First, the repetitive use of the term “megakaryocytic hyperplasia” in the paper by Ciurea et al1 should be replaced by “neoplastic accumulation of megakaryocytes.” The process is an accumulation of neoplastic, not hyperplastic, megakaryocytes as a result of an increased clonal production of megakaryocytes and a decrease in apoptotic signaling in the neoplastic megakaryocytes, as they have skillfully confirmed. The correct use of the term hyperplasia in nonclonal accumulations of megakaryocytes, such as in immune thrombocytopenia, reflects a quite different process from neoplasia. The distinctions among hyperplasia/aplasia, hyperplasia, metaplasia, dysplasia, and neoplasia should retain their classical pathobiologic meaning.2,3 Of course, these experts understand these distinctions but it is important to consider the specificity of language for those trying to learn about these processes. Likewise, the structural aberrations in clonal myeloid disorders may result in the dysmophia of neoplasia, but they are not “dysplastic.” Similarly, extramedullary neoplastic (clonal) hematopoietic cells or tumors, distant from the primary site (marrow) represent metastases, not “metaplasia,” probably the result, in part, of high concentrations of circulating clonal CD34+ cells capable of local extramedullary proliferation and partial differentiation.

Second, the centrality of neoplastic megakaryocytes in the disease is not unexpected, since it is, in fact, chronic megakaryocytic leukemia, as has been argued in a Commentary in the journal Leukemia.4

The members of the WHO committee who are responsible for nomenclature of the clonal myeloid diseases should permit nosology to advance with the science it is meant to encapsulate. The paper by Ciurea et al1 adds important evidence to the centrality of neoplastic megakaryocytes in this disorder.

Marshall A. Lichtman
Conflict-of-interest disclosure: The author declares no competing financial interests.
Correspondence: Marshall A. Lichtman, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642; e-mail: mal@urmc.rochester.edu.

References

To the editor:

Philadelphia-negative clonal hematopoiesis is a significant feature of dasatinib therapy for chronic myeloid leukemia

Descriptions of chromosomally abnormal Philadelphia-negative (Ph−) clones in chronic myeloid leukemia (CML) patients treated with chemotherapy,1 interferon-alpha,2 and, most recently, imatinib3-5 suggest that such events arise indirectly as a result of suppression of the BCR-ABL–positive cell population. However, the incidence and significance of karyotypic abnormalities in Ph− cells of patients receiving alternative tyrosine kinase inhibitors (TKIs) to imatinib have not yet been explored. We assessed a series of 35 patients with imatinib-resistant CML who then received dasatinib, for the emergence of cytogenetic abnormalities in Ph− cells. After commencing dasatinib treatment, 3 (8.6%) of the 35 patients acquired cytogenetic abnormalities in Ph− cells, namely trisomy 8, disomy Y, and ins(22;3)(q11; q26;q21) (Table 1 patients 1 to 3, respectively). G-band data and retrospective interphase fluorescence in situ hybridization (FISH) analysis of fixed bone marrow preparations from these 3 patients

Table 1. Karyotypes and clinical characteristics of dasatinib-treated CML patients with clonal cytogenetic abnormalities in Ph− cells

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Previous treatment</th>
<th>Length of follow-up on dasatinib, mo</th>
<th>Representative karyotype with Ph− clone†</th>
<th>Time from start of dasatinib to appearance of Ph− clone, m</th>
<th>Duration of Ph− clone, m‡</th>
<th>Best response on dasatinib, %¶</th>
<th>Karyotype of most recent sample</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HU, IFN, IM</td>
<td>23</td>
<td>47.XY, +8[29]/46.XY, t(9;22)(q34;q11.2)[2]</td>
<td>6</td>
<td>8</td>
<td>Trilineage dysplasia†</td>
<td>46,XY,i(9;22)[9q34]; q11[30]</td>
<td>A, loss of CyR</td>
</tr>
<tr>
<td>2</td>
<td>HU, IFN, IM</td>
<td>22</td>
<td>47.XY, +Y[36]/46.XY[4]</td>
<td>7</td>
<td>15+</td>
<td>No</td>
<td>46,XY, +Y[3][46], XY[27]</td>
<td>A, CyCMy</td>
</tr>
<tr>
<td>3</td>
<td>HU, IFN, IM</td>
<td>21</td>
<td>46,XX,ins(22;3)[q11; q26q21][12]</td>
<td>8.5</td>
<td>12</td>
<td>No</td>
<td>46,XX,i(9;22)[q34]; q11[30]</td>
<td>A, loss of CyR</td>
</tr>
</tbody>
</table>

Ph− indicates Philadelphia chromosome negative; MDS, myelodysplastic syndrome; HU, hydroxyurea; IFN, interferon alpha; IM, imatinib mesylate; A, Alive; CyR, cytogenetic response; AP, accelerated phase; and CyCyR, complete cytogenetic response.

*Patients were identified from a series of 35 cases of dasatinib-treated CML. Cytogenetic analysis was carried out on 30 metaphases from each of 151 bone marrow samples, with a median of 4 analyses per patient (range, 1-10). Nineteen patients were in chronic phase, 10 in accelerated phase, and 6 in blast crisis. The median follow-up from start of dasatinib was 16 months (range, 4-23 months). Twenty-seven patients (77%) showed at least a 5% reduction in Philadelphia-positive metaphases over the course of dasatinib treatment, of which 8 patients (23%) demonstrated minimal or minor CyR and 19 patients (54%) showed partial or complete CyR.
†Karyotype of the sample with the highest level of chromosomally abnormal Ph− clone is presented. Ph− clone is shown in bold.
‡Calculated as the time between the first and last sample with a Ph− clone.
¶Consistent with the World Health Organization Criteria and corresponding with the emergence of the Ph− clone.
§Consistent with the World Health Organization Criteria and corresponding to the emergence of the Ph− clone.
||The myelodysplastic features observed in patient 1 disappeared at the same time point as the +8 clone, coinciding with loss of CyR.
Figure 1. G-band and FISH analysis of an ins(22;3) in Ph− cells of a CML patient undergoing dasatinib treatment (patient 3). (A) Partial G-band karyotype showing the products of the ins(22;3)(q11;q26q21) (right) beside the normal chromosome homologues (left). Part of the q-arm of chromosome 3 is seen, inverted, within the structure of the abnormal chromosome 22. A simplified diagrammatic representation of the structural rearrangement resulting in this abnormality is shown (inset). (B) Structure of the EVI1 locus together with the composition and relative coverage of the dual-color EVI1 FISH probe (Kreatech Biotechnology, Amsterdam, The Netherlands). The probe system employs 2 separate components specific for the 5′ and 3′ portions of the locus, labeled in red and green, respectively. In cells without a 3q26 rearrangement, 2 red/green fusion signals are produced. In contrast, physical separation of 5′ and 3′ hybridization signals, or splitting of 1 of these component signals, is visible in cells with rearrangement of this region. (C) Analysis of an ins(22;3)–positive bone marrow metaphase cell from patient 3 using the dual-color EVI1 probe. One intact red/green fusion signal is observed on the normal chromosome 3 homologue, marking the unrearranged EVI1 locus. In contrast, the second EVI1 hybridization signal reveals relocation and rearrangement of this region, with part of the red 5′ signal hybridized toward the distal end of the der(22), whereas the remains of the red 5′ signal together with the green 3′ signal are present toward the centromere of this marker. This finding is consistent with an inversion of chromosome 3 involving a break distal to EVI1 and a subsequent insertion of 3q material, including the inverted region, into chromosome 22.

excluded the presence of the Ph− clones prior to dasatinib treatment.

Patients 1 to 3 are the first reported cases of de novo clonal cytogenetic aberrations arising in Ph− cells during dasatinib treatment of CML. The incidence and type of karyotypic abnormalities in Ph− cells after dasatinib were similar to those reported in patients responding to imatinib, in which the majority of aberrations resemble recurrent primary cytogenetic abnormalities of other myeloid malignancies.3,4 The ins(22;3)(q11;q26q21) in patient 3 resulted in rearrangement of the EVI1 oncogene, demonstrable by FISH (Figure 1). Importantly, acquisition of EVI1-associated chromosome rearrangement in Ph− cells has previously been reported in 2 CML patients with a t(3;21) who rapidly developed Ph− myelodysplastic syndrome (MDS).5,6 The absence of myelodysplastic features in patient 3 demonstrates that, despite being a poor prognostic indicator in other myeloid malignancies, EVI1 rearrangement can exist in an apparently benign clone in CML patients without overt progression to secondary neoplasia.

The biologic mechanism behind the genesis of Ph− clones remains elusive. It has been suggested that the karyotypic abnormalities provide evidence of a 2-step process of CML pathogenesis in which a monoclonal preleukemic stage exists that favors the acquisition of either the Philadelphia rearrangement or other chromosome aberrations. This notion has gained support from the recent observation that some patients responding to imatinib retain specific cytogenetic abnormalities other than the Ph chromosome that were present originally in Ph+ cells.6 Alternatively it is possible that Ph− clones arise as a result of increased pressure on normal hematopoietic stem cells to expand rapidly to replace the BCR-ABL–positive population. Such an environment would favor any BCR-ABL–negative cell that acquired a mechanism of selective advantage such as one that might be conferred by a cytogenetic abnormality. It is tempting to speculate that the latter effect might be compounded in those patients experiencing cytopenias, a side effect observed in around 50% of CML patients undergoing TKI therapy.7 However, of the 3 patients described here, cytopenia was detected only in patients 1 and 3 prior to the acquisition of the Ph− aberrant clone, thus excluding a consistent link between dasatinib-induced myelosuppression and Ph− clonal chromosome abnormalities.

Our findings demonstrate that Philadelphia-negative clonal hematopoiesis is not only a feature of imatinib therapy but also likely to be a general phenomenon associated with TKI-induced suppression of BCR-ABL–positive cells. These data emphasize the importance of conventional cytogenetic analysis as part of routine patient management for CML patients undergoing TKI therapy, even after induction of complete cytogenetic remission.

Valeria A. S. De Melo, Dragana Miliøjkovic, Jamshid S. Khorashad, David Marin, John M. Goldman, Jane F. Apperley, and Alistair G. Reid

Conflict of interest: The authors declare no competing financial interests.

Correspondence: Alistair G. Reid, Department of Haematology, Imperial College London, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK; e-mail: a.reid@imperial.ac.uk.

References

Philadelphia-negative clonal hematopoiesis is a significant feature of dasatinib therapy for chronic myeloid leukemia

Valeria A. S. De Melo, Dragana Milojkovic, Jamshid S. Khorashad, David Marin, John M. Goldman, Jane F. Apperley and Alistair G. Reid