

situations should and can be avoided. G-CSF is a safe and effective drug to stimulate myelopoiesis and allows for continued Imatinib therapy in CML patients at risk for disease progression.

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Simultaneous occurrence of a t(9;22) (Ph) with a t(2;11) in a patient with CML and emergence of a new clone with the t(2;11) alone after imatinib mesylate treatment

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TO THE EDITOR

Chronic myeloid leukemia (CML) is characterized by the translocation t(9;22), called Philadelphia (Ph) chromosome. It is an acquired abnormality of hematopoietic stem cells, which is present in all dividing granulocytic, erythroid, and megakaryocytic cells in the marrow and also in some B and probably a minority of T lymphocytes. Fusion of the *ABL* gene on 9q34 to the *BCR* gene on 22q11 results in a constitutive activation of the ABL tyrosine kinase, which plays a major role in leukemogenesis.¹ Although a multistep model for the development of CML has been proposed with the acquisition of the Ph chromosome as a second event,^{2–4} the expression of *Bcr/Abl* as a sole oncogenic event in mice results in the development of leukemia, establishing *Bcr/Abl* as the molecular pathogenic event in CML.^{5,6} The tyrosine kinase activity of the fusion protein is critical for the transforming ability, making it an attractive target for antitumor drug development. The novel molecular, targeted anticancer agent imatinib mesylate, also called STI571 or Gleevec (Novartis), specifically inhibits the p210^{BCR/ABL} tyrosine kinase activity, the *in vitro* growth of *BCR/ABL* positive cells, and eradicates *Bcr/Abl* positive tumors in nude mice.^{7,8} Phase I and II clinical trials of patients with chronic phase CML produced encouraging results with a complete hematologic response in a large proportion of patients (for a recent review, see Capdeville *et al*).⁸

Here we describe a patient with a Ph⁺ CML in chronic phase, with a second translocation present in all cells at the time of the first cytogenetic analysis. The patient, a 63-year-old male, was initially treated with hydroxyurea (HU) for 7 weeks, and a cytoreduction from 93.8 leukocytes/nl to 8.9/nl with 4% metamyelocytes was achieved. Then, therapy with imatinib mesylate (400 mg/day) was started in the context of a randomized phase III study and a

complete hematological remission (CHR) was observed after 1 week, lasting until today (22 months of therapy).

The first cytogenetic analysis was performed before starting the imatinib mesylate therapy and during therapy every 3 months. At least 20–22 metaphases were fully karyotyped. Two translocations, t(2;11)(p21;q23) and t(9;22)(q34;q11), were found in all cells analyzed. To exclude a complex variant translocation involving all four chromosomes, we used whole-chromosome paints for these four chromosomes, confirming the presence of two independent reciprocal translocations. A FISH analysis using the 1S-FISH dual color translocation probe (ONCOR, Gaithersburg, MD, USA) was performed on meta- and interphases and a regular *BCR/ABL* rearrangement without amplification was apparent (not shown). As assessed by multiplex RT-PCR the patient had the b3a2 variant of the *BCR/ABL* mRNA.

During imatinib mesylate treatment, cells containing both translocations gradually decreased. After 2½ months of therapy, only four cells had both abnormalities and a clone with the t(2;11) as the sole abnormality became apparent. In the most recent analysis, at 22 months of imatinib mesylate therapy, both translocations were seen in 1/20 cells and the t(2;11) in 19/20 cells. Table 1 shows the results of the consecutive analyses. Quantitative real-time RT-PCR on peripheral blood (PB) was used to follow the treatment response on a molecular level. We found a continuous reduction of the *BCR/ABL:G6PDH* ratios from 3.53 to 0.0027% during treatment, indicating a good molecular response of the disease. This correlates with a CHR and a major cytogenetic response with 0/20 Ph⁺ metaphases at 17 and 1/20 at 22 months of therapy (Table 1).

As the t(2;11) was still present in a high number of the analyzed cells, it seems possible that the patient carried this translocation constitutionally. This was tested by the analysis of PB. No metaphases were obtained from unstimulated cells, but 18 out of 101 PHA stimulated cells carried the t(2;11), indicating the presence of this translocation in at least a precursor for T cells. We also analyzed fibroblasts from a skin biopsy of the patient, but the t(2;11) was not found in 200 cells. This suggests that he does not carry it constitutionally; however, a constitutional mosaicism limited to

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hematopoietic precursor cells cannot be excluded. Taken together, it seems likely that the presence of this translocation is associated with the hematological disorder.

To test whether the *MLL* gene is involved in the t(2;11), the commercial (11q23, FITC labelled) DNA probe was used. In Figure 1a, it can be seen that the *MLL* probe hybridized to the normal chromosome 11q and a second signal was seen on the der(11) chromosome. Therefore, the translocation breakpoint does not disrupt the *MLL* gene. In order to study whether the breakpoint occurred close to the *MLL* gene, we isolated two overlapping BACs containing the *MLL* gene and one mapping immediately telomeric to *MLL*. These were hybridized individually, labelled in red, together with a 11q telomere BAC in green. The 11q23.3 probes span about 538 kb. We detected one red 11q23 BAC signal on the normal and one on the der(11), and one green 11q telomere BAC signal on the normal and one on the der(2). Neither splitting nor a translocation of the red 11q23 BAC signal to chromosome 2 was observed, suggesting that the breakpoint does not map close to the *MLL* gene. This indicates that a gene more telomeric is involved (schematic diagram of the result in Figure 1b). The breakpoint in 2p21 was analyzed using two overlapping BACs covering the protein kinase $C\epsilon$ (PRKCE) gene (RZPD in Berlin, Germany). The region analyzed spans about 450 kb. In all metaphases analyzed, the 2p21 BAC signals remained on the der(2), indicating that the breakpoint maps telomeric to PRKCE on 2p. One 2p telomeric BAC signal was found on the der(11), indicating that all metaphases analyzed contained the t(2;11).

The appearance of cells with this translocation alone after therapy suggests that it was the primary alteration and the Ph translocation occurred later as a second event. It is possible that the t(2;11) conferred a growth advantage of a pluripotent hematopoietic stem cell resulting in clonal expansion of cells in which the Ph translocation occurred as a second event. Therefore, cells with the t(2;11) alone may correspond to the initial Ph⁻ stage in the development of CML.

The t(2;11) is a recurrent translocation in hematopoietic malignancies that can occur as a sole abnormality or in complex karyotypes in association with other aberrations (for a summary, see Fleischman *et al.*⁹). The breakpoints differ slightly in the reports and exact breakpoints on either chromosome are not known. The molecular cloning of this translocation has not been described so far, and therefore it is presently unknown whether all involve or

occur in the same gene/s on chromosome 2 and 11. The 11q23 breakpoint was analyzed in three cases, and two mapped to the *MLL* gene whereas one did not.⁹ Various forms of myelodysplastic syndrome, acute myeloid leukemia (AML), acute lymphoblastic leukemia, and acquired idiopathic sideroblastic anemia (AISA) were diagnosed. To our knowledge no case with a t(2;11) in CML has been described so far. As the t(2;11) was found either alone or in combination with other chromosomal abnormalities, this would suggest that an early hematopoietic precursor cell with the t(2;11) can acquire several different secondary abnormalities determining the cell type involvement in the ensuing hematological disorders. The patient described here is the only case where the t(2;11) alone did not confer a clinical phenotype; all other literature cases with this translocation as a sole abnormality had an apparent hematological disease (RA, RAEB, M1-AML, early progenitor AML, AISA). Some of these patients responded well to therapy, whereas others had a rapid disease progression with this translocation as the sole abnormality and died. Of note is that the same translocation was not seen in 20 000 patients cytogenetically studied in the laboratory of de la Chapelle *et al.*,¹⁰ indicating that it is not a recurrent 'normal' variant.

In the case described here, the imatinib mesylate treatment eliminated cells with both translocations, whereas a new clone with the t(2;11) alone emerged and survived. Recently, several articles described the eradication of Ph⁺ clones and the emergence of clones with new aberrations.^{11,12} This was observed after IFN- α as well as after imatinib mesylate therapy. A summary of all cases described in the literature was presented in Andersen *et al.*¹² with an excess of new clones with trisomy 8. Most cases developed new Ph⁻ clonal aberrations not seen at first analysis. Only two of the 16 cases who developed new Ph⁻ clonal aberrations had a complex karyotype at first analysis; however, in both cases the Ph⁺ cells with other abnormalities disappeared after imatinib mesylate therapy, and Ph⁻ clones with new, different alterations appeared. Brazier *et al.*¹¹ observed one case with a Ph⁺ clone containing an additional translocation t(7;8), which was extremely sensitive to imatinib mesylate. In this patient Ph⁺ clones without the t(7;8), which were resistant to therapy, emerged. All these cases are different from the case described here, where at the first analysis all cells contained two translocations, the clone with both aberrations was eradicated, and Ph⁻ cells with the second translocation only emerged and remained until today. This clearly demonstrates that the t(2;11) was present in this patient before the Ph⁺ translocation occurred.

Table 1 Cytogenetic and PCR data during imatinib mesylate treatment and correlation to the clinical course of the patient

Sample date	Cytogenetic results	PCRb3a2 ^a	Treatment	Hematological remission state
8/18/2000	46, XY, t(2;11)(p21;q23), t(9;22)(q34;q11) [20]	3.53	Imatinib mesylate treatment begins at 8/24/2000	Leukocyte count: 8.6/nl 4% metamyelocytes
11/10/2000	46, XY, t(2;11)(p21;q23) [14], 46, idem, t(9;22)(q34;q11) [4], 46, XY [3]	ND	Imatinib mesylate	CHR
2/8/2001	46, XY, t(2;11)(p21;q23) [15], 46, XY [5],	0.394	Imatinib mesylate	CHR
5/2/2001	46, XY, t(2;11)(p21;q23) [14], 46, idem, t(9;22)(q34;q11) [2], 46, XY [4]	2.482	Imatinib mesylate	CHR
8/2/2001	46, XY, t(2;11)(p21;q23) [14], 46, idem, t(9;22)(q34;q11) [2]/46 XY [4]	0.1533	Imatinib mesylate	CHR
10/18/2001 Pb (PHA)	46, XY, t(2;11)(p21;q23) [3], 46, XY [17]	ND	Imatinib mesylate	CHR
1/11/2002	46, XY, t(2;11)(p21;q23) [18], 46, XY [2]	0.0963	Imatinib mesylate	CHR
2/26/2002 skin biopsy	46, XY [200]	ND	Imatinib mesylate	CHR
6/28/2002	46, XY, t(2;11)(p21;q23) [19]/46, idem, t(9;22)(q34;q11) [1]	0.0027	Imatinib mesylate	CHR

CHR: complete hematological remission.

^a%BCR/ABL:G6PDH ratio.

ND: not determined.

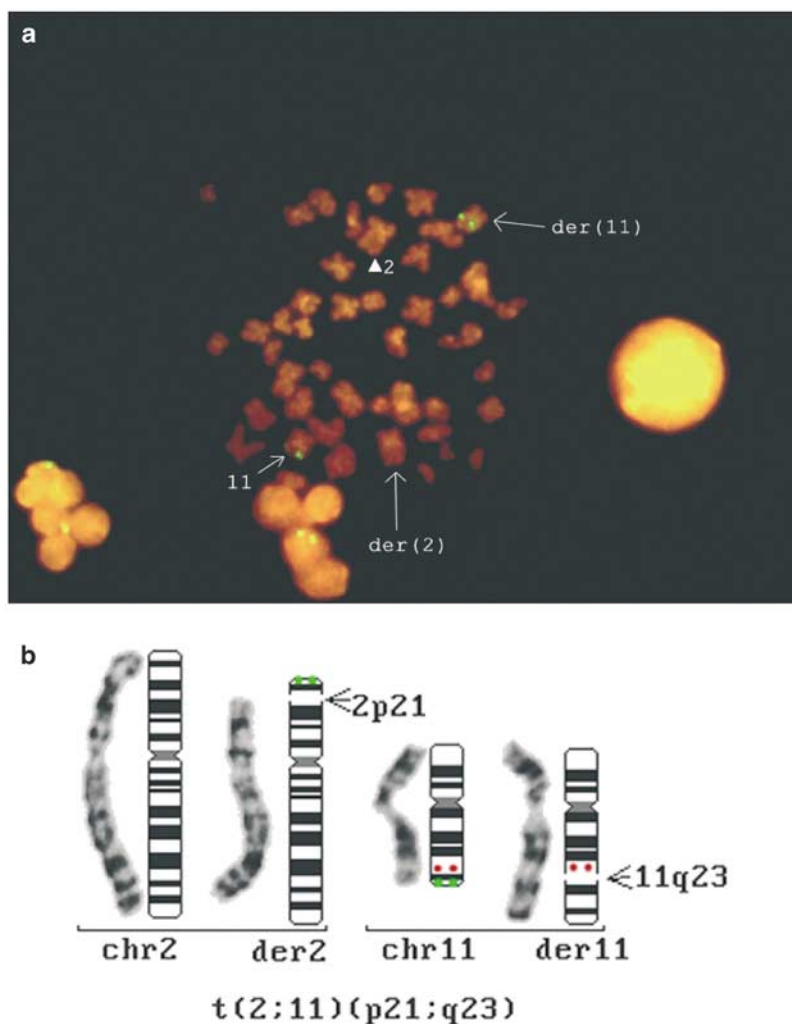


Figure 1 FISH hybridization with the *MLL* probe. (a) Hybridization of the *MLL* probe (green) is seen on the normal chromosome 11 close to the telomere and on the der(11) (white arrow). The normal chromosome 2 is labelled with an arrow head and the der(2) with an arrow. (b) Schematic diagram of the translocation with location of the *MLL* probe (green) and the 11q telomere BAC (red) on the normal and derivative chromosomes.

Imatinib mesylate specifically targets Ph^+ cells and Ph^- leukemic progenitors may survive. This phenomenon is now observed in several patients but was also described previously in IFN α -treated patients (summarized in Andersen *et al*¹²). The patient described here did not have a previous course of IFN α and was only shortly treated with HU, further supporting the notion that these new clonal aberrations are not the result of a previous leukemogenic treatment, but rather represent preleukemic clones as was suggested as a first step in the development of CML.^{2,3}

Therefore, it is important to study more cases with Ph and additional abnormalities in order to determine whether imatinib mesylate can eradicate all Ph^+ cells irrespective of other alterations or whether some specific aberrations render them resistant. In all cases described so far, the additional cytogenetic abnormalities did not confer a resistance to imatinib mesylate. Furthermore, the persistence of a clone with the $t(2;11)$ alone in our patient or the emergence of new Ph^- unrelated clones as reported in the literature^{11,12} demonstrates the importance of classical cytogenetics for disease monitoring of patients treated with imatinib mesylate before and during therapy. The clinical significance of the new Ph^- clones remains to be elucidated, but it seems possible that they

represent a reservoir for disease development (not necessarily CML) in these patients.

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