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

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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 (Million *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

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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 granulocytopoiesis, but the maturation arrest was less pronounced. There were 4% blasts, 3·5% 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. Cytogenetic 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

Table I. Peripheral blood values, interphase findings and G-banding karyotype of the present CML patient during imatinib mesylate treatment.

Days after treatment	WBC ($\times 10^9$/l)	Neutrophils ($\times 10^9$/l)	Basophils ($\times 10^9$/l)	Blasts ($\times 10^9$/l)	Platelets ($\times 10^9$/l)	Hb (g/dl)	Nuclei with rearranged *ABL1* signals (%)	G-banded karyotype
0	4·8	2·7	0·2	0·1	47	8·5	56	45,XY,−7,t(9;12)(q34;q13)[18]/46,XY[6]
15	3·0	1·7	< 0·1	0·4	32	8·4	25	ND
30	2·4	1·3	0·2	0	52	9·4	22	ND
44	3·5	1·7	0·2	0	82	9·7	17	ND
57	3·8	2·4	0·2	0	102	10·3	14	ND
71	4·2	3·0	< 0·1	0	98	11·5	11	ND
92	4·5	3·1	< 0·1	0	114	13·3	21	45,XY,−7,t(9;12)[2]/45,idem,t(12;13)(p12;q13)[10]/46,XY[13]
126	59	2·2	3·2	21	19	96	ND	ND
127	66	2·7	4·0	27	18	84	ND	ND
128	72	1·2	5·0	37	14	80	74	45,XY,−7,t(9;12),t(12;13)[10]

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

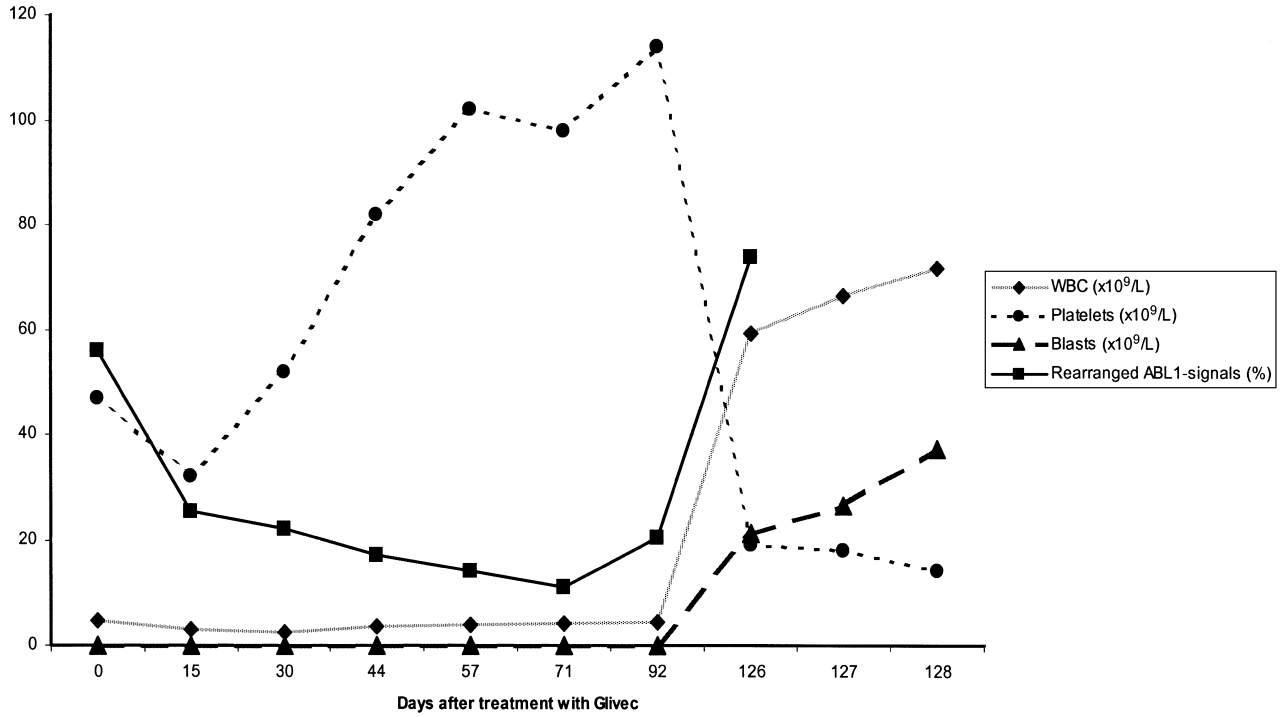

Fig 1. Peripheral blood values and interphase FISH findings during the course of imatinib mesylate treatment.

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 cosmid 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 CYTO VISION 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 one st9q probe on the normal chromosome 9 and

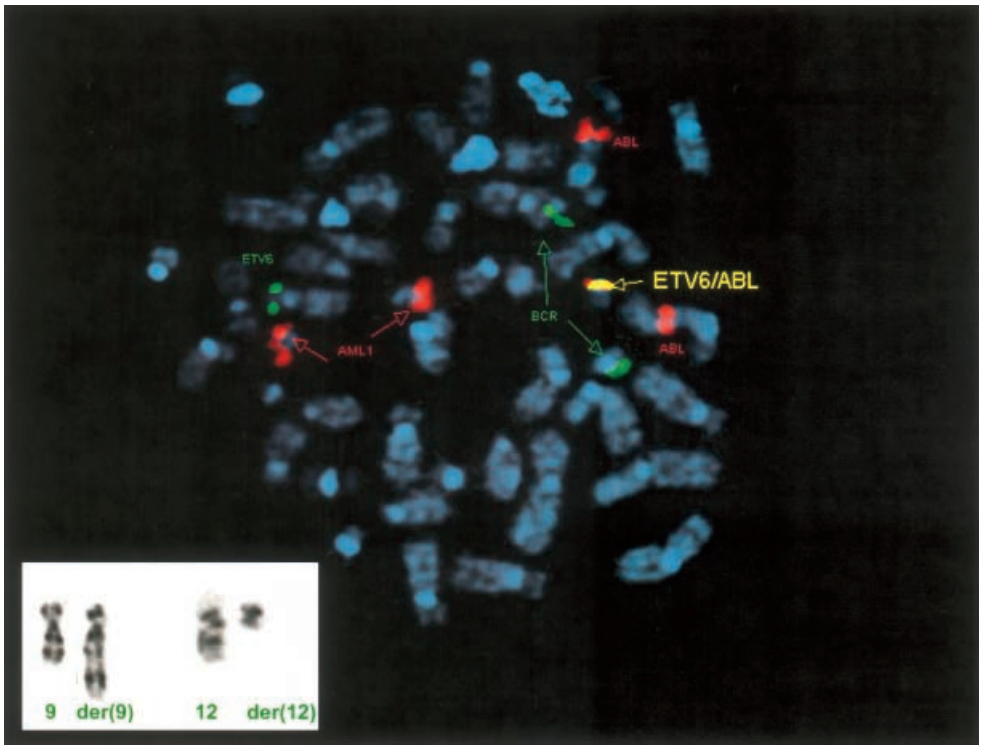

Fig 2. FISH analyses of the der(9) and der(12). *ABL1* and *RUNX1* (also designated *CBFA2* or *AML1*) signals (Vysis *BCR/ABL* and *TEL/AML1* probes) are shown in red, and *TEL* (also designated *ETV6*) and *BCR* signals are shown in green. The two red signals of the *AML1* probe and the two green signals 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).

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)(q34;q11). FISH analysis of the t(12;13)(p12;q13) 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.

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

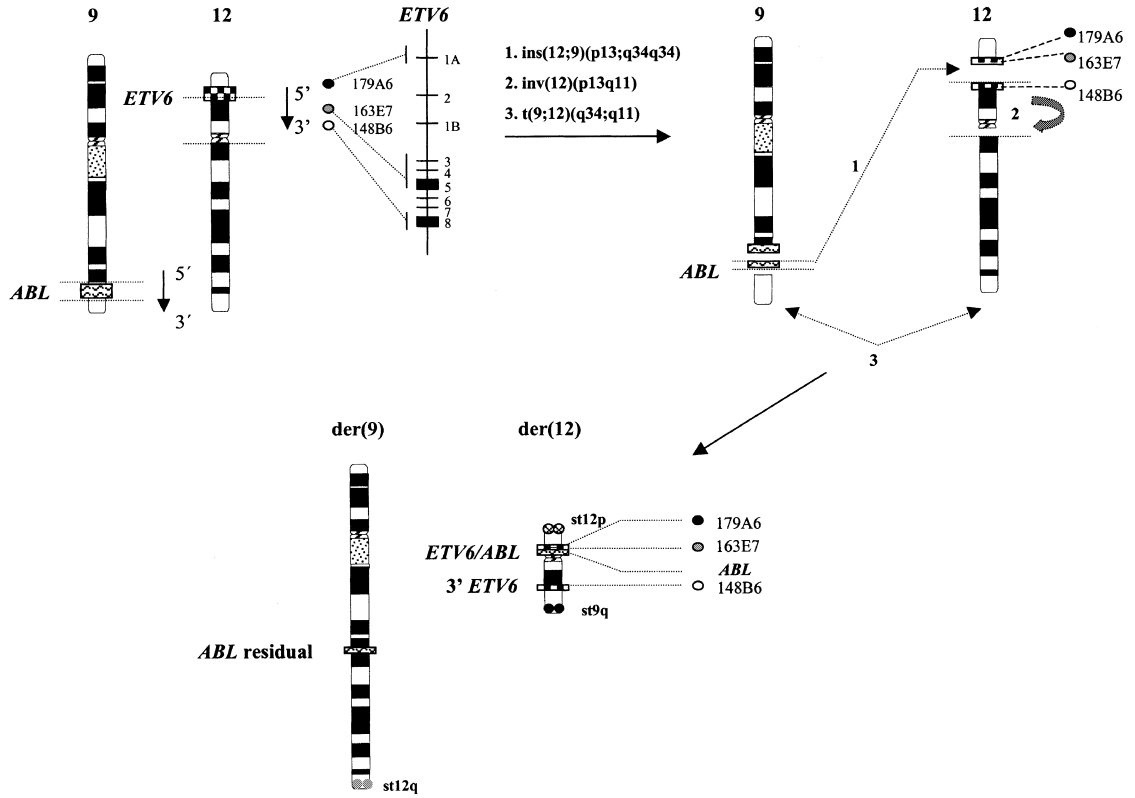

Fig 3. Schematic representation of a possible mechanism for the 9;12 rearrangement. Following four breaks – one 5′ and one 3′ of *ABL*1 exon 2 and 11, respectively, one 3′ of *ETV6* exon 5, and a fourth breakpoint in 12q11 – the *ABL1* gene is inserted into the *ETV6* locus, which is followed by an inv(12)(p13q11) (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.

Table II. Primers used for PCR and sequencing.

Designation	Sequence (5′ → 3′)	Direction	Position (nt)	Exon	Gene (GenBank accession number)
TEL143F	GCCGGAGGTCATACTGCATCAG	Forward	453–474	4	*ETV6* (U11732) (Andreasson *et al*, 1997)
ABL3478R	ACCATTCCCCATTGTGATTAT	Reverse	332–352	3	*ABL1* (NM_007313) (Andreasson *et al*, 1997)
973U23	CACATCATGGTCTCTGTCTCCCC	Forward	973–996	5	*ETV6* (U11732)
3140L24	AGCTCCTTTTCCACTTCGTCTGAG	Reverse	1559–1583	9	*ABL1* (NM_007313)
2290U24	AGCGCAACAAGCCCACTGTCTATG	Forward	709–733	4	*ABL1* (NM_007313)
929U22	AGCCCATCAACCTCTCTCATCG	Forward	929–951	5	*ETV6* (U11732)
2291L21	TAGACAGTGGGCTTGTTGCGC	Reverse	710–731	4	*ABL1* (NM_007313)
1294L23	GTTCGGCCACTCATGATTTCATC	Reverse	1294–1317	7	*ETV6* (U11732)
975U23	CATCATGGTCTCTGTCTCCCCGC	Forward	975–998	5	*ETV6* (U11732)
2848L21	CCAGGCTCTCGGGTGCAGTCC	Reverse	1267–1288	7	*ABL1* (NM_007313)
AKTIN F	CCTCGCCTTTGCCGATCC	Forward	25–42	1	β-actin (NM_001101) (Raff *et al*, 1997)
AKTIN R	GGATCTTCATGAGGTAGTCAGTC	Reverse	650–628	4	β-actin (NM_001101) (Raff *et al*, 1997)

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 inframe with nt 134 (exon 2) of *ABL1* (GenBank accession nos. U11732 and NM_007313 respectively).

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 3·3 × XL buffer,

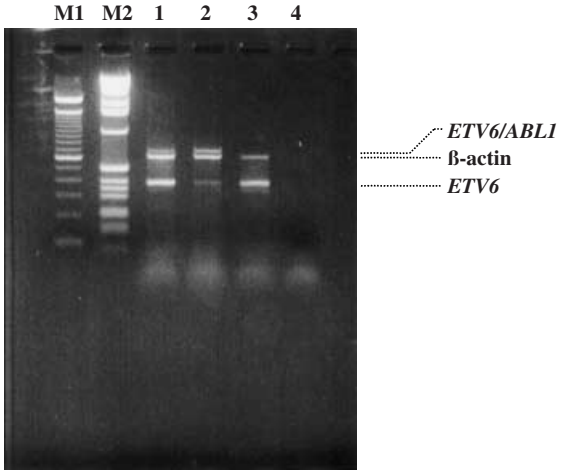

Fig 4. Multiplex RT-PCR assay for quantification of *ETV6* expression in relation to *ETV6/ABL1* using β-actin as internal control. The 700 bp fragment of *ETV6/ABL1* was amplified with the primer pair 929U22/2291L21, the 630 bp fragment of β-actin was amplified with the primer pair AKTIN F/AKTIN R, 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 rT*th* 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 (Andreasson *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 (Andreasson *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; Andreasson *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 (Sharrocks, 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 imitanib (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; Sirulink *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 I). Interestingly, ALL cases with t(12;21)/*ETV6-RUNX1*(*CBFA2*) 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 (Poirel *et al,* 1998). It has been suggested that normal ETV6 dimerizes with the ETV6/RUNX1(CBFA2) 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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