To the editor:

Prognostic significance of high-level FLT3 expression in MLL-rearranged infant acute lymphoblastic leukemia

Recently, we have demonstrated that in primary MLL-rearranged infant acute lymphoblastic leukemia (ALL) samples, high-level expression of wild-type FLT3 is associated with FLT3 phosphorylation (ie, constitutive activation) and sensitivity toward the small-molecule FLT3 inhibitor PKC412.1,2 Similarly, Brown et al reported that MLL-rearranged ALL cells with high-level FLT3 expression are selectively killed by the FLT3 inhibitor CEP-701.3

These findings suggest the existence of a threshold level of FLT3 expression above which activated FLT3 becomes apparent and “targetable.” Exploring this possibility, we have analyzed the prognosis of our recently published cohort of patients with MLL-rearranged infant ALL in relation to FLT3 expression. For 32 of 41 samples for which the level of FLT3 expression was determined using quantitative RT-PCR, clinical data were available. All of these samples tested negative for the presence of activating FLT3/tyrosine kinase domain (TKD) mutations or FLT3/internal tandem duplications (ITDs). Among these 32 patients with MLL-rearranged infant ALL, FLT3 expression relative to the housekeeping gene GAPDH ranged from 0.46% to 17.4%, with a median and mean expression level of 1.8% and 3.5%, respectively. Moreover, all patients were uniformly treated according the Interfant-99 protocol.

Using the mean FLT3 expression as the cut-off value to divide patients into 2 groups expressing low or high levels of FLT3, we found that high-level FLT3 expression is likely associated with a poor treatment outcome (Figure 1) within this subtype of ALL that is already characterized by a poor prognosis.4–6 The 1-year event-free survival (EFS) estimate is 71% for patients expressing low levels of FLT3, compared with 36% for patients displaying high-level FLT3 expression. These data are in concordance with data published by Ozeki et al,7 who showed that high-level expression of wild-type FLT3 is an unfavorable prognostic factor for overall survival in acute myeloid leukemia (AML).

We conclude that constitutively activated FLT3 as a result of increased expression may contribute to the dismal prognosis of MLL-rearranged infant ALL. However, this should be confirmed in a larger prospective study. Targeting FLT3 overexpression in MLL-rearranged infant ALL using the FLT3 inhibitors PKC412 and CEP-701 has been shown to be effective in vitro and in vivo in a mouse model.1–3 Based on these findings, The Childhood Oncology Group in the United States, and the Interfant Study Group worldwide are planning clinical studies including these FLT3 inhibitors. The finding we present here emphasizes the value of these clinical trials, as we show that the patients that are most likely to respond to FLT3 inhibitors are patients at very high risk of treatment failure.

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References

Cytoplasmic localization of phosphorylated STAT5 in human acute myeloid leukemia is inversely correlated with Flt3-ITD

The fms-related tyrosine kinase-3 internal tandem duplication (Flt3-ITD) confers growth-factor independence on acute myeloid leukemia (AML) cells and is associated with an unfavorable prognosis. Signal transducer and activator of transcription 5 (STAT5) is a downstream mediator of Flt3 signaling in AML; however, flow cytometry for phosphorylated STAT5 (pSTAT5) did not show specific association with Flt3-ITD. Since a recent study published in Blood by Harir et al. reported pSTAT5 in the cytoplasm of leukemic samples, we wanted to determine whether this localization to the cytoplasm might be influenced by Flt3-ITD status and whether nuclear pSTAT5 would be a more informative end point. Using a routine immunohistochemistry technique that we previously reported for detecting activated STAT5 in chronic myeloproliferative disease, we have now analyzed pSTAT5 staining in myeloid blasts in bone marrow biopsies from patients with AML.

STAT5 tyrosine phosphorylation was detected immunohistochemically with the anti-phosphoSTAT5a/b (Y694/99) mouse monoclonal antibody (AX1; Advantex Bioreagents) as described by others in breast cancer. In our study, we analyzed a total of 40 patients with AML for nuclear versus cytoplasmic staining of pSTAT5 using the AX1 antibody. Total pSTAT5 was seen in 15 of 40 patients, while the Flt3-ITD was detected in 8 (20%) patients. Interestingly, as shown in Figure 1, Flt3-ITD samples had predominant nuclear pSTAT5, as would be expected from increased transcriptional activation. However, the Flt3-ITD samples showed some nuclear staining, but had much more prominent cytoplasmic pSTAT5 staining with significant variation between groups (Kruskal-Wallis test, P = .03). A total of 2 patients showed predominant cytoplasmic staining and no nuclear staining. The distribution of cytoplasmic staining was significantly different between Flt3-ITD+ and Flt3-ITD− patients (Mann-Whitney test, P = .04).

The study by Harir et al. demonstrated in mouse and human samples that STAT5 may potentially have cytoplasmic functions that promote PI3-kinase activation of Akt. Our data confirm the cytoplasmic localization of pSTAT5 in a larger set of patient samples and further suggests that this localization is modulated by the presence of Flt3-ITD. This is an important finding since it suggests that competing biological mechanisms are responsible for the nuclear versus cytoplasmic distribution of pSTAT5 in AML. Cytoplasmic pSTAT5 may not be directed efficiently to the nucleus in the absence of essential interactions provided by the Flt3-ITD scaffold. Alternatively, the hyperphosphorylation of STAT5 mediated by Flt3-ITD may override other mechanisms that may sequester pSTAT5 in the cytoplasm. This finding could be analogous to the requirement for JAK2V617F to interact with type I cytokine receptors to promote STAT5 transcriptional activation and cytokine-independent growth. Finally, our data also demonstrate that detection of nuclear, but not total pSTAT5 could be the most meaningful test for rapidly identifying patients who may respond to Flt3 or other tyrosine kinase inhibitors.

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