

EDITORIAL

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Multidrug Resistance Proteins in Leukaemias*

Białka oporności wielolekowej w białaczkach

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Abstract

The development of resistance to many cytostatic agents in response to a treatment with a single agent is defined as a multidrug resistance (MDR). In leukaemia MDR is often attributed to the function of drug transporter proteins present in the plasma membrane. Several transporters have been implicated in resistance to chemotherapy in acute leukemia, of which P-glycoprotein (Pgp) seems to play the most important role. In the majority of reports investigating *de novo* or secondary acute leukaemia, Pgp is an independent prognostic variable associated with reduced remission probability and overall survival. Overexpression of other transporter proteins, such as multidrug resistance protein (MRP1), breast cancer resistance protein (BCRP) and lung resistance related protein (LRP) have been shown in acute leukemia, but their prognostic value still is not clear. Many therapeutic strategies targeting the MDR mechanisms are being developed in order to improve outcomes and survival of leukaemia patients. Four generations of Pgp inhibitors have been tested in an attempt to reverse its expression or function (**Adv Clin Exp Med 2005, 14, 3, 407–416**).

Key words: multidrug resistance, acute leukaemia, prognosis.

Streszczenie

Pojawienie się oporności nowotworu w stosunku do kilku różnych cytostatyków w odpowiedzi na terapię pojedynczym cytostatykiem jest określane mianem oporności wielolekowej (MDR – *multidrug resistance*). W białaczkach powstanie oporności wielolekowej jest uwarunkowane głównie przez nieprawidłową, podwyższoną ekspresję obecnych w błonie komórkowej białek transportowych, aktywnie usuwających leki z komórki. Wśród błonowych białek transportowych najważniejszą rolę pełni glikoproteina P (Pgp). Większość prac eksperymentalnych i klinicznych dotyczących ostrych białaczek podaje, że podwyższona ekspresja tego białka jest uznawana za niekorzystny czynnik rokowniczy zarówno dla uzyskania całkowitej remisji, jak i czasu przeżycia pacjentów. Znaczenie prognostyczne innych białek transportowych, takich jak: MRP1 (*multidrug resistance protein*), BCRP (*breast cancer resistance protein*) i LRP (*lung resistance related protein*) nie jest jeszcze w pełni poznane. Istnieje wiele strategii mających na celu zniesienie lekooporności, polegających głównie na blokowaniu aktywności białek oporności wielolekowej (**Adv Clin Exp Med 2005, 14, 3, 407–416**).

Słowa kluczowe: oporność wielolekowa, ostra białaczka, rokowanie.

Introduction

The goal of administering a chemotherapeutic drug is to reach the neoplastic cellular target and cause cellular damage and subsequent cell death. However, as the result of chemotherapy, cancer cells often develop mechanisms to survive that

treatment, resulting in resistance not only to the previously administered agents, but also to multiple other chemotherapeutic or biologic agents that may be used in subsequent treatments. This development of resistance to multiple therapies in response to a single treatment is defined as multidrug resistance (MDR).

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If a malignancy displays resistance to chemotherapy right from the outset of the treatment, this is termed intrinsic resistance, implying the innate resistance property of the cells. If the cancer cells are initially sensitive to chemotherapy and then commences the resistance, despite ongoing treatment, and thereafter this is referred to as acquired resistance.

The MDR phenotype refers to any cell that can express resistance simultaneously to many different agents as a consequence of a single biochemical change. Many cellular mechanisms may contribute to the development of MDR. These mechanisms include the decreased drug uptake and its metabolic activation, the increased efflux of administered drug, or the increased catabolism and drug detoxification. Also possible is the alteration of drug target or the more efficient repair of damaged DNA in tumor cells. In addition, there is mounting evidence that defective or deregulated apoptosis programs may also contribute to drug resistance (Fig. 1). Resistance due to the increased drug efflux seems to be the most important of all those mentioned above. In leukaemic cells it is often attributed to the function of drug transporter proteins present in the plasma membrane. Most of them are members of the ATP-binding cassette (ABC) transporter family, which act as ATP-dependent efflux pumps that decrease the intracellular concentration of anticancer agents.

Resistance to chemotherapy is the major factor limiting treatment outcome in patients with leukaemia, especially acute myeloid leukaemia (AML). Although almost 80% of AML patients younger than 60 years initially achieve a complete remission (CR), the 5-year leukaemia free survival (LFS) is only 25–35% with intensive consolidation

treatment. With subsequent allogeneic or autologous bone marrow transplantation the long-term survival rate is 50–60% and 45–55%, respectively [1]. In patients older than 60 years the CR rates are less than 50% and the median overall survival (OS) with intensive treatment is usually limited to approximately 6 months. Moreover, 25% of younger and 40–50% of patients older than 60 years with *de novo* AML have intrinsic resistance to chemotherapy. Additionally, many patients, who respond to initial treatment relapse within 1–2 years after the diagnosis [1].

In this review the clinical significance of various MDR phenotypes and the pharmacological strategies to reverse MDR in leukaemia will be focused and discussed.

Drug Resistance Mechanisms in Haematological Malignancies

Several transporters have been identified to have a role in cancer and acute leukaemia during the last decade. The first identified and most extensively studied transporter is P-glycoprotein (Pgp), a member of the ATP-binding cassette (ABC) superfamily of transporter proteins. *MDR1* gene that encodes Pgp is located on chromosome 7 (7q21) and its 170 kDa product consists of two structurally homologous units, which are probably the result of internal gene duplication. Each unit possess six hydrophobic transmembrane domains (*TMDs*), one nucleotide-binding site (*NBSs*) and the highly conserved Walker A and Walker B motifs directly engaged in ATP hydrolysis (Fig. 2). Within the TMDs there

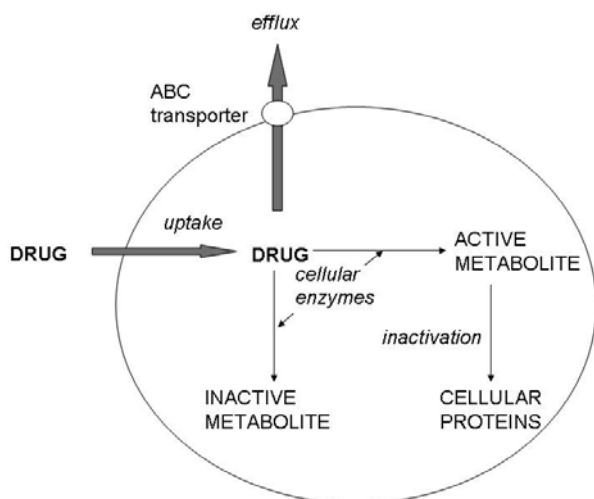


Fig. 1. Potential pathways for antineoplastic drug disposition within tumor cells

Ryc. 1. Prawdopodobne losy cytostatyku w komórce nowotworowej

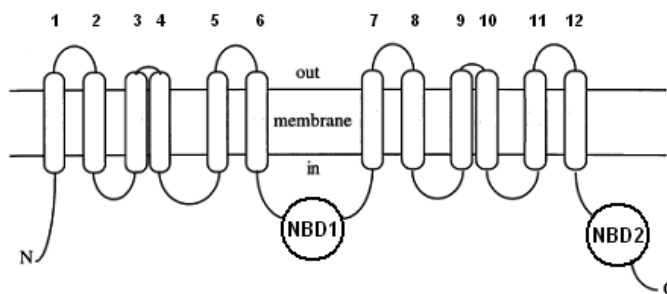


Fig. 2. The linear secondary structure of P-glycoprotein, with numbered putative α -helices and the two ATP-binding cassettes represented as NBD1 and NBD2

Ryc. 2. Liniowa drugorzędowa struktura glikoproteiny P. Poszczególne α -helisy zostały oznaczone numerami 1–12. Dwa miejsca wiążące ATP oznaczono jako NBD1 i NBD2

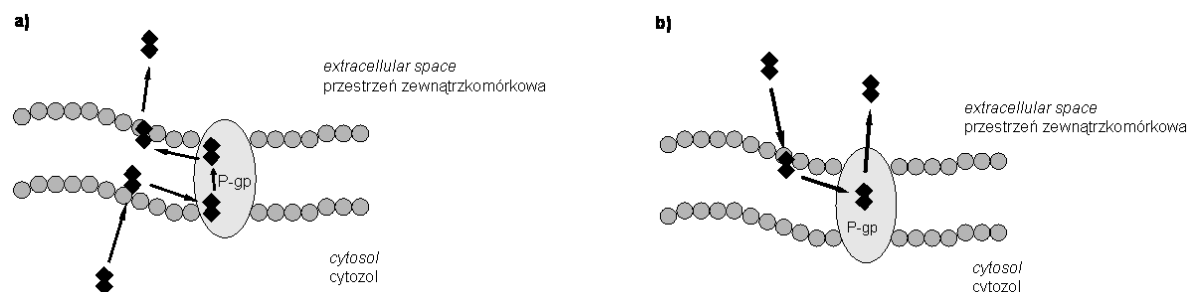


Fig. 3. Possible mechanisms of drug efflux by P-glycoprotein: **a** – the flippase model, **b** – the hydrophobic “vacuum cleaner” model

Ryc. 3. Hipotetyczne modele transportu leków z udziałem P-gp: **a** – model flipazy, **b** – hydrofobowy „odkurzacz molekularny”

exist at least two drug binding sites (*DBSs*) – one for vincristin and verapamil and the second for the other drugs [see: 2, 3]. The *MDR1* gene promoter lacks a consensus TATA box found on many protein-encoding genes, and contains instead an inverted CCAAT and a GC-rich element. *MDR1* can be upregulated by chemotherapeutic agents, other xenobiotics, or ultraviolet radiation. The transcriptional upregulation mechanism involves the activation of the transcription factors YB-1 or NF-Y (binding to a GC-rich element), and epigenetic changes such as hypomethylation of CpG sites in the *MDR1* promoter and/or increased acetylation of histones. The use of the hypomethylating agent 5-azacytidine in a human epidermal cancer cell line resulted in the upregulation of *MDR1* transcription [2].

The wide range of compounds that are actively transported by Pgp include hydrophobic and amphipatic drugs, calcium channel blockers, antihistamines, peptides and steroids. Pgp decreases the cellular concentration of anthracyclines (doxorubicin and daunorubicin), *Vinca* alkaloids (vinblastine and vincristine), actinomycin-D, taxol, etoposide, topotecan, mitomycin-C, colchicine and puromycin. How exactly Pgp removes drugs from the cell, still remains unclear. One proposed mechanism is that Pgp detects and ejects drugs before they reach the cytoplasm by removing the drugs directly from the plasma membrane. Another possibility is that Pgp acts as a flippase, carrying its substrate from the inner leaflet of the lipid bilayer to the outer leaflet (Fig. 3).

Pgp is expressed at high levels in the apical membrane of epithelial cells of the gastrointestinal tract, kidney and the parenchymal cells of the liver (bile canaliculi). In this localization, these transporter pumps absorb substrate drugs or xenobiotics back into the gut lumen, urine or into the bile, effectively contributing to the clearance of these substances. Pgp is also present at lower levels in tissues such as capillary endothelium of lungs, brain, heart and testis, in cortex and medulla of

adrenal glands and in the epithelial cell lining of pancreas. Pgp expressed in the placenta plays an important role in the protection of the developing fetus against toxic substances. Pgp has also been found on hematopoietic stem cells (HSCs), CD4⁺ and CD8⁺ T lymphocytes, B lymphocytes and natural killer (NK) cells. There is now evidence that Pgp may be involved in the secretion of cytokines, particularly IL-1 β , IL-2, IL-4 and IFN- γ , but the mechanism of their transport still remains unknown. It has been suggested that CD8⁺ and NK-cell cytotoxicity requires Pgp function for the efflux of lytic products. The inhibition of Pgp resulted in a decreased cytotoxicity of NK cells [4]. Pgp plays probably a role in differentiation and proliferation of HSCs by influencing some regulatory substances. The fact that Pgp is normally expressed on the surface of CD34⁺ hematopoietic precursor cells brings the possibility that just the re-expression of this transporter protein in malignant leukaemia may result in the drug resistance of hematological malignancies.

Other members of the ABC transporter family, identified as being capable of transporting chemotherapeutic drugs are the multidrug resistance protein (MRP1, ABCC1) and the breast cancer resistance protein (BCRP, ABCG2). The MRP family consists of six transporter proteins, of whose only MRP1 is involved in drug transport, MRP2 (c-MOAT) is engaged in phosphatidylcholine transport and MRP4 is a transporter of pyrimidine analogues [5, 6]. Unlike most ABC transporters the MRP1 gene is located on chromosome 16 (16p13.1). MRP1 has been found both on the plasma membrane and membranes of intracellular compartments. It is expressed in many types of normal cells and tissues including erythrocytes, hepatocytes and mastocytoma cells. Its overexpression has been observed in lung cancer, colon cancer, neuroblastoma and AML, and correlated with resistance to varied anticancer drugs. Cell lines in which MRP has been deleted were more sensitive

to the anthracyclines, *Vinca* alkaloids and epipodophyllotoxins [7]. Pgp and MRP confer resistance to a similar, but not identical spectrum of anticancer agents. However, the important difference between Pgp and MRP is that MRP transports cationic and neutral compound only in the presence of glutathione (GSH) or its conjugates [8].

BCRP, the second member of the G subfamily of half-transporters within the ABC transporter superfamily, was identified in 1998 in MCF-7 human breast carcinoma cells [9]. *BCRP* gene maps to chromosome 4q22 and encodes a product of 655 aa, but there is an evidence that the functional BCRP protein is a homodimer [10]. Owing to tissue localization in the placenta, biliary ductules in the liver, colon, small bowel and brain, BCRP, like Pgp, may play a role in protecting the organism from potentially harmful xenobiotics [10]. Overexpression of BCRP in human cancer cell lines result in resistance to a variety of cytostatic agents. The spectrum of anticancer drugs effluxed by BCRP includes mitoxanthrone, doxorubicin, daunorubicin, camptothecin-derivatives and indolocarbazole topoizomerase I inhibitors, methotrexate, flavopiridol and quinazoline ErbB1 inhibitors [9–11]. Studies are in progress to evaluate the role of BCRP expression in drug resistance of clinical cancers.

Opposite to Pgp, MRP and BCRP, lung resistance related protein (LRP) is not an ABC transporter but it is a major vault protein involved in nuclear-cytoplasmic transport. The *LRP* gene is located on chromosome 16 (16p13.2) proximal to the *MRP* gene and encodes a 110 kDa protein. For the first time LRP was isolated from Pgp negative drug-resistant lung cancer cells [12], but it is also expressed in normal tissues such as colon epithelial cells, renal proximal tubules, adrenal cortex, keratinocytes and macrophages, where it probably play a role in protection against toxic and harmful substances, however, its physiological function remains to be evaluated [12]. Recently, it became evident that LRP is present in a variety of human cancer cell lines, for example in stomach, ovary and bronchial cancer, myeloma, and childhood as well as adult AML. In these cell lines, the expression of LRP correlated with intrinsic resistance to doxorubicin, vincristine, carboplatin, cisplatin and melphalan. LRP is thus believed to contribute to drug resistance and cancer progression [13]. Co-expression of LRP and Pgp or LRP and MRP was often observed in adult and childhood AML [14]. This complexity of the drug resistance phenotype is a main reason why oncologists fail their fight to limit this phenomenon.

Pgp Expression and Prognosis in Leukaemia

Overexpression of *MDR1*/Pgp in cell lines *in vitro* confers cellular resistance to a wide variety of anticancer drugs, including many of those used in the treatment of AML. Hematological malignancies such as AML, ALL, lymphomas, and multiple myeloma usually present low levels of Pgp at diagnosis, and are initially chemotherapy-sensitive, but show increased levels of Pgp and drug resistance development at relapse [15]. In some studies, increased Pgp expression was observed at the time of initial diagnosis or relapse in AML, and Pgp expression seems to increase with age and is predictive of poor outcome in terms of disease-free and overall survival.

In many studies employing various assays for the assessment of Pgp expression, patients with Pgp-positive leukaemic cells have a significantly lower probability of achieving a complete remission (CR) as compared to those with Pgp-negative leukaemic cells [8, 15–18]. Filipits et al [18] examined 111 patients with *de novo* AML and demonstrated the association of Pgp expression with a poor response to chemotherapy. For patients with low, intermediate and high Pgp expression, the CR rates were 77, 68 and 38%, respectively. In another study *MDR1* RNA levels in blast cells were determined at diagnosis and correlated with treatment outcome in 63 AML patients. *MDR1* RNA was not found in 29% and positive were 71% of the patients. The complete remission rate in response to induction chemotherapy was 89% for *MDR1* RNA negative patients and 53% for positive [19].

In contrast to these results, a few studies found no correlation between Pgp expression and CR rates in AML [7, 20]. Therefore, there is a general agreement on the necessity of standardization of the assays that are used for Pgp analysis in AML specimens. Even with highly specific assays it is not clear if low numbers of Pgp expressing cells could contribute to a poor outcome of the treatment. However, te Boekhorst et al. [21] showed that even presence of low percentage of Pgp-positive blast cells ($1 \pm 5\%$) represented an increased risk of refractory disease. These data suggest that Pgp positive cells are relevant for the response to treatment in *de novo* AML, and that assays should be developed that are capable of detecting Pgp activity even in small cell fractions.

In some studies it was found that Pgp overexpression at diagnosis has a negative impact not only on CR rate, but also on remission time and overall survival in patients with AML [18, 22–24]. In above mentioned study by Filipits et al. [18] median over-

all survival was 1.4, 0.8 and 0.4 years for patients with low, intermediate and high expression of Pgp, respectively. Wood et al. [22] examined Pgp expression in 54 newly diagnosed AML patients. CR rates were significantly lower in the Pgp positive group than that in the Pgp negative group (60 *versus* 92%). The overall survival for the Pgp positive patients was markedly shorter: 329 *versus* 543 days for patients classified as Pgp negative. The disease-free survival for the Pgp positive patients was also shorter, but the difference was not statistically significant. Leith et al. [24] demonstrated that elder AML patients have a higher probability of Pgp expression and, consequently, enhanced drug efflux. In these patients, Pgp expression was associated with a lower chance of achieving CR and also with shorter survival. These data confirm the hypothesis that Pgp overexpression is an independent prognostic variable for patients response in AML and that it is independent from age, cytogenetic profile and proportion of CD34⁺ AML cells [16].

The clinical significance of Pgp expression in ALL is less clear than in AML. Pgp was observed in 38% of ALL cases [6]. Although single studies showed that Pgp expression may be related to a poor prognosis in both children and adults [25], other reports gave no indication that Pgp expression could be clinically important in ALL. Wattel et al. [26] analyzed Pgp expression and its correlation with outcome in 50 cases of newly diagnosed adult ALL. Pgp expression was evaluated by flow cytometry and immunochemistry. No difference was found in CR rate and disease-free survival in Pgp-positive and Pgp-negative cases. In studies by den Boer et al. [7] Pgp expression was analyzed in 141 cases of children with ALL. No evidence was found that Pgp contributes to drug resistance in childhood ALL.

In CML 20–50% of patients have Pgp positive phenotype. The protein is expressed by leukaemic cells more often in the terminal disease stages [27]. Sequential studies show that Pgp positive cells often disappear from the peripheral blood (PB) during the course of chemotherapy. Nevertheless, Pgp expression has some prognostic value in CML blast crisis (BC), predicting shorter BC [28, 29]. Turkina et al. [30] postulate that Pgp may be regarded as the unfavorable prognostic factor in BC CML.

Other Membrane Transport Proteins: Relevance for Clinical Prognosis

Although Pgp may confer clinical resistance to many patients with leukaemia, other mechanisms of MDR seem to be involved as well. MRP expres-

sion has been reported in a variety of untreated and refractory hematological malignancies including AML and chronic leukaemia [31–33]. The frequency of MRP expression in untreated AML at a level surpassing that of normal blood leukocytes was approximately 50% [31]. The expression in relapsed AML patients is equal or slightly higher than that in untreated cases [32, 33]. The clinical relevance of MRP in AML is still a matter of discussion. Early studies indicated that the expression of MRP in AML increased with disease progression [32, 33], but more recent studies have found no correlation between MRP expression and clinical response in AML [23, 34, 35]. Another study by Legrand et al. [36] on 56 AML patients revealed that MRP functional activity, but not MRP1 protein expression, is an unfavorable prognostic factor for the achievement of CR. The same group presented a study in which a correlation is described between simultaneous activities of MRP and Pgp and *in vivo* resistance in AML [37] <http://clincancerres.aacr-journals.org/cgi/content/full/6/8/3205> – B30. Van der Kolk [8] recently demonstrated that although MRP activity is not an independent prognostic factor for CR achievement, patients with both high Pgp and MRP activity show a lower CR rate after one cycle of chemotherapy than patients with low Pgp and MRP activities.

LRP expression was observed in 35–50% of AML patients [14, 24, 38, 39] and has been reported by many groups to be an adverse prognostic factor for AML [7, 14, 38–40]. Filipits et al. [18] studied the expression of LRP, MDR1 and MRP in 111 patients with *de novo* AML. Expression of LRP and Pgp, but not MRP, was associated with poor response to chemotherapy. Patients LRP-negative had a CR of 79% but those with positive LRP of only 53%. LRP was the only drug resistance factor with independent prognostic significance in terms of disease-free and overall survival. There are also several studies that indicate a clinical relevance of LRP in ALL. Oh et al. [41] analyzed MDR parameters (Pgp, LRP MRP) in 86 ALL patients, and they found that Pgp and LRP, but not MRP, were associated with poor clinical outcome. RT-PCR analysis of Pgp, LRP and MRP expression in 30 children with ALL showed that only the increased expression of LRP was related to the worsened event-free survival [42]. However, other studies have shown no relationship between LRP expression and clinical outcome [16, 24, 36]. Leith et al. [16] found that overexpression of Pgp, MRP, or LRP was not seen in patients whose blast cells had cyclosporin-resistant drug efflux *in vitro*. An additional research is required into the role of LRP in drug resistance in leukaemia. Little is known about the other physiological mechanisms of drug resistance and their rel-

evance in leukaemia. However, in recent years clinical significance of BCRP has increased as a result of many studies on its role in multidrug resistance.

The role of BCRP expression in AML is still controversial. Relatively high expression of BCRP was observed in approximately 30% of a group of high-risk AML patients, and did not correlate with the expression of Pgp, suggesting that BCRP may prompt resistance to therapeutic agents in the absence of Pgp [43]. In a very recent study by Brender et al. [44] the expression of BCRP and Pgp was analyzed in 149 AML patients. BCRP was a prognostic factor for CR achievement (43% in positive patients and 69% in negative patients), the 4-year disease-free survival (12 *versus* 33%) and 4-year overall survival (19 *versus* 38%). But these were the patients expressing both BCRP and Pgp, who had the poorest prognosis. Steinbach et al. [11] using RT-PCR technique found that BCRP expression correlates with poor prognosis in childhood AML. In studies of 20 paired samples taken at diagnosis and at relapse, BCRP mRNA expression was found to increase in relapsed or refractory AML [45]. On the other hand, some studies using functional assays or immunological detection of BCRP protein found low BCRP expression in AML blast cells and no increase in BCRP expression at time of relapse in 20 paired samples [46, 47]. Interestingly, these investigators also found that BCRP expression correlated with an immature cellular phenotype and a higher percentage of CD34 positive cells, as found in hematopoietic stem cells and also in AML [46, 47]. In ALL, the role of BCRP is not assessed yet. Recently, Suvannasankha et al. [48] examined blasts from 30 ALL patients for BCRP mRNA by RT-PCR, BCRP protein level, evaluated with the use of three different antibodies and BCRP functional assay measured by flow cytometry. BCRP function was seen in 21 cases (70%), but correlated poorly with antibody staining. Poor correlations between mRNA presence, protein level and functional activity indicate the complex role of BCRP in ALL. More prospective studies are needed, preferably combining BCRP protein or mRNA quantification with functional assays, in order to determine the contribution of BCRP to drug resistance and clinical outcome in AML and ALL.

Modulation of Multidrug Resistance in Leukaemia Patients

The ways of overcoming MDR by MDR transporters inhibition, and especially Pgp, have been intensively studied for more than two decades.

There are many agents that modulate Pgp, including calcium channel blockers, calmodulin antagonists, steroidal agents, protein kinase C inhibitors, immunosuppressive drugs, antibiotics and surfactants.

In patients with AML, several trials have been conducted in order to evaluate the effect of Pgp reversal. Since approximately 50% of untreated AML patients reveal Pgp expression in their blast cells, they represent a unique population for studying the effect of Pgp reversal on a condition when no other pathways of resistance have been induced by exposure of the patients to chemotherapy. This is in contrast to most relapse models in solid tumors, when many patients may have high levels of Pgp expression in combination with other, unknown mechanisms of clinical refractoriness. In addition, most anti-leukaemic agents, except cytarabine, belong to the natural product class of cytostatic drugs. These conditions make AML a suitable model to study MDR reversal.

Cyclosporin-A (CsA) and quinine, first-generation Pgp inhibitors, and also its second-generation, non-immunosuppressive cyclosporin-A analog, PSC833 (cyclosporin D), have completed extensive testing in combination with cytotoxic agents in high-risk AML yielding diverse clinical results. PSC 833 has 10-fold greater potency for Pgp inhibition than CsA. Although PSC 833 lacks intrinsic renal toxicity, both cyclosporines delay the hepatic elimination of bilirubin and natural product-derived anti-cancer agents. Because of this pharmacokinetic interaction, considerable effort was invested during phase I trials to estimate appropriate reductions in anti-neoplastic drug dosage when co-administered with PSC 833 to approximate conventional drug exposure and limit toxicity [6]. The cyclosporines have shown some benefit in phase I and phase II trials in AML. These initial trials had a patient group with a high blasts MDR level occurring through multiple mechanisms, along with adverse cytogenetic characteristics and myelodysplastic syndrome (MDS) [see: 2]. However, in opposite to suggested benefits of Pgp-blocking agents, two large cooperative groups (CALGB 9621 and CALGB 9420) phase I studies on PSC833, and some randomized phase III clinical trials have shown no benefits of this treatment for AML patients. Only a single phase III trial conducted by SWOG in relapsed/refractory poor-risk AML patients showed some benefit [see: 2]. Outcomes from these trials and other studies with MDR modulators in other cancers indicate that an MDR inhibitor should be used only in the case of patients who show high MDR level and, therefore, are not really suitable for all patients.

In recent years, a third-generation of Pgp blockers has been developed, such as zosuquidar (LY3335979), laniquidar (R101933), tariquidar (XR9576) and ONT-093 (OC144-093). These agents are highly specific for Pgp, and have modest or minimal effects on the clearance of chemotherapeutic agents. Clinical trials with the use of these new modulators are in progress. Zosuquidar and laniquidar are potent and selective inhibitors of Pgp, rapidly and effectively inhibiting drug efflux. A phase I study of zosuquidar in combination with doxorubicin revealed only a modest decrease in doxorubicin clearance at higher doses of this agent, indicating that it can be safely co-administered with doxorubicin. No dose-limiting toxicity of zosuquidar was observed [see: 2]. Fracasso et al. [49] examined the combination of zosuquidar and docetaxel in a phase I trial in patients with resistant solid tumors. This combination was well tolerated. Zosuquidar only minimally altered the pharmacokinetics of docetaxel, allowing full dose docetaxel to be given with this Pgp modulator. The phase II studies with this combination in advanced cancers are underway. A phase I trial of oral laniquidar in combination with docetaxel revealed that effective Pgp-blocking plasma concentrations of laniquidar were achieved with no alteration in the plasma pharmacokinetics of docetaxel. Subsequent studies on intravenous administration of laniquidar performed by the same group also showed lack of pharmacokinetic interaction [see: 2]. ONT-093 is a potential candidate for use in cancer therapy and exhibits potent biological activity when combined with anticancer agents such as paclitaxel (Taxol). Drug interaction studies of orally administered ONT-093 demonstrated that ONT-093 inhibited the CYP3A mediated metabolism of paclitaxel at high concentrations only; furthermore, ONT-093 had no effect on the clearance of paclitaxel given intravenously, but did affect orally administered paclitaxel clearance, which was consistent with blockade of Pgp function in the gut [see: 2]. Third generation Pgp inhibitors appeared to be promising in first clinical trials. Further investigations on this subject are in progress. The continued development of these agents may establish the real therapeutic potential of Pgp-mediated MDR reversal.

Future Directions of MDR Reversal in Leukaemia

Reports linking overexpression of Pgp to adverse treatment outcome in adult leukaemia provided the evidence necessary to implicate this

multidrug resistance phenotype as an important biologic target for pharmacologic modulation. Many MDR inhibitors have been tested in an attempt to reverse Pgp expression or function.

Although second-generation Pgp inhibitors are significantly more efficacious than the first-generation modulators, they also often have the drawback of inhibiting the metabolism and excretion of toxic substances transported by Pgp in normal tissues, leading to high toxicity of treatment, diminished drug clearance, and the required reduction of the dose of the chemotherapeutic agents. Often, there is competition between cytotoxic agents and Pgp inhibitors for cytochrome P450 3A4 (CYP3A4) leading to complicated and unpredictable pharmacokinetics, raising serum concentration of cytotoxic drugs and leading to overexposure to cytotoxic drugs. Third-generation Pgp-inhibitors have been developed using structure-activity relationships that specifically block Pgp while precluding inhibition of other transporters. These drugs minimally affect the clearance of co-administered chemotherapeutic drugs, do not inhibit cytochrome P450 at the concentrations used, and are currently in clinical trials. Also new agents inhibiting multiple ABC MDR transporters such as birocodar (VX7100) that inhibits Pgp, MRP1, and BCRP, may prove efficacious if multiple transport mechanisms are found to influence leukaemia outcome, particularly if these transporters are expressed in the self-renewing cancer/leukaemia stem cell population [see: 2].

The next, fourth-generation Pgp inhibitors are emerging from efforts to delineate structural interactions with Pgp its transcriptional regulators. One class of these compounds, the farnesyl protein transferase inhibitors (FTIs), has already entered the clinic. In a cell line engineered to overexpress human Pgp, the FTI SCH66336 (lonafarnib; Sarasar; Schering-Plough, Kenilworth, NJ) inhibited xenobiotics export with a potency comparable to CsA. SCH66336 impedes ATP utilization by Pgp by interacting directly with the substrate binding site. Optimally, compounds that inhibit ATPase activity of ABC transporters or the utilization of ATP by drug-resistant cells offer the prospect to disrupt multiple mechanisms of cell defense while enhancing selectivity for malignant cells. One such approach conjugates conventional antineoplastic drugs to polyethylene-derived block copolymers and has shown initial success with its ability to circumvent multiple ABC transporters in resistant cells [see: 50].

Other new technologies such as the use of antisense RNA and RNA interference to reduce the expression of the MDR1 gene are now investigated in preclinical studies.

Conclusions

The development of drug resistance is a serious problem in acute and chronic leukaemia and affects the outcome of chemotherapeutic regimens. This resistance has been associated with rapid drug efflux mediated by P-glycoprotein, and more recently with expression of other multidrug resistance proteins, such as MRP, BCRP and LRP. All these proteins confer cross-resistance to a wide variety of structurally unrelated antineoplastic drugs used in the therapy of leukaemia. In the vast majority of reports investigating *de novo* or secondary adult AML, Pgp is an independent prognostic variable associated with reduced remission probability and overall survival. Predictive power for resistance outcome is proportional to the surface density of multidrug resistance proteins. In ALL, Pgp expression is probably of less signifi-

cance, as most of the studies have demonstrated no relationship of Pgp expression and poor prognosis in both children and adults. There is enough evidence that also LRP and BCRP have prognostic value in AML and particularly the co-expression of LRP and Pgp can be an important factor associated with treatment outcome. Pgp modulators such as cyclosporine A, its non immunosuppressive analogue PSC833 and the third generation of Pgp inhibitors can reverse MDR1-mediated resistance *in vitro* and *in vivo*. Large clinical trials have been developed to assess the benefits of incorporation of these agents into AML therapy.

The more is learned regarding the ABC transporters and other mechanisms involved in the native and acquired drug resistance of leukaemia, the better therapeutic strategies targeting these mechanisms can be developed to improve outcomes and survival of leukaemia patients.

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