To the editor:

BCR-ABL expression in leukemic progenitors and primitive stem cells of patients with chronic myeloid leukemia

Kumari and colleagues have recently reported a detailed analysis of *BCR-ABL* expression in CFU-Cs (colony forming units in culture) of patients with chronic myeloid leukemia (CML).[1] Using quantitative reverse-transcription PCR on individual Ph1 hematopoietic colonies, they demonstrated that CFU-Cs from patients in major molecular response (MMR) displayed lower *BCR-ABL* mRNA expression than CFU-Cs from patients at diagnosis. In addition, the authors observed a large variability of *BCR-ABL/ABL* ratios in homogeneous subpopulations of CFU-Cs. These interesting findings were evaluated in the progenitor compartment and not in the more primitive stem cell compartment, including long-term-culture initiating cell (LTC-IC)–derived CFU-Cs.

We have previously analyzed *BCR-ABL*–expressing leukemic progenitors and stem cells, either in the context of imatinib resistance[2] or that of sustained undetectable molecular residual disease (UMRD).[3] In the light of the data presented by Kumari et al, we wished to examine the *BCR-ABL* mRNA levels in individual progenitors and LTC-IC derived CFU-Cs from our experiments. In the first study of a patient with imatinib-resistant CML,[2] *BCR-ABL/ABL* ratios in CFU-Cs and LTC-IC-derived CFU-Cs were found to be similar, with a median value of 52.1% and 51% respectively, comparable with the ratio (68%) measured in peripheral blood (Figure 1). In the second work,[3] we have analyzed the *BCR-ABL* mRNA expression in 6 patients with sustained UMRD (> 3 years). We have detected persistent *BCR-ABL*–expressing stem cells not only in the patient on targeted therapy, but also in the other 5 patients, in whom IFN-α or imatinib treatments were discontinued for 2-13 years. The analysis of *BCR-ABL/ABL* ratios in hematopoietic colonies showed a large expression variability from one CFU-C to another, in accordance with Kumari et al.[1] Moreover, a significant difference was observed between the amount of *BCR-ABL* mRNA transcript in CFU-Cs and LTC-IC–derived progeny in the context of UMRD (Figure 1). Interestingly, primitive stem cells expressed significantly less *BCR-ABL* mRNA than committed progenitors (median *BCR-ABL/ABL* ratios of 0.7% and 12%, respectively).

The persistence of primitive leukemic stem cells in CML patients with UMRD has been recently documented,[3,4] demonstrating that, despite a major success in the eradication of bulk leukemic cells, imatinib therapy does not fully eradicate leukemic stem cells. Several biologic explanations for the intrinsic refractoriness of CML stem cells against imatinib have been proposed,[5,6] but a mechanism involving low *BCR-ABL* expression in these primary cells had not been suggested before. The variable *BCR-ABL* transcription by Ph1 progenitors has previously been described in patients with chronic phase CML.[7,8] Kumari and coworkers observed a lower *BCR-ABL* expression in individual hematopoietic progenitor colonies from patients with MMR, and suggested a relationship between this phenomenon and the persistence of leukemic stem cells resistant to imatinib. Our data, based on patients with sustained UMRD, show that low amounts of *BCR-ABL* mRNA transcripts are observed not only in hematopoietic progenitors but also in primitive stem cells with a major reduction in the latter. Therefore, the weak level of *BCR-ABL* expression in persistent leukemic stem cells could be an additional mechanism explaining their innate resistance toward imatinib and perhaps to other tyrosine kinase inhibitors. In addition, in imatinib discontinuation context, correlation studies between levels of *BCR-ABL* transcripts in residual leukemic stem cells and molecular relapse could be of major interest.

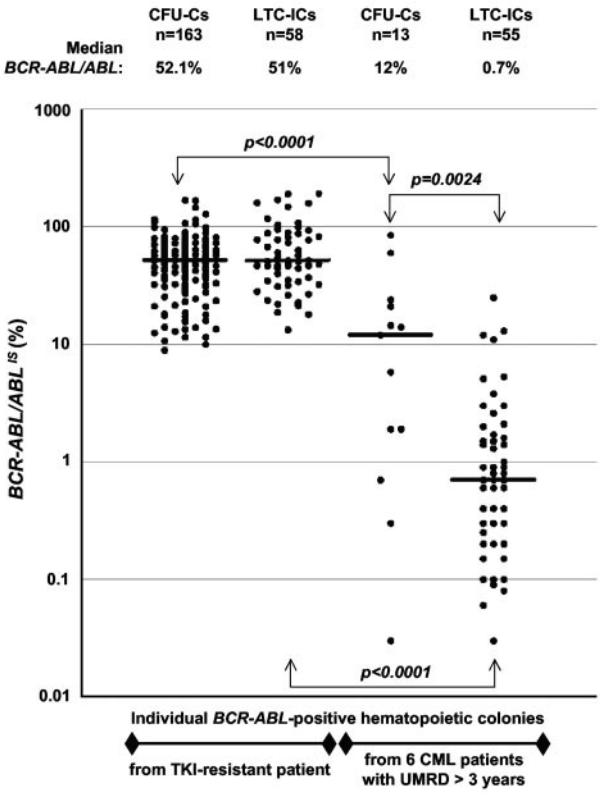

Figure 1. *BCR-ABL* expression in individual *BCR-ABL*-positive hematopoietic colonies. The determination of *BCR-ABL/ABL* ratios was performed according to the international scale (IS) standardization by quantitative RT-PCR on individual hematopoietic colonies from CFU-C (colony forming unit-cell) and LTC-IC (long-term culture-initiating cell) assays. Dots represent *BCR-ABL/ABL* ratios for single colonies (CFU-Cs or LTC-ICs) from a patient resistant to tyrosine kinase inhibitor therapy and from 6 patients with undetectable molecular residual disease (UMRD) for more than 3 years. Mann Whitney *U* tests were used to estimate differences between groups. Significant differences are reported after Bonferroni correction to account for multiple testing. The horizontal lines indicate median *BCR-ABL/ABL* ratios, and the number of *BCR-ABL*–positive CFU-Cs and LTC-ICs analyzed in the study is shown.

Jean-Claude Chomel
Service d'Hématologie et Oncologie Biologique,
Centre Hospitalier Universitaire (CHU) de Poitiers,
Inserm U935, Université de Poitiers,
Poitiers, France

Nathalie Sorel
Service d'Hématologie et Oncologie Biologique, CHU de Poitiers, Inserm U935, Université de Poitiers, Poitiers, France

Joëlle Guilhot
Inserm CIC 0802, CHU de Poitiers, Poitiers, France

François Guilhot
Inserm U935, Université de Poitiers, Inserm CIC 0802, CHU de Poitiers, Poitiers, France

Ali G. Turhan
Service d'Hématologie et Oncologie Biologique, CHU de Poitiers, Inserm U935, Université de Poitiers, Poitiers, France

Acknowledgments: This work was supported by grants from INCa (Institut National du Cancer), Cancéropôle Grand Ouest, Université de Poitiers, Inserm, the association Laurette Fugain (Project ALF 09-04), and a supplementary contribution from Novartis Pharma SAS France.

Contribution: J.C.C. and N.S. performed experiments; J.G. computerized expression data and performed statistical analyses; F.G. collected and analyzed clinical data; J.C.C. and A.G.T. analyzed the data and wrote the manuscript; and all authors read and approved the manuscript in its final version.

Conflict-of-interest disclosure: F.G. has received consulting fees and research funding from Novartis Oncology and Bristol Myers Squibb. A.G.T has received lecture fees and research funding from Novartis Oncology and Bristol Myers Squibb. The remaining authors declare no competing financial interests.

Correspondence: Dr J. C. Chomel, Service d'Hématologie et Oncologie Biologique–Inserm U935, CHU de Poitiers, 2 rue de la Milétrie, 86021 Poitiers Cedex, France; e-mail: j.c.chomel@chu-poitiers.fr.

References

1. Kumari A, Brendel C, Hochhaus A, Neubauer A, Burchert A. Low BCR-ABL expression levels in hematopoietic precursor cells enable persistence of chronic myeloid leukemia under imatinib. *Blood.* 2012;119(2):530-539.
2. Chomel JC, Sorel N, Bonnet ML et al. Extensive analysis of the T315I substitution and detection of additional ABL mutations in progenitors and primitive stem cell compartment in a patient with tyrosine kinase inhibitor-resistant chronic myeloid leukemia. *Leuk Lymphoma.* 2010;51(11):2103-2111.
3. Chomel JC, Bonnet ML, Sorel N et al. Leukemic stem cell persistence in chronic myeloid leukemia patients with sustained undetectable molecular residual disease. *Blood.* 2011;118(13):3657-3660.
4. Chu S, McDonald T, Lin A et al. Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment. *Blood.* 2011;118(20):5565-5572.
5. Graham SM, Jorgensen HG, Allan E et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. *Blood.* 2002;99(1):319-325.
6. Jiang X, Zhao Y, Smith C et al. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. *Leukemia.* 2007;21(5):926-935.
7. Bedi A, Zehnbauer BA, Collector MI et al. BCR-ABL gene rearrangement and expression of primitive hematopoietic progenitors in chronic myeloid leukemia. *Blood.* 1993;81(11):2898-2902.
8. Keating A, Wang XH, Laraya P. Variable transcription of BCR-ABL by Ph+ cells arising from hematopoietic progenitors in chronic myeloid leukemia. *Blood.* 1994;83(7):1744-1749.

Response:

Too much BCR-ABL to live on, but too little BCR-ABL to die on?

The mechanistic understanding of persistence of leukemic stem cells during tyrosine kinase inhibitor therapy is an important unmet prerequisite for targeting residual CML and eradicating the disease. Unfortunately, the rarity of BCR-ABL–positive cells during major and complete molecular remission (MMR; CMR) makes it notoriously difficult to investigate genetic or biologic features of CML clones that persist in vivo under imatinib. The analysis by Chomel and colleagues on hundreds of single residual CML stem and progenitor cell clones, derived from precious marrow samples of yearlong-followed CML patients deserves respect—also for the elaborate translational approach.[1]

In keeping with our referenced finding,[2] Chomel et al demonstrate that persisting CML stem cells (in vitro referred to as long-term culture initiating cells, LTC-IC), but also committed precursor cells express substantially less *BCR-ABL* mRNA than their imatinib-resistant counterparts. These results can be interpreted as follows: high-level BCR-ABL expression is incompatible with persistence under imatinib, whereas low BCR-ABL levels contribute to intrinsic BCR-ABL kinase inhibitor resistance.

This has 2 important implications. First, if low BCR-ABL expression is a hallmark of persisting stem and progenitor cells under kinase inhibitor therapy in vivo, then 20-300 times more potent second generation BCR-ABL inhibitors such as nilotinib and dasatinib will presumably not be more potent in eradicating persistent CML. CML eradication concepts should consequently target BCR-ABL independent pathways.

Secondly, the data by Chomel and colleagues lend support to the conclusion that the genetic pressure to mutate BCR-ABL is low in persistent CML.[1] For example, oncogene-initiated signal flux must exceed a critical threshold to trigger cell intrinsic tumor suppressive responses via activation of Arf/p53.[2,3] Hence, low-level oncogenic BCR-ABL may not suffice to induce oncogene addiction in the first place. As a consequence, even potent BCR-ABL inhibition by imatinib at the stem cell level[4] would not generate a genetic pressure toward mutating BCR-ABL in persistent residual CML cells, because the resolution of this pressure (eg, by kinase mutations) would not result in a growth/survival advantage. Indeed, this presumption is evidenced by the clinical observation that relapsing CMR patients retain imatinib sensitivity after imatinib discontinuation.[5] Altogether, this is good news for the patients, because the data imply that we can "afford to let sleeping dogs lie" (see Deininger and Holyoake[7]), as long as imatinib therapy continues.

As Chomel et al point out, it would now be important to study BCR-ABL expression regulation in CMR patients after imatinib discontinuation.[1] However, this may not only enable the prediction relapse candidates as Chomel et al suppose, but could also lead to the discovery of signaling molecules and pathways that stimulate BCR-ABL expression. Their inhibition would specifically attack BCR-ABL–positive persistent disease and represent a novel approach to eradicate residual CML.

Andreas Burchert
Universitätsklinikum Marburg, Marburg, Germany

Andreas Neubauer
Universitätsklinikum Marburg, Marburg, Germany

Andreas Hochhaus
Universitätsklinikum Jena, Jena, Germany