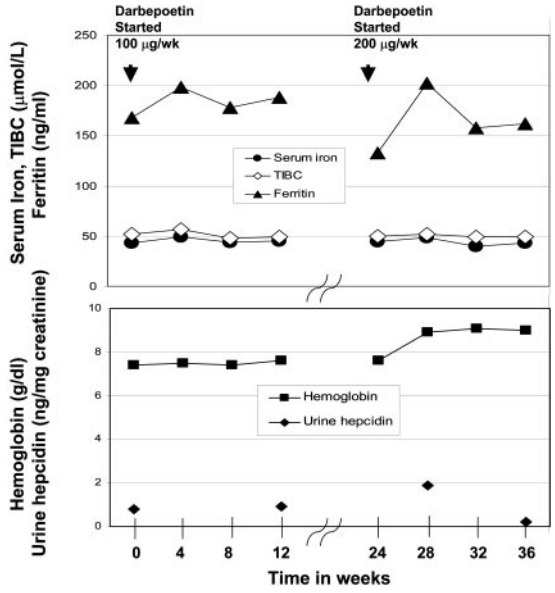

Figure 1. Response of hematologic parameters to darbepoetin. Blood and urine samples of the patient were obtained with informed consent. The ethics committee of the Palacky University Hospital approved the study. Darbepoetin was administered as a subcutaneous injection beginning at a dose of 100 μg (1.67 μg/kg) every week and continued for 3 months. There was a 3-month break in therapy, and darbepoetin was reinstituted at a dose of 200 μg weekly, where it remained throughout the course of the study. Hepcidin assay was performed as previously described[5] and urine hepcidin concentration was expressed as nanograms hepcidin per milligrams creatinine. Normal ranges for hematologic parameters are as follows: serum iron level, 14.5-26 μM/L; total iron-binding capacity (TIBC), 44.8-71.6 μM/L; ferritin level, 20-150 μg/L (20-150 ng/mL); hemoglobin level, 120-155 g/L (12-15.5 g/dL); and urine hepcidin level, 10-200 ng/mg creatinine.

Patients with anemia due to *DMT1* mutations are critically dependent on iron delivery to developing erythrocytes; increased transferrin saturation may be essential to deliver iron when DMT1 activity is diminished. Thus, even if medications that increase hepcidin levels become available for treatment of iron overload, they may not benefit DMT1 patients because increased plasma hepcidin level would limit release of iron into the bloodstream, decreasing serum iron level and transferrin saturation, further limiting available iron for hemoglobin synthesis. Removing excess iron is probably the only viable treatment for iron overload in these patients.

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To the editor:

More on prognostic significance of FLT3/ITD size in acute myeloid leukemia (AML)

We read with great interest the paper by Stirewalt et al[1] analyzing the prognostic significance of different Fms-like tyrosine kinase (FLT3) gene internal tandem duplication (ITD) size in acute myeloid leukemia (AML). There is a considerable variability in ITD size, and Stirewalt et al[1] suggest that with increasing size of ITD there is a more significant loss of autoinhibitory function of FLT3, translating into inferior prognosis. We would like to comment on this issue based on our results, which may suggest different conclusions.

We have analyzed 86 consecutive patients with AML, 37 men and 49 women, with a median age of 57 years (range, 18-85 years), by reverse transcriptase–polymerase chain reaction (RT-PCR) for FLT3/ITD mutation.[2] We have found 18 (20.9%) patients with FLT3/ITD mutations, with the highest frequency (3/8; 37.5%) in acute promyelocytic leukemia (APL). ITD was associated with higher white blood cell (WBC) count (67 vs 26 $\times$ 10^9/L, $P = .013$) and somewhat inferior survival (hazard ratio [HR] = 1.98; confidence interval [CI] 1.11-6.11; $P = .02$, when we exclude patients with APL). There was no significant difference in age, sex, cytogenetic risk, and other hematologic parameters between FLT3/ITD-mutated and unmutated patients.

Median ITD size was 70 bp (range, 30-100 bp). ITD size was negatively correlated with age ($R = -0.51$; $P < .05$) and cytogenetic risk ($R = -0.58$; $P < .05$) and was not correlated with WBC count, sex, or other hematologic parameters. We have divided our patients' FLT3/ITD mutations into long (≥ 70 bp; 11/18, 61.1%) and short ITD (< 70 bp; 7/18, 38.9%) subgroups. We found better survival in the long-ITD subgroup (HR 4.07, CI 2.28-56.32; $P = .002$)[3] (Figure 1), even without patients with APL (HR 3.18, CI 1.31-27.28; $P = .02$). In the multivariate Cox proportional hazard model, with ITD size as continuous variable, in overall series higher age, WBC count, and poor cytogenetic risk were

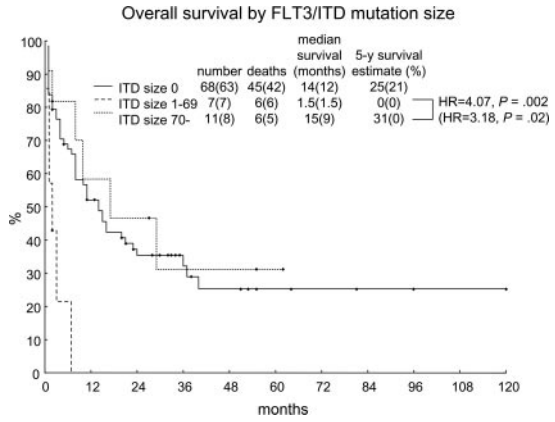

Figure 1. Overall survival by FLT3/ITD size for 86 patients with AML. Kaplan-Meier estimates are shown. Marks represent censored cases. Values in parentheses are results with patients with APL excluded.

associated with poor prognosis. However, in FLT3/ITD-mutated patients, only age and WBC count were associated with prognosis, but when we excluded patients with APL, ITD size also had independent prognostic significance ($P = .05$).

In our series of patients with AML, association with prognosis was opposite to that of Stirewalt et al.[1] It is unlikely that different ITD amplification methods using cDNA[2] versus DNA templates[1] or different cutoff values (40 vs 70 bp) are critical. When we used the same cutoff (40 bp), in the short-ITD subgroup there were only 5 patients but the survival was still significantly shorter ($P = .02$). In our group, ITD size was negatively associated with age and cytogenetic risk—which is not observed by Stirewalt et al,[1] although values for major parameters were quite similar. Also in our group with FLT3/ITD mutation there were 3 patients with APL, all with long ITDs, but the difference was still significant when we excluded patients with APL.

Quantitatively equal activating potential of both FLT3 mutation classes (ITD and tyrosine kinase domain) and activation of additional signaling pathways, including STAT5 pathway, by FLT3/ITD may suggest qualitative differences as the basis for different biologic effects of FLT3 mutations.[4] Thus the observed different biologic effects of different FLT3/ITDs may relate to a different activation of these additional signaling pathways. FLT3 modeling and functional studies including signaling as well as further clinical studies are warranted to resolve these puzzling and conflicting results.

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Response:

Does FLT3/ITD size matter?

In this issue of *Blood*, Kusec et al describe their results examining the potential prognostic significance of FMS-like tyrosine kinase 3/internal tandem duplication (FLT3/ITD) size in 86 patients with acute myeloid leukemia (AML). Contrary to our recent publication,[1] these investigators found that patients with AML with smaller FLT3/ITDs had a worse prognosis. The exact reason for the disparity of the results between the 2 studies is uncertain. These investigators used a different screening technique, which may not be as sensitive as the method in our analyses (polymerase chain reaction/single-strand conformation polymorphism [PCR/SSCP]).[2] Using a less sensitive screening method will reduce the detection of the smallest ITDs, and if only the smallest ITDs are associated with favorable outcomes, the potential benefits of these small ITDs would be lost in their analyses. In addition, it was not clear from the analyses what type of therapy these patients received or if all patients received the same treatment. Differences in therapy between the 2 studies or within their study could have significant impact on their results and be responsible for some of the differences. For example, if patients receiving aggressive therapies such as transplantation were included in the analyses, these therapies must be examined as potential confounding variables. Although the findings are interesting and potentially informative, we believe that larger studies examining more patients remain necessary in order to either confirm or disprove that patients with AML with large FLT3/ITDs have an overall worse prognosis than similar patients with small FLT3/ITDs.

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