Perspectives of P-Glycoprotein Modulating Agents in Oncology and Neurodegenerative Diseases: Pharmaceutical, Biological, and Diagnostic Potentials

Nicola Antonio Colabufo,* Francesco Berardi, Mariangela Cantore, Marialessandra Contino, Carmela Inglese, Mauro Niso, and Roberto Perrone

Dipartimento Farmacochimico, Universitá degli Studi di Bari, Facoltá di Farmacia, Via Orabona 4, 70125, Bari, Italy

1. ABC Transporters: P-gp and Other Multidrug Efflux Pumps (MDEs)

Human ATP binding cassette (ABC[a]) transporters belong to a family of 49 genes classified into seven subfamilies: ABC-A, ABC-B, ABC-C, ABC-D, ABC-E, ABC-F, ABC-G.[1,2] Some of these transporters are involved in multidrug resistance (MDR), in particular ABC-B1, better known as P-glycoprotein (P-gp), ABC-G2, better known as breast cancer resistance protein (BCRP), and ABC-C1-6, also known as multidrug resistance associated proteins (MRP$_{1-6}$).[3] These transporters are overexpressed in several tumor cell lines and are responsible for drug efflux out of the cells.[4,5] They use the energy of ATP hydrolysis to extrude compounds by a complex translocation process.[6] Three models for P-gp translocation have been suggested: (1) pore, (2) flippase, and (3) hydrophobic vacuum cleaner models.[7,8] In the pore model, drugs binding P-gp to the cytosol are transported out through a protein channel. In the flippase model, P-gp flips drugs that are transported from the inner to the outer compartment of the plasma membrane against a concentration gradient. In the hydrophobic vacuum cleaner model, molecules recognized by P-gp in the lipid bilayer enter the protein from the membranous site and exit through the central cavity.

P-gp contains 12 transmembrane helices organized in two membrane spanning domains (MSDs), each containing six transmembrane helices, and two nucleotide binding domains (NBDs) responsible for ATP binding. BCRP is a "half transporter" because it is formed by only one MSD and one NBD although it dimerizes to be fully active. MRPs differ from P-gp because they display three MSDs, and the additional domain contains five transmembrane domains.[3] This review will focus on the physiological and pathological role of P-gp and will highlight the involvement of this protein both in MDR of tumors and in the physiological function of several barriers.

P-gp overexpression is a significant factor in chemotherapy failure due to the ability of this pump to limit the cell accumulation of antineoplastic drugs. Moreover, P-gp is expressed in barriers such as the blood–brain barrier (BBB), blood–cerebro spinal fluid (B-CSF) barrier, and blood–testis barrier (BTB). It modulates the absorption and excretion of xenobiotics across these barriers.[9]

P-gp is localized at the apical membranes of liver, kidney, placenta, and the villus tip of enterocytes in the gut.[10,11] In the gut, P-gp displays a strategic activity modulating access of drugs to the CYP3A4 enzyme, thereby regulating drug metabolism and absorption.

1.1. P-gp Involvement in Cancer and CNS Diseases. MDR is a complex phenomenon that is caused by tumor microenvironment changes or cancer cell-specific factors. Cancer cell-specific factors can occur at different levels: (i) increased drug efflux or decreased drug influx; (ii) drug inactivation; (iii) drug target modification; (iv) apoptosis evasion. The first of these mechanisms can be mediated by plasma membrane transporters such as P-gp.[12] As mentioned above, high levels of P-gp are reported in the luminal membrane of the endothelial cells constituting the BBB, B-CSF, and BTB.[11] This strategic localization gives P-gp a crucial physiologically role in keeping drugs in the blood. P-gp exerts a protective function in the BBB; indeed, recent studies have reported a potential correlation between P-gp activity and/or expression in CNS disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and epilepsy.

AD, a neurodegenerative disorder characterized by a progressive loss of cognitive function, evolves as several forms of dementia that display insoluble β-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs). Aβ secretion is

*To whom correspondence should be addressed. Phone: +39-080-5442727. Fax +39-080-5442231. E-mail: colabufo@farmchim.uniba.it.

^{a} Abbreviations: Aβ, β-amyloid; AB, apical–basolateral; ABC, ATP binding cassette; AD, Alzheimer's disease; AML, acute myelogenous leukemia; ATP, adenosine 5′-triphosphate; BA, basolateral–apical; B_{max}, maximal bound; BBB, blood–brain barrier; BCRP, breast cancer resistance protein; B-CSF, blood–cerebro spinal fluid; BTB, blood-–testis barrier; Caco-2, human colonic carcinoma; calcein-AM, calcein acethoxymethyl ester; CD56+, peripheral blood mononuclear cells; CHOP, cyclophosphamide, hydroxydaunorubicin (adriamycin), oncovin (vincristine), prednisone/prednisolone; CNS, central nervous system; ^{11}C-PIB, ^{11}C-Pittsburgh compound B; CYP3A4, cytochrome P-450-3A4; DV, distribution volume; FDA, Food and Drug Administration; ^{18}F-FDDNP, ^{18}F- (2-(1-{6-[(2-[^{18}F]fluoroethyl(methyl)amino]-2-naphthyl}ethylidene)malononitrile; FMZ, flumazenil; GI, gastrointestinal; HCT-8, human colorectale adenocarcinoma; ISF, interstitial fluid; K_d, dissociation constant; LBs, Lewy bodies; LLC-PK1, porcine kidney cell line; LRP1, low density lipoprotein receptor-related protein; MDCK, Madin–Darby canine kidney; MDE, multidrug efflux; MDR, multidrug resistance; MPP+, 1-methyl-4-phenylpyridinium; MRP, multidrug resistance associated proteins; MSA, multisystem atrophy; MSD, membrane spanning domain; MTD, maximum tolerated dose; MTL, medial temporal lobe; NBD, nucleotide binding domain; NFTs, neurofibrillary tangles; NSCLC, non-small-cell lung cancer; PAHG, hippocampus, parahippocampal, ambient gyrus; P_{app}, apparent permeability; PD, Parkinson's disease; PET, positron emission tomography; P-gp, P-glycoprotein; PSP, progressive supranuclear palsy; SCLC, small-cell lung cancer; SNP, single nucleotide polymorphism; SPECT, single photon emission computed tomography; T8, T-suppressor cells; TLE, temporal lobe epilepsy.

the initial event in the pathogenesis of AD, whereas Aβ-aggregation is implicated in subsequent neurodegeneration.[13] Interestingly, in AD brain the levels of Aβ deposition correlated inversely with the level of P-gp in brain vasculature, as confirmed by immunohistochemistry studies.[14] It has been suggested that P-gp exerts a critical role in the elimination of Aβ from the brain. The transporter could be considered as a target for the prevention and for the treatment of this neurodegenerative disorder.[15]

PD, characterized by the loss of dopaminergic cells in the substantia nigra, displays the presence of protein aggregates known as Lewy bodies (LBs) composed mainly of α-synuclein, a soluble neuronal protein that in pathological conditions evolves into insoluble oligomers.[16] Although it is still unknown if α-synuclein is a P-gp substrate, it has been widely demonstrated that P-gp is able to efflux some pesticides[17] and toxins considered responsible for PD onset.[18] Indeed, MPP+, a neurotoxin involved in Parkinsonian syndrome, is reported to be a P-gp substrate.[19] Moreover, it has been recently demonstrated that several pesticides and metals that interact with α-synuclein promote its fibrillation in vitro[20] and so induce sporadic PD.[21]

Thus, a dysfunction of P-gp activity in the BBB is considered one of the causes of the onset of PD[22,23] and AD,[14] since a correlation between aging and the decreased function of the pump in the BBB has been established in vivo.[24] This study led to the identification of brain regions (white matter and orbitofrontal regions) in which P-gp function was decreased. Bartels and co-workers demonstrated that the reduction in P-gp function was age-dependent establishing a close relationship between aging and the susceptibility to the onset of PD.[24]

It is reported that some antiepileptic drugs such as phenytoin and carbamazepine are P-gp substrates. Consequently, intrinsic or acquired overexpression of this protein in the BBB can lead to resistance to these antiepileptic drugs by limiting drug penetration into the brain.[25] P-gp overexpression can be due to the effects of epilepsy, drug treatment, or polymorphisms in the gene encoding P-gp (ABC-B1).[26] It is reported that carbamazepine is able to induce the expression of drug efflux pumps and drug-metabolizing enzymes in the intestine, thereby reducing its own absorption.[27,28] Gottesman and co-workers reported that the C3435T polymorphism in exon 27, which affects the timing of P-gp folding and insertion in the membrane, resulted in a P-gp with an altered drug interaction sites[29] reducing the oral bioavailability of some antiepileptic drugs. The link between intestinal P-gp expression and epilepsy was also demonstrated by the influence of the genotype in positions 3435 and 2677 of the ABC-B1 gene on phenytoin, valproate, and carbamazepine dose requirements in the treatment of the disease.[30]

P-gp is also present in high level in the vessels of the BBB and in the parenchymal cells (astrocytes and neurons) in drug refractory epilepsy.[31,32] P-gp overexpression in the BBB vessels, which affects access of antiepileptic drugs to the brain, can be considered as one of the causes of drug resistance in epilepsy. The overexpression of this protein in the parenchyma could exert a cytoprotective role, reducing the apoptotic response induced by growth factor withdrawal,[33] the cytotoxicity mediated by the complement,[34] and the cell death due to caspases activation.[35]

2. P-gp Substrate, Modulator, and Inhibitor Characterization

The large number of compounds interacting with P-gp led to the hypothesis of the presence of several binding sites.[36,37]

The presence of at least four distinct interaction sites on P-gp was detected using equilibrium and kinetic radioligand binding assays. Three sites (I−III) have been characterized for the transport because they interact with substrates (vinblastine, paclitaxel, rhodamine-123, and Hoechst33342) and modulators (XR9576, XR9051). By contrast, site IV is a regulatory site where elacridar (**5**) and nicardipine act as modulators. The four binding sites were able to allosterically communicate in a negative heterotropic manner, and the binding to one of sites switched the other sites to a low-affinity conformation.[36]

The physiological role of P-gp is as a first line of defense in several barriers, and its mechanism of interaction with drugs is the key to predict its pharmacological activity and to determine their pharmacokinetic profile. The FDA therefore requires drug interaction studies with P-gp for drug approval.[38]

Compounds that interact with P-gp have unrelated structures that can be classified into three groups: substrates, modulators, and inhibitors. Substrates are molecules actively transported by the protein and therefore have a higher concentration outside the cell with respect to the cytosol.[36] Modulators interact at the binding sites, therefore reducing substrate binding through a negative allosteric interaction. They have been shown through radioligand studies to alter substrate binding in a noncompetitive manner, as the maximal receptor density (B_{max}) for substrate binding, but not the dissociation equilibrium constant (K_d), was reduced. This finding demonstrates that the modulators interact with P-gp at a site distinct from that of the substrate. Moreover, it suggests allosteric communication between substrate and modulator binding sites.[7]

Inhibitor activity has improved our understanding of the translocation mechanism of P-gp.[7] The initial step of the translocation process involves the binding of drugs to a high affinity site and simultaneously the binding of ATP at the NBDs. Drug and ATP binding are coupled as shown by the ability of drugs to stimulate ATP hydrolysis and the requirement for two functional NBDs for drug translocation. The coupling of substrate and nucleotide binding causes the reorientation of binding site, and drug release occurs. Inhibitors interfere with the substrate or nucleotide binding step, thereby blocking P-gp translocation. The interaction mechanisms of substrate, modulators, and inhibitors with P-gp molecule are depicted in Figure 1.

Modulators and inhibitors exert the same final biological effect, restoring cell sensitivity to chemotherapeutic agents. Therefore, in the oncology field chemotherapeutic agents are coadministered with a modulator or an inhibitor, with the aim to reverse MDR as reported in clinical trials section.

In neurodegenerative diseases, substrates, modulators, and inhibitors are suitable tools to investigate the functionality and expression of P-gp in the biological barriers. Substrates administered either alone or in combination with a modulator are required to evaluate P-gp activity. Inhibitors can be employed as radiotracers to detect P-gp expression. They are administered either alone or in the presence of a substance binding to the same target site (e.g., an excess of the unlabeled test compound). Therefore, it is important to establish if a compound interacts with P-gp as a substrate, a modulator, or an inhibitor by specific biological assays.[38] A wide range of methodologies have been used to characterize drug interaction with P-gp. These methods can employ intact cells or purified protein, and a combination of different approaches are often required to identify the mechanism of interaction.
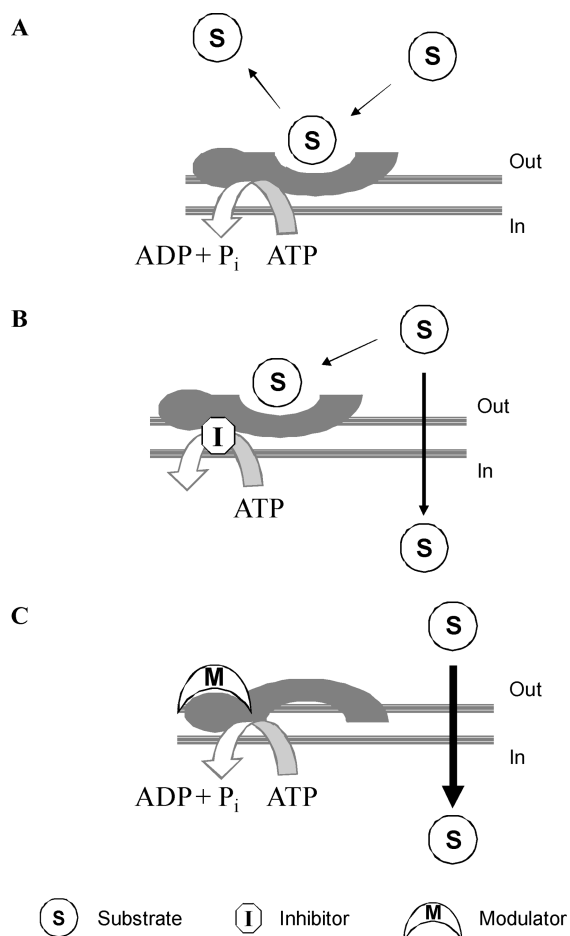
A

B

C

$$S \quad \text{Substrate} \qquad I \quad \text{Inhibitor} \qquad M \quad \text{Modulator}$$

Figure 1. Mechanism of P-gp interaction with substrates (A), inhibitors (B), and modulators (C).

A number of P-gp expressing cell lines can be used to assess the interaction and transport of new chemical entities with the protein including: human colonic carcinoma cells (Caco-2), Madin−Darby canine kidney cells (MDCK), T8, LLC-PK1, and HCT-8 cells.[38] In particular, the transfected MDCK-P-gp cell line displays a high expression level of the transporter and these cells can be easily manipulated for overexpression and coexpression of other MDE pumps. Compounds interacting with P-gp can also be radioactively or fluorescently labeled which can be detected by HPLC or liquid chromatography with tandem mass spectrometry (LC/MS/MS).

MDCK cells are a versatile tool for overexpressing ABC transporters and in particular MDCK-P-gp stably transfected lines are the commonly employed in these biological assays. These transfected cells have several advantages with respect to other cell lines, such as Caco-2 cells, used in similar biological assays. They differentiate into columnar epithelium forming tight-junctions more rapidly. The P-gp expression level in these cell lines are higher and more stable, and the flux data are more reproducible. However, a limitation could be the expression of canine P-gp (81% structural similarity to human P-gp) that could complicate the interpretation of the data.[38]

The presence of P-gp on the apical membrane of Caco-2 cells permits us to consider this cell line as a model for the gastrointestinal tract absorption of food and drugs.[39] Caco-2 cells are derived from human colonic carcinoma displaying high levels of P-gp expression, although they are not a good model for drug efflux in the normal human colon.[39]

Moreover, the presence of other transporters such as MRP2[40] and BCRP[41] can affect the data interpretation.[42] In these cells the tested compound could be employed as a radiotracer to evaluate the radioactivity inside the cell, in bilayer membranes, or out of the endocellular compartment. The low intracellular accumulation and high extracellular concentration of the probe lead to the determination of the compound as a P-gp substrate. The radioactivity found in the bilayer membrane represents the amount of probe employed as a mass balance.

The inhibitor evaluation is carried out by radioactivity measurement in the bilayer membranes both in the absence and in the presence of cold inhibitor. In the presence of unlabeled compound, the radioactivity decreases with respect to the amount found with radiotracer alone. Moreover, the radioactivity into and out of the cells is employed as a mass balance. A modulator could be better studied in coadministration with a radiolabeled substrate, and in this way its efficacy is related to the increased concentration of intracellular substrate.

This kind of study could also be performed by employing an inhibitor in coadministration with a substrate. However, the in vivo results demonstrated that this coadministration was less efficacious than expected because the inhibitor was able to block the pump translocation but was unable to modify the high affinity substrate sites.[43]

The fluorescent measurements can be performed by employing a compound with adequate spectroscopic properties or functionalized with an appropriate fluorescent moiety. In both cases, the biological evaluation of substrates and inhibitors is the same as reported for the radiolabeled compound. The evaluation of modulators was carried out by using a fluorescent prodrug such as acetoxymethyl ester of calcein (calcein-AM) that is a known P-gp substrate.[44] In the presence of a modulator, calcein-AM diffuses into the cytosol where it is hydrolyzed to the fluorescent dye calcein and no longer able to be transported by P-gp. This investigation could be affected through interference if the tested compound displays similar spectroscopic properties to that of calcein. However, compounds that cannot be radiolabeled, that do not display fluorescent properties, or that cannot be functionalized with a fluorescent moiety can be analyzed by quantitative HPLC and/or LC−MS. These methodologies allow the identification of metabolite formation and how they interact with P-gp.

Pharmacokinetic properties, such as the apparent permeability (P_{app}), are useful parameters for the discrimination of substrates, modulators, and inhibitors for P-gp. These properties are determined in a suitable cell model for the study of the basolateral−apical flux (BA) and apical−basolateral flux (AB). The first flux represents passive diffusion, while the second represents active P-gp-modulated transport. The BA/AB ratio identifies substrates (BA/AB from 18 to 20) and inhibitors (BA/AB < 2), while modulators display an intermediate BA/AB ratio (ranging from 2 to 18). Previously, Polli et al. considered the same parameter and classified P-gp interacting agents as substrates or inhibitors while modulator category was not suggested.[45]

2.1. P-gp and CYP Activities by ex Vivo Biological Assays. Drug penetration across the small intestine is influenced by the presence of the efflux pump P-gp and the metabolizing enzyme CYP3A4 that modifies drugs bioavailability. P-gp and CYP3A4 synergistically act to modulate presystemic drug metabolism partly because of the extensive overlap in their substrate specificity.[46] The intestinal metabolism and

active transport might therefore affect the rate of trans-epithelial drug transport, and mediating drug–drug and drug–food interactions could cause variable drug disposition, decreased efficiency of transport, or unwanted side effects.[47]

In gut metabolism studies, in vitro models are more often used than in vivo investigations. With respect to hepatic microsomes, the preparation of intestinal microsomes appears to be more difficult and their enzymatic activity considerably depends on the preparation techniques.[48]

Moreover, prediction of the in vivo metabolism, extrapolated from the rates of metabolism by isolated microsomes, hepatocytes, or intestinal cells, remains difficult because the rate of metabolism is dependent on many physiological factors, not least the rate of transport of drugs into the cells and the cell integrity. Caco-2 and other intestinal cells in culture have also been used to study intestinal metabolism and related drug interactions, but normal Caco-2 cells display a very low expression of the metabolizing enzymes.[49,50] Caco-2 cells lack the CYP450 isozymes and in particular CYP3A4, which is normally expressed at high levels in the intestine.

The Ussing chamber method is employed[51] to measure the in vitro passive transport of xenobiotics across the intestinal mucosa suitably performed for side-by-side diffusion cells.[52] In this method a correlation between the absorption percentage and the membrane permeability can be established, and with respect to Caco-2 cells, this correlation is considered a more reliable prediction. Moreover, by use of the Ussing chamber method, the role of P-gp in drug absorption can be carried out but the same result cannot be obtained using Caco-2 cells.[53]

The everted gut sac experiment is another ex vivo model that uses the intestinal epithelium for the studies of transport properties. Since this isolated organ expresses both CYP3A4 and P-gp, the method is valuable for studying various aspects of drug absorption[54] including P-gp activity[55] and intestinal CYP3A4-mediated metabolism.[56] The metabolism data obtained by the everted gut sac are reflective of the physiological response, as they are derived from the interaction of substrates with CYP enzymes inside enterocytes during the passage through the cells.[57]

The role of P-gp in controlling the intestinal CYP3A4-mediated drug metabolism is performed in the single-pass rat intestinal perfusion model. This in situ assay, based on the difference between the solute concentration entering and leaving a specific region, offers a direct way to measure human intestinal permeability. The model integrates aspects of drug transport and metabolism in all the physiological factors influencing drug passage.[58,52]

The in vitro models reported above are also used to predict BBB permeation, although this barrier differs from the GI tract by the presence of other MDE pumps, amino acid carriers, and active solute transporters. Moreover, the GI tract differs from BBB by the presence of CYP3A4 activity that can alter the permeability and the metabolism of xenobiotics.

Considering the constitution of the BBB, a more suitable biological assay for evaluating the permeation of xenobiotics across the BBB is needed. The in situ brain perfusion technique is a simple and accurate method to determine permeability of the BBB for drugs.[59] In this assay, the blood–brain flux is measured by infusion of artificial blood or plasma, or saline, dressed with tested compound, into the heart or the carotid arteries, calculating the amount of solute in brain at several times. From these measurements, the kinetics of brain uptake are analyzed and appropriate transport or permeability constants are calculated. This approach permits the study of the BBB permeability of xenobiotics because they are unaffected by metabolizing activity. In addition, the concentration of compound and the coadministrated potential competitor can be addressed in the perfusion method so that the method is predictive for the in vivo characterization in kinetic studies of saturable transport across the BBB and for the effects of plasma protein binding.[60]

3. Development of P-gp Modulating Agents: SAR Studies

Verapamil (**1**), a calcium channel blocker, was found able to reverse MDR,[61] and it has become the reference compound for developing other P-gp modulating agents.[62] However, reversing MDR by compound **1** was associated with toxic cardiovascular side effects. Similarly, the immunosuppressive agent cyclosporin A (**2**) was found to be a potent MDR modulator but unfortunately also interfered with metabolizing enzyme CYP3A4 activity (Figure 2).[63]

Drugs such as calmodulin antagonists, the antimalarial quinine, and antisteroid tamoxifen also belong to the first generation P-gp inhibitors.[64,65] These drugs were characterized by low potency and specificity, due to their primary activity, so that high doses were required and unfortunately resulted in several side effects.[66,67] Moreover, because they were substrates for other transporters and inhibitors for CYP450A4 enzyme, numerous pharmacokinetic interactions occurred.[68]

The second generation of inhibitors included analogues of compound **1** displaying lower cardiovascular activity (dexverapamil and dexiniguldipine), valspodar (**3**) (a compound **2** analogue), and compound **4** (a tacrolimus derivative) (Figure 2).[66] These P-gp modulators were more potent compared to first generation modulators, although they showed critical pharmacokinetic interactions due to inhibition of CYP450 and reduction of biliary excretion leading to increased toxicity.[69,70] This led to a reduction of drug doses resulting in inadequate levels of chemotherapeutic agent and reduced overall efficacy. However, some inhibitors such as compounds **3** and **4** are not selective because they bind P-gp and MRP1.[71]

The third generation inhibitors, designed to overcome the limitations reported above, are more powerful and selective than the second. They consist of compounds lacking significant pharmacokinetic interactions with antineoplastic agents, and many of these are undergoing clinical trials.

The acridonecarboxamide **5**, the antranilamide **6**, the cyclopropyldibenzosuberane **7**, the farnesyl transferase inhibitor **8**, the diarylimidazole **9**, and compound **10** are some third generation inhibitors (Figure 3). Moreover, several tyrosine kinase inhibitors such as imatinib mesylate or gleevec have been evaluated as selective P-gp inhibitors not able to interact with MRP1.[72] Other new modulators are 1,3-dioxolane, 1,3-dioxane, and small molecules such as dihydropirroloquinoline PGP-4008 and dihydropyridine DP7.[73–75]

The design of third generation P-gp inhibitors, characterized by suitable physicochemical properties, has been carried out by SAR studies and by better knowledge of how substrates, modulators, and inhibitors interact with P-gp.[36]

Early studies developed derivatives of compound **1** such as isoindolines and tetrahydroisoquinolines which incorporated

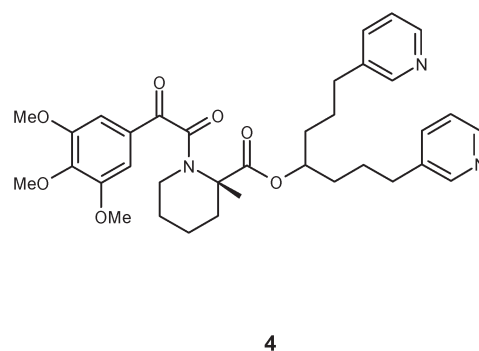**First Generation**
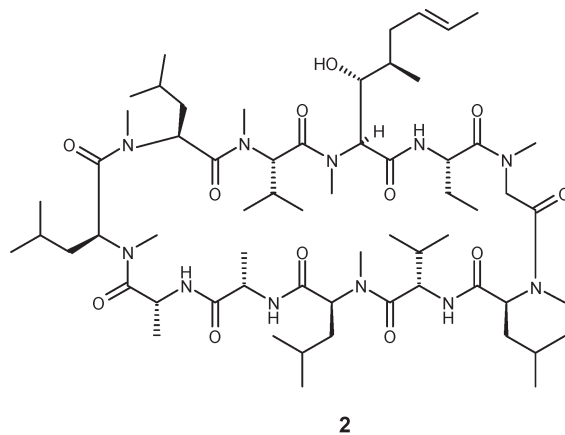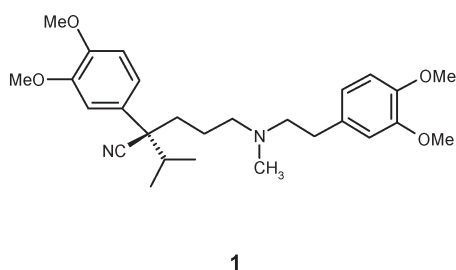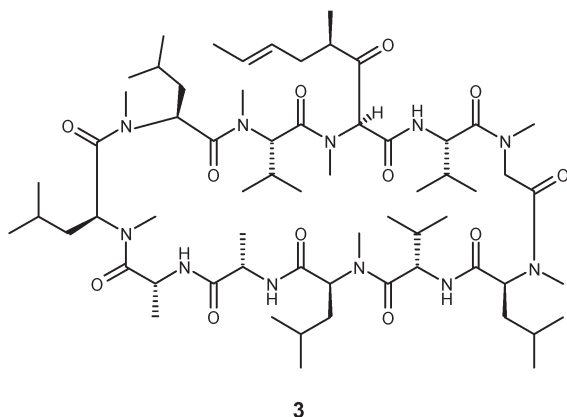

Second Generation

Figure 2. First and second generation of P-gp interacting agents: **1** (verapamil, substrate), **2** (cyclosporin A, modulator), **3** (valspodar, inhibitor), and **4** (biricodar, inhibitor).

a nitrogen atom into the heterocyclic nucleus. These findings demonstrated the importance of a hydrogen bond acceptor group such as an alkoxy substituent, the critical role of a hydrophobic cyclohexyl ring and of an alkyl spacer.[76] Furthermore, the replacement of the dimethoxyphenyl-4-cyano-4-isopropylbutyl group of compound **1** with an azapentacyclic moiety[77] demonstrated the fundamental role played by the overlapping of π orbitals of aromatic residues in binding to P-gp. These findings led to the introduction of aromatic rings and to the development of several compounds that were strong P-gp inhibitors without cardiovascular side effects.[78] It was therefore suggested that the nature of linker was not critical but it must provide a protonated nitrogen atom at the optimal distance between the two aromatic residues to recognize the binding site.[62] Moreover, a partial reduction of flexibility enhances good absorption and selectivity.[79]

Trifluoperazine also displayed MDR reversing activity, and therefore, phenothiazine bioisosteres such as phenoxazine and acridones were developed.[80] In both classes the presence of a tertiary amine linked to tricyclic nucleus through methylene chain increased P-gp inhibiting activity. SAR studies on tricyclic ring structures allowed the development of third generation inhibitors such as lonafarnib (**8**), zosuquidar (**7**), and its derivative laniquidar (**10**). In addition, dibenzosuberane derivatives contained a quinoline nucleus such as quinine, belonging to the first generation inhibitors.[81]

Compound **5**, an acridone derivative, displayed P-gp and BCRP inhibitory activity; therefore, common structural determinants for P-gp and BCRP recognition have been evaluated in SAR studies.[82] In particular, the importance of isoquinoline moiety and *N*-anilide fragment linked to *N*-azaxanthone nucleus has been investigated. BCRP activity was affected by the presence and the position of substituents on *N*-azaxanthone moiety, whereas carboxamide spacer did not influence BCRP activity.[83] In addition, the presence of a piperazine or piperidine nucleus binding to *N*-azaxanthone increased the P-gp reverting effect because the basic nucleus, in protonated form at physiological pH, facilitated cell accumulation of the drug.[83]

Compound **6** is an antranilamide derivative, and its MDR reversing activity is due to the presence of both tetrahydroisoquinoline and anthranilamide moieties on a phenyl ring.[84] Analogues of compound **6** bearing a tetrahydroisoquinolinethylphenylamine group[85] showed activity towards both P-gp and BCRP. Moreover, more water-soluble compounds than compound **6**, characterized by methoxylation of the anthranilamide moiety, displayed unchanged activity.[62]

The alkyl spacer was evaluated by inserting pyrrolidinyl, cyclohexyl, arylpiperazinyl rings, and the best results have been obtained in the presence of arylpiperazinyl moiety and methoxy-substituted anthranilamide nucleus.[86,87]

At present, research efforts are directed toward the identification of new compounds, with particular attention to natural food and herbal extracts. Curcumas and curcumin are natural sources that might regulate the function of P-gp in completely opposite ways. Curcumas increased P-gp activity by up-regulating the expressions of P-gp protein and mRNA
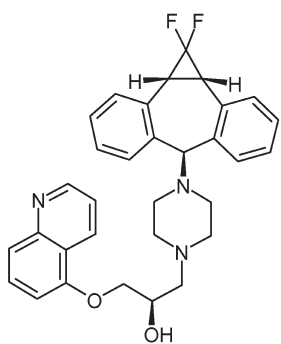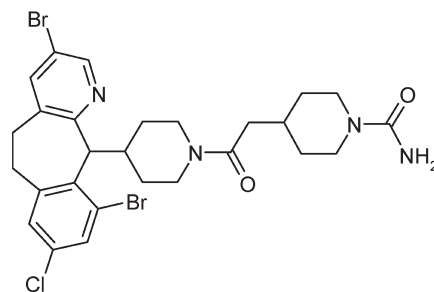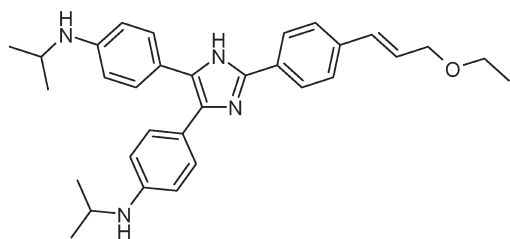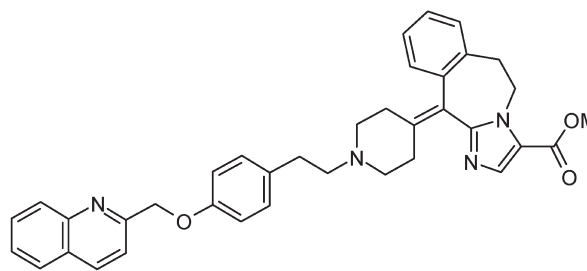
Third Generation
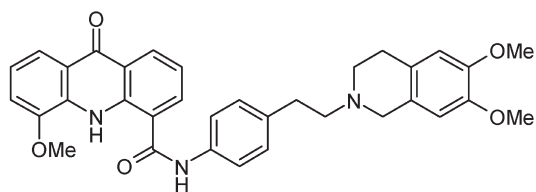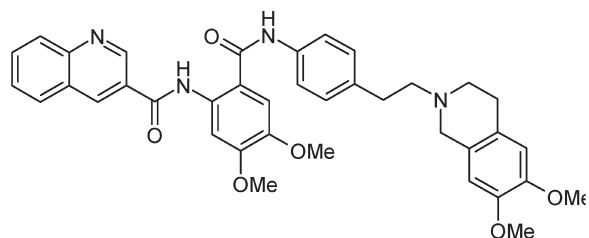
Figure 3. Third generation of P-gp inhibitors.

levels, whereas curcumin is a P-gp inhibitor that decreases P-gp mRNA expression levels.[88] Recently, it was demonstrated that grapefruit juice and its components (flavonoids and furanocoumarins such as bergamottin and naringenin) inhibit CYP3A4 but are also able to modulate P-gp.[89]

An in vitro evaluation of CYP450 inhibition and P-gp interaction of goldenseal, *Ginkgo biloba*, grape seed, milk thistle, and ginseng extracts has demonstrated that the clearance of many drugs is decreased by CYP450 inhibition more than by P-gp-mediated effects.[90] Furthermore, although natural compounds are not potent, they are able to influence the bioavailability of drugs and through appropriate chemical modifications may become new selective P-gp inhibitors.

Flavonoids, polyphenolic components of many fruits and vegetables, olive oil, tea, and red wine, also represent a natural class of P-gp modulators. Flavonoids are characterized by a common phenylcromanone nucleus that differs in oxidation state within the different subclasses including flavones, isoflavones, flavanones, flavonols, flavanols, and chalcones (Figure 4). They have instigated SAR studies to determine structural requirements for inhibiting MDR and to generate synthetic derivatives such as dimers of dietary flavonoids.[91]

4. Potential Application of P-gp Inhibitors

This section will focus on the results of clinical trials for the most promising P-gp inhibitors such as compounds **3** and **4** belonging to second generation and compounds **5, 6, 7, 10** to third generation. The strategy for overcoming MDR by P-gp and antineoplastic agent coadministration remains a hot topic, but clinical applications remain distant. Another potential application of these inhibitors involves diagnostic imaging with PET or SPECT. In fact, promising results have been obtained in animal in vivo models to monitor the activity and or the expression of P-gp in several biological barriers, in particular BBB. The diagnosis of several neurodegenerative diseases could be better supported ab initio by this noninvasive investigation.

4.1. Second Generation of P-gp Inhibitors. 4.1.1. Biricodar.
It displays a dual P-gp and MRP-1 blocking activity. In a phase I study compound **4** coadministered with doxorubicin showed no pharmacokinetic or pharmacodynamic limitations and the coadministration was well tolerated.[92] Compound **4** has been evaluated in a phase II study with doxorubicin and vincristine in patients with refractory SCLC.[93] This combination showed a higher incidence and severity of neutropenia with respect to results from the phase
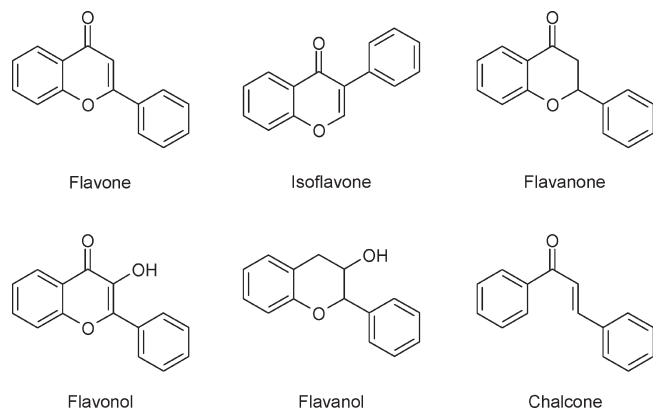
Figure 4. Flavonoids subclasses.

I study.[92] Furthermore, phase II studies of compound **4** coadministrated with paclitaxel in patients with advanced breast cancer or refractory ovarian cancer reported a high incidence of neutropenia, and this effect is probably due to an increased myelosuppression by vincristine. These results indicate that the coadministration of compound **4** with doxorubicin and vincristine did not improve the response rate, and because coadministration caused elevated toxicity, no studies are presently ongoing. Moreover, compound **4** inhibits both P-gp and MRP1, but P-gp levels in SCLC are lower than MRP expression so that alternative transporters could be expressed in recurrent SCLC, resulting in a low efficacy of compound **4**. Further studies should establish MDR transporter expression using biopsies to select patients with a high probability of obtaining clinical benefit.

4.1.2. Valspodar. It is a non-nephrotoxic and non-immuno-suppressive analogue of compound **2**. In phase I studies, combinations of compound **3** with etoposide, doxorubicin, paclitaxel, or vinblastine caused high toxicity consisting of reversible cerebellar ataxia, myelosuppression, and hyperbilirubinemia.[94−96]

Furthermore, compound **3** interacted with bile salt export protein and CYP450 decreasing the clearance of anticancer agents, and these interactions required dose reductions of drugs.[94−96] Moreover, coadministration of oral compound **3** plus infusional vinblastine in patients with advanced renal carcinoma (phase I) resulted in complete or partial response.[94] In a phase I/II study coexposure of intravenously administered infusional compound **3** and vinblastine were evaluated, and findings demonstrated that a lower dose than those previously recommended was sufficient to inhibit P-gp-mediated transport. This reduction permitted the use of higher doses of chemotherapeutic agents as a result of reduced inhibition of drug metabolism, principally mediated by CYP450.[94] Compound **3** has also been evaluated (phase I) in combination with pegylated liposomal doxorubicin in advanced malignancies showing moderate pharmacokinetic interaction and a partial response in patients with breast and ovarian carcinoma.[97]

Moreover, it has been coadministered with idarubicin, cytarabine, and etoposide in refractory AML, and although a pharmacokinetic interaction might have been expected, compound **3** did not significantly alter the disposition of idarubicin.[98] In a phase II trial, compound **3** in combination with paclitaxel in the treatment of metastatic carcinoma of the breast was studied,[99] and although this combination was tolerable, the coadministration did not

increase paclitaxel exposure; moreover, other studies are ongoing.[100]

Valpsodar coadministered with daunorubicin and cytarabine (phase II) in the treatment of elderly patients with previously untreated AML did not significantly improve response, disease-free survival, or overall survival.[101] To date, some clinical studies have been terminated because of high toxicity[102] and other studies are still ongoing in AML and myelodysplastic syndrome (phase II and phase III, respectively).[103,104]

In a phase III study, compound **3** combined with paclitaxel and carboplatin did not show promising results and was more toxic in ovarian and peritoneal cancer compared with carboplatin alone.[105] Moreover, the coadministration of vincristine, doxorubicin, and dexamethasone in patients with recurring or refractory multiple myeloma did not improve treatment outcome and was related to increased toxicity.[106]

4.2. Third Generation of P-gp Inhibitors. 4.2.1. Tariquidar. It has been characterized as a potent and noncompetitive inhibitor displaying high specificity for P-gp transporter. In vitro, compound **6** reverted resistance to doxorubicin, vincristine, and paclitaxel with greater potency than compounds such as compound **3**.[36] Compound **6** (phase I) has been evaluated in vinorelbine coadministration employing [99mTc]-sestaMIBI scintigraphy to visualize P-gp function both in tumors and in normal tissues of patients with metastatic cancer.[107] An increase of [99mTc]-sestaMIBI uptake in normal liver was found (128%) in the presence of compound **6**, and the ratio of radiotracer accumulation in tumor and heart was increased from 36% to 263%.[107] To date, a study using compound **6** and [94mTc]-sestaMIBI for patients with cancer is ongoing.[108]

Compound **6** with vinorelbine (phase I) has been also evaluated in adults with refractory solid tumors,[109] while a pediatric (phase I) study with vinorelbine, doxorubicin, or docetaxel is ongoing.[110] The results demonstrated that a compound **6**−vinorelbine coadministration is well tolerated and no dose limiting toxicity was observed because of the absence of pharmacokinetic interactions. Only in bone marrow stem cells granulocyte toxicity has been observed because of a higher vinorelbine exposure.[109]

In phase II trials, compound **6** was evaluated in combination with docetaxel in patients with recurrent or metastatic lung, ovarian, cervical cancer, or kidney cancer but the results are not yet available.[111] Similarly, coadministration of doxorubicin−compound **6** was tested in adults with refractory solid tumors and the study was completed, but the results have not yet been reported.[109] Furthermore, studies with patients displaying breast cancer have demonstrated that compound **6** failed to restore cell sensitivity to anthracycline or taxane chemotherapy.[112]

In phase III, trials of compound **6** with paclitaxel/carboplatin or with vinorelbine in patients with non-small-cell lung cancer (NSCLC) were terminated because the coadministration displayed high toxicity due to pharmacodynamic limitations.[12] Furthermore, clinical investigations of compound **6**−mitotane, −doxorubicin, −vincristine, or −etoposide coadministration in adrenocortical cancer and compound **6**−docetaxel in lung, ovarian, and cervical cancer are in progress.[111,113]

Recently, it has been demonstrated that [11C]**6** is captured by P-gp in the BBB in rats. Blockade of P-gp by a saturating dose of unlabeled compound **6** appeared to result in a

breakthrough of activity into brain parenchyma, thereby suggesting specific interaction of [^{11}C]**6** with P-gp in the BBB. The usefulness of [^{11}C]**6** for the visualization and quantification of cerebral P-gp merits further investigation.[114]

4.2.2. Zosuquidar. It displays both a suitable pharmacodynamic and pharmacokinetic profile, as it does not inhibit transporters involved in MDR and the CYP3A4 enzyme, respectively. It is administrated both orally and intravenously; however, an infusion into the blood is safer than oral administration which causes severe cerebellar toxicity associated with hallucinations.[115]

In phase I clinical trials, decreased vinorelbine or doxorubicin clearance in compound **7** coadministration was observed resulting in leukopenia and thrombocytopenia, although these effects are considered to have little clinical significance.[116] Moreover, no pharmacokinetic interactions between compound **7**−anthracyclines coadministration were observed.[117]

Because of the short half-life of compound **7**, coadministration with cytarabine or daunorubicin was tested in AML patients (phase I), initially in short[118] and then in continuous administration.[119] After 12 h of coadministration compound **7**−daunorubicin completely restored drug sensitivity and no severe inhibition of daunorubicin clearance occurred.

In phase I/II trials, compound **7** in combination with cyclophosphamide, hydroxydaunorubicin or adriamycin, oncovin or vincristine, prednisone/prednisolone (CHOP) regimen to treat non-Hodgkin's lymphoma caused low toxicity, no significant pharmacokinetic interactions with doxorubicin, and only moderate effects on the pharmacokinetics of vincristine.[120] The combination of compound **7** and docetaxel (phase II) was also evaluated in patients with metastatic breast cancer.[121] Although this combination was safe, a significant increase in neutropenic fever was reported, but this was unrelated to compound **7** exposure. Unfortunately, no difference in progression-free survival, overall survival, or response rate was reported in this study.[121]

4.2.3. Elacridar. It is a dual P-gp/BCRP inhibitor and can be orally administered. It was tested in combination with doxorubicin in patients with advanced solid tumors (phase I).[122] At the recommended dose of doxorubicin, a pharmacologic hematological toxicity was observed, mainly consisting of leukocytopenia and granulocytopenia. However, coadministration protocols with other inhibitors demonstrated that doxorubicin could be administered at a dose considered clinically active in most tumors with minimal side effects.[122]

Compound **5** was coadministrated with topotecan,[123] a P-gp and BCRP substrate used in the treatment of small cell lung cancer (SCLC) and metastatic ovarian cancer (phase I) to observe the dual blocking activity.[124] No dose-limiting diarrhea with respect to topotecan oral monotherapy was found, probably because of a local topotecan activity on the intestinal mucosa.[125] Compound **5**−topotecan oral coadministration led to a complete apparent bioavailability of topotecan estimated higher than 100%, and this overestimation was probably due to a slightly decreased clearance of topotecan.[126] By contrast, intravenous administration of topotecan caused side effects such as neutropenia and thrombocytopenia.[123] Additional studies (phase II) should therefore elucidate the safety and efficacy of compound **5**−topotecan coadministration.

Recently, good diagnostic results have been obtained with [^{11}C]**5** that appears to be specific for P-gp at the BBB, although its utility for the visualization of cerebral P-gp merits further investigation.[127]

4.2.4. Laniquidar and ONT-093 (9). Laniquidar (**10**) has been studied in oral coadministration with docetaxel (phase I). The results indicated unaltered docetaxel clearance but large interpatient variability and low bioavailability of compound **10**. Moreover, compound **10** has also been evaluated by intravenous coadministration with docetaxel and the combination appeared safe. Compound **10** in combination with docetaxel or paclitaxel in breast cancer has been evaluated, but the results have not yet been published.[128]

It has been reported that 2-[4-[3-ethoxy-1(*E*)-propenyl]-phenyl]-4,5-bis[4-(isopropylamino)phenyl]-1*H*-imidazole (**9**) does not interact with CYP3A4 and the results in coadministration with docetaxel were not concluded and not published.[129]

Recently, radiosynthesis and biodistribution of [^{11}C]**10** as a tracer of P-gp in rats have been developed. Further kinetic studies are needed to investigate the antagonistic behavior of [^{11}C]**10** at tracer level.[130]

5. Perspectives of P-gp Ligands in Diagnosis of CNS Diseases

The aim of this section is to highlight the potentials of P-gp inhibitors both in the reversal of MDR by coadministration with chemotherapeutic agents and in the diagnosis of some CNS diseases, using imaging techniques such as PET and SPECT. Recent papers[131,14,132] reported that P-gp function decreases at the advanced stages of neurodegenerative diseases such as PD and AD. P-gp dysfunction can both induce the increased accumulation of toxins, as in PD, and decrease the ability of the brain to efflux proteins, as in AD. Since these pathologies are characterized by aberrant polymerization and accumulation of proteins into the brain, P-gp function and expression at the BBB attract attention as a potential target involved in the pathogenesis of these neurodegenerative disorders. Moreover, P-gp overexpression is detected during other neurodegenerative diseases such as epilepsy, in which P-gp drug efflux restricts and/or prevents CNS drugs from entering the brain.

With this in mind, P-gp inhibitors represent innovative and novel therapeutic strategies for neurodegenerative disorders and for the treatment of chemoresistant tumors. Meanwhile, radiolabeled P-gp inhibitors for PET and SPECT are important tools to investigate both the activity and the expression of P-gp (with a P-gp substrate and P-gp inhibitor, respectively) in several neurodegenerative diseases such as PD, AD, and epilepsy.[133−138]

5.1. Parkinson's Disease Diagnosis. Exposure to environmental toxins, genetic predisposition, and advancing age increases the risk for developing PD. It has been reported that the C3435T polymorphism of the *MDR1* gene, responsible for decreased P-gp function at the BBB,[22] increases the exposure to neurotoxic substances.

PET is a useful tool to study BBB P-gp function in vivo, using the tracer [^{11}C]**1**. The distribution volume (DV) of radiotracer in the brain is an inverse measure of P-gp function at the BBB. A higher [^{11}C]**1** uptake indicates either reduced P-gp mediated efflux or an increased passive diffusion across the vascular endothelium.

Although [^{11}C]**1** is commonly used as a PET probe for imaging P-gp function in the brain, it has some limitations,

such as a low basal signal in normal condition and a relatively large intersubject variation in tracer uptake.

In a pilot PET study using racemic mixture [^{11}C]**1**, decreased P-gp efflux was found in the midbrain of 5 PD patients compared to 5 healthy subjects.[23] However, the same results were not observed in 10 early-stage PD patients scanned with (*R*)-[^{11}C]**1**. No significant differences in DV of (*R*)-[^{11}C]**1** between PD patients and control subjects were found. Moreover, no correlation between different *MDR1* C3435T polymorphism and higher DV of (*R*)-[^{11}C]**1** was evident.[136]

An in vivo study, carried out on 17 volunteers (age 18−86), shows a specific regional decrease of P-gp function in orbitofrontal (comprising the olfactory bulb) and posterior internal capsule regions of older subjects.[139] Moreover, decreased BBB P-gp function is observed in white matter regions that are thought to contribute to the functional denervation of cortical areas.[24]

Another in vivo PET study compares regional P-gp function in patients with different Parkinsonian pathology, including early and advanced stage PD, progressive supranuclear palsy (PSP), multisystem atrophy (MSA), and healthy control subjects. The results show high [^{11}C]**1** uptake in the pituitary, ventricles, and skin moderate uptake in gray matter and low uptake in white matter and bone. No substantial differences in PD, PSP, and MSA are evident. Advanced PD patients show a significant increase of [^{11}C]**1** in frontal white matter regions compared to the control subjects. By contrast, the early PD patients display lower uptake in midbrain and frontal regions and with respect to control, they do not show "clusters" with higher intensity signal. Higher [^{11}C]**1** uptake is found in the anterior cingulate cortex and in the head of the caudate of advanced PD patients compared to subjects with early PD. In PSP and MSA patients, a significant [^{11}C]**1** uptake is seen in basal ganglia regions, while in PSP patients increased [^{11}C]**1** uptake in frontal regions is found.[131]

Compared to healtly subjects advanced PD patients show a decreased P-gp function in frontal regions. This reduced frontal P-gp activity, in advanced PD and PSP, reflects the frontostriatal neuronal dysfunction found in these diseases. Increased [^{11}C]**1** uptake is found in the white and in gray regions rather than in the cortex. Although the cerebellum is linked to midbrain nuclei, lower [^{11}C]**1** uptake of both advanced and early PD patients is found. The results of these studies indicate that P-gp function is regionally decreased in the advanced stages of several parkinsonian diseases. Apparently, reduced P-gp efflux does not play a primary role in the initiation of PD but the function of P-gp decreases with aging.

To better understand the role of *MDR1* gene in the progression of PD, post-mortem human and rodent tissues have been analyzed by in situ hybridization to characterize *MDR1* mRNA expression.[140] Expression of *MDR1* mRNA is observed in both rat and human physiological barriers, such as the endothelial cells lining blood vessels. Expression in the meninges and BBB is critical for controlling the entry of drugs and xenobiotics into the CNS. Indeed, rats that lack one or both *mdr1a/b* genes show increased brain drug accumulation.[141] In this study, a reduced expression level of *MDR1* mRNA was detected in the striatum of PD patients compared to controls. This leads to reduced activity of the *MDR1* gene product, particularly in elderly patients.[142]

P-gp function evaluation, using [^{11}C]**1** as radiotracer, is an exciting diagnostic possibility to assess the location and

progression of pathology in PD patients. Increase in the [^{11}C]**1** uptake in the brain of PD patients will probably correspond to decreased P-gp function. Prevention of the accumulation of toxic drugs that are responsible for some neurodegenerative diseases by increasing P-gp activity at the BBB could be an additional therapeutic goal. A possible treatment strategy to increase P-gp expression could be the use of a specific inducer (e.g., grapefruit juice, St. John's wort, rifampicin)[143] or an endogenous drug (e.g., progesterone).[144]

5.2. Alzheimer's Disease Diagnosis. Accumulation of amyloid-*β* (A*β*) is an AD hallmark. Normally, A*β* proteins, secreted from neurons into the interstitial fluid (ISF) of the brain, can be eliminated in three ways: (1) by proteolytic degradation; (2) by passive efflux; (3) by active transport across the BBB.[145] The low density lipoprotein receptor-related protein (LRP1) is mostly involved in the active efflux of A*β* at the BBB,[146] but other proteins do also mediate A*β* efflux including P-gp.[147]

It has been reported that P-gp plays an important role in A*β* removal from the brain, and the inhibition or the lack of expression of this protein leads to increased A*β* levels in the ISF.[145] These findings support the hypothesis that a dysfunction of P-gp in the BBB could play an important role in the progression of AD. Indeed, an up-regulation of P-gp expression has been observed in the early pathogenesis of AD while in later stages of the disease, P-gp expression was dramatically decreased.[14]

Moreover, it was demonstrated that A*β* is a P-gp substrate in vitro. In healthy, elderly humans, P-gp expression was inversely correlated with the amount of A*β* plaques,[14] while in AD, decreased efflux of A*β* is the cause of myelin damage and determines white matter injury.

Since AD diagnosis is usually based on clinical parameters that have a poor sensitivity and specificity in early stages of the disorder, the development of techniques that can identify incipient AD is urgently required. Decreased P-gp function in the BBB, associated with a genetic predisposition for neurodegenerative disease,[24] could be useful for AD diagnosis by neuroimaging techniques in vivo.

Indeed, it would be useful to use P-gp substrates and inhibitors with adequate pharmacodynamic and pharmacokinetic properties as PET tracers to detect P-gp activity and expression. Among P-gp ligands, (*R*)-[^{11}C]**1** is the most widely used PET probe to assess the functionality of P-gp in disorders of the CNS.[24]

Currently, AD diagnosis by PET is performed using ^{11}C-Pittsburgh compound B (^{11}C-PIB)[148] and ^{18}F-(2-(1-{6-[(2-[^{18}F]fluoroethyl(methyl)amino]-2-naphthyl}ethylidene)-malononitrile) (^{18}F-FDDNP)[149] for amyloid deposition measurement.

5.3. Epilepsy Diagnosis. In chronic epilepsy, about 20−30% of patients become resistant to antiepileptic drug treatment. Two hypotheses have been put forward to explain pharmacoresistance: (1) "the drug target hypothesis" that postulates a target alteration; (2) "the multidrug transporter hypothesis" that is based on the overexpression of certain efflux pumps at the BBB. Both mechanisms could lead to a reduction in the responsiveness of patients to treatment with antiepileptic drug. The second hypothesis suggests P-gp as a potential biomarker for detection of the onset of epilepsy.

In a PET study, (*R*)-[^{11}C]**1** was employed to assess P-gp functionality in the brain of patients with a form of focal epilepsy refractory to antiepileptic treatment (TLE). In this

study, different brain regions involved in seizure generation such as the hippocampus, parahippocampal ambient gyrus (PAHG), and amygdala were analyzed.[150] Regional increases in the radiotracer DV suggested an impairment of the BBB. The most interesting epileptogenic area, the hippocampus, was not studied. A spillover of radioactivity from the adjacent choroid plexus, due to P-gp expression in its epithelium, led to active transport of P-gp substrates such as compound **1** from blood into CSF.[151] (R)-[11C]**1** kinetics were compared in homologous brain volume of interest located ipsilaterally and controlaterally to the seizure focus. In the area adjacent to the hippocampus (implicated in seizure generation and propagation), increased influx and efflux rate constants of (R)-[11C]**1** were ipsilaterally observed.

These asymmetries were most prominent in the PAHG, amygdala, and medial and lateral anterior temporal lobes and were minimal in a brain region localized outside the temporal lobe. This finding demonstrated that the asymmetries were present only in epileptic brain regions. Moreover, since some antiepileptic drugs used in therapy are P-gp substrates, it is important to note that in some patients of this study the coadministrated antiepileptic agent might compete with (R)-[11C]**1** for P-gp transport. This competition could lead to a modest DV decrease of (R)-[11C]**1** in epileptic brain tissue. Thus, it would be of great interest to acquire (R)-[11C]**1** PET scans in epileptic patients during administration of antiepileptic drugs in order to monitor a possible occupancy of P-gp by antiepileptic drugs.

Furthermore, in animal models it has been demonstrated that seizures and antiepileptic drug treatment could induce *ABCB1* gene expression. Indeed, the "multidrug transporter hypothesis" suggests that refractory epilepsy could be due to a localized P-gp overexpression that limits the transport of some antiepileptic drugs across the BBB and leads to therapy resistance. This theory is based on three pieces of evidence: (i) P-gp overexpression is exclusively localized in the seizure focus, allowing access of antiepileptic drug to other brain regions where they may cause side effects; (ii) P-gp overexpression occurs only in pharmacoresistant epilepsy patients; (iii) some antiepileptic drugs are P-gp substrates. The expression and functionality of P-gp are influenced by *ABCB1* gene polymorphisms.[152] Recently, a correlation of the *ABCB1* exon 26 C3435T polymorphism and multidrug resistance has been found in epilepsy patients.[29] In addition, a correlation has been demonstrated between *ABCB1* single nucleotide polymorphisms (SNPs) in exons 12 (C1236T), 21 (G2677T), and 26 (C3435T) and drug resistance in patients with TLE, with a greater risk of drug resistance for homozygous carriers of the CGC haplotype (1236C, 2677G, 3435C).[153] Therefore, since regionally enhanced P-gp activity is involved in drug resistance, the development of P-gp inhibitors as antiepileptic comedication[154] is a potential strategy to reverse epilepsy pharmacoresistance. Third-generation P-gp inhibitors, initially developed as MDR reversing agents in oncology, were not tested in clinical trials in epilepsy patients.[155] In future studies with P-gp modulators, PET scans using (R)-[11C] **1** could be useful to characterize enhanced cerebral P-gp activity. Patients with other forms of epilepsy, such as cortical dysplasia, could be examined for direct analysis of epileptogenic brain regions to deepen our understanding of the role of P-gp in refractory epilepsy. At the moment, (R)-[11C]**1** has been largely employed as a radiotracer in PET analysis to measure P-gp-mediated transport at the BBB in animals[156] and humans.[142] The limitation of this probe is its faster peripheral metabolism in epileptic patients due to CYP3A4A activity induced by antiepileptic drugs, which leads to reduced concentrations of intact radiotracers. This finding shows that induction or inhibition of hepatic enzymes by the coadministered drugs could lead to pharmacokinetic interactions with (R)-[11C]**1**.[157] Therefore, different rates of (R)-[11C]**1** metabolism could be important for the comparison of PET-derived kinetic parameters in epileptic patients and healthy controls.

A recent in vivo PET study, performed in amygdala kindled animals, employed the radiotracer [11C]flumazenil (FMZ).[158] The binding of this probe was decreased, and this finding is consistent with increased transport of FMZ across the BBB. Because FMZ is not a P-gp substrate, its reduced concentration in the brain could be due to efflux mediated by a non-ABC transporter such as the RLIP76.[159] This protein is known to be regionally up-regulated in the brain of epileptic patients.

6. Conclusions

P-gp is a target in oncology for restoring chemotherapeutic cell accumulation in resistant tumors. This protein is overexpressed on the apical membrane of tumor cells and causes the failure of cancer therapy by antineoplastic efflux. On the other hand, this transporter plays a crucial role at several biological barriers such as BBB, B-CSF, and intestinal barrier. This pump affects the flux of xenobiotics in and out of the cells modulating their metabolism and the efflux of their metabolites. The characterization of the activity and expression of this transporter is the goal of sophisticated imaging techniques such as PET and SPECT. Radiolabeled P-gp substrates can be employed to monitor P-gp activity in the absence and presence of a P-gp modulator or inhibitor visualizing how the block of this pump increases the amount of radiotracer in the cells or after the biological barriers. P-gp expression can also be visualized by a radiolabeled inhibitor that binds to the pump in the high affinity state, thereby blocking the translocation caused by the pump. In the oncology field, MDR is a critical clinical aspect and the most employed strategy to revert it is to coadminister a P-gp modulator or inhibitor with chemotherapeutic agents. Several clinical trials studies have been carried out, associating third generation P-gp inhibitors such as compounds **3**, **5**, **6** with some chemotherapeutic drugs known as P-gp substrates. At present, the results are not very encouraging because pharmacokinetic limitations of several coadministrated antineoplastic drugs have been reported. In addition, pharmacodynamic interactions have been observed resulting in high systemic toxicity.

By contrast, P-gp substrates and inhibitors are potential novel tools in the diagnosis of CNS diseases by PET and SPECT. In particular, PD and AD patients can be monitored in an early stage and during disease progression with radiolabeled compounds that interact with P-gp. Several studies have been carried out in animal models that highlight these possibilities. Taking into account the good potential of P-gp tracers in diseases of the CNS, new molecules should be designed, preferentially "small molecules", that can be radiolabeled in a single, final step of the synthesis. These objectives match medicinal chemistry research that should suggest novel molecules displaying favorable pharmacodynamic and pharmacokinetic profiles and being accessible for radiolabeling with 11C- or 18F at several points.

Biographies

Nicola Antonio Colabufo received his Ph.D. in Medicinal Chemistry in 1996 from University of Bari, Italy. After working as a Postdoctoral Fellow in the synthesis of GPCRs ligands, he worked in biological evaluation of GPCRs and σ receptors ligands. Since 2005 he has been Associate Professor and was involved in the development of new compounds for reversal of MDR. He is author and coauthor of numerous papers in the MDR field, edited a textbook on MDR in antitumor treatment (Research SignPost), and was Guest Editor of a special issue on the role of ABC pumps in drug transport (CTMC). Currently, he is developing potential PET radiotracers to visualize P-gp expression and function in tumor progression and in CNS disorders.

Francesco Berardi is Professor of Medicinal Chemistry at the University of Bari, Italy, where he has taught since 1979. He received a degree (1976) in Medicinal Chemistry and Technology from the University of Bari. Prof. Berardi is the author or coauthor of numerous publications and patents in the field of dopaminergic, serotonergic, and σ receptors ligands and more recently in P-gp ligands and MDR topics. He took part as author in the preparation of a book on multidrug resistance in antitumor treatment and a special issue on the role of ABC pumps in drug transport.

Mariangela Cantore completed her Ph.D. in Medicinal Chemistry in 2009 at the University of Bari, Italy, working on design and synthesis of ABC transporters modulators for reverting multidrug resistance. She now works as a Postdoctoral Fellow in the development of P-glycoprotein inhibitors suitable as radiotracers in PET and SPECT analysis.

Marialessandra Contino received her Ph.D. in Medicinal Chemistry in 2004 at the University of Bari, Italy, developing biological methods for the evaluation of σ-1 and σ-2 ligands in organ bath experiments (ex vivo) and in tumor cell lines (in vitro). In 2002 she acquired expertise in cell lines handling at IGBMC, Strasbourg, France. From 2004 to 2008, she was involved, as Postdoctoral Fellow, in the biological evaluation of the affinity and activity of ligands for some CNS receptors (5-HT$_7$, 5-HT$_{1A}$, D$_2$, D$_3$) and receptors involved in cancer progression and development (σ and EBP binding sites). Since 2008 she has been a Postdoctoral Fellow studing ligands for the reversion of MDR.

Carmela Inglese received her Ph.D. in Medicinal Chemistry in 2009 from University of Bari, Italy, working on the diagnosis of tumors with fluorescent, [^{3}H], and [^{11}C] probes for σ-2 receptors. She now works as a Postdoctoral Fellow in the evaluation of the affinity and activity of compounds active toward serotonergic, dopaminergic, and σ receptors. In addition, she evaluates ligands able to revert multidrug resistance and interacting with CYP3A4.

Mauro Niso received his Ph.D. in Medicinal Chemistry in 2006 from University of Bari, Italy, studying biochemical and pharmacological events induced by σ receptor ligands in tumor cell lines and tissues. He obtained a scholarship by PerkinElmer Italia s.p.a. for the development of fluorescent ligands for pharmacological assays. Now as Postdoctoral Fellow, he works on analyzing compounds that interact with some serotonergic, dopaminergic, and σ receptors and ligands involved in the reversion of MDR in tumor cell lines.

Roberto Perrone has been Professor of Medicinal Chemistry at the University of Bari since 1990. His scientific interest is the design, synthesis, and pharmacological in vitro evaluation of drugs toward CNS receptors. He is author and coauthor of numerous publications and patents in the field of dopaminergic, serotonergic, and σ receptors ligands and more recently in P-gp ligands and multidrug resistance topics. He is author of a book on multidrug resistance in antitumor treatment and of a special issue on the role of ABC pumps in drug transport.

References

(1) Ross, D. D.; Doyle, L. A. Mining our ABCs: pharmacogenomic approach for evaluating transporter function in cancer drug resistance. *Cancer Cell* **2004**, *6*, 105–107.

(2) Pharm, A.-N.; Penchala, S.; Graf, R. A.; Wang, J.; Huang, Y. Pharmacogenomic Characterization of ABC Transporters Involved in Multidrug Resistance. In *Multidrug Resistance: Biological and Pharmaceutical Advance in the Antitumour Treatment*; Colabufo, N. A., Ed.; Research Signpost: Kerala, India, 2008; pp 19–62.

(3) Giménez-Bonafé, P.; Guillén Canovas, A.; Ambrosio, S.; Tortosa, A.; Pérez-Tomás, R. Drugs Modulating MDR. In *Multidrug Resistance: Biological and Pharmaceutical Advance in the Antitumour Treatment*; Colabufo, N. A., Ed.; Research Signpost: Kerala, India, 2008; pp 63–99.

(4) Pérez-Tomás, R. Multidrug resistance: retrospect and prospects in anti-cancer drug treatment. *Curr. Med. Chem.* **2006**, *13*, 1859–1876.

(5) Teodori, E.; Dei, S.; Martelli, S.; Scapecchi, F.; 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.

(6) Avendaño, C.; Menéndez, J. C. Inhibitors of multidrug resistance to antitumor agents (MDR). *Curr. Med. Chem.* **2002**, *9*, 159–193.

(7) Callaghan, R.; Ford, R. C.; Kerr, I. D. The translocation mechanism of P-glycoprotein. *FEBS Lett.* **2006**, *580*, 1056–1063.

(8) Constantinides, P. P.; Wasan, K. M. Lipid formulation strategies for enhancing intestinal transport and absorption of P-glycoprotein (P-gp) substrate drugs: in vitro/in vivo case studies. *J. Pharm. Sci.* **2007**, *96*, 235–248.

(9) Ohtsuki, S.; Terasaki, T. Contribution of carrier-mediated transport systems to the blood–brain barrier as a supporting and protecting interface for the brain; importance for CNS drug discovery and development. *Pharm. Res.* **2007**, *24*, 1745–1758.

(10) Chan, L. M.; Lowes, S.; Hirst, B. H. The ABCs of drug transport in intestine and liver: efflux proteins limiting drug absorption and bioavailability. *Eur. J. Pharm. Sci.* **2004**, *21*, 25–51.

(11) Colabufo, N. A.; Berardi, F.; Contino, M.; Niso, M.; Perrone, R. ABC pumps and their role in active drug transport. *Curr. Top. Med. Chem.* **2009**, *9*, 119–129.

(12) Nobili, S.; Landini, I.; Giglioni, B.; Mini, E. Pharmacological strategies for overcoming multidrug resistance. *Curr. Drug Targets* **2006**, *7*, 861–879.

(13) Arriagada, P. V.; Marzloff, K.; Hyman, B. T. Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. *Neurology* **1992**, *42*, 1681–1688.

(14) Vogelgesang, S.; Cascorbi, I.; Schroeder, E.; Pahnke, J.; Kroemer, H. K.; Siegmund, W.; Kunert-Keil, C.; Walker, L. C.; Warzok, R. W. Deposition of Alzheimer's beta-amyloid is inversely correlated with P-glycoprotein expression in the brains of elderly nondemented humans. *Pharmacogenetics* **2002**, *12*, 535–541.

(15) Kuhnke, D.; Jedlitschky, G.; Grube, M.; Krohn, M.; Jucker, M.; Mosyagin, I.; Cascorbi, I.; Walker, L. C.; Kroemer, H. K.; Warzok, R. W.; Vogelgesang, S. MDR1-P-glycoprotein (ABCB1) mediates transport of Alzheimer's amyloid-beta peptides–implications for the mechanisms of Abeta clearance at the blood–brain barrier. *Brain Pathol.* **2007**, *17*, 347–353.

(16) Wood-Kaczmar, A.; Gandhi, S.; Wood, N. W. Understanding the molecular causes of Parkinson's disease. *Trends Mol. Med.* **2006**, *12*, 521–528.

(17) Bain, L. J.; LeBlanc, G. A. Interaction of structurally diverse pesticides with the human MDR1 gene product P-glycoprotein. *Toxicol. Appl. Pharmacol.* **1996**, *141*, 288–298.

(18) Rajput, A. H. Environmental toxins accelerate Parkinson's disease onset. *Neurology* **2001**, *56*, 4–5.

(19) Martel, F.; Calhau, C.; Soares-da-Silva, P.; Azevedo, I. Transport of [H-3]MPP+ in an immortalized rat brain microvessel endothelial cell line (RBE 4). *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2001**, *363*, 1–10.

(20) Uversky, V. N.; Li, J.; Fink, A. L. Metal-triggered structural transformations, aggregation, and fibrillation of human α-synuclein. *J. Biol. Chem.* **2001**, *276*, 44284–44296.

(21) Di Monte, D. A. The environment and Parkinson's disease: is the nigrostriatal system preferentially targeted by neurotoxins? *Lancet Neurol.* **2003**, *2*, 531–538.

(22) Drozdzik, M.; Bialecka, M.; Mysliwiec, K.; Honczarenko, K.; Stankiewicz, J.; Sych, Z. Polymorphism in the P-glycoprotein drug transporter MDR1 gene: a possible link between environmental and genetic factors in Parkinson's disease. *Pharmacogenetics* **2003**, *13*, 259–263.

(23) Kortekaas, R.; Leenders, K. L.; van Oostrom, J. C. H.; Vaalburg, W.; Bart, J.; Willemsen, A. T. M.; Hendrikse, N. H. Blood−brain barrier dysfunction in Parkinsonian midbrain in vivo. *Ann. Neurol.* **2005**, *57*, 176–179.

(24) Bartels, A. L.; Kortekaas, R.; Bart, J.; Willemsen, A. T. M.; de Klerk, O. L.; de Vries, J. J.; van Oostrom, J. C. H.; Leenders, K. L. Blood−brain barrier P-glycoprotein function decreases in specific brain regions with aging: a possible role in progressive neurodegeneration. *Neurobiol. Aging* **2009**, *30*, 1818–1824.

(25) Kwan, P.; Brodie, M. J. Potential role of drug transporters in the pathogenesis of medically intractable epilepsy. *Epilepsia* **2005**, *46*, 224–235.

(26) Löscher, W.; Klotz, U.; Zimprich, F.; Schmidt, D. The clinical impact of pharmacogenetics on the treatment of epilepsy. *Epilepsia* **2009**, *50*, 1–23.

(27) Giessmann, T.; May, K.; Modess, C.; Wegner, D.; Hecker, U.; Zschiesche, M.; Dazert, P.; Grube, M.; Schroeder, E.; Warzok, R.; Cascorbi, I.; Kroemer, H. K.; Siegmund, W. Carbamazepine regulates intestinal P-glycoprotein and multidrug resistance protein MRP2 and influences disposition of talinolol in humans. *Clin. Pharmacol. Ther.* **2004**, *76*, 192–200.

(28) Christians, U.; Schmitz, V.; Haschke, M. Functional interactions between P-glycoprotein and CYP3A in drug metabolism. *Expert Opin. Drug Metab. Toxicol.* **2005**, *1*, 641–654.

(29) 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*, 525–528.

(30) Lazarowski, A.; Czornyj, L.; Lubienieki, F.; Girardi, E.; Vazquez, S.; D'Giano, C. ABC transporters during epilepsy and mechanisms underlying multidrug resistance in refractory epilepsy. *Epilepsia* **2007**, *48*, 140–149.

(31) Marchi, N.; Hallene, K. L.; Kight, K. M.; Cucullo, L.; Moddel, G.; Bingaman, W.; Dini, G.; Vezzani, A.; Janigro, D. Significance of MDR1 and multiple drug resistance in refractory human epileptic brain. *BMC Med.* **2004**, *2*, 37–46.

(32) Aronica, E.; Gorter, J. A.; Ramkema, M.; Redeker, S.; Ozbas-Gerçeker, F.; van Vliet, E. A.; Scheffer, G. L.; Scheper, R. J.; van der Valk, P.; Baayen, J. C.; Troost, D. Expression and cellular distribution of multidrug resistance-related proteins in the hippocampus of patients with mesial temporal lobe epilepsy. *Epilepsia* **2004**, *45*, 441–451.

(33) Robinson, L. J.; Roberts, W. K.; Ling, T. T.; Lamming, D.; Sternberg, S. S.; Roepe, P. D. Human MDR 1 protein overexpression delays the apoptotic cascade in Chinese hamster ovary fibroblasts. *Biochemistry* **1997**, *36*, 11169–11178.

(34) Weisburg, J. H.; Curcio, M.; Caron, P. C.; Raghu, G.; Mechetner, E. B.; Roepe, P. D.; Scheinberg, D. A. The multidrug resistance phenotype confers immunological resistance. *J. Exp. Med.* **1996**, *183*, 2699–2704.

(35) Smyth, M. J.; Krasovskis, E.; Sutton, V. R.; Johnstone, R. W. The drug efflux protein, P-glycoprotein, additionally protects drug-resistant tumor cells from multiple forms of caspase-dependent apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7024–7029.

(36) Martin, C.; Berridge, G.; Mistry, P.; Higgins, C.; Charlton, P.; Callaghan, R. Drug binding sites on P-glycoprotein are altered by ATP binding prior to nucleotide hydrolysis. *Biochemistry* **2000**, *39*, 11901–11906.

(37) Aller, S. G.; Yu, J.; Ward, A.; Weng, Y.; Chittaboina, S.; Zhuo, R.; Harrell, P. M.; Trinh, Y. T.; Zhang, Q.; Urbatsch, I. L.; Chang, G. Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* **2009**, *323*, 1718–1722.

(38) Sharom, F. J.; Siarheyeva, A. Functional Assays for Identification of Compounds That Interact with P-gp. In *Multidrug Resistance: Biological and Pharmaceutical Advance in the Antitumour Treatment*; Colabufo, N. A., Ed.; Research Signpost: Kerala, India, 2008; pp 261−290.

(39) Stephens, R. H.; O'Neill, C. A.; Warhurst, A.; Carlson, G. L.; Rowland, M.; Warhurst, G. Kinetic profiling of P-glycoprotein-mediated drug efflux in rat and human intestinal epithelia. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 584–591.

(40) Terlouw, S. A.; Masereeuw, R.; Van den Broek, P. H. H.; Notenboom, S.; Russel, F. G. M. Role of multidrug resistance protein 2 (MRP2) in glutathione−bimane efflux from Caco-2 and rat renal proximal tubule cells. *Br. J. Pharmacol.* **2001**, *134*, 931–938.

(41) Xia, C. Q.; Liu, N.; Yang, D.; Miwa, G.; Gan, L. S. Expression, localization, and functional characteristics of breast cancer resistance protein in Caco-2 cells. *Drug Metab. Dispos.* **2005**, *33*, 637–643.

(42) Taipalensuu, J.; Törnblom, H.; Lindberg, G.; Einarsson, C.; Sjöqvist, F.; Melhus, H.; Garberg, P.; Sjöström, B.; Lundgren, B.; Artursson, P. Correlation of gene expression of ten drug efflux proteins of the ATP-binding cassette transporter family in normal human jejunum and in human intestinal epithelial Caco-2 cell monolayers. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 164–170.

(43) van Waarde, A.; Ramakrishnan, N.; Rybczynska, A.; Elsinga, P.; Berardi, F.; De Jong, J.; Kwizera, C.; Perrone, R.; Cantore, M.; Sijbesma, J.; Dierckx, R.; Colabufo, N. A. Synthesis and preclinical evaluation of novel PET probes for P-glycoprotein function and expression. *J. Med. Chem.* **2009**, *52*, 4524–4532.

(44) Feng, B.; Mills, J. B.; Davidson, R. E.; Mireles, R. J.; Janiszewski, J. S.; Troutman, M. D.; de Morais, S. M. In vitro P-glycoprotein assays to predict the in vivo interactions of P-glycoprotein with drugs in the central nervous system. *Drug Metab. Dispos.* **2008**, *36*, 268–275.

(45) Polli, J. W.; Wring, S. A.; Humphreys, J. E.; Huang, L.; Morgan, J. B.; Webster, L. O.; Serabjit-Singh, C. S. Rational use of in vitro P-glycoprotein assays in drug discovery. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 620–628.

(46) Benet, L. Z.; Izumi, T.; Zhang, Y.; Silverman, J. A.; Wacher, V. J. Intestinal MDR transport proteins and P-450 enzymes as barriers to oral drug delivery. *J. Controlled Release* **1999**, *62*, 25–31.

(47) Custodio, J. M.; Wu, C.; Benet, L. Z. Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. *Adv. Drug Delivery Rev.* **2008**, *60*, 717–733.

(48) Mohri, K.; Uesawa, Y. Enzymatic activities in the microsomes prepared from rat small intestinal epithelial cells by differential procedures. *Pharm. Res.* **2001**, *18*, 1232–1236.

(49) Engman, H. A.; Lennernäs, H.; Taipalensuu, J.; Otter, C.; Leidvik, B.; Artursson, P. CYP3A4, CYP3A5, and MDR1 in human small and large intestinal cell lines suitable for drug transport studies. *J. Pharm. Sci.* **2001**, *90*, 1736–1751.

(50) Fayer, J. L.; Petullo, D. M.; Ring, B. J.; Wrighton, S. A.; Ruterbories, K. J. A novel testosterone 6 beta-hydroxylase activity assay for the study of CYP3A-mediated metabolism, inhibition, and induction in vitro. *J. Pharmacol. Toxicol. Methods* **2001**, *46*, 117–123.

(51) Grass, G. M.; Sweetana, S. A. In vitro measurement of gastrointestinal tissue permeability using a new diffusion cell. *Pharm. Res.* **1988**, *5*, 372–376.

(52) Castella, M. E.; Reist, M.; Mayer, J. M.; Turban, J. J.; Testa, B.; Boursier-Neyret, C.; Walther, B.; Delbos, J. M.; Carrupt, P. A. Development of an in vitro rat intestine segmental perfusion model to investigate permeability and predict oral fraction absorbed. *Pharm. Res.* **2006**, *23*, 1543–1553.

(53) Gotoh, Y.; Kamada, N.; Momose, D. The advantages of the Ussing chamber in drug absorption studies. *J. Biomol. Screening* **2005**, *10*, 517–523.

(54) Barthe, L.; Woodley, J. F.; Kenworthy, S.; Houin, G. An improved everted gut sac as a simple and accurate technique to measure paracellular transport across the small intestine. *Eur. J. Drug Metab. Pharmacokinet.* **1998**, *23*, 313–323.

(55) Barthe, L.; Bessouet, M.; Woodley, J. F.; Houin, G. The improved everted gut sac: a simple method to study intestinal Pglycoprotein. *Int. J. Pharm.* **1998**, *173*, 255–258.

(56) Bouer, R.; Barthe, L.; Philibert, C.; Tournaire, C.; Woodley, J.; Houin, G. The roles of P-glycoprotein and intracellular metabolism in the intestinal absorption of methadone: in vitro studies using the rat everted intestinal sac. *Fundam. Clin. Pharmacol.* **1999**, *13*, 494–500.

(57) Arellano, C.; Philibert, C.; Vachoux, C.; Woodley, J.; Houin, G. The metabolism of midazolam and comparison with other CYP enzyme substrates during intestinal absorption: in vitro studies with rat everted gut sacs. *J. Pharm. Pharm. Sci.* **2007**, *10*, 26–36.

(58) Mols, R.; Brouwers, J.; Schinkel, A. H.; Annaert, P.; Augustijns, P. Intestinal perfusion with mesenteric blood sampling in wild-type and knockout mice: evaluation of a novel tool in biopharmaceutical drug profiling. *Drug Metab. Dispos.* **2009**, *37*, 1334–1337.

(59) Zhao, R.; Kalvass, J. C.; Pollack, G. M. Assessment of blood−brain barrier permeability using the in situ mouse brain perfusion technique. *Pharm. Res.* **2009**, *26*, 1657–1664.

(60) Smith, Q. R.; Allen, D. D. In situ brain perfusion technique. *Methods Mol. Med.* **2003**, *89*, 209–218.

(61) Cano-Gauci, D. F.; Riordan, J. R. Action of calcium antagonists on multidrug resistant cells. Specific cytotoxicity independent of increased cancer drug accumulation. *Biochem. Pharmacol.* **1987**, *36*, 2115–2123.

(62) Teodori, E.; Dei, S.; Martelli, C.; Scapecchi, S.; Gualtieri, F. Multidrug Resistance (MDR) Modulators: Verapamil Offsprings and Heterocyclic Derivatives. In *Multidrug Resistance: Biological and Pharmaceutical Advance in the Antitumour Treatment*; Colabufo, N. A., Ed.; Research Signpost: Kerala, India, 2008; pp 141−169.

(63) Foxwell, B. M.; Mackie, A.; Ling, V.; Ryffel, B. Identification of the multidrug resistance-related P-glycoprotein as a cyclosporine binding protein. *Mol. Pharmacol.* **1989**, *36*, 543–546.

(64) Solary, E.; Velay, I.; Chauffert, B.; Caillot, D.; Bidan, J. M.; Dumas, M.; Casasnovas, O.; Guy, H. Quinine circumvents the doxorubicin resistance of a multidrug resistant human leukemic cell-line, K562/DXR. *Nouv. Rev. Fr. Hematol.* **1990**, *32*, 361–363.

(65) Hu, X. F.; Nadalin, G.; De Luise, M.; Martin, T. J.; Wakeling, A.; Huggins, R.; Zalcberg, J. R. Circumvention of doxorubicin resistance in multi-drug resistant human leukaemia and lung cancer cells by the pure antioestrogen ICI 164384. *Eur. J. Cancer* **1991**, *27*, 773–777.

(66) Krishna, R.; Mayer, L. D. Multidrug resistance (MDR) in cancer. Mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. *Eur. J. Pharm. Sci.* **2000**, *11*, 265–283.

(67) Thomas, H.; Coley, H. M. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting P-glycoprotein. *Cancer Control* **2003**, *10*, 159–165.

(68) Modok, S.; Mellor, H. R.; Callaghan, R. Modulation of multidrug resistance efflux pump activity to overcome chemoresistance in cancer. *Curr. Opin. Pharmacol.* **2006**, *6*, 350–354.

(69) Stupp, R.; Bauer, J.; Pagani, O.; Gerard, B.; Cerny, T.; Sessa, C.; Bastian, G.; Sarkany, M.; Schläpfer, J.; Giroux, B.; Leyvraz, S. Ventricular arrhythmia and torsade de pointe: dose limiting toxicities of the MDR-modulator S9788 in a phase I trial. *Ann. Oncol.* **1998**, *9*, 1233–1242.

(70) Wandel, C.; Kim, R. B.; Kajiji, S.; Guengerich, P.; Wilkinson, G. R.; Wood, A. J. P-glycoprotein and cytochrome P-450 3A inhibition: dissociation of inhibitory potencies. *Cancer Res.* **1999**, *59*, 3944–3948.

(71) Minderman, H.; O'Loughlin, K. L.; Pendyala, L.; Baer, M. R. VX-710 (biricodar) increases drug retention and enhances chemosensitivity in resistant cells overexpressing P-glycoprotein, multidrug resistance protein, and breast cancer resistance protein. *Clin. Cancer Res.* **2004**, *10*, 1826–1834.

(72) Brendel, C.; Scharenberg, C.; Dohse, M.; Robey, R. W.; Bates, S. E.; Shukla, S.; Ambudkar, S. V.; Wang, Y.; Wennemuth, G.; Burchert, A.; Boudriot, U.; Neubauer, A. Imatinib mesylate and nilotinib (AMN107) exhibit high-affinity interaction with ABCG2 on primitive hematopoietic stem cells. *Leukemia* **2007**, *21*, 1267–1275.

(73) Schmidt, M.; Ungvári, J.; Glöde, J.; Dobner, B.; Langner, A. New 1,3-dioxolane and 1,3-dioxane derivatives as effective modulators to overcome multidrug resistance. *Bioorg. Med. Chem.* **2007**, *15*, 2283–2297.

(74) Lee, B. D.; Li, Z.; French, K. J.; Zhuang, Y.; Xia, Z.; Smith, C. D. Synthesis and evaluation of dihydropyrroloquinolines that selectively antagonize P-glycoprotein. *J. Med. Chem.* **2004**, *47*, 1413–1422.

(75) D'Elia, P.; De Matteis, F.; Dragoni, S.; Shah, A.; Sgaragli, G.; Valoti, M. DP7, a novel dihydropyridine multidrug resistance reverter, shows only weak inhibitory activity on human CYP 3A enzyme(s). *Eur. J. Pharmacol.* **2009**, *614*, 7–13.

(76) Berger, D.; Citarella, R.; Dutia, M.; Greenberger, L.; Hallett, W.; Paul, R.; Powell, D. Novel multidrug resistance reversal agents. *J. Med. Chem.* **1999**, *42*, 2145–2461.

(77) Bisi, A.; Gobbi, S.; Rampa, A.; Belluti, F.; Piazzi, L.; Valenti, P.; Gyemant, N.; Molnár, J. New potent P-glycoprotein inhibitors carrying a polycyclic scaffold. *J. Med. Chem.* **2006**, *49*, 3049–3051.

(78) Dei, S.; Budriesi, R.; Sudwan, P.; Ferraroni, M.; Chiarini, A.; Garnier-Suillerot, A.; Manetti, D.; Martelli, C.; Scapecchi, S.; Teodori, E. Diphenylcyclohexylamine derivatives as new potent multidrug resistance (MDR) modulators. *Bioorg. Med. Chem.* **2005**, *13*, 985–998.

(79) Teodori, E.; Martelli, C.; Salerno, M.; Darghal, N.; Dei, S.; Garnier-Suillerot, A.; Gualtieri, F.; Manetti, D.; Scapecchi, S.; Romanelli, M. N. Isomeric *N*,*N*-bis(cyclohexanol)amine aryl esters: the discovery of a new class of highly potent P-glycoprotein (Pgp)-dependent multidrug resistance (MDR) inhibitors. *J. Med. Chem.* **2007**, *50*, 599–602.

(80) Ford, J. M.; Prozialeck, W. C.; Hait, W. N. Structural features determining activity of phenothiazines and related drugs for inhibition of cell growth and reversal of multidrug resistance. *Mol. Pharmacol.* **1989**, *35*, 105–115.

(81) Teodori, E.; Dei, S.; Scapecchi, S.; Gualtieri, F. The medicinal chemistry of multidrug resistance (MDR) reversing drugs. *Farmaco* **2002**, *57*, 385–415.

(82) Boumendjel, A.; Macalou, S.; Ahmed-Belkacem, A.; Blanc, M.; Di Pietro, A. Acridone derivatives: design, synthesis, and inhibition of breast cancer resistance protein ABCG2. *Bioorg. Med. Chem.* **2007**, *15*, 2892–2897.

(83) Colabufo, N. A.; Berardi, F.; Perrone, M. G.; Cantore, M.; Niso, M.; Perrone, R. Small Molecules and Acridone Derivatives as P-gp Inhibitors. In *Multidrug Resistance: Biological and Pharmaceutical Advance in the Antitumour Treatment*; Colabufo, N. A., Ed.; Research Signpost: Kerala, India, 2008; pp 203−222.

(84) Globisch, C.; Pajeva, I. K.; Wiese, M. Structure−activity relationships of a series of tariquidar analogs as multidrug resistance modulators. *Bioorg. Med. Chem.* **2006**, *14*, 1588–1598.

(85) Jekerle, V.; Klinkhammer, W.; Reilly, R. M.; Piquette-Miller, M.; Wiese, M. Novel tetrahydroisoquinolin-ethyl-phenylamine based multidrug resistance inhibitors with broad-spectrum modulating properties. *Cancer Chemother. Pharmacol.* **2007**, *59*, 61–69.

(86) Labrie, P.; Maddaford, S. P.; Lacroix, J.; Catalano, C.; Lee, D. K.; Rakhit, S.; Gaudreault, R. C. In vitro activity of novel dual action MDR anthranilamide modulators with inhibitory activity at CYP-450. *Bioorg. Med. Chem.* **2006**, *14*, 7972–7987.

(87) Labrie, P.; Maddaford, S. P.; Lacroix, J.; Catalano, C.; Lee, D. K.; Rakhit, S.; Gaudreault, R. C. In vitro activity of novel dual action MDR anthranilamide modulators with inhibitory activity on CYP-450 (Part 2). *Bioorg. Med. Chem.* **2007**, *15*, 3854–3868.

(88) Hou, X. L.; Takahashi, K.; Tanaka, K.; Tougou, K.; Qiu, F.; Komatsu, K.; Takahashi, K.; Azuma, J. Curcuma drugs and curcumin regulate the expression and function of P-gp in Caco-2 cells in completely opposite ways. *Int. J. Pharm.* **2008**, *358*, 224–229.

(89) de Castro, W. V.; Mertens-Talcott, S.; Derendorf, H.; Butterweck, V. Grapefruit juice−drug interactions: grapefruit juice and its components inhibit P-glycoprotein (ABCB1) mediated transport of talinolol in Caco-2 cells. *J. Pharm. Sci.* **2007**, *96*, 2808–2817.

(90) Etheridge, A. S.; Black, S. R.; Patel, P. R.; So, J.; Mathews, J. M. An in vitro evaluation of cytochrome P450 inhibition and P-glycoprotein interaction with goldenseal, *Ginkgo biloba*, grape seed, milk thistle, and ginseng extracts and their constituents. *Planta Med.* **2007**, *73*, 731–741.

(91) Colabufo, N. A.; Berardi, F.; Contino, M.; Inglese, C.; Perrone, R. Flavonoids as MDR Modulating Agents: SAR Studies. In *Multidrug Resistance: Biological and Pharmaceutical Advance in the Antitumour Treatment*; Colabufo, N. A., Ed.; Research Signpost: Kerala, India, 2008; pp 171−201.

(92) Peck, R. A.; Hewett, J.; Harding, M. W.; Wang, Y. M.; Chaturvedi, P. R.; Bhatnagar, A.; Ziessman, H.; Atkins, F.; Hawkins, M. J. Phase I and pharmacokinetic study of the novel MDR1 and MRP1 inhibitor biricodar administered alone and in combination with doxorubicin. *J. Clin. Oncol.* **2001**, *19*, 3130–3141.

(93) 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*, 924–932.

(94) Bates, S. E.; Bakke, S.; Kang, M.; Robey, R. W.; Zhai, S.; Thambi, P.; Chen, C. C.; Patil, S.; Smith, T.; Steinberg, S. M.; Merino, M.; Goldspiel, B.; Meadows, B.; Stein, W. D.; Choyke, P.; Balis, F.; Figg, W. D.; Fojo, T. A phase I/II study of infusional vinblastine with the P-glycoprotein antagonist valspodar (PSC 833) in renal cell carcinoma. *Clin. Cancer Res.* **2004**, *10*, 4724–4733.

(95) Chico, I.; Kang, M. H.; Bergan, R.; Abraham, J.; Bakke, S.; Meadows, B.; Rutt, A.; Robey, R.; Choyke, P.; Merino, M.; Goldspiel, B.; Smith, T.; Steinberg, S.; Figg, W. D.; Fojo, T.; Bates, S. Phase I study of infusional paclitaxel in combination with the P-glycoprotein antagonist PSC 833. *J. Clin. Oncol.* **2001**, *19*, 832–842.

(96) Minami, H.; Ohtsu, T.; Fujii, H.; Igarashi, T.; Itoh, K.; Uchiyama-Kokubu, N.; Aizawa, T.; Watanabe, T.; Uda, Y.; Tanigawara, Y.; Sasaki, Y. Phase I study of intravenous PSC-833 and doxorubicin: reversal of multidrug resistance. *Jpn. J. Cancer Res.* **2001**, *92*, 220–230.

(97) Fracasso, P. M.; Blum, K. A.; Ma, M. K.; Tan, B. R.; Wright, L. P.; Goodner, S. A.; Fears, C. L.; Hou, W.; Arquette, M. A.; Picus,

J.; Denes, A.; Mortimer, J. E.; Ratner, L.; Ivy, S. P.; McLeod, H. L. Phase I study of pegylated liposomal doxorubicin and the multidrug-resistance modulator, valspodar. *Br. J. Cancer* **2005**, *93*, 46–53.

(98) Bauer, K. S.; Karp, J. E.; Garimella, T. S.; Wu, S.; Tan, M.; Ross, D. D. A phase I and pharmacologic study of idarubicin, cytarabine, etoposide, and the multidrug resistance protein (MDR1/Pgp) inhibitor PSC-833 in patients with refractory leukemia. *Leuk. Res.* **2005**, *29*, 263–271.

(99) Carlson, R. W.; O'Neill, A. M.; Goldstein, L. J.; Sikic, B. I.; Abramson, N.; Stewart, J. A.; Davidson, N. E.; Wood, W. C. Eastern Cooperative Oncology Group. A pilot phase II trial of valspodar modulation of multidrug resistance to paclitaxel in the treatment of metastatic carcinoma of the breast (E1195): a trial of the Eastern Cooperative Oncology Group. *Cancer Invest.* **2006**, *24*, 677–681.

(100) www.clinicaltrials.gov/NCT00002937.

(101) van der Holt, B.; Löwenberg, B.; Burnett, A. K.; Knauf, W. U.; Shepherd, J.; Piccaluga, P. P.; Ossenkoppele, G. J.; Verhoef, G. E.; Ferrant, A.; Crump, M.; Selleslag, D.; Theobald, M.; Fey, M. F.; Vellenga, E.; Dugan, M.; Sonneveld, P. The value of the MDR1 reversal agent PSC-833 in addition to daunorubicin and cytarabine in the treatment of elderly patients with previously untreated acute myeloid leukemia (AML), in relation to MDR1 status at diagnosis. *Blood* **2005**, *106*, 2646–2654.

(102) Greenberg, P. L.; Lee, S. J.; Advani, R.; Tallman, M. S.; Sikic, B. I.; Letendre, L.; Dugan, K.; Lum, B.; Chin, D. L; Dewald, G.; Paietta, E.; Bennett, J. M.; Rowe, J. M. Mitoxantrone, etoposide, and cytarabine with or without valspodar in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome: a phase III trial (E2995). *J. Clin. Oncol.* **2004**, *22*, 1078–1086.

(103) www.clinicaltrials.gov/NCT00004217.

(104) www.clinicaltrials.gov/NCT00005823.

(105) Lhommé, C.; Joly, F.; Walker, J. L.; Lissoni, A. A.; Nicoletto, M. O.; Manikhas, G. M.; Baekelandt, M. M.; Gordon, A. N.; Fracasso, P. M.; Mietlowski, W. L.; Jones, G. J.; Dugan, M. H. Phase III study of valspodar (PSC 833) combined with paclitaxel and carboplatin compared with paclitaxel and carboplatin alone in patients with stage IV or suboptimally debulked stage III epithelial ovarian cancer or primary peritoneal cancer. *J. Clin. Oncol.* **2008**, *26*, 2674–2682.

(106) Friedenberg, W. R.; Rue, M.; Blood, E. A.; Dalton, W. S.; Shustik, C.; Larson, R. A.; Sonneveld, P.; Greipp, P. R. Phase III study of PSC-833 (valspodar) in combination with vincristine, doxorubicin, and dexamethasone (valspodar/VAD) versus VAD alone in patients with recurring or refractory multiple myeloma (E1A95): a trial of the Eastern Cooperative Oncology Group. *Cancer* **2006**, *106*, 830–838.

(107) Agrawal, M.; Abraham, J.; Balis, F. M.; Edgerly, M.; Stein, W. D.; Bates, S.; Fojo, T.; Chen, C. C. Increased 99mTc-sestamibi accumulation in normal liver and drug-resistant tumors after the administration of the glycoprotein inhibitor, XR9576. *Clin. Cancer Res.* **2003**, *9*, 650–656.

(108) www.clinicaltrials.gov/NCT00086853.

(109) Fox, E.; Bates, S. E. Tariquidar (XR9576): a P-glycoprotein drug efflux pump inhibitor. *Expert Rev. Anticancer Ther.* **2007**, *7*, 447–459.

(110) www.clinicaltrials.gov/NCT00020514.

(111) www.clinicaltrials.gov/NCT00072202.

(112) Pusztai, L.; Wagner, P.; Ibrahim, N.; Rivera, E.; Theriault, R.; Booser, D.; Symmans, F. W.; Wong, F.; Blumenschein, G.; Fleming, D. R.; Rouzier, R.; Boniface, G.; Hortobagyi, G. N. Phase II study of tariquidar, a selective P-glycoprotein inhibitor, in patients with chemotherapy-resistant, advanced breast carcinoma. *Cancer* **2005**, *104*, 682–691.

(113) www.clinicaltrials.gov/NCT00073996.

(114) Bauer, F.; Mairinger, S.; Dörner, B.; Kuntner, C.; Stundner, G.; Bankstahl, J. P.; Wanek, T.; Stanek, J.; Müller, M.; Langer, O.; Erker, T. Synthesis and µPET evaluation of the radiolabelled P-glycoprotein inhibitor [11C]tariquidar. *Annu. Congr. Eur. Assoc. Nucl. Med.* **2009** No. OP339.

(115) Rubin, E. H.; de Alwis, D. P.; Pouliquen, I.; Green, L.; Marder, P.; Lin, Y.; Musanti, R.; Grospe, S. L.; Smith, S. L.; Toppmeyer, D. L.; Much, J.; Kane, M.; Chaudhary, A.; Jordan, C.; Burgess, M.; Slapak, C. A. A phase I trial of a potent P-glycoprotein inhibitor, zosuquidar·3HCl trihydrochloride (LY335979), administered orally in combination with doxorubicin in patients with advanced malignancies. *Clin. Cancer Res.* **2002**, *8*, 3710–3717.

(116) Sandler, A. B.; Johnson, D. H.; Herbst, R. S. Anti-vascular endothelial growth factor monoclonals in non-small cell lung cancer. *Clin. Cancer Res.* **2004**, *10*, 4258s–4262s.

(117) Tang, R.; Faussat, A. M.; Perrot, J. Y.; Marjanovic, Z.; Cohen, S.; Storme, T.; Morjani, H.; Legrand, O.; Marie, J. P. Zosuquidar restores drug sensitivity in P-glycoprotein expressing acute myeloid leukemia (AML). *BMC Cancer* **2008**, *8*, 51–59.

(118) Gerrard, G.; Payne, E.; Baker, R. J.; Jones, D. T.; Potter, M.; Prentice, H. G.; Ethell, M.; McCullough, H.; Burgess, M.; Mehta, A. B.; Ganeshaguru, K. Clinical effects and P-glycoprotein inhibition in patients with acute myeloid leukemia treated with zosuquidar trihydrochloride, daunorubicin and cytarabine. *Haematologica* **2004**, *89*, 782–790.

(119) Lancet, J. E.; Baer, M. R.; Duran, G. E.; List, A. F.; Fielding, R.; Marcelletti, J. F.; Multani, P. S.; Sikic, B. I. A phase I trial of continuous infusion of the multidrug resistance inhibitor zosuquidar with daunorubicin and cytarabine in acute myeloid leukemia. *Leuk. Res.* **2009**, *33*, 1055–1061.

(120) Morschhauser, F.; Zinzani, P. L.; Burgess, M.; Sloots, L.; Bouafia, F.; Dumontet, C. Phase I/II trial of a P-glycoprotein inhibitor, zosuquidar·3HCl trihydrochloride (LY335979), given orally in combination with the CHOP regimen in patients with non-Hodgkin's lymphoma. *Leuk. Lymphoma* **2007**, *48*, 708–715.

(121) Ruff, P.; Vorobiof, D. A.; Jordaan, J. P.; Demetriou, G. S.; Moodley, S. D.; Nosworthy, A. L.; Werner, I. D.; Raats, J.; Burgess, L. J. A randomized, placebo-controlled, double-blind phase 2 study of docetaxel compared to docetaxel plus zosuquidar (LY335979) in women with metastatic or locally recurrent breast cancer who have received one prior chemotherapy regimen. *Cancer Chemother. Pharmacol.* **2009**, *64*, 763–768.

(122) Planting, A. S.; Sonneveld, P.; van der Gaast, A.; Sparreboom, A.; van der Burg, M. E.; Luyten, G. P.; de Leeuw, K.; de Boer-Dennert, M.; Wissel, P. S.; Jewell, R. C.; Paul, E. M.; Purvis, N. B., Jr.; Verweij, J. A phase I and pharmacologic in combination with doxorubicin with advanced solid tumors. *Cancer Chemother. Pharmacol.* **2005**, *55*, 91–99.

(123) Kuppens, I. E.; Witteveen, E. O.; Jewell, R. C.; Radema, S. A.; Paul, E. M.; Mangum, S. G.; Beijnen, J. H.; Voest, E. E.; Schellens, J. H. A phase I, randomized, open-label, parallel-cohort, dose-finding study of elacridar (GF120918) and oral topotecan in cancer patients. *Clin. Cancer Res.* **2007**, *13*, 3276–3285.

(124) de Vries, N. A.; Zhao, J.; Kroon, E.; Buckle, T.; Beijnen, J. H.; van Tellingen, O. P-glycoprotein and breast cancer resistance protein: two dominant transporters working together in limiting the brain penetration of topotecan. *Clin. Cancer Res.* **2007**, *13*, 6440–6449.

(125) Gerrits, C. J.; Burris, H.; Schellens, J. H.; Eckardt, J. R.; Planting, A. S.; van der Burg, M. E.; Rodriguez, G. I.; Loos, W. J.; van Beurden, V.; Hudson, I.; Fields, S.; Von Hoff, D. D.; Verweij, J. Oral topotecan given once or twice daily for ten days: a phase I pharmacology study in adult patients with solid tumors. *Clin. Cancer Res.* **1998**, *4*, 1153–1158.

(126) Kruijtzer, C. M.; Schellens, J. H.; Mezger, J.; Scheulen, M. E.; Keilholz, U.; Beijnen, J. H.; Rosing, H.; Mathôt, R. A.; Marcus, S.; van Tinteren, H.; Baas, P. Phase II and pharmacologic study of weekly oral paclitaxel plus cyclosporine in patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.* **2002**, *20*, 4508–4516.

(127) Dörner, B.; Kuntner, C.; Bankstahl, J. P.; Bankstahl, M.; Stanek, J.; Wanek, T.; Stundner, G.; Mairinger, S.; Löscher, W.; Müller, M.; Langer, O.; Erker, T. Synthesis and small-animal positron emission tomography evaluation of [(11)C]-elacridar as a radiotracer to assess the distribution of P-glycoprotein at the blood−brain barrier. *J. Med. Chem.* **2009**, *52*, 6073–6082.

(128) www.clinicaltrials.gov/NCT00028873.

(129) Kuppens, I. E.; Bosch, T. M.; van Maanen, M. J.; Rosing, H.; Fitzpatrick, A.; Beijnen, J. H.; Schellens, J. H. Oral bioavailability of docetaxel in combination with OC144-093 (ONT-093). *Cancer Chemother. Pharmacol.* **2005**, *55*, 72–78.

(130) Luurtsema, G.; Schuit, R. C.; Klok, R. P.; Verbeek, J.; Leysen, J. E.; Lammertsma, A. A.; Windhorst, A. D. Evaluation of [11C]laniquidar as a tracer of P-glycoprotein: radiosynthesis and biodistribution in rats. *Nucl. Med. Biol.* **2009**, *36*, 643–649.

(131) Bartels, A. L.; Willemsen, A. T. M.; Kortekaas, R.; de Jong, B. M.; de Vries, R.; de Klerk, O.; van Oostrom, J. C. H.; Portman, A.; Leenders, K. L. Decreased blood−brain barrier P-glycoprotein function in the progression of Parkinson's disease, PSP and MSA. *J. Neural Transm.* **2008**, *115*, 1001–1009.

(132) Rapposelli, S.; Digiacomo, M.; Balsamo, A. P-gp transporter and its role in neurodegenerative diseases. *Curr. Top. Med. Chem.* **2009**, *9*, 209–217.

(133) Lewis, J. S.; Dearling, J. L.; Sosabowski, J. K.; Zweit, J.; Carnochan, P.; Kelland, L. R.; Coley, H. M.; Blower, P. J. Copper bis(diphosphine) complexes: radiopharmaceuticals for the detection of multi-drug resistance in tumours by PET. *Eur. J. Nucl. Med.* **2000**, *27*, 638–646.

(134) Sharma, V.; Beatty, A.; Wey, S. P.; Dahlheimer, J.; Pica, C. M.; Crankshaw, C. L.; Bass, L.; Green, M. A.; Welch, M. J.; Piwnica-Worms, D. Novel gallium(III) complexes transported by MDR1 P-glycoprotein: potential PET imaging agents for probing P-glycoprotein-mediated transport activity in vivo. *Chem. Biol.* **2000**, *7*, 335–343.

(135) Hendrikse, N. H.; de Vries, E. G.; Franssen, E. J.; Vaalburg, W.; van der Graaf, W. T. In vivo measurement of [^{11}C]verapamil kinetics in human tissues. *Eur. J. Clin. Pharmacol.* **2001**, *56*, 827–829.

(136) Bartels, A. L.; van Berckel, B. N.; Lubberink, M.; Luurtsema, G.; Lammertsma, A. A.; Leenders, K. L. Blood−brain barrier P-glycoprotein function is not impaired in early Parkinson′s disease. *Parkinsonism Relat. Disord.* **2008**, *14*, 505–508.

(137) Zoghbi, S. S.; Liow, J. S.; Yasuno, F.; Hong, J.; Tuan, E.; Lazarova, N.; Gladding, R. L.; Pike, V. W.; Innis, R. B. [^{11}C]Loperamide and its *N*-desmethyl radiometabolite are avid substrates for brain permeability−glycoprotein efflux. *J. Nucl. Med.* **2008**, *49*, 649–656.

(138) Mehta, B. M.; Rosa, E.; Biedler, J. L.; Larson, S. M. In vivo uptake of carbon-14-colchicine for identification of tumor multi-drug resistance. *J. Nucl. Med.* **1994**, *35*, 1179–1184.

(139) Salat, D. H.; Tuch, D. S.; Greve, D. N.; van der Kouwe, A. J.; Hevelone, N. D.; Zaleta, A. K.; Rosen, B. R.; Fischl, B.; Corkin, S.; Rosas, H. D.; Dale, A. M. Age-related alterations in white matter microstructure measured by diffusion tensor imaging. *Neurobiol. Aging* **2005**, *26*, 1215–1227.

(140) Westerlund, M.; Belin, A. C.; Olson, L.; Galter, D. Expression of multi-drug resistance 1 mRNA in human and rodent tissues: reduced levels in Parkinson patients. *Cell Tissue Res.* **2008**, *334*, 179–185.

(141) Liu, J.; Liu, Y.; Powell, D. A.; Waalkes, M. P.; Klaassen, C. D. Multidrug-resistance mdr1a/1b double knockout mice are more sensitive than wild type mice to acute arsenic toxicity, with higher arsenic accumulation in tissues. *Toxicology* **2002**, *170*, 55–62.

(142) Toornvliet, R.; van Berckel, B. N.; Luurtsema, G.; Lubberink, M.; Geldof, A. A.; Bosch, T. M.; Oerlemans, R.; Lammertsma, A. A.; Franssen, E. J. Effect of age on functional P-glycoprotein in the blood−brain barrier measured by use of (*R*)-[(11)C]verapamil and positron emission tomography. *Clin. Pharmacol. Ther.* **2006**, *79*, 540–548.

(143) Hennessy, M.; Kelleher, D.; Spiers, J. P.; Barry, M.; Kavanagh, P.; Back, D.; Mulcahy, F.; Feely, J. St Johns wort increases expression of P-glycoprotein: implications for drug interactions. *Br. J. Clin. Pharmacol.* **2002**, *53*, 75–82.

(144) Kim, W. Y.; Benet, L. Z. P-glycoprotein (P-gp/MDR1)-mediated efflux of sex-steroid hormones and modulation of P-gp expression in vitro. *Pharm. Res.* **2004**, *21*, 1284–1293.

(145) Cirrito, J. R.; Deane, R.; Fagan, A. M.; Spinner, M. L.; Parsadanian, M.; Finn, M. B.; Jiang, H.; Prior, J. L.; Sagare, A.; Bales, K. R.; Paul, S. M.; Zlokovic, B. V.; Piwnica-Worms, D.; Holtzman, D. M. P-glycoprotein deficiency at the blood−brain barrier increases amyloid-beta deposition in an Alzheimer disease mouse model. *J. Clin. Invest.* **2005**, *115*, 3285–3290.

(146) Zlokovic, B. V. Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends Neurosci.* **2005**, *28*, 202–208.

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

(148) Klunk, W. E.; Engler, H.; Nordberg, A.; Wang, Y.; Blomqvist, G.; Holt, D. P.; Bergström, M.; Savitcheva, I.; Huang, G. F.; Estrada, S.; Ausén, B.; Debnath, M. L.; Barletta, J.; Price, J. C.; Sandell, J.; Lopresti, B. J.; Wall, A.; Koivisto, P.; Antoni, G.; Mathis, C. A.; Langström, B. Imaging brain amyloid in Alzheimer's disease with Pittsburgh compound-B. *Ann. Neurol.* **2004**, *55*, 306–319.

(149) Small, G. W.; Kepe, V.; Ercoli, L. M.; Siddarth, P.; Bookheimer, S. Y.; Miller, K. J.; Lavretsky, H.; Burggren, A. C.; Cole, G. M.; Vinters, H. V.; Thompson, P. M.; Huang, S. C.; Satyamurthy, N.; Phelps, M. E.; Barrio, J. R. PET of brain amyloid and tau in mild cognitive impairment. *N. Engl. J. Med.* **2006**, *355*, 2652–2663.

(150) Langer, O.; Bauer, M.; Haers, A.; Karch, R.; Pataraia, E.; Koepp, M. J.; Abrahim, A.; Luurtsema, G.; Brunner, M.; Sunder-Plassmann, R.; Zimprich, F.; Joukhadar, C.; Gentzsch, S.; Dudczak, R.; Kletter, K.; Müller, M.; Baumgartner, C. Pharmacoresistance in epilepsy: a pilot PET study with the P-glycoprotein substrate *R*-[^{11}C]verapamil. *Epilepsia* **2007**, *48*, 1774–1784.

(151) Rao, V. V.; Dahlheimer, J. L.; Bardgett, M. E.; Snyder, A. Z.; Finch, R. A.; Sartorelli, A. C.; Piwnica-Worms, D. Choroid plexus epithelial expression of MDR1 P glycoprotein and multi-drug resistance-associated protein contribute to the blood-cerebrospinal-fluid drug-permeability barrier. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3900–3905.

(152) Hoffmeyer, S.; Burk, O.; von Richter, O.; Arnold, H. P.; Brockmöller, 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. U.S.A.* **2000**, *97*, 3473–3478.

(153) Zimprich, F.; Sunder-Plassmann, R.; Stogmann, E.; Gleiss, A.; Dal-Bianco, A.; Zimprich, A.; Plumer, S.; Baumgartner, C.; Mannhalter, C. Association of an ABCB1 gene haplotype with pharmacoresistance in temporal lobe epilepsy. *Neurology* **2004**, *63*, 1087–1089.

(154) Sisodiya, S. M.; Bates, S. E. Treatment of drug resistance in epilepsy: one step at a time. *Lancet Neurol.* **2006**, *5*, 380–381.

(155) Bates, S. F.; Chen, C.; Robey, R.; Kang, M; Figg, W. D.; Fojo, T. Reversal of multidrug resistance: lessons from clinical oncology. *Novartis Found. Symp.* **2002**, *243*, 83–96.

(156) Lee, Y. J.; Maeda, J.; Kusuhara, H.; Okauchi, T.; Inaji, M.; Nagai, Y.; Obayashi, S.; Nakao, R.; Suzuki, K.; Sugiyama, Y.; Suhara, T. In vivo evaluation of P-glycoprotein function at the blood−brain barrier in nonhuman primates using [^{11}C]verapamil. *J. Pharmacol. Exp. Ther.* **2006**, *316*, 647–653.

(157) Thummel, K. E.; Wilkinson, G. R. In vitro and in vivo drug interactions involving human CYP3A. *Annu. Rev. Pharmacol. Toxicol.* **1998**, *38*, 389–430.

(158) Liefaard, L. C.; Ploeger, B. A.; Molthoff, C. F.; de Jong, H. W.; Dijkstra, J.; van der Weerd, L.; Lammertsma, A. A.; Danhof, M.; Voskuyl, R. A. Changes in GABAA receptor properties in amygdala kindled animals: in vivo studies using [^{11}C]flumazenil and positron emission tomography. *Epilepsia* **2009**, *50*, 88–98.

(159) Awasthi, S.; Hallene, K. L.; Fazio, V.; Singhal, S. S.; Cucullo, L.; Awasthi, Y. C.; Dini, G.; Janigro, D. RLIP76, a non-ABC transporter, and drug resistance in epilepsy. *BMC Neurosci.* **2005**, *6*, 61–71.