

## Multidrug Resistance and Stem Cells in Acute Myeloid Leukemia

Branimir I. Sikic

The article by Raaijmakers et al. (1) in this issue reports a provocative study with important implications for therapeutic strategies in acute myelogenous leukemia (AML). Expression of the *MDR1* (*ABCB1*) gene, encoding the multidrug transporter P-glycoprotein (P-gp), is a major negative prognostic factor in adult patients with AMLs (2–4). Attempts to improve clinical outcomes in AML by modulation of P-gp have yielded variable results and limited benefit (3, 5–8).

Raaijmakers et al. studied the efflux of mitoxantrone and its inhibition by verapamil and PSC-833 in leukemic and normal bone marrow stem cells (defined as CD34<sup>+</sup> and CD38<sup>-</sup>). Active efflux of mitoxantrone was shown in both leukemic and normal stem cells from all 15 patients, but efflux was not inhibitable in the leukemic stem cells. The investigators hypothesize that the differences between the leukemic and normal stem cells are caused by additional transport mechanisms in the leukemic cells and that modulation of P-gp in leukemic patients may therefore preferentially target normal bone marrow stem cells.

These results highlight a number of questions in leukemia therapy.

- How do leukemic stem cells differ among different subtypes and individuals with AML?
- What are the underlying mechanisms controlling *ABCB1*/P-gp expression in this diverse disease?
- What is the significance of P-gp expression in the bulk of the leukemic cell population versus the stem cells?
- What are the relevant, redundant mechanisms of resistance in AML cells expressing *ABCB1*?
- Should diagnostic, prognostic, and predictive assays in AML specimens analyze the stem cell component specifically?
- What are the best assays to assess *ABCB1* and other resistance mechanisms?
- Have clinical trials of multidrug resistance modulation selectively targeted normal stem cells?
- Can effective therapies specific for leukemic stem cell therapies be developed?

The scope of this editorial does not allow for a detailed discussion of these issues. The implications of the study should be placed in the context of its methodologic limitations,

however. First, the number of patients in the study, 15, is quite low, and the median age of 50 years is far below the median age for adults with AML. To what extent are these findings applicable to the large majority of AML patients (i.e., those over age 60 years), to secondary AML, and to the blastic transformation of chronic myelogenous leukemia? Second, mitoxantrone is a relevant substrate for transport studies but may behave differently both in preclinical and clinical trials with multidrug resistance modulators than with daunorubicin (9). It would have been of interest to replicate the efflux studies with daunorubicin as a substrate and with the relatively P-gp specific fluorescent substrate dye DiOC<sub>2</sub>. In addition, studies of the cytotoxic efficacy *in vitro* of mitoxantrone and daunorubicin in the presence or absence of P-gp inhibitors would have added important information. Third, no information is provided about the expression of other transporters and potential mechanisms of resistance in these specimens. The investigators hypothesize that *ABCG2* (*BCRP*) may be differentially expressed in the leukemic versus normal marrow stem cells, and it is disappointing that they did not provide definitive evidence for their hypothesis. Fourth, no information is reported on the rest of the leukemic cell population from these patients, usually CD33 positive, with regard to P-gp expression and function. Nonetheless, detailed studies in leukemic stem cell populations are technically demanding, and the investigators are to be commended for their efforts.

The identity and nature of leukemic stem cells is an area of intense research interest and activity (10–15). Clearly, not all AML stem cells are created equal. The pathogenetic heterogeneity of this disease is highlighted by its diverse cytogenetic and molecular oncogenic characteristics, as well as the varied differentiation status of the bulk leukemic populations in subtypes of AML (14). A thorough understanding of leukemia biology and its implications for clinical behavior, however, should include consideration of other leukemic subpopulations with more limited reproductive capacity. Even the limited proliferation capacity of the non-stem cell leukemic population may have dire clinical consequences, and elimination of those cells by current cytotoxic therapies results in major benefits to patients.

Can one achieve complete remission and cure of AML without specific stem cell-directed therapy? The answer to both questions is clearly yes. Therapies specific for leukemic stem cells currently do not exist, with the possible exception of the high-dose alkylating agents and radiation used in stem cell transplantation regimens. Nonetheless, complete remissions are relatively common and cures are achievable without stem cell transplants. In childhood AML, P-gp is detectable in fewer than 15% of cases assessing the entire population of leukemic cells and only 2% express P-gp at levels considered positive in adult trials (5). Do the leukemic stem cells in children with AML also express the resistance mechanisms identifiable in

**Author's Affiliation:** Oncology Division, Department of Medicine, Stanford University School of Medicine, Stanford, California  
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**Requests for reprints:** Branimir I. Sikic, Oncology Division, CCSR 1105, Stanford Medical Center, Stanford, CA 94305-5151. Phone: 650-725-6427; Fax: 650-736-1454; E-mail: brandy@stanford.edu.

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adults, including P-gp? Even a therapy which targets the CD33 antigen, which is absent in stem cells, produces a complete remission rate of 26% in relapsed patients with some durable remissions (16).

P-gp expression and function are variably expressed in AML populations, increasing in frequency with patient age, prior chemotherapy, secondary leukemias, and blast transformation of chronic myelogenous leukemia (2–4). Almost certainly, the underlying mechanisms for the regulation of *ABCB1* expression vary in these different clinical settings.

There are many genetic and cellular determinants of both sensitivity and resistance to anticancer drugs. Information about these mechanisms in AML specimens at diagnosis and at relapse is limited (2–4, 17) but newer molecular analytic techniques such as quantitative reverse transcription-PCR and

microarrays should shed light on this important area. List has observed conversion from P-gp-positive to P-gp-negative status in the majority of AML specimens from patients who relapse after achieving complete remission with daunorubicin and a P-gp inhibitor (18).

The presence of other non-*ABCB1* mechanisms of resistance in AML may help explain the limitations of P-gp inhibition as a treatment strategy but does not eliminate its potential significance. Ultimately, the rational application of this strategy may require the development of reliable predictive assays for both P-gp and other determinants of therapeutic response, prospective selection of patients most likely to benefit, concurrent inhibition of other resistance mechanisms or the use of non-cross-resistant agents, and the optimal dosing and scheduling of the modulating agents.

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