Chronic Myelogenous Leukemia

Complex variant Philadelphia translocations involving the short arm of chromosome 6 in chronic myeloid leukemia

Roberta La Starza,* Nicoletta Testoni,°
Marina Lafage-Pochitaloff,# Deborah Ruggeri,°
Emanuela Ottaviani,° Gianni Perla,@
Massimo Fabrizio Martelli,* Peter Marynen,^
Cristina Mecucci*

Correspondence: Cristina Mecucci MD, PhD, Hematology and Bone Marrow Transplantation Unit, University of Perugia, Policlinico Monteluce, via Brunamonti, Perugia, Italy.
Phone: international +39.075.5783808. Fax: international +39.075.5783691. E-mail: crimecux@unipg.it

*Hematology and Bone Marrow Transplantation Unit, University of Perugia, Italy; °Institute of Hematology and Medical Oncology "Seragnoli", University of Bologna, Italy; #Institut Paoli Calmettes, INSERM U119, Marseille, France; @Hematology, Opera Padre Pio, Ospedale S. Giovanni Rotondo, Italy; ^Center for Human Genetics, University of Leuven and Flanders Interuniversity Institute for Biotechnology, Leuven, Belgium

Background and Objectives. Around 5% of chronic myeloid leukemias (CML) are characterized by complex variant Philadelphia (Ph) translocations involving one or more chromosomal regions in addition to 9 and 22. The BCR/ABL1 fusion gene is usually found on der(22). The additional gene(s) involved in complex variant Ph rearrangements have not been characterized.

Design and Methods. We performed fluorescent *in situ* hybridization (FISH) in three complex variant Ph translocations involving the short arm of chromosome 6 in addition to 9 and 22. The BCR/ABL1 D-FISH probe was applied to localize the BCR/ABL1 fusion gene as well as the 5'ABL1 and the 3'BCR. Locus-specific probes were used to narrow the 6p breakpoint.

Results. In all cases the BCR/ABL1 fusion gene was located on the Ph chromosome whereas the reciprocal ABL1/BCR gene was detected only in patient #2. On 6p, breakpoints were narrowed to three different regions: centromeric to the human major histocompatibility complex (MHC), between PAC 524E15 and PAC162J16, in the first patient, and telomeric to the MHC, between PAC 329A5 and PAC 145H9, and between PAC 136B1 and PAC 206F19, in the second and third patients, respectively. In patients #2 and 3 a chromosomal rearrangement different from a true complex variant was discovered. In both cases, a classical t(9;22) was associated with an additional translocation involving the der(9)t(9;22).

Interpretation and Conclusions. Rearrangements at 6p in complex Ph aberrations involve more than one gene/locus. Classical t(9;22), masked by additional chromosomal rearrangements, can resemble complex variant Ph translocations, and can be detected only using appropriate FISH probes.
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Key words: Ph rearrangement, complex variant translocation, 6p chromosome.

Masked or variant Philadelphia (Ph) rearrangements characterize 5-8% of chronic myeloid leukemia (CML).[1] Masked Ph chromosomes can be found in cases with a normal karyotype, as a result of a cryptic rearrangement, or in cases with a complex karyotype in which the typical t(9;22) is not detectable at karyotypic level. Variant Ph translocations are cytogenetically classified as simple variants, involving chromosome 22 and a chromosome other than 9, and complex variants, involving chromosomes 9, 22, and one or more other chromosomes. The common molecular event underlying classical, masked, and variant Ph translocations, is the production of the BCR/ABL1 fusion gene. This chimeric gene is located on der(22) or, less frequently, in one of the other chromosomes participating in the translocation.

Evaluation of prognostic features in a limited number of CMLs with classical or variant Ph rearrangements gave controversial results.[2,3] Cytogenetically, a higher frequency of additional structural abnormalities have been shown in patients carrying variant Ph.[4] Whether variant Ph translocations originate from sequential events, or from a one-step mechanism with multiple chromosomal breaks, at once, has not been definitively established. Data supporting both hypotheses have been

reported.[1]

We performed a fluorescent *in situ* hybridization (FISH) study in three CML patients with complex changes involving a 6p region with the aim of identifying the molecular events underlying the BCR/ABL1 fusion, and of narrowing the 6p breakpoint participating in the variant Ph.

Design and Methods

Patients

Three patients with a diagnosis of CML and variant Ph translocations involving the short arm of chromosome 6 were selected. The following Institutions participated in the study: the Hematology and Oncology Department of the University of Bologna, the Laboratoire de Cytogénétique Hématologique, Institute of Paoli Calmettes, in Marseille, and the Hematology Unit of the University of Perugia.

Cytogenetics and reverse transcription polymerase chain reaction (RT-PCR)

Cytogenetic studies were done on bone marrow samples in blastic phase in patient #1, and in chronic phase, during treatment with α-interpheron (α-IFN), in patients #2 and 3. Chromosomes were G-banded after short-term cultures (24 or 48 hours). Karyotypes were described according to the *International System for Human Cytogenetic Nomenclature*.[5] Mononuclear cells from bone marrow were obtained by Ficoll-Hypaque density gradient centrifugation and stored at –80°C in guanidium thiocyanate. Total cellular RNA extraction and qualitative RT-PCR were performed at diagnosis as previously described.[6,7]

FISH

FISH was performed as already described elsewhere.[8] The BCR/ABL1 rearrangement was studied by applying the BCR/ABL1 D-FISH probe (BCR, 500 Kb in red, and ABL1 600 Kb in green, Oncor, Gaithersburg, MD, USA). Paintings for chromosomes 6p, 20, and 22 (ListarFish, Milan, Italy; Oncor, Gaithersburg, MD, USA), labeled with biotin or digoxigenin, were used in patient #2. Breakpoints at 6p were narrowed with BAC (b) and PAC (p) clones, labeled with biotin, mapped from telomere to centromere as follows: p409K9-p476O18-b82M9-p206F19-p136B1-p145H9-p90J20-p430A16-p153G14-p329A5-p162J16-p524E15-p50J22-p1043E3. A centromeric probe for chromosome 6 (D6Z1, kindly provided by Dr. M. Stul, University of Leuven), labeled with digoxigenin was added in the experiments with 6p locus-specific probes. At least seven abnormal cells were analyzed by FISH plus G-banding. Data were collected on a fluorescence microscope Olympus (Provis) equipped with a CCD camera (Sensys) run by PathVysion software (Vysis).

Results

Patient #1 was a 56-year old female who refused α-IFN therapy and underwent treatment with hydroxyurea until she died, in blastic phase, 39 months after diagnosis. Patient #2 was a 49-year old female who underwent α-IFN treatment from diagnosis. She was alive at last follow-up 9 years after diagnosis. Patient #3 was a 68-year old man who was treated with α-IFN achieving complete cytogenetic remission 16 months after diagnosis. After 24 months, following discontinuation of α-IFN, Ph-positive cells reappeared. He is still in chronic phase, treated with hydroxyurea, 5 years from diagnosis.

Cytogenetics and RT-PCR

In patient #1 all the analyzed cells were abnormal, showing the following karyotype: 46,XX,t(6;9;22)(p21;q34;q11)[3/42]; 47,XX,t(6;9;22)(p21;q34;q11),+8,i(17)(q10) [39/42]. In patient #2, the karyotype was: 46,XX[11/25]/46,XX,t(6;9;22)(p21;q34;q11), add(20)(p)[14/25]; in patient #3: the karyotype was: 46,XY[19/26]/45,X,-Y,t(6;9;22)(p24;q34;q11)[7/26]. Patients #1 and #3 showed a b3a2 transcript, whereas patient #2 had a b2a2 transcript.

FISH

The BCR/ABL1 D-FISH probe[9] in metaphases with t(9;22)(q34;q11) gives a BCR/ABL1 fusion signal on the Ph chromosome, an ABL1/BCR fusion signal on the der(9)(q34), a BCR signal (red) on the normal 22, and an ABL1 signal (green) on the normal chromosome 9. In patient #1, the BCR/ABL1 fusion signal was located on the Ph chromosome, an ABL1 signal was present on the normal 9 and on the der(9), and a BCR signal was present on the normal 22 and on the der(6) (Figure 1A). In patient #2, the BCR/ABL1 fusion signal was located on the Ph chromosome and the ABL1/BCR fusion signal was detected on the der(9)(q34). An ABL1 signal was present on the normal 9, and a BCR signal on the normal 22, as expected (Figure 1B). In patient #3, the BCR/ABL1 fusion signal was located on the Ph chromosome. An ABL1 signal was present on the normal 9 and on the der(6). A BCR signal was present on the normal 22, while the other BCR signal was missing (Figure 1C).

In patient #2, painting 6p labeled the short arm of the normal 6, partially the der(6), and the
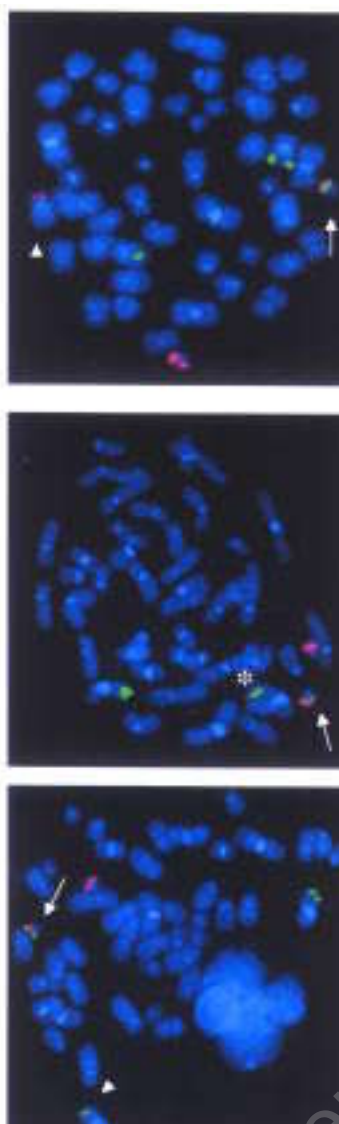
Figure 1. Examples of FISH experiments with a double color BCR/ABL1 D-FISH probe. In all three patients the BCR/ABL1 fusion is located on der(22) (arrow). A) In patient #1, a BCR signal (red) is present on the normal 22 and on der(6) (arrowhead), and an ABL1 signal (green) is present on the normal 9 and on der(9). B) In patient #2, a fusion signal corresponding to the ABL1/BCR fusion gene is present on der (9) (asterisk), a BCR signal on the normal 22, and an ABL1 signal on the normal 9. C) In patient #3, a BCR signal is present on the normal 22, and an ABL1 signal is present on the normal 9, and translocated to der(6) (arrowhead). The signal corresponding to the 3′BCR is missing.

ing the short arm of chromosome 6 are reported.

This FISH study showed that chromosomal changes in patients #2 and #3 did not fit the definition of complex variant Ph translocation as derived from conventional cytogenetics. In both cases, a classical t(9;22) with the fusion gene between 5'BCR and 3'ABL1 on der(22) was associated with a second exchange involving an additional breakpoint on the der(9), derived from the t(9;22). Moreover, while in patient #2 a reciprocal chimeric gene ABL1/BCR was present on chromosome 9, in patient #3, the 3′BCR was lost. Altogether, these data, plus results from specific paintings, showed that exchange of material between chromosome 9, 6, and 20, likely followed the Ph translocation in the second patient (Pt #2 of Figure 2), with an additional breakpoint occurring within the 22 material translocated, as usual, next to the 5'ABL1 on der(9). This case was the only one in this series with a chimeric 5'ABL1/3'BCR fusion gene on the der(9). This event was found in around 70% of CML cases by Melo *et al.*[10] In patient #3, there was an independent translocation between the same chromosome 9 involved in the Ph rearrangement and chromosome 6. The second breakpoint on 9q fell upstream of the 5'ABL1 gene which moved to the 6p arm (Pt #3 of Figure 2). Whether all breaks occurred simultaneously, or at different times, during the origin of Ph in this patient, cannot be determined at present. In our series, despite the cytogenetic appearance, only case #1 resulted to be a true variant Ph with three chromosomal breakpoints leading to the BCR/ABL1 fusion on der(22), translocation of the 3′BCR to 6p, rather than to the der(9), and juxtaposition of 6p material to the 5' of ABL1 gene on der(9) (Pt #1 of Figure 2).

We wish to emphasize that, without application of a double color FISH probe capable of detecting both the BCR/ABL1 and the ABL1/BCR fusion genes, complex Ph translocations may not be distinguished from complex changes superimposing a simple t(9;22).

Deletions at the t(9;22) breakpoints, involving the 5'ABL1 and/or the 3'BCR locus on derivative 9, represent an independent negative prognostic factor.[11,12] A significant correlation with variant Ph-chromosomes has been reported, and deletions have been hypothesized to be the consequence of complex recombination events.[11] In our series, deletion of the 3'BCR locus was detected only in patient #3 with a classical t(9;22) followed by an additional rearrangement of the der(9). The CML in this case responded well to α-IFN as documented

add(20)(p). Painting 20 labeled the normal 20, the der(20), and the most telomeric part of the long arm of der(9). Painting 22 marked the normal 22 and the der(22), as expected, as well as the der(9) and the der(6). Breakpoints on 6p were narrowed down to three different regions: in patient #1, the breakpoint fell between PAC 524E22 and PAC162J16, which was the first pac translocated to the der(9); in patient #2 it was between PAC 329A5 and PAC 145H9, which moved to the add(20)(p); in patient #3 it was between PAC 136B1, kept on der(6), and PAC 206f19, translocated to der(9).

Discussion

Cytogenetic, molecular, and FISH studies in three CML cases with complex Ph translocations involv-
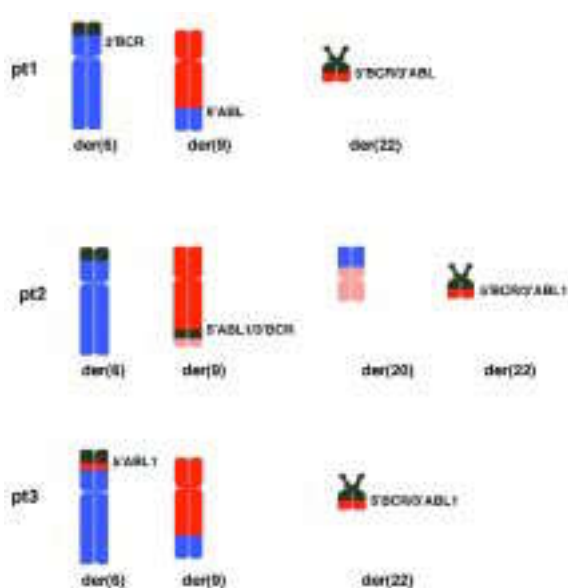
Figure 2. Schematic representation of chromosomal changes occurring in patients (Pt) #1, 2, and 3 based on FISH results. Pt #1: FISH with the BCR/ABL1 D-FISH probe was consistent with a true complex variant Ph-translocation showing the BCR/ABL1 fusion gene on the der(22), the 5'ABL1 kept on der(9), and the 3'BCR translocated to the der(6). Pt #2: The BCR/ABL1 D-FISH probe detected two fusion signals on der(22) and on der(9). Paintings for the short arm of chromosome 6, for chromosome 20, and 22 revealed a reciprocal exchange between chromosomes 6, 9, and 20. Pt #3: The BCR/ABL1 probe showed a fusion signal on der(22), the 5'ABL translocated to the der(6), while the 3'BCR was missing. Translocation of the 5'ABL1 to the der(6) is the result of a reciprocal exchange between chromosome 6 and der(9) in which the breakpoint was centromeric to the ABL1 gene.

by a long-term follow-up. So far, involvement of 6p in Ph translocations has been found in three CML cases with a three-way variant, i.e., t(6;9;22), and in two CML with four-way variants involving either chromosome 12q or 11q.[13-16] In four out of five published cases and in our patients #1 and #2, the breakpoint on 6p was assigned to band 6p21. The 6p21 breakpoint in our true variant Ph mapped centromeric to the MHC and was flanked by PAC 524E15 and PAC 162J16 in a segment of DNA of about 150 kb, containing two genes encoding for two kinases, i.e., MAPK14 and MAPK13. Unfortunately, material was not available to investigate whether one of these genes was fused to the 3′ of BCR. As far as we know, this is the third case in which molecular information is available on the chromosomal breakpoint involved in a true variant. Dierlamm et al.[17] reported on one case of CML with

a t(Y;9;22)(q12;q34;q11) variant, in which the breakpoint on chromosome Yq12 was located, by FISH, centromerically to the SYBL1 gene. Koduru et al.[18] in a case of CML with a Ph variant involving chromosome 11q13, i.e., t(9;22;11) (q34;q11;q13), showed that the 3'-half of the glutathione S-transferase Pi (GST-Pi) gene, at 11q13, was fused to the 3'-part of BCR. However, mRNA from fusion between GST-Pi and BCR was not identified.

With respect to 6p involvement in Ph-positive leukemic cells, we found a spreading of chromosomal breakpoints in two bands: at 6p21, namely at the centromeric (patient #1) or telomeric (patient #2) side of the MHC, and at 6p24, in a region of around 400Kb, between PAC 136B1 and PAC 206F19 (patient #3).

In conclusion, the present study shows that more than one locus/gene is involved in 6p rearrangements occurring in Ph-positive cells. Moreover, for the first time, a clear distinction emerged between true variant translocations and classical Ph masked by additional structural anomalies. While in the former group of changes breakpoints at ABL1, BCR, and additional loci occur simultaneously, in the latter group the typical Ph rearrangement is independent and likely precedes the other changes. In order to elucidate the biological and clinical significance(s) of different types of complex changes in Ph-positive leukemic cells, a FISH approach is strongly recommended to identify classical Ph translocations plus additional anomalies among variant Ph involving either 6p or other chromosomal regions.

Disclosures

Conflict of interest: none.
Redundant publications: no substantial overlapping with previous papers.

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Peer Review Outcomes

What is already known on this topic

There are 4 types of chromosomal aberrations associated with CML. The first class which occurs in nearly all CML patients is the Philadelphia chromosome (Ph) which is associated with the translocation t(9;22)(q34;q11). The second class which occurs in the minority of patients, is a variant Ph-translocation. In the variant translocation, the Ph-chromosome can be recognized, and the reversed translocation segment 22q11>qter is translocated to a third chromosome, which in turn translocates its segment to 9q34. The third class of CML patients are those with Ph-negativity and the fourth class are the complex aberrations which represent clonal evolution.

What this study adds

Starza *et al.* describe 3 cases of variant translocation involving the short arm of chromosome 6.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Dina Ben-Yehuda, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Prof. Ben-Yehuda and the Editors. Manuscript received August 3, 2001; accepted October 6, 2001.

Potential implications for clinical practice

FISH analysis should be used to distinguish between variant and complex translocations, the latter representing clonal evolution. Patients with a proven *true* variant translocation at diagnosis have the same prognostic features as patients with simple Ph-translocation.

Dina Ben-Yehuda, Associate Editor