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LETTERS TO THE EDITOR

Retention of $CD34^+$ CML stem/progenitor cells during imatinib treatment and rapid decline after treatment with second-generation BCR–ABL inhibitors

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ABL-tyrosine kinase inhibitor (TKI), imatinib (IM) is suggested to be effective for proliferating leukemic cells, not quiescent chronic myeloid leukemia (CML) stem cells.[1–3] Recent clinical trials suggest that CML treatment can be improved with more potent BCR–ABL inhibition during the second generation of ABL-tyrosine kinase inhibitors (2nd-TKIs) such as dasatinib and nilotinib.[4] To comparatively examine the effects of IM and 2nd-TKIs on the BCR–ABL-positive hematopoietic stem cells (HSC) and progenitors, we investigated 47 CML-chronic phase cases using methods previously reported with *FACSAria* and quantitative real-time-PCR of *BCR–ABL* among each sorted population: total mononuclear cells, HSC/Thy-1^+, HSC/Thy-1^-, common myeloid progenitors, granulocyte macrophage progenitors and megakaryocyte erythroid progenitors (detailed in Supplementary Materials and Methods).[5,6] By using this method in the HSC population, more than 30% of cells are supposed to have stem cell potential, probably as long-term culture-initiating cells.[7]

In optimal responders to IM therapy, *BCR–ABL* transcripts in the HSC population tended to be more retentive than other populations, while a gradual reduction was observed during the first 12 months in all populations (Figure 1a). After 2- or 3-year of treatment, *BCR–ABL* transcripts in the total mononuclear cells continued to decrease, but were more retentive in the HSC and progenitor populations showing a greater discrepancy (about 2 log difference) (Figure 1b). After longer treatment with IM, even when *BCR–ABL* transcripts were undetectable in total mononuclear cells, residual transcripts were observed in the HSC population with around 2-log discrepancy of the averages (Supplementary Table 1). There was no significant difference between Thy-1^+ and Thy-1^- in the HSC population, and among the progenitor population common myeloid progenitors were most retentive.

We also prospectively investigated *BCR–ABL* transcripts in each population of 27 IM-resistant or -intolerant cases during treatment with the 2nd-TKIs, dasatinib or nilotinib. In optimal responders to nilotinib therapy for IM-intolerance, *BCR–ABL* transcripts in total mononuclear cells after 6 to 12 months decreased to the equivalent level after 2-, or 3-year IM treatment (Figure 2a). In this situation with IM therapy, retention of *BCR–ABL* transcripts in the $CD34^+$ populations was observed. However, there was no significant difference in minimal residual disease among each population. Also in optimal responders to dasatinib therapy for IM-intolerance, we observed a rapid decline of *BCR–ABL* transcripts even in the $CD34^+38^-$ population (Figure 2b). Although we continued to examine with longer-treated patients, there was a methodological limitation in subtle quantitative evaluation around the complete molecular response during 2nd-TKI treatments (data not shown).

For deeper interpretation of our results, we collaborated with mathematicians. Based on the pivotal IRIS (International Randomized Study of Interferon and STI571) trial involving 1106 patients with CML-chronic phase, they made a standardized formula about the manner of decline of *BCR–ABL* transcripts, consisting of bi-exponential phases: α-slope with initial rapid decline and β-slope corresponding to kinetics of more residual cells.[8] Our results were similar, with biphasic decreasing in the $CD34^+38^-$ population. Combined with the results, we developed a hypothesis that the β-slope corresponds mainly to the partial (quiescent, IM-insensitive stem cells) $CD34^+38^-$ population, not the entire one. Our results showed treatment with 2nd-TKI induced at least steeper α-slope in comparison with IM treatment. To evaluate the β-slope properly,

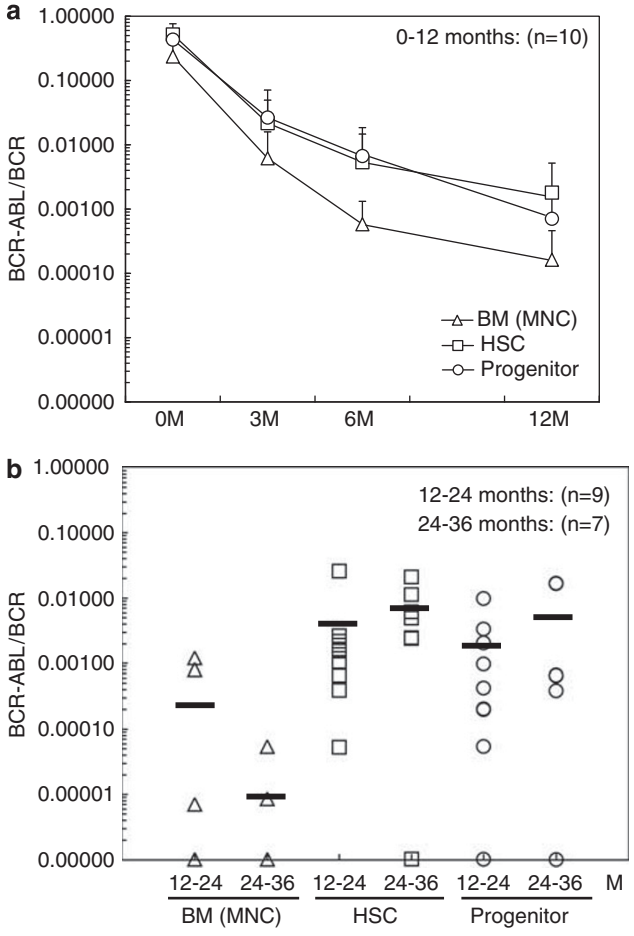

Figure 1. Retention of *BCR–ABL* transcripts in primitive populations during optimal response to imatinib. (**a**) Imatinib-treated cohort ($n = 10$) for the first 12 months. (**b**) Imatinib-treated cohort for 2 years ($n = 9$) and 3 years ($n = 7$).

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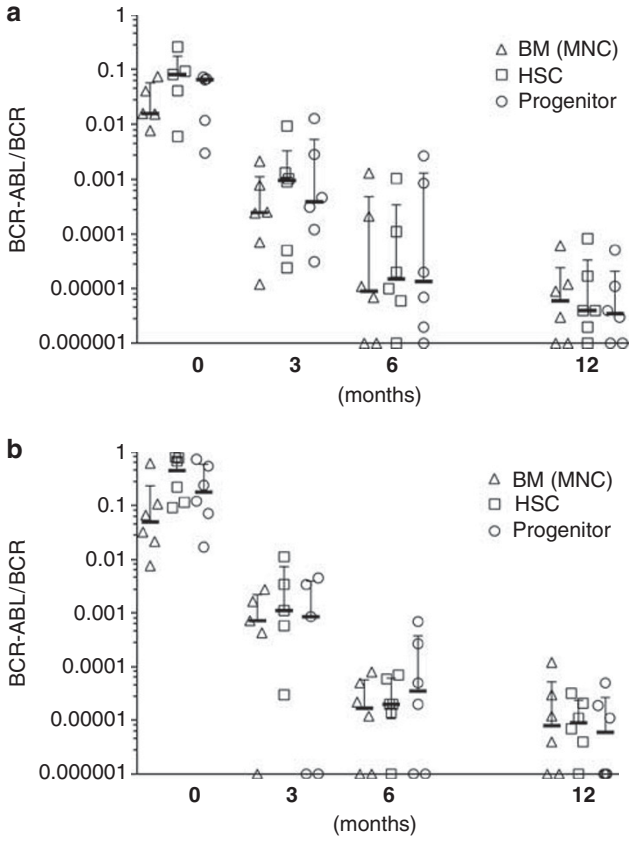

Figure 2. *BCR–ABL* transcripts during optimal response to 2nd-TKI therapy for imatinib-intolerant CML-chronic phase patients. (**a**) Nilotinib-treated cohort ($n = 6$). (**b**) Dasatinib-treated cohort ($n = 6$).

examination of 2nd-TKIs as 1st-line setting and development of a more accurate qPCR method are also warranted.

Our results implied that treatment with 2nd-TKI was more effective even on populations with more quiescent property. Transient potent BCR–ABL inhibition is sufficient to commit CML cells irreversibly to apoptosis.[9–11] Such pro-apoptotic effects due to more potent BCR–ABL inhibition during treatment with 2nd-TKIs might work even on the reduction of BCR–ABL-positive primitive cells. Future efforts toward cure in CML patients who are responding well to kinase inhibitors, but continue to show evidence of minimal residual disease, should focus on understanding the mechanisms of proliferating arrest and dormancy on oncogene inactivation in the CML stem cell population and also aim to target BCR–ABL kinase-independent survival pathways that remain active in these cells or are activated on kinase inhibition.[3]

In conclusion, 2nd-TKI therapy can be a more promising approach than IM treatment for early reduction of CML stem cells.

CONFLICT OF INTEREST

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)