ABCG2 Overexpression and Deoxyadenosine Analogue Activity in Acute Myeloid Leukemia

We have read with great interest the article by Jabbour et al[1] entitled "A Randomized Phase 2 Study of Idarubicin and Cytarabine With Clofarabine or Fludarabine in Patients With Newly Diagnosed Acute Myeloid Leukemia." The authors reported that both clofarabine and fludarabine were associated with high complete remission rates and a 2-year overall survival rate higher than 50% and that, in comparison with treatment with idarubicin and cytarabine alone, fludarabine was associated with superior outcomes for younger patients. Despite these encouraging results, which are in line with what has been achieved in other studies using these 2 purine nucleoside analogues[2,3] or cladribine,[4] disease relapse is still the major cause of poor long-term outcomes for acute myeloid leukemia (AML) patients. Although disease-free survival is not clearly reported in Jabbour et al's article, the median event-free survival with both clofarabine and fludarabine is approximately 12 months.

Among the mechanisms of resistance of AML blasts to anticancer therapy, the role of multidrug resistance proteins is well established. In particular, the overexpression of ATP-binding cassette subfamily G member 2 (ABCG2) is associated with higher rates of failure to achieve remission[5] and shorter disease-free survival.[6] We have previously reported that fludarabine-based induction therapy does not overcome the negative impact of high ABCG2 levels on disease-free survival.[7] To investigate the possible influence of ABCG2 on clofarabine activity, we tested clofarabine toxicity in 3 different cell lines: RPMI8266 (overexpressing ABCG2), A549 (heterozygous for the commonest amino acid variant in whites [Q141K] that alters protein function and modifies sensitivity to many anticancer drugs),[8] and A498 (homozygous for the same polymorphism). HL60 (no ABC protein overexpression) and its ABCB1-overexpressing subline HL60-DNR were used to assess the effect of clofarabine in the presence of classic P-glycoprotein–mediated multidrug resistance. In parallel, experiments in the same cellular settings were performed with fludarabine.

Fludarabine fully overcame the negative effect of ABCB1 overexpression (with inhibition dose 50 [ID_{50}] values of 30 and 24 ng/mL in HL60 and HL60-DNR, respectively). An almost equal effect was obtained with clofarabine (with ID_{50} values of 14 and 7.5 ng/mL in HL60 and HL60-DNR, respectively). Completely different was the impact of high levels of ABCG2 on the toxicity of the 2 drugs: in RPMI-8266, ID_{50} for fludarabine was 6000 ng/mL (ie, 200-fold higher than the value in HL60), and this supports our previous observation of a worse prognosis for ABCG2-overexpressing AML patients treated with fludarabine-based induction therapy. ID_{50} for clofarabine in ABCG2-overexpressing cells (650 ng/mL) was lower than the value for fludarabine but still almost 50 times higher than the value for the negative control. With respect to the ABCG2 polymorphism, no significant differences were observed for fludarabine, whereas in the presence of the Q141K variant, both in homozygosity and in heterozygosity, the negative impact on clofarabine was nearly completely overcome.

In line with data published by Nagai et al,[9] our findings confirm a possible role of ABCG2 overexpression as a cause of resistance to clofarabine, just as for fludarabine. ABCG2 seems to be responsible for an increased rate of AML relapse, maybe because of its expression in a blast cell population with a stemlike phenotype that could constitute a reservoir responsible for relapse.[10] Deoxyadenosine analogues are certainly effective drugs for AML when they are used both in induction chemotherapy and in the setting of relapsed or refractory disease,[11] but various mechanisms can be responsible for treatment failure. An evaluation of ABCG2 expression should be considered both at diagnosis and in the case of AML recurrence because ABCG2-negative patients could gain the maximal benefit from clofarabine and fludarabine.

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Reply to ABCG2 Overexpression and Deoxyadenosine Analogue Activity in Acute Myeloid Leukemia

We are grateful for the opportunity to respond to the letter by Tiribelli et al regarding the potential role of drug efflux pumps, particularly ATP-binding cassette subfamily G member 2 (ABCG2; also known as BCRP), as mechanisms of resistance in our study of clofarabine- or fludarabine-based chemotherapy for patients with acute myeloid leukemia (AML).[1] Several authors have previously investigated the role of these pumps in resistance to antileukemia therapy. These studies have varied in their estimation of the proportion of newly diagnosed AML patients with sufficiently high levels of ABCG2 expression to cause resistance (values have ranged from 7% to 55% according to the cutoff used).[2,3] Nevertheless, these efflux pumps have been correlated with worse outcomes in a number of studies, including studies with patients who received a fludarabine-based regimen.[4,5] In their letter, Tiribelli et al present in vitro cell line data suggesting the ABCG2 overexpression in AML blasts may mediate resistance to both clofarabine and fludarabine. Given the wealth of data showing that ABCG2 and other drug efflux pumps play an integral role in AML resistance to cytotoxic agents, we agree with them that such transporters certainly may have mediated resistance for these nucleoside analogue–based therapies in our study, at least in a subset of patients in whom ABCG2 was overexpressed.

Because of the number of ways in which leukemic cells are capable of developing resistance to chemotherapy, this raises an important question about how these resistance mechanisms can best be bypassed to improve the outcomes of our patients with AML and other leukemias. With the exception of younger patients with favorable-risk disease features, there is likely a ceiling of benefit that intensification of chemotherapy can achieve in AML.[6] Perhaps for these younger patients who tend to have chemosensitive disease, an evaluation of drug efflux pump expression and strategies for targeting these transporters may be beneficial. However, for the many patients with AML who do not fit into this group (eg, older patients and those with unfavorable cytogenetics or mutations), novel agents and combination strategies are needed.

Improvements in AML outcomes will require continued molecular classification of AML to identify targetable pathogenic mutations. For example, the addition of FMS-like tyrosine kinase 3 (FLT3) inhibitors to chemotherapy has been shown to improve survival for patients with *FLT3*–internal tandem duplication mutations.[7] Similarly, the promising results seen with isocitrate dehydrogenase (IDH) inhibitors in the relapsed/refractory setting[8] raise the question whether these agents should also be incorporated into frontline regimens for patients with *IDH* mutations. Monoclonal antibodies conjugated with toxins or bispecific antibody constructs (CD3 with CD33 or CD123) may also provide effective anti-AML activity.[9]