. Med.*, **2007**, 58, 267-84.

[97] Dean, M.; Fojo, T.; Bates S. Tumour stem cells and drug resistance. *Nat. Rev. Cancer*, **2005**, *5*, 275-84.

[98] Uchida, N.; Leung, F.Y.; Eaves, C.J. Liver and marrow of adult mdr-1a/1b(-/-) mice show normal generation, function, and multitissue trafficking of primitive hematopoietic cells. *Exp. Hematol*., **2002**, *30*, 862-9.

[99] Goodell, M.A.; Rosenweig, M.; Kim, H.; Marks, D.F.; DeMaria, M.; Paradis, G.; Grupp, S.A.; Sieff, C.A.; Mulligan, R.C.; Johnson, R.P. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. *Nat. Med.*, **1997**, *3*, 1337-45.

[100] Sorrentino, B.P.; McDonagh, K.T.; Woods, D.; Orlic, D. Expression of retroviral vectors containing the human multidrug resistance 1 cDNA in hematopoietic cells of transplanted mice. *Blood*, **1995**, *86*, 491-501.

[101] Zhou, S.; Morris, J.J.; Barnes, Y.; Lan, L.; Schuetz, J.D.; Sorrentino, B.P. Bcrp1 gene expression is required for normal numbers of side population stem cells in mice, and confers relative protection to mitoxantrone in hematopoietic cells *in vivo*. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 12339-44.

[102] Zhou, S.; Schuetz, J.D.; Bunting, K.D.; Colapietro, A.M.; Sampath, J.; Morris, J.J.; Lagutina, I.; Grosveld, G.C.; Osawa, M.; Nakauchi, H.; Sorrentino, B.P. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat. Med*., **2001**, 7, 1028-34.

[103] Huss, W.J.; Gray, D.R.; Greenberg, N.M.; Mohler, J.L.; Smith, G.J. Breast cancer resistance protein-mediated efflux of androgen in putative benign and malignant prostate stem cells. *Cancer Res*., **2005**, *65*, 6640-50.

[104] Bleau, A.M.; Hambardzumyan, D.; Ozawa, T.; Fomchenko, E.I.; Huse, J.T.; Brennan, C.W.; Holland, E.C. PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. *Cell Stem Cell*, **2009**, 4, 226-35.

[105] Schatton, T.; Murphy, G.F.; Frank, N.Y.; Yamaura, K.; Waaga-Gasser, A.M.; Gasser, M.; Zhan, Q.; Jordan, S.; Duncan, L.M.; Weishaupt, C.; Fuhlbrigge, R.C.; Kupper, T.S.; Sayegh, M.H.; Frank, M.H. Identification of cells initiating human melanomas. *Nature*, **2008**, 451, 345-9.

[106] Frank, N.Y.; Pendse, S.S.; Lapchak, P.H.; Margaryan, A.; Shlain, D.; Doeing, C.; Sayegh, M.H.; Frank, M.H. Regulation of progenitor cell fusion by ABCB5 P-glycoprotein, a novel human ATP-binding cassette transporter. *J. Biol. Chem*., **2003**, 278, 47156-65.

[107] Frank, N.Y.; Margaryan, A.; Huang, Y.; Schatton, T.; Waaga-Gasser, A.M.; Gasser, M.; Sayegh, M.H.; Sadee, W.; Frank, M.H. ABCB5-mediated doxorubicin transport and chemoresistance in human malignant melanoma. *Cancer Res*., **2005**, *65,* 4320-33.

[108] Efferth, T.; Volm, M. Pharmacogenetics for individualized cancer chemotherapy. *Pharmacol. Ther.*, **2005**, *107*, 155-76.

[109] Cascorbi, I. Role of pharmacogenetics of ATP-binding cassette transporters in the pharmacokinetics of drugs. *Pharmacol Ther*, **2006**, *112*, 457-73.

[110] Cropp, C.D.; Yee, S.W.; Giacomini, K.M. Genetic variation in drug transporters in ethnic populations. *Clin. Pharmacol. Ther*., **2008**, *84*, 412-6.

[111] Deeken, J.F.; Figg, W.D.; Bates, S.E.; Sparreboom, A. Toward individualized treatment: prediction of anticancer drug disposition and toxicity with pharmacogenetics. *Anticancer Drugs*, **2007**, *18*, 111-26.

[112] Ishikawa, T.; Tamura, A.; Saito, H.; Wakabayashi, K.; Nakagawa, H. Pharmacogenomics of the human ABC transporter ABCG2: from functional evaluation to drug molecular design. *Naturwissenschaften*, **2005**, *92*, 451-63.

[113] Schellens, J.H.; Maliepaard, M.; Scheper, R.J.; Scheffer, G.L.; Jonker, J.W.; Smit, J.W.; Beijnen, J.H.; Schinkel, A.H. Transport of topoisomerase I inhibitors by the breast cancer resistance protein. Potential clinical implications. *Ann. N.Y. Acad. Sci.*, **2000**, *922*, 188-94.

[114] Hoffmeyer, S.; Burk, O.; von Richter, O.; Arnold, H.P.; Brockmoller, J.; Johne, A.; Cascorbi, I.; Gerloff, T.; Roots, I.; Eichelbaum, M.; Brinkmann, U. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 3473-8.

[115] Kimchi-Sarfaty, C.; Oh, J.M.; Kim, I.W.; Sauna, Z.E.; Calcagno, A.M.; Ambudkar, S.V.; Gottesman, M.M. A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science,* **2007**, *315*, 525-8.

[116] Conseil, G.; Deeley, R.G.; Cole, S.P. Polymorphisms of MRP1 (ABCC1) and related ATP-dependent drug transporters. *Pharmacogenet. Genomics*, **2005**, *15*, 523-33.

Received: 17 December, 2008 **Revised: 07 April, 2009** **Accepted: 08 April, 2009**