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To the editor:

Telomere length in peripheral blood granulocytes reflects response to treatment with imatinib in patients with chronic myeloid leukemia

Telomeres are composed of TTAGGG repeats and associated proteins.[1] In somatic cells, telomere repeats are lost with each cell division, eventually leading to genetic instability and cellular senescence.[2] In previous studies, we and others described substantial and disease stage–specific telomere shortening in Philadelphia chromosome–positive (Ph^+) peripheral blood (PB) leukocytes from patients with chronic myeloid leukemia (CML).[3,4]

The selective tyrosine kinase inhibitor imatinib blocks phosphorylation of tyrosine residues by occupying the adenosine triphosphate (ATP) site of BCR-ABL.[5] Clinical phase 2 studies in CML revealed that the drug is capable of inducing major cytogenetic remissions in 60% of chronic phase (CP) patients previously treated with interferon α[6], and even in about 26% of patients in accelerated phase (AP)[7] and in 15% in myeloid blast crisis (BC).[8]

In the current study, we sought to determine whether age-adjusted telomere length in PB granulocytes (ΔTEL_{gran}) is correlated with response to treatment with imatinib.

A total of 517 samples from 206 patients in CP, AP, and BC before and up to 706 days after initiation of imatinib therapy (median, 144 days) were analyzed by fluorescence in situ hybridization and flow cytometry (flow-FISH), telomere fluorescence was expressed in molecular equivalents of soluble fluorochrome units (MESF).[9] Age-adjusted telomere length decreased dependent on disease stage from samples derived from patients in CP (median, −1.1 kMESF; 25-75 percentile; −3.3 to 1.2 kMESF), AP (−1.6 kMESF; −3.9 to 1.3 kMESF), and BC (−1.8 kMESF; −3.7 to 0.5 kMESF). However, the degree of telomere shortening was substantially less than what we had observed in previous studies performed in the "pre-imatinib era" of CML treatment.[3] Therefore, we investigated the correlation between the duration of imatinib treatment and telomere length in the PB. Telomere length in samples from start of treatment up to day 144 was significantly shorter (mean ± SE; −1.5 ± 0.3 kMESF) compared with samples from patients treated for more than 144 days (−0.8 ± 0.3 kMESF, $P = .035$).

In order to analyze whether the increase in telomere length observed during imatinib treatment was due to a shift from Ph^+ to Ph^- cells in the PB of these patients, samples were grouped based on the degree of remission achieved either in the bone marrow (BM) measured by conventional cytogenetics (Figure 1A) or in the PB by quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) (Figure 1B). Telomere length in samples from patients in major or complete cytogenetic remission (median, −0.3 kMESF; 25-75 percentile; −2.8 to 2.6 kMESF; n = 58) was found to be longer compared with samples from patients with minor (−1.4 kMESF; −3.4 to 1.2 kMESF; n = 44) or without cytogenetic response (−1.7 kMESF; −3.9 to 0.6 kMESF; n = 144, $P < .05$ for difference between major and no cytogenetic response; Figure 1A). When the samples were grouped according to molecular remission[10] (Figure 1B), median telomere length in samples from patients in

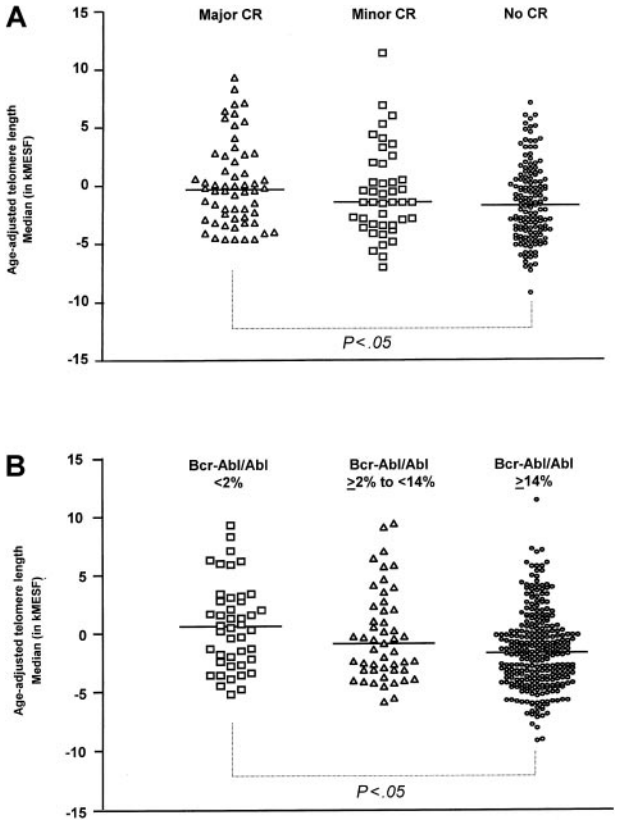

Figure 1. Age-adjusted telomere length in patients with CML. (A) Cytogenetic remission in the BM and (B) molecular response in the PB by quantitative RT-PCR.

good molecular response condition (BCR-ABL/ABL ratio < 2%, 0.6 kMESF; −2.3 to 3.0 kMESF; n = 44) was not different from age-adjusted controls but differed significantly from samples obtained from patients with no molecular response (BCR-ABL/ABL ratio > 14%, −1.8 kMESF; −4.1 to 0.3 kMESF; n = 278, $P < .05$). Patients with intermediate molecular response (BCR-ABL/ABL ratio 2%-14%) showed an intermediate degree of telomere reduction (−0.9 kMESF; −3.2 to 2.1 kMESF; n = 49).

In summary, our observations reflect a steadily increasing fraction of Ph^- cells (with normal or only slightly reduced telomere length) contributing to the peripheral blood cell pool in patients receiving imatinib treatment. Cytogenetic and molecular responses achieved during imatinib therapy are associated with a normalization of previously shortened telomere length arguing against a preexisting telomere length deficit in normal hematopoietic stem cells from patients with CML at the time of malignant transformation.

Tim H. Brümmendorf, Inci Ersöz, Ulrike Hartmann, Kerol Bartolovic, Stefan Balabanov, Alexandra Wahl, Peter Paschka, Sebastian Kreil, Tanja Lahaye, Ute Berger, Harald Gschaidmeier, Carsten Bokemeyer, Rüdiger Hehlmann, Klaus Dietz, Peter M. Lansdorp, Lothar Kanz, and Andreas Hochhaus

Correspondence: Tim H. Brümmendorf, Department of Hematology and Oncology, University Medical Center II, Otfried-Müller-Str 10, 72076 Tübingen, Germany; e-mail: tim.bruemmendorf@med.uni-tuebingen.de

This study was supported by fortune project No 697-0-0 from the University of Tübingen, the Sonderforschungsbereich 510 (Teilprojekt A6) of the Deutsche Forschungsgemeinschaft as well as a grant from the Deutsche Krebshilfe (no. Br 70-2746) and by a grant from the German Ministry of Education and Research (BMBF), Kompetenznetz Akute und chronische Leukämien-01 GI9980/6.

One of the authors (H.G.) has declared a financial interest in a company whose product was studied in the present work.

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To the editor:

The role of interleukin-3 and stem cell factor in classical Hodgkin disease

We have read with great interest the comprehensive review by Skinnider and Mak that summarizes the current literature regarding the expression and activity of cytokines in classical Hodgkin disease (cHD), a unique pathologic condition in which a minority of clonal neoplastic cells are embedded in a heterogeneous background of nonmalignant cells.[1] As stated by the authors, given the peculiar features of HD, bona fide HD-derived cell lines remain to date invaluable tools for investigating the cytokine-dependent interactions of Hodgkin–Reed Sternberg (H-RS) cells. Nevertheless, information from these studies should be always confirmed and validated by other studies carried out in the context of fresh HD-involved tissues or involving primary H-RS cells. By using such an approach, we have described 2 cytokine circuitries allegedly operating in HD that involve interleukin-3 (IL-3), stem cell factor (SCF), and their corresponding receptors (Rs).[2-5]

As opposed to data reported by Skinnider and Mak,[1] our evidence seems to suggest a role for IL-3 in H-RS cell proliferation and survival.[2] In this regard, we demonstrated that IL-3 can promote the clonogenic growth of HD-derived cells and is able to partially rescue them from apoptosis induced by serum deprivation.[2] Accordingly, HD-derived cell lines express mRNA and protein of the IL-3Rαβ complex, whereas primary H-RS cells from all cHD cases tested can be stained by anti–IL-3Rα antibodies.[2]

On the other hand, data reported by Skinnider and Mak indicating the lack of IL-3 production by HD-derived cell lines[1] are consistent with our findings obtained by using a technical approach as sensitive as reverse transcriptase–polymerase chain reaction.[2]

Moreover, the notion that IL-3 mRNA was detected by Northern analysis in some cases of cHD-involved tissues, as reported by Skinnider and Mak,[1] is in keeping with data from our group demonstrating the ability of HD-derived cells to modulate the production of IL-3 by T cells.[3] In fact, preactivated purified T cells, when cultured along with paraformaldehyde-fixed HD-derived cells, release significantly higher amounts of IL-3 than in cultures carried out without fixed HD-derived cells.[3]

The expression of SCF receptor (SCFR)/c-kit by the neoplastic component of cHD has been described by us some years ago.[4] This data, given the notion that fibrosis is a common feature of cHD-involved tissues[6] and SCF is produced by several stomal cells including fibroblasts,[7] strongly suggested a role of SCF/SCFR pair in cHD. The formal proof of this has been recently provided.[5] By using HD-derived cell lines and fibroblasts from HD-involved lymph nodes (HDF), we demonstrated the expression of c-kit in HD-derived cells and of SCF in primary HDF.[5] Moreover, functional experiments have shown the in vitro adhesion of HD-derived cells to HDF through c-kit/SCF interactions, as well as the capability of SCF to increase the growth/survival of HD-derived cells, and to partially rescue HD-derived cells from apoptosis induced by serum starvation.[5]