Cancer multidrug resistance: A review of recent drug discovery research
Mordechai Liscovitch1* and Yaakoy Lavie2

Address
1Department of Biological Regulation
Weizmann Institute of Science
Rehovot 76100
Israel
Email: moti.liscovitch@weizmann.ac.il
2Steralx Pharmaceuticals Ltd
DN Mischav 20179
Israel

*To whom correspondence should be addressed

IDrugs 2002 5(4): © PharmaPress Ltd ISSN 1369-7056

Conventional cancer chemotherapy is seriously limited by tumor cells exhibiting multidrug resistance (MDR), caused by changes in the level or activity of membrane transporters that mediate energy-dependent drug efflux and of other proteins that affect drug metabolism and/or drug action. Many inhibitors of MDR transporters have been identified and some are undergoing clinical trials, but currently none are in clinical use. Here we briefly review the status of MDR drugs, outline novel approaches designed to suppress or circumvent MDR mechanisms and discuss the future of MDR therapy in oncology.

Introduction
Multidrug resistance (MDR) is defined as resistance of tumor cells to the cytostatic or cytotoxic actions of multiple, structurally dissimilar and functionally divergent drugs commonly used in cancer chemotherapy [1•]. MDR is termed ‘intrinsic’ when the disease is refractory to chemotherapy from the outset, or acquired when the disease becomes insensitive to treatment upon relapse. MDR is responsible for the overall poor efficacy of cancer chemotherapy. Attempts to develop drugs targeting proteins that mediate MDR were initiated as soon as such proteins began to be identified [2]. This effort continues, with third-generation drugs currently being tested in advanced clinical trials, but clinically useful drugs have yet to make it to the market. In this brief review, we shall outline the known molecular mechanisms of MDR, the various approaches taken to block or bypass these mechanisms and the drugs that are being developed to treat patients with chemotherapy-resistant cancer.

Drug transporters as mediators of MDR

P-glycoprotein (P-gp), a prototypical MDR protein, was originally identified as a 170-kDa glycoprotein abundantly expressed in MDR cells, that later was purified, cloned and found to mediate unidirectional ATP-dependent drug efflux [1,3]. A product of the human MDR1 gene, P-gp belongs to the ATP-binding cassette (ABC) superfamily of small molecule and ion transporters [4]. Other members of the ABC superfamily have also been implicated in cancer MDR, including multidrug resistance-associated protein-1 (MRP1), its homologs MRP2-6 that transport glutathione, glucuronate and sulfate-conjugated drugs [5•], and the breast cancer resistance protein (BCRP) [6]. The high expression of P-gp and certain other MDR transporters in the epithelia of liver bile ducts, kidney and colon and in the microvasculature comprising blood-organ barriers, suggests that they are normally involved in detoxification and removal of xenobiotics [7]. Other roles in regulation of cell proliferation, differentiation and apoptosis have also emerged [8•]. Tumors arising from cells that highly express P-gp or other MDR-related transporters are often intrinsically resistant to chemotherapy. Other tumor cells acquire high MDR transporter expression upon drug treatment via gene induction or DNA amplification. Based on ample experimental evidence, it is generally believed that these transporters mediate MDR by effecting an energy-dependent export of drugs (or drug-conjugates) thus reducing cellular drug levels and efficacy. The structure, regulation and mode of action of P-gp and other MDR-related transporters have been reviewed [5•,9].

Other MDR mechanisms

Forced expression of recombinant MDR-related transporters in tumor cells confers drug resistance, supporting their role in mediating MDR. However, the degree of drug resistance thus obtained is rarely equivalent to that observed in intrinsic or drug-selected MDR cells, indicating that additional mechanisms must contribute to a full-fledged MDR phenotype. Indeed, MDR cells sometimes exhibit altered kinetics of cellular drug uptake linked to changes in membrane permeability, and increased drug detoxification linked to overexpression of enzymes such as glutathione-S-transferase or elevated intracellular glutathione concentrations [10]. Changes in drug effectiveness are also documented, eg, increased DNA damage repair via alterations in O6-methylguanine DNA methyl transferase or changes in topoisomerase II activity. The often-observed pleiotropic resistance of MDR cells to other apoptosis-inducing agents (eg, oxidative stress, radiation, Fas ligand, tumor necrosis factor (TNF)-α) indicates that defects in apoptotic pathways (eg, through overexpression of anti-apoptotic proteins such as Bcl-2) are also efficient drug-resistance mechanisms [10]. The involvement of multiple mechanisms of MDR, particularly in tumors that comprise a heterogenous population of cells, makes the development of effective therapies for multidrug resistant cancer a formidable task.

Small molecule MDR inhibitors

First- and second-generation MDR drugs

The relative promiscuity of drug transport by P-gp and other MDR-associated transporters inspired a wide search for compounds that would not be cytotoxic themselves but would inhibit MDR transporters. The initial demonstration of verapamil as a P-gp inhibitor [11•] was followed by many additional compounds reported to inhibit drug transport and thus sensitize MDR cells to chemotherapeutic drugs. Various called chemosensitizers, MDR reversal agents, modulators or convertors, these ‘first-generation’ MDR drugs included compounds of diverse structure and
function such as calcium channel blockers (eg, verapamil), immunosuppressants (eg, cyclosporin A), antibiotics (eg, erythromycin), antimalarials (eg, quinine), psychotropic phenothiazines and indole alkaloids (eg, fluphenazine and reserpine), steroid hormones and anti-steroids (eg, progesterone and tamoxifen) and detergents (eg, cremophor EL) [2]. Although the structure of these compounds is very different, see Figure 1, many are amphipathic molecules with a ternary nitrogen and a planar ring or ring system.

First-generation MDR drugs were not specifically developed for inhibiting MDR. They often had other pharmacological activities, as well as a relatively low affinity for MDR transporters, and thus were limited in application. Clinical trials with first-generation MDR drugs failed for various reasons, often due to side effects resulting from adverse reactions to the MDR drug itself [12]. Second-generation drugs were based on the first generation, but were specifically selected or designed to reduce the side effects of the latter by eliminating their non-MDR pharmacological actions. For example, the R-enantiomers of verapamil (R-verapamil) and the dihydropyridine nifedipine (dextenidine) were much weaker calcium channel blockers but nearly equally effective as the L-enantiomers in blocking P-gp [13]. Unfortunately, these compounds did not fare any better as MDR drugs in clinical studies, most likely because their affinity towards P-gp still fell short of producing significant inhibition of MDR in vivo at tolerable doses [12]. However, the early clinical trials were useful in that they indicated the complexity of clinical drug resistance, compared with in vitro MDR models and highlighted conceptual problems and study design issues that must be addressed in future studies [14,15]. Among the more important lessons to be learnt was the need to determine which MDR protein is upregulated in the patient population (eg, P-gp) and to utilize an anticancer drug that would most clearly benefit from inhibition of that protein (eg, a taxane). Another lesson was that the plasma concentrations of the tested MDR drug must be monitored in order to verify that an effective inhibitory concentration was in fact achieved in vivo. Finally, and perhaps most important, is the need to avoid pharmacokinetic interactions between the MDR drug and the anticancer drug(s) used in the study; co-administration of an MDR drug may significantly elevate plasma concentrations of an anticancer drug by interfering with its clearance (eg, via biliary elimination) or metabolism (eg, via the cytochrome P450 system). This would result in an increase in the area under the curve (AUC) leading to unacceptable side effects, necessitating dose reductions down to pharmacologically ineffective levels. Because of these problems, van Zuylen et al stated that “…administration of MDR-convertors is unlikely to improve the therapeutic index of anticancer drugs unless such agents lack significant pharmacokinetic interactions” [15]. Some of the design issues and recommendations for clinical studies with MDR drugs are summarized in Box 1.

Figure 1. First-generation MDR drugs.
Clinical studies with prospective MDR drugs have helped to unravel the complex nature of clinical drug resistance and the problems associated with combination chemotherapy of anticancer drug(s) together with an MDR inhibitor. Among the factors that must be considered before embarking on clinical trials of MDR drugs are: (i) the identity of the MDR protein involved; (ii) the concentration and in vivo effectiveness of the MDR drug; (iii) the pharmacokinetic interaction between the anticancer drug(s) and the MDR inhibitor; and, (iv) novel side-effects that may result from inhibition of drug transporters in organ-tissue barriers [14,15••]. It is therefore recommended that the design of clinical trials should address the following issues:

1. Determination of MDR mechanism/protein in the patient's tumor cells; the anticancer drug(s) utilized should match the MDR protein being inhibited.
2. Determination of plasma concentrations of the MDR drug to confirm that it attains the required levels; surrogate assays should be used to verify effectiveness in vivo or ex vivo already in phase I.
3. Determination of pharmacokinetic interactions between anticancer drug/metabolite and MDR drug already in phase I studies; MDR drugs that exhibit a pharmacokinetic interaction should be avoided as this would require a reduction in anticancer drug dosage to maintain constant AUC.
4. Monitoring side effects due to modulation of MDR protein in normal tissues; special attention should be given to possible neurological responses.
5. Performance of phase II studies in a randomized and controlled manner in patients with proven progression to drug. However, as these are difficult in accrual, it has been suggested that phase III studies should follow phase I studies [15••].

Third-generation MDR drugs

Third-generation MDR drugs are characterized by high affinity to P-gp (and/or other MDR transporters) enabling inhibition at low nanomolar concentrations in in vitro models of MDR. Several such compounds originating from drug development programs are currently undergoing clinical trials in specific forms of advanced cancer (see Table 1/Figure 2).

Results from previously completed clinical trials were reviewed recently [15••,16]. The first of these drugs to be studied was PSC-833 (Valspodar; Novartis AG; Figure 2), a non-immunosuppressive cyclosporin D derivative. While early trials with PSC-833 were encouraging, further work revealed potentially significant pharmacokinetic interactions with several anticancer drugs and, furthermore, possible inhibition of non-MDR-related transporters. Novartis subsequently discontinued development of this compound, although it is currently being examined in additional phase III studies in acute myeloid leukemia (AML), multiple myeloma and myelodysplastic syndrome sponsored by the National Cancer Institute (NCI) [17-20].

MS-209 (Figure 2) is a quinolone derivative under development by Schering AG (formerly Mitsui Pharmaceuticals). Currently, MS-209 is in phase III trials in Japan for the treatment of breast cancer and phase I trials in breast and lung cancer in Europe [21].

### Table 1. Some third-generation MDR drugs in clinical development.

<table>
<thead>
<tr>
<th>Sponsor/Company</th>
<th>Drug</th>
<th>Analog</th>
<th>Target</th>
<th>Phase</th>
<th>Cancer type</th>
<th>Anticancer drugs</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI</td>
<td>PSC-833; valspodar</td>
<td>cyclosporin D</td>
<td>P-gp</td>
<td>III</td>
<td>Acute myeloid leukemia, multiple myeloma and myelodysplastic syndrome</td>
<td>Busulfan, cytarabine, Daunorubicin, dexamethasone, doxorubicin, etoposide, filgrastim, hydroxyurea, idarubicin, IL-2, mitoxantrone, thioguanine, tretonin, vinblastine, vincristine</td>
<td>[17-20]</td>
</tr>
<tr>
<td>Schering/ EORTC</td>
<td>MS-209</td>
<td>quinoline</td>
<td>P-gp MRP</td>
<td>III</td>
<td>Breast cancer</td>
<td>Docetaxel</td>
<td>[21]</td>
</tr>
<tr>
<td>NCI/ Xenova/ QLT</td>
<td>XR-9576; tariquidar</td>
<td>anthranilamide</td>
<td>P-gp MRP</td>
<td>II</td>
<td>Refractory solid tumors</td>
<td>Docetaxel, vinorelbine, docetaxel</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertex</td>
<td>VX-710; biricodar; Incel</td>
<td>pipercolinate</td>
<td>P-gp MRP</td>
<td>II</td>
<td>Prostate, lung, ovarian, breast carcinomas</td>
<td>Paclitaxel</td>
<td>[24]</td>
</tr>
<tr>
<td>NCI/ EORTC</td>
<td>R-101933</td>
<td>-</td>
<td>P-gp</td>
<td>II</td>
<td>Metastatic refractory breast cancer</td>
<td>Paclitaxel or docetaxel</td>
<td>[25]</td>
</tr>
<tr>
<td>NCI</td>
<td>mitotane; NSC-38721</td>
<td>2,4-dichlorodiaryl diphenylidyilorothane</td>
<td>P-gp</td>
<td>II</td>
<td>Adrenal cortical carcinoma</td>
<td>Docetaxel, vincristine, etoposide</td>
<td>[26]</td>
</tr>
<tr>
<td>Ontogen</td>
<td>OC-144-093</td>
<td>diarylimidazole</td>
<td>P-gp</td>
<td>II</td>
<td>Metastatic breast cancer</td>
<td>Paclitaxel</td>
<td>[27]</td>
</tr>
<tr>
<td>Eli Lilly</td>
<td>LY-335979</td>
<td>cyclopropyldibenzol suberane</td>
<td>P-gp</td>
<td>II</td>
<td>Advanced solid tumors</td>
<td>NA</td>
<td>[28]</td>
</tr>
<tr>
<td>Antigenics</td>
<td>Annamycin</td>
<td>anthracycline</td>
<td>NA</td>
<td>II</td>
<td>Refractory or relapsed leukemia</td>
<td>NA</td>
<td>[30]</td>
</tr>
</tbody>
</table>

Sources: www.biospace.com and clinicaltrials.gov. NA not available
All other prospective MDR drugs are still in phase II or phase I trials. XR-9576 (tariquidar; Xenova Group plc/QLT Inc; Figure 2) has successfully concluded phase II studies with paclitaxel and vinorelbine in ovarian cancer. US FDA approval for the initiation of phase III trials has been granted, and the trials are expected to commence in mid-2002 [22]. By April 2002, the NCI was enrolling a total of 24 patients for a phase I trial and pharmacokinetic study of XR-9576 in children with refractory solid tumors including brain tumors [23]. VX-710 (biricodar, Incel; Vertex Pharmaceuticals Inc; Figure 2), a high-affinity P-gp and MRP inhibitor, appears to have no pharmacokinetic interactions with doxorubicin and is currently undergoing phase II trials in solid tumors [24].

R-101933 (Janssen Pharmaceutica NV; Figure 2) exhibits desirable pharmacokinetic characteristics with respect to taxols and is also now undergoing a European Organisation for Research and Treatment of Cancer (EORTC)/NCI sponsored phase II study in metastatic breast cancer in combination with these anticancer drugs [25]. Another drug that is not typically third-generation is mitotane (Figure 2), long utilized for treatment of adrenocortical carcinoma and recently found to inhibit P-gp, is now similarly being studied in combination with anticancer drugs in an NCI-sponsored phase II study [26].

Additional candidate MDR drugs in phase II studies are listed in Table 1 [27,28]. Many more compounds are undoubtedly in phase I and preclinical development. Noteworthy among those is GF-120918 (elacridar; GlaxoSmithKline plc; Figure 2), initially characterized as a P-gp inhibitor but now known to inhibit also BCRP, which has completed phase I studies and shows no pharmacokinetic interactions with doxorubicin [29]. Annamycin (Antigenics Inc; Figure 2) is an exception, as it is not a P-gp inhibitor but an anthracycline that is not transported by P-gp (see below) [30].
Novel approaches to MDR therapy

The discussion so far has focused on direct inhibitors of MDR transporters, mainly P-gp inhibitors. The difficulties encountered in applying these drugs in the clinic and the emerging complexity of the MDR phenotype have engendered several alternative approaches to MDR therapy, designed either to inhibit MDR in novel ways or to cleverly circumvent MDR mechanisms altogether.

Inhibiting MDR mechanisms

Downregulation of MDR transporters by antisense oligonucleotides has been suggested as an alternative and more specific way to overcome MDR than the use of conventional small molecule pharmacological inhibitors [31]. Recent advances in antisense oligonucleotide technologies are reflected in patents such as those of Isis Pharmaceuticals [101] and Hybridon [102], claiming methods to suppress P-gp expression using antisense oligonucleotides with improved stability and cellular permeability. Another proposed approach is to exploit or target physiological mechanisms involved in regulation of MDR proteins. Induction of MDR1 gene expression in tumor cells occurs upon treatment with cytotoxic drugs, whereas this response is inhibited by pharmacological inhibitors of calcium-dependent signaling in what could be a novel therapeutic strategy, as proposed in a recent patent by the University of Illinois [103]. MDR1 and cytochrome P450 3A4 (CYP3A4) gene expression can also be stimulated by anticancer drugs such as taxol via its direct interaction with and activation of the nuclear steroid and xenobiotic receptor (SRX), leading to increased drug resistance and faster drug clearance. Hence, antagonists of SRX such as ET-743 (PharmaMar SA/Ortho Biotech Inc; Figure 3) may be utilized in conjunction with anticancer drugs to counteract the induction of MDR1 and CYP3A4 [104].

Figure 3.

Acquisition of the MDR phenotype is often associated with upregulation of glucosylceramide (GlcCer) [32-34], which results from elevated GlcCer synthase activity [32,34]. Overexpression of recombinant GlcCer synthase confers resistance to adriamycin and to ceramide in human breast cancer cells, suggesting that drug resistance in GlcCer synthase-transfected cells is related to stimulation of glucosylation of ceramide and the resultant inhibition of drug-induced apoptotic signaling [35]. The role of GlcCer synthase in drug resistance was demonstrated directly by antisense suppression of GlcCer synthase expression in MDR cells [36]. These results are consistent with the hypothesis that GlcCer synthase contributes to drug resistance in MDR cells by attenuating drug-induced formation of apoptotic ceramide and indicate that GlcCer synthase may represent a novel drug target in cancer MDR [37]. A recent US patent application from Shayman et al describes novel amino-ceramide analogs that inhibit GlcCer synthase and thereby may elevate ceramide production in MDR cells, enhancing drug-induced apoptosis [105].

Circumventing MDR mechanisms

MDR mechanisms reflect the innate adaptive potential of living cells and may thus prove to be intractable. Therefore, researchers have looked for various ways to circumvent rather than directly inhibit MDR mechanisms. One approach has focused on developing anticancer drugs that are poor substrates for MDR transporters. Examples include anthracyclines such as idarubicin and annamycin. The latter has reached phase II development with Antigenics, which is currently evaluating its development program. Another anticancer drug with such properties is an olivacine derivative, S16020-2 (Servier; Figure 4), that is similarly able to bypass P-gp-mediated resistance possibly because of its rapid uptake kinetics compared with standard anticancer agents [38].

Figure 4.

Tumors require an adequate blood supply in order to grow and are capable of inducing the formation of new blood vessels that provide them with oxygen and nutrients, a phenomenon called angiogenesis [39]. The angiogenic response requires proliferation of vascular endothelial cells which depends on angiogenic factors, and it can be inhibited by anti-angiogenic factors. Recent work has shown that the latter can effectively inhibit tumor growth and hence anti-angiogenic therapy may become an important anticancer treatment modality [40]. As anti-angiogenic factors do not target the tumor cells themselves but rather the endothelial cells, anti-angiogenic therapy should, in principle, be equally effective toward non-MDR and MDR tumors. A possible proof of feasibility for this therapeutic strategy is provided by recent clinical studies showing the effectiveness of thalidomide (Celgene Corp; Figure 5), an anti-angiogenic drug, in treating patients with refractory multiple myeloma [41,42]. However, the action of thalidomide may additionally reflect modalities other than anti-angiogenesis, since it also induces apoptosis in drug resistant multiple myeloma cells in vitro [43].

Figure 5.
A novel procedure for circumventing MDR mechanisms, involving immunization with an autologous tumor cell vaccine, was proposed by Shtil et al [44]. This work shows that vaccination with irradiated myeloma cells engineered to express GM-CSF, elicits a strong cytotoxic T-lymphocyte response leading to > 90% graft rejection. Significantly, similar response rates were obtained with drug resistant myeloma cells, indicating that cell killing bypasses the resistant apoptotic pathway(s). Additional studies suggest that the T-lymphocyte cytotoxicity occurs by perforin-induced necrosis, although granzyme B-induced apoptosis may also be involved [44]. This strategy holds great promise but the road to clinical implementation is still long and arduous. A conceptually related approach involves the use of rituximab (Genentech Inc/IDEC Pharmaceuticals Corp), an apoptosis-inducing monoclonal antibody directed against the CD20 receptor. Rituximab induces apoptosis in drug sensitive cells and may modulate the threshold for drug-induced apoptosis in MDR cells. Recent clinical studies show that rituximab improves the efficacy of chemotherapy [45].

Finally, a recent patent outlines an elegant way to overcome MDR simply by elevating the anticancer drug dosage. The problem is to achieve this without complete eradication of bone marrow stem cells, which is considered to be a major dose-limiting toxicity factor. Gottesman et al thus suggest upregulating drug resistance of autologous bone marrow stem cells by transfection with vectors carrying the MDR1 cDNA. This would result in multidrug resistant bone marrow cells which may be employed to reconstitute a lethally irradiated endogenous hemopoietic system, providing protection against a chemotherapeutic regimen at otherwise unacceptable doses, and thus overcoming MDR [106].

Conclusion
So far, the complexity and versatility of cellular MDR mechanisms have hindered the search for effective and clinically applicable MDR therapies. Yet, in the last two decades we have learnt much about the required properties of putative MDR drugs and how best to evaluate them. A few good candidates have emerged that may soon make it into the clinic. Novel inhibitors of MDR transporters are continually being discovered, including many natural products derived from rare plants and marine fauna (eg, WO-00149279 [107] and US-06087370 [108]). In addition, novel approaches are being devised in order to bypass rather than block MDR mechanisms. With these advances in sight there is ground for cautious optimism that improvements in the efficacy of cancer chemotherapy may be expected in the not too distant future.

Acknowledgements
We thank Dr Elke Burgermeister and Dana Ravid for helpful discussions. We are grateful to Yona Eli and Tovi Harel-Orbital for excellent technical assistance. Our work is supported in part by grants from the Ministry of Science, Culture and Sports and the Deutches Krebsforschungszentrum, the Mary Ralph Desigated Philantropic Fund of the Jewish Community Endowment Fund, San Francisco, and the Harry Levine Foundation. Mordechai Liscovitch is the incumbent of the Harold L. Korda Professorial Chair in Biology.

Associated references

- of outstanding interest
- of special interest

- An excellent entry point into the MDR field, focusing on earlier studies of P-gp, its discovery, cloning and inhibition.
- A comprehensive overview of MDR proteins belonging to the MRP family.
- A healthy dose of P-gp physiology in a field dominated by oncology. Covers all the roles of P-gp in normal tissues.
- Opening the floodgates, this paper showed that verapamil could inhibit MDR, indicating that off-the-shelf biochemicals could be utilized to inhibit P-gp.
- An outstanding review of recent results from clinical studies of candidate MDR drugs, providing a thorough and lucid presentation of the problems involved in translation of in vitro studies on MDR into clinical reality.
- An excellent entry point into the MDR field, focusing on earlier studies of P-gp, its discovery, cloning and inhibition.


References to patent literature


