JAK2-V617F mutation in a patient with Philadelphia-chromosome-positive chronic myeloid leukaemia

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In July, 2000, a 50-year-old man presented with leucocytosis and splenomegaly (21 cm). Leucocyte concentration was 93×10⁹/L, haemoglobin 150 g/L, and platelets 345×10⁹/L (figure 1). A differential blood count showed 54% neutrophils, 2% lymphocytes, 13% myelocytes, 7% metamyelocytes, 2% promyelocytes, 1% blasts, and 7% basophils. Lactate dehydrogenase (LDH) concentration was increased at 484 U/L. 17 months previously, the blood count had been in the normal range. Bone-marrow aspiration was dry and bone-marrow biopsy showed marked myeloid hyperplasia, increased megalakaryopoesis, and the beginning of fibrosis. Cytogenetic analysis revealed a chromosome translocation (t[9;22][q34;q11]) in all ten metaphases examined. Expression of B3A2 BCR-ABL mRNA was detected by reverse transcriptase polymerase chain reaction (RT-PCR) in peripheral-blood leucocytes. A diagnosis of BCR-ABL-positive chronic myeloid leukaemia (CML) was made and treatment with hydroxyurea was started, resulting in rapid normalisation of peripheral-blood leucocytes. After 3 weeks, treatment was changed to imatinib (400 mg/day). Within 1 month, a complete haematological response was recorded. Cytogenetic and interphase fluorescence in-situ hybridisation at 6 months after the start of imatinib showed a complete cytogenetic response. The BCR-ABL:ABL ratio dropped from 90% at diagnosis to 0·021% after 6 months. 16 months after starting imatinib, the BCR-ABL:ABL ratio was already present at initial diagnosis of JAK2-V617F mutation in a patient with Philadelphia-positive CML (figure 2). Repeated cytogenetic and quantitative BCR-ABL RT-PCR analyses showed a continuing complete cytogenetic and molecular response, as shown in figure 2. However, in 2003, an unexpected decrease in platelet count and a continuous increase in serum LDH concentration was noted (figure 1). In 2004, immature myeloid cells began to appear in the peripheral blood and a bone-marrow biopsy in May, 2004, revealed advanced fibrosis. In 2005, leucocyte concentration continually rose above the upper normal limit. The spleen was enlarged to 18 cm. Cytogenetic analysis identified a normal karyotype and BCR-ABL transcripts were still undetectable. Therefore, a JAK2-V617F mutational analysis was done, which highlighted the presence of a heterozygous JAK2-V617F mutation, leading to the diagnosis of idiopathic myelofibrosis (IMF) according to the WHO diagnostic criteria. For JAK2 genotyping, DNA was amplified and PCR products were directly sequenced using the PCR primers JAK2-1F (5'-TGCTGAAAGTAGGAGAAAGTGCAT-3') and JAK2-1R (5'-TCCTACAGTGTGTTCAGTCA-3').

The proportion of mutant JAK2-V617F alleles was quantified using pyrosequencing as described. Additionally, the allelic ratio of JAK2-V617F was confirmed by a newly established quantitative real-time (RQ) PCR assay based on LightCycler technology (Roche Diagnostics, Mannheim, Germany). DNA was extracted from peripheral blood leucocytes after red-cell lysis by standard procedures. Total JAK2 was established using a forward primer, 5'-AGGCTACATCCATCTACCTCAC-3', a reverse primer, 5'-CCTAGCTGTGATCCTGAAACTG-3', and the hybridisation probes 5'-ACAGGCTTGACCCATAAGACCTGAAATA GAG-3' and 5'-GAGTGGTACAGGAA TCATGAAATAGGCACGTCA-3' (Tib Molbiol, Berlin, Germany). Mutant JAK2-V617F alleles were quantified using the same forward primer and probes in combination with a mutation-specific reverse primer, 5'-TTTACTTACTCTGTCCTCCACAGAA-3' (MWG Biotech, Ebersberg, Germany). The allele copy number was identified using a plasmid standard curve. JAK2-V617F positivity was expressed as the ratio between mutant JAK2-V617F and total JAK2. Dilution experiments showed an assay sensitivity of 1%.

Retrospective analysis of frozen samples of peripheral-blood leucocytes showed that the JAK2-V617F mutation was already present at initial diagnosis of BCR-ABL-positive CML (figure 3). Consistent with the presence of an acquired somatic mutation, the JAK2-V617F mutation was absent in purified CD3+ T lymphocytes (figure 3).
and buccal cells (data not shown). As demonstrated by two independent methods—JAK2-V617F pyrosequencing and PCR—the proportion of the mutant allele stayed roughly constant from diagnosis of CML in July, 2000, to February, 2006 (figure 2). Repeated cytogenetic analysis in May, 2006 again showed a normal karyotype.

JAK2 is a tyrosine kinase that has an important role in the signalling pathways of many haemopoietic growth-factor receptors. The single acquired point mutation V617F (1849G>T) in JAK2 occurs in 50–97% of patients with IMF, essential thrombocytosis, and polycythemia vera.2,3 By contrast, the JAK2-V617F mutation has never been identified in a patient with BCR-ABL-positive CML.7 This is the first description of a patient, to our knowledge, in whom the BCR-ABL fusion gene and an acquired somatic JAK2-V617F mutation could be detected contemporaneously, with IMF becoming clinically relevant after successful treatment of CML by imatinib.

Several conclusions can be drawn from these data. First, bone-marrow fibrosis was not a consequence of progressive CML. Rather, myelofibrosis developed as a second myeloproliferative disorder (MPD) during complete cytogenetic and molecular remission of CML. Although the BCR-ABL fusion gene might represent the initiating lesion in most cases of CML, clinical manifestations of the disease can be variable. Specifically, bone-marrow fibrosis can occur, sometimes varying greatly during the course of the disease, and has been used to categorise patients into different groups with distinctive survival characteristics.8 Fibrogenesis in MPD is generally assumed to be mediated by the abnormal release of transforming growth factor-β and platelet-derived growth factor (PDGF). Imatinib, a selective inhibitor of ABL, KIT, and PDGF receptor tyrosine kinases, and which is not active against JAK2, has been seen to reduce the content of bone-marrow fibre in patients with CML.9 Regression of myelofibrosis by imatinib is thought to be caused either by a direct PDGF-antagonistic effect or by normalisation of megakaryopoiesis from which abnormal concentrations of PDGF are released. However, expression of JAK2-V617F in murine haematopoietic cells leads to MPD associated with myelofibrosis.10 Moreover, expression of a BCR-JAK2 fusion gene has been described in a patient with atypical CML and bone-marrow fibrosis,11 and the PCM1-JAK2 fusion is associated with the development of myelofibrosis in patients with chronic and acute leukaemias.12 These findings suggest that JAK2-V617F contributes to the development of myelofibrosis in MPD,13,14 and might have been responsible for the induction of myelofibrosis in our patient which, consequently, was independent of the presence of BCR-ABL and did not respond to imatinib treatment. This assumption is supported by the absence of clinical and molecular responses of patients with IMF and polycythemia vera treated with imatinib.15,16 If treatment with imatinib had not been initiated, the clinical course of the patient could only have been speculated upon. Treatment and response to imatinib might have accelerated the outgrowth of the IMF.

Second, our data suggest that in our patient, the JAK2-V617F mutation had occurred before the acquisition of the Philadelphia (Ph)-chromosome. Development of a Ph-positive CML has rarely been reported in patients with MPD.17 In the few cases that have been reported, clinical manifestations of the disease can be variable. Specifically, bone-marrow fibrosis can occur, sometimes varying greatly during the course of the disease, and has been used to categorise patients into different groups with distinctive survival characteristics.8 Fibrogenesis in MPD is generally assumed to be mediated by the abnormal release of transforming growth factor-β and platelet-derived growth factor (PDGF). Imatinib, a selective inhibitor of ABL, KIT, and PDGF receptor tyrosine kinases, and which is not active against JAK2, has been seen to reduce the content of bone-marrow fibre in patients with CML.9 Regression of myelofibrosis by imatinib is thought to be caused either by a direct PDGF-antagonistic effect or by normalisation of megakaryopoiesis from which abnormal concentrations of PDGF are released. However, expression of JAK2-V617F in murine haematopoietic cells leads to MPD associated with myelofibrosis.10 Moreover, expression of a BCR-JAK2 fusion gene has been described in a patient with atypical CML and bone-marrow fibrosis,11 and the PCM1-JAK2 fusion is associated with the development of myelofibrosis in patients with chronic and acute leukaemias.12 These findings suggest that JAK2-V617F contributes to the development of myelofibrosis in MPD,13,14 and might have been responsible for the induction of myelofibrosis in our patient which, consequently, was independent of the presence of BCR-ABL and did not respond to imatinib treatment. This assumption is supported by the absence of clinical and molecular responses of patients with IMF and polycythemia vera treated with imatinib.15,16 If treatment with imatinib had not been initiated, the
there is a debate as to whether MPD and CML arise separately from each other, representing independent transformation of a normal stem cell, or whether the Ph-chromosome arises in a stem cell that was part of the MPD clone. In our patient, JAK2-V617F was detectable at an almost constant allele frequency in all peripheral-blood samples examined (figure 2). The most likely explanation for this finding is the presence of a heterozygous JAK2-V617F mutation in most of the peripheral-blood leucocytes, implicating the simultaneous presence of the BCR-ABL fusion gene and the JAK2-V617F mutation in white blood cells. Because BCR-ABL became undetectable after treatment with imatinib, but the JAK2-V617F allele frequency remained virtually unchanged, the conclusion can be drawn that the Ph-chromosome was acquired from a haematopoietic cell carrying the JAK2-V617F mutation. Several findings suggest that MPD are caused by a mutation in an, as yet, unknown gene that precedes the acquisition of either a JAK2-V617F mutation or a BCR-ABL fusion gene. The most reasonable explanation for the findings presented here is that BCR-ABL can, in rare cases, occur on the background of the JAK2-V617F mutation, possibly augmented by an elusive initial mutation predisposing to the acquisition of both a JAK2-V617F mutation and a BCR-ABL translocation.

Conflicts of interest
The authors declared no conflicts of interest.

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References