Variant Philadelphia t(X;9;22)(q22?;q34;q11.2) can be successfully treated with second generation tyrosine kinase inhibitors: A case report and literature review

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Abstract. Chronic myeloid leukemia (CML) is characterized by the reciprocal translocation between chromosomes 9 and 22: t(9;22)(q34;q11). However, 5-10% of patients with CML have complex variant translocations involving at least a third chromosome; only a few cases affect the X chromosome. Therefore, the data available regarding their features and the response to treatment is limited. In the present report, a case of a variant Philadelphia translocation t(X;9;22)(q22?;q34;q11.2) identified in a 51-year-old female with a newly diagnosed CML is described. The patient was treated with nilotinib. A major molecular response was observed after 12 months of starting treatment. Deep molecular response was obtained 20 months later and maintained after the 110-month follow-up. Additionally, a literature review was performed, with the aim of comprehending the complex clinical and biological characteristics of CML cytogenetic variants involving the X chromosome.

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm in which granulocytes are the major proliferative component (1). The annual incidence of CML in Europe is 0.7-1 cases per 100,000 individuals, with a male predominance (male/female ratio: 1.2-1.7) and an average age at presentation of 57-60 years (2).

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CML is characterized by a reciprocal chromosomal translocation between the chromosomes 9 and 22: t(9;22)(q34;q11), which results in the formation of the Philadelphia (Ph) chromosome, generating the fusion of *BCR* and *ABL1* genes (3). *BCR-ABL1* chimeric gene produces a protein with increased tyrosine kinase activity, and this results in constitutively activated cell-signaling pathways, causing aberrant proliferation, apoptosis and cellular adhesion. Thus, tyrosine kinase inhibitors (TKIs) are the treatment of choice for these patients (3).

Nevertheless, *BCR-ABL1* rearrangements are not exclusive to CML, and they may also be found in acute lymphoblastic leukemia and acute myeloid leukemia (4). At the time of diagnosis, 5-10% of patients with CML possess a variant Ph chromosome translocation, involving chromosome 22 and a chromosome other than chromosome 9 (simple variant translocation), or one or more chromosomes in addition to chromosomes 9 and 22 (complex variant translocation) (5). Their breakpoints are frequently located in the G-light bands, in the CG-richest regions (6). Different mechanisms have been proposed to explain the variant Ph translocations (6). However, the specifics remain to be determined. The prognosis of these patients has been analyzed in case reports or small series, which have reported variable outcomes (7,8).

Despite the fact that there are reports describing the involvement of every chromosome in variant translocations with different breakpoints, their frequency is highly variable (6,9). The X chromosome(s) in particular, are very rarely affected.

In the present report, the case of a patient with CML with a variant translocation involving the X chromosome is described. Additionally, a review of the cases previously reported, including their clinical and genetic features, the treatment they received and the response to it was performed.

Materials and methods

Approval and consent. Human material and clinical information were obtained in accordance with the Declaration of Helsinki (10). Informed consent was obtained from the patient and the present study was approved by the Hospital Clínico San Carlos Ethics Committee.

Literature review. A review of the literature was performed using the PubMed database using the following search terms: 'variant Philadelphia translocation involving X chromosome', 'variant Philadelphia translocation' and 'variant translocation in chronic myeloid leukemia'. All the studies that were found describing CML in chronic phase with variant Ph translocation involving the X chromosome, and were written in English, were included. Cases that were in the CML accelerated phase or blast crisis, and acute leukemia with variant Ph translocations were excluded.

Karyotype. Based on the white blood cell counts, an appropriate amount of heparinized bone marrow (~300 μl) was added to 5 ml RPMI-1640 culture medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 2 mM L-glutamine (Biological Industries; Sartorius AG), 30% FBS (Gibco; Thermo Fisher Scientific, Inc.), 15 mM HEPES (Gibco; Thermo Fisher Scientific, Inc.), and 75 U/ml Penicillin-75 μg/ml Streptomycin (Gibco; Thermo Fisher Scientific, Inc.) per a culture tube. Bone marrow cells were cultured in this mitogen-free media at 37˚C. After 24 or 48 h, 100 μl Colcemid was added to the sample and incubated for 60 min at 37˚C. Then, the cells were centrifuged for 10 min at room temperature at 500 x g, the supernatant was removed, 5 ml KCl 0.075 M was added and the sample was incubated for a further 30 min at 37˚C. Samples were centrifuged again at room temperature centrifugation at 500 x g, the supernatant was removed and 5 ml Carnoy's solution was added gradually to resuspend the pellet. The previous steps were repeated 2-3 times, until the pellet was clear. Pellet smears were prepared on glass slides. Each slide was incubated at 37˚C for 4 sec in trypsin solution prepared with 270 ml DPBS (Gibco; Thermo Fisher Scientific, Inc.; Thermo Fisher Scientific, Inc) and 30 ml Trypsin (Lonza Group, Ltd.). Next, the samples were stained for 90 sec at room temperature with Leishman stain prepared with 3 ml pH 6.8 buffer solution made with 1 Buffer tablet (Merck KGaA) in 1,000 ml distilled water and 1 ml Leishman stain stock [1.5 g Leishman's eosin methylene blue (Merck KGaA) in 1,000 ml methanol]. Finally, G-banded cytogenetic analysis of 20 metaphases was performed.

Fluorescence in situ hybridization (FISH). FISH was performed on cells from bone marrow samples using a Vysis LSI BCR/ABL1/ASS1 Tri-Color Dual Fusion FISH Probe kit and Vysis Whole Chromosome Painting for chromosome X (WCP X) SpectrumGreen Probe (both from Abbott Laboratories) according to the manufacturer's protocol. Briefly, the slides were immersed in the denaturation solution (formamide) for 5 min at 73±1˚C. Then, they were dehydrated for 1 min in 70% ethanol, followed by 1 min in 85% ethanol, and 1 min in 100% ethanol. A total of 10 μl probe mixture (7 μl LSI/WCP hybridization buffer, 1 μl probe, 2 μl purified water), pre-heated at 73±1˚C in a water bath for 5 min, was applied to one target area and the coverslip was immediately added. The slides were placed in a pre-warmed humidified box at 37˚C for 16 h. The coverslip was removed and the slide was washed for 2 min in 0.4X saline-sodium citrate (SSC) buffer (0.3 M sodium chloride and 30 mM trisodium citrate, adjusted to pH 7.0 with HCl) at 73±1˚C, and then for 5-8 sec in 2X SSC at room temperature; 10 μl DAPI II counterstaining solution was applied at room temperature and the coverslips were added. A minimum of 200 interphases were observed for each Vysis probe.

Array-comparative genomic hybridization (CGH). The sample was hybridized against a female human reference commercial DNA sample (Promega Corporation) using a 60k KaryoNIM® array-CGH, designed by NIMGenetics® for the detection of copy number variations (CNV) (Agilent Technologies, Inc.). Data analysis was performed using hg19 genomic build and the ADM-2 statistic (set as 6). At least five probes were accepted as the threshold for detected CNVs. Analytical validation of this platform confirms an informative range of 350 kb across the whole genome. Additionally, the syndromic regions included in this design were covered with an estimated average resolution of ~100 kb. The interpretation of the CNVs was performed as described by Vermeesch *et al* (11).

RNA extraction and reverse transcription. RNA was isolated from bone marrow mononuclear cells or peripheral blood total leukocytes using TRIzol® reagent (Sigma-Aldrich; Merck KGaA) according to the manufacturer's protocol. RNA concentration was quantified using spectrophotometry on a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Inc.) and 2 μg RNA was reverse transcribed to cDNA using SuperScript IV Reverse Transcriptase with random hexamers according to the manufacturer's protocol (Invitrogen; Thermo Fisher Scientific, Inc.).

Reverse-transcription qualitative PCR (RT-PCR). RT-PCR analysis of bone marrow mononuclear cells, obtained by Ficoll (Lymphoflot; Bio-Rad Laboratories, Inc.) gradient centrifugation at 500 x g for 30 min at room temperature, was performed in accordance with the BIOMED I protocols (12), using 50 ng cDNA for each assay and ampliTaq Gold DNA polymerase for amplification (Applied Biosystems; Thermo Fisher Scientific, Inc.). *BCR/ABL1 MBCR* fusion transcripts were amplified with the primers *BCR*-b1-A, 5'-GAAGTGTTTCAGAAGCTTCTCC-3' and *ABL*-a3-B, 5'-GTTTGGGCTTCACACCATTCC-3' (12). *ABL1* control was amplified with *ABL1* forward, 5'-CTTCTCGCTGGACCCAGTGA-3' and reverse, 5'-TGTGATTATAGCCTAAGACCCGGAG-3'. Amplified products were viewed using 2% agarose gel electrophoresis using DNA Phi-X-174 Hae III digested molecular weight marker (Sigma-Aldrich) and ethidium bromide-staining.

Reverse transcription-quantitative PCR (RT-qPCR). A total of 9 ml peripheral blood was collected in EDTA tubes. Total leukocytes were obtained by centrifugation at 2,000 x g for 15 min at 4˚C, and red blood cells were removed using Erythrocyte Lysis Buffer-Buffer EL (Qiagen GmbH).

BCR-ABL1 expression was measured using RT-qPCR as described previously by the Europe Against Cancer Program (13). *ABL1* was used as a reference gene (14). The reaction was performed in a 20 μl reaction mixture containing TaqMan Universal PCR MasterMix or TaqMan Fast Universal PCR MasterMix (Applied Biosystems; Thermo Fisher Scientific, Inc.) with 100 ng cDNA for each assay in duplicate, either in an ABI Prism 7700 Sequence Detection system or a 7500 Fast Real Time PCR system (Applied Biosystems;

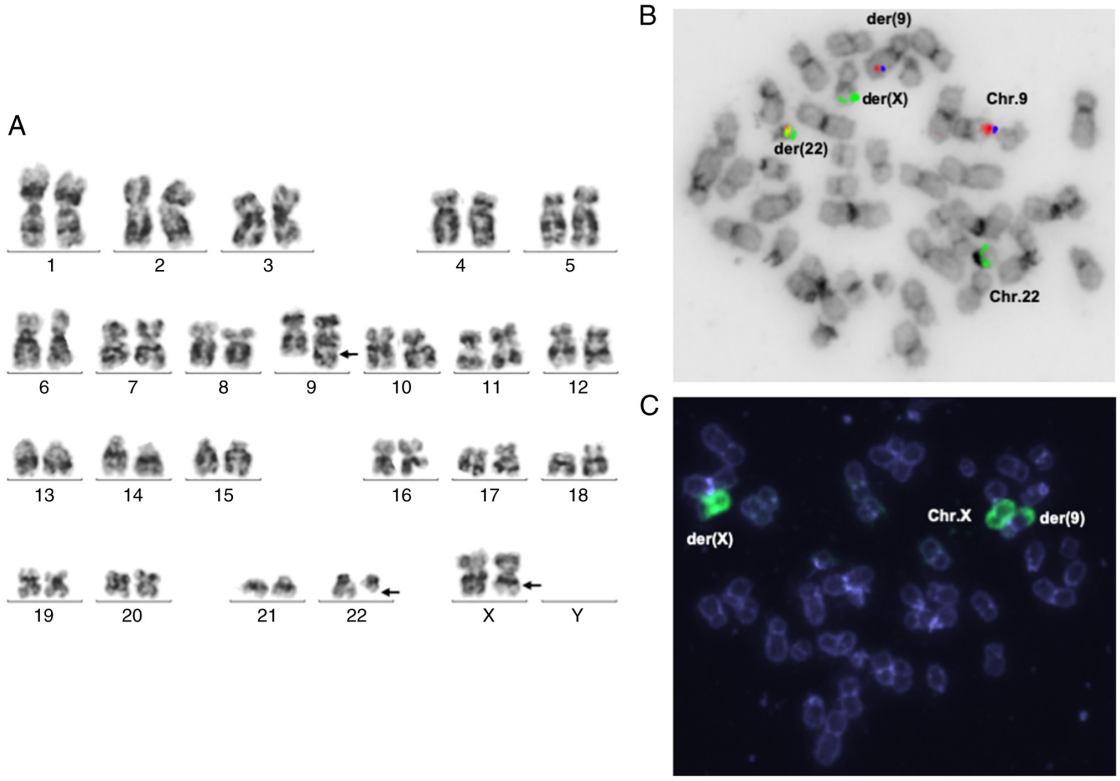

Figure 1. Cytogenetic analysis of the case with Ph variant involving the X chromosome. (A) G-banded karyotype showing 46,X,t(X;9;22)(q22?;q34;q11.2)[10]. The der(X),der(9) and der(22) are indicated by arrows. (B) Metaphase FISH analysis using a LSI *BCR/ABL1/ASS1* Tri-Color Dual Fusion probe showed a normal orange and blue signal (*ABL1* and *ASSI*; 9q34), normal green signal (*BCR*; 22q11.2), an orange/green fusion (yellow) signal (*BCR-ABL1* rearrangement; Ph), a green signal that was half the size of a normal signal [*BCR*; der(X)] and an orange signal that was half the size of a normal signal and normal blue signal [*ABL1* and *ASSI*; der(9)]. (C) Metaphase FISH analysis using the WCP X SpectrumGreen probe showing chromosome X, der(X) and der(9) in green. FISH, fluorescence *in situ* hybridization; Ph, Philadelphia chromosome.

Thermo Fisher Scientific, Inc.). Quantification was expressed on the International Scale (IS) as a percentage of *BCR-ABL1* copy number using *ABL1* as a reference gene (15), using a *BCR-ABL1* Mbcr IS-Major Molecular Response (MMR) kit (Qiagen GmbH).

Case report

A 51-year-old female consulted her GP due to the presence of abnormal bruises on her limbs. Laboratory results revealed a leucocyte count of 90,300/μl, 12 g/dl hemoglobin and a platelet count of 270,000/μl. Peripheral blood smears showed neutrophilia with granulocytes at various stages of differentiation: 49% neutrophils, 17% metamyelocytes, 13% myelocytes, 1% promyelocytes, 9% lymphocytes, 5% eosinophils and 6% basophils. Bone marrow aspiration morphology showed hypercellularity with granulocytic proliferation without maturational hiatus, a substantially increased myeloid: erythroid ratio, sea-blue histiocytes, and augmented basophils and eosinophils. The proportion of megakaryocytes was also increased; several of which were small and exhibited hypolobulated nuclei. A hematoma in the right upper limb was observed in the physical examination. Abdominal ultrasound revealed a mild splenomegaly of 13.3 cm.

G-banded cytogenetic analysis of bone marrow cells showed the following karyotype: 46,X,t(X;9;22)(q22?,q34;q11.2) (Fig. 1A). FISH analysis of cells from the bone marrow showed an atypical pattern of *BCR-ABL1* rearrangement in >90% cells (Fig. 1B and C). Analysis using array-CGH 60k detected no deletions (Fig. S1). RT-PCR analysis of bone marrow mononuclear cells demonstrated the expression of e13a2 (b2a2) *BCR-ABL1* RNA (Fig. 2A).

Given the above findings, the patient was diagnosed with chronic phase CML with a variant Ph translocation. The risk group for this patient was low, according to Sokal and European Treatment and Outcome Study for CML (EUTOS) scores, and intermediate using the Hasford Score (16-18). The patient was treated with nilotinib 300 mg twice a day, and exhibited a complete hematologic response after 2 weeks of treatment, and a complete cytogenetic response after 3 months. The patient was monitored by RT-qPCR following the Europe Against Cancer Program (13), using *ABL1* as a reference gene (14) and expressed on the IS as a percentage of *BCR-ABL1* (15). The patient had an optimal molecular response according to the ELN criteria (15): *BCR-ABL1* ≤10% after 3 months of treatment (1.12%) and a MMR after 12 months (0.02%), that was subsequently maintained. A deep molecular response was assessed following EUTOS laboratory recommendations (19), and this was achieved after 32 months of starting treatment, and has been maintained even after 9 years of follow-up (Fig. 2B and C).

Discussion

CML is characterized by the presence of a t(9;22)(q34;q11.2) translocation, which results in the Ph chromosome. Of CML cases, >85% of patients possess a classic translocation, 5-10%

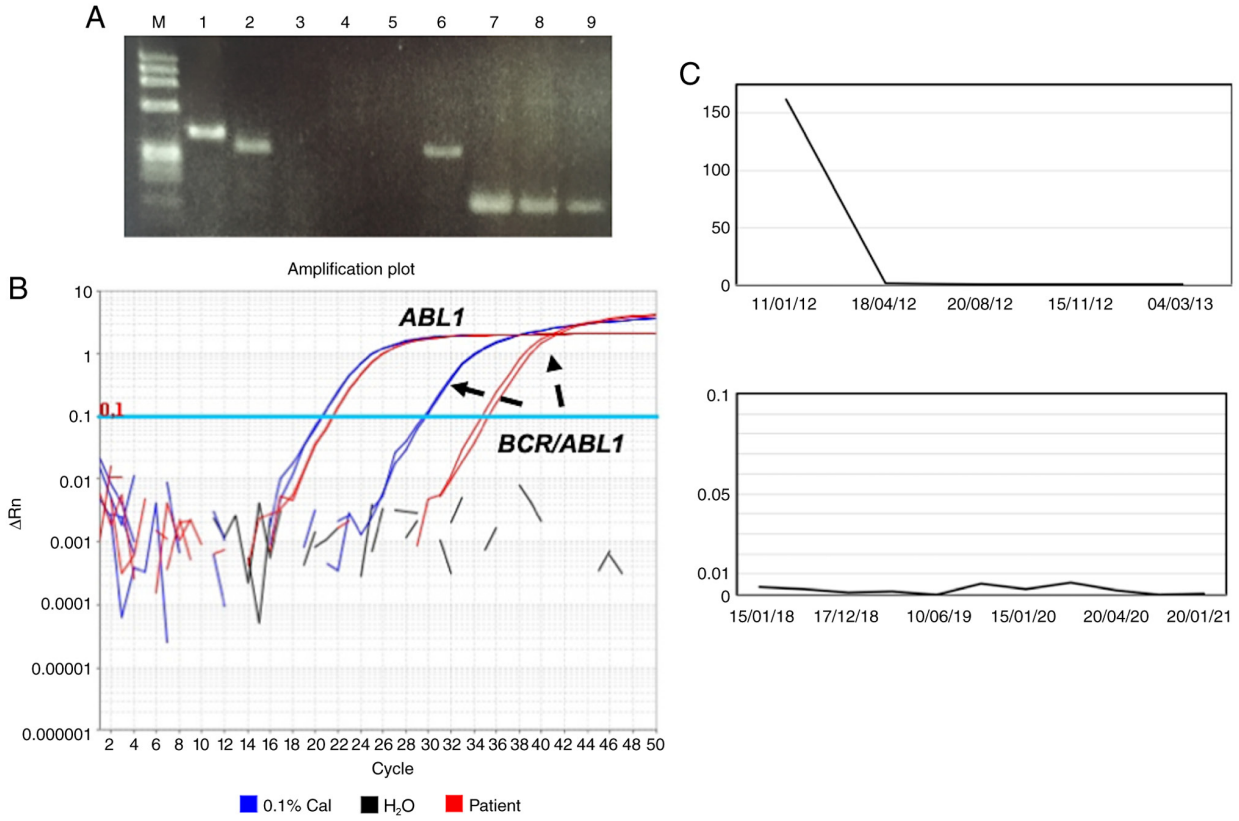

Figure 2. RT-qPCR follow-up shows a good response to a second generation tyrosine kinase inhibitor in the patient with variant Ph involving X chromosome. (A) Ethidium bromide-stained agarose gel showing electrophoresis of *BCR-ABL1* RT-PCR (lanes 1-6) and *ABL1* control (lanes 7-9). Lane descriptions: M, molecular weight marker DNA Phi-X-174 Hae III digested; 1, corresponds to a patient who expressed e14a2 (b3a2) *BCR-ABL1* transcript; 2, shows the *BCR/ABL1* amplification product of the case described in the present study that possessed an e13a2 (b2a2) transcript; 3, negative case; 4 and 5, blank negative controls (water); 6, e13a2 (b2a2) transcript positive control; 7-9, *ABL1* control amplification of patients 1-3, respectively. (B) Representative real time amplification plot of the last year of follow-up showing the patient results in red, water control in black and the 0.1% (major molecular response) IS calibrator in blue. PCR duplicates are shown, the curves on the left correspond to *ABL1* amplification with similar results in the patient and the calibrator. The curves on the right are *BCR-ABL1* data showing the difference between the patient and the calibrator. (C) *BCR-ABL1* RT-qPCR monitoring expressed on the IS as a percentage of *BCR-ABL1/ABL1* normalized copy number (Y axis), only a selection of the dates are indicated at the X axis to facilitate the reading. The first year follow-up is shown at the top; the last 3 years, showing deep molecular response, can be seen at the bottom. RT-qPCR, Reverse transcription-quantitative PCR; RT-PCR, Reverse transcription-qualitative PCR; IS, International Scale; ΔRn, Δ normalized reporter value, where Rn is the reporter fluorescence signal normalized to the signal of the passive reference dye: ΔRn=Rn - baseline.

have a variant translocation and <5% possess a masked Ph translocation that is not detected by conventional cytogenetic analysis; however, the *BCR-ABL1* fusion gene is identified by FISH and/or PCR (20).

The involvement of the X chromosome in the variant Ph has seldom been described. It has been detected in case series; however, in the majority of these, the description of clinical features, the treatment administered, the response to treatment and other data are not individually available (reviewed in Table I).

In the present report, the case of a new patient with X chromosome-variant Ph CML is described, and a review of cases reported in the literature is provided. A total of 16 cases were identified (Table I) (20-35). Using this data, it was estimated that the frequency of variant Ph CML involving the X chromosome is <1% of all CML cases. Bonifacio *et al* (33), in the most recent and largest study identified on this topic, described only 2 patients with complex variant translocations involving the X chromosome amongst 3,361 newly diagnosed CML cases; thus, it only accounted for 0.06% of cases (Table I). The mean age of patients with CML with the variant Ph translocation involving the X chromosome, including just the cases for which data are available (7 cases out of 16), was 36 years (range 13-54 years; Table I).

The reported breakpoints were Xp22, Xp11.2, Xq24, Xq28, Xp22, Xp11.2, Xq11, Xp11, Xq13.1, Xp11, Xq28, Xq13 and Xq24, with Xp11 being the most frequent breakpoint (four cases). In three cases, the breakpoint was unknown. Most cases were complex three-way variant Ph; cases 5 and 12 were simple variant Ph and cases 7 and 11 were four-way variant Ph (Table I).

The treatment administered is detailed in a few cases (7 out of 16 cases): Hydroxyurea-interferon α for case 3, busulfan for case 5, aspirin and pipobroman, thrombocytapheresis, nitrogen mustard and busulfan for case 8, imatinib for cases 4, 6 and 9, and hydroxyurea and imatinib for case 15. The response to treatment was specified in 3 cases (3, 4 and 15). In case 3, a minor cytogenetic response was described 1 year after starting therapy, whereas case 4 presented a complete cytogenetic response after 6, 12 and 18 months, and case 15 achieved complete a hematologic response, although follow-up cytogenetic studies were not possible as the patient passed away 4 months after diagnosis. Survival was only reported in case 15 (Table I).

Table I. Description of previously published cases of a variant translocation involving chromosome X focusing on chronic phase CML.

Patient no.	Sex/age	Diagnosis	Description of the studies	Variant karyotype	Treatment and response	Survival	Refs
1	NA	CML CP	336 CML cases: 25 cases in CP with vPh (7.4%), 1 of them involving X (0.29%). No deletion on der (9).	46,XY,t(X;9;22) (p22;q34;q11.2)[20]	NA	NA	(21)
2	M/33	CML	1 ALL Ph+ case, 1 AML Ph+ case and 5 CML cases (2 cases with masked Ph and 3 cases with vPh), 1 of them with vPh involving X.	46,Y,t(X;9;22) (p11.2;q34;q11.2)[3]/ 47,idem,+der(9)t(X;9;22) (p11.2;q34;q11.2)[17]	NA	NA	(20)
3	NA	CML	22 CML cases with vPh, 1 of them involving X.	46,X,t(X;9;22)(q24;q34;q11.2)[20]	HU-IFNα; mCgR at 1 year.	NA	(22)
4	F/13	CML CP	93 CML CP cases treated with imatinib: 8 with vPh, 1 of them involving X. Hb, 11 g/dl; WBC, 134,000/μl; platelet count, 222,000/μl. Sokal score, low.	46,X,t(X;9;22)(q28;q34;q11.2)[20]	Imatinib; CCgR after 6, 12 and 18 months.	NA	(23)
5	M/54	CML	2 CML cases with vPh: 1 involving X. Hb, 12 g/dl; WBC, 76,500/μl; platelet count, 134,000/μl.	47,Y,t(X;22)(p22;q11), +19	Busulfan	NA	(24)
6	NA	CML	721 CML cases treated with imatinib: 44 cases with vPh (6.1%), 1 of them involving X (0.13%).	46,X,t(9;22;X)(q34;q11.2;p11.2)[20]	Imatinib	NA	(25)
7	NA	CML	94 cases with vPh: 3 involving X; 1 simple[a], 1 complex three-way[b] and 1 complex four-way.	t(X;9;17;22)	NA	NA	(26,27)
8	F/27	CML from ET	5 CML cases with vPh: 1 case involving X. Hb, 13 g/dl; WBC, 12,200/μl; platelets, 2,030,000/μl.	46,X,t(X;9;22)(q11;q34;q11)[14]	Aspirin and pipobroman, no response. Thrombocytapheresis, nitrogen mustard and busulfan.	NA	(28,29)
9	NA	CML CP	615 CML CP cases: 72 with vPh (11.7%), 1 of them involving X (0.16%).	46,Y,t(X;9;22)(p11;q34;q11)	Imatinib	NA	(30)
10	NA	CML CP	452 CML CP cases: 43 with vPh (9.5%), 1 of them involving X (0.22%), presented del (9)(q33q34).	t(9;X;22)(q34;q13.1;q11)	NA	NA	(31)
11 and	M/54	CML CP	7 CML CP cases with vPh: 2 of them involving X,	46,Y,t(X;9;11;22) (p11;q34;q12;q11)[20]	NA	NA	(32)
12	M/25		1 complex and 1 simple.	46,Y,t(X;22)(q28;q11)[12]	NA	NA	(32)
13 and 14	NA	CML	3,361 newly diagnosed CML: 109 vPh (3.2%), 2 of them involving X (0.06%).	NA	NA	NA	(33)
15	F/48	CML CP	4 CML cases with vPh: 1 case involving X. Hb, 9 g/dl; WBC, 4,800/μl; platelets, 9,150/μl.	46,XX,t(X;9;22)(q13;q34;q11.2)[10]	HU and Imatinib, CHR	4 months	(34)
16	NA	CML	507 CML: 28 vPh (5.5%); 1 of them involving X (0.19%).	Xq24	NA	NA	(35)

CML, chronic myeloid leukemia; Ph, Philadelphia chromosome; vPh, variant Philadelphia translocation; F, female; M, male; AML, acute myeloid leukemia; NA, not-available; X, chromosome X; CP, chronic phase; ALL, acute lymphoblastic leukemia; HU, hydroxyurea; IFNα, interferon α; CHR, complete hematologic response; CCgR, complete cytogenetic response; mCgR, minor cytogenetic response; Hb, hemoglobin; WBC, white blood cell count; AP, accelerated phase; BC, blast crisis; ET, essential thrombocythemia. aThis patient is case number 5. bThis patient was in BC.

Data concerning prognosis of these patients is controversial. Certain studies have shown that patients with a variant Ph chromosome translocation have a worse response to treatment compared with those with the classic Ph chromosome (8,22), whereas several other studies have reported no differences with regard to treatment outcomes (7,25,36). Certain studies have described a higher frequency of deletions on the derivative chromosome 9 in patients with variant translocations, which may be responsible for the worse prognosis reported (21,22). Case 10 presented a deletion on derivative chromosome 9, but the treatment administrated, the response to it and the survival information is not available.

In summary, the literature review revealed that the studied translocation is a very rare rearrangement, as only 16 cases involving chromosome X have been described thus far. Additionally, it does not offer enough data to allow drawing definitive conclusions on the prognosis of patients with variant translocations involving the X chromosome.

The mechanism of production of the variant translocation may represent a clonal evolution of the malignant cells, a phenomenon which is known to precede or to coincide with the progression of the disease (22), and therefore may be associated with the worse prognosis of some of these patients. However, the origin of the variant translocations is not well understood, and it may be heterogeneous in nature. The patient described here did not present a deletion of derivative chromosome 9, nor progression of the disease to accelerated phase or blast crisis in a long follow-up period. In fact, the patient achieved ELN optimal molecular response (MR) after 3 and 12 months and has remained in deep MR, *BCR-ABL1* expression on the IS ≥4 $\log_{10}$ reduction from IRIS baseline -MR^4 or more (19), 110 months after starting treatment. This shows that some patients with variant translocations can be successfully treated with second generation TKIs.

The 2020 ELN recommendations have recently established that generic imatinib is a cost effective first line treatment for chronic phase CML with low toxicity, when compared with second generation TKIs. This guide also gives clear rules as to when the therapy should be discontinued (37). However, when the patient in the present report was diagnosed 9 years ago, the treatment selection was based on the quicker and deeper MR achieved with second generation TKIs, which was considered a potential advantage with more options for treatment discontinuation, that requires the maintained deep MR (38,39). Although this option is particularly recommended for women of fertile age, this patient had several decades of life expectancy, and the best opportunity to cease treatment was prioritized. This means of clinical reasoning is still under consideration as a possible exception in first line treatment choice (40).

The case in the present report had a good response to second generation TKIs. However, clinical behavior of these variant translocations requires further characterization, as well as additional investigation on their prognostic impacts, particularly with regard to sensitivity to imatinib and second generation TKIs.

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Availability of data and materials

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

Authors' contributions

AI, RO, CC and EA performed experiments and participated in writing the manuscript. EA edited the manuscript. All authors have read and approved the final manuscript. All authors confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Human material and clinical information were obtained in accordance with the Declaration of Helsinki, informed consent was obtained from the patient and the present study was approved by the Hospital Clínico San Carlos Ethics Committee.

Patient consent for publication

The patient provided written informed consent for the publication of the data.

Competing interests

The authors declare that they have no competing interests.

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