



## Novel mechanisms of drug resistance in leukemia

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**A key issue in the treatment of acute leukemia is the development of resistance to chemotherapeutic drugs. Several mechanisms may account for this phenomenon, including failure of the cell to undergo apoptosis in response to chemotherapy, or failure of the drug to reach and/or affect its intracellular target. This review focuses on the latter mechanism, and on intracellular drug transport resistance mechanisms in particular. Expression of the ATP-binding cassette (ABC) transporter P-glycoprotein (Pgp) has generally been reported to correlate with prognosis in acute myeloid leukemia (AML). Additionally, but more controversial, expression of the ABC transporter multidrug resistance protein (MRP) and the vault-transporter lung resistance protein (LRP) have been correlated with outcome in AML. Despite these findings, functional efflux assays indicate the presence of non-Pgp, non-MRP transporters in AML. Recently, a novel ABC transporter, breast cancer resistance protein (BCRP) was cloned and sequenced in our laboratory. Transfection and overexpression of BCRP in drug-sensitive cells confers drug-resistance to the cells. BCRP is a half-transporter, and may homodimerize or form heterodimers (with a yet unknown half-transporter) to produce an active transport complex. Relatively high expression of BCRP mRNA is observed in approximately 30% of AML cases, suggesting a potential role for this new transporter in drug resistance in leukemia. *Leukemia* (2000) 14, 467–473.**

**Keywords:** drug resistance; P-glycoprotein; leukemia

### Introduction

Multidrug resistance is recognized clinically as the development of tumor resistance to a wide variety of anticancer drugs following exposure to a single drug. Anticancer treatments such as drugs, biologics, or radiation must hit their cellular targets and then cause some form of cellular alteration or damage. Rather than killing the cancer cell directly, it is thought that in most cases the damage inflicted by the anticancer agent triggers the process of programmed cell death, or apoptosis. Hence, resistance to multiple drugs could arise from cellular defenses that broadly limit access of the agent to a cellular target (for example, drug transporters), or prevent the cell from entering apoptosis following injury. This review is concerned with multidrug resistance in leukemia, with a focus on the rather narrow category of transport-based drug resistance.

### P-glycoprotein (Pgp)

Great excitement followed the discovery of the membrane-associated transport protein, P-glycoprotein (Pgp), because Pgp provided a laboratory model that emulated the clinically observed phenomenon of multidrug resistance.<sup>1,2</sup> Pgp, the

product of the *MDR1* gene, is a 170-kilodalton (kDa) member of the ATP binding cassette (ABC) superfamily of transport proteins. Pgp is resident in plasma membranes and functions as an efflux transporter of natural-product lipophilic xenobiotics. Overexpression of Pgp imparts cellular resistance to a wide variety of anticancer drugs, including anthracyclines, mitoxantrone, taxanes, epipodophyllotoxins, and vinca alkaloids, many of which are used in the treatment of acute leukemia. It is of note that cytosine arabinoside, an important drug in the treatment of acute myeloid leukemia (AML), is not transported by Pgp.

Several solid tumors, including colon cancer, renal cell carcinoma, hepatoma, non-small cell lung cancer, gliomas, and Kaposi's sarcoma, are generally known to be drug resistant, even at diagnosis. These tumors frequently express high levels of Pgp.<sup>1,2</sup> In other, more chemotherapy-sensitive tumor types, such as AML, acute lymphoblastic leukemia, myeloma, lymphomas, breast and ovarian cancers, expression of Pgp is relatively low at time of diagnosis, but increases after treatment at the time of relapse.<sup>1–4</sup> Many studies have indicated that overexpression of Pgp in AML tends to correlate with poor treatment response in terms of obtaining complete remission, and in some cases, overall survival.<sup>3–11</sup> A recent investigation of Pgp expression in *de novo* AML patients under the age of 60 found the expression of functional Pgp in approximately 35% of cases.<sup>4</sup> In elderly patients (>60 years of age), the frequency of Pgp expression increases to 71%.<sup>3</sup> Moreover, Pgp expression is higher in typically drug-resistant leukemias such as secondary leukemias and leukemias arising from myelodysplastic disorders.<sup>3</sup> In childhood AML, however, Pgp expression is not associated with a poor prognostic group.<sup>12</sup> In contrast to AML, the consensus among studies of acute lymphoblastic leukemia is less as to the prognostic significance of Pgp expression, with some studies reporting Pgp as an adverse prognostic factor<sup>13,14</sup> and others not.<sup>15–18</sup>

A number of agents have been identified that can inhibit Pgp drug efflux.<sup>2</sup> The older, first-generation drugs used in this context include extant drugs found to be substrates for Pgp, including verapamil, cyclosporin A, tamoxifen, and the phenothiazines. Novel agents have been developed to target Pgp specifically, such as dexverapamil and a nonimmunosuppressive analogue of cyclosporin A, PSC833, which is a highly potent inhibitor of Pgp. Thus far, clinical trials with the Pgp antagonists have yielded disappointing results. Overall, no significant change in clinical drug resistance has been demonstrated in solid tumors.<sup>1</sup> In the more responsive tumors, leukemia, lymphoma, and myeloma, there are better than expected response rates reported in trials with Pgp modulators, but in general, no long-term disease-free survival has been demonstrated to date.<sup>19–21</sup> Recently, however, the Southwest Oncology Group completed a large phase III trial of cyclosporin A as a Pgp antagonist in induction therapy of relapsed and refractory AML patients, and found a significant and favorable impact of the Pgp modulator on overall survival and the remission duration of these patients.<sup>22</sup> Importantly, there was a more favorable impact of cyclosporin treatment on the

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median survival of patients whose blast cells had high Pgp expression, suggesting true modulation of Pgp-mediated resistance.

### Non-Pgp transport resistance mechanisms: MRP

Since it is likely that many cellular events in addition or complementary to Pgp overexpression contribute to the clinical multidrug resistance phenotype, it is not surprising that Pgp antagonists have not made a significant impact on clinical drug resistance. Although Pgp is an adverse prognosis factor in AML, many other cellular adaptations in favor of drug resistance may occur, such as changes in the apoptotic pathway and alterations in the drug target itself. For example, recent studies of blast cells from patients with poor prognosis forms of AML found an association of autonomous cell growth *in vitro* with co-overexpression of Pgp and the antiapoptosis gene *BclXL*.<sup>23</sup> Additionally, another recent study in AML found that while the percentage of patients whose blast cells overexpressed Pgp increased between initial diagnosis and relapse, the predictive value of Pgp expression for nonresponse to therapy decreased among patients in late relapse.<sup>16</sup> This could be interpreted as the acquisition of resistance mechanisms in addition to Pgp in late-relapsing disease. These changes, enhanced by the genomic instability of the cancer cell, all contribute to making a given cell able to withstand the maximally tolerated dose of chemotherapy. Hence, if one attempts to improve therapeutic outcome, there is a need to unravel these complex and often interlaced cellular drug resistance mechanisms. The search for other transporters in addition to Pgp has led to the discovery of the multidrug resistance protein (MRP) transporter family, the lung cancer resistance protein (LRP, which actually is not a transporter, but part of a complex that results in intracellular sequestration of drug), and finally, the breast cancer resistance protein, which was cloned and characterized at the University of Maryland Greenebaum Cancer Center.

An ATP-dependent drug efflux resistance phenotype without Pgp overexpression was reported independently by two groups of investigators who selected HL-60 human leukemia cells for resistance with doxorubicin.<sup>24–26</sup> The mysterious transporter in the HL-60 cells was elucidated with the subsequent cloning of the MRP from multidrug-resistant human lung cancer cells.<sup>27</sup> The 190-kDa MRP, unlike Pgp, can be localized on both the plasma and intracytoplasmic membranes, including the endoplasmic reticulum and Golgi apparatus.<sup>28</sup> Because of its intracellular localization, MRP can cause intracellular or cytoplasmic sequestration of drug, thus preventing pharmaceuticals from reaching their cellular targets.

The spectrum of resistance imparted by MRP overexpression is very similar to that of Pgp, with the exception of taxanes and mitoxantrone, which are effluxed by Pgp, but not by MRP.<sup>28</sup> MRP transport is dependent on the presence of glutathione (GSH).<sup>28</sup> Drug substrates for MRP are transported either as GSH conjugates, or are co-transported with GSH. Depletion of GSH sensitizes MRP overexpressing cells to drugs that are transported by MRP.<sup>28</sup>

Initial investigations into the relationship of MRP and treatment outcome in AML showed low MRP expression at presentation,<sup>29–31</sup> with an increase in expression at disease relapse.<sup>30</sup> Recent large studies, however, have been unable to detect any relationship between MRP expression and clinical response in AML.<sup>4,32,33</sup> However, the simultaneous activity of MRP and Pgp in AML blast cells has been correlated with drug resist-

ance.<sup>34,35</sup> MRP is located on chromosome 16, and is deleted in the 'inverse 16' translocation. This may explain the more favorable prognosis in leukemias associated with this genotype.<sup>36</sup>

Since its discovery, MRP is now known to be one of at least six genes that comprise a family of multispecific organic anion transporters (MOAT).<sup>37–42</sup> The originally described p190 MRP has been designated as MRP1. To date, two of the other MRP family members, MRP2 (also called canalicular multiorganic anion transporter, cMOAT) and MRP3 (also known as MOAT-D) have been cloned and transfected into drug-sensitive cells. Enforced expression of MRP2 resulted in resistance to cisplatin, anthracyclines, etoposide, and methotrexate;<sup>43–45</sup> enforced expression of MRP3 caused resistance to vinca alkaloids, etoposide, and methotrexate.<sup>46,47</sup>

### Lung resistance-related protein

Contrary to its name, lung resistance-related protein (LRP) is not prognostic of resistance in lung cancer; rather, the name is derived from the initial isolation of LRP from a lung cancer cell line.<sup>48</sup> The phenotype associated with LRP overexpression includes resistance to natural products, including doxorubicin, mitoxantrone, vincristine, and etoposide. LRP has been shown to be identical to the major vault protein.<sup>49</sup> Vaults are cytoplasmic ribonucleoprotein organelles that constitute the transporter core of the nuclear pore complex.<sup>50</sup> Hence, LRP is involved with intracellular transport, but unlike Pgp and MRP, is not an ABC transporter. Vaults are present in many cells, but appear to be upregulated in certain cancer cells that overexpress LRP. The enforced expression of LRP in drug-sensitive cells does not result in drug resistance<sup>49</sup> because the overexpression LRP alone does not increase the number of vaults. The overexpression of intracellular vaults seems to result in the sequestration of drug in the vaults, preventing the drug from reaching its intracellular target. Reports are divided as to the significance of LRP expression in predicting treatment outcome. Overexpression of LRP, as measured by the LRP-56 antibody, has been found in some reports to be an adverse prognostic factor in AML,<sup>51,52</sup> in ovarian cancer,<sup>53</sup> and in multiple myeloma treated with conventional doses of melphalan.<sup>54</sup> In contrast, other reports do not ascribe prognostic significance to LRP expression in acute leukemia<sup>4,55</sup> or in myelodysplastic syndrome.<sup>56</sup>

### Non-Pgp, non-MRP, non-LRP transporters

Studies of acute leukemia indicated that the overexpression of Pgp and/or MRP in patient-derived blast cells was insufficient to account for the observed frequency of a drug efflux-based resistance phenotype, suggesting the existence of other resistance mechanisms.<sup>57,58</sup> This is confirmed by a recent study of 352 newly diagnosed AML patients that reported, 'a distinct and nonoverlapping phenotype was detected in 18% of these cases: cyclosporine-resistant efflux not associated with *MDR1*, *MRP1*, or LRP expression, implying the existence of other as yet undefined efflux mechanisms in AML'.<sup>4</sup> Laboratory evidence for a non-Pgp, non-MRP transporter also came from the recognition that *in vitro* selection of a variety of cancer cell lines with mitoxantrone produced a phenotype characterized by ATP-dependent drug efflux, and resistance to mitoxantrone, anthracyclines, and topoisomerase I inhibitors.<sup>59–64</sup> Additionally, investigators at the National Cancer Institute, in

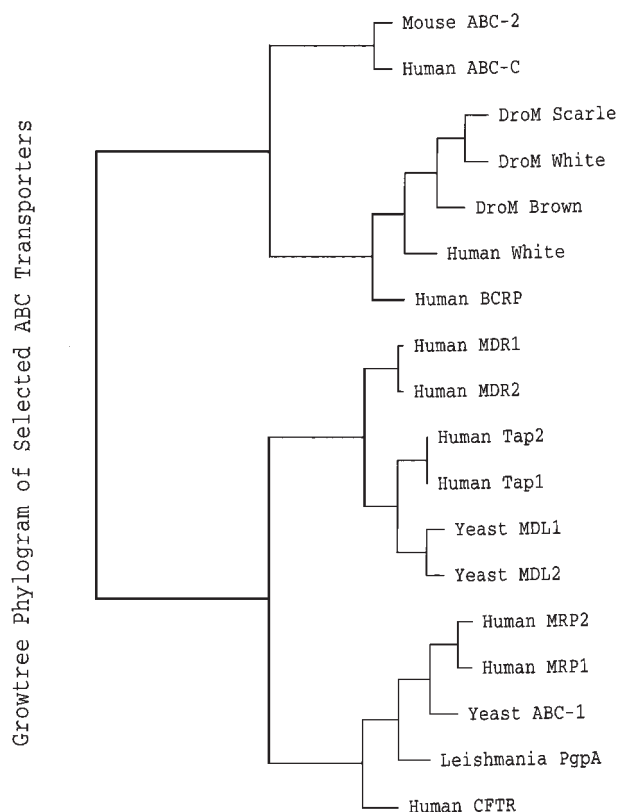
search of non-Pgp mechanisms of resistance, selected human breast cancer MCF-7 cells in the presence of doxorubicin and verapamil, an inhibitor of Pgp.<sup>65</sup> The resulting MCF-7/AdrVp cells demonstrated ATP-dependent drug efflux with a phenotype almost identical to that of the cell lines selected with mitoxantrone, and also without overexpression of Pgp or MRP.<sup>66,67</sup>

Our laboratory became interested in the novel transporter-based drug resistance phenotype manifested by MCF-7/AdrVp cells. Using the technique of differential display hybridization,<sup>68</sup> we compared the mRNA repertoire of parental MCF-7 cells to the resistant MCF-7/AdrVp cells. A 2.4 kb mRNA was identified that was overexpressed in the AdrVp cells, and expressed at low levels in the parental cells. The overexpressed mRNA contained an open reading frame that encoded a novel 655-amino acid, 72.6-kDa protein, with motifs characteristic of an ABC half transporter. Hence, we designated the new transporter breast cancer resistance protein (or BCRP), because it was isolated from multidrug resistant breast cancer cells.<sup>69</sup> A portion of BCRP cDNA is homologous to a human expressed sequence tag (U66681/EST157481) that was predicted to represent one of 21 new human ABC transporter genes identified by the use of the human EST database.<sup>37</sup> Subsequent to our findings, two other groups have independently isolated BCRP cDNA and have confirmed our sequence data.<sup>70,71</sup> These groups have termed the transporter the mitoxantrone resistance gene, *MXR1*,<sup>70</sup> and the human placental ABC transporter, ABC-P.<sup>71</sup> The GenBank accession number for BCRP is AF098951.

BCRP has the greatest sequence similarity to the product of the human homologue of the white gene of *Drosophila*. In the fruit fly, the white gene is involved in the transport of eye pigments.<sup>72</sup> The human white gene is not overexpressed in MCF-7/AdrVp cells, however. BCRP is in the white family of transporter proteins, along with the human homologue of white, and the *Drosophila* genes white, brown, and scarlet.<sup>72-74</sup> BCRP is evolutionarily distinct from the families that contain Pgp and MRP, which are on a completely separate limb of the phylogenetic tree (Figure 1). Compared to the full transporters Pgp or MRP, white gene family members are approximately half the size of the full transporters, and have a single hydrophilic ATP binding region (nucleotide binding domain, NBD1) at the amino terminus. The hydrophobic region containing the transmembrane domains is at the carboxyl terminus of BCRP (Figure 2).

The white, brown, and scarlet genes of *Drosophila* are, like BCRP, half transporters. These half transporters form heterodimers to constitute an active transmembrane conductance channel.<sup>72</sup> The white/brown heterodimer transports guanine, an eye pigment precursor. Likewise, the white/scarlet combination produces a transporter for the pigment precursor tryptophan. There are no known transport complexes formed by white, brown, or scarlet protein homodimers, or a scarlet/brown heterodimer. The products of the human *Tap* genes, *Tap1* and *Tap2*, also half transporters, form a heterodimeric *Tap1/Tap2* complex for the transport of peptides of major histocompatibility class I.<sup>72</sup> There are no known active transport complexes formed by homodimers of *Tap1* or *Tap2*. Unlike BCRP, the *Tap* half-transporter proteins are in the Pgp family of ABC transporters.

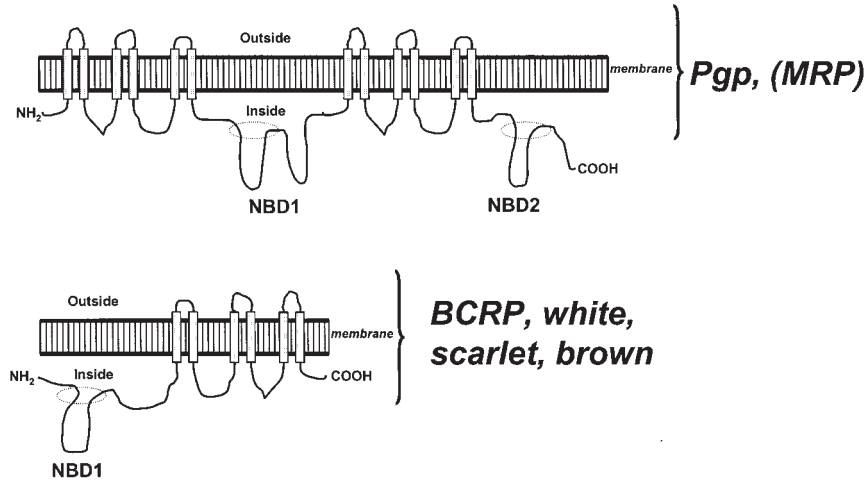
Conclusive proof that BCRP is responsible for the drug resistance phenotype of MCF-7/AdrVp cells can only be obtained by demonstrating that the enforced expression of BCRP in drug-sensitive cells confers the resistance phenotype of MCF-7/AdrVp cells. Furthermore, because BCRP is a half



**Figure 1** Evolution of the amino acid sequence of BCRP relative to certain other members of the ABC transporter family. This phylogram was produced with the multiple sequence alignment programs PILEUP, DISTANCES, and GROWTREE, which are part of the Genetics Computer Group software package (version 8, Genetics Computer Group, Madison, WI, USA). The figure is reproduced from Ref. 69, with permission from the National Academy of Sciences of the United States of America.

transporter, transfection studies may shed light on the requirement for other yet unidentified half transporters for BCRP activity. For this reason, we placed a high priority on the transfection studies. We chose the pcDNA3 expression vector to enforce high constitutive expression of BCRP in drug-sensitive MCF-7 cells. Indeed, the BCRP-transfected cells recapitulated the phenotype of the MCF-7/AdrVp cells, including ATP-dependent drug efflux, diminished intracellular accumulation and retention of drug, and resistance to mitoxantrone, daunorubicin, and doxorubicin, and retained sensitivity to platinum, paclitaxel, and vincristine (Table 1).<sup>69</sup> Additionally, we have found that the BCRP-transfected MCF-7 cells are resistant to inhibitors of DNA topoisomerase I, including topotecan<sup>75</sup> and SN-38, the active form of irinotecan (Table 1).

The transfection studies have three possible interpretations as to the functional BCRP transporter configuration: (1) active BCRP is a homodimer or homomultimer. If this were the case, it would be somewhat unique because most known functional half transporter complexes are heterodimers; (2) BCRP partners with an unknown half transporter that is constitutively expressed in MCF-7 cells. To date, we have been unable to detect such a half transporter in MCF-7 cells; (3) BCRP is active as a monomer. This seems unlikely, based on extensive mutational studies done with Pgp<sup>76</sup> that demonstrated an absolute requirement for two ATP binding domains and extensive transmembrane regions. Nevertheless, if a mammalian analogy to the white/brown/scarlet system exists, it is reason-



**Figure 2** Schematic representation of some physical characteristics of the white or P-glycoprotein families of ABC transporters. True distances are only approximate in the figure, and the actual orientation of the transporter in the membrane is not shown. In cell membranes, the transporter transmembrane domains probably form a cylindrical aggregate to constitute a conductance channel. Note that the full transporter Pgp is composed of two similar halves, each half having six transmembrane regions and an intracellular region containing the nucleotide binding domain (NBD). In Pgp family members, the transmembrane domains of each half are located toward the amino terminus, relative to the NBD. MRP family members are similar in orientation to the Pgp family, however, MRP contains 18 transmembrane domains, with the transmembrane region of MRP closest to the amino terminus having 12 transmembrane domains. White family members have a single NBD and transmembrane region containing six predicted transmembrane domains, with the NBD oriented towards the amino terminus of the molecule. In the figure, the approximate location of the Walker A and Walker B nucleotide binding motifs is indicated by an oval.

**Table 1** Drug resistance phenotype of MCF-7 cells with enforced overexpression of BCRP

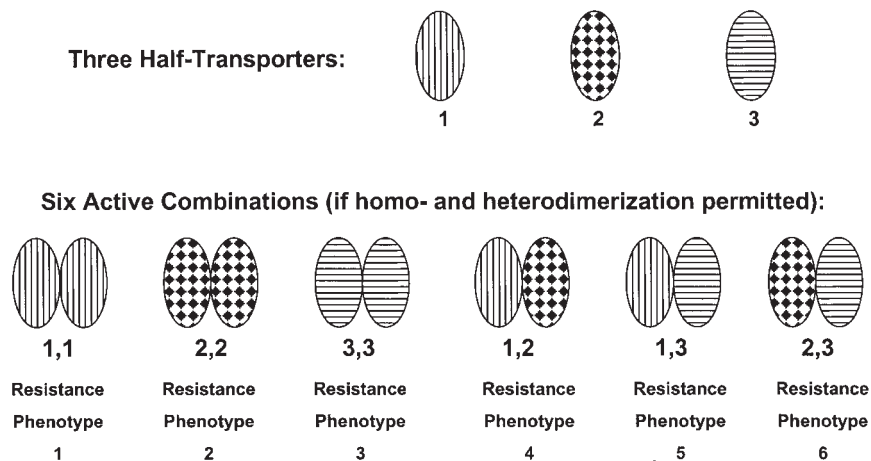
Resistance to:	Sensitive to:
Mitoxantrone	Vincristine
Daunorubicin	Cisplatin
Doxorubicin	Taxol
Topotecan	
SN-38	

(Figure 3). Clearly, the identification of potential partner transporters for BCRP is of great importance.

In normal human tissues, BCRP expression is quite distinct from that of Pgp and MRP. BCRP mRNA levels are highest in the placenta<sup>69,71</sup> and in certain areas of the midbrain (putamen). In comparison, the expression of BCRP in normal adult tissues and fetal tissues is relatively low, although the highest expressing adult organs are liver, small intestine, and colon. Because of the relatively low expression of BCRP in normal tissues, BCRP expression in tumor samples may be useful as a tumor marker.

able to postulate that neoplastic cells may use a versatile combinatorial strategy to custom-tailor transport for a given anti-neoplastic agent (Figure 3). Under this hypothesis, three half transporters could generate up to six functional dimeric units, if one allows both homo- and heterodimers in the hypothesis

Since many human cancer cell lines selected with mitoxantrone developed a non-Pgp, non-MRP transport-based resistance phenotype similar to that manifested by MCF-7/AdrVp cells, we investigated whether BCRP was overexpressed in these mitoxantrone cell lines.<sup>77</sup> We found overexpression of



**Figure 3** The half transporter hypothesis. Based on the possibility that a BCRP homodimer constitutes a functional transporter complex, the hypothesis states that cells may defend themselves from a given xenobiotic by assembling homo- or heterodimers of half transporters that are optimal for the transport of that xenobiotic. This permits the cell to assemble six functionally different transporter complexes from only three half transporter molecules.

BCRP in most of the mitoxantrone-selected cell lines studied.<sup>59–64</sup> In some cases, marked amplification of the BCRP gene was also noted. BCRP overexpression was associated with multidrug resistance in mitoxantrone-selected cell lines derived from human breast, gastric, and colon cancers, as well as from fibrosarcoma and multiple myeloma cells.<sup>77</sup>

The finding of elevated expression of BCRP mRNA in one of the mitoxantrone-selected cell lines (human colon carcinoma S1-M1–3.2 cells) is of particular significance because of the recent report of a novel and specific inhibitor of the transporter identified in S1-M1–3.2 cells, fumitremorgin C.<sup>63</sup> Fumitremorgin C, a natural product isolated from *Aspergillus fumigatus*, does not reverse resistance in cells that overexpress Pgp or MRP.<sup>63</sup> In studies of our BCRP-transfected MCF-7 cells, fumitremorgin C was also able to enhance drug accumulation and cause marked sensitization of the cells to mitoxantrone and anthracyclines.<sup>75</sup> Further development of fumitremorgin C is warranted for use in functional assays of BCRP activity and possibly as a clinical adjunct to chemotherapy. Interestingly, classical inhibitors of Pgp such as cyclosporin A are not effective in reversing drug resistance in BCRP overexpressing cells.

A transport-mediated drug resistance phenotype similar to that observed in mitoxantrone-selected cell lines has been reported recently in IGROV1 human ovarian carcinoma cells following selection with topotecan.<sup>78</sup> The resulting drug-resistant cells are cross-resistant to mitoxantrone, SN-38, and 9-aminocamptothecin, have diminished accumulation and retention of topotecan, do not overexpress Pgp or MRP, and have no alteration in the catalytic activity or expression of DNA topoisomerase I.<sup>78</sup> In contrast to the mitoxantrone-selected cells described above, the topotecan-selected IGROV1 cells are not cross-resistant to doxorubicin or daunorubicin. Recently, marked overexpression of BCRP was reported in the topotecan-selected IGROV1 cells, confirming that selection with topotecan can induce BCRP as a cellular defense mechanism.<sup>79</sup>

In exploratory studies to determine the frequency of expression of BCRP in acute leukemia, we measured the expression of BCRP mRNA in blast cells from 14 AML patients by means of a quantitative reverse-transcription polymerase chain reaction (RT-PCR) method.<sup>80</sup> Although most of the patients studied were newly diagnosed, the majority had unfavorable cytogenetic alterations. Relatively high expression of BCRP mRNA was observed in marrow from four patients with drug-resistant disease in this group of 14 patients (29%).<sup>80</sup> The remaining patients had low or barely detectable expression of BCRP mRNA. The overexpression of BCRP in blast cells of some but not all AML patients indicates a need for further studies of the diagnostic and prognostic significance of BCRP expression in AML blast cells, particularly in regard to drug treatment response and the expression of other markers of drug resistance. Preferably, future studies will incorporate assays of BCRP protein and function. Functional assays for BCRP will be facilitated by the use of the specific BCRP inhibitor, fumitremorgin C. Perhaps BCRP can account for some or all of the recently described subset of AML patients whose blast cells had cyclosporin-resistant drug efflux not associated with the overexpression of Pgp, MRP or LRP.<sup>4</sup>

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#### References

- Murren JR, DeVita VT. Another look at multidrug resistance. *Principles Practice Oncol Updates* 1995; **9**: 1–12.
- Fisher GA, Sikic BI (eds). Drug resistance in clinical oncology and hematology. *Hematol Oncol Clin N Am* 1995; **9**: 239–382.
- Leith CP, Kopecky KJ, Godwin JE, McConnell T, Slovak M, Chen IM, Head DR, Appelbaum F, Willman CL. Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. *Blood* 1997; **89**: 3323–3329.
- Leith CP, Kopecky KJ, Chen IM, Eijdemis L, Slovak ML, McConnell TS, Head DR, Weick J, Grever MR, Appelbaum FR, Willman CL. Frequency and clinical significance of the expression of the multidrug resistance proteins MDR1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia. A Southwest Oncology Group study. *Blood* 1999; **94**: 1086–1099.
- Samdani A, Vijapurkar U, Grimm MA, Spier CS, Grogan TM, Glinsmann-Gibson BJ, List AF. Cytogenetics and P-glycoprotein (PGP) are independent predictors of treatment outcome in acute myeloid leukemia (AML). *Leukemia Res* 1996; **20**: 175–180.
- Guerci A, Merlin JL, Missoum N, Feldmann L, Marchal S, Witz F, Rose C, Guerci O. Predictive value for treatment outcome in acute myeloid leukemia of cellular daunorubicin accumulation and P-glycoprotein expression simultaneously determined by flow cytometry. *Blood* 1995; **85**: 2147–2153.
- Wood P, Burgess R, MacGregor A, Yin JA. P-glycoprotein expression on acute myeloid leukemia blast cells at diagnosis predicts response to chemotherapy and survival. *Br J Haematol* 1994; **87**: 509–514.
- Lamy T, Goasguen JE, Mordelet E, Grulois I, Dauriac C, Drenou B, Chaperon J, Fauchet R, le Prise PY. P-glycoprotein (P-170) and CD34 expression in adult acute myeloid leukemia (AML). *Leukemia* 1994; **8**: 1879–1883.
- Del Poeta G, Venditti A, Aronica G, Stasi R, Cox MC, Buccisano F, Bruno A, Tamburini A, Suppo G, Simone MD, Epiceno AM, Del Moro B, Masi M, Papa G, Amadori S. P-glycoprotein expression in *de novo* acute myeloid leukemia. *Leuk Lymphoma* 1997; **27**: 257–274.
- Senent L, Jarque I, Martin G, Sempere A, Gonzalez-Garcia Y, Gomis F, Perez-Srvent M, De La Rubia J, Sanz MA. P-glycoprotein expression and prognostic value in acute myeloid leukemia. *Haematologica* 1998; **83**: 783–787.
- Zochbauer S, Gsur A, Brunner R, Kyrle PA, Lechner K, Pirker R. P-glycoprotein expression as unfavorable prognostic factor in acute myeloid leukemia. *Leukemia* 1994; **8**: 974–977.
- Sievers EL, Smith FO, Woods WG, Lee JW, Bleyer WA, Willman CL, Bernstein ID. Cell surface expression of the multidrug resistance P-glycoprotein (P-170) as detected by monoclonal antibody MRK-16 in pediatric acute myeloid leukemia fails to define a poor prognostic group: a report from the Children's Cancer Group. *Leukemia* 1995; **9**: 2042–2048.
- Ivy SP, Olshefski RS, Taylor BJ, Patel KM, Reaman G. Correlation of P-glycoprotein expression and function in childhood acute leukemia: a Children's Cancer Group study. *Blood* 1996; **88**: 309–318.
- Dhooge C, De Moerloose B, Laureys G, Kint J, Ferster A, De Bacquer D, Philippe J, Beniot Y. P-glycoprotein is an independent prognostic factor predicting relapse in childhood acute lymphoblastic leukemia: results of a 6-year prospective study. *Br J Haematol* 1999; **105**: 676–683.
- Wattel E, Lepelley P, Merlat A, Sartiaux C, Bauters F, Jouet JP, Fenaux P. Expression of the multidrug resistance P glycoprotein in newly diagnosed adult acute lymphoblastic leukemia: absence of correlation with response to treatment. *Leukemia* 1995; **9**: 1870–1874.
- Nussler V, Pelka-Fleischer R, Zwierzina H, Nerl C, Beckert B, Gieseler F, Diem H, Ledderose G, Gullis E, Sauer H, Wilmanns W.

- P-glycoprotein expression in patients with acute leukemia-clinical relevance. *Leukemia* 1996; **10** (Suppl. 3): S23-S31.
- 17 den Boer ML, Pieters R, Kazemier KM, Rottier MM, Zwaan CM, Kaspers GJ, Janka-Schaub G, Henze G, Creutzig U, Scheper RJ, Veerman AJ. Relationship between major vault protein/lung resistance protein, multidrug resistance-associated protein, P-glycoprotein expression and drug resistance in childhood leukemia. *Blood* 1998; **91**: 2092-2098.
  - 18 Kanerva J, Tiirikainen M, Makiperna A, Riikonen P, Mottonen M, Salmi TT, Krusius T, Saarinen-Pihkala UM. Multiple drug resistance mediated by P-glycoprotein is not a major factor in a slow response to therapy in childhood ALL. *Pediatr Hematol Oncol* 1998; **15**: 11-21.
  - 19 Salmon SE, Dalton WS, Grogan TM, Plezia P, Lehnert M, Roe DJ, Miller TP. Multidrug resistant myeloma: Laboratory and clinical effects of verapamil as a chemosensitizer. *Blood* 1991; **78**: 44-50.
  - 20 Wilson WH, Bates SE, Fojo A, Bryant G, Zhan Z, Regis J, Wittes RE, Jaffe ES, Steinberg SM, Hearsh J *et al*. Controlled trial of dexverapamil, a modulator of multidrug resistance, in lymphomas refractory to EPOCH chemotherapy. *J Clin Oncol* 1995; **13**: 1995-2004.
  - 21 List AF, Spier C, Greer J, Wolff S, Hutter J, Dorr R, Salmon S, Fitcher B, Baier M, Dalton W. Phase I/II trial of cyclosporine as a chemotherapy-resistance modifier in acute leukemia. *J Clin Oncol* 1993; **11**: 1652-1660.
  - 22 List AF, Kopecy KJ, Willman CL, Spier C, Dorr R, Appelbaum F, Hynes H. Benefit of cyclosporine (CsA) modulation of anthracycline resistance in high-risk AML: A Southwest Oncology Group study. *Blood* 1998; **92** (Suppl. 1): 312a.
  - 23 Pallis M, Zhu YM, Russell NH. BclXL is heterogeneously expressed by acute myeloblastic leukaemia cells and is associated with autonomous growth *in vitro* and with P-glycoprotein expression. *Leukemia* 1997; **11**: 945-949.
  - 24 Bhalla K, Hindenberg A, Taub RN, Grant S. Isolation and characterization of an anthracycline-resistant human leukemic cell line. *Cancer Res* 1985; **45**: 3657-3662.
  - 25 Gervasoni JE Jr, Taub RN, Rosado M, Krishna S, Stewart VJ, Knowles DM, Bhalla K, Ross DD, Baker MA, Lutsky J, Hindenberg AA. Membrane glycoprotein changes associated with anthracycline resistance in HL-60 cells. *Cancer Chemother Pharmacol* 1991; **28**: 93-101.
  - 26 Marquardt D, McCrone S, Center MS. Mechanisms of multidrug resistance in HL-60 cells: detection of resistance-associated proteins with antibodies against synthetic peptides that correspond to the deduced sequence of P-glycoprotein. *Cancer Res* 1990; **50**: 1426-1430.
  - 27 Cole SPC, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AMV, Deeley RG. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* 1992; **258**: 1650-1654.
  - 28 Lautier D, Canitrot Y, Deeley RG, Cole SPC. Multidrug resistance mediated by the multidrug resistance protein (MRP) gene. *Biochem Pharmacol* 1996; **52**: 967-977.
  - 29 Hart SM, Ganeshaguru K, Hoffbrand AV, Prentice HG, Mehta AB. Expression of the multidrug resistance-associated protein (MRP) in acute leukemia. *Leukemia* 1994; **8**: 2163-2168.
  - 30 Schneider E, Cowan KH, Bader H, Toomey S, Schwartz GN, Karp JE, Burke PJ, Kaufmann SH. Increased expression of the multidrug resistance-associated protein gene in relapsed acute leukemia. *Blood* 1995; **85**: 186-193.
  - 31 Zhou DC, Zittoun R, Marie JP. Expression of multidrug resistance-associated protein (MRP) and multidrug resistance (MDR1) genes in acute myeloid leukemia. *Leukemia* 1995; **9**: 1661-1666.
  - 32 Filipits M, Suchomel RW, Zochbauer S, Brunner R, Lechner K, Pirker R. Multidrug resistance-associated protein in acute myeloid leukemia: no impact on treatment outcome. *Clin Cancer Res* 1997; **3**: 1419-1425.
  - 33 Filipits M, Stranzl T, Pohl G, Suchomel RW, Zochbauer S, Brunner R, Lechner K, Pirker R. MRP expression in acute myeloid leukemia. An update. *Adv Exp Med Biol* 1999; **47**: 141-150.
  - 34 Schuurhuis GJ, Broxterman HJ, Ossenkoppele GJ, Baak JP, Eekman CA, Kuiper CM, Feller N, van Heijningen TH, Klumper E, Pieters *et al*. Functional multidrug resistance phenotype associated with combined overexpression of Pgp/MDR1 and MRP together with 1-beta-D-arabinofuranosylcytosine sensitivity may predict clinical response in acute myeloid leukemia. *Clin Cancer Res* 1995; **1**: 81-93.
  - 35 Legrand O, Simonin G, Beauchamp-Nicoud A, Zittoun R, Marie JP. Simultaneous activity of MRP1 and Pgp is correlated with *in vitro* resistance to daunorubicin and with *in vivo* resistance in acute myeloid leukemia. *Blood* 1999; **94**: 1046-1056.
  - 36 Kuss BJ, Deeley RG, Cole SP, Willman CL, Kopecy KJ, Wolman SR, Eyre HJ, Lane SA, Nancarrow JK, Whitmore SA, Callen DF. Deletion of gene for multidrug resistance in acute myeloid leukaemia with inversion in chromosome 16: prognostic implications. *Lancet* 1994; **343**: 1531-1534.
  - 37 Allikmets R, Gerrard B, Hutchinson A, Dean M. Characterization of the human ABC superfamily: Isolation and mapping of 21 new genes using the expressed sequence tags database. *Hum Mol Genet* 1996; **5**: 1649-1655.
  - 38 Buchler M, Konig J, Brom M, Kartenbeck J, Spring H, Horie T, Keppler D. cDNA cloning of the hepatocyte canalicular isoform of the multidrug resistance protein, cMrp, reveals a novel conjugate export pump deficient in hyperbilirubinemic mutant rats. *J Biol Chem* 1996; **271**: 15091-15098.
  - 39 Kool M, de Haas M, Scheffer GL, Scheper RJ, van Eijk MJ, Juijn JA, Baas F, Borst P. Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. *Cancer Res* 1997; **57**: 3537-3547.
  - 40 Belinsky MG, Bain LJ, Balsara BB, Testa JR, Kruh GD. Characterization of MOAT-C and MOAT-D, new members of the MRP/cMOAT subfamily of transporter proteins. *J Natl Cancer Inst* 1998; **90**: 1735-1741.
  - 41 Lee K, Belinsky MG, Bell DW, Testa JR, Kruh GD. Isolation of MOAT-B, a widely expressed multidrug resistance-associated protein/canalicular multispecific organic anion transporter-related transporter. *Cancer Res* 1998; **58**: 2741-2747.
  - 42 Belinsky M, Kruh G. MOAT-E (ARA) is a full-length MRP/cMOAT subfamily transporter expressed in kidney and liver. *Br J Cancer* 1999; **80**: 1342-1349.
  - 43 Cui Y, Konig J, Buchholz JK, Spring H, Leier I, Keppler D. Drug resistance and ATP-dependent conjugate transport mediated by the apical multidrug resistance protein, MRP2, permanently expressed in human and canine cells. *Mol Pharmacol* 1999; **55**: 929-937.
  - 44 Koike K, Kwabe T, Tanaka T, Toh S, Uchiumi T, Wada M, Akiyama S, Ono M, Kuwano M. A canalicular multispecific organic anion transporter (cMOAT) antisense cDNA enhances drug sensitivity in human hepatic cancer cells. *Cancer Res* 1997; **57**: 5475-5479.
  - 45 Hooijberg JH, Broxterman HJ, Kool M, Assaraf YG, Peters GJ, Noordhuis P, Scheper RJ, Borst P, Pinedo HM, Jansen G. Antifolate resistance mediated by the multidrug resistance proteins MRP1 and MRP2. *Cancer Res* 1999; **59**: 2532-2535.
  - 46 Zeng H, Bain LJ, Belinsky MG, Kruh GD. Expression of MRP-3 in HEK293 cells confers resistance to anticancer agents. *Cancer Res* (in press).
  - 47 Kool M, van der Linden M, de Haas M, Scheffer GL, de Vree JM, Smith AJ, Jansen G, Peters GJ, Ponne N, Scheper RJ, Elferink RP, Baas F, Borst P. MRP3, an organic anion transporter able to transport anti-cancer drugs. *Proc Natl Acad Sci USA* 1999; **96**: 694-699.
  - 48 Scheper RJ, Broxterman HJ, Scheffer GL, Kaaijk P, Dalton WS, van Heijningen THM, van Kalken CK, Slovak ML, de Vries EGE, van der Valk P, Meijer, CJLM, Pinedo HM. Overexpression of a Mr 110,000 vesicular protein in non-P-glycoprotein-mediated multidrug resistance. *Cancer Res* 1993; **53**: 1475-1479.
  - 49 Scheffer GL, Wijngaard PLJ, Flens MJ, Izquierdo MA, Slovak ML, Pinedo HM, Meijer CJLM, Clevers HC, Scheper RJ. The drug resistance-related protein LRP is the human major vault protein. *Nature Med* 1995; **1**: 578-582.
  - 50 Chugani DC, Rome LH, Kedersha NL. Evidence that vault ribonucleoprotein particles localize to the nuclear pore complex. *J Cell Sci* 1993; **106**: 23-29.
  - 51 List AF, Spier CS, Grogan TM, Johnson C, Greer JP, Wolff SN, Broxterman HJ, Scheffer GL, Scheper RJ, Dalton WS. Overexpression of the major vault transporter protein lung-resistance protein predicts treatment outcome in acute myeloid leukemia. *Blood* 1996; **87**: 2464-2469.

- 52 Filipits M, Pohl G, Stranzl T, Suchomel RW, Scheper RJ, Jager U, Geissler K, Lechner K, Pirker R. Expression of the lung resistance protein predicts poor outcome in *de novo* acute myeloid leukemia. *Blood* 1998; **91**: 1508–1513.
- 53 Izquierdo MA, van der Zee A, Vermoken J, van der Valk P, Belien JAM, Giaccone G, Scheffer GL, Flens MJ, Pinedo HM, Kenemans P, Meijer CJLM, de Vries EGE, Scheper RJ. Expression of the new drug resistance-associated marker LRP in ovarian carcinoma predicts poor response to chemotherapy and shorter survival. *J Natl Cancer Inst* 1995; **87**: 1230–1237.
- 54 Raaijmakers HG, Izquierdo MA, Lokhorst HM, de Leeuw C, Belien JA, Bloem AC, Dekker AW, Scheper RJ, Sonneveld P. Lung-resistance-related protein expression is a negative predictive factor for response to conventional low but not to intensified dose alkylating chemotherapy in multiple myeloma. *Blood* 1998; **91**: 1029–1036.
- 55 Michieli M, Damiani D, Ermacora A, Masolini P, Raspadori D, Visani G, Scheper RJ, Baccarani M. P-glycoprotein, lung resistance-related protein and multidrug resistance associated protein in *de novo* acute non-lymphocytic leukaemias: biological and clinical implications. *Br J Haematol* 1999; **104**: 328–335.
- 56 Lepelley P, Poulain S, Gardel N, Preudhomme C, Cosson A, Fenaux P. Expression of lung resistance protein and correlation with other drug resistance proteins and outcome in myelodysplastic syndromes. *Leuk Lymphoma* 1998; **29**: 547–551.
- 57 Leith CP, Chen I-M, Kopecky KJ, Appelbaum FR, Head DR, Godwin JE, Weick JK, Willman CL. Correlation of multidrug resistance (MDR1) protein expression with functional dye/drug efflux in acute myeloid leukemia by multiparameter flow cytometry: Identification of discordant MDR–/Efflux+ and MDR1+/Efflux– cases. *Blood* 1995; **86**: 2329–2342.
- 58 Ross DD, Doyle LA, Schiffer CA, Lee EJ, Grant CE, Cole SPC, Deeley RG, Yang W, Tong Y. Expression of multidrug resistance-associated protein (MRP) mRNA in blast cells from acute myeloid leukemia (AML) patients. *Leukemia* 1996; **10**: 48–55.
- 59 Taylor CW, Dalton WS, Parrish PR, Gleason MC, Bellamy WT, Thompson FH, Roe DJ, Trent JM. Different mechanisms of decreased drug accumulation in doxorubicin and mitoxantrone resistant variants of the MCF-7 human breast cancer cell line. *Br J Cancer* 1991; **63**: 923–929.
- 60 Futcher BW, Abbaszadegan MR, Domann F, Dalton WS. Analysis of MRP mRNA in mitoxantrone-selected, multidrug-resistant human tumor cells. *Biochem Pharm* 1994; **47**: 1601–1606.
- 61 Nakagawa M, Schneider E, Dixon KH, Horton J, Kelley K, Morrow C, Cowan KH. Reduced intracellular drug accumulation in the absence of P-glycoprotein (mdr1) overexpression in mitoxantrone-resistant human MCF-7 breast cancer cells. *Cancer Res* 1992; **52**: 6175–6181.
- 62 Yang C-HJ, Cowan K, Schneider E. Reselection of a mitoxantrone-resistant breast carcinoma cell line with mitoxantrone results in a parallel increase in cross-resistance to camptothecin analogues. *Proc Amer Assoc Cancer Res* 1996; **37**: 308 (Abstr.).
- 63 Rabindran SK, He H, Singh M, Brown E, Collins KI, Annable T, Greenberger LM. Reversal of a novel multidrug resistance mechanism in human colon carcinoma cells by fumitremorgin C. *Cancer Res* 1998; **58**: 5850–5858.
- 64 Dietel M, Arps H, Lage H, Niendorf A. Membrane vesicle formation due to acquired mitoxantrone resistance in human gastric carcinoma cell line EPG85–257. *Cancer Res* 1990; **50**: 6100–6106.
- 65 Chen Y-N, Mickley LA, Schwartz AM, Acton EM, Hwang J, Fojo AT. Characterization of adriamycin-resistant human breast cancer cells which display overexpression of a novel resistance-related membrane protein. *J Biol Chem* 1990; **265**: 10073–10080.
- 66 Doyle LA, Ross DD, Sridhara R, Fojo AT, Kaufmann SH, Lee EJ, Schiffer CA. Expression of a 95 kDa membrane protein is associated with low daunorubicin accumulation in leukaemic blast cells. *Br J Cancer* 1995; **71**: 52–58.
- 67 Lee JS, Scala S, Matsumoto Y, Dickstein B, Robey R, Zhan Z, Altenberg G, Bates SE. Reduced drug accumulation and multidrug resistance in human breast cancer cells without associated P-glycoprotein or MRP overexpression. *J Cell Biochem* 1997; **65**: 513–526.
- 68 Liang P, Pardee AB. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* 1992; **257**: 967–971.
- 69 Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, Ross DD. A novel multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci USA* 1998; **95**: 15665–15670.
- 70 Miyake K, Mickley L, Litman T, Zhan Z, Robey R, Cristensen B, Brangi M, Greenberger L, Dean M, Fojo T, Bates SE. Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. *Cancer Res* 1999; **59**: 8–13.
- 71 Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, Dean M. A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. *Cancer Res* 1998; **58**: 5337–5339.
- 72 Griffith J, Sansom C. *The Transporter Fact Book*. Academic Press, Harcourt Brace & Company: San Diego, 1998, pp 115–120.
- 73 Dreesen TD, Johnson DH, Henikoff S. The brown protein of *Drosophila melanogaster* is similar to the white protein and to components of active transport complexes. *Mol Cell Biol* 1988; **8**: 5206–5215.
- 74 Tearle RG, Belotte JM, McKeown M, Baker BS, Howells AJ. Cloning and characterization of the scarlet gene of *Drosophila melanogaster*. *Genetics* 1989; **122**: 595–606.
- 75 Rabindran SK, He H, Singh M, Ross DD, Doyle LA, Yang W, Bates SE, Greenberger LM. Fumitremorgin C reverses a novel multidrug resistance mechanism in mitoxantrone-selected cells. *Proc Amer Assoc Cancer Res* 1999; **40**: 315(abstr).
- 76 Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Ann Rev Pharmacol Toxicol* 1999; **39**: 361–398.
- 77 Ross DD, Yang W, Abruzzo LV, Dalton WS, Schneider E, Lage H, Dietel M, Greenberger L, Cole SPC, Doyle LA. Atypical multidrug resistance: breast cancer resistance protein messenger RNA expression in mitoxantrone-selected cell lines. *J Natl Cancer Inst* 1999; **91**: 429–433.
- 78 Ma J, Maliapaard M, Nooter K, Loos WJ, Kolker HJ, Verweij J, Stoter G, Schellens JH. Reduced cellular accumulation of topotecan: a novel mechanism of resistance in a human ovarian cancer cell line. *Br J Cancer* 1998; **77**: 1645–1652.
- 79 Maliapaard M, VanGastelen MA, DeJong LA, Pluim D, van Waardenburg RCAM, Ruevekamp-Helmers MC, Floot BGJ, Schellens JHM. Overexpression of the BCRP/MXR/ABCP gene in a topotecan-selected ovarian tumor cell line. *Cancer Res* 1999; **59**: 4559–4563.
- 80 Ross DD, Karp J, Yang W, Gao Y, Abruzzo LV, Doyle LA. Expression of breast cancer resistance protein (BCRP) in blast cells from patients with acute myeloid leukemia (AML). *Blood* 1998; **92**: 386a.