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Perspective

Multidrug Resistance Reversal Agents

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The development of pharmacological agents able to counteract the mechanisms of drug resistance in oncology has remained a major goal for the past 10 years. When the mechanisms of multidrug resistance have been identified, the hope of identifying molecules able to reverse simultaneously the resistance to a number of unrelated drugs has stimulated research in this field. In particular, the discovery of efflux pumps that could be inhibited with high or low selectivity by small interfering molecules was of major importance. Hundreds of compounds were selected by different approaches, with the properties of inhibiting P-glycoprotein, the most studied among these efflux pumps, at least in vitro. It is acknowledged that the promise of this field of investigations was not fulfilled; there are currently no reversal agents clinically available. In this Perspective, we will attempt to unravel the reasons underlying this failure and suggest new tracks that could be followed for a successful development of the approach of multidrug resistance reversal. Recent reviews of the mechanisms are available and will not be reproduced here.[1−5] A comprehensive listing of the molecules tested and developed to 1990 has been published.[6] Only those compounds still under exploration at the clinical level will be mentioned in this article.

1. Reasons for the Lack of Clinical Success of Multidrug Resistant (MDR) Inhibitors

The clinical activity of multidrug resistance reversal agents is dependent on several conditions that should be borne in mind when a translation from in vitro identification to clinical trials is considered: (1) The tumors to be treated must be resistant to chemotherapy, at least in a significant part, through the MDR mechanism that is targeted. (2) The inhibition of P-glycoprotein (or another pump) should be feasible in tumor cells in vivo without deleterious effects in normal tissues expressing the pump. (3) The compounds used as MDR reversal agents should not have toxicity preventing a safe usage; it should be within the limits of the toxicities acceptable for anticancer treatments.

The failures that have been encountered during the development of MDR reverters have arisen mostly because one or several of these conditions were not met, as can be elaborated as follows.

1.a. Tumors treated were not truly resistant to chemotherapy or were not resistant through the mechanism targeted. At the beginning of the clinical development of MDR reverters, many drugs were tested in "refractory patients", generally in patients who had received a successful treatment 6 months earlier and who had relapsed afterward. The proof of clinical resistance was therefore not definitively provided in these trials. It is well-known that resuming a treatment after a delay of several months after its failure may recruit new responses to this treatment. The clinical trials should be performed on patients who have a documented situation of resistance to a given protocol of chemotherapy in the recent weeks before the introduction of the reversing agent, to determine whether this adjunct therapy modifies tumor response.

At the time when the first clinical trials with MDR reversal agents were implemented, the diagnosis of MDR was quite difficult and there existed large inter-

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laboratory differences that prevented any consistent view of the effect of these drugs on the MDR phenotype. This dramatic situation was claimed by reversal groups, including ours, working at the definition of standardized and agreed-upon techniques allowing homogeneous interpretations of MDR phenotyping of tumors.[7−10] Under these conditions, it appears impossible to interpret correctly the data from the clinical trials in the absence of a credible determination of the MDR phenotype of the tumor treated.

When it is clear that an MDR reversal agent is being tested on a truly drug-resistant cancer expressing the target mechanism (most often P-glycoprotein), the question arises as to whether this mechanism is unique and responsible for the drug resistance of the tumor. This may not be always the case. Let us consider, for instance, the fact that anthracyclines and taxanes, which share a common P-glycoprotein-mediated efflux mechanism, have almost additive effects in eliciting tumor responses in metastatic breast cancer.[11] It is not likely that P-glycoprotein-mediated multidrug resistance plays a significant role in breast cancer resistance to treatment. Under these conditions, drug resistance reversing agents should not be tried for this cancer but rather for cancers for which resistance is actually mediated by P-glycoprotein. Any situation of clinical cross-resistance between anthracyclines and taxanes would, in contrast, be a good one to explore the MDR reverters. This might be the case for ovarian cancers[12] or for hematological malignancies.[13] Defining the tumor type for drug development should be, therefore, the most important step for this type of approach. Non-Hodgkin's lymphomas appear paradigmatic in this type of approach because MDR is early on the major cause of drug resistance in this disease.[12,13] In the case of acute myeloid leukemia, it is clear that the expression of P-glycoprotein is at the origin of drug resistance. Studies by the Southwest Oncology Group have shown that MDR1 expression was accompanied by a markedly reduced response rate[14] and that only MDR activity was predictive of response to chemotherapy.[15]

1.b. Inhibition of P-glycoprotein in tumor cells should be feasible in vivo without deleterious effect on normal tissues expressing the pump. The efficacy of reversal agents has been generally well documented on in vitro models (direct interference with P-glycoprotein, restoration of drug accumulation, etc.), but the in vitro conditions do not correctly mimic the in vivo requirements. For instance, cell cultures most often grow in the presence of 10% fetal calf serum, which represents a 10% protein content in their environment compared to the in vivo conditions. As a consequence, protein binding is quite different and the fraction of unbound drug is not the same in in vitro and in vivo situations. It is not known whether protein binding is a key factor for MDR reversal agents activity, but if it is, then the behavior of these compounds in vivo cannot be predicted by in vitro experiments alone. It is obvious that we lack in vivo models of multidrug resistance. For many years, human tumor xenografts have been the ideal tool for anticancer drug development, but MDR cells, which are so easy to obtain and maintain in vitro, often lose their tumorogenic properties and cannot be easily transplanted in laboratory animals. However, a number of MDR tumors have been developed for growth in vivo[16−18] and have allowed the demonstration that only a small number of the MDR reverters that work in vitro can significantly reverse MDR in vivo. Such a demonstration is relatively difficult to accomplish for large-scale screening of potential reverters because of the delays in tumor growth and in establishing schedules for treatment in the absence and presence of the reversing agent. However, this step is necessarily required between the in vitro screening steps and the introduction of the MDR reverter in the clinical setting. It is emphasized that a clear demonstration of an in vivo MDR reversal activity was not made for the first molecules, such as verapamil, that were tested for MDR reversal in clinical trials.

Another useful in vivo model suitable for large-scale screening has been proposed by the group of Gottesman in the 1990s. Galski et al.[19] had produced a strain of transgenic mice overexpressing the human MDR1 gene in their bone marrow. The advantage of such a model is the possibility of restoring the hematological toxicity of an MDR drug (vinblastine, doxorubicin) by combination with a reverter, thus eliminating the requirement of a tumor model and maintaining the advantages of an in vivo exploration.[20−22] However, this invaluable tool progressively lost transgene expression and has never allowed the identification of new reverters active in vivo. A second unsuccessful inititative was published in 1998 by Evans[23] and a similar failure occurred in our laboratory (Laurand et al., unpublished results). It is likely, therefore, that such a mouse strain will be very difficult to obtain and maintain as a pharmacological tool.

An absence of deleterious effects of the reversal agent on normal tissues expressing P-glycoprotein should also be shown. It is clear that the physiological function of P-glycoprotein is to detoxify the organism from potentially toxic components present in our environment. The presence of P-glycoprotein in the liver, kidney, and gut is a good indication of this function. However, P-glycoprotein is also present in the endothelial cells of vessels present in the central nervous system and the testis, with a clear role of protection of these organs from the compounds present in the blood stream.[24] It has been shown that compounds normally devoid of neurotoxicity such as ivermectin become highly neurotoxic in mouse in which both mdr genes have been knocked out.[25] Under these conditions, it may seem dangerous to administer a potentially neurotoxic drug combined with a strong inhibitor of P-glycoprotein. In addition, such an inhibition of hepatic P-glycoprotein may lead to a major alteration of drug disposition, with gross pharmacokinetic changes that may increase the general toxicity of the anticancer treatment. There is, therefore, a relatively narrow window for the use of MDR reversal agents, the most active ones on tumor cells being also those that increase most of the general toxicity of the anticancer agent.

1.c. Compounds used for MDR reversal should not have an intrinsic toxicity preventing safe usage. This important condition was put at the first level by pharmaceutical companies developing MDR reversal agents. This explains the successive steps that were followed and the three generations of compounds that were developed. The first step was the use of

compounds already used clinically for other specific therapeutic applications. Obviously, these compounds had an intrinsic toxicity because they were pharmacologically active, and this hindered the use of effective MDR reversal doses of these compounds. This was the case for verapamil, cyclosporin A, and quinidine, which cannot be used safely at the dosages required for MDR reversal. The second step was to identify analogues of those compounds that were devoid of the pharmacological properties of the original molecule. This approach was quite interesting and should have led to the identification of clinically useful compounds. However, because no toxicity was accepted for these compounds in the clinical trials, the stringent conditions followed have prevented the complete development of such drugs. For instance, dextroverapamil (one of the two enantiomers of racemic verapamil with a 200-fold lower effect on calcium channels) was withdrawn from the clinical trial despite quite conclusive results as an MDR reverter.[26] Another compound, valspodar, a nonimmunosuppressive analogue of cyclosporin, has been followed further, up to phase III studies (see below).

The third generation of MDR reversing compounds constitutes novel molecules first selected on the basis of structural features (lipophilicity, positive charge at neutral pH, presence of aromatic rings) and then submitted to in vitro screening. There again, the challenge of a complete absence of proper toxicity has prevented the full evaluation of compounds presenting major MDR reversal properties. This is the case for S9788, a triazine piperidinyl compound that was withdrawn from the trials because of cardiac toxicity occurring at the highest dosage utilized (see below).

The difficulties in clinical development of combinations of an active drug with a modulator of its activity have somewhat discouraged the pharmaceutical industry from fully analyzing the efficacy of compounds such as GF120918, an acridone carboxamide derivative, active on both MDR1 and BCRP gene products,[27] and MS-209, another bifunctional compound active on both MDR1 and MRP1 gene products.[28] The requirements of high efficacy and total lack of toxicity may have been set at too high a level for a positive identification of clinically useful compounds. Dealing with a disease for which the positive effect of a drug or a combination is evaluated in months of survival should have led to the acceptance of some toxicity balanced against the gain in survival. As a consequence, despite the fact that the "proof of principle" has been given in several instances at the phase II level for several drugs, very few phase III trials have been conducted and not a single compound has been approved for routine use or is close to such an approval. This is unfortunate because it appears that it would have been possible to bring valuable compounds to the clinics if a better understanding among clinicians, pharmacologists, and the pharmaceutical industry had existed.

2. P-glycoprotein Drug-Binding Sites for Transport and Modulation

One of the key issues for the design and/or discovery of modulators of multidrug resistance is the identification of the target sites on P-glycoprotein. This has been a relatively difficult task because P-glycoprotein does not offer a simple and well-defined active site that could be easily modeled. Three major difficulties arise when considering the study of P-glycoprotein drug-binding sites: (1) P-glycoprotein is a membrane protein strongly embedded in the lipid phase of plasma membranes and cannot be purified at a sufficient level for crystallization studies. (2) P-glycoprotein has a very wide specificity for the substrates transported, and it was not clear whether the modulators were also substrates for transport. (3) P-glycoprotein is also an ATPase, and the molecules interfering with ATP binding and cleavage can also present modulating properties. We will discuss in this section the present knowledge of the P-glycoprotein−modulator interaction(s) and the clues that may exist for the discovery of novel modulators. Two different approaches have been developed: those starting from the structure of P-glycoprotein to define the drug-binding sites and those starting from the structure−activity relationships existing among the wide variety of P-glycoprotein-interfering drugs, substrates, or modulators.

2.a. P-glycoprotein Drug-Binding Sites. Drug-binding and photoaffinity labeling studies have established well a direct interaction between P-glycoprotein and its substrates.[29−34] From these studies and from the sequencing of P-glycoprotein from cells with altered resistance profiles, it became clear that the predicted transmembrane domains (TM) played a critical role in the recognition and transport of substrates. These domains even appear to be sufficient to mediate drug binding in deletion mutants lacking the nucleotide-binding domains.[35] Mutational studies in transmembrane domains have clearly shown that amino acid substitutions at precise sites lead to alterations in substrate specificity of P-glycoprotein (for review, see ref 36). In particular, Loo and Clarke have developed a series of studies aimed at the "molecular dissection" of P-glycoprotein[37] by combining molecular biology with protein chemistry. The drug binding site or sites appear to be effectively located in transmembrane domains, especially but not exclusively TM6 and TM12, and systematic mutational studies have allowed the identification of the amino acids of these domains that were involved in drug transport. The corresponding amino acids in TM6 and TM12 play a symmetrical role, and the interface between them constitutes a drug-binding pocket. This result has been confirmed by cross-linking experiments,[38,39] which have shown that these TM segments are close to each other and undergo conformational changes during the reaction cycle.

The existence of one or several drug-binding sites is still controversial. Shapiro and Ling[40] described a cooperative interaction between the transport of two dyes, Hoechst 33342 and rhodamine 123, and concluded the existence of two drug-binding sites, H and R, characterized by specific recognition of each of the two dyes. A third drug-binding site was even hypothesized by the same group.[41] The group of Orlowski[42] also characterized two binding sites on P-glycoprotein, the first one able to bind verapamil, cyclosporin A, and actinomycin D, whereas the second one binds vinblastine. Molecular modeling has allowed the recognition of structural similarities between the drugs binding each site.[43] However, the binding sites of P-glycoprotein

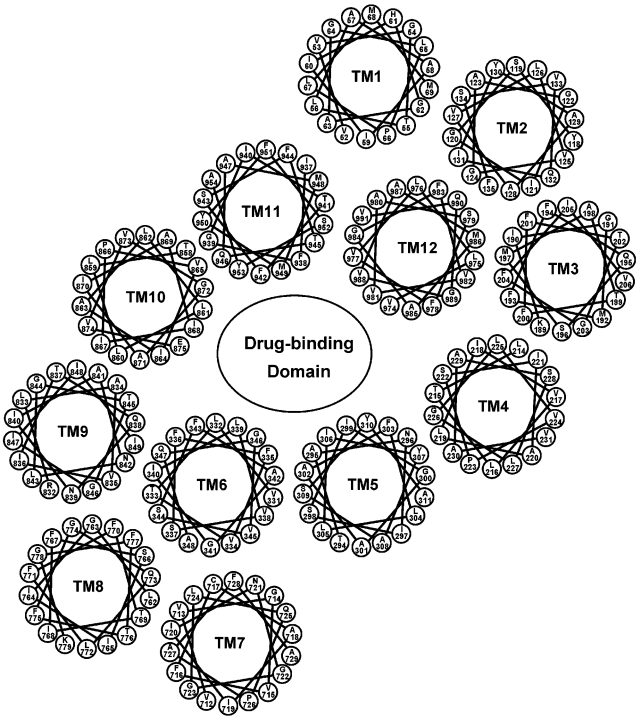

Figure 1. Topography of the transmembrane domains of P-glycoprotein delimiting the drug-binding site, as represented from the studies of Loo and Clarke.[44,45] P-glycoprotein residues within the predicted transmembrane domains (TM1−12), arranged as α-helical wheels, are shown and viewed from the cytoplasmic side of the membrane. Reprinted with permission from *Journal of Biological Chemistry*.[45] Copyright 2002 The American Society for Biochemistry and Molecular Biology.

are clearly overlapping; mutual exclusion (competitive binding between modulators) would result from steric constraints caused by this overlapping. In other words, size-related interactions between drugs able to bind at different sites may occur, the larger molecules competing with most other molecules and two molecules being able to bind simultaneously to P-glycoprotein if they are small enough.[43]

This view of the drug-binding sites on P-glycoprotein is not contradictory with the model presently defended by Loo and Clarke, who describe P-glycoprotein−drug interactions at the level of only one binding site. In this model, according to their precise sizes and structures, the various substrates would interfere with different portions of the transmembrane pore delimited by the transmembrane domains.[44,45] Figure 1 shows the assembly of the transmembrane domains to form a drug-binding site, as viewed from the mutational studies performed by Loo and Clarke. In fact, the substrates would even "create" their own binding site by using a combination of residues from different transmembrane domains to form a particular drug-binding site. This would be possible because of the mobility of transmembrane domains within the lipid bilayer, which has been shown to occur by Loo and Clarke.[46,47] Different combinations of residues would be possible, explaining why P-glycoprotein can bind such a wide variety of molecules and why a different affinity for each substrate can be measured. The mutations in the residues present in the transmembrane domains would change the interactions of the drug-binding site with definite substrates. The shape and size of the drug-binding domain can be estimated from cross-linking studies using thiol-specific methane thiosulfonate cross-linkers containing spacer arms of 2−17 atoms as "molecular rulers".[48] According to this approach, the drug-binding domain would be funnel-shaped, narrow at the cytoplasmic site, larger in the middle (0.9−2.5 nm), and wider at the extracellular surface.

The precise knowledge of the drug-binding site or sites on P-glycoprotein allow a fair understanding of drug−protein interactions and may be used for the design of new modulators. In addition, the ATP-binding sites should not be ignored, even if the MDR modulators identified today interfere with the hydrophobic drug-binding site(s) rather than with the hydrophilic nucleotide-binding sites. There is a good proportionality between the ability of P-glycoprotein to bind and transport its substrates and the stimulation of ATPase activity,[49] suggesting an interaction between the two types of active domains on P-glycoprotein. However, until now, only little attention has been paid to the means of interfering with ATP binding in order to modulate P-glycoprotein activity.

2.b. Structure−Activity Relationships among P-glycoprotein Modulators. The structural diversity of the molecules recognized and eventually transported by P-glycoprotein has been of interest for many years and has stimulated considerable effort in the identification of any common characteristics on these molecules. Some obvious features were rapidly recognized, such as the amphiphilic character of the molecules, the presence of aromatic rings, and the positive charge at neutral pH.[50] A number of studies establishing structure−activity relationships among single structural classes of MDR modulators have been published (reviewed in ref 5), but only a few studies tried to extend such approaches across different chemical families.[51] As previously noted by Pajeva and Wiese,[5] little is known about the 3D structure of P-glycoprotein and other transport proteins involved in MDR. Therefore, indirect methods have to be used by the MDR (Q)SAR investigators.

Studying the structural features shared by P-glycoprotein modulators, Seelig[52] was able to distinguish, in a wide variety of P-glycoprotein-interfering drugs, the presence of specific recognition patterns consisting of hydrogen bond acceptor (or electron donor) groups (e.g., carbonyl, ether, hydroxy, or halide groups) with precise spatial separation. Two types of patterns were defined according to the spatial separation of two electron donor groups able to accept hydrogen bonding, i.e., 0.25 ± 0.03 nm (type I) and 0.46 ± 0.06 nm (type II). In addition, the presence of a larger number of these "functional units" increases P-glycoprotein binding. As a consequence, this author suggested that P-glycoprotein modulation was based on the number and strength of electron donor groups separated by fixed distances of 0.25 or 0.46 nm in relation to the formation of hydrogen bonds. Indeed, the transmembrane domains on P-glycoprotein contain several amino acids with hydrogen bonding donor side chains, especially TM4-6 and TM11-12.[53] Most of these transmembrane groups are precisely those already shown to be responsible for drug binding and transport by studies on P-glycoprotein structure, and mutations in these domains are followed by impor-

tant modifications of substrate recognition and transport.[38,39,44,45]

The functions of transport and modulation can hardly be separated for most compounds interacting with P-glycoprotein. It was recognized by the group of Bates[54] that one could discriminate between the two properties, the best transported molecules being poor inhibitors and vice versa. The approach developed by Seelig[52,53] may explain this phenomenon, taking into account the fact that the number of P-glycoprotein-interacting groups on a molecule determines the strength of the binding; a high potential to form hydrogen bonds would correspond to a high P-glycoprotein inhibitory property, while a low potential to form these bonds would correspond to a weaker inhibitory property associated with easier transport of the molecules through the channel formed between transmembrane domains.

A quite different approach was developed by Klopman et al.[55] and Bakken and Jurs[56] to analyze the chemical features involved in P-glycoprotein-modulating properties. After determining the potency of compounds for P-glycoprotein modulation with a model of doxorubicin-resistant cells, they defined a series of "topological descriptors" such as the number of oxygen or nitrogen atoms, the number of aromatic bonds, the electrotopological character, etc. Using linear discriminant analysis of these features, these authors were able to predict with acceptable accuracy the activity of untested compounds for P-glycoprotein modulation. In this type of approach, no precise mechanistic conclusions on protein−ligand interaction can be drawn from the definition of the molecular features characterizing MDR reversal, but they may prove to be very useful for the design and/or the selection of compounds to be tested in the clinical setting.

Using in vitro data and the Catalyst software, Ekins et al.[57] built three-dimensional quantitative structure−activity relationship (3D-QSAR) models that rank and predict IC_{50} values for P-glycoprotein inhibitors. These models were obtained with data derived from several biological tests of P-glycoprotein modulation (inhibition of digoxin transport in Caco-2 cells, inhibition of vinblastine accumulation, and binding to plasma-membrane-enriched vesicles, etc.). It was thus possible to generate a pharmacophore that consisted of one hydrogen bond acceptor, one aromatic ring feature, and two hydrophobic residues. These models overlap only partially, suggesting again the presence of several drug-binding sites on P-glycoprotein, a problem discussed in the previous section. Using these statistical approaches does not allow the determination of the molecular structure of these sites, but they may be of great value for selecting new compounds for MDR reversal.

Finally, a general pharmacophore model was recently proposed by Pajeva and Wiese.[58] It is based on the study of the molecular characteristics of 19 compounds belonging to different structural classes, some studied in their enantiomeric forms. This was achieved using the GASP software (genetic algorithm similarity program). The structure proposed for the pattern of recognition by P-glycoprotein is much more complex than the simple hydrogen bond donor model proposed by Seelig[52] and, as a consequence, much more informative for the design of new modulators. It involves two hydrophobic planes, three hydrogen bond acceptors, and one hydrogen bond donor, with distances and angles evaluated with good accuracy. Figure 2 presents the molecular organization of this pharmacophore. The conclusions drawn from this modeling study of P-glycoprotein modulators are not different from those drawn from the modeling study of P-glycoprotein itself: the drug-binding site of P-glycoprotein has several points that can participate in hydrophobic and hydrogen bond interactions, and different drugs can interact with different receptor points in different binding domains.

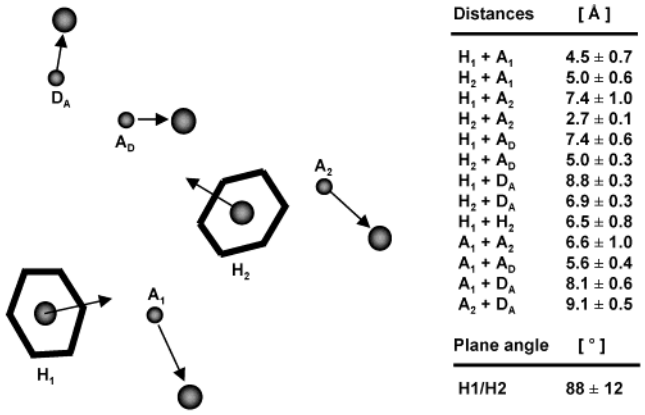

Figure 2. General pharmacophore pattern of drugs interacting with P-glycoprotein at the drug-binding site, as proposed by Pajeva and Wiese.[58] H_1 and H_2 are hydrophobic points located around the centers of the aromatic rings. A_1, A_2, and A_D are hydrogen bond acceptor points, and D_A is a hydrogen bond donor point. The arrows show directions of the hydrophobic and hydrogen bond interactions. Reprinted with permission from *Journal of Medicinal Chemistry*.[58] Copyright 2002 The American Chemical Society.

The absence of narrow specificity for P-glycoprotein substrates now appears to be well understood because of the complementary approaches targeted at both the P-glycoprotein site structure and the modulator structure. We now have in hand the tools to screen known drugs for their potential as P-glycoprotein inhibitors and to design new drugs with the desired inhibitor properties.

3. Present Status of MDR Reversing Agents

It is not possible to gather in a single list all of the compounds that have been tested in vitro and recognized as potent MDR reversing agents. We have tried in Table 1 to present the compounds that have been tested in the clinical setting; the three-generation classification has been used to distribute these compounds, but this does not preclude a high interest for molecules of the second generation. The reasons that compounds have not been selected for routine use and developed for approval are tentatively indicated in this table when they have been identified. The structures of verapamil and quinine, as well as those of the original compounds that were developed for clinical use, are presented in Figure 3. With the exception of cyclosporin A and its analogues, the classical common structural features are obvious; the presence of aromatic rings and the presence of at least one protonatable nitrogen are the most striking ones.

3.a. Verapamil and Dextroverapamil. As early as 1987, results from the first clinical trial of MDR reversal

Table 1. List of Compounds That Have Been Tested in the Clinics for Multidrug Resistance Reversal

	pharmacological properties	reason for stopping trials
first-generation compounds[a]	medical use	
verapamil[59−68,70,134]	coronary vasodilator	too toxic by itself
nifedipine[135]	coronary vasodilator	not active enough
trifluoperazine[136−138]	antipsychotic	not active enough
cyclosporine A[76−79,81,82,84−88,139,140]	immunosuppressive	too toxic by itself
quinidine[106]	antiarrhythmic	too toxic by itself
quinine[107,109,141]	antimalarial	not active enough
tamoxifen[69,142−144]	antiestrogen	not active enough
progesterone[145]	progestative	not active enough
dipyridamole[146,147]	coronary vasodilator	not active enough
amiodarone[148]	coronary vasodilator	not active enough
bepridil[149]	coronary vasodilator	not active enough
second-generation compounds[a]	analogy to	
dexverapamil[26,71−75]	verapamil	too toxic by itself
dexniguldipine[150]	nifedipine	not active enough
trans-flupentixol	trifluoperazine	not yet tested
valspodar (psc-833)[91−105]	cyclosporine a	still in trials
cinchonine[110]	quinine	still in trials
MS-209	quinine	not yet tested
toremifene[111]	tamoxifen	still in trials
BIBW22BS	dipyridamole	not yet tested
third-generation compounds[a]	chemical structure	
VX-710 (biricodar)[112,113]	piperidine carboxylate	still in trials
S-9788[116−117]	triazinopiperidine	too toxic by itself
GF-120918[119]	acridone carboxamide	no objective reason
LY-335979[99]	dibenzosuberane	still in trials
XR-9576[126]	anthranilamide	still in trials

[a] Definition of generation is as follows. First generation: drugs already in current use in clinics for other indications. Second generation: analogues of the first-generation drugs. Third generation: drugs of original structure developed for the purpose of MDR reversal.

in ovarian cancer with verapamil in combination with doxorubicin were published.[59] Because of significant cardiac toxicity, the study was discontinued. In myeloma and non-Hodgkin's lymphomas, verapamil was clearly shown to be active in situations of resistance to standard protocols with anthracyclines and/or Vinca alkaloids.[60−63] However, patient survival was not increased in a phase III study combining verapamil and cytotoxic drugs in the treatment of multiple myeloma.[64] In contrast to hematological malignancies, refractory solid tumors were never shown to respond to verapamil when this reverter was added to classical chemotherapy, likely because the doses administered were too low as a consequence of the fear of cardiac toxicity.[65−67] The encouraging results obtained in no-small-cell lung cancer and pediatric cancers were not confirmed.[68,69] An interesting randomized study performed in anthracycline-resistant metastatic breast cancer patients treated by vindesine with or without verapamil revealed a significant increase of survival of patients receiving verapamil.[70]

Proof of activity of dextroverapamil was found in a phase I study[71] and in lymphoma[26] and breast cancer[72] patients in phase II studies but not in colorectal[73] or renal[74] cancer patients. Despite its potential interest, this drug has not been developed because its cardiac toxicity was judged to be unacceptable.[75]

3.b. Cyclosporins. Cyclosporin A entered very early into trials of reversal of multidrug resistance, despite the fact that its pharmacological properties could lead to unacceptable toxicity symptoms. It was rapidly clear that cyclosporin A exerted a major pharmacokinetic effect; there is an almost doubling of the area under the concentration−time curve of the anticancer agent when combined with cyclosporin A,[76−78] preventing a clear analysis of the effects seen. The proof of reversing activity of cyclosporin A was found in phase II studies with myeloma[79] and acute leukemias,[80] but no responders were recruited among colorectal[81] or renal[82] adenocarcinomas and no benefit was observed in non-Hodgkin's lymphoma.[83] However, even in hematological malignancies, when randomized phase III studies were conducted, no effects of cyclosporin A on the overall response rate, progression-free survival, and overall survival were detected in advanced refractory myeloma patients,[84] whereas only one study[85] out of four[86−88] showed a positive effect of cyclosporin A in acute myeloblastic leukemia.

Valspodar (PSC-833, Novartis) is certainly the compound that has been the most extensively studied. This is a cyclosporin analogue that has been selected among others because of the absence of immunosuppressive properties.[89] In preclinical models, it shows a 10-fold higher potency in MDR reversal together with lower renal toxicity.[90] During the phase I studies conducted in several countries, an important effect of this compound on the pharmacokinetics of the associated anticancer drug was shown, the anticancer drug being etoposide,[91] doxorubicin,[92] mitoxantrone,[93] or paclitaxel.[94] In most cases, either a doubling of the time−plasma concentration area under the curve or an important increase in elimination half-life was found. Therefore, it was not possible to definitely separate the *pharmacokinetic* effects of valspodar (increase in exposure to the anticancer drug) from its *pharmacodynamic* effects (increase in cancer cell uptake of anticancer drug). As a consequence, the proposal of reducing by 30−50% the dose of the anticancer drug was made by Novartis. However, despite the pharmacological rationale of such a reduction, the clinicians remained relatively reluctant to participate in such trials because of the risk of underdosing the patients.

In acute myeloid leukemia, after several phase I or I/II studies,[93,95−98] two phase III studies were under-

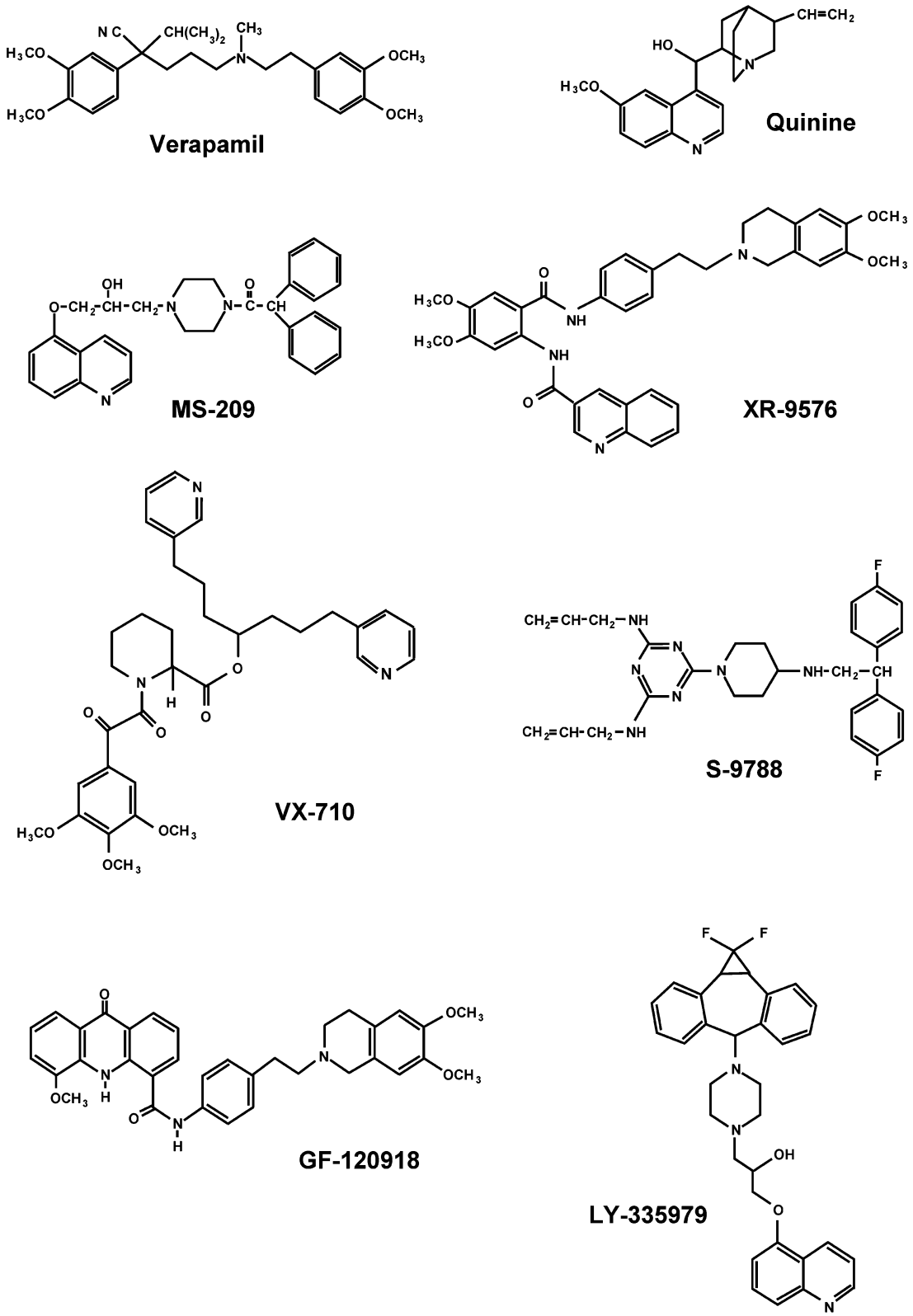

Figure 3. Structural formulas of some of the compounds that have been developed for multidrug resistance reversal and have entered clinical trials.

taken. The results of the one conducted by the cancer and leukemia group B have been recently published.[99] In this study, valspodar was administered as a continuous infusion at a dose of 10 mg/kg per day in combination with a regimen containing daunorubicin, cytarabine, and etoposide. The doses of the two MDR-related drugs had been reduced in the valspodar-containing arm from 60 to 40 mg/m^2 per day for daunorubicin and from 100 to 60 mg/m^2 per day for etoposide. The study was closed earlier than planned because of excessive early mortality in the valspodar-containing arm, confirming the high toxicity of anticancer drugs to normal tissues no longer protected by P-glycoprotein. However, this study showed that in patients whose cells exhibited in

vitro a valspodar-modulated drug efflux, the median disease-free survival was increased from 5 months to 14 months when valspodar was added to the treatment. It appears at this stage that only tumors presenting a documented MDR phenotype should be included in future trials. The results of the second phase III study conducted in acute myeloid leukemia by the Eastern Cooperative Oncology Group, after the phase I and phase II study,[93] have only been published as an abstract.[100]

In ovarian cancer, only phase II studies have been conducted until now with a combination of either paclitaxel[101] or doxorubicin and cisplatin[102] with valspodar. The recruitment of a limited number of new responders in a situation of documented resistance to regimen containing the anticancer drug(s) alone warrants the implementation of randomized phase III studies in ovarian cancer.

The effect of cyclosporin A or valspodar in other malignancies treated with MDR drugs has not been assessed with enough studies to warrant further development. In particular, the absence of effect of cyclosporin A on the response rate, progression-free survival, and overall survival in myeloma patients treated by the combination of vincristine, doxorubicin, and dexamethasone[84] does not encourage any additional trials for this disease. To our knowledge, no phase II or phase III trial combining valspodar with an anthracycline, a Vinca alkaloid, or a taxane has ever been attempted. However, the modalities of combination of valspodar with vinblastine,[103] paclitaxel,[104] or doxorubicin[105] in solid tumors such as renal cell or ovarian carcinoma are still under study and warrant further attention. The pharmacokinetic interaction of cyclosporin A or valspodar with the cytotoxic drugs has considerably hampered the clinical development of these drugs as MDR modulators. The cyclosporin family appears to be the only one to present this interaction that might well be related to the specific inhibition of another hepatic ABC protein involved in biliary elimination of drugs. The inhibitory effect of cyclosporins on the bile salt export protein (BSEP, ABCB11) should be borne in mind because it may explain the fact that only cyclosporines exhibit pharmacokinetic interactions with the cytotoxic drugs.

3.c. Quinolines. Quinidine entered very early in a randomized trial in breast cancer, aimed at reversing anthracycline resistance.[106] No improvement over epirubicin alone resulted from the combination. The potential cardiac toxicity of quinidine did not encourage further evaluation.

Quinine is much less toxic than quinidine and can be used at higher doses. In a phase II study, Solary et al.[107] showed that its association with mitoxantrone and cytarabine could improve the response rate of acute leukemias with poor prognosis. This was not confirmed in a phase III randomized study.[108] Quinine was also devoid of any effect on the resistance of non-Hodgkin's lymphomas to paclitaxel,[109] which may not be surprising because this drug has no major effect on lymphomas. We have undertaken a phase II study of a combination of quinine to CEOP (cyclophosphamide, epirubicin, vincristine, and prednisone) protocol in non-Hodgkin's lymphoma patients (Soubeyran and Robert, unpublished results). The patients were selected for true resistance to CEOP reintroduction after failure of two lines of chemotherapy. Addition of quinine immediately after two courses of treatment allowed the recruitment of a significant number of responders, bringing the proof of activity of this drug as a MDR reverter in non-Hodgkin's lymphoma patients. Phase III studies remain to be performed, with an early introduction of the modulator in the therapeutic strategy.

Cinchonine is a demethoxy derivative of quinidine that has shown interesting reversing properties in vitro and in vivo. A phase I trial has recently been completed, showing no interaction with the pharmacokinetics of the anticancer drug but presenting a dose-limiting cardiac toxicity.[110]

MS-209 was developed initially as a potential multifunctional reverter because it was able to inhibit both P-glycoprotein and MRP1.[28] It has proven in vivo activity against xenografts of solid tumors in nude mice[111] but has not yet been tested in the clinics.

3.d. Third-Generation Drugs. Among the hundreds of drugs belonging to original families, only a very few were selected for clinical trials and, as mentioned before, none of them has been yet approved.

Biricodar (VX-710) was studied in two phase I trials in combination with doxorubicin[112] or paclitaxel.[113] This drug had been shown to reverse MDR in vitro and in vivo by acting on both P-glycoprotein and MRP1.[114,115] The phase I studies of this compound have shown an acceptable toxicity together with an absence of effect on doxorubicin pharmacokinetics but with a reduction in the clearance of paclitaxel. An increase in ^{99m}Tc-Sestamibi hepatic uptake and retention was observed in all patients, bringing good arguments for further evaluation of biricodar.

S-9788 was selected among thousands of compounds because of its important action against MDR cells in vitro and in vivo.[116] Phase I studies revealed cardiac toxicity at relatively high doses. Despite its potential interest and the absence of data on the frequency and lethal risk of this toxicity, this compound has not been further developed.[117]

Elacridar (GF-120918) was selected by theoretical considerations of characteristics of its structure.[27] It is probably one of the most active compounds in vitro. It has also been shown to be active against another ABC pump, BCRP (or MXR),[118] which is especially expressed in leukemias. However, it is not active on MRP1. A phase I study has been completed showing no major pharmacokinetic interaction with doxorubicin,[119] but to our knowledge no phase II study has been undertaken with the aim of reversing multidrug resistance. However, its use as an enhancer of intestinal uptake of oral paclitaxel or topotecan might be an interesting clinical application.[120,121]

Zosuquidar (LY 335979), a difluorocyclopropyldibenzosuberane derivative, is also active in the nanomolar range for inhibition of P-glycoprotein in vitro and in vivo.[122] It was developed for its high affinity for P-glycoprotein and appears devoid of other pharmacological properties on MRP1- or BCRP-mediated drug resistance.[123] Phase I results were recently reported[124] showing some risk of neurotoxicity at high dosage and no pharmacokinetic interaction with doxorubicin. The

fact that it does not alter the pharmacokinetics of the anticancer drug used in combination may be viewed either as a positive feature (no need for dose reduction of the cytotoxin, no problem in result interpretation) or as a negative one (no effect on hepatic or renal P-glycoprotein may signify no effect on tumor cell P-glycoprotein). Zosuquidar is presently in phase III trials in acute myelogenous leukemia as a first line therapy in combination with chemotherapy with daunorubicin and cytarabine.

Tariquidar (XR9576), an anthranilic acid derivative, is also active in the nanomolar range and is also devoid of pharmacokinetic interactions with paclitaxel.[125] A phase I study in healthy volunteers has shown activity on rhodamine 123 uptake by P-glycoprotein expressing lymphocytes and without toxic symptoms.[126] It is presently in phase III trials in non-small-cell lung cancer as first line therapy in combination either with paclitaxel plus carboplatin or with vinorelbine. The results of these trials are eagerly awaited by the scientific community.

4. Perspective

It appears from this clinically oriented overview that the reversal of multidrug resistance has not yet reached the level of routine clinical applications. The future of this potential therapeutic area remains uncertain. This is not for lack of molecules, since hundreds of compounds have been selected or designed with comprehensive studies on structure−activity relationships in several chemical families. Rather, the reason for this failure originates from the inadequate design of clinical trials or from the exaggeratedly high requirements by the clinicians for the reverters. It may well be that the perfect reverter does not exist. This compound, for instance, should not alter the pharmacokinetics of the cytotoxic drug in the combination; however, the presence of P-glycoprotein at the canalicular pole of the hepatocytes as well as at the luminal side of the renal tubular cells should be necessary, followed by an alteration of the distribution of the anticancer drug if it is a good substrate of P-glycoprotein. An ideal reverter should also be completely devoid of toxicity. This may be impossible, since the simple blockade of P-glycoprotein may have deleterious consequences in the tissues where it is expressed. The pharmacological and toxicological properties of these drugs should have been taken into consideration with those of the anticancer drugs in terms of the benefit/risk ratio of the combination. This has never been the case, and despite 15 years of clinical experience with MDR modulators, we still do not know much about their actual potential in the clinical setting.

Apart from the classical approach of small molecules interfering with P-glycoprotein or another pumping system, other approaches of multidrug resistance reversal have been considered in the past: the use of P-glycoprotein targeted antibodies such as UIC2;[127] the use of antisense strategies targeting the MDR1 messenger RNA.[128] More recently, the development of transcriptional regulators[129] appears promising. However, since thus far we do not know whether the MDR reversal strategy is worthy of development, these approaches appear even less realistic than the "small molecule" approach. The encapsulation of anticancer drugs in liposomes[130] or nanospheres[131] has also been claimed to be able to circumvent multidrug resistance. Among the various formulations of liposomal anthracyclines that have been studied and even marketed, none appears able to recruit responders outside the usual field of anthracycline activity. Finally, a very recent optimistic note is worth a mention:[132] Lehne et al.[133] have shown in vivo that the simple inhibition of P-glycoprotein by valspodar may lead to the direct elimination of MDR cells perhaps because this membrane pump is also involved in malignancy as well as in drug resistance. This observation warrants further exploration, and a careful reexamination of the clinical trials already performed may shed some light upon this.

Biographies

Jacques Robert obtained his M.D. and Ph.D. degrees from Université Louis Pasteur, Strasbourg, France. He moved to Bordeaux in 1978 to set up a laboratory dedicated to the study of anticancer drug pharmacology. He is presently Professor of Experimental Oncology at Université Victor Segalen, Bordeaux, France, and Head of the Pharmacology Department of Institut Bergonié, the comprehensive cancer center of Bordeaux. His main interests are the clinical, cellular, and molecular pharmacology of anthracyclines and camptothecins, with special emphasis on the molecular determinants of drug activity and toxicity in patients. He is President-Elect of the French Cancer Society and Officer of the Pharmacology and Metabolism Group of the European Organisation for Research and Treatment of Cancer (EORTC PAMM Group).

Christian Jarry, born in La Charité, France, in 1947, started his studies in chemistry at the Faculté des Sciences, Bordeaux, France, where he graduated with a Ph.D. in 1970. Then he switched to pharmacy and he obtained a Doctorat d'Etatès Sciences Pharmaceutiques in 1983. Since 1990, he is Professor of Physical Chemistry at Université Victor Segalen Bordeaux 2, France. His scientific interests included the synthesis of heterocyclic compounds for biological screening and pharmacokinetic studies for drug monitoring. In more recent years, his research team is focused on medicinal chemistry, especially on the physicochemical properties applied to drug design and molecular pharmacology.

References

(1) Avendano, C.; Menendez, J. C. Inhibitors of multidrug resistance to antitumor agents (MDR). *Curr. Med. Chem.* **2002**, *9*, 159−193.
(2) Gottesman, M. M. Mechanisms of cancer drug resistance. *Annu. Rev. Med.* **2002**, *53*, 615−627.
(3) Robert, J. Multidrug resistance in oncology: diagnostic and therapeutic approaches. *Eur. J. Clin. Invest.* **1999**, *29*, 536−545.
(4) Sikic, B. I.; Fisher, G. A.; Lum, B. L.; Halsey, J.; Beketic-Oreskovic, L.; Chen, G. Modulation and prevention of multidrug resistance by inhibitors of P-glycoprotein. *Cancer Chemother. Pharmacol.* **1997**, *40* (Suppl.), S13−S19.
(5) Wiese, M.; Pajeva, I. K. Structure−activity relationships of multidrug resistance reversers. *Curr. Med. Chem.* **2001**, *8*, 685−713.
(6) Ford, J. M.; Hait, W. N. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol. Rev.* **1990**, *42*, 155−199.
(7) Beck, W. T.; Grogan, T. M.; Willman, C. L.; Cordon-Cardo, C.; Parham, D. M.; Kuttesch, J. F.; et al. Methods to detect P-glycoprotein-associated multidrug resistance in patients' tumors: consensus recommendations. *Cancer Res.* **1996**, *56*, 3010−3020.
(8) Chevillard, S.; Vielh, P.; Validire, P.; Marie, J. P.; Faussat, A. M.; Barbu, V.; et al. French multicentric evaluation of MDR1 gene expression by RT-PCR in leukemia and solid tumours. Standardization of RT-PCR and preliminary comparisons between RT-PCR and immunohistochemistry in solid tumours. French Network of the Drug Resistance Intergroup, and Drug Resistance Network of Assistance Publique-Hôpitaux de Paris. *Leukemia* **1997**, *11*, 1095−1106.

(9) Huet, S.; Marie, J. P.; Gualde, N.; Robert, J. Reference method for detection of Pgp mediated multidrug resistance in human hematological malignancies: a method validated by the laboratories of the French Drug Resistance Network. *Cytometry* **1998**, *34*, 248−256.
(10) Marie, J. P.; Huet, S.; Faussat, A. M.; Perrot, J. Y.; Chevillard, S.; Barbu, V.; et al. Multicentric evaluation of the MDR phenotype in leukemia. French Network of the Drug Resistance Intergroup, and Drug Resistance Network of Assistance Publique-Hopitaux de Paris. *Leukemia* **1997**, *11*, 1086−1094.
(11) Gianni, L.; Munzone, E.; Capri, G.; Fulfaro, F.; Tarenzi, E.; Villani, F.; et al. Paclitaxel by 3-hour infusion in combination with bolus doxorubicin in women with untreated metastatic breast cancer: high antitumor efficacy and cardiac effects in a dose-finding and sequence-finding study. *J. Clin. Oncol.* **1995**, *13*, 2688−2699.
(12) Yuen, A. R.; Sikic, B. I. Multidrug resistance in lymphomas. *J. Clin. Oncol.* **1994**, *12*, 2453−2459.
(13) Kao, C. H.; Tsai, S. C.; Wang, J. J.; Ho, Y. J.; Ho, S. T.; Changlai, S. P. Technetium-99m-sestamethoxyisobutylisonitrile scan as a predictor of chemotherapy response in malignant lymphomas compared with P-glycoprotein expression, multidrug resistance-related protein expression and other prognosis factors. *Br. J. Haematol.* **2001**, *113*, 369−374.
(14) Leith, C. P.; Kopecky, K. J.; Godwin, J.; McConnell, T.; Slovak, M. L.; Chen, I. M.; et al. Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. *Blood* **1997**, *89*, 3323−3329.
(15) Leith, C. P.; Kopecky, K. J.; Chen, I. M.; Eijdems, L.; Slovak, M. L.; McConnell, T. S.; et al. Frequency and clinical significance of the expression of the multidrug resistance proteins MDR1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia: a Southwest Oncology Group Study. *Blood* **1999**, *94*, 1086−1099.
(16) Bellamy, W. T.; Odeleye, A.; Finley, P.; Huizenga, B.; Dalton, W. S.; Weinstein, R. S.; et al. An in vivo model of human multidrug-resistant multiple myeloma in SCID mice. *Am. J. Pathol.* **1993**, *142*, 691−698.
(17) Leveille-Webster, C. R.; Arias, I. A. Establishment and serial quantification of intrahepatic xenografts of human hepatocellular carcinoma in severe combined immunodeficiency mice, and development of therapeutic strategies to overcome multidrug resistance. *Clin. Cancer Res.* **1996**, *2*, 695−706.
(18) van de Vrie, W.; Marquet, R. L.; Stoter, G.; De Bruijn, E. A.; Eggermont, A. M. In vivo model systems in P-glycoprotein-mediated multidrug resistance. *Crit. Rev. Clin. Lab. Sci.* **1998**, *35*, 1−57.
(19) Galski, H.; Sullivan, M.; Willingham, M. C.; Chin, K. V.; Gottesman, M. M.; Pastan, I.; et al. Expression of a human multidrug resistance cDNA (MDR1) in the bone marrow of transgenic mice: resistance to daunomycin-induced leukopenia. *Mol. Cell. Biol.* **1989**, *9*, 4357−4363.
(20) Mickisch, G. H.; Merlino, G. T.; Galski, H.; Gottesman, M. M.; Pastan, I. Transgenic mice that express the human multidrug-resistance gene in bone marrow enable a rapid identification of agents that reverse drug resistance. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 547−551.
(21) Mickisch, G. H.; Pastan, I.; Gottesman, M. M. Multidrug resistant transgenic mice as a novel pharmacologic tool. *BioEssays* **1991**, *13*, 381−387.
(22) Mickisch, G. H.; Rahman, A.; Pastan, I.; Gottesman, M. M. Increased effectiveness of liposome-encapsulated doxorubicin in multidrug-resistant-transgenic mice compared with free doxorubicin. *J. Natl. Cancer Inst.* **1992**, *84*, 804−805.
(23) Evans, G. L. Construction and analysis of multidrug resistance transgenic mice. *Methods Enzymol.* **1998**, *292*, 572−594.
(24) Thiebaut, F.; Tsuruo, T.; Hamada, H.; Gottesman, M. M.; Pastan, I.; Willingham, M. C. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7735−7738.
(25) Schinkel, A. H.; Smit, J. J.; van Tellingen, O.; Beijnen, J. H.; Wagenaar, E.; van Deemter, L.; et al. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood−brain barrier and to increased sensitivity to drugs. *Cell* **1994**, *77*, 491−502.
(26) Wilson, W. H.; Bates, S. E.; Fojo, A.; Bryant, G.; Zhan, Z.; Regis, J.; et al. Controlled trial of dexverapamil, a modulator of multidrug resistance, in lymphomas refractory to EPOCH chemotherapy. *J. Clin. Oncol.* **1995**, *13*, 1995−2004.
(27) Hyafil, F.; Vergely, C.; Du, V. P.; Grand-Perret, T. In vitro and in vivo reversal of multidrug resistance by GF120918, an acridonecarboxamide derivative. *Cancer Res.* **1993**, *53*, 4595−4602.
(28) Sato, W.; Fukazawa, N.; Nakanishi, O.; Baba, M.; Suzuki, T.; Yano, O.; et al. Reversal of multidrug resistance by a novel quinoline derivative, MS-209. *Cancer Chemother. Pharmacol.* **1995**, *35*, 271−277.
(29) Safa, A. R. Photoaffinity labeling of the multidrug-resistance-related P-glycoprotein with photoactive analogs of verapamil. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 7187−7191.
(30) Safa, A. R.; Mehta, N. D.; Agresti, M. Photoaffinity labeling of P-glycoprotein in multidrug resistant cells with photoactive analogs of colchicine. *Biochem. Biophys. Res. Commun.* **1989**, *162*, 1402−1408.
(31) Bruggemann, E. P.; Germann, U. A.; Gottesmann, M. M.; Pastan, I. Two different regions of P-glycoprotein are photoaffinity-labeled by azidopine. *J. Biol. Chem.* **1989**, *264*, 15483−15488.
(32) Demmer, A.; Thole, H.; Kubesch, P.; Brandt, T.; Raida, M.; Fislage, R.; Tummler, B. Localization of the iodomycin binding site in hamster P-glycoprotein. *J. Biol. Chem.* **1997**, *272*, 20913−20919.
(33) Greenberger, L. M. Major photoaffinity drug labeling sites for iodoaryl azidoprazosin in P-glycoprotein are within, or immediately C-terminal to, transmembrane domain-6 and domain-12. *J. Biol. Chem.* **1993**, *268*, 11417−11425.
(34) Morris, D. I.; Greenberger, L. M.; Bruggemann, E. P.; Cardarelli, C.; Gottesman, M. M.; Pastan, I.; Seamon, K. B. Localization of the forskolin labeling sites to both halves of P-glycoprotein: similarity of the sites labeled by forskolin and prazosin. *Mol. Pharmacol.* **1994**, *46*, 329−337.
(35) Loo, T. W.; Clarke, D. M. The transmembrane domains of the human multidrug resistance P-glycoprotein are sufficient to mediate drug binding and trafficking to the cell surface. *J. Biol. Chem.* **1999**, *274*, 24759−24765.
(36) Ambudkar, S. V.; Dey, S.; Hrycyna, C. A.; Ramachandra, M.; Pastan, I.; Gottesman, M. M. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Annu. Rev. Pharmacol. Toxicol.* **1999**, *39*, 361−398.
(37) Loo, T. W.; Clarke, D. M. Molecular dissection of the human multidrug resistance P-glycoprotein. *Biochem. Cell. Biol.* **1999**, *77*, 11−23.
(38) Loo, T. W.; Clarke, D. M. Inhibition of oxidative cross-linking between engineered cysteine residues at positions 332 in predicted transmembrane segment (TM) 6 and 975 in predicted TM12 of human P-glycoprotein by drug substrates. *J. Biol. Chem.* **1996**, *271*, 27482−27487.
(39) Loo, T. W.; Clarke, D. M. Identification of residues within the drug-binding domain of the human multidrug resistance p-glycoprotein by cysteine-scanning mutagenesis and reaction with dibromobimane. *J. Biol. Chem.* **2000**, *275*, 39272−39278.
(40) Shapiro, A. B.; Ling, V. Positively cooperative sites for drug transport by P-glycoprotein with distinct drug specificities. *Eur. J. Biochem.* **1997**, *250*, 130−137.
(41) Shapiro, A. B.; Fox, K.; Lam, P.; Ling, V. Stimulation of P-glycoprotein-mediated drug transport by prazosin and progesterone—evidence for a third drug-binding site. *Eur. J. Biochem.* **1999**, *259*, 841−850.
(42) Pascaud, C.; Garrigos, M.; Orlowski, S. Multidrug resistance transporter P-glycoprotein has distinct but interacting binding sites for cytotoxic drugs and reversing agents. *Biochem. J.* **1998**, *333*, 351−358.
(43) Garrigues, A.; Loiseau, N.; Delaforge, M.; Ferte, J.; Garrigos, M.; Andre, F.; Orlowski, S. Characterization of two pharmacophores on the multidrug transporter P-glycoprotein. *Mol. Pharmacol.* **2002**, *62*, 1288−1298.
(44) Loo, T. W.; Clarke, D. M. Defining the drug-binding site in the human multidrug resistance P-glycoprotein using a methanesulfonate analog of verapamil, MTS-verapamil. *J. Biol. Chem.* **2001**, *276*, 14972−14979.
(45) Loo, T. W.; Clarke, D. M. Location of the rhodamine-binding site in the human multidrug resistance P-glycoprotein. *J. Biol. Chem.* **2002**, *277*, 44332−44338.
(46) Loo, T. W.; Clarke, D. M. The packing of the transmembrane segments of human multidrug resistance P-glycoprotein is revealed by disulfide cross-linking analysis. *J. Biol. Chem.* **2000**, *275*, 5253−5256.
(47) Loo, T. W.; Clarke, D. M. Drug-stimulated ATPase activity of human P-glycoprotein is blocked by disulfide cross-linking between the nucleotide-binding sites. *J. Biol. Chem.* **2000**, *275*, 19435−19438.
(48) Loo, T. W.; Clarke, D. M. Determining the dimensions of the drug-binding domain of human P-glycoprotein using thiol cross-linking compounds as molecular rulers. *J. Biol. Chem.* **2001**, *276*, 36877−36880.
(49) Senior, A. E. Catalytic mechanism of P-glycoprotein. *Acta Physiol. Scand.* **1998**, *163*, 213−218.
(50) Zamora, J. M.; Pearse, H. L.; Beck, W. T. Physical−chemical properties shared by compounds that modulate multidrug resistance in human leukemic cells. *Mol. Pharmacol.* **1988**, *33*, 454−462.
(51) Stouch, T. R.; Gudmundsson, A. Progress in understanding the structure−activity relationships of P-glycoprotein. *Adv. Drug Delivery Rev.* **2002**, *54*, 315−328.
(52) Seelig, A. A general pattern for substrate recognition by P-glycoprotein. *Eur. J. Biochem.* **1998**, *251*, 252−261.

(53) Seelig, A.; Landwojtowicz, E. Structure−activity relationship of P-glycoprotein substrates and modifiers. *Eur. J. Pharm. Sci.* **2000**, *12*, 31−40.
(54) Scala, S.; Akhmed, N.; Rao, U. S.; Paull, K.; Lan, L. B.; Dickstein, B.; Lee J. S.; et al. P-glycoprotein substrates and antagonists cluster into two distinct groups. *Mol. Pharmacol.* **1997**, *51*, 1024−1033.
(55) Klopman, G.; Shi, L. M.; Ramu, A. Quantitative structure−activity relationship of multidrug resistance reversal agents. *Mol. Pharmacol.* **1997**, *52*, 323−334.
(56) Bakken, G. A.; Jurs, P. C. Classification of multidrug-resistance reversal agents using structure-based descriptors and linear discriminant analysis. *J. Med. Chem.* **2000**, *43*, 4534−4541.
(57) Ekins, S.; Kim, R. B.; Leake, B. F.; Dantzig, A. H.; Schuetz, E. G.; Lan, L. B.; et al. Three-dimensional quantitative structure−activity relationships of inhibitors of P-glycoprotein. *Mol. Pharmacol.* **2002**, *61*, 964−973.
(58) Pajeva, I. K.; Wiese, M. Pharmacophore model of drugs involved in P-glycoprotein multidrug resistance: explanation of structural variety (hypothesis). *J. Med. Chem.* **2002**, *45*, 5671−5686.
(59) Ozols, R. F.; Cunnion, R. E.; Klecker, R. W. J.; Hamilton, T. C.; Ostchega, Y.; Parrillo, J. E.; et al. Verapamil and adriamycin in the treatment of drug-resistant ovarian cancer patients. *J. Clin. Oncol.* **1987**, *5*, 641−647.
(60) Dalton, W. S.; Grogan, T. M.; Meltzer, P. S.; Scheper, R. J.; Durie, B. G.; Taylor, C. W.; et al. Drug-resistance in multiple myeloma and non-Hodgkin's lymphoma: detection of P-glycoprotein and potential circumvention by addition of verapamil to chemotherapy. *J. Clin. Oncol.* **1989**, *7*, 415−424.
(61) Durie, B. G.; Dalton, W. S. Reversal of drug-resistance in multiple myeloma with verapamil. *Br. J. Haematol.* **1988**, *68*, 203−206.
(62) Miller, T. P.; Grogan, T. M.; Dalton, W. S.; Spier, C. M.; Scheper, R. J.; Salmon, S. E. P-glycoprotein expression in malignant lymphoma and reversal of clinical drug resistance with chemotherapy plus high-dose verapamil. *J. Clin. Oncol.* **1991**, *9*, 17−24.
(63) Salmon, S. E.; Dalton, W. S.; Grogan, T. M.; Plezia, P.; Lehnert, M.; Roe, D. J.; et al. Multidrug-resistant myeloma: laboratory and clinical effects of verapamil as a chemosensitizer. *Blood* **1991**, *78*, 44−50.
(64) Dalton, W. S.; Crowley, J. J.; Salmon, S. S.; Grogan, T. M.; Laufman, L. R.; Weiss, G. R.; et al. A phase III randomized study of oral verapamil as a chemosensitizer to reverse drug resistance in patients with refractory myeloma. A Southwest Oncology Group study. *Cancer* **1995**, *75*, 815−820.
(65) Demicheli, R.; Jirillo, A.; Bonciarelli, G.; Lonardi, F.; Balli, M.; Bandello, A. 4′-Epidoxorubicin plus verapamil in anthracycline-refractory cancer patients. *Tumori* **1989**, *75*, 245−247.
(66) Langenbuch, T.; Mross, K.; Jonat, W.; Hossfeld, D. K. A phase II study of intensive-dose epirubicin/verapamil as induction therapy followed by intensive-dose ifosfamide for advanced breast cancer. *Cancer Chemother. Pharmacol.* **1990**, *26* (Suppl.), S93−S96.
(67) Mross, K.; Bohn, C.; Edler, L.; Jonat, W.; Queisser, W.; Heidemann, E.; et al. Randomized phase II study of single-agent epirubicin ± verapamil in patients with advanced metastatic breast cancer. An AIO clinical trial. Arbeitsgemeinschaft Internistische Onkologie of the German Cancer Society. *Ann. Oncol.* **1993**, *4*, 45−50.
(68) Cairo, M. S.; Siegel, S.; Anas, N.; Sender, L. Clinical trial of continuous infusion verapamil, bolus vinblastine, and continuous infusion VP-16 in drug-resistant pediatric tumors. *Cancer Res.* **1989**, *49*, 1063−1066.
(69) Millward, M. J.; Cantwell, B. M.; Lien, E. A.; Carmichael, J.; Harris, A. L. Intermittent high-dose tamoxifen as a potential modifier of multidrug resistance. *Eur. J. Cancer* **1992**, *28A*, 805−810.
(70) Belpomme, D.; Gauthier, S.; Pujade-Lauraine, E.; Facchini, T.; Goudier, M. J.; Krakowski, I.; et al. Verapamil increases the survival of patients with anthracycline-resistant metastatic breast carcinoma. *Ann. Oncol.* **2000**, *11*, 1471−1476.
(71) Bissett, D.; Kerr, D. J.; Cassidy, J.; Meredith, P.; Traugott, U.; Kaye, S. B. Phase I and pharmacokinetic study of D-verapamil and doxorubicin. *Br. J. Cancer* **1991**, *64*, 1168−1171.
(72) Lehnert, M.; Mross, K.; Schueller, J.; Thuerlimann, B.; Kroeger, N.; Kupper, H. Phase II trial of dexverapamil and epirubicin in patients with non-responsive metastatic breast cancer. *Br. J. Cancer* **1998**, *77*, 1155−1163.
(73) Scheithauer, W.; Kornek, G.; Kastner, J.; Raderer, M.; Locker, G.; Depisch, D.; et al. Phase II study of D-verapamil and doxorubicin in patients with metastatic colorectal cancer. *Eur. J. Cancer* **1993**, *29A*, 2337−2338.
(74) Motzer, R. J.; Lyn, P.; Fischer, P.; Lianes, P.; Ngo, R. L.; Cordon-Cardo, C.; et al. Phase I/II trial of dexverapamil plus vinblastine for patients with advanced renal cell carcinoma. *J. Clin. Oncol.* **1995**, *13*, 1958−1965.
(75) Warner, E.; Hedley, D.; Andrulis, I.; Myers, R.; Trudeau, M.; Warr, D.; et al. Phase II study of dexverapamil plus anthracycline in patients with metastatic breast cancer who have progressed on the same anthracycline regimen. *Clin. Cancer Res.* **1998**, *4*, 1451−1457.
(76) Fisher, G. A.; Bartlett, N. L.; Lum, N. A.; Brophy, N. A.; Duran, G. E.; Ehsan, M. N.; et al. Phase I trial of Taxol with high-dose cyclosporin A as a modulator of multidrug resistance. *Proc. Am. Soc. Clin. Oncol.* **1994**, *13*, 144, Abstr. 369.
(77) Lum, B. L.; Kaubisch, S.; Yahanda, A. M.; Adler, K. M.; Jew, L.; Ehsan, M. N.; et al. Alteration of etoposide pharmacokinetics and pharmacodynamics by cyclosporine in a phase I trial to modulate multidrug resistance. *J. Clin. Oncol.* **1992**, *10*, 1635−1642.
(78) Rushing, D. A.; Raber, S. R.; Rodvold, K. A.; Piscitelli, S. C.; Plank, G. S.; Tewksbury, D. A. The effects of cyclosporine on the pharmacokinetics of doxorubicin in patients with small cell lung cancer. *Cancer* **1994**, *74*, 834−841.
(79) Sonneveld, P.; Durie, B. G.; Lokhorst, H. M.; Marie, J. P.; Solbu, G.; Suciu, S.; et al. Modulation of multidrug-resistant multiple myeloma by cyclosporin. The Leukaemia Group of the EORTC and the HOVON. *Lancet* **1992**, *340*, 255−259.
(80) List, A. F.; Spier, C.; Greer, J.; Wolff, S.; Hutter, J.; Dorr, R.; et al. Phase I/II trial of cyclosporine as a chemotherapy-resistance modifier in acute leukemia. *J. Clin. Oncol.* **1993**, *11*, 1652−1660.
(81) Verweij, J.; Herweijer, H.; Oosterom, R.; van der Burg, M. E.; Planting, A. S.; Seynaeve, C.; et al. A phase II study of epidoxorubicin in colorectal cancer and the use of cyclosporin-A in an attempt to reverse multidrug resistance. *Br. J. Cancer* **1991**, *64*, 361−364.
(82) Rodenburg, C. J.; Nooter, K.; Herweijer, H.; Seynaeve, C.; Oosterom, R.; Stoter, G.; et al. Phase II study of combining vinblastine and cyclosporin-A to circumvent multidrug resistance in renal cell cancer. *Ann. Oncol.* **1991**, *2*, 305−306.
(83) Sarris, A. H.; Younes, A.; McLaughlin, P.; Moore, D.; Hagemeister, F.; Swan, F.; et al. Cyclosporin A does not reverse clinical resistance to paclitaxel in patients with relapsed non-Hodgkin's lymphoma. *J. Clin. Oncol.* **1996**, *14*, 233−239.
(84) Sonneveld, P.; Suciu, S.; Weijermans, P.; Beksac, M.; Neuwirtova, R.; Solbu, G.; et al. Cyclosporin A combined with vincristine, doxorubicin and dexamethasone (VAD) compared with VAD alone in patients with advanced refractory multiple myeloma: an EORTC-HOVON randomized phase III study (06914). *Br. J. Haematol.* **2001**, *115*, 895−902.
(85) List, A. F.; Kopecky, K. J.; Willman, C. L.; Head, D. R.; Persons, D. L.; Slovak, M. L.; et al. Benefit of cyclosporine modulation of drug resistance in patients with poor-risk acute myeloid leukemia: a Southwest Oncology Group study. *Blood* **2001**, *98*, 3212−3220.
(86) Dahl, G. V.; Lacayo, N. J.; Brophy, N.; Dunussi-Joannopoulos, K.; Weinstein, H. J.; Chang, M.; et al. Mitoxantrone, etoposide, and cyclosporine therapy in pediatric patients with recurrent or refractory acute myeloid leukemia. *J. Clin. Oncol.* **2000**, *18*, 1867−1875.
(87) Liu, Y. J.; Wheatley, K.; Rees, J. K.; Burnett, A. K. Comparison of "sequential" versus "standard" chemotherapy as re-induction treatment, with or without cyclosporine, in refractory/relapsed acute myeloid leukaemia (AML): results of the UK Medical Research Council AML-R trial. *Br. J. Haematol.* **2001**, *113*, 713−726.
(88) Tallman, M. S.; Lee, S.; Sikic, B. I.; Paietta, E.; Wiernik, P. H.; Bennett, J. M.; et al. Mitoxantrone, etoposide, and cytarabine plus cyclosporine for patients with relapsed or refractory acute myeloid leukemia: an Eastern Cooperative Oncology Group pilot study. *Cancer* **1999**, *85*, 358−367.
(89) Twentyman, P. R.; Bleehen, N. M. Resistance modification by PSC-833, a novel non-immunosuppressive cyclosporin. *Eur. J. Cancer* **1991**, *27*, 1639−1642.
(90) Boesch, D.; Gaveriaux, C.; Jachez, B.; Pourtier-Manzanedo, A.; Bollinger, P.; Loor, F. In vivo circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. *Cancer Res.* **1991**, *51*, 4226−4233.
(91) Boote, D. J.; Dennis, I. F.; Twentyman, P. R.; Osborne, R. J.; Laburte, C.; Hensel, S.; et al. Phase I study of etoposide with SDZ PSC 833 as a modulator of multidrug resistance in patients with cancer. *J. Clin. Oncol.* **1996**, *14*, 610−618.
(92) Giaccone, G.; Linn, S. C.; Welink, J.; Catimel, G.; Stieltjes, H.; van der Vijgh, W. J.; et al. A dose-finding and pharmacokinetic study of reversal of multidrug resistance with SDZ PSC 833 in combination with doxorubicin in patients with solid tumors. *Clin. Cancer Res.* **1997**, *3*, 2005−2015.
(93) Advani, R.; Saba, H. I.; Tallman, M. S.; Rowe, J. M.; Wiernik, P. H.; Ramek, J.; et al. Treatment of refractory and relapsed acute myelogenous leukemia with combination chemotherapy plus the multidrug resistance modulator PSC 833 (Valspodar). *Blood* **1999**, *93*, 787−795.

(94) Fracasso, P. M.; Westervelt, P.; Fears, C. L.; Rosen, D. M.; Zuhowski, E. G.; Cazenave, L. A.; et al. Phase I study of paclitaxel in combination with a multidrug resistance modulator, PSC 833 (Valspodar), in refractory malignancies. *J. Clin. Oncol.* **2000**, *18*, 1124−1134.

(95) Chauncey, T. R.; Rankin, C.; Anderson, J. E.; Chen, I.; Kopecky, K. J.; Godwin, J. E.; et al. A phase I study of induction chemotherapy for older patients with newly diagnosed acute myeloid leukemia (AML) using mitoxantrone, etoposide, and the MDR modulator PSC 833: a southwest oncology group study 9617. *Leuk. Res.* **2000**, *24*, 567−574.

(96) Dorr, R.; Karanes, C.; Spier, C.; Grogan, T.; Greer, J.; Moore, J.; et al. Phase I/II study of the P-glycoprotein modulator PSC 833 in patients with acute myeloid leukemia. *J. Clin. Oncol.* **2001**, *19*, 1589−1599.

(97) Lee, E. J.; George, S. L.; Caligiuri, M.; Szatrowski, T. P.; Powell, B. L.; Lemke, S.; et al. Parallel phase I studies of daunorubicin given with cytarabine and etoposide with or without the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age or older with acute myeloid leukemia: results of cancer and leukemia group B study 9420. *J. Clin. Oncol.* **1999**, *17*, 2831−2839.

(98) Visani, G.; Milligan, D.; Leoni, F.; Chang, J.; Kelsey, S.; Marcus, R.; et al. Combined action of PSC 833 (Valspodar), a novel MDR reversing agent, with mitoxantrone, etoposide and cytarabine in poor-prognosis acute myeloid leukemia. *Leukemia* **2001**, *15*, 764−771.

(99) Baer, M. R.; George, S. L.; Dodge, R. K.; O'Loughlin, K. L.; Minderman, H.; Caligiuri, M. A.; et al. Phase 3 study of the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age and older with acute myeloid leukemia: cancer and leukemia group B study 9720. *Blood* **2002**, *100*, 1224−1232.

(100) Greenberg, P.; Advani, R.; Tallman, M.; Letendre, L.; Saba, H.; Dugan, K.; et al. Treatment of refractory/relapsed AML with PSC-833 plus mitoxantrone, etoposide, cytarabine (PSC-MEC) vs MEC: randomized phase III trial (E2995). *Blood* **1999**, *94*, 383a (Abstract 1703).

(101) Fracasso, P. M.; Brady, M. F.; Moore, D. H.; Walker, J. L.; Rose, P. G.; Letvak, L.; et al. Phase II study of paclitaxel and valspodar (PSC 833) in refractory ovarian carcinoma: a gynecologic oncology group study. *J. Clin. Oncol.* **2001**, *19*, 2975−2982.

(102) Baekelandt, M.; Lehne, G.; Trope, C. G.; Szanto, I.; Pfeiffer, P.; Gustavssson, B.; et al. Phase I/II trial of the multidrug-resistance modulator valspodar combined with cisplatin and doxorubicin in refractory ovarian cancer. *J. Clin. Oncol.* **2001**, *19*, 2983−2993.

(103) Bates, S.; Kang, M.; Meadows, B.; Bakke, S.; Choyke, P.; Merino, M.; et al. A phase I study of infusional vinblastine in combination with the P-glycoprotein antagonist PSC 833 (valspodar). *Cancer* **2001**, *92*, 1577−1590.

(104) Chico, I.; Kang, M. H.; Bergan, R.; Abraham, J.; Bakke, S.; Meadows, B.; et al. Phase I study of infusional paclitaxel in combination with the P-glycoprotein antagonist PSC 833. *J. Clin. Oncol.* **2001**, *19*, 832−842.

(105) Minami, H.; Ohtsu, T.; Fujii, H.; Igarashi, T.; Itoh, K.; Uchiyama-Kokubu, N.; et al. Phase I study of intravenous PSC-833 and doxorubicin: reversal of multidrug resistance. *Jpn. J. Cancer Res.* **2001**, *92*, 220−230.

(106) Wishart, G. C.; Bissett, D.; Paul, J.; Jodrell, D.; Harnett, A.; Habeshaw, T.; et al. Quinidine as a resistance modulator of epirubicin in advanced breast cancer: mature results of a placebo-controlled randomized trial. *J. Clin. Oncol.* **1994**, *12*, 1771−1777.

(107) Solary, E.; Caillot, D.; Chauffert, B.; Casasnovas, R. O.; Dumas, M.; Maynadie, M.; et al. Feasibility of using quinine, a potential multidrug resistance-reversing agent, in combination with mitoxantrone and cytarabine for the treatment of acute leukemia. *J. Clin. Oncol.* **1992**, *10*, 1730−1736.

(108) Solary, E.; Witz, B.; Caillot, D.; Moreau, P.; Desablens, B.; Cahn, J. Y.; et al. Combination of quinine as a potential reversing agent with mitoxantrone and cytarabine for the treatment of acute leukemias: a randomized multicenter study. *Blood* **1996**, *88*, 1198−1205.

(109) Miller, T. P.; Chase, E. M.; Dorr, R.; Dalton, W. S.; Lam, K. S.; Salmon, S. E. A phase I/II trial of paclitaxel for non-Hodgkin's lymphoma followed by paclitaxel plus quinine in drug-resistant disease. *Anti-Cancer Drugs* **1998**, *9*, 135−140.

(110) Solary, E.; Mannone, L.; Moreau, D.; Caillot, D.; Casasnovas, R. O.; Guy, H.; et al. Phase I study of cinchonine, a multidrug resistance reversing agent, combined with the CHVP regimen in relapsed and refractory lymphoproliferative syndromes. *Leukemia* **2000**, *14*, 2085−2094.

(111) Naito, M.; Matsuba, Y.; Sato, S.; Hirata, H.; Tsuruo, T. MS-209, a quinoline-type reversal agent, potentiates antitumor efficacy of docetaxel in multidrug-resistant solid tumor xenograft models. *Clin. Cancer Res.* **2002**, *8*, 582−588.

(112) Peck, R. A.; Hewett, J.; Harding, M. W.; Wang, Y. M.; Chaturvedi, P. R.; Bhatnagar, A.; et al. 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.

(113) Rowinsky, E. K.; Smith, L.; Wang, Y. M.; Chaturvedi, P.; Villalona, M.; Campbell, E.; et al. Phase I and pharmacokinetic study of paclitaxel in combination with biricodar, a novel agent that reverses multidrug resistance conferred by overexpression of both MDR1 and MRP. *J. Clin. Oncol.* **1998**, *16*, 2964−2976.

(114) Germann, U. A.; Shlyakhter, D.; Mason, V. S.; Zelle, R. E.; Duffy, J. P.; Galullo, V.; et al. Cellular and biochemical characterization of VX-710 as a chemosensitizer: reversal of P-glycoprotein-mediated multidrug resistance in vitro. *Anti-Cancer Drugs* **1997**, *8*, 125−140.

(115) Germann, U. A.; Ford, P. J.; Shlyakhter, D.; Mason, V. S.; Harding, M. W. Chemosensitization and drug accumulation effects of VX-710, verapamil, cyclosporin A, MS-209 and GF120918 in multidrug resistant HL60/ADR cells expressing the multidrug resistance-associated protein MRP. *Anti-Cancer Drugs* **1997**, *8*, 141−155.

(116) Pierre, A.; Dunn, T. A.; Kraus-Berthier, L.; Leonce, S.; Saint-Dizier, D.; Regnier, G.; et al. In vitro and in vivo circumvention of multidrug resistance by Servier 9788, a novel triazinoaminopiperidine derivative. *Invest. New Drugs* **1992**, *10*, 137−148.

(117) Punt, C. J.; Voest, E. E.; Tueni, E.; Van Oosterom, A. T.; Backx, A.; De Mulder, P. H.; et al. Phase IB study of doxorubicin in combination with the multidrug resistance reversing agent S9788 in advanced colorectal and renal cell cancer. *Br. J. Cancer* **1997**, *76*, 1376−1381.

(118) de Bruin, M.; Miyake, K.; Litman, T.; Robey, R.; Bates, S. E. Reversal of resistance by GF120918 in cell lines expressing the ABC half-transporter, MXR. *Cancer Lett.* **1999**, *146*, 117−126.

(119) Sparreboom, A.; Planting, A. S.; Jewell, R. C.; van der Burg, M. E.; van der Gaast, A.; de Bruijn, P.; et al. Clinical pharmacokinetics of doxorubicin in combination with GF120918, a potent inhibitor of MDR1 P-glycoprotein. *Anti-Cancer Drugs* **1999**, *10*, 719−728.

(120) Kruijtzer, C. M.; Beijnen, J. H.; Rosing, H.; Ten Bokkel, H.; Schot, M.; Jewell, R. C.; et al. Increased oral bioavailability of topotecan in combination with the breast cancer resistance protein and P-glycoprotein inhibitor GF120918. *J. Clin. Oncol.* **2002**, *20*, 2943−2950.

(121) Malingre, M. M.; Beijnen, J. H.; Rosing, H.; Koopman, F. J.; Jewell, R. C.; Paul, E. M.; et al. Co-administration of GF120918 significantly increases the systemic exposure to oral paclitaxel in cancer patients. *Br. J. Cancer* **2001**, *84*, 42−47.

(122) Dantzig, A. H.; Law, K. L.; Cao, J.; Starling, J. J. Reversal of multidrug resistance by the P-glycoprotein modulator, LY335979, from the bench to the clinic. *Curr. Med. Chem.* **2001**, *8*, 39−50.

(123) Shepard, R. L.; Cao, J.; Starling, J. J.; Dantzig, A. H. Modulation of P-glycoprotein but not MRP1- or BRCP-mediated drug resistance by LY335979. *Int. J. Cancer* **2003**, *103*, 121−125.

(124) Rubin, E. H.; De Alwis, D. P.; Pouliquen, I.; Green, L.; Marder, P.; Lin, Y.; et al. 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.

(125) Mistry, P.; Stewart, A. J.; Dangerfield, W.; Okiji, S.; Liddle, C.; Bootle, D.; et al. In vitro and in vivo reversal of P-glycoprotein-mediated multidrug resistance by a novel potent modulator, XR9576. *Cancer Res.* **2001**, *61*, 749−758.

(126) Stewart, A.; Steiner, J.; Mellows, G.; Laguda, B.; Norris, D.; Bevan, P. Phase I trial of XR9576 in healthy volunteers demonstrates modulation of P-glycoprotein in CD56+ lymphocytes after oral and intravenous administration. *Clin. Cancer Res.* **2000**, *6*, 4186−4191.

(127) Mechetner, E. B.; Roninson, I. B. Efficient inhibition of P-glycoprotein-mediated multidrug resistance with a monoclonal antibody. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5824−5828.

(128) Alahari, S. K.; Dean, N. M.; Fisher, M. H.; Delong, R.; Manoharan, M.; Tivel, K. L.; et al. Inhibition of expression of the multidrug resistance-associated P-glycoprotein of by phosphorothioate and 5′ cholesterol-conjugated phosphorothioate antisense oligonucleotides. *Mol. Pharmacol.* **1996**, *50*, 808−819.

(129) Labialle, S.; Gayet, L.; Marthinet, E.; Rigal, D.; Baggetto, L. G. Transcriptional regulators of the human multidrug resistance 1 gene: recent views. *Biochem. Pharmacol.* **2002**, *64*, 943−948.

(130) Thierry, A. R.; Vige, D.; Coughlin, S. S.; Belli, J. A.; Dritschilo, A.; Rahman, A. Modulation of doxorubicin resistance in multidrug-resistant cells by liposomes. *FASEB J.* **1993**, *7*, 572−579.

(131) Cuvier, C.; Roblot-Treupel, L.; Millot, J. M.; Lizard, G.; Chevillard, S.; Manfait, M.; et al. Doxorubicin-loaded nanospheres bypass tumor cell multidrug resistance. *Biochem. Pharmacol.* **1992**, *44*, 509−517.

(132) Shtil, A. A. P-glycoprotein as a therapeutic target: good news. *Leukemia* **2002**, *16*, 2169−2170.

(133) Lehne, G.; Sorensen, D. R.; Tjonnfjord, G. E.; Beiske, C.; Hagve, T. A.; Rugstad, H. E.; et al. The cyclosporin PSC 833 increases survival and delays engraftment of human multidrug-resistant leukemia cells in xenotransplanted NOD-SCID mice. *Leukemia* **2002**, *16*, 2388−2394.
(134) Millward, M. J.; Cantwell, B. M.; Munro, N. C.; Robinson, A.; Corris, P. A.; Harris, A. L. Oral verapamil with chemotherapy for advanced non-small cell lung cancer: a randomised study. *Br. J. Cancer* **1993**, *67*, 1031−1035.
(135) Philip, P. A.; Joel, S.; Monkman, S. C.; Dolega-Ossowski, E.; Tonkin, K.; Carmichael, J.; et al. A phase I study on the reversal of multidrug resistance (MDR) in vivo: nifedipine plus etoposide. *Br. J. Cancer* **1992**, *65*, 267−270.
(136) Budd, G. T.; Bukowski, R. M.; Lichtin, A.; Bauer, L.; Van Kirk, P.; Ganapathi, R. Phase II trial of doxorubicin and trifluoperazine in metastatic breast cancer. *Invest. New Drugs* **1993**, *11*, 75−79.
(137) Miller, R. L.; Bukowski, R. M.; Budd, G. T.; Purvis, J.; Weick, J. K.; Shepard, K.; et al. Clinical modulation of doxorubicin resistance by the calmodulin-inhibitor, trifluoperazine: a phase I/II trial. *J. Clin. Oncol.* **1988**, *6*, 880−888.
(138) Murren, J. R.; Durivage, H. J.; Buzaid, A. C.; Reiss, M.; Flynn, S. D.; Carter, D.; et al. Trifluoperazine as a modulator of multidrug resistance in refractory breast cancer. *Cancer Chemother. Pharmacol.* **1996**, *38*, 65−70.
(139) List, A. F.; Spier, C.; Greer, J.; Wolff, S.; Hutter, J.; Dorr, R.; et al. Phase I/II trial of cyclosporine as a chemotherapy-resistance modifier in acute leukemia. *J. Clin. Oncol.* **1993**, *11*, 1652−1660.
(140) Sarris, A. H.; Younes, A.; McLaughlin, P.; Moore, D.; Hagemeister, F.; Swan, F.; et al. Cyclosporin A does not reverse clinical resistance to paclitaxel in patients with relapsed non-Hodgkin's lymphoma. *J. Clin. Oncol.* **1996**, *14*, 233−239.
(141) Solary, E.; Witz, B.; Caillot, D.; Moreau, P.; Desablens, B.; Cahn, J. Y.; et al. Combination of quinine as a potential reversing agent with mitoxantrone and cytarabine for the treatment of acute leukemias: a randomized multicenter study. *Blood* **1996**, *88*, 1198−1205.
(142) Berman, E.; McBride, M.; Lin, S.; Menedez-Botet, C.; Tong, W. Phase I trial of high-dose tamoxifen as a modulator of drug resistance in combination with daunorubicin in patients with relapsed or refractory acute leukemia. *Leukemia* **1995**, *9*, 1631−1637.
(143) Samuels, B. L.; Hollis, D. R.; Rosner, G. L.; Trump, D. L.; Shapiro, C. L.; Vogelzang, N. J.; et al. Modulation of vinblastine resistance in metastatic renal cell carcinoma with cyclosporine A or tamoxifen: a cancer and leukemia group B study. *Clin. Cancer Res.* **1997**, *3*, 1977−1984.
(144) Trump, D. L.; Smith, D. C.; Ellis, P. G.; Rogers, M. P.; Schold, S. C.; Winer, E. P.; et al. High-dose oral tamoxifen, a potential multidrug-resistance-reversal agent: phase I trial in combination with vinblastine. *J. Natl. Cancer Inst.* **1992**, *84*, 1811−1816.
(145) Christen, R. D.; McClay, E. F.; Plaxe, S. C.; Yen, S. S.; Kim, S.; Kirmani, S.; et al. Phase I/pharmacokinetic study of high-dose progesterone and doxorubicin. *J. Clin. Oncol.* **1993**, *11*, 2417−2426.
(146) Isonishi, S.; Kirmani, S.; Kim, S.; Plaxe, S. C.; Braly, P. S.; McClay, E. F.; et al. Phase I and pharmacokinetic trial of intraperitoneal etoposide in combination with the multidrug-resistance-modulating agent dipyridamole. *J. Natl. Cancer Inst.* **1991**, *83*, 621−626.
(147) Murphy, B. R.; Rynard, S. M.; Pennington, K. L.; Grosh, W.; Loehrer, P. J. A phase II trial of vinblastine plus dipyridamole in advanced renal cell carcinoma. A Hoosier oncology group study. *Am. J. Clin. Oncol.* **1994**, *17*, 10−13.
(148) Bates, S. E.; Meadows, B.; Goldspiel, B. R.; Denicoff, A.; Le, T. B.; Tucker, E.; et al. A pilot study of amiodarone with infusional doxorubicin or vinblastine in refractory breast cancer. *Cancer Chemother. Pharmacol.* **1995**, *35*, 457−463.
(149) Linn, S. C.; van Kalken, C. K.; van Tellingen, O.; van der Valk, P.; van Groeningen, C. J.; Kuiper, C. M.; et al. Clinical and pharmacologic study of multidrug resistance reversal with vinblastine and bepridil. *J. Clin. Oncol.* **1994**, *12*, 812−819.
(150) Ukena, D.; Boewer, C.; Oldenkott, B.; Rathgeb, F.; Wurst, W.; Zech, K.; et al. Tolerance, safety, and kinetics of the new antineoplastic compound dexniguldipine·HCl after oral administration: a phase I dose-escalation trial. *Cancer Chemother. Pharmacol.* **1995**, *36*, 160−164.
(151) Braybrooke, J. P.; Vallis, K. A.; Houlbrook, S.; Rockett, H.; Ellmen, J.; Anttila, M.; et al. Evaluation of toremifene for reversal of multidrug resistance in renal cell cancer patients treated with vinblastine. *Cancer Chemother. Pharmacol.* **2000**, *46*, 27−34.

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