

# Conventional and molecular cytogenetic studies to characterize 32 complex variant Philadelphia translocations in patients with chronic myeloid leukemia

DOLORS COSTA<sup>1</sup>, JAVIER GRAU<sup>2</sup>, BLANCA ESPINET<sup>3</sup>, AMPARO ARIAS<sup>1</sup>, CÁNDIDA GÓMEZ<sup>1</sup>,  
MÓNICA LÓPEZ-GUERRA<sup>1</sup>, MERITXELL NOMDEDEU<sup>4</sup> and FRANCISCO CERVANTES<sup>5</sup>

<sup>1</sup>Hematopathology Section, Hospital Clínic, Barcelona, Catalonia 08036; <sup>2</sup>Hospital Germans Trias i Pujol, Badalona, Catalonia 08916; <sup>3</sup>Molecular Cytogenetics Laboratory, Pathology Service, Hospital del Mar, Barcelona, Catalonia 08003; <sup>4</sup>Department of Hematology, Hospital Plató, Barcelona, Catalonia 08006; <sup>5</sup>Department of Hematology, Hospital Clínic, IDIBAPS, Barcelona, Catalonia 08036, Spain

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**Abstract.** *BCR/ABL1* gene fusion is the hallmark of chronic myeloid leukemia (CML), and is generated in 5-10% of patients by a variant translocation involving 9q34, 22q11.2 and one or more additional genomic regions. The objective of the present study was to characterize, by conventional and molecular cytogenetics, 32 complex variant Philadelphia (Ph) translocations present at diagnosis in patients with CML. The chromosomes most frequently involved were 1 and 5, and the breakpoint most frequently involved was 12p13. The q-chromosome arm was more frequently involved (60%) than the p-arm. The breakpoints were located in the G-light bands in the majority of cases (85%). Additional chromosomal abnormalities were observed in 6 out of 32 (19%) patients. In conclusion, the combination of conventional and molecular cytogenetics studies has allowed us to: i) Detect and quantify the *BCR/ABL1* fusion gene; ii) characterize the complex variant translocations and detect cryptic translocations; iii) confirm that the breakpoints are commonly localized in the G-light bands; (iv) confirm that the genesis of variant translocations could be via either the one-step or two-step mechanisms; and v) to report new cases of complex variant translocations.

## Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the proliferation and accumulation of mature myeloid cells and their progenitors (1). The hallmark of the disease is the presence of the reciprocal translocation

(9;22)(q34;q11.2), resulting in a *BCR/ABL1* gene fusion on the derivative chromosome 22, the so-called Philadelphia (Ph) chromosome (1). Variant translocations are identified in 5-10% of patients with newly diagnosed CML (1). The translocation can be observed either in a simple form (involving 22q11.2 and one additional breakpoint) or in a complex form, involving 9q34, 22q11.2, and at least one additional breakpoint (2,3). Although all chromosomes have been reported to participate in variants, the distribution of breakpoints clearly exhibits a non-random pattern, with a marked clustering to specific chromosome bands (2,3).

Fluorescence *in situ* hybridization (FISH) has been commonly used to detect the presence of the *BCR/ABL1* fusion gene at disease diagnosis and also to monitor its evolution during therapy. Different FISH probes can be combined to accurately determine the complex variant translocations involving more than two chromosomes when observed by cytogenetic analysis (3).

We report herein the characterization, by conventional and molecular cytogenetics, of 32 cases with complex variant Ph translocations, diagnosed in 32 patients with CML.

## Materials and methods

**Patients' initial features.** From 1990 to 2015, 693 patients with CML were diagnosed in three different centers: Hospital Clínic de Barcelona, Hospital del Mar de Barcelona, and Hospital Trias i Pujol de Badalona. Among these, 32 (5%) CML patients exhibited complex variant Ph translocations. The primary clinical and hematological parameters of the patients are outlined in Table I. The patients were comprised of 15 females and 17 males, ranging in age from 23 to 81 years. The ethical approval for the present study, including the written informed consent of the patients, was granted following the guidelines of the Ethics Committee of the Hospital Clínic de Barcelona, Hospital del Mar de Barcelona, and Hospital Trias i Pujol de Badalona.

**Conventional cytogenetics.** Bone marrow samples were processed for cytogenetic and FISH analysis. Cytogenetic

*Correspondence to:* Dr Dolores Costa, Hematopathology Section, Hospital Clínic, 170 Villarroel, Barcelona, Catalonia 08036, Spain  
E-mail: dcosta@clinic.cat

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Table I. Main clinical and hematological data of 32 CML patients at the time of diagnosis.

No.	Age	Palpable spleen	WBC (x10 <sup>9</sup> /l)	Hb (g/l)	Platelets (x10 <sup>9</sup> /l)	Therapy	Survival (months)
1	27	NO	67	97	745	HU, IFN, ALLO-SCT	9
2	81	NO	18.5	112	884	IMATINIB	60
3	NA	NA	NA	NA	NA	NA	NA
4	38	YES	57.9	150	237	HU, IFN, ALLO-SCT	22
5	51	YES	254	90	498	HU, IFN, ALLO-SCT	23
6	NA	NA	NA	NA	NA	NA	NA
7	67	YES	15	111	1,075	IMATINIB, DASATINIB	NA
8	39	NO	41	145	126	IMATINIB, DASATINIB	55
9	23	NA	49	95	349	NA	NA
10	NA	NA	NA	NA	NA	NA	NA
11	NA	NA	NA	NA	NA	NA	NA
12	67	NO	42.6	146	326	IMATINIB	108
13	72	YES	7.9	124	249	IMATINIB, DASATINIB, BOSUTINIB	108
14	27	NO	24	133	378	DASATINIB, PONATINIB, ALLO-SCT	36
15	36	YES	537	93	503	HU, IFN ALLO-SCT	24
16	NA	NO	NA	NA	NA	NA	NA
17	48	YES	234	68	69	HU, DASATINIB, BOSUTINIB	24
18	61	YES	73	93	30	IMATINIB, DASATINIB, ALLO-SCT	12
19	50	NO	149	95	466	IMATINIB	120
20	45	YES	93	111	250	IMATINIB, DASATINIB ALLO-SCT, PONATINIB	24
21	53	NO	18	108	208	IMATINIB, NILOTINIB	96
22	43	NA	12	116	1294	HU, IFN	121
23	34	YES	228	90	297	HU, IFN, ALLO-SCT	27
24	39	NA	12.9	81	16	IMATINIB, ALLO-SCT DASATINIB	60
25	74	NO	27	142	347	IMATINIB, DASATINIB	59
26	41	NO	178	108	282	IMATINIB	96
27	50	NO	88	144	336	IMATINIB	132
28	44	YES	130	110	394	NILOTINIB	32
29	42	NA	126	116	260	IMATINIB	108
30	50	NA	21.6	110	72	NA	NA
31	38	YES	212	92	122	HU, IMATINIB	10
32	64	NA	6.7	171	206	IMATINIB, NILOTINIB	144

Patients 1, 4, 5, 15, 22, 23 and 31 have been investigated in a previous study (5). WBC, white blood cell count; Hb, hemoglobin; HU, hydroxy-urea; IFN, alpha-interferon; ALLO-SCT, allogeneic stem cell transplantation; NA, not available.

studies were carried out on G-banded chromosomes obtained from 24 h un-stimulated bone marrow cultures. Karyotypes were described according to An International System for Human Cytogenomic Nomenclature (4). A300-band ideogram was considered as the standard level of resolution for the purpose of the present study. Given that three laboratories were involved in the present study, with a different chromosome quality, it was agreed that translocations with breakpoints differing in one band would be considered as the same translocation.

**Molecular cytogenetics (FISH).** FISH probes were used to establish whether the *BCR/ABL1* rearrangement was present, as well as its location, and to characterize the complex variant translocations. Two different FISH probes were used in order to detect the *BCR/ABL1* rearrangements: LSI *BCR/ABL1*.ES

and LSI *BCR/ABL1* DCDF, as described previously (5). For the characterization of complex variant translocations, whole chromosome paint (WCP) probes for chromosomes 1, 5, 11, 12, 20 and 22; Centromeric (CEP) probes for chromosome 9.

All probes were provided by VYSIS (Abbott Products Operations AG, Allschwil, Switzerland) and the hybridization and detection were performed according to the manufacturer's protocols. Images were captured and processed with a Cytovision Ultra System (v.5.1.22; Leica Biosystems, Wetzlar, Germany).

***BCR-ABL* determination by the reverse transcription quantitative polymerase chain reaction (RT-qPCR).** White blood cells were isolated from peripheral blood or bone marrow samples with a lysis buffer containing 0.144 M NH<sub>4</sub>Cl and 0.01 M NH<sub>4</sub>HCO<sub>3</sub>. Total RNA was extracted using TRIzol reagent

Table II. Karyotype, chromosomal region of the additional chromosome/s involved in the complex variant Ph translocation and its location in a G-light band for the 32 patients with CML.

Case	Karyotype at diagnosis	BP	G-light BP
1	46,XX,del(22)(q11.2)[19]/46,XX[1]// <sup>a</sup> 46,XX,t(1;9;22)(p36.1;q34;q11.2) cryptic	1p36	Yes
2	46,XX,t(1;9;22)(p36.1;q34;q11.2)[20]	1p36	Yes
3	46,XX,t(1;9;22)(p11;q34;q11.2)[19]/46,XX[1]	1p11	Cen
4	46,XY,t(1;9;22)(q21;q34;q11.2)[9]/46,XY[1]	1q21	Yes
5	46,XX,t(1;9;22)(q21;q34;q11.2)[13]// <sup>a</sup> 46,XX, der(1)ins(9;1)(q34;q23q44), der(9)t(9;22)(q34;q11.2)ins(9;1)	1q21	Yes
6	46,XX,t(1;9;22)(q32;q34;q11.2)[16]	1q32	Yes
7	46,XX,t(9;22)(q34;q11.2)[5]/46,XX,t(1;9;22)(q42;q34;q11.2)[2]/47, idem, +der(22)t(1;9;22)(q42.1;q34;q11.2)[16]/46,XX[4]	1q42	Yes
8	46,XY,t(2;9;22)(p13;q34;q11.2)[19]/46,XY[1]	2p13	No
9	46,XY,t(2;9;22)(p13;q34;q11.2)[20]	2p13	Yes
10	46,XX,t(3;9;22)(p21;q34;q11.2)[20]	3p21	Yes
11	46,XY,t(3;9;22)(p13;q34;q11.2)[20]	3p13	Yes
12	46,XY,der(3)del(3)(p25)t(3;9;22)(q27;q34;q11.2)[20]	3q27	Yes
13	46,XX,t(5;9;22)(q12;q34;q11.2)[19]/47,XX,t(5;9;22)(q12;q34;q11.2), +der(22)t(5;9;22)(q12;q34;q11.2)[1]	5q12	No
14	46,XY,t(5;9;22)(q31;q34;q11.2)[14]/46,XY[6]	5q31	Yes
15	46,XY,t(5;9;22)(q31;q34;q11.2),-21,+mar[20]/46,XY[1]	5q31	Yes
16	47,XX,add(1)(q42),t(5;9;22)(q35;q34;q11.2),+8[20]	5q35	Yes
17	46,XY,t(5;9;22)(q35;q34;q11.2)[20]	5q35	Yes
18	45,X,-Y[13]/46,XY,t(6;9;22)(p23;q34;q11.2)[7]	6p23	Yes
19	46,XY,t(6;9;22)(p21;q34;q11.2)[20]	6p21	Yes
20	46,XX,t(7;9;22)(q36;q34;q11.2)[20]	7q36	Yes
21	46,XX,t(9;22;11)(q34;q11.2;q11)[20]	11q11	Cen
22	46,XX,t(9;22;11)(q34;q11.2;q13)[20]	11q13	Yes
23	46,XX,t(9;22;11)(q34;q11.2;q13)[15]	11q13	Yes
24	44,XY,dic(7;9)(q11;q11),t(9;22;12)(q34;q11.2;p13),-18, add[20](q13)(18)/46,XY[2]	12p13	Yes
25	46,XY,t(9;22;12)(q34;q11.2;p13)[3]	12p13	Yes
26	46,XY,t(9;22;13)(q34;q11.2;q14)[20]	13q14	Yes
27	46,XY,t(9;22;15)(q34;q11.2;q22)[20]	15q22	Yes
28	46,XY,t(9;22;15)(q34;q11.2;q22)[6]	15q22	Yes
29	46,XX,t(9;22;17)(q34;q11.2;q12)[20]	17q12	Yes
30	47,XX,+8,t(9;22;19)(q34;q11.2;p13)[15]	19p13	Yes
31	46,XY,t(9;22;12)(q34;q11.2;p13)[20]// <sup>a</sup> 46,XY,t(9;22;20;12)(q34;q11.2;q12;p13)	20q12/12p13	Yes/yes
32	46,XY,t(9;22;21)(q34;q11.2;q21)[25]	21q21	No

Patients 1, 4, 5, 15, 22, 23, and 31 have been previously reported (5). BP: breakpoint. <sup>a</sup>Karyotypes modified after FISH studies. Cen, centromere.

(Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. Reverse transcription was performed on 1 µg of RNA with the Moloney murine leukemia virus (M-MLV) reverse transcriptase (Thermo Fisher Scientific, Inc.) and random hexamer primers. Briefly, 1 µg of RNA in 19 µl of RNase-free water was incubated at 65°C for 5 min. Samples were cooled on ice and the following reagents were added to a final volume of 40 µl: 8 µl 5x RT buffer (250 mM Tris-HCl pH 8.3, 375 mM KCl, 15 mM KCl, 15 nM MgCl<sub>2</sub>; Thermo Fisher Scientific, Inc.), 0.4 µl DTT (0.1 M; Thermo Fisher Scientific, Inc.); 1.6 µl dNTPs (25 mM; GE Healthcare) 1.2 µl pdN6 hexanucleotides (10X; Roche Diagnostics, Basel, Switzerland), 1.5 µl RT enzyme M-MLV (200 U/µl; Thermo

Fisher Scientific, Inc.), 0.75 µl RNasin (40 U/µl; Thermo Fisher Scientific, Inc.) and 7.55 µl RNase-free water. Samples were then incubated at 37°C for 80 min, at 65°C for 10 min and 4°C at the end of RT step. Subsequently, qPCR was run from 2 µl cDNA as described by Van Dongen *et al* (6).

## Results

All variant chromosome Ph translocations were complex and involved 3 chromosomes, except in case 31 where the translocation included 4 chromosomes. The karyotypes are described in Table II. The karyotypes of patients 1, 4, 5, 15, 22, 23 and 31 have already reported in a previous study (5).

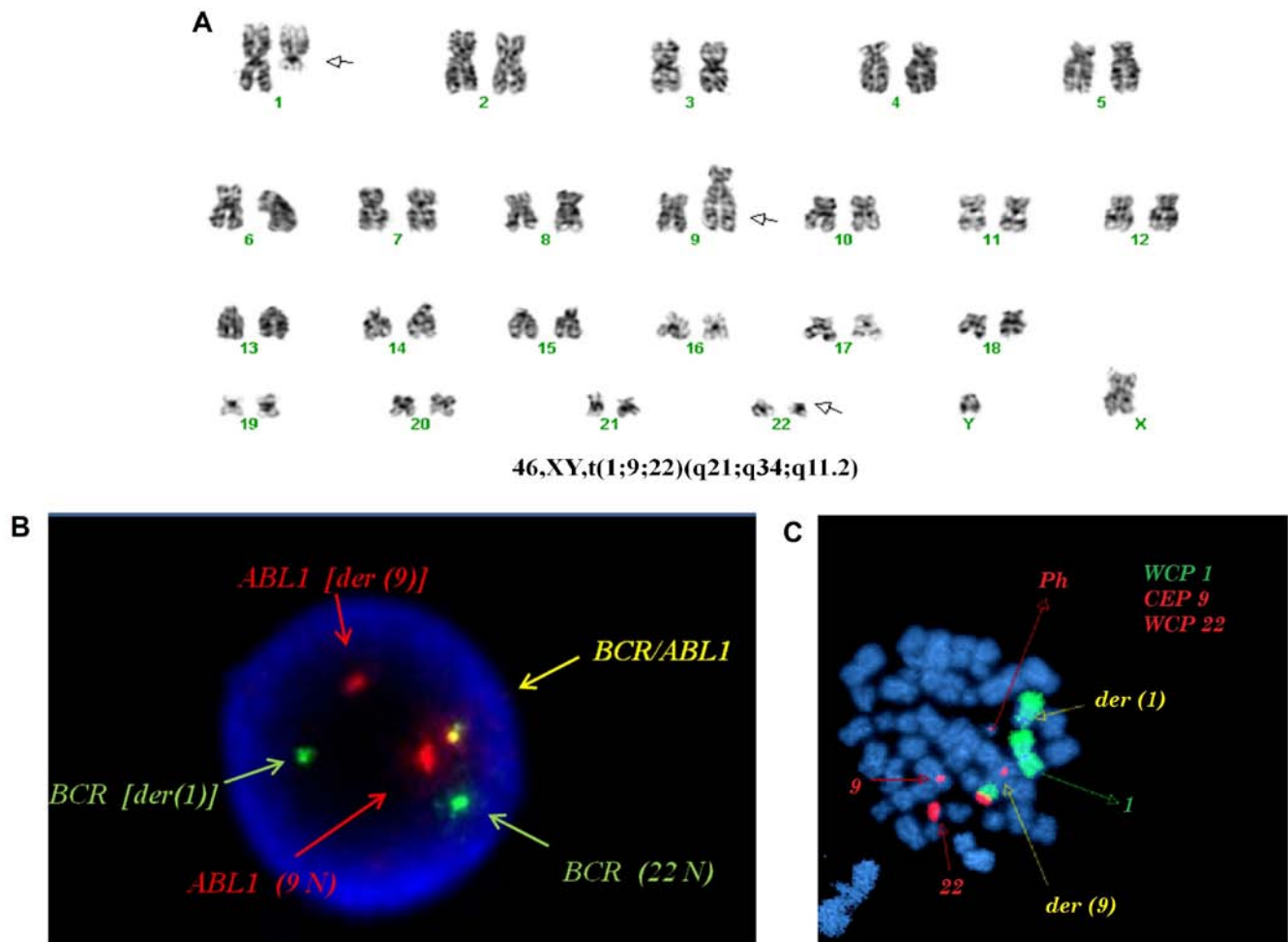


Figure 1. From patient 4: (A) Giemsa banded karyotype. The der(1),der(9) and der(22) chromosomes are indicated by arrows. (B) Interphase FISH, showing a normal red signal (ABL1; 9q34), normal green signal (BCR; 22q11.2), yellow signal (BCR/ABL1 rearrangement; Ph), and one green and red signal that was half the size of a normal signal (BCR; der(1) and ABL1; der(9), respectively). (C) Metaphase FISH showing chromosome 1 and der(1) in green, the centromere of the chromosome 9 and the chromosome 22 in red. The der(9) shows red in the centromere, and attached to 9q34 green material from chromosome 1 and red material from chromosome 22. FISH, fluorescence *in situ* hybridization.

The most frequent variants were t(1;9;22) (p36;q34;q11.2), t(1;9;22)(q21;q34;q11.2), t(2;9;22)(p13;q34;q11.2), t(5;9;22)(q31;q34;q11.2), t(5;9;22)(q35;q34;q11.2), t(9;22;11)(q34;q11.2;q13), t(9;22;12)(q34;q11.2;p13) and t(9;22;15)(q34;q11.2;q22), as they were identified twice.

The chromosomes included in the translocations were: 1 (n=7), 5 (n=5), 3, 11 and 12 (n=3), 2, 6 and 15 (n=2), and 7, 13, 17, 19, 20 and 21 (one each). Chromosomes 4, 8, 9, 10, 14, 16, 18, 22, X and Y were not included in any translocations. A total of 33 breakpoints were described in 32 translocations, and 17 of those were recurrent, being 12p13 (n=3), and 1p36, 1q21, 2p13, 5q31, 5q35, 11q13 and 15q22 (n=2). The q chromosome arm was more frequently involved in the translocations (n=20; 60%) than the p arm. The breakpoints were located in the G-light bands in the majority of cases (n=28; 85%), while the remaining breakpoints were in the dark bands (5q12, 17q12 and 21q21) and in the centromeric areas (1p11 and 11q11) (Table II).

Additional chromosomal abnormalities were observed in 6 out of 32 (19%) patients, including: der(22)/ der(22)/ -21,+mar/ add(1)(q42), +8/dic(7;9)(q11;q11),-18 and add(20)(q13)/ +8. Clinical information on possible progression was available in 3 out of the 6 cases with additional chromosomal abnormalities, (cases 7, 15 and 30), and none of these patients were in the blast crisis phase (Table II).

FISH studies using the LSI BCR/ABL1 were performed in 23 out of 32 cases, allowing for the detection of the BCR/ABL1 fusion gene in the Ph chromosome in all cases. In the 7 cases (cases 1, 5, 14, 15, 16, 31 and 32) where WCP and CEP FISH probes were used, the complex variant translocations were confirmed. Characterization of the t(1;9;22)(q21;q34;q11.2), using G-banded karyotype and LSI and WCP FISH probes for chromosomes 1, 9 and 22 in interphase nuclei and metaphases, are shown in Fig. 1.

Molecular studies (RT-PCR) revealed e14a2 chimeric BCR/ABL mRNA in 15 cases and e13a2 chimeric BCR/ABL

mRNA in 12 cases. In the remaining 5 cases the molecular studies were not performed.

## Discussion

The karyotype and the combination of different FISH probes are essential to characterize complex variant Ph translocations (3,5). The use of the FISH probe for detecting the *BCR/ABL1* rearrangement in interphase nuclei and metaphases is crucial to determine not only the presence of the rearrangement and its localization, but also whether further events have occurred, such as the presence of a double fusion gene, which may be relevant to interpret the clinical course and the prognosis of the disease. In our series, conventional and molecular cytogenetic studies have allowed the characterization of the 32 complex variant Ph translocations.

At present, in spite of its high genetic complexity, it is widely accepted that the clinical, prognostic and hematological features of patients with CML with complex variant translocations are not different from those with the classical t(9;22) translocation because it is accepted that the key pathological event is the formation of the *BCR/ABL1* fusion gene (2).

Although all chromosomes have been described in the complex variants, some regions are more frequently involved (3). In our series of experiments, the chromosomes most frequently involved were chromosomes 1 and 5, while the more frequent breakpoint was 12p13. The q chromosome arm participated more frequently (60%) than the p-arm. It may be hypothesized that the longer the arm, the higher the probability of recombination. Chromosomes 4, 8, 9, 10, 14, 16, 18, 22, X and Y were not identified in our translocations. All 32 variant translocations identified in our experiments have been previously described in complex variant Ph translocations (3,7).

The present study observed that the breakpoints of the variant 9,22 translocations locate preferentially, with 85% of them in the G-light bands (CG-rich areas). Fisher *et al* (8), reported this association in relation with that the CG richness areas reflect increases in the density of the CpG islands, genes, repetitive elements, and recombination.

Variant translocations may be caused by different mechanisms. Some variants are originated by multiple simultaneous breaks (one-step) and some arise as a result of two, or even more, genetic events in close succession (two-step or multiple-step) (9-11). In our series, the complex variant t(9;22;V) was identified in 30 out of 32 cases at the time of diagnosis suggesting that the t(9;22;V) originated in a stem cell, probably as the result of a one-step translocation. In two cases (cases 5 and 7), a two-step translocation could explain the complex variant translocations. In case 5, the insertion of material from chromosome 1 into the der (9) involved a second breakpoint in 9q34. In case 7, the identification of the two cell lines, t(9;22) and t(1;9;22), at the time of diagnosis suggests a two-step translocation.

Clonal evolution typically coincides with or precedes the accelerate phase or blast crisis of CML (9,10). Therefore, an inherent implication of the two-step mechanism is that variant translocations might be associated with a poorer prognosis (9,10).

Secondary abnormalities in the chronic phase of CML have been reported between 10-20% of cases, with the frequency being similar in t(9;22) or its variants. In our series,

19% of the cases have a secondary abnormality, which is concordant with the reported rates (1).

In conclusion, the combination of conventional and molecular cytogenetics studies has allowed us: i) To detect and quantify the *BCR/ABL1* fusion gene; ii) to characterize the complex variant translocations and detect cryptic translocations; iii) to confirm that the breakpoints are commonly localized in the CG-rich regions of the genome; (iv) to confirm that the genesis of variant translocations could be via either the one-step or two-step mechanisms; and v) to report new cases of complex variant translocations, which can involve new breakpoints that can eventually be recurrent and important for the understanding of this leukemia.

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## Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

## Authors' contributions

DC conceived and designed the study. JG, BE and DC were responsible for the data acquisition, selection and analysis. AA, CG and MLG were responsible for the analysis and interpretation of the conventional (karyotype) and molecular (FISH and RT-qPCR) studies. MN and FC were responsible for the analysis and interpretation of data, and critically revised the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the Hospital Clínic de Barcelona, Hospital del Mar de Barcelona and Hospital Trias i Pujol de Badalona. Written informed consent was obtained from all patients.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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