Leukemia (2005) 19, 671–673

www.nature.com/leu

SPOTLIGHT CORRESPONDENCE

Chronic myeloid leukaemia with BCR–ABL fusion genes located to both chromosomes 9, cyclic leukocytosis and nodal T-lymphoblastic transformation – durable complete remission following imatinib therapy

Leukemia (2005) **19,** 671–673. doi:10.1038/sj.leu.2403662
Published online 10 February 2005

TO THE EDITOR

The causative molecular event in chronic myeloid leukaemia (CML) is the formation of a BCR–ABL fusion gene, leading to expression of a constitutively active BCR–ABL tyrosine kinase. In most cases, the BCR–ABL fusion gene results from the translocation t(9;22), which creates a shortened chromosome 22 known as the Philadelphia (Ph) chromosome. Clinically, CML is characterised by three phases: chronic, accelerated and blastic phase. In one-third of blastic phases, the blast cells are lymphoid, usually of B-cell type. T-lymphoblastic transformation is a rare event.[1]

The tyrosine kinase inhibitor imatinib is a potent inhibitor of the BCR–ABL tyrosine kinase. The drug has effect in the blastic phase of CML, but responses in lymphoid blast crisis have been of short duration.

We report a patient with an unusual Ph translocation and spontaneous oscillation in peripheral blood counts who developed a nodal T-lymphoblastic transformation and obtained a complete and durable response to treatment with imatinib. A different splicing pattern was found for BCR–ABL in blood and affected lymph node tissue.

The patient was a 56-year-old man who presented with fever for 2 months and a white blood cell count of 14×10^9/l. The bone marrow microscopy was compatible with chronic phase CML. Conventional cytogenetic analysis of bone marrow cells identified a normal male karyotype (15/15 metaphases). However, FISH revealed two BCR–ABL fusion genes in all cells (15/15 metaphases), located at chromosome bands 9q34. One chromosome 22 showed a BCR signal while the other was negative for BCR (Figure 1a). Apart from 3 months treatment with hydroxyurea, the patient was followed without treatment for 26 months as his peripheral blood counts cycled spontaneously with a median period of 60 days (range 56–63 days). The neutrophil count varied between 3.3×10^9 and 89.3×10^9/l, and lymphocytes and CD34-positive cells varied in parallel, with lymphocytes between 1.6×10^9 and 7.9×10^9/l and CD34-positive cells between <1 and 120×10^3/ml. The platelet count varied inversely between 136 and 370×10^9/l. Haemoglobin varied between 6.8 and 8.8 mmol/l (Figure 2).

At 2 years after diagnosis the patient experienced fatigue, fever and night sweats and had enlarged axillary and inguinal glands, and enlarged glands in relation to the left iliac vessels. The bone marrow showed chronic phase CML. Cytogenetic analysis including FISH for BCR–ABL showed no change. Histological and immunological examination of a lymph node was compatible with lymphoblastic lymphoma of the T-cell type. The tumour cells were positive for CD3, CD5, Mib1 and TdT. FISH analysis of the lymph node tissue showed BCR–ABL positivity in most of the cells. In many cells, two fusion signals were found, compatible with the transformed cells having the same variant Ph translocation as the marrow cells (Figure 1b).

BCR–ABL fusion-transcripts were measured by real-time quantitative PCR as previously described,[2] but the levels of BCR–ABL were related to the 'house keeping' transcript beta-glucuronidase (GUS) instead of BCR.[3] The actual ratio BCR–ABL (b2a2 + b3a2)/GUS was 0.99 in blood and 1.66 in lymph node tissue, respectively. In blood, the ratio between b3a2 and b2a2 was 0.92 while b3a2 was expressed at a level 260 times higher compared with b2a2 in lymph node tissue at transformation.

The patient was started on treatment with imatinib 600 mg daily. After 3 weeks, he was clinically well without lymphadenopathy and after 5 weeks he was in complete haematologic remission. After 2 weeks, treatment with imatinib was temporarily stopped because of grade III nonhaematologic toxicity. A week later, imatinib was restarted at 400 mg daily with no recurrence of side effects. No evident cyclical changes in peripheral blood counts were seen during imatinib treatment. A major cytogenetic response (5% Ph+ cells, 1/20 metaphases) was obtained after 6 months. However, complete cytogenetic remission was not obtained after 2 years and the dose of imatinib was increased to 600 mg daily which led to complete cytogenetic remission. The ratio BCR–ABL/GUS in peripheral blood after 35 months treatment with imatinib was 1.6×10^{-4}, an almost 4 log reduction compared with the level at diagnosis. Only b3a2 transcripts were found at this time, while b2a2 transcripts were below the level of detection.

The patient is still in complete clinical, haematologic and cytogenetic remission 39+ months following start of treatment with imatinib.

The reported patient had spontaneous oscillation in peripheral blood neutrophil and lymphocyte counts, CD34-positive cells and platelet counts, indicating deregulation at the stem cell level. The mechanisms involved are largely unknown but abnormal responses to growth factors or accelerated cell loss through apoptosis may play an important role. Cycling was noted also during treatment with hydroxyurea but was no longer evident during treatment with imatinib. Cyclic changes in blood counts have been described in CML and it is well known that hydroxyurea does not abolish spontaneous oscillation in blood counts whereas treatment with noncell-cycle-specific drugs may abolish cycling.

At the time of nodal transformation, the marrow morphology was compatible with chronic phase CML. In 10 of 26 reported patients with T-lymphoblastic transformation of CML, a predominant extramedullary component was found.[4]

Treatment of lymphoid blast crisis in CML with imatinib has resulted in complete haematologic response in 20%, but most

Correspondence: Dr IH Dufva, Department of Haematology, Herlev Hospital, University of Copenhagen, Herlev Ringvej 75, 2730 Herlev, Denmark; Fax: +45 44535067;
E-mail: inhd@herlevhosp.kbhamt.dk
Received 11 October 2004; accepted 16 December 2004; Published online 10 February 2005

SPOTLIGHT

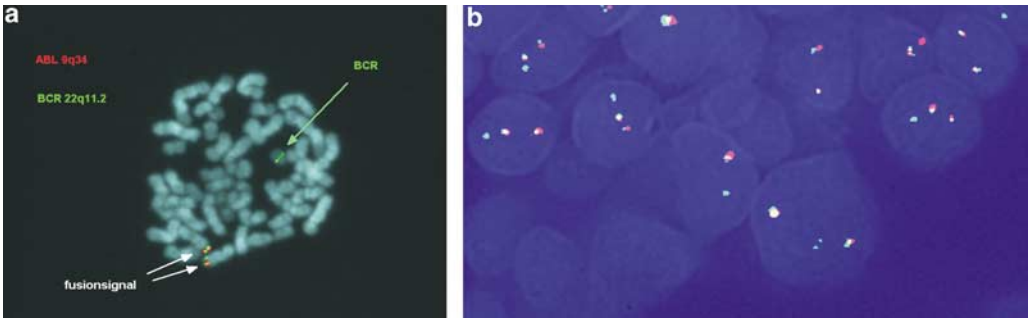

Figure 1 (a) FISH analysis of metaphase cells from bone marrow showing the two BCR–ABL fusion signals on chromosome 9 and one BCR signal on chromosome 22. (b) FISH image of interphase cells of lymph node tissue showing two BCR–ABL fusion signals (red/green or yellow) and one green signal representing native BCR. The analysis was performed using a BCR–ABL probe (LSI bcr/abl extra signal, Vysis, Downers Grove, IL, USA) as recommended by the manufacturer. Signals were visualised by an epifluorescence microscope (Zeiss Axioscop, Oberkochen, Germany), and images captured by the Quips Smart Capture FISH Imaging Software (Vysis).

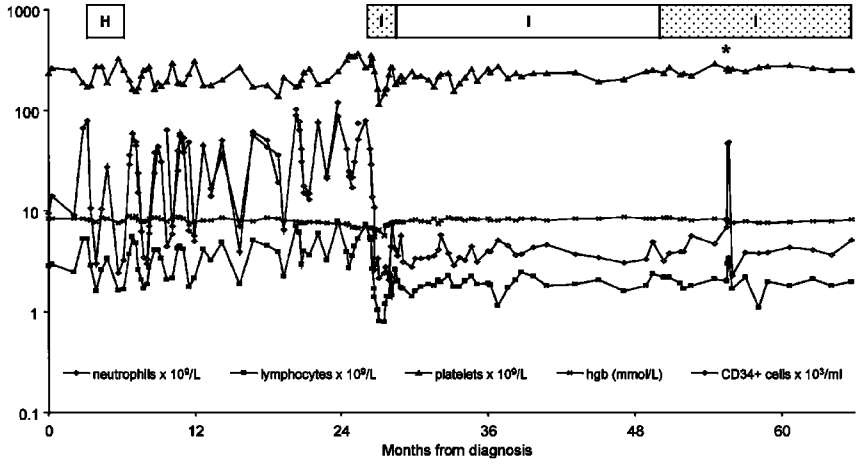

Figure 2 Haemoglobin (hgb), peripheral blood counts and CD34-positive cells in peripheral blood during the course of the disease. Spontaneous oscillations are seen before treatment with imatinib. Treatment with hydroxyurea is shown by the box marked H. Treatment with imatinib is shown by boxes marked I. Dotted boxes correspond to imatinib 600 mg daily, while the white box corresponds to 400 mg daily. Asterisk marks treatment with G-CSF for four days before autologous peripheral stem cell harvest.

responders relapse quickly. This is a report of the first patient with isolated nodal T-cell lymphoid blast crisis treated with imatinib. The patient is in complete clinical, haematologic and cytogenetic remission following 39+ months treatment. A case of relapsed T-cell lymphoid blast crisis of CML affecting both marrow and lymph nodes has recently been described. This patient also obtained a durable complete response on imatinib (26+ months).[1] In the present patient, low levels of BCR–ABL can still be detected in peripheral blood. In newly diagnosed patients with CML (treated with imatinib or interferon-α+Ara-C) who achieved a complete cytogenetic response, undetectable levels of BCR–ABL transcripts were only seen in four per cent of patients during the first 18 month's follow up.[5]

Of special interest, the reported patient had two BCR–ABL fusion genes in each cell located at 9q34. Five similar Ph-negative cases with double BCR–ABL fusion genes have been published previously;[6–9] three were in chronic phase,[6,8] two were in myeloid blast crisis.[7,9] The described variant Ph-translocation could be the result of either an insertion of the 5′ part of the BCR gene within or at the 5′ side of the ABL gene on chromosome 9 or by two successive translocations, a classic t(9;22) followed by a second translocation between both derivatives 9q+ and 22q−, masking the first chromosomal exchange. In two patients, FISH results indicated that the former mechanism was involved[6,7] although the latter mechanism could not be completely ruled out.[6] For one patient, it was shown that duplication of the BCR/ABL fusion gene occurred by mitotic recombination of part of chromosome 9.[6] Interestingly, one of the patients in chronic phase had highly variable leukocyte counts, which were difficult to stabilise with hydroxyurea. However it is not described whether the patient had cyclic leukocytosis.[6] None of the previously reported patients were treated with imatinib.

A different splicing pattern for the BCR–ABL transcripts was observed between peripheral blood dominated by myeloid cells expressing equal amounts of b2a2 and b3a2 transcripts, and T-lymphoblasts in lymph node tissue almost exclusively expressing b3a2. To our knowledge, a heterogeneous expression of alternatively spliced BCR–ABL fusion transcripts in different cell types has not been previously described. It is well known that splice-site selection can be determined by the cell in a tissue-specific manner. The different splicing pattern observed might represent tissue-specific regulation of splicing of the BCR/ABL mRNA. It is unknown if the alternative expression of

BCR–ABL in lymph node tissue had any impact on the process of transformation. Earlier studies have not been able to demonstrate a difference in clinical course between patients with b2a2 and b3a2 transcripts in CML.

The effect of imatinib in T-lymphoblastic transformation of CML should be further investigated in a larger series of patients.

IH Dufva[1]
H Karle[1]
K Brondum-Nielsen[2]
MK Andersen[3]
HO Madsen[4]
HE Johnsen[1]

[1]*Department of Haematology, Herlev Hospital, University of Copenhagen, Herlev, Denmark;* [2]*John F. Kennedy Institute, Glostrup, Denmark;* [3]*Department of Clinical Genetics, The Cytogenetic Laboratory, Section of Haematology/Oncology, Rigshospitalet, Copenhagen, Denmark; and* [4]*Tissue Typing Laboratory, Department of Clinical Immunology, Rigshospitalet, Copenhagen, Denmark*

References

1 Atallah E, Talpaz M, O'Brien S, Rios MB, Guo JQ, Arlinghaus R *et al.* Chronic myelogenous leukemia in T cell lymphoid blastic phase achieving durable complete cytogenetic and molecular remission with imatinib mesylate (STI571; Gleevec) therapy. *Cancer* 2002; **94**: 2996–2999.

2 Branford S, Hughes TP, Rudzki Z. Monitoring chronic myeloid leukaemia therapy by real-time quantitative PCR in blood is a reliable alternative to bone marrow cytogenetics. *Br J Haematol* 1999; **107**: 587–599.

3 Beillard E, Pallisgaard N, van der Velden VH, Bi W, Dee R, van der Schoot E *et al.* Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) - a Europe against cancer program. *Leukemia* 2003; **17**: 2474–2486.

4 Ye CC, Echeverri C, Anderson JE, Smith JL, Glassman A, Gulley ML *et al.* T-cell blast crisis of chronic myelogenous leukemia manifesting as a large mediastinal tumor. *Hum Pathol* 2002; **33**: 770–773.

5 Hughes TP, Kaeda J, Branford S, Rudzki Z, Hochhaus A, Hensley ML *et al.* Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med* 2003; **349**: 1423–1432.

6 Hagemeijer A, Buijs A, Smit E, Janssen B, Creemers GJ, Van der Plas D *et al.* Translocation of BCR to chromosome 9: a new cytogenetic variant detected by FISH in two Ph-negative, BCR-positive patients with chronic myeloid leukemia. *Genes Chromosomes Cancer* 1993; **8**: 237–245.

7 Vieira L, Alves AC, Marques B, Reis I, Jorge G, Ambrosio AP *et al.* Insertion of the 5′ part of BCR within the ABL gene at 9q34 in a Philadelphia-negative chronic myeloid leukemia. *Cancer Genet Cytogenet* 1999; **114**: 17–21.

8 Michalova K, Zemanova Z, Bkezinova J, Moravcova J, Oltova A, Sobotka J *et al.* Location of the BCR/ABL fusion genes on both chromosomes 9q34 in Ph negative chronic myeloid leukemia. *Leuk Lymphoma* 2002; **43**: 1695–1700.

9 Takahashi N, Miura I, Ohshima A, Utsumi S, Nimura T, Hashimoto K *et al.* Duplication of chromosome 9 carrying a BCR/ABL chimeric gene in Philadelphia chromosome negative chronic myeloid leukemia. *Cancer Genet Cytogenet* 1996; **89**: 166–169.