5. Tonon L, Bergamaschi G, Dellavecchia C, Rosti V, Lucotti C, Malabarba L, et al. Unbalanced X-chromosome inactivation in haemopoietic cells from normal women. Br J Haematol 1998; 102:996-1003.
6. Busque L, Mio R, Mattioli J, Brais E, Blais N, Lalonde Y, et al. Nonrandom X-inactivation patterns in normal females: iyonization ratios vary with age. Blood 1996;88:59-65.
7. Christensen K, Kristiansen M, Hagen-Larsen H, Skytthe A, Bathum L, Jeune B, et al. X-linked genetic factors regulate hematopoietic stem-cell kinetics in females. Blood 2000; 95: 2449-51.
8. Furuyama K, Fujita H, Nagai T, Yomogida K, Munakata H, Kondo M, et al. Pyridoxine refractory X-linked sideroblastic anemia caused by a point mutation in the erythroid 5-aminolevulinate synthase gene. Blood 1997;90:822-30.
9. Aivado M, Gattermann N, Bottomley S. X chromosome inactivation ratios in female carriers of X-linked sideroblastic anemia. Blood 2001;97:4000-1.
10. Plenge RM, Hendrich BD, Schwartz C, Arena JF, Naumova A, Sapienza C, et al. A promoter mutation in the XIST gene in two unrelated families with skewed X-chromosome inactivation. Nat Gen 1997;17:353-6.

Chronic Myeloproliferative Disorders

Lack of response to imatinib mesylate in a patient with accelerated phase myeloproliferative disorder with rearrangement of the platelet-derived growth factor receptor β-gene

Imatinib mesylate has been reported to produce positive results in atypical chronic myeloproliferative disorders (CMD) with chromosomal translocations that disrupt the platelet-derived growth factor receptor β gene (*PDGFRB*). We used imatinib to treat a 49-year old man with atypical CMD in accelerated phase and the *H4 (D10S170)-PDGFRB* fusion gene. After 3 months of treatment, we observed grade 4 hematologic toxicity and a lack of response.

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In September 1999, a 49-year old man presented with asthenia and huge splenomegaly. Blood counts performed 2 years previously had already shown hyperleukocytosis and eosinophilia that were not investigated. The blood count at presentation showed hemoglobin 68 g/L, white blood cell count (WBC) of 7.4×10^9/L with 64% neutrophils, 19% lymphocytes, 6% monocytes, 6% eosinophils, 1% basophils, 2% metamyelocytes, 1% myelocytes, 1% promyelocytes, 1% erythroblasts and a platelet count of 63×10^9/L. Bone marrow aspiration was difficult and showed granulocytic hyperplasia without excess of blast cells and few megakaryocytes. The bone marrow biopsy, stained with hematoxylin-eosin and May-Grünwald Giemsa, showed granulocytic hyperplasia, established myelofibrosis (grade III reticulin) and no evidence of blastic transformation. Cytogenetic analysis on bone marrow cells showed: 46,XY, t(5;10) (q33;q21) [18] /46,XY [2]. Reverse transcription polymerase chain reaction (RT-PCR) did not detect *BCR-ABL* transcripts. RT-PCR to detect the *H4-PDGFRB* fusion gene was performed on bone marrow cells as previously described.[1] The fusion junction was identical to that found in the two previously reported cases (Figure 1). The patient was given hydroxyurea which controlled the leukocytosis but the anemia and thrombocytopenia worsened. The spleen enlarged and a splenectomy was performed. The *in vitro* sensitivity of mononuclear (MN) cells to imatinib was studied. Bone marrow MN cells were cultured as previously reported[2] and compared to CML cells. The viability of MN cells exposed or not to 1 μM of imatinib was determined and found to be 50% versus 70% at day 2 and 35% versus 55% at day 3. It should, however, be noted that 100% of cells were dead by the 6th day of culture in the presence or absence of imatinib (Figure 2). In February 2002, the patient began therapy with imatinib mesylate at 400 mg daily. At this time cytogenetics analysis showed additional abnormalities of t(5;10) in 2 mitoses: add(3)(p21) and monosomy 15.

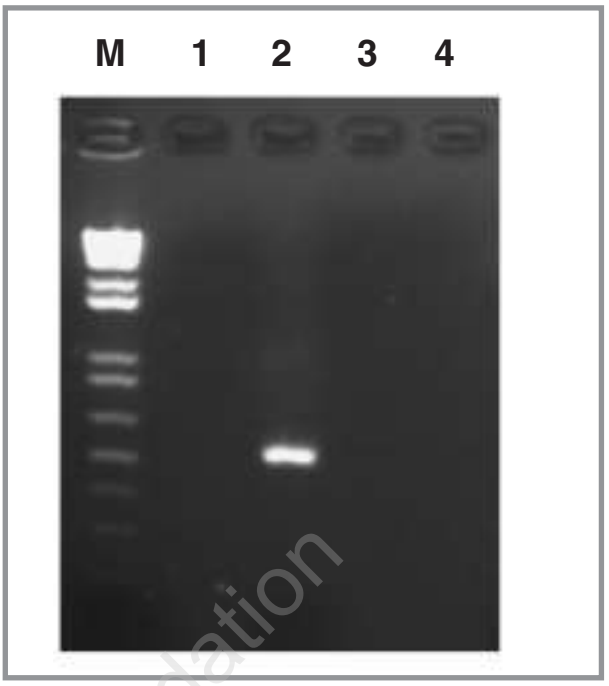

Figure 1. RT-PCR analysis to detect the *H4-PDGFRB* fusion. M is the 1kb+ marker (Invitrogen), lanes 1 and 3 represent negative control patients, lane 2 represents the t(5;10) patient and lane 4 is a negative PCR control.

Imatinib was stopped after 3 months of therapy because of severe hematologic toxicity with the patient requiring platelet and red blood cell transfusions. In the six months following discontinuation of imatinib, hematologic recovery was observed. A second trial of imatinib immediately led to a worsening of thrombocytopenia. Imatinib was definitively stopped and blood counts again recovered. Cytogenetic analysis, repeated after each course of imatinib, showed the persistence of t(5;10) in all the mitoses and *H4-PDGFRB* transcript was still detectable by RT-PCR. Hydroxyurea was subsequently given with persisting good hematologic control until now.

Abnormal activation of *PDGFRB* was first described as a consequence of the t(5;12)(q33;p13), which fuses the 5'end of *ETV6* to the 3'end of *PDGFRB* including the entire tyrosine kinase domain,[3] and complete and durable responses to Imatinib were reported in four patients with t(5;12) translocation.[2] Other translocations involving the same region of *PDGFRB* have been reported:[4] t(5;10) (q33;q21) translocation fusing *PDGFRB* to *H4(D10S170)*, a gene encoding for a 585-amino acid protein with no significant homology to known genes and with unknown function, has been reported in 3 patients.[5,6,7] *H4* is fused to the *ret* gene as a result of an inv(10)(q22q21) in a subset of papillary thyroid carcinomas. The H4-ret fusion protein is a constitutively active tyrosine

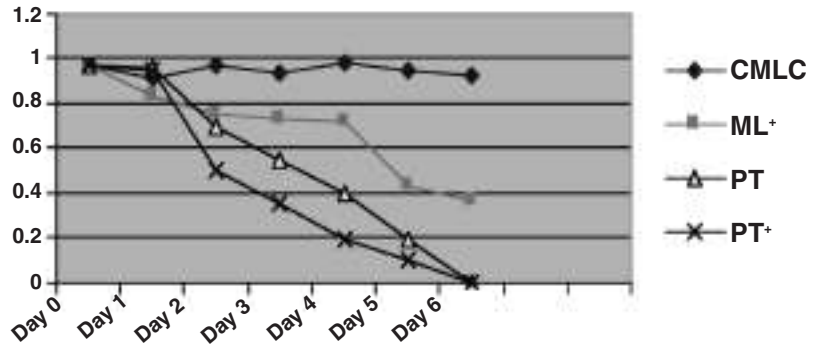

Figure 2. Viability of mononuclear cells from the patient (PT) and from 5 CML other patients exposed or not to 1 µM of Imatinib (STI).

kinase.[8] One patient with a t (5;10) was treated at diagnosis with imatinib and achieved clinical and cytogenetic responses after 3 weeks.[7] In contrast our patient did not respond to imatinib. It must be noted that in our case treatment with imatinib was begun 29 months after diagnosis and 5 years after the first signs of the disease. Moreover several clinical and biological signs including anemia, thrombocytopenia, marked reticulin fibrosis, prominent splenomegaly at diagnosis, and additional chromosome abnormalities at the time of treatment with imatinib suggested that the patient had an accelerated phase of disease. Imatinib was given at the dose of 400 mg/day, which is lower than the dose recommended for accelerated phase CML. Some CML patients have imatinib-resistant disease as a consequence of acquired mutations in the ATP binding pocket of *BCR-ABL*.[9] Direct sequencing of the *H4-PDGFRB* kinase domain (corresponding to amino acids 478-886 of *PDGFRB*) was performed. The sequence of the kinase domain showed no coding changes. We hypothesize that other mechanisms of resistance were responsible for the lack of response observed in our patient. Drugs with activity against the imatinib-resistant variants are currently being developed and will be therapeutically important.[10]

Jean-Noël Bastie, Isabel Garcia,° Christine Terré,° Nicholas C. P. Cross,# Francois-Xavier Mahon,@ Sylvie Castaigne**

**Department of Hematology and Oncology, °Department of Biochemistry, Haematology and Cytogenetics, #Wessex Regional Genetics Laboratory, Salisbury District Hospital, UK; @Unité CNRS 2617, Université Bordeaux 2, France*

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Key words: atypical chronic myeloproliferative disorder, PDGFRB, t(5;10), imatinib mesylate.

Correspondence: Sylvie Castaigne, Department of Hematology and Oncology, Centre Hospitalier de Versailles, Le Chesnay, 78157, France. E-mail: scastaigne@ch-versailles.fr

References

1. Cross NCP, Melo JV, Feng L, Goldman JM. An optimized multiplex polymerase chain reaction (PCR) for detection of BCR-ABL fusion mRNAs in hematological disorders. Leukemia 1994; 8:186-9.
2. Apperley JF, Gardembas M, Melo JV,Russel-Jones R, Bain BJ, Baxter EJ, e al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor β. N Engl J Med 2002; 347:481-7.
3. Golub TR, Barker GF, Lovett M, Gilliland DG. Fusion of PDGF receptor β to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. Cell 1994;77:307-16.
4. Sterr EJ, Cross NCP. Myeloproliferative disorders with translocations of chromosome 5q31-35: role of the platelet-derived growth factor receptor β. Acta Haematol 2002;107:113-22.
5. Siena S, Sammarelli G, Grimoldi MG, Schiavo R, Nozza A, Roncalli M, et al. New reciprocal translocation t(5;10)(q33;q22) associated with atypical chronic myeloid leukemia. Haematologica 1999;84:369-72.
6. Kulkarni S, Heath C, Parker S, Chase A, Iqbal S, Pocock CF, et al. Fusion of H4/D10S170 to the platelet-derived growth factor receptor β in BCR-ABL-negative myeloproliferative disorders with a t(5;10)(q33;q21). Cancer Res 2000;60:3592-8.
7. Garcia JL, Font de Mora J, Hernandez Jesus M, Queizan JA, Gutierrez NC, Hernandez Jose M, et al. Imatinib mesylate elicits positive clinical response in atypical chronic myeloid leukemia involving the platelet-derived growth factor receptor β. Blood 2003;102:2699-700.
8. Schwaller J, Anastasiadou E, Cain D, Kutok J, Wojiski S, Williams IR, et al. H4(D10S170), a gene frequently rearranged in papillary thyroid carcinoma, is fused to the platelet-derived growth factor receptor β gene in atypical chronic myeloid leukemia with t(5;10)(q33;q22). Blood 2001;97:3910-8.
9. Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J, et al. Detection of BCR-ABL mutations in patients treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood 2003;102:276-83.
10. Shawver LK, Slamon D, Ullrich A. Smart drugs: tyrosine kinase inhibitors in cancer therapy. Cancer Cell 2002;2:117-25.

Chronic Myeloproliferative Disorders

The effects of hydroxyurea on *PRV-1* expression in patients with essential thrombocythemia and polycythemia vera

The study was designed to investigate the influence of hydroxyurea (HU) treatment on *PRV-1* expression. Eighteen newly diagnosed patients with essential thrombocythemia (ET) or polycythemia vera (PV) were included. HU significantly increased *PRV-1* gene expression in the early stage of treatment.

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Hopes of finding a pathognomonic molecular marker for the diagnosis of polycythemia vera (PV) were raised when the polycythemia rubra vera-1 (*PRV-1*) gene was