ABCB1 Modulation Does Not Circumvent Drug Extrusion from Primitive Leukemic Progenitor Cells and May Preferentially Target Residual Normal Cells in Acute Myelogenous Leukemia


Abstract

**Purpose:** Acute myelogenous leukemia (AML) is a disease originating from normal hematopoietic CD34⁺CD38⁻ progenitor cells. Modulation of the multidrug ATP-binding cassette transporter ABCB1 has not resulted in improved outcome in AML, raising the question whether leukemic CD34⁺CD38⁻ cells are targeted by this strategy.

**Experimental Design:** ABCB1-mediated transport in leukemic CD34⁺CD38⁻ cells compared with their normal counterparts was assessed by quantitating the effect of specific ABCB1 modulators (verapamil and PSC-833) on mitoxantrone retention [defined as efflux index (EI), intracellular mitoxantrone fluorescence intensity in the presence/absence of inhibitor].

**Results:** ABCB1 was the major drug transporter in CD34⁺CD38⁻ cells in normal bone marrow (n = 16), as shown by the abrogation of mitoxantrone extrusion by ABCB1 modulators (EI, 1.99 ± 0.08). Surprisingly, ABCB1-mediated drug extrusion was invariably reduced in CD34⁺CD38⁻ cells in AML (n = 15; EI, 1.21 ± 0.05; P < 0.001), which resulted in increased intracellular mitoxantrone retention in these cells (mitoxantrone fluorescence intensity, 4.54 ± 0.46 versus 3.08 ± 0.23; P = 0.004). Active drug extrusion from these cells occurred in the presence of ABCB1 modulators in the majority of samples, pointing in the direction of redundant drug extrusion mechanisms. Residual normal CD34⁺CD38⁻ cells could be identified by their conserved ABCB1-mediated extrusion capacity.

**Conclusion:** ABCB1-mediated drug extrusion is reduced in leukemic CD34⁺CD38⁻ progenitor cells compared with their residual normal counterparts. Redundant drug transport mechanisms confer mitoxantrone transport from leukemic progenitors. These data argue that ABCB1 modulation is not an effective strategy to circumvent drug extrusion from primitive leukemic progenitor cells and may preferentially target residual normal progenitors in AML.

Acute myelogenous leukemia (AML) is considered a disease originating from CD34⁺CD38⁻ hematopoietic stem cells (1). This has been conclusively shown in studies using the nonobese diabetic-severe combined immunodeficient mouse model showing that cells with leukemic engraftment and self-renewal potential in AML are found in the CD34⁺CD38⁻ subpopulation (2). This CD34⁺CD38⁻ phenotype of leukemia-initiating cells was observed regardless of the lineage markers expressed by the leukemic blasts, percentage of cells expressing the CD34 surface antigen, or the French-American-British subtype. These studies stress that leukemia-initiating transformation and progression-associated genetic events occur at the level of these primitive CD34⁺CD38⁻ cells. Consequently, incomplete chemotherapeutic eradication of these cells may ultimately result in disease relapse. Therefore, elucidation of the mechanisms conferring resistance against chemotherapy to these cells is of crucial importance (3).

The ABCB1-encoded P-glycoprotein is a highly conserved membrane-bound ATP-binding cassette (ABC) transporter, which extrudes a wide variety of structurally unrelated chemotherapeutic compounds across the cell membrane (4), conferring the multidrug resistance phenotype in cancer cells. ABCB1 is highly expressed in normal hematopoietic CD34⁺CD38⁻ stem cells (5, 6).

The observation that it is frequently expressed in AML and that expression is associated with adverse treatment outcome (7) has led to the introduction of ABCB1 modulators to enhance chemotoxicity of various chemotherapeutic agents in clinical trials. Phase III clinical trials using PSC-833 (valspodar), the most potent and specific inhibitor of ABCB1 function today, however, failed to reach their intended end points of improved complete remission and survival and have been complicated by increased toxicity (8–12). In contrast, a clinical trial using cyclosporin A, which is a broad-spectrum ABC
transporter inhibitor, showed clinical benefit (13). The reasons for the lack of benefit of ABCB1 modulation in AML remain to be elucidated. In interpreting these negative results, it is important to know whether the leukemia-initiating CD34+CD38- hematopoietic subpopulation is targeted by ABCB1 modulation and whether additional transporters confer drug extrusion from these cells, which may explain the beneficial effect of broad-spectrum inhibition. The vast amount of studies on ABCB1 expression and function in AML has been done on general blast populations. The goal of the current study was to investigate ABCB1 expression and function in leukemia CD34+CD38- cells and the effect of ABCB1 modulators on drug retention in these cells compared with their normal counterparts.

Materials and Methods

Bone marrow samples. Bone marrow was obtained after informed consent from healthy bone marrow donors and patients with AML at diagnosis. Mononuclear cells were isolated by Ficoll (1.077 g/mL density centrifugation; Pharmacy Biotech, Uppsala, Sweden). Isolation, cryopreservation, and thawing procedures of cells have been described previously (14) and were identical for normal and leukemic bone marrow samples. CD34+CD38- hematopoietic cells. CD34+CD38- cells were defined flow cytometrically as described previously (15) and are shown in Fig. 1A. Flow cytometric assessment of ABCB1 expression. ABCB1 expression was analyzed using the ABCB1-specific antibody MRK16, recognizing an external epitope of the protein in three-color flow cytometric assays as described previously (7). ABCB1 protein expression was quantitated as the median fluorescence channel shift (MRK16/IgG2a isotype control) in CD34+CD38-, CD34+CD38+, and CD34- cell populations. Differences in fluorescence are assessed using the Kolmogorov-Smirnov statistic, denoted as D. This method accurately identifies small differences in fluorescence and is useful in detection of low-level ABCB1 expression (7). MRK16 staining intensity is categorized as follows: negative (D < 0.10), dim (0.10 < D < 0.15), moderate (0.15 < D < 0.25), and bright (D > 0.25).

Flow cytometric assessment of ABCB1-mediated mitoxantrone transport. Cells were stained with CD34-FITC and CD38-PE membrane markers, washed in HBSS 1% FCS, and preincubated with or without verapamil (20 µg/mL; Knoll AG, Ludwigshafen, Germany; ref. 16) or PSC-833 (2 µmol/L; Novartis Pharma AG, Basel, Switzerland; ref. 17) as inhibitors for ABCB1-mediated transport for 20 minutes in Iscove's modified Dulbecco's medium supplemented with 1% FCS. Verapamil at the concentration used in this study is regarded ABCB1 specific without inhibition of ABCG2 [breast cancer resistance protein (BCRP); ref. 18] and ABCG1 (multidrug resistance protein; ref. 19). Although inhibition of ABCG2 by verapamil has been described at higher concentrations (20). Mitoxantrone (Novantrone; 10 µmol/L; Lederle, Etten-Leur, the Netherlands) was added, and cells were incubated for 2 hours at 37°C, 5% CO2, with or without verapamil or PSC-833. Subsequently, cells were allowed an additional 1-hour efflux in drug-free medium with or without inhibitor. The combined uptake and efflux with or without inhibitor assay was chosen because it has higher sensitivity for ABC transporter function compared with assays using only uptake with or without inhibitor (15).

Cellular mitoxantrone fluorescence was measured at a single time point after the additional hour of efflux in drug-free medium on a flow cytometer (Coulter Elite, Beckman Coulter, Fullerton, CA) equipped with an argon laser. Fluorescence was assessed at an excitation wavelength of 635 nm through a 670-nm bandpass filter in a three-color protocol with CD34-FITC and CD38-PE. At least 200 CD34+CD38- cells were analyzed in each sample. ABCB1-mediated transport was depicted as the ratio of intracellular mitoxantrone fluorescence intensity (MFI) in the presence or absence of inhibitor [defined as efflux index (EI)]. To assess interexperimental reproducibility, ABCB1-mediated transport in CD34+CD38- cells was assessed in a normal bone marrow sample in completely independent experiments done on separate days (n = 7). ABCB1-mediated EI was 2.10 ± 0.09 (mean ± SE), indicating good reproducibility of the assay. This sample was subsequently included in other experiments as a control. Preliminary experiments showed similar ABCB1-mediated efflux in cryopreserved samples when compared with fresh cells in normal bone marrow and AML, in line with a previous report addressing this issue (21).
Single-cell image analysis for assessment of ABCB1-mediated transport of rhodamine 123 in CD34+CD38- cells. ABCB1-mediated transport of rhodamine 123 was assessed in CD34+CD38- cells sorted on adhesive biolayers as comprehensively described elsewhere (22). Briefly, 500 CD34+CD38- cells were sorted in 8 μL washing buffer solution containing 0.9% NaCl supplemented with sodium diphasate (6.6%, v/v), albumin (1%, v/v), and glucose (1%, v/v) applied on a glass coverslip coated with mussel adhesive protein (10 μL/cm²). Cells were allowed to adhere for 10 minutes in a perfusion chamber constructed to enable cell environment manipulation (incubation and washing steps) within the microscope environment. The microscope was placed in an incubator to allow all procedures to run at 37°C, which is essential for physiologic efflux studies.

After immobilization, cells were incubated with rhodamine 123 dissolved Inscoe’s modified Dulbecco’s medium containing 0.5% (v/v) heat-inactivated FCS (Hyclone, Logan, UT) with or without efflux modulator during 30 minutes. Cells were washed with washing buffer at 37°C, again in the presence or absence of efflux modulators, and allowed to efflux the dye for an additional 15 minutes. Fluorescence images were acquired after 15 minutes of efflux. Cells were excited with a mercury arc lamp using bandpass filter of 440 to 490 nm for rhodamine 123 or bandpass filter of 510 to 560 nm for propidium iodide. Emission was measured with bandpass filter of 515 to 565 nm and longpass filter of 610 nm. Cell viability typically exceeded 90% of cells. Dead cells were excluded from analysis.

Fluorescence intensity analysis of individual cells was done on TCellImage 4.6 software package (TNO, Delft, the Netherlands). At least 30 cells were evaluated per sample. Fluorescence intensity of the overall cell population is expressed as MFI and depicted in arbitrary fluorescence units. ABCB1-mediated efflux was quantitated as the ratio of mean fluorescence intensity in the presence or absence of verapamil and depicted as “El.”

Fluorescence in situ hybridization. CD34+CD38- cells were sorted and lysed in 15 μL KCI (75 μmol/L) on a glass coverslip. Cells were fixed in methanol/acetic acid and stored at 4°C until analysis. Fluorescence in situ hybridization was done using the LSI AML1/ETO dual color, dual-fusion (8:21) probe, or LSI EGR1 (5q31) dual-color probe (Vysis, Downers Grove, IL) according to the manufacturer’s instructions. Cells (100) were analyzed microscopically for (8:21) or chromosome 5 (5q31) cytogenetic abnormalities as appropriate.

Adapted real-time quantitative reverse transcription-PCR for assessment of CBFB-MYH11 gene expression in CD34+CD38- cells. A linear real-time quantitative reverse transcription-PCR approach has been developed for quantitation of gene expression in low-frequency hematopoietic stem cells and was described comprehensively elsewhere (6). Primer and probe sequences were as follows: glyceraldehyde-3-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase control reagents (Applied Biosystems, Foster City, CA; part 402869); CBFB-MYH11 (type D) fusion gene, 5'-CATTCAGGAAACCAGCCCTTTGA-3' (forward), 5'-CCTCGTTAAGCATCCCTGTGA-3' (reverse), and TET-ATAAGACAGGTCATCG (probe).

Normalized gene expression to the internal standard glyceraldehyde-3-phosphate dehydrogenase is given by the following equation: To/Ro(Xn) = K (1 + E)(CT- - CTn), where To is the initial number of target gene copies, Ro is the initial number of standard gene copies, E is the efficiency of amplification, CTn is the threshold cycle of target gene, CT is the threshold cycle of standard gene, and K is constant.

Statistical analysis. Differences in ABCB1 protein expression (D of MRK16/isotype control) and function (E) between different normal and leukemic cell populations were calculated using the Student’s t test with a level of significance of P < 0.05.

Results

ABCB1 is the major determinant of mitoxantrone extrusion in CD34+CD38- cells in normal bone marrow. CD34+CD38- hematopoietic cells were flow cytometrically defined as shown in Fig. 1A. ABCB1 protein expression was assessed in hematopoietic cell populations using the monoclonal antibody MRK16. ABCB1 was differentially expressed in CD34+CD38- cells compared with more differentiated cell populations (Fig. 2). A decrease in ABCB1 protein expression in CD34+CD38- and CD34- cells was observed in all samples examined.

CD34+CD38- cells in normal bone marrow were mitoxantrone “dull” compared with CD34+CD38+ cells (MFI, 3.08 ± 0.23 versus 7.29 ± 0.50; n = 16; P < 0.001). Modulation of ABCB1-mediated transport by verapamil significantly increased mitoxantrone fluorescence in CD34+CD38- cells (Fig. 1B). In line with expression profiles, ABCB1-mediated efflux was invariably higher in CD34+CD38- cells (mean E1, 1.99 ± 0.08; range, 1.64-2.92) compared with more differentiated CD34+CD38+ cells (1.45 ± 0.06; Fig. 3A). Serial measurements of mitoxantrone fluorescence in CD34+CD38- cells both after 2-hour exposure with mitoxantrone and after an additional hour of efflux in drug-free medium done in a subpanel of samples (n = 7) showed complete abrogation of mitoxantrone extrusion by ABCB1 modulation (Fig. 4A).

Verapamil is regarded ABCB1 specific, although inhibition of ABCG2 (BCRP) has been observed at concentrations considerably higher than used in our experiments. To definitely confirm that the drug efflux from normal CD34+CD38- cells was indeed ABCB1 mediated, additional experiments were done.

First, similar results were found when the ABCB1-specific inhibitor PSC-833 was used in a subgroup of samples (n = 4; mean E1 ± SE, 2.03 ± 0.12 and 1.38 ± 0.09 for CD34+CD38- and CD34+CD38+ cells, respectively; Fig. 1B), showing that the observed efflux is ABCB1 rather than BCRP mediated because PSC833 is not an inhibitor of BCRP. This was further confirmed using rhodamine 123 as a substrate, which is not a substrate for BCRP (23), in an independent assay in single CD34+CD38- cells sorted on an adhesive biolayer (Fig. 1C). Rhodamine 123 retention was significantly lower in CD34+CD38- cells compared with CD34+CD38+ cells in normal bone marrow (P < 0.001), similar to the mitoxantrone dull phenotype of CD34+CD38- cells. Blocking of ABCB1-mediated transport by verapamil increased rhodamine 123 fluorescence significantly.

Figure 2. ABCB1 is differentially expressed in CD34+CD38- cells in both normal bone marrow and AML. ABCB1 expression was assessed flow cytometrically using the MRK16 monoclonal antibody and valued using the Kolmogorov-Smirnov statistic, denoted as D, as described in the Materials and Methods. ABCB1 was preferentially expressed in CD34+CD38- cells compared with more differentiated CD34+CD38+ progenitors in all normal (n = 10; black dots) and leukemic (n = 10; white dots) samples examined. MRK16 staining intensity is categorized as follows: negative (D < 0.10), dim (0.10 < D < 0.15), moderate (0.15 < D < 0.25), and bright (D > 0.25). No difference existed in levels of expression in CD34+CD38- cells between normal bone marrow and AML (median D, 0.13 ± 0.03 and 0.12 ± 0.04, respectively).
Reduced ABCB1 Function in Leukemic Progenitor Cells

Figure 3. Reduced ABCB1-mediated drug transport in CD34+CD38- cells is a biological commonality in AML. ABCB1-mediated transport in hematopoietic CD34+CD38- cells from normal bone marrow and AML samples depicted as an EI of substrate fluorescence in the presence or absence of verapamil. Black dot, each sample. Average ABCB1 efflux index ± SEM is indicated. A, ABCB1-mediated mitoxantrone transport as determined by flow cytometry. B, ABCB1-mediated rhodamine 123 transport as determined by single-cell image analysis.

ABC1-mediated efflux was found in all examined (n = 5) CD34+CD38- samples (mean El, 3.74 ± 1.81; Fig. 3B). Together, these results show that ABC1-mediated transport is the major determinant of the mitoxantrone dull phenotype of CD34+CD38- cells in human normal bone marrow and that its modulation completely abrogates mitoxantrone extrusion from these cells.

Abcb1-mediated transport is reduced in CD34+CD38- hematopoietic cells in AML. Conserved Abcb1-mediated drug extrusion from CD34+CD38- cells after malignant transformation would be an important obstacle for chemotherapeutic eradication of these cells in AML. We therefore investigated Abcb1 expression and function of this cell population in AML compared with their counterparts in normal bone marrow.

Investigations were done on a panel of bone marrow samples of 20 untreated AML patients (Table 1). The panel consisted predominantly of CD34+ leukemias, with CD34+ cells median percentage of 31% (range, 1-91). CD34+CD38- cells in AML were immunophenotypically defined as indicated in Fig. 1A. Fluorescence in situ hybridization analyses in AML patients who carried a cytogenetic abnormality and from whom sufficient cells were available for analysis showed a predominantly leukemic character of CD34+CD38- cells in these samples (n = 5; Table 2) as described previously (24).

Abcb1 expression in CD34+CD38- cells in AML as assessed by the MRK16 monoclonal antibody was comparable with expression of their counterparts in normal bone marrow (Fig. 2). Similar to the expression in normal bone marrow, decreased protein expression in CD34+CD38- and CD34- subsets was observed in all samples. Surprisingly, Abcb1-mediated mitoxantrone transport was invariably reduced in CD34+CD38- cells in AML compared with their counterparts in normal bone marrow as shown by a decreased effect of Abcb1 modulation by verapamil on intracellular mitoxantrone fluorescence (mean El, 1.21 ± 0.05; range, 0.93-1.51; P < 0.0001; Figs. 1B and 3A). Similar results were found when PSC-833 was used as an inhibitor (n = 4; mean El in CD34+CD38- cells, 1.23 ± 0.16; Fig. 1B). Reduced Abcb1 function was not restricted to CD34+CD38- cells in AML; reduced Abcb1 function compared with normal counterparts was also found for CD34+CD38- cells (mean El, 1.17 ± 0.03; range, 1.05-1.31; P < 0.001). Reduced Abcb1-mediated transport in CD34+CD38- cells in AML was confirmed using rhodamine 123 as a substrate in single cells (Figs. 1C and 3B). CD34+CD38- cells in AML (n = 8) were rhodamine 123 dull compared with the more differentiated CD34+CD38- cells, but Abcb1 modulation by verapamil did not increase intracellular retention (mean El, 0.99 ± 0.07; Fig. 3B). Of interest, analysis of mitoxantrone efflux mediated by the ABC transporters Abcg2 (BCRP), done in a series of normal and leukemic bone marrow samples, including those described in this article, using the Abcg2-specific fumitremorgin C analogue KO143 as inhibitor, showed no difference between normal and leukemic CD34+CD38- cells (15), further showing that the observed decrease in mitoxantrone efflux is indeed due to deficient Abcb1 rather than Abcg2 activity.

Serial measurements both after 2 hours of drug exposure and after an additional hour in drug-free medium in a subpanel of AML samples (n = 10; Fig. 4B) showed significant extrusion of mitoxantrone in the presence of verapamil in the majority of patients, pointing in the direction of redundant drug extrusion mechanisms.

These redundant drug transport mechanisms, however, did not compensate for the reduced Abcb1-mediated transport from leukemic CD34+CD38- cells as suggested by the significantly higher mitoxantrone retention in leukemic CD34+CD38- cells compared with their counterparts in normal bone marrow after drug exposure and subsequent efflux in drug-free medium (MFI, 5.45 ± 0.46 versus 3.08 ± 0.23; P = 0.004). These findings strongly suggest that the decreased efficacy of Abcb1 modulation on mitoxantrone retention of leukemic CD34+CD38- cells is indeed due to reduced Abcb1 activity rather than increased activity of other transport mechanisms, which would have resulted in similar or lower retention of mitoxantrone compared with their normal CD34+CD38- counterparts.

Translated to the clinical context, these data indicate that modulation of Abcb1 has limited effect on mitoxantrone retention in leukemic CD34+CD38- cells due to reduced activity of Abcb1 in leukemic CD34+CD38- cells. Drug efflux from these cells occurs in the presence of Abcb1 modulation due to the activity of promiscuous transport mechanisms.
ABC1-mediated drug transport identifies a subpopulation of residual normal CD34+CD38+ hematopoietic stem cells in a patient with CBFB-MYH11-positive AML. The finding that impairment of ABC1 function is biological commonality in CD34+CD38+ cells in AML compared with normal bone marrow implies that, in AML, residual normal CD34+CD38+ hematopoietic cells may be identified by their conserved ABC1-mediated transport capacity.

Indeed, during additional monitoring, one patient was identified with a clearly discernable CD34+CD38+ mitoxantrone null population caused by ABC1-mediated drug efflux within the range observed in normal bone marrow (Fig. 5). Because the intracellular presence of mitoxantrone precluded the use of fluorescence in situ hybridization, we used real-time quantitative reverse transcription-PCR to quantify the number of CBFB-MYH11 transcripts in these CD34+CD38+ subpopulations. The number of transcripts in the CD34+CD38+ ABC1 efflux ± cell population was similar to that in the positive control (blast population from CBFB-MYH11, type D−positive patient; Xn, 4.3 × 10⁻³ versus 3.5 × 10⁻³ copies CBFB-MYH11/copy glyceraldehyde-3-phosphate dehydrogenase, respectively), whereas no transcripts were detected in the CD34+CD38− ABC1 efflux ++ subpopulation. This sample provides proof of principle that conserved ABC1-mediated drug efflux may indeed identify a subpopulation of CD34+CD38− cells that harbor residual normal cells at least in a subgroup of AML patients. This implies that modulation of ABC1 preferentially targets these residual normal cells as shown in Fig. 5.

Discussion

In this report, we show that ABC1-mediated drug transport is reduced in leukemic CD34+CD38+ progenitor cells compared with their normal counterparts in AML.

This finding was initially unanticipated because AML is generally considered to be an ABC1-overexpressing malignancy with conserved physiologic function (25). This assumption, however, is based on the observation that ABC1 is preferentially expressed in CD34+ blasts in AML (26) similar to preferential expression of ABC1 in these cells in normal bone marrow (27), thus reflecting similar patterns of expression rather than direct comparison of ABC1 expression and function of normal and malignant cell subpopulations. Expression data in our study confirm the preferential expression of ABC1 in CD34+ cells in AML (26) at levels comparable with those observed in normal bone marrow. ABC1 transport capacity, however, was markedly impaired in both CD34+CD38− and CD34+CD38+ cells in AML.

The occurrence of ABC1− protein− efflux− leukemic blasts has been observed in a minority of AML cases (28) earlier, pointing in the direction of the presence of nonfunctional forms of the ABC1 protein, but the current report, aimed at directly comparing ABC1 function between normal and leukemic primitive progenitor cells, shows that impaired ABC1 function is a biological commonality rather than an occasional finding in AML.

The molecular determinants behind impaired ABC1-mediated transport in hematopoietic cells in AML remain to be elucidated and may comprise both downstream event of oncoproteins central in leukemogenesis, such as AML1-ETO (29) and TEL-AML1 (30), and factors governed by the cellular environment, such as disruption of cellular ATP metabolism, membrane integrity, or cell cycle regulation (31).

Table 1. Patient characteristics

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<td>Sex</td>
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<td>% CD34</td>
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Regardless of the underlying mechanism, the finding that ABCB1-mediated transport is impaired in CD34⁺CD38⁻/C₀ cells in AML has important clinical implications. The recognition of AML as a disease originating from these cells dictates a paradigm shift in the treatment toward the eradication of this crucial stem cell population in AML.

Our results indicate that ABCB1 modulation does not circumvent drug extrusion from these cells and that additional, redundant drug extrusion mechanisms determine the relatively dull mitoxantrone appearance of these cells. We have recently documented ABCG2/BCRP as one of these additional drug transporters in leukemic CD34⁺CD38⁻ cells (15). Additionally, using low-density real-time PCR arrays, we recently showed differential expression of all 13 ABC transporters currently associated with drug extrusion in CD34⁺CD38⁻ cells in AML (32). These studies, however, did not show higher expression or function of these transporters in leukemic CD34⁺CD38⁻ cells compared with their normal counterparts. This may explain the observation in this study that intracellular retention of mitoxantrone is increased in leukemic CD34⁺CD38⁻ cells compared with their normal counterparts, suggesting that redundant mechanisms confer drug extrusion from leukemic

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<th>Mitoxantrone [1.99 (1.64-2.94)*]</th>
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Abbreviation: NA, material not available for analysis.

*Values normal bone marrow: mean (range).

Figure 5. ABCB1-mediated mitoxantrone transport identifies a subpopulation that harbors residual normal CD34⁺CD38⁻ cells in a patient with CBFB-MYH11 AML. A patient was identified in which mitoxantrone discriminated a population of CD34⁺CD38⁻ mitoxantrone dull cells. The dull phenotype was determined by strong ABCB1-mediated efflux (CD34⁺CD38⁻ ABCB1 efflux ++) within the range observed in CD34⁺CD38⁻ cells observed in normal bone marrow (EI 2.50). Real-time PCR confirmed the residual normal character of these cells.
cells but that this redundant action does not completely compensate for the reduced ABCB1 function in these cells.

The combined data suggest that the prognostic value of ABCB1, one of the main rationales for ABCB1 targeting in AML, may be pleiotropic, identifying leukemias with a primitive CD34+ (CD38-) character and innate drug resistance due to differential expression of many, promiscuous ABC transporters and possibly other mechanisms protecting primitive progenitor cells. Modulation of additional transporters is therefore likely to be required to abrogate drug extrusion and to enhance drug-induced eradication of leukemic stem cells in AML. These laboratory findings provide an alternative explanation for the poor results of ABCB1 modulation by the highly potent ABCB1 inhibitor PSC-833 and other inhibitors on long-term disease outcome in clinical trials (8–10). The report of improved clinical outcome in a trial using cyclosporin as an ABCB1 inhibitor in AML (13) may reflect these findings because cyclosporin is a more promiscuous ABC transporter inhibitor with effects on ABCB1 and other ABC transporters (33).

Additionally, the current study shows that residual normal CD34+CD38- cells are likely to harbor within the ABCB1 efflux ++ subpopulation. This seems congruent with the report (34) that the capacity to efflux Hoechst 33342 (side population) identifies a subpopulation of CD34+CD38- residual normal cells in AML, whereas CD34+CD38- Hoechst bright cells were leukemic. Although these authors could not relate Hoechst 33342 efflux with ABCB1 protein expression in the overall side population of cells, this was not investigated in the CD34+CD38- subset. The current report shows that differences in ABCB1-mediated efflux discriminate normal from leukemic CD34+CD38- cells. This finding implies that ABCB1 modulation may preferentially target residual normal hematopoietic cells, whereas drug extrusion from leukemic CD34+CD38- cells occurs through the activity of redundant mechanisms. Together, these data argue against the use of ABCB1 modulation as a strategy to eradicate leukemic stem cells in AML but prompt further research to elucidate the mechanisms involved in the extrusion of drugs from leukemic CD34+CD38- cells to provide novel targets for the eradication of this crucial cell population in AML.

Acknowledgments

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