LETTER TO THE EDITOR

Patients with chronic myeloid leukemia treated with imatinib who showed the appearance of clonal cytogenetic abnormalities in Philadelphia chromosome-negative cells

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder, characterized by the presence of the Philadelphia (Ph) chromosome, a consequence of a reciprocal translocation between chromosomes 9 and 22, resulting in the fusion of the genes BCR and ABL. The BCR–ABL fusion gene encodes a constitutively active leukemogenic protein tyrosine kinase. BCR–ABL kinase activity is inhibited by the selective activity of imatinib, a target agent that has demonstrated remarkable efficacy and tolerability. It has been shown that imatinib blocks cell proliferation and induces apoptosis in BCR–ABL-expressing hematopoietic cells. Imatinib has been used as a first-line therapy for CML patients. Different patterns of response to imatinib treatment have been recognized, ranging from best-case scenarios of rapid and unresponsive response to difficult situations of intolerance and resistance, either primary or secondary. In general, most patients under imatinib achieve a major or complete cytogenetic response (CCyR), with restoration of the Ph chromosome-negative cells, which frequency varies greatly, ranging from 2 to 15%. Among these patients, 74 had the Ph chromosome as sole chromosomal abnormality (74%) and 26 had Ph chromosome and additional chromosomal abnormalities (26%) before beginning the treatment. Patients only with Ph chromosome before the treatment were more frequently in CP than in AP and BC (81% versus 19%, P < 0.001). Also, the patients with only the Ph-positive cells had a significant higher rate of major cytogenetic response than the patients with Ph and additional chromosomal abnormalities (90% versus 10%, P < 0.001). Based on clinical results from randomized trials, imatinib is approved for all phases of CML, but best results of this treatment is observed in CML patients at the initial phase of the disease, as observed in our study and others.

From the total of 100 patients studied, we observed the appearance of clonal chromosomal alterations during the imatinib treatment in four cases. The chromosome Ph was the only karyotypic abnormality. The molecular analysis showed the presence of the fusion transcripts: b2a2 in patients 1, 2 and 3; the b3a2 in patient 4. The patients were in CP and they received 400mg per day of imatinib after interferon-α resistance/intolerance. After the patients had achieved a CCyR, with Ph-negative cells, which was observed by G-banding and FISH analysis, it was detected the presence of clonal cytogenetic abnormalities. Three patients showed the gain of chromosome Y (Figure 1) and one patient acquired trisomy 8. The patients achieved hematologic, cytogenetic and molecular responses. They showed no clinical symptoms of CML and the imatinib treatment was continued. The mean survival of these four patients was 60 months, since the imatinib treatment had been started. The clinical, cytogenetic and molecular characteristics of the four patients are shown in Table 1.

A cytogenetic phenomenon that has showed considerable interest in the present era of imatinib treatment is the appearance of clonal cytogenetic alterations in Ph-negative cells, which frequency varies greatly, ranging from 2 to 15%. In our study, this frequency was 4%. The mean time of...
appearance of clonal cytogenetic abnormalities was 46 months after imatinib therapy started. A review of the literature showed that this period may vary from 3 to 47 months. The most common changes observed in Ph-negative cells are: +7, +8, +5 and +Y. Such changes are also presented in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), which has raised concerns about their clinical implication. The frequency of MDS/AML in such patients is in order of 2–10%.

Analyzing the bone marrow of our four patients, they did not show the morphologic features of MDS/AML. Lin et al. also showed no association with MDS/AML. Jabbour et al. suggested that patients with CML require continued monitoring with cytogenetic analysis, but in the absence of additional clinical complications (for example, trilineage dysplasia) a change in treatment strategy is not necessarily indicated.

In our study, we observed an acquisition of an extra chromosome Y in three cases and in one case the trisomy 8. The trisomy 8 is well documented in the literature. Nevertheless, an extra chromosome Y is a rare phenomenon in leukemia. It was described by Watanabe et al. in one case of T-cell acute lymphoblastic leukemia, as a sole chromosome abnormality and the patient achieved complete remission by chemotherapy. Interesting, Deininger et al. studied 532 patients with CML treated with imatinib. They related that the most frequently involved chromosomes were Y and 8 in cases with Ph-negative cells in response to imatinib, with a frequency of 37% and 27%, respectively. But, Deininger et al. also detected an extra chromosome Y in three CML patients with Ph-negative cells in response to imatinib treatment. Nevertheless, these findings were not yet discussed in the literature, as most cases with the involvement of chromosome Y are related to loss of this chromosome and not the gain. The loss of the chromosome Y in hematologic neoplasms is frequently associated with a favorable prognosis. Although, Lippert et al. related that the loss of Y chromosome in Ph-positive cells predicts a poor response of CML patients to imatinib therapy. These findings raise the question: Which genes are localized in Y chromosome that may be associated with imatinib response? Analyzing the genes localized in chromosome Y we can find: SRY (sex determining region Y), TGIF2Ly (TGFB-induced factor homeobox 2-like), PCDH11Y (protocadherin 11), PRKY (protein kinase), TSPY 1 (testis-specific protein), USP9Y (ubiquitin-specific peptidase 9), DDX3Y (DEAD box polypeptide 3), CD24 (CD24 molecule), RBMY1A1 (RNA-binding motif protein). It may be possible that some of these genes, such as: TGIF2Ly, PRKY, RBMY1A1, may be influencing the

Table 1 Clinical, cytogenetic and molecular characteristics of the four patients with clonal chromosomal abnormalities in Ph-negative cells during imatinib therapy

| Patient | Sex/age (years) | Peripheral blood counts | Cytogenetic alterations in Ph-negative cells | Molecular analysis | CCyR (months) | Cytogenetic alterations in Ph-negative cells | Time of detection (months) | Status (follow-up, in months) |
|---------|----------------|-------------------------|-----------------------------------------------|-------------------|--------------|-----------------------------------------------|---------------------------|-----------------------------|
| 1       | M/36           | 7.97 106 409            | 46,XYt(9;22)(q34;q11)[25] b2a2                | G-banding         | 6            | 47,XY,+Y[5]/46,XY[25]                          | 72                        | Alive (84)                  |
| 2       | M/35           | 10.2 89.9 676           | 46,XYt(9;22)(q34;q11)[20] b2a2                | G-banding         | 12           | 47,XY,+Y[6]/46,XY[19]                          | 44                        | Alive (72)                  |
| 3       | M/57           | 13.4 42.7 531           | 46,XYt(9;22)(q34;q11)[51] b2a2                | G-banding         | 12           | 47,XY,+Y[3]/46,XY[17]                          | 48                        | Alive (60)                  |
| 4       | F/47           | 9.0 83.8 348            | 46,XXt(8;22)(q34;q11)[20] b2a2                | G-banding         | 15           | 47,XX,+8[10]/46,XX[10]                         | 21                        | Alive (24)                  |

Abbreviations: CCyR, complete cytogenetic response; F, female; Hb, hemoglobin; M, male.
transductional signal in imatinib response. So, they may be candidates of future studies.

Despite the increased number of reports of Ph-negative clones, the significance and potential causes for Ph-negative clonal cytogenetic abnormalities in hematopoiesis remain unclear. Some studies associated the development of clonal cytogenetic abnormalities by previous treatment like hydroxyurea or IFN-α. Nevertheless, some studies have been shown the clonal cytogenetic alterations in larger series of patients receiving only imatinib treatment. But, in fact, more studies with longer follow-up in larger patient cohorts are necessary to enable researchers to answer the significance of the acquired clonal chromosome abnormalities during imatinib therapy, their correlation into the basic CML pathogenesis and their clinical impact.

**Conflict of interest**

The authors declare no conflict of interest.

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