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MINIREVIEW

Drug Efflux Transporters and Multidrug Resistance in Acute Leukemia: Therapeutic Impact and Novel Approaches to Mediation

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ABSTRACT

Multidrug resistance (MDR), which is mediated by multiple drug efflux ATP-binding cassette (ABC) transporters, is a critical issue in the treatment of acute leukemia, with permeability glycoprotein (P-gp), multidrug resistance-associated protein 1, and breast cancer resistance protein (i.e., ABCG2) consistently being shown to be key effectors of MDR in cell line studies. Studies have demonstrated that intrinsic MDR can arise as a result of specific gene expression profiles and that drug-induced overexpression of P-gp and other MDR proteins can result in acquired resistance, with multiple ABC transporters having been shown to be overexpressed in cell lines selected for resistance to multiple drugs used to treat acute leukemia. Furthermore, numerous anticancer drugs, including agents commonly used for the treatment of acute leukemia (e.g., doxorubicin, vincristine, mitoxantrone, and methotrexate), have been shown to be P-gp substrates or to be susceptible to efflux mediated by other MDR proteins, and multiple clinical studies have demonstrated associations between P-gp or other MDR protein expression and responses to therapy or survival rates in acute leukemia. Here we review the importance of MDR in cancer, with a focus on acute leukemia, and we highlight the need for rapid accurate assessment of MDR status for optimal treatment selection. We also address the latest research on overcoming MDR, from inhibition of P-gp and other MDR proteins through various approaches (including direct antagonism and gene silencing) to the design of novel agents or novel delivery systems for existing therapeutic agents, to evade cellular efflux.

Introduction

Drug resistance is a critical issue in the treatment of cancer, notably acute leukemias. Research performed in the previous 25 years showed that this resistance may be mediated by multiple multidrug resistance (MDR) proteins, with 48 ATP-binding cassette (ABC) transporters having been identified as facilitating the efflux of various substrates, including anticancer drugs, from cells (Steinbach and Legrand, 2007). Permeability glycoprotein (P-gp) was identified as the first ABC transporter associated with drug resistance (Kartner et al., 1983; Campos et al., 1992), but multiple additional transporters, which confer resistance to a wide range of drugs, have since been identified (Szakács et al., 2004). The three most-studied MDR proteins are P-gp (encoded by the *MDR1* gene), multidrug resistance-associated protein 1 (MRP1), and breast cancer resistance protein (BCRP) (i.e., ABCG2), which have been shown consistently in studies with cancer cell lines to mediate the primary mechanism of MDR (Ambudkar et al., 1999; Hipfner et al., 1999; Abbott, 2003;

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ABBREVIATIONS: MDR, multidrug resistance; ABC, ATP-binding cassette; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BCRP, breast cancer resistance protein; COX, cyclooxygenase; CR, complete remission; EFS, event-free survival; GCS, glucosylceramide synthase; LRP, lung resistance protein; MRP, multidrug resistance-associated protein; CML, chronic myeloid leukemia; ITD, internal tandem duplication; OS, overall survival; P-gp, permeability glycoprotein; shRNA, short hairpin RNA; siRNA, small interfering RNA; SNP, single-nucleotide polymorphism; RT, reverse transcription; PCR, polymerase chain reaction; hOCT1, human organic cation transporter 1; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide; FG020326, (*E*)-methyl-3-[4-[4,5-bis(4-[isopropyl(methyl)amino]phenyl)-1*H*-imidazol-2-yl]phenyl]acrylate; Genz-123346, (1*R*,2*R*)-nonanoic acid-[2-(2′,3′-dihydrobenzo[1,4]dioxin-6′-yl)-2-hydroxy-1-pyrrolidin-1-yl-methylethyl-]amide L-tartaric acid salt; MZ3, 4-(4-bromophenyl)-2,3-dihydro-*N*,3-bis(3,4,5-trimethoxyphenyl)-2-oxoidmidazole-1-carboxamide.

Szakács et al., 2004). The genes responsible for encoding these proteins, as well as other genes that encode ABC transporters known to be involved in anticancer drug resistance, are shown in Table 1, along with the known drug substrates for each MDR protein (Szakács et al., 2006; Moitra et al., 2011). Non-ABC transporter proteins with known roles in MDR are also listed. The 48 genes encoding the ABC transporters are subdivided into seven families, A to G. As shown in Table 1, a large number of proteins encoded by the B and C families in particular were shown to confer resistance through efflux, which highlights their importance in cancer (Dean et al., 2001).

A number of studies demonstrated that intrinsic MDR can arise as a result of specific gene expression profiles. For example, increased MDR gene expression (*MDR1* and *ABCG2*) was associated with poorer overall survival (OS) rates in a gene expression profiling study among adults with acute myeloid leukemia (AML) (Wilson et al., 2006). Elevated P-gp expression was identified more frequently among older versus younger patients with AML (Erba, 2007), which reflects the greater resistance to therapy and the poorer prognosis seen for older patients with AML. Because of the importance of MDR gene expression, the contributions of genetic polymorphisms to intrinsic MDR have been investigated extensively, to determine whether specific genotypes or haplotypes are associated with responses to therapy (Leschziner et al., 2007). Individual studies evaluated specific *MDR1* polymorphisms and P-gp expression in acute leukemias, with inconsistent results. In one study, no significant effect on P-gp-mediated drug resistance among patients with acute leukemia was associated with *MDR1* C3435T, G2677T, or T−129C polymorphisms (Kaya et al., 2005); in other studies, C3435T polymorphisms were associated with poor prognoses for childhood but not adult acute lymphoblastic lymphoma and not adult AML (Jamroziak et al., 2004, 2005, 2006). In another study, however, the C/C and G/G genotypes of C3435T were associated with higher probabilities of complete remission and longer event-free survival (EFS) times (Kim et al., 2006). Further work is required in this area (Leschziner et al., 2007).

Additional studies demonstrated that drug-induced overexpression of P-gp and other MDR proteins could result in acquired resistance, with multiple ABC transporters being overexpressed in cell lines selected for resistance to multiple AML drugs (Ambudkar et al., 1999; Szakács et al., 2006). For example, doxorubicin induces overexpression of MDR1 in HL-60 AML cells (Puhlmann et al., 2005), and up-regulated expression of both *MDR1* and *MRP1* was demonstrated in doxorubicin-resistant HL-60 cells (HL-60/DOX cells) (Baran et al., 2007). Likewise, cytarabine was shown to up-regulate *MDR1* gene and P-gp protein expression in HL-60 cells (Prenkert et al., 2009).

This review addresses the important issue of MDR in AML and other cancers and highlights the critical need for rapid accurate assessment of MDR status for optimal treatment selection, on the basis of known resistance to various agents. We discuss the specific aspects of MDR status and their prognostic significance for AML and other cancers, and we address the latest research on overcoming MDR, from P-gp inhibition to the design of novel agents to evade cellular efflux.

Assessment of MDR Status

Numerous anticancer drugs, including agents commonly used for the treatment of acute leukemia, such as doxorubicin, vincristine, mitoxantrone, and methotrexate, have been shown to be P-gp substrates or to be susceptible to efflux through other MDR proteins (Table 1). Therefore, it is important to assess MDR status, to facilitate appropriate treatment selection. Multiple methods for assessment of MDR in cell lines and among patients have been investigated, with various recent developments offering the potential for accurate identification of gene overexpression or protein up-regulation.

TABLE 1
ABC transporters involved in anticancer drug resistance (Dean et al., 2001; Szakacs et al., 2006; Moitra et al., 2011)

Gene	Protein	Roles in Drug Resistance	Anticancer Drug Substrates/Inducers
ABCA2	ABC2	Drug transport	Estramustine, mitoxantrone
ABCA3	ABC3	Surfactant lipid transporter, lysosomal drug sequestration (Chapuy et al., 2008)	Doxorubicin, daunorubicin (Steinbach et al., 2006), imatinib (Chapuy et al., 2009)
ABCB1	P-gp/MDR1	Drug transport	Multiple, including *Vinca* alkaloids, anthracyclines, etoposide, taxanes, imatinib, irinotecan, methotrexate, mitoxantrone
ABCB4	PGY3/MDR3	Phosphatidylcholine and drug transport, bile acid secretion	Vinblastine (Wang et al., 2008), doxorubicin (Turton et al., 2001)
ABCB5	ABC19	Drug transport	5-Fluorouracil (Wilson et al., 2011), doxorubicin (Frank et al., 2005)
ABCB11	SPGP	Bile salt and drug transport	Paclitaxel
ABCC1	MRP1	Drug transport	Multiple, including *Vinca* alkaloids, anthracyclines, etoposide, imatinib, irinotecan, methotrexate, mitoxantrone
ABCC2	MRP2	Organic anion efflux, drug transport	Multiple, including *Vinca* alkaloids, anthracyclines, etoposide, taxanes, irinotecan, cisplatin, methotrexate, mitoxantrone
ABCC3	MRP3	Drug transport	Etoposide
ABCC4	MRP4	Nucleoside and drug transport	Irinotecan, thiopurines, methotrexate
ABCC5	MRP5	Nucleoside and drug transport	Thiopurines, cisplatin, methotrexate
ABCC6	MRP6	Drug transport	Anthracyclines, etoposide, cisplatin, gemcitabine (Ikeda et al., 2011)
ABCC10	MRP7	Drug transport	*Vinca* alkaloids, taxanes
ABCC11	MRP8	Drug transport	5-Fluorouracil
ABCC12	MRP9	Drug transport	None identified
ABCG2	ABCP/BCRP1	Toxin efflux, drug transport	Multiple, including anthracyclines, etoposide, imatinib, flavopiridol, irinotecan, methotrexate, mitoxantrone
LRP	LRP	Major vault transporter protein (Scheffer et al., 1995)	AML induction chemotherapy (List et al., 1996; Huh et al., 2006)

For example, semiquantitative RT-PCR methods were used in studies of *MDR1* expression among patients with AML (Balatzenko et al., 2002; Trnková et al., 2007) and, although higher levels of expression in bone marrow were correlated with lower rates of complete remission (CR) induction in one study, there was no association with OS rates (Trnková et al., 2007). RT-PCR assays with fluorescent hybridization probes were used to evaluate the expression of BCRP among patients with acute leukemia (Nakanishi et al., 2003). Furthermore, rapid detection of P-gp, MRP1, and BCRP with a technique involving an automated cell counter with fluorescence detection capabilities was demonstrated (Robey et al., 2011), BCRP and MRP2 activities were assessed by using membrane vesicle-based assays (Elsby et al., 2011), and MRP1 expression was analyzed by using capillary electrophoresis immunoassays (Mbuna et al., 2011). A novel technique, i.e., reverse-phase protein microarray assays, for identification of MDR leukemia cells on the basis of Akt1 activity or phosphorylation was reported, with higher Akt1 activity being demonstrated in MDR cells (Maraldi et al., 2011). Other novel techniques studied for assessment of P-gp-mediated transport activity include the use of gallium-labeled metalloprobes (Sivapackiam et al., 2010) and single-photon emission computed tomography with other radiolabeled metal complexes (Piwnica-Worms and Sharma, 2010).

Among older techniques, fluorometric assays of calcein accumulation or uptake, in conjunction with flow cytometry, provide a method for measurement of P-gp functional activity, because calcein undergoes efflux mediated by P-gp and uptake levels are significantly lower in P-gp-expressing cells, compared with control cells (Holló et al., 1994; Homolya et al., 1996). This technique was used to demonstrate correlations between P-gp and MRP1 expression and activity in pediatric acute lymphoblastic leukemia (ALL) and adult AML (Legrand et al., 1998; Fazlina et al., 2008). Low calcein uptake was shown to be a marker of poor prognosis in AML (Legrand et al., 1998). MDR among patients with AML also was assessed by using efflux assays with rhodamine 123 (Lamy et al., 1995), 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) (Legrand et al., 2001), 3,3′-diethyloxacarbocyanine iodide (Leith et al., 1999), and daunorubicin (Kim et al., 2005), all of which are substrates of P-gp, as well as the MDR1-specific antibody MRK16 (Leith et al., 1997). Higher daunorubicin efflux levels were significantly predictive of lower CR and OS rates among patients with AML, and results were more reliable than *MDR1* RT-PCR or P-gp expression findings (Kim et al., 2005), which indicates the importance of evaluating functional activity rather than gene or protein expression alone. Rhodamine 123 efflux was correlated with P-gp expression, and both were predictive of CR and OS rates among patients with AML or ALL; however, some patients showed efflux without P-gp expression, which indicates the importance of other MDR efflux pumps (Lamy et al., 1995).

Positron emission tomography using [^{18}F]fluoroethyl compounds (Kawamura et al., 2011) and ^{99m}Tc-hexakis-2-methoxyisobutylisonitrile scintigraphy (Dizdarevic and Peters, 2011) were used recently to assess the in vivo function of P-gp and BCRP, whereas another study suggested that MDR could be assessed by using a carbon nanotube-drug supramolecular nanocomposite electrochemical sensor, as demonstrated with sensitive and MDR K562 leukemia cells (Zhang et al., 2011a). Finally, the combination of single-photon emission computed tomography, positron emission tomography, and other imaging techniques with genetic data, guided by the findings of preclinical and clinical studies of MDR, may prove important for the selection of optimal treatments for patients who demonstrate particular MDR phenotypes (Dizdarevic and Peters, 2011).

Among the methods highlighted here, RT-PCR assays represent the most convenient assays for assessment of MDR gene expression. However, it remains difficult to correlate differences in levels of MDR mRNA expression with differences in MDR protein levels or function. Although protein microarray assays are now available, more data are required to demonstrate a relationship between protein levels and functionality. Cell-based functional assays, such as rhodamine 123 and doxorubicin efflux assays, and fluorescent cell-counting and membrane vesicle-based assays can directly reflect MDR activity but present some technical challenges associated with the preparation of live cells or membrane vesicles. In vivo imaging assays offer the best indication of the clinical significance of MDR; however, their broad use remains challenging because of the limited availability of imaging agents. Given the limitations associated with each method, recommendations might be to use multiple assays and, until definitive links between assay results and activity are demonstrated, to interpret findings with caution.

MDR Proteins Conferring Resistance in Preclinical In Vitro Models

Numerous in vitro studies have highlighted the importance of P-gp in AML resistance (Pallis et al., 2002). P-gp is associated with resistance to a range of drugs in AML cell lines (Table 1), and it was suggested that P-gp plays a role in the development of an apoptosis-resistant phenotype (Pallis et al., 2002; Guenova et al., 2010). As noted earlier, *MDR1* expression was shown to be up-regulated in doxorubicin-resistant HL-60/DOX AML cells (Baran et al., 2007) and to be associated with in vitro sensitivity to daunorubicin in cells from adults with acute leukemia (Marie et al., 1991). Likewise, P-gp overexpression resulted in resistance to gemtuzumab ozogamicin in HL-60 cells (Cianfriglia et al., 2010) and reduced sensitivity to FLT3 inhibitors in *FLT3*-ITD$^+$ primary AML blasts (Hunter et al., 2004). P-gp activity was identified as a possible mechanism mediating the sensitivity of leukemia cell lines to 17-*N*-allylamino-17-demethoxygeldanamycin (Napper and Sollars, 2010). In studies of 13 cell lines, including some leukemia cell lines, P-gp expression was associated with the inhibitory effects of cyclosporine A on rhodamine 123, daunorubicin, and calcein acetomethoxy ester uptake (Legrand et al., 1998), whereas P-gp overexpression in ALL cells was associated with resistance to silvestrol, a translation initiation inhibitor (Gupta et al., 2011). Clofarabine cytotoxicity in AML cells was shown to be reduced through P-gp-mediated efflux, which was influenced by deoxycytidine kinase; deoxycytidine kinase is responsible for clofarabine activation through monophosphorylation, and P-gp mediated the efflux of clofarabine more readily than that of its monophosphate (Nagai et al., 2011).

In contrast to the aforementioned findings for *MDR1* expression, analysis of blast cell samples from patients with acute leukemia showed that *BCRP* but not *MDR1* expression was correlated with cell viability and induction of apoptosis by flavopiridol (Nakanishi et al., 2003). In another report, BCRP and other transporters were shown to mediate drug efflux in leukemia cell line studies (Raaijmakers et al., 2005). As with P-gp, multiple studies showed that the expression of MRP1 is associated with resistance in AML cell lines. For example, studies with MRP$^+$ NB4 and HL-60 cells showed that gemtuzumab ozogamicin-induced cytotoxicity was attenuated with MRP1 expression (Walter et al., 2003). Furthermore, MRP1 expression was shown to reduce DNA intercalation of daunorubicin and idarubicin (Smeets et al., 1999) and to be up-regulated in AML-2/DX300 (Kweon et al., 2010) and HL60/DOX (Baran et al., 2007) doxorubicin-resistant AML cells. MRP1 was found to be overexpressed in an arsenic trioxide-resistant human leukemia cell line, K562/AS-3 (Seo et al., 2007). MRP4 was shown to be possibly involved in AML cell proliferation and differentiation through the efflux of cAMP, which plays a key role in cell maturation (Copsel et al., 2011). Studies of pediatric ALL and AML cells did not demonstrate a definitive link between P-gp and BCRP expression and drug resistance in vitro (Svirnovski et al., 2009). However, studies with leukemia cell lines and severe combined immunodeficiency mouse xenograft models demonstrated that P-gp overexpression may be associated with enhanced leukemia cell invasiveness (Hu et al., 2011).

Effects of MDR Protein Expression on Clinical Outcomes

MDR in Acute Myeloid Leukemia and Other Acute Leukemias. Several clinical studies demonstrated associations between P-gp expression or function/activity and responses to therapy or survival times for AML and other acute leukemias (Pallis et al., 2002; Trnková et al., 2007). MDR1 expression was evaluated in a number of studies. Huh et al. (2006) demonstrated poorer 2-year survival rates among patients with ALL or AML with high MDR1 mRNA expression levels. Reduced responses to induction therapy were seen among patients with high MDR1 expression levels in another study of adult patients with acute leukemia (Marie et al., 1991), MDR1 expression was prognostic for poorer responses to induction therapy and shorter OS times in a study of 331 adult patients with AML (Schaich et al., 2005), and high MDR1 expression levels were associated with a significantly poorer EFS rate in a study of 49 pediatric patients with ALL (Kourti et al., 2007). MDR1 expression was associated with lower CR rates but not decreased OS rates in a study of 405 patients with AML (Illmer et al., 2002); the homozygous wild-type genotype was associated with a decreased OS rate and an increased risk of relapse, which suggests that mechanisms in addition to P-gp expression are involved. An analysis of MDR1 expression and *FLT3*-ITD mutation status among 166 adult patients with AML demonstrated shorter times to relapse for MDR1-overexpressing patients and poor disease-free survival rates for patients with both MDR1 overexpression and *FLT3*-ITD$^+$ status (Tiribelli et al., 2011).

The parameter of P-gp expression also has been shown to be associated with responses and outcomes. P-gp expression was associated with a significantly lower CR rate, as well as resistant disease, among elderly patients with AML who were enrolled in a Southwest Oncology Group study (Leith et al., 1997), and P-gp expression levels were prognostic for OS times in a study of 121 adults with de novo AML (Wuchter et al., 2000). P-gp expression was prognostic for not achieving CR among 53 patients with AML who were treated in two European Organization for the Research and Treatment of Cancer study protocols (Legrand et al., 1998) and was associated with a lower CR rate in a study of 200 adult patients with ALL (Tafuri et al., 2002). Venditti et al. (2004) demonstrated that patients with newly diagnosed AML who expressed both Bcl-2 and P-gp exhibited a significantly lower CR rate in response to standard induction therapy than did patients who expressed only one or neither of those proteins (Venditti et al., 2004). Larger proportions of older versus younger patients with AML demonstrated MDR and an antiapoptotic phenotype, which was associated with a greater incidence of homogeneous CD34$^+$ blast cell populations among older patients; the blast cells exhibited elevated levels of P-gp and Bcl-2 expression (Suárez et al., 2005). Elevated levels of P-gp and Bcl-2 expression also were reported for CD34$^+$ versus CD34$^-$ childhood AML leukemia cells (Shman et al., 2008). P-gp expression on the surface of acute nonlymphoblastic lymphoma cells obtained at the time of diagnosis was associated with significantly lower CR rates and shorter survival times in a study of 150 patients (Campos et al., 1992). Likewise, P-gp activity identified with a rhodamine efflux assay was associated with significantly shorter OS times for pediatric ALL in one study (Brozek et al., 2009) and with responses to induction, relapse rates, and OS times for adult AML but not newly diagnosed pediatric ALL in another study (Wuchter et al., 2000).

P-gp is not the only MDR transporter to be associated with poorer responses to therapy and survival rates for acute leukemia. Multiple studies have associated *BCRP* gene expression and BCRP protein expression and/or function with poor responses and prognoses for adult (Benderra et al., 2004, 2005; Uggla et al., 2005; Damiani et al., 2006) and pediatric (Steinbach et al., 2002) AML. One study showed that the adverse impact of BCRP on disease-free survival rates was not overcome with fludarabine-based induction therapy (Damiani et al., 2010). Among older patients with AML, coexpression of MDR1 and BCRP was shown to be associated with a clinically resistant phenotype (van den Heuvel-Eibrink et al., 2007). Likewise, elevated levels of expression of MDR1 and/or BCRP in CD34$^+$/CD38$^-$ AML cells were correlated with negative responses to chemotherapy among patients and at the cellular level (Ho et al., 2008).

In contrast to other findings (Legrand et al., 1998; Laupeze et al., 2002; Schaich et al., 2005), a number of studies did not demonstrate a prognostic impact of MRP1 expression in AML (Leith et al., 1999; van der Kolk et al., 2000). MRP1, MRP2, MRP3, MRP5, and MRP6 expression levels were all shown to be associated with poorer relapse-free survival rates in pediatric and adult ALL (Plasschaert et al., 2005). In other studies, MRP3 expression was associated with poor prognoses for pediatric ALL (Steinbach et al., 2003b) and pediatric (Steinbach et al., 2003a) and adult (Benderra et al., 2005) AML. Lung resistance protein (LRP) expression also has been associated with therapeutic efficacy. List et al. (1996) demonstrated that LRP overexpression was associated with poorer responses to induction therapy and a trend toward shorter response durations and OS times in a study of 66 patients with AML, whereas Huh et al. (2006) showed that LRP

mRNA expression was associated with resistance to induction chemotherapy among patients with acute leukemia, MRP1 mRNA expression was associated with poorer 2-year survival rates, and expression of both MRP1 and LRP identified patients with very poor 2-year survival rates. Similar findings were reported from a study of 34 pediatric patients with ALL, with MRP1 and LRP mRNA expression being associated with lower CR rates and poorer 2-year survival rates (El-Sharnouby et al., 2010). Finally, a phase 2 study of gemcitabine and mitoxantrone treatment for patients with AML at first relapse suggested that higher levels of expression of total MRP4 and solute carrier family 29 member 2 were associated with not achieving CR (Advani et al., 2010); high levels of expression of glutathione transferase P (encoded by *GSTP1*) were also seen.

Prognostic Effects of Specific Gene Polymorphisms in Acute Leukemias. With numerous studies demonstrating the adverse prognostic impact of up-regulated MDR1 transcription or P-gp expression/activity in AML and other acute leukemias, multiple analyses have been performed to determine whether specific *MDR1* gene polymorphisms are associated with poorer responses to treatment and overall outcomes, with mixed findings (Leschziner et al., 2007), as summarized in Table 2. A number of studies reported positive associations between specific *MDR1* polymorphisms and responses and/or outcomes (van den Heuvel-Eibrink et al., 2001; Monzo et al., 2006). For example, a study of the three most-frequent single-nucleotide polymorphisms (SNPs) of the *MDR1* gene, i.e., C1236T, G2677T, and C3435T, among 405 patients with AML demonstrated that, although the C/C genotype of C3435T was associated with lower MDR1 expression levels, it was also significantly associated with the highest probability of relapse and poor OS rates (Illmer et al., 2002). Consistent with these findings, the C/C genotype of *MDR1* C3435T was associated with lower EFS and OS rates in pediatric ALL, whereas the T/T genotype was associated with the risk of developing ALL (Jamroziak et al., 2004); similar findings were reported from a study of 147 Indian patients with ALL (Rao et al., 2010) and a study of 105 Taiwanese pediatric patients with ALL (Yang et al., 2010). In contrast, a study of 101 Asian patients with AML showed that the C/C genotype of *MDR1* C3435T, although associated with lower levels of P-gp expression in leukemic blasts, compared with the C/T and T/T genotypes, was associated with better 3-year EFS but not OS rates; the G/G genotype of G2677T was also associated with better 3-year EFS rates (Kim et al., 2006).

A number of studies reported an absence of associations between genotypes and responses and/or outcomes. For example, van der Holt et al. (2006) reported no associations between genotypes involving *MDR1* C1236T, G2677T, or C3435T polymorphisms and P-gp expression and function in leukemic blasts, MDR1 expression, CR rates, or survival rates in a study of 150 patients ≥60 years of age with AML who were treated in a phase 3 study. Other studies reported no associations between *MDR1* C3435T polymorphisms and P-gp function in leukemic blasts (Jamroziak et al., 2005, 2006; Hur et al., 2008) or responses and long-term outcomes among patients with AML (Jamroziak et al., 2005; Hur et al., 2008). In addition, the C/C genotype of C3435T was not associated with prognoses in a study of 143 Indian patients with AML (Rao et al., 2010). Likewise, a single-center retrospective study of 262 patients with AML did not identify any *MDR1* polymorphisms that were associated with survival rates (Hampras et al., 2010), and a study of 45 Turkish patients with AML or ALL showed no significant effects of C3435T, G2677T, and T−129C polymorphisms on P-gp-mediated drug resistance (Kaya et al., 2005). In contrast to studies described earlier, a study of 53 patients with ALL identified no associations between the *MDR1* C3435T polymorphism and ALL resistance or prognosis (Efferth et al., 2003).

Studies also assessed the prognostic impact of *BCRP, MRP1*, and other MDR gene polymorphisms in AML (Table 2). A single-center retrospective study identified a SNP in the *BCRP* gene that was associated with improved OS rates, compared with the wild-type genotype, as well as increased risk of toxicity (Hampras et al., 2010). In a study of 112 Israeli patients with AML, the *ABCC3* C−211T polymorphism and *GSTM1*-null genotype were associated with poor prognoses (Müller et al., 2008). In contrast, a study of 111 patients with AML or ALL showed no significant associations between any of the genotypes with *MRP1* T2684C, C2007T, C2012T, or C2665T polymorphisms and MRP1 expression and chemosensitivity, despite high levels of MRP1 expression being associated with MDR in both AML and ALL (Mahjoubi et al., 2008).

The effects of genetic variations in drug transporter genes associated with phenotypical consequences are still controversial, because contradictory results have been reported. Most published studies reported experiences with small sample sizes in relation to the allelic and genotypic frequencies of the studied variant, and results might have been affected by potentially confounding factors related to the patient population and the probe drug. Transporters interact with drug-metabolizing enzymes and are regulated by several nuclear receptors. Probe drugs usually are substrates for multiple transporters and metabolizing enzymes. Therefore, to evaluate the genetic component of drug transporter function, a more-integrated approach, considering several genes involved in specific functional units and pathways, is necessary. Given the presence of linkage disequilibrium, which exists for many SNPs investigated to date, studies of the effects of haplotypes, rather than SNPs, are increasing. Factors such as lifestyle, concomitant medication use, and comorbidities must be considered in addition to patients' genetic features.

Notable Examples of Prognostic Effects of the Expression and Polymorphisms of MDR Genes in Other Cancers. The expression and function of *MDR1* and other MDR genes were reported to be of prognostic relevance in multiple other cancers, including colorectal cancer (Balcerczak et al., 2010), esophageal squamous cell carcinoma (Yamasaki et al., 2011), gastric cancer (Zhang and Fan, 2010), chronic lymphoproliferative disorders (Drain et al., 2010), and breast cancer (Germano and O'Driscoll, 2009). Furthermore, prognostic effects of MDR gene polymorphisms were reported for other cancers. For example, *MDR1* C1236T, G2677T, and C3435T polymorphisms and the *BCRP* G/G genotype (rs2231137) were shown to affect resistance to imatinib among patients with chronic myeloid leukemia (CML) (Dulucq et al., 2008; Kim et al., 2009; Ni et al., 2011), whereas some *MDR1* and *MRP1* polymorphisms were shown to have effects on response rates, progression-free survival times, and OS rates among patients with relapsed multiple myeloma who were treated with bortezomib plus pegylated

TABLE 2
MDR gene polymorphisms and associations with clinical outcomes in acute leukemia and other cancers

MDR Gene	Polymorphism	Genotype	Cancer Type	Associations with Clinical Outcomes
MDR1	C1236T		Untreated AML	None reported (Illmer et al., 2002; Hampras et al., 2010)
			Untreated AML, age ≥60 yr	No associations with CR or survival rates reported (van der Holt et al., 2006)
		TT vs. CT vs. CC	CML	Higher rate of major molecular responses to imatinib (85 vs. 53 vs. 41%; $P = 0.008$) (Dulucq et al., 2008)
		TT vs. CT/CC	CML	Higher rate of resistance to imatinib (75 vs. 31%; $P = 0.004$) (Ni et al., 2011)
		TT vs. CT/CC	Colorectal cancer	Decreased risk of death (HR, 0.26; $P = 0.0424$) (Balcerczak et al., 2010)
	G2677T	GG/TT vs. GT	Relapsed/refractory AML	Shorter relapse-free interval ($P = 0.002$), poorer survival rate ($P = 0.02$) (van den Heuvel-Eibrink et al., 2001)
		GG vs. GT/TT	Untreated AML	Higher probability of CR ($P = 0.04$), higher 3-yr EFS rate (61 vs. 22%; $P = 0.0241$), no OS rate difference (Kim et al., 2006)
			Untreated AML	None reported (Illmer et al., 2002; Kaya et al., 2005; Hampras et al., 2010)
			Untreated AML, age ≥60 yr	No association with CR or survival rates reported (van der Holt et al., 2006)
		GG	Pediatric ALL	Reduced EFS rate (HR, 6.8; $P = 0.01$) (Yang et al., 2010)
	G2677T/A	AG/AT/AA vs. TT/GT/GG	CML	Higher rate of complete cytogenetic remission with imatinib ($P = 0.02$) (Ni et al., 2011)
		TT/TA vs. GG/GT/GA	CML	Higher rate of major molecular responses with imatinib (OR, 3.94; $P = 0.018$) (Dulucq et al., 2008)
	C3435T	CC/CT vs. TT	Untreated, intermediate-risk AML	Increased probability of relapse (84 vs. 45%, $P = 0.02$; multivariate analysis RR, 2.4; $P = 0.02$), lower OS rate (14 vs. 37%; $P = 0.1$; multivariate analysis RR, 2.1; $P = 0.02$) (Monzo et al., 2006)
		CC vs. CT/TT	Untreated AML	Increased risk of relapse ($P < 0.001$), poorer OS rate ($P < 0.01$) (Illmer et al., 2002)
		CC vs. CT/TT	Untreated AML	Higher probability of CR ($P = 0.05$), higher EFS rate ($P = 0.0139$), no OS rate difference (Kim et al., 2006)
			Untreated AML, age ≥60 yr	No association with CR or survival rates reported (van der Holt et al., 2006)
			Untreated AML	None reported (Illmer et al., 2002; Jamroziak et al., 2005; Kaya et al., 2005; Hur et al., 2008;Hampras et al., 2010; Rao et al., 2010)
		CC vs. CT/TT	Pediatric ALL	Lower EFS probability (62 vs. 87%; $P = 0.007$; HR, 3.9; $P = 0.008$) and OS probability (72 vs. 91%; $P = 0.006$; HR, 3.3; $P = 0.02$) (Jamroziak et al., 2004)
		CC	Pediatric ALL	Reduced EFS probability (HR, 21.7; $P = 0.009$) (Yang et al., 2010)
			Adult ALL	None reported (Jamroziak et al., 2005)
		CC vs. CT/TT	CML	Lower rate of resistance to imatinib (25 vs. 59%; $P = 0.023$) (Ni et al., 2011)
	rs1045642	CC/CT vs. TT	CML	Better OS rate with imatinib (HR, 3.70; $P = 0.04$) (Kim et al., 2009)
		TT vs. CC vs. CT	MM	Better PFS rate ($P = 0.0578$), response rate ($P = 0.0782$), and TTP ($P = 0.0601$) for patients treated with bortezomib plus pegylated liposomal doxorubicin (Buda et al., 2010)
BCRP/ ABCG2	G34A	AG/AA vs. GG	Untreated AML	Improved OS rate (HR, 0.44; 95% CI, 0.25–0.79) (Hampras et al., 2010)
	rs2231137	GG vs. AG/ AA	CML	Adverse impact on achievement of major cytogenetic response (HR, 0.68; $P = 0.05$) or complete cytogenetic response (HR, 0.63; $P = 0.02$) to imatinib (Kim et al., 2009)
	rs2231142	AA vs. AC/CC	CML	Adverse impact on achievement of major molecular response (HR, 0.40; $P = 0.004$) or complete molecular response (HR, 0.42; $P = 0.006$) to imatinib (Kim et al., 2009)
ABCC3	C−211T		Untreated AML	Adverse prognostic significance (treatment response and survival rates) (Müller et al., 2008)
GSTM	Null alleles		Untreated AML	Adverse prognostic significance (treatment response and survival rates) (Müller et al., 2008)
MRP1	T2684C, C2007T, C2012T, C2665T		AML/ALL	No impact on responses to therapy (Mahjoubi et al., 2008)
	R723Q	GG vs. AG	MM	Improved TTP ($P = 0.0008$), PFS rate ($P = 0.0006$), and OS rate ($P = 0.0045$) for patients treated with bortezomib plus pegylated liposomal doxorubicin (Buda et al., 2010)
MRP2	G40A	GG	Pancreatic cancer	Poor histological responses to chemoradiotherapy ($P = 0.028$) and reduced OS rate ($P = 0.097$) (Tanaka et al., 2011)
MRP5	A−2G	AA	Pancreatic cancer	Poor OS rate (HR, 1.65; $P = 0.01$) (Tanaka et al., 2011)

MM, multiple myeloma; PFS, progression-free survival; OR, odds ratio; RR, relative risk; HR, hazard ratio; CI, confidence interval; TTP, time to progression.

liposomal doxorubicin (Buda et al., 2010). A review of studies that reported outcomes for patients with solid tumors according to *MDR1* polymorphisms identified some associations with outcomes after paclitaxel/carboplatin treatment for patients with ovarian cancer but yielded inconsistent results for other tumor types (Hamidovic et al., 2010). *MDR1* polymorphisms were shown to be associated with rates of toxicity with 5-fluorouracil- and capecitabine-based therapy among patients with colorectal cancer (Gonzalez-Haba et al., 2010). *MDR1* polymorphisms also were shown to be possible prognostic factors in colorectal cancer (Balcerczak et al., 2010), and *MRP2* and *MRP5* polymorphisms were associated with poorer responses to therapy and OS rates for pancreatic cancer (Tanaka et al., 2011).

Overcoming MDR Arising from Drug Efflux

P-gp and Other MDR Protein Inhibitors. In the past few decades, a large number of putative inhibitors of P-gp have been investigated in both preclinical and clinical studies. Although preclinical investigations have validated the approach of P-gp inhibition, such inhibitors have generally met with little success clinically, likely because of the complexity of the MDR phenotype and potency and specificity issues (Dantzig et al., 2003; Szakács et al., 2006; Yang et al., 2008). The first generation of P-gp inhibitors, representing currently available drugs found to have P-gp-inhibitory properties, included verapamil (Pereira et al., 1994; Belpomme et al., 2000), quinine (Wattel et al., 1999; Solary et al., 2003), and cyclosporine (List et al., 2001; Becton et al., 2006). Some studies provided evidence of the feasibility and utility of P-gp inhibition with these compounds. For example, the addition of quinine to mitoxantrone and cytarabine therapy for patients with high-risk myelodysplastic syndromes resulted in improved OS rates among P-gp$^+$ patients (Wattel et al., 1999), the addition of cyclosporine to daunorubicin and cytarabine therapy for patients with poor-risk AML resulted in improved OS rates (List et al., 2001), and cyclosporine plus daunorubicin increased the CR rate for patients with AML (Li et al., 2009).

On the basis of these promising initial results, second-generation inhibitors, which were based on the first-generation inhibitors but were designed to have improved toxicity profiles, were developed. For example, the nonimmunosuppressive cyclosporine analog valspodar (PSC-833) was studied with standard agents in previously untreated AML (Baer et al., 2002; Kolitz et al., 2004, 2010), AML among elderly patients (van der Holt et al., 2005), relapsed/refractory AML (Greenberg et al., 2004), and relapsed/refractory pediatric acute leukemia (O'Brien et al., 2010); however, there was limited evidence of benefits in terms of CR or OS rates. Likewise, biricodar (VX-710) demonstrated limited success in phase 3 trials and its use was discontinued, like that of valspodar (Goldman, 2003). A key reason why these agents were not successful involved their pharmacokinetic interactions with chemotherapeutic drugs. These interactions arose as a result of non–drug transporter inhibition and altered biotransformation and tissue distribution, which resulted in reduced systemic clearance, reduced metabolism of the chemotherapeutic agents, and thus decreased maximal tolerated doses (Goldman, 2003; Bates et al., 2004; Pein et al., 2007; Patel and Tannock, 2009).

To overcome these problems, third-generation P-gp inhibitors were designed to be more selective for transporter inhibition, with high affinities for efflux transporters, and to have fewer systemic pharmacokinetic interactions (Martin et al., 1999; Mistry et al., 2001; Globisch et al., 2006; Fox and Bates, 2007; Yang et al., 2008). They are noncompetitive inhibitors and inhibit P-gp activity by binding to the transporter protein without themselves being substrates (Martin et al., 1999; Mistry et al., 2001; Shepard et al., 2003; Di Nicolantonio et al., 2004). Some of the newer agents are inhibitors of P-gp and/or other transporters (Gardner et al., 2009; Lagas et al., 2009), which may extend the range of tumor types in which they have beneficial effects. Preclinical studies demonstrated the effectiveness of these agents in reversing or overcoming MDR in leukemia cells. For example, zosuquidar restored drug sensitivity in P-gp-expressing leukemia cell lines and enhanced anthracycline cytotoxicity in P-gp-active primary AML blasts (Tang et al., 2008). Tariquidar was shown to be a highly effective P-gp inhibitor (Fox and Bates, 2007), increasing paclitaxel concentrations in the brain (Hubensack et al., 2008) and reversing MDR in both in vitro and in vivo studies (Mistry et al., 2001). Likewise, the imidazole derivative (*E*)-methyl-3-[4-[4,5-bis(4-[isopropyl(methyl)amino]phenyl)-1*H*-imidazol-2-yl]phenyl]acrylate (FG020326) potentiated paclitaxel, doxorubicin, and vincristine activity in P-gp-overexpressing cell lines and enhanced paclitaxel and vincristine antitumor activities in vivo (Dai et al., 2009).

Unfortunately, the findings from clinical studies with these agents have not always reflected the promising preclinical data, possibly because of multiple factors such as the presence of multiple mechanisms of MDR (rather than just the specific targets of these agents) among patients, the tolerability of MDR protein inhibitors, and the poor pharmacokinetic characteristics of MDR protein inhibitors. For example, although zosuquidar was shown to inhibit P-gp-mediated rhodamine 123 efflux from AML cells from patients in a phase 1 study (Gerrard et al., 2004), the addition of zosuquidar to standard cytarabine and daunorubicin induction therapy in a randomized study of patients >60 years of age with newly diagnosed AML or myelodysplastic syndromes did not result in improved outcomes (Cripe et al., 2010). In a phase 1 study of the use of tariquidar in combination with vinorelbine, a modest reduction in the maximal tolerated dose of vinorelbine was seen, compared with the standard therapeutic dose (Abraham et al., 2009).

It might be argued that suboptimal study design contributed to the failure of these clinical studies of MDR protein inhibitors (van Zuylen et al., 2000). In particular, although multiple assays have been developed and used for the evaluation of efflux pump activity, a definitive link between assay results and activity remains to be established for specific assays and specific MDR proteins. Consequently, the anticipated effect size in clinical trials would be difficult to predict. Furthermore, numerous trials did not make use of surrogate markers for MDR protein activity, and no patient selection criteria (such as selection of only patients with P-gp$^+$ tumors) were applied.

Despite these potential mitigating factors with regard to the outcomes of the clinical trials, it seems that the strategy of efflux pump inhibition is no longer a favored approach for overcoming MDR. In the absence of potent, selective, efflux pump inhibitors with associated validated assays, the development of efflux pump inhibitors seems to be declining because of multiple factors, including the pharmacokinetic complexity associated with these agents. This strategy may be confined to history, given the substantially greater interest in other current approaches, including the development of novel compounds that are not efflux pump substrates, as discussed below.

Novel Anticancer Agents that Inhibit MDR Protein Function and Expression. Numerous other agents and approaches are being investigated with the aim of improving MDR protein inhibition. For example, multiple novel, targeted, anticancer agents have been shown to have inhibitory properties against P-gp and other MDR protein activities, through direct inhibition, through activities as competitive

transporter substrates, or through downstream signaling effects resulting from target inhibition.

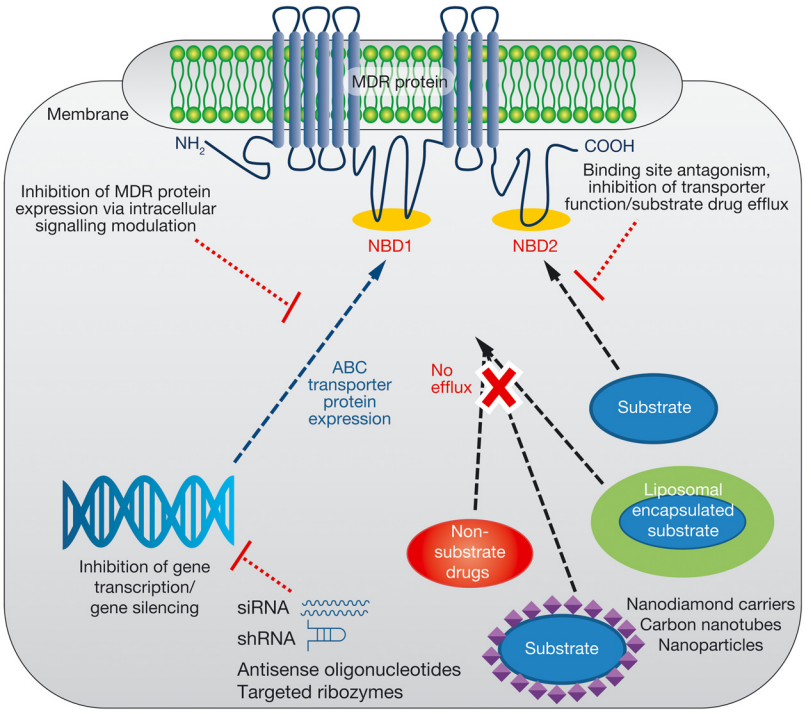
A number of farnesyltransferase and tyrosine kinase inhibitors have demonstrated the ability to reverse MDR. Tipifarnib significantly inhibited daunorubicin efflux in leukemia cell lines overexpressing P-gp and showed synergistic proliferation inhibition and apoptosis induction (Medeiros et al., 2007), whereas lapatinib, erlotinib, and nilotinib were shown to inhibit the efflux activity of P-gp and BCRP by functioning as substrates for those transporters (Shi et al., 2007, 2009; Dai et al., 2008; Dohse et al., 2010). Nintedanib, an inhibitor of vascular endothelial growth factor receptors, platelet-derived growth factor receptors, and fibroblast growth factor receptor tyrosine kinases, inhibited P-gp activity in P-gp-overexpressing cancer cells and enhanced doxorubicin and paclitaxel cytotoxicity (Xiang et al., 2011). A number of studies suggested the utility of phosphodiesterase 5 inhibitors as inhibitors of MDR protein-mediated efflux through their roles as substrates for those pumps. For example, sildenafil was shown to inhibit the transporter functions of P-gp and BCRP, to stimulate their ATPase activities, and thus to sensitize MDR cells to chemotherapeutic drugs (Shi et al., 2011a,b). Likewise, vardenafil was shown to block the drug efflux role of P-gp and to stimulate its ATPase activity in a MDR human epidermoid carcinoma cell line (Ding et al., 2011), which indicates that vardenafil is a transport substrate of P-gp. The evidence is less consistent for the usefulness of histone deacetylase inhibitors in overcoming MDR. Histone deacetylase inhibitors were shown to down-regulate MRP2 protein expression, but not MDR1 and BCRP expression, in the MDR KBV20C cell line (Kim et al., 2011), an effect that was possibly mediated by histone deacetylase inhibitor-induced expression of interleukin 6-type cytokine receptors (Blanchard et al., 2002), as with oncostatin M (Le Vee et al., 2011). Other studies of histone deacetylase inhibitors in AML cells showed that such agents, including suberoylanilide hydroxamic acid and valproate, in combination with various chemotherapeutic agents induced the activity of MDR1, BCRP, MRP7, and MRP8, which resulted in reduced apoptosis and resistance (Hauswald et al., 2009).

A number of studies have shown that targeted agents inhibiting specific pathways may induce downstream effects on MDR as a consequence of signaling inhibition. For example, the doxorubicin-induced overexpression of MDR1 in HL-60 AML cells was suggested to be regulated by the cyclooxygenase (COX) system, particularly COX-2, which indicates a potential role for COX-2 inhibitors in ameliorating induced resistance (Puhlmann et al., 2005). In a recent report, the COX-2 inhibitor SC236 and the nonsteroidal anti-inflammatory drug indomethacin were shown to inhibit P-gp and MRP1 expression and thus to enhance doxorubicin cytotoxicity in a MDR hepatocellular carcinoma cell line (Ye et al., 2011). Another therapeutic target in MDR leukemia may be STAT3 signaling; a recent study showed that STAT3 was overexpressed in MDR K562/AO2 leukemia cells and inhibition of STAT3 activation resulted in down-regulation of *MDR1* transcription and P-gp expression (Zhang et al., 2011c).

Multiple cell line studies have demonstrated that numerous novel compounds have the ability to inhibit MDR protein function, although no clinical studies have been reported. For example, curcumin was shown to have inhibitory activity against MDR1 expression in leukemia cells from patients (Anuchapreeda et al., 2006), and the combretastatin A-4 analog 4-(4-bromophenyl)-2,3-dihydro-*N*,3-bis(3,4,5-trimethoxyphenyl)-2-oxoidmidazole-1-carboxamide (MZ3) overcame MDR in leukemia cells by down-regulating *MDR1* transcription and antiapoptotic protein expression (Xu et al., 2008). Two milbemycin compounds (Gao et al., 2011), two novel acrylonitrile derivatives (Yamazaki et al., 2011), and a number of benzo(*a*)quinolizin-4-ones (Kanintronkul et al., 2011) showed chemosensitizing properties attributable to modulation of P-gp, whereas X-shaped poly(ethylene oxide)-poly(propylene oxide) block copolymers (poloxamines) inhibited P-gp and BCRP in hepatic carcinoma cell lines (Cuestas et al., 2011). A number of flavonoid compounds from various plant species were shown to inhibit BCRP function (Versiani et al., 2011) and to inhibit vinblastine-stimulated P-gp activity but to promote daunorubicin-stimulated P-gp activity in leukemic T cells (Tran et al., 2011). Limonin and other citrus compounds enhanced doxorubicin cytotoxicity in MDR CEM/ADR5000 leukemia cells (El-Readi et al., 2010). Finally, the use of an ATP analog that was shown to interact with the drug and ATP binding sites of P-gp resulted in reduced P-gp efflux activity (Ohnuma et al., 2011).

Nonchemical MDR Protein Inhibition. Multiple additional approaches to MDR protein inhibition have been investigated (Fig. 1). For example, small interfering RNAs (siRNAs), including short hairpin RNAs (shRNAs), targeted at MDR genes were shown to be effective in a number of studies (Wu et al., 2008). shRNAs/siRNAs targeting *MDR1* were shown to be effective in inhibiting P-gp expression and resensitizing cells to harringtonine and curcumin when they were transfected into MDR HT9 leukemia cells (Shao et al., 2010), and they were shown to down-regulate P-gp expression and to increase drug sensitivity in MDR K562/Adr leukemia cells (Lim et al., 2007). A combination of daunorubicin-conjugated magnetic Fe_3O_4 nanoparticles and shRNA expression vector aimed at MDR1 mRNA overcame resistance in MDR K562/AO2 leukemia cells (Chen et al., 2010). A potential interaction between the glucosylceramide synthase (GCS) gene and *MDR1* was indicated when GCS siRNA resulted in down-regulation of not only GCS mRNA but also MDR1 mRNA in K562/AO2 cells (Zhang et al., 2011d); this relationship was reinforced by studies that showed that chemosensitization with the GCS inhibitor (1*R*,2*R*)-nonanoic acid-[2-(2′,3′-dihydrobenzo[1,4]dioxin-6′-yl)-2-hydroxy-1-pyrrolidin-1-yl-methylethyl]amide L-tartaric acid salt (Genz-123346) was mediated through P-gp inhibition (Chai et al., 2011).

Alternative approaches to gene silencing, including the use of antisense oligonucleotides (Kang et al., 2004), transcriptional regulation (Xu et al., 2002), and targeted ribozymes (Kowalski et al., 2002), also have been studied (Wu et al., 2008). P-gp down-regulation mediated by RNA interference gene silencing was demonstrated to be effective (Abbasi et al., 2011a,b), with an antisense oligonucleotide against MDR1 mRNA resulting in decreased P-gp and mRNA expression (i.e., reversal of the MDR phenotype) in leukemia cells (Nadali et al., 2007). In a novel approach to overcoming MDR, xanthones were studied in MRP1-overexpressing cells and were shown to induce apoptosis through activation of MRP1-mediated glutathione efflux, an effect



NBD, nucleotide binding domain

Fig. 1. Current approaches to eliminating drug resistance through efflux.

that was not seen in non-MDR cells (Genoux-Bastide et al., 2011). This property of "collateral sensitivity" (Hall et al., 2009) also was reported for the first-generation P-gp inhibitor verapamil (Trompier et al., 2004) and the propanoylglycine derivative tiopronin (Goldsborough et al., 2011).

New Agents with Reduced Drug Efflux Properties. As an alternative approach to inhibiting the activity of P-gp and other drug efflux pumps, new therapeutic agents might be designed to avoid these efflux mechanisms and thus to achieve high concentrations in cancer cells, which might result in enhanced cell death. For example, existing chemotherapeutic agents might be modified so that they no longer would be substrates for P-gp or other MDR proteins and thus could evade the efflux mechanism (Nobili et al., 2011). One example of a new therapeutic agent with reduced drug efflux properties is amonafide, a novel topoisomerase II inhibitor. Although the topoisomerase II inhibitors daunorubicin, doxorubicin, idarubicin, and others are substrates of P-gp, which leads to their rapid efflux from leukemia cells, amonafide was shown to be neither a substrate nor an inhibitor of P-gp (Chau et al., 2008). Consequently, amonafide was suggested as a possible agent for the treatment of AML (Allen and Lundberg, 2011). Likewise, the recently approved taxane cabazitaxel, a dimethyloxy derivative of docetaxel, has no affinity for P-gp and can cross the blood-brain barrier, unlike docetaxel and paclitaxel (Paller and Antonarakis, 2011). Other novel agents that were shown not to be substrates of P-gp or other efflux pumps include the glutathione transferase inhibitor 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio)hexanol (Ascione et al., 2009) and a series of pyrrolo-1,5-benzoxazepine compounds (Nathwani et al., 2010).

Alternatively, agents may be designed to be more lipophilic and thus to undergo influx more readily. Encapsulation of agents in liposomes may help overcome MDR, as reported for pegylated liposomal doxorubicin (Riganti et al., 2011) and stealthy liposomal encapsulation of vincristine and quinacrine (Liang et al., 2008). These approaches, which increase the passive lipid permeability of compounds, yield improved passive diffusion, and prevent the development of large concentration gradients, may alleviate resistance attributable to efflux transporters whether or not the compounds are substrates (Raub, 2006). This concept may be demonstrated (in reverse) through related work on uptake transporters with imatinib, the standard treatment for CML, and the second-generation agent nilotinib. Both agents are substrates for MDR efflux transporters as well as various solute carrier family transporters, including the human organic cation transporter 1 (hOCT1) influx protein (Minematsu and Giacomini, 2011); however, nilotinib is more hydrophobic than imatinib and enters cells more rapidly. Imatinib uptake was decreased when hOCT1 activity was low (Crossman et al., 2005; White et al., 2006), which resulted in poorer responses among patients with CML (White et al., 2007; Engler et al., 2011), but nilotinib uptake was unaffected by hOCT1 activity levels (White et al., 2006; Davies et al., 2009).

Influx and efflux kinetic characteristics for doxorubicin were shown to be altered to enhance cytotoxicity in MDR KD30 leukemia cells through the linking of doxorubicin with a hybrid cell-penetrating and drug-binding peptide (Zheng et al., 2010). Likewise, nanotechnology has been shown to be a promising approach to help therapeutic agents evade efflux. Doxorubicin attached to 2- to 8-nm nanodiamond carriers was shown to increase apoptosis, compared with free doxorubicin, in MDR liver cancer both in vitro and in vivo (Merkel and DeSimone, 2011). Anti-P-gp antibody-functionalized, single-walled, carbon nanotubes loaded with doxorubicin demonstrated enhanced cytotoxicity in MDR K562R leukemia cells, compared with free doxorubicin, and overcame the resistance of those cells (Li et al., 2010). Multifunctional nanoassemblies carrying vincristine sulfate yielded higher

levels of vincristine uptake in P-gp-overexpressing cells and overcame efflux and vincristine MDR (Zhang et al., 2011b), whereas nanoparticle-mediated delivery of paclitaxel and tariquidar demonstrated significantly enhanced cytotoxicity in drug-resistant tumor cells (Patil et al., 2009).

Future Directions

This review has highlighted the importance of MDR in cancer and particularly in acute leukemia. Given the potential impact of MDR on the efficacy of anticancer therapeutic agents, this is clearly a key issue to be considered during the development of novel therapeutic agents. As described above, there is a substantial body of research on P-gp inhibition as a means of improving the efficacy of therapeutic agents that are ABC transporter substrates, and there are a large number of potential inhibitors in development. For successful MDR modulation in acute leukemia, particularly AML, these inhibitors must be specific for the ABC transporters known to be associated with the patient's MDR (for example, targeting both P-gp and BCRP), to avoid adverse effects arising from off-target inhibitory properties. Even with targeted inhibition of the key mediators of MDR, however, inhibition may not represent a feasible therapeutic approach because of the complexity of MDR in AML and other cancers, as suggested by the results of clinical trials with third-generation agents. Therefore, the alternative approach of developing novel agents with reduced efflux properties may prove to be the most promising way to improve on the efficacy of existing agents for AML. It is hoped that exploitation of the available resources and tools to identify novel compounds that are toxic to MDR cancer cell lines and are not substrates of P-gp or other transporters (Szakács et al., 2004) will facilitate the development of novel therapeutic agents for acute leukemia and other cancers that will help overcome the established adverse prognostic impact of MDR in these diseases.

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