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Clinical and genetic studies of ETV6/ABL1-positive chronic myeloid leukaemia in blast crisis treated with imatinib mesylate

Aikaterini Barbouti,1 Tomas Ahlgren,2 Bertil Johansson,1 Mattias Höglund,1 Carin Lassen,1 Ingemar Turesson,2 Felix Mitelman1 and Thoas Fioretos1 1Department of Clinical Genetics, Lund University Hospital, and 2Department of Medicine, Section of Haematology, Malmö University Hospital, Sweden

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Summary. Most chronic myeloid leukaemia (CML) patients are genetically characterized by the t(9;22)(q34;q11), generating the BCR/ABL1 fusion gene. However, a few CML patients with rearrangements of 9q34 and 12p13, leading to ETV6/ABL1 chimaeras, have also been reported. Here we describe the clinical and genetic response to imatinib mesylate treatment of an ETV6/ABL1-positive CML patient diagnosed in blast crisis (BC). A chronic phase was achieved after acute myeloid leukaemia induction therapy. Then, treatment with imatinib mesylate (600 mg/d) was initiated and the effect was assessed clinically as well as genetically, including by repeated interphase fluorescence in situ hybridization studies. Until d 71 of imatinib mesylate therapy, stable improvements in the clinical and laboratory features were noted, and the frequency of ABL1-rearranged peripheral blood cells decreased from 56% to 11%. At d 92, an additional t(12;13)(p12;q13), with the 12p breakpoint proximal to ETV6, was found. The patient relapsed into BC 126 d after the start of the imatinib mesylate treatment and succumbed to the disease shortly afterwards. No mutations in the tyrosine kinase domain of ABL1 of the ETV6/ABL1 fusion were identified in the second BC. However, whereas the ETV6/ABL1 expression was seemingly the same at diagnosis and at second BC, the expression of ETV6 was markedly lower at the second BC. This decreased expression of wild-type ETV6 may have been a contributory factor for the relapse.

Keywords: chronic myeloid leukaemia, blast crisis, ETV6/ABL1, imatinib mesylate.

Chronic myeloid leukaemia (CML) is clinically characterized by three disease phases – a rather indolent chronic phase (CP), an accelerated phase (AP) and an aggressive, usually fatal, blast crisis (BC) – and genetically by the t(9;22)(q34;q11), which generates the BCR/ABL1 fusion gene encoding a protein with increased tyrosine kinase activity and transforming properties (Faderl et al, 1999). Whereas BCR/ABL1 is strongly implicated as an initiating event in the development of CML, the genetic mechanisms underlying disease progression are poorly understood, although several cytogenetic and molecular genetic changes occurring during, or even prior to, AP and BC have been identified (Deininger et al, 2000; Johansson et al, 2002).

To date, however, five CML patients and five acute leukaemias with variant ABL1 rearrangements, involving 9q34 and 12p13, but not 22q11, and resulting in ETV6/ABL1 chimaeras, have been reported (Papadopoulos et al, 1995; Brunel et al, 1996; Andreasson et al, 1997; Golub et al, 1997; Van Limbergen et al, 2001; La Starza et al, 2002; Lin et al, 2002; O’Brien et al, 2002). ETV6, encoding a nuclear transcriptional repressor which plays a role in yolk-sac angiogenesis and in the establishment of adult haematopoiesis (Wang et al, 1997, 1998), is now known to be fused to approximately 20 different genes, which mainly code for tyrosine kinases or transcription factors, in various haematological malignancies (Mitelman et al, 2002).

The ETV6/ABL1 fusion gives rise to a tyrosine kinase with a cytoplasmic localization and elevated enzymatic activity, which is able to transform factor-dependent cell lines to factor independent (Golub et al, 1996; Hannemann et al, 1998) and to induce myeloproliferative disease in mice (Millon et al, 2002). Recently, a patient with ETV6/ABL1-positive acute leukaemia was reported in whom treatment with imatinib mesylate (previously known as ST1-571, Glivec), a tyrosine kinase inhibitor of BCR/ABL1 and ABL (Druker, 2002), resulted in a transient clinical response (O’Brien et al, 2002).

In the present study, we present a more durable, albeit transient, clinical and genetic response to imatinib mesylate.
treatment of a 36-year-old man with an ETV6/ABL1-positive CML, diagnosed in BC. In addition, possible molecular genetic mechanisms underlying disease relapse and drug resistance were investigated, and a detailed fluorescence in situ hybridization (FISH) investigation, characterizing the complex chromosomal rearrangements that resulted in the ETV6/ABL1 fusion, was performed.

PATIENT AND METHODS

Case history and cytogenetic features. A 36-year-old man was admitted to the hospital in December 2000 with a few weeks’ history of fatigue, weight loss, thoracic pain and upper airway infection. At the age of 16 years, he had undergone surgery for Crohn’s disease, and he had a previous history of intravenous drug abuse and was hepatitis C positive. At admission, splenomegaly was noted and the peripheral blood values were as follows: haemoglobin (Hb) 9.6 g/dl, white blood cells (WBC) 238 × 10^9/l and platelets 88 × 10^9/l. The bone marrow smears were hypercellular with 31% blasts, 8% promyelocytes, 4% basophils and 13% eosinophils. The blasts stained positively for myeloperoxidase and no Auer rods could be seen. There were no dysplastic features in the neutrophils. The erythropoiesis was normoblastic without any dysplastic features. Few megakaryocytes were present. The bone marrow picture was compatible with acute myeloid leukaemia and, because of the marked basophilia and eosinophilia, CML in myeloid BC was considered. However, the cytogenetic analysis showed no t(9;22)(q34;q11). The karyotype was 45,XY, 7,t(9;12)(q34;q13)[20], but further FISH and molecular genetic characterizations of the t(9;12) showed that it was a complex rearrangement resulting in an ETV6/ABL1 fusion (see below).

Initially, leukapheresis was performed and treatment with intermittent low-dose cytarabine and etoposide was initiated, but a few days after admission the patient suffered multiple cerebral bleeds. During his recovery from the cerebral bleeds, repeated infusions of 200–400 mg cytosine arabinoside (Ara-C) and 100 mg etoposide were given to maintain the WBC below 100 × 10^9/l. AML induction therapy, consisting of mitoxantrone (12 mg/m^2) d 1–4, Ara-C (1000 mg/m^2 twice daily) d 1–4 and etoposide (100 mg/m^2) d 1–4, was eventually started in March 2001. The bone marrow examined 37 d post treatment was still hypercellular and dominated by granulocytopenia, but the maturation arrest was less pronounced. There were 4% blasts, 5% promyelocytes and 3% basophils. Megakaryocytes were now more abundant, most of them having normal nuclear segmentation, and only few micromegakaryocytes were present. The findings were consistent with CML CP, the only exception being the megakaryocyte morphology. Cyto genetic analysis revealed a minor cytogenetic response, with six out of 24 metaphases displaying a normal karyotype. Permission for the ‘compassionate use’ of imatinib mesylate was obtained from Novartis (Basel, Switzerland). Treatment was started in April 2001, at a dose of 600 mg/d, and the effect, summarized in Table I and Fig 1, was assessed every 2 weeks.

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>Neutrophils (× 10^9/l)</th>
<th>Blasts (× 10^9/l)</th>
<th>Basophils (× 10^9/l)</th>
<th>Platelets (× 10^9/l)</th>
<th>G-banded karyotype</th>
<th>G-banded karyotype with rearranged ABL1 signals (%)</th>
<th>C-band karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19</td>
<td>0.4</td>
<td>0.1</td>
<td>47</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>1.7</td>
<td>0.3</td>
<td>82</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
</tr>
<tr>
<td>10</td>
<td>2.3</td>
<td>2.4</td>
<td>0.2</td>
<td>62</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
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</tr>
<tr>
<td>20</td>
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<td>1.7</td>
<td>2.4</td>
<td>57</td>
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<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
</tr>
<tr>
<td>30</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
<td>57</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
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<tr>
<td>35</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
<td>57</td>
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<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
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<tr>
<td>40</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>57</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
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<tr>
<td>45</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
<td>57</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
<td>45,X,Y,7,t(9;12)(q34;q13)[18]/46,XY[6] 56,42,4,5 N D</td>
</tr>
</tbody>
</table>

WBC: white blood cells; Hb, haemoglobin; ND, not determined.

15 d, including by peripheral blood values and interphase FISH analyses (see below). Until d 71 of imatinib mesylate therapy, the white blood cell counts remained stable, the haemoglobin and platelet levels increased, and the frequency of ABL1-rearranged peripheral blood cells decreased from 56% to 11%. At d 92, however, the cytogenetic analysis revealed clonal evolution, with the karyotype being 45,XY,−7,t(9;12)[2]/45.idem,t(12;13)[p12;q13][10]/46,XY[13]. At this time, the bone marrow smears were hypocellular with a normal morphology, but the number of peripheral aberrant cells, as assessed by interphase FISH, had increased to 21%. Subsequently, at d 126 after starting imatinib mesylate, the patient relapsed into a second BC, with all metaphases analysed being abnormal, 45,XY,−7,t(9;12),t(12;13)[10], and he died shortly afterwards.

**FISH probes.** The following probes were used to characterize the t(9;12)(q34;q13) and, to some extent, the t(12;13)(p12;q13): whole chromosome painting (wcp) probes for chromosomes 9 and 12 (Vysis, Downers Grove, IL, USA), partial chromosome painting (pcp) probes for 12p and 12q (ALTechnologies, Arlington, VA, USA), a centromeric chromosome 12 probe (Vysis), subtelomeric (st) 9q and 12p probes (TelVysion DNA probes; Vysis), BCR/ABL1 and ETV6/RUNX1 dual-colour probes (LSI bcr/abl ES and LSI TEL/AML1 ES; Vysis), and the cosmide probes 179A6, 163E7, 148B6 and 244E8 covering the ETV6 gene from telomere to centromere (kindly provided by Dr P. Marynen, Leuven, Belgium), and YAC 964c10 that contains ETV6 and part of CDKN1B. The ABL1 component of the LSI bcr/abl ES probe (Vysis) was also used, in interphase FISH analyses, to evaluate the size of the malignant clone.

**Metaphase FISH analyses.** The bone marrow culturing and metaphase FISH procedures were performed as described previously (Barbouti et al. 2002). Biotinylated probes were detected either by 1 μg/ml avidin–Cy3 (Amersham Plase, UK) or Cy5, and digoxigenin-labelled probes were detected with antidigoxigenin fluorescein isothiocyanate (FITC). Chromosomes were counterstained with 4,6-diamidino-2-phenyl-indole (DAPI) and analysed in an Axioplan 2 microscope (Zeiss, Oberkochen, Germany) coupled to a cooled charge-coupled device camera and a 12-position filter wheel. The images were captured using the cytovision chromofluor system (Applied Imaging, Newcastle, UK). Whenever possible, and in the great majority of the analyses, at least 10 metaphases were evaluated.

**Metaphase FISH results.** Hybridizations with wcp9 and wcp12 showed that a large part of chromosome 12 was located on the tip of the q-arm of the der(9), as expected from the chromosome banding analysis, and that a small part of chromosome 9 was located on the tip of one of the arms of the der(12). FISH, using the commercial locus-specific probes for ABL1 and ETV6, showed three signals of ABL1, one on the normal chromosome 9, one on the der(9) and one on the der(12) on which the ABL1 probe co-localized with the ETV6 probe, indicating an ETV6/ABL1 fusion on the der(12) (Fig 2). Using pcp12p and pcp12q, it was shown that that 12q participated in the formation of der(9), while the der(12) consisted only of 12p material. Analyses with subtelomeric probes for 9q and 12p showed only st9q probe on the normal chromosome 9 and

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one on der(12), and one st12p probe on the normal chromosome 12 and one on the one tip of the der(12). In order to delineate further the breakpoints within ETV6, the cosmid probes 179A6, 163E7 and 148B6 were combined with wcp9 or wcp12 in three-colour FISH experiments. These analyses showed that the cosmids 179A6 and 163E7 co-localized on one arm of the der(12), whereas wcp9 and cosmid 148B6 co-localized on the other arm. Finally, hybridizations with ABL1 and wcp9 revealed that the signals of these probes were located on the opposite arms of the der(12). As outlined in Fig 3, these findings suggested that the seemingly simple and reciprocal t(9;12)(q34;q13) was, in fact, a very complex rearrangement, which should be designated as der(9)t(9;12)(q34;q11) and der(12)ins(12;9)(p13; q34q34)inv(12)(p13q11)t(9;12)(q34q11). FISH analysis of the t(12;13)(p12q13) occurring during clonal evolution, using the cosmid probes 179A6, 163E7, 148B6 and 244E8, and the YAC 964c10, showed that the 12p breakpoint was proximal to cosmid 244E8 and YAC 964c10 and thus centromeric to the ETV6 locus.

**Fig 2.** FISH analyses of the der(9) and der(12). ABL1 and RUNX1 (also designated CBFA2 or AML1) signals (Vysis BCR/ABL and TEL/AML1 probes) are shown in red, and TEL (also designated ETV6) and BCR signals are shown in green. The two red signals of the AML1 probe and the two green signals of the BCR probe are present on the two normal chromosomes 21 and 22 respectively. ABL1 signals (red) are present on the der(9) and on the normal chromosome 9. A normal ETV6 signal (green) is present on the normal chromosome 12. The yellow signal on the der(12) is generated by the co-localization of ETV6 (green) and ABL1 signals (red). A partial G-banding karyotype of the normal and derivative chromosomes 9 and 12 is shown (insert, bottom left).

Interphase FISH analyses. The ABL1 component of the BCR/ABL1 probe was used on uncultured peripheral blood samples. Hybridization of the BCR/ABL1 probe to normal interphase nuclei generated two red signals for ABL1 and two green signals for BCR, whereas three ABL1 signals and two BCR signals were seen in ETV6/ABL1-rearranged nuclei. More than 1000 interphase nuclei were scored in each investigation. Control analyses revealed a false-positive background rate of split ABL1 signals in < 5% of normal interphase nuclei. As shown in Table I, the frequencies of ABL1-rearranged peripheral blood cells decreased from 56% to 11% until d 71 of imatinib mesylate treatment. Then, the frequencies increased and, at the time of the second BC, almost 75% of the nuclei were abnormal.

Reverse transcription-polymerase chain reaction (RT-PCR) analyses of ETV6/ABL1. Total RNA, obtained from bone marrow cells at diagnosis and during the second BC, was extracted using the Trizol reagent, according to the manufacturer’s instructions (GibcoBRL, Life Technologies, Stockholm, Sweden). Five micrograms of total RNA were reverse transcribed and PCR amplified under identical conditions, as described in Fioretos et al (2001). The primers used for PCR amplification and the GenBank accession numbers on which they are based are listed in Table II. To detect a putative ETV6/ABL1 fusion transcript, RT-PCR was performed with the primer pairs TEL143F (exon 4 of ETV6) and ABL3478R (exon 3 of ABL1) (Andreasson et al, 1997) and 929U22 (exon 5 of ETV6) and 2291L21 (exon 4 of ABL1). Fragments of approximately 850 bp and 700 bp, respectively, were obtained in
the patient’s sample (Fig 4). The amplified products were purified and directly sequenced using the Big Dye sequencing kit (PE Applied Biosystems, Warrington, UK). The analysis revealed that nucleotide (nt) 1033 (exon 5) of ETV6 was fused in frame with nt 134 (exon 2) of ABL1 (GenBank accession nos. U11732 and NM_007313 respectively).

**Table II.** Primers used for PCR and sequencing.

<table>
<thead>
<tr>
<th>Designation</th>
<th>Sequence (5’→ 3’)</th>
<th>Direction</th>
<th>Position (nt)</th>
<th>Exon</th>
<th>Gene (GenBank accession number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEL143F</td>
<td>GCCGGAGGTCATACTGCATCAG</td>
<td>Forward</td>
<td>453–474</td>
<td>4</td>
<td>ETV6 (U11732) (Andreasson et al, 1997)</td>
</tr>
<tr>
<td>ABL3478R</td>
<td>ACCATCCCCATTGTGATAT</td>
<td>Reverse</td>
<td>312–352</td>
<td>3</td>
<td>ABL1 (NM_007313) (Andreasson et al, 1997)</td>
</tr>
<tr>
<td>973U23</td>
<td>CACATCATGGTCTCTGTCTCCCC</td>
<td>Forward</td>
<td>973–996</td>
<td>5</td>
<td>ETV6 (U11732)</td>
</tr>
<tr>
<td>3140L24</td>
<td>AGCTCCTTTTCCACTTGCGAG</td>
<td>Reverse</td>
<td>1559–1583</td>
<td>9</td>
<td>ABL1 (NM_007313)</td>
</tr>
<tr>
<td>2290U24</td>
<td>AGCCCAACAGCCACTGCTATG</td>
<td>Forward</td>
<td>709–733</td>
<td>4</td>
<td>ABL1 (NM_007313)</td>
</tr>
<tr>
<td>2291L21</td>
<td>TAGACAGTGCGCTTTGGCGCC</td>
<td>Reverse</td>
<td>710–731</td>
<td>4</td>
<td>ABL1 (NM_007313)</td>
</tr>
<tr>
<td>1294L23</td>
<td>GTTCGCGCCTCATGATTTCCATC</td>
<td>Reverse</td>
<td>1294–1317</td>
<td>7</td>
<td>ETV6 (U11732)</td>
</tr>
<tr>
<td>975U23</td>
<td>CATCATGTCCTGCTCTCCCGC</td>
<td>Forward</td>
<td>975–998</td>
<td>5</td>
<td>ETV6 (U11732)</td>
</tr>
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<td>2848L21</td>
<td>CCAGCTCTGGGTGCGAGTCC</td>
<td>Reverse</td>
<td>1267–1288</td>
<td>7</td>
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<tr>
<td>AKTIN F</td>
<td>CCTGCGCTTTGCCGATTC</td>
<td>Forward</td>
<td>25–42</td>
<td>1</td>
<td>β-actin (NM_001101) (Raff et al, 1997)</td>
</tr>
<tr>
<td>AKTIN R</td>
<td>GGATCTGGAGGATGTCAGTC</td>
<td>Reverse</td>
<td>650–628</td>
<td>4</td>
<td>β-actin (NM_001101) (Raff et al, 1997)</td>
</tr>
</tbody>
</table>

The primers 973U23 and 3140L24 (Table I) were used in an extra-long (XL) PCR approach (PE Applied Biosystems, Foster City, CA, USA) for the amplification of an ETV6/ABL1 fusion junction containing the tyrosine kinase domain of ABL1. The reactions were carried out in 100 µl of 1:3 diluted 3× XL buffer.

**Fig 3.** Schematic representation of a possible mechanism for the 9;12 rearrangement. Following four breaks – one 5’ and one 3’ of ABL1 exon 2 and 11, respectively, one 3’ of ETV6 exon 5, and a fourth breakpoint in 12q11 – the ABL1 gene is inserted into the ETV6 locus, which is followed by an inv(12)(p13q11) (inverting the 3’ end of ETV6) and a translocation between the long arms of chromosomes 9 and 12 respectively. For abbreviations and probes used, see text.

**Mutation analysis of the tyrosine kinase domain of ABL1 in the ETV6/ABL1 fusion at relapse.** The primers 973U23 and 3140L24 (Table I) were used in an extra-long (XL) PCR approach (PE Applied Biosystems, Foster City, CA, USA) for the amplification of an ETV6/ABL1 fusion junction containing the tyrosine kinase domain of ABL1. The reactions were carried out in 100 µl of 1:3 diluted 3× XL buffer.
ETV6/ABL1
β-actin
ETV6

Fig 4. Multiplex RT-PCR assay for quantification of ETV6 expression in relation to ETV6/ABL1 using β-actin as internal control. The 700 bp fragment of ETV6/ABL1 was amplified with the primer pair 929U22/2291L21, the 630 bp fragment of β-actin was amplified with the primer pair AKTIN F/AKTIN R, and the 400 bp fragment of ETV6 was amplified with the primer pair 929U22/1294L23. M1, 100 bp ladder; M2, 1 kb ladder; lane 1, sample in first BC; lane 2, sample in second BC; lane 3, the Ph-positive cell line K562; lane 4, blank.

1-1 mmol/l Mg(OAc)₂, 0.2 mmol/l of each dNTP, 1 unit of rTth DNA polymerase XL, 0.4 μmol/l of each of the forward and reverse primers, and 3 μl of cDNA. The condition for XL-PCR was set up as follows: denaturation for 1 min at 94°C, 32 cycles of 15 s at 94°C, and 10 min at 68°C, followed by a final extension for 10 min at 72°C. PCR-amplified fragments were excised from the gels, purified and directly sequenced. An approximately 2000 bp product, retaining the tyrosine kinase domain of ABL1, i.e. nt 778–1533 (NM_007313), was amplified. The primers 2290U24 and 3140L24 were used for direct sequencing of the tyrosine kinase domain of ABL1. No mutations were identified in the 817 bp fragment sequenced (corresponding to nt 734–1550).

Expression analyses of ETV6/ABL1 and ETV6. Semiquantitative analysis was performed, using a multiplex RT-PCR assay, in order to compare the expression levels of ETV6/ABL1 and ETV6 at diagnosis and during the second BC, with actin expression levels being used as an internal control in the same RT-PCR reaction. Dilution series were made to ensure that the multiplex RT-PCR did not reach saturation. The PCR mixture contained a common forward primer, 929U22, for amplification of both ETV6/ABL1 and ETV6, and the reverse primers 2291L21 and 1294L23 for ETV6/ABL1 and ETV6 respectively. The primer pair AKTIN F/AKTIN R, for amplification of β-actin, were added after the 10 first cycles of the PCR reaction.

The amplified products were approximately 700 bp for ETV6/ABL1, 630 bp for β-actin and 400 bp for ETV6. To quantify the relative levels of gene expressions, the amplified products were separated on 1.8% agarose gels and visualized using Vistra Green Nucleic Acid Stain gel (Amersham Pharmacia Biotech, Uppsala, Sweden) and the fluorescent image analyser FLA-3000 (Fujifilm, Stockholm, Sweden). No marked differences in ETV6/ABL1 expression were seen between the first and second BC, whereas the ETV6 expression was clearly lower at second BC in comparison to the first BC (Fig 4).

DISCUSSION

The formation of an ETV6/ABL1 fusion gene in haematological malignancies has to date only been reported in 10 patients: two acute lymphoblastic leukaemias (ALL) (Papadopoulos et al, 1995; Van Limbergen et al, 2001), three acute myeloid leukaemias (AML) (Golub et al, 1996; La Starza et al, 2002), four CML (Andreasen et al, 1997; Van Limbergen et al, 2001; Lin et al, 2002; O’Brien et al, 2002) and one atypical CML (Brunel et al, 1996). The rarity of this abnormality is further supported by studies that used RT-PCR and FISH to screen for this fusion gene in large numbers of ALL and chronic myeloid disorders, without detecting a single patient with this transcript (Janssen et al, 1995; Nilsson et al, 1998). The rare occurrence of ETV6/ABL1 has been explained by the opposite orientation of the two genes with respect to the centromere (Andreasen et al, 1997). Thus, at least three breaks are required for the formation of an inframe ETV6/ABL1 fusion gene. A similar rare incidence of the variant fusion genes MLL/T10(AF10) in AML and ALL, and of EWSR1/ERG in Ewing’s sarcoma, has also been attributed to their opposite orientation with respect to the centromere (Desmaze et al, 1997; Van Limbergen et al, 2002).

In the present patient, the simplest mechanism, requiring the least number of breaks, would be an insertion of the ABL1 gene (including at least exons 2–11) into the ETV6 locus (between exons 5 and 6) with a simultaneous inversion of chromosome 12 – inv(12)(p13q11) – and a translocation t(9;12)(q34;q11) (outlined in Fig 3).

Direct sequencing of the fragments amplified by RT-PCR revealed the fusion of exon 5 of ETV6 to exon 2 of ABL1. Among the nine informative cases described previously, two patients showed an identical inframe fusion product (Golub et al, 1996; Andreasen et al, 1997), six patients displayed an additional ETV6 exon 4/ABL1 exon 2 fusion (Van Limbergen et al, 2001; La Starza et al, 2002; Lin et al, 2002; O’Brien et al, 2002), whereas one patient showed an inframe fusion of ETV6 exon 4 to ABL1 exon 2 (Papadopoulos et al, 1995). The ETV6/ABL1 gene fuses the pointed (PNT) domain of ETV6, which is involved in the oligomerization of ETV6 and also interacts with other proteins involved in transcriptional repression (Sharrock, 2001), inframe with ABL1, which retains the tyrosine kinase domain.

Several observations suggest that ETV6/ABL1 and BCR/ABL1 may confer similar functions to the leukaemic cells. Both ETV6 and BCR contain oligomerization domains in their N-terminal parts that are included in the chimaeric genes, and this seems to be the mechanism by which the tyrosine kinase activity, contained within ABL1, becomes activated (McWhirter et al, 1993; Golub et al, 1996).
Moreover, both fusion proteins have been shown to activate similar signal transduction pathways, show comparable transforming activity and are inhibited by imatinib (Golub et al., 1996; Carroll et al., 1997; Voss et al., 2000).

In light of the biological similarities of ETV6/ABL1 and BCR/ABL1 and the now well-established effect of imatinib mesylate treatment in CML CP and BC (Druker et al., 2001; Sawyers et al., 2002), permission was obtained to treat the patient with this drug. After an initial treatment course with chemotherapy, CML CP with a partial cytogenetic response was achieved. Following imatinib mesylate treatment only, the patient obtained a complete haematological response and a steady decrease in the frequency of ABL1-rearranged peripheral blood cells, from an initial 56% to 11%. He remained in haematological remission for more than 92 d, clearly indicating a potent and sustained effect of imatinib mesylate on the leukaemic cells. Very recently, a similar case of a patient with ETV6/ABL1-positive leukaemia was described in whom treatment with imatinib mesylate showed beneficial effects, lending further support to our conclusion (O'Brien et al., 2002). This patient, also diagnosed in CML BC, was treated for 10 d with imatinib mesylate, resulting in a prompt initial effect with reduction of lymphadenopathy and WBC counts. However, the response was only transient and conventional chemotherapy had to be instituted at d 9 because of rapid clinical deterioration. Different secondary genetic aberrations or the fact that our patient obtained chemotherapy before administration of imatinib mesylate may be one of several explanations for the more durable remission in our patient. It seems, however, highly unlikely that the cytostatic treatment alone in our patient would result in the steady decline of genetically aberrant cells, not least because this treatment only resulted in a partial cytogenetic response.

The patient relapsed into a second BC 126 d after treatment initiation with imatinib mesylate. Because mutations in the catalytic part of the tyrosine kinase domain of ABL1 have been described in several CML BC patients developing resistance to imatinib mesylate (Gorre et al., 2001; Branford et al., 2002; von Bubnoff et al., 2002), we sequenced this region but did not identify any mutations. Other mechanisms of resistance to imatinib mesylate in BCR/ABL1-positive leukaemia include amplification or overexpression of BCR/ABL1 (le Coutre et al., 2000; Gorre et al., 2001; Sirulnik et al., 2001; Roche-Lestienne et al., 2002), but again no such alterations were observed in the present patient.

Before the emergence of clinically evident BC, an additional chromosomal abnormality, t(12;13)(p12;q13), was seen at d 92. The size of this aberrant clone increased from 10/25 metaphase cells at d 92, when an elevation in the number of ABL1-rearranged peripheral blood cells (21%) also was noted, to 10/10 at d 128 (Table 1). Interestingly, ALL cases with t(12;21)/ETV6-RUNX1(CBF) fusion show a deletion of the second ETV6 allele in 25–77% of the patients (Cave et al., 1997) and loss of expression at the protein level (Foirer et al., 1998). It has been suggested that normal ETV6 dimerizes with the ETV6/RUNX1(CBF) fusion protein and that deletion of normal ETV6 results in enhanced activity of the fusion protein (Golub et al., 1996; Cave et al., 1997). Because the additional t(12;13) observed before the emergence of the second BC showed a breakpoint in 12p13, corresponding to the location of ETV6, we performed FISH analysis to investigate whether ETV6 was disrupted by this translocation. However, the breakpoint was found to lie centromeric to the ETV6 locus and no gross deletion, as determined by FISH, was seen. In spite of this, the expression of ETV6 could still have been affected by the translocation and we, therefore, evaluated normal ETV6 and ETV6/ABL1 expression at both the first and second BC using a multiplex-semiquantitative PCR approach. While the level of ETV6/ABL1 expression was similar on both occasions, expression of ETV6 was markedly lower in the second BC (Fig 4), with the levels detected possibly originating from normal bone marrow cells. Hence, the decreased expression of wild-type ETV6 may very likely have been a contributory factor for the relapse.

A remarkable clinical effect of imatinib mesylate has now been documented in BCR/ABL1 (Kantarjian et al., 2002; Sawyers et al., 2002), ETV6/ABL1 (this report and O'Brien et al., 2002) and more recently also in ETV6/PDGFRB-positive leukaemia (Apperley et al., 2002). Hence, the detection of such fusion genes at diagnosis, or of variant fusion genes involving ABL1 or PDGFRB, and the consideration of imatinib mesylate treatment in these instances seem increasingly important. Furthermore, detailed genetic studies of individual patients failing treatment with imatinib mesylate may reveal important insights into how to alleviate resistance to this drug.

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