

An unusual translocation, t(1;11)(q21;q23), in a case of chronic myeloid leukemia with a cryptic Philadelphia chromosome

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Abstract. Chronic myeloid leukemia (CML) is characterized by the translocation t(9;22)(q34;q11) [Philadelphia (Ph) chromosome]. Although not frequently occurring, additional chromosome abnormalities (ACAs) can be detected at diagnosis and a number have been associated with an adverse cytogenetic and molecular outcome. The present study reports a case of CML presenting with the translocation t(1;11)(q21;q23) and a cryptic Ph chromosome. The presence of ACAs could generate greater genetic instability, promoting the emergence of further alterations. The present findings suggest that t(1;11)(q21;q23) can prevent a good response to tyrosine kinase inhibitor (TKI) therapy developing a primary resistance. In the present patient, at a recent follow-up, the T315I mutation was detected. This mutation confers full resistance to all available TKI, except ponatinib, which was not a therapeutic option due to comorbidities.

Introduction

Chronic myeloid leukemia (CML) is a clonal disease of pluripotent hematopoietic stem cells that is characterized by the Philadelphia (Ph) chromosome with the reciprocal translocation t(9;22)(q34;q11). At diagnosis, the majority of cases (85%) exhibit the typical Ph chromosome as the only

cytogenetic finding. A few cases either harbor variant translocations [t(v;22)] or cryptic rearrangement (1).

However, additional chromosome abnormalities (ACAs) are a recurring event associated with clonal evolution. An extra Ph, +8, i(17q) and +19 have been described as the most common secondary changes (known as the major route), whereas others infrequent changes have been labeled as the minor route. The major route of ACAs is usually detected during disease progression into the accelerated phase or blast crisis (2). At diagnosis, this has been documented with low frequency (<5% of cases) (3). These aberrations have been reported as an independent prognostic factor with a negative impact on the cytogenetic and molecular response (4,5). The t(1;11)(q21;q23) translocation has been described at diagnosis in certain cases of pediatric acute myeloid leukemia or myelomonocytic leukemia, with a poor prognosis, but the subgroup with this rearrangement suggests an uncertain impact in the outcome of acute leukemia (6).

The present study reports a rare case of CML with a cryptic Ph chromosome and a t(1;11)(q21;q23) translocation at diagnosis, with unfavourable responses, even though the patient received appropriate treatments. Furthermore, a T315I mutation was later detected in the patient.

Case report

In June 2000, a 47-year-old female with a CML diagnosis was referred to the Hematology Research Institute 'Mariano R. Castex', National Academy of Medicine (Buenos Aires, Argentina) for further studies and treatment. The clinical records of the patient indicated the onset of the disease in 1998, when the patient was 45 years old, with a cytogenetic study showing the translocation t(1;11)(q21;q23). The patient was treated with hydroxyurea (1 g/day).

In the first consultation at the current institute, the patient presented with fatigue, weakness, fever and splenomegaly (4 cm below the costal margin). Laboratory results were as follows: Hematocrit, 36% (normal range, 36-46%); haemoglobin, 11 g/dl (normal range, 11.5-15.5 g/dl); white blood cells (WBC), $11.2 \times 10^9/l$ (normal range, $4.5-10.5 \times 10^9/l$); platelets,

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5.90x10¹¹/l (normal range, 1.5-4.0x10¹¹/l); reticulocytes, 1.2% (normal range, 0.9-5.5%); LDH, 205 U/l (normal range, 100-200 U/l). Bone marrow biopsy revealed hyper-cellularity with granulocytic hyperplasia, which was consistent with a diagnosis of chronic phase CML.

The cytogenetic analysis by G banding (7) confirmed t(1;11)(q21;q23), add(9)(q34) and a cryptic Ph chromosome (Fig. 1) [detected by fluorescence *in situ* hybridization (FISH) simple fusion using DAKO DNA Probes; Dako, Glostrup, Denmark]. The molecular analysis by reverse transcription-polymerase chain reaction (RT-PCR) (8) showed the chimeric breakpoint cluster region-Abelson murine leukemia viral oncogene homolog 1 (*BCR-ABL1*) mRNA transcripts. To determine whether the chromosome marker t(1;11) was of constitutional origin, a phytohemagglutinin-stimulated peripheral blood culture was performed. A normal karyotype was detected, which excluded the constitutional origin and indicated clonality.

The patient started treatment with hydroxyurea (1 and 2 g on alternate days) and interferon α (4.5 MU/day) until 2002 when interferon therapy was discontinued due to intolerance. With these treatments, the patient only achieved a complete haematological response. On September 2005, the patient started treatment with imatinib (400 mg/day) and mild adverse events were observed, including facial and lower limb edema, and a minor cutaneous rash.

The patient achieved a hematological response without a cytogenetic and molecular response after 8 months of therapy. In October 2006, a laboratory analysis showed the following: Hematocrit, 41.2%; hemoglobin, 12.5%; WBC, 9.3x10⁹/l; and platelets, 9.50x10¹¹/l. The dose of imatinib was increased to 600 mg/day and then to 800 mg/day in 2007 for better control of the thrombocytosis and to enhance the responses.

In May 2009, due to increased platelets counts of 1.39x10¹²/l and the absence of a major cytogenetic response, the treatment was switched to 800 mg/day nilotinib. After 15 months of treatment, the patient achieved a complete hematological response, and FISH studies showed 69% of cells positive for *BCR-ABL1* (*BCR-ABL1* dual fusion-dual color, LIVE Probes; Lexel, Buenos Aires, Argentina) (Fig. 2A). Quantification by RT-quantitative (q)PCR (MolecularMD® One-Step qRT-PCR *BCR-ABL* kit), according to the International Scale, gave a result of 8.5%, representing a minimal molecular response.

In October 2010, following adequate treatment adherence, the patient maintained the complete hematological response and exhibited reduced levels of *BCR-ABL1* transcripts at 0.84% (minor molecular response). Treatment was sustained between 2011 and 2013 with good tolerance, but without significant changes in cytogenetic and molecular responses (values range from 20.0-24.5% of Ph-positive cells by FISH analysis and 3.23-5.00% of *BCR-ABL1* transcripts by RT-qPCR). At the end of 2013, pegylated interferon (80 μ cg/15 days) was added to treatment with nilotinib in order to improve responses, but not better outcome was observed.

In 2014, the patient's condition worsened, with levels of *BCR-ABL1* transcript reaching of 12.3% and with 71% Ph-positive cells, as determined by FISH. The treatment was switched to dasatinib (50 mg/day). In June 2015, cytogenetic control follow-up showed the karyotype 46,XX,t(1;11)

(q21;q23), add(9)(q34) in all metaphases and RT-qPCR detected 100% of *BCR-ABL1* transcripts.

An obstructed carotid artery was diagnosed during the control follow-up. After 2 months, the screening for mutations in the *ABL1* gene by direct sequencing (9) detected the T315I mutation. Treatment with tyrosine kinase inhibitor (TKI) was suspended. A carotid artery angioplasty was performed and omacetaxine was prescribed at an induction dose (1.25 mg/m²) by subcutaneous injection twice daily for 14 consecutive days. Grade 3 anemia and grade 2 thrombocytopenia were observed, and the patient received blood red cells transfusions. With a delay of 6 days, a second cycle was administered at the same dosage but for 9 days only. To date, the patient has progressed to blast crisis and is receiving transfusions and supportive care for febrile neutropenia, and chemotherapy is under evaluation. Bone marrow transplantation was not an option, as the only sibling available as a donor for stem cell transplant was unfit due to histocompatibility and limiting comorbidities.

In order to assess if *MLL* [also known as lysine methyltransferase 2A (*KMT2A*)] was affected by the t(1;11)(q21;q23) translocation, FISH was performed using a dual color-split signal probes (Cytocell Aquarius probes; Cytocell, Cambridge, UK). The pattern of signal observed was not consistent with alterations in this gene (data not shown), indicating that the *MLL* gene was not split by the t(1;11) translocation.

Finally, to determine if the cryptic Ph chromosome was the result of a variant translocation, involving chromosomes 1, 9, 11 and 22, a whole chromosome painting (WCP) of chromosomes 1 and 11 was performed (WCP LIVE Probes; Lexel), as previously described (10). The results indicated that the t(1;11)(q21;q23) translocation and cryptic Ph chromosome had an independent origin (Fig. 2B). The FISH analysis could not determine the nature of the extra material in add(9q).

The experiments performed in this study were approved by the Ethics Committee of the Institutes of the National Academy of Medicine (Buenos Aires, Argentina) and the patient provided written informed consent for the publication of the study.

Discussion

In CML patients, ACAs in Ph-positive cells affect the progression and response to treatment according to the chromosome aberration and time of appearance. The European LeukemiaNet guidelines suggest that the presence of ACAs at diagnosis may represent a 'warning' feature, requiring careful monitoring (11). ACAs at diagnosis have been observed in ~5% of cases and are associated with clonal evolution, the mechanism of resistance and an adverse prognosis under treatment with imatinib (3). A previous study showed that the presence of ACAs in the early chronic phase was one of the independent adverse predictors for progression within a year (12). Fabarius *et al* reported that only the major route ACAs [+8, +Ph and i(17q)] at diagnosis are associated with a negative impact on progression-free and overall survival times (13). However, the impact of ACAs at diagnosis and their prognostic significance are issues that remain under discussion.

The present study reports the case of a CML patient who presented with a reciprocal translocation between chromosomes 1 and 11 [t(1;11)(q21;q23)], add(9q34) and a cryptic Ph

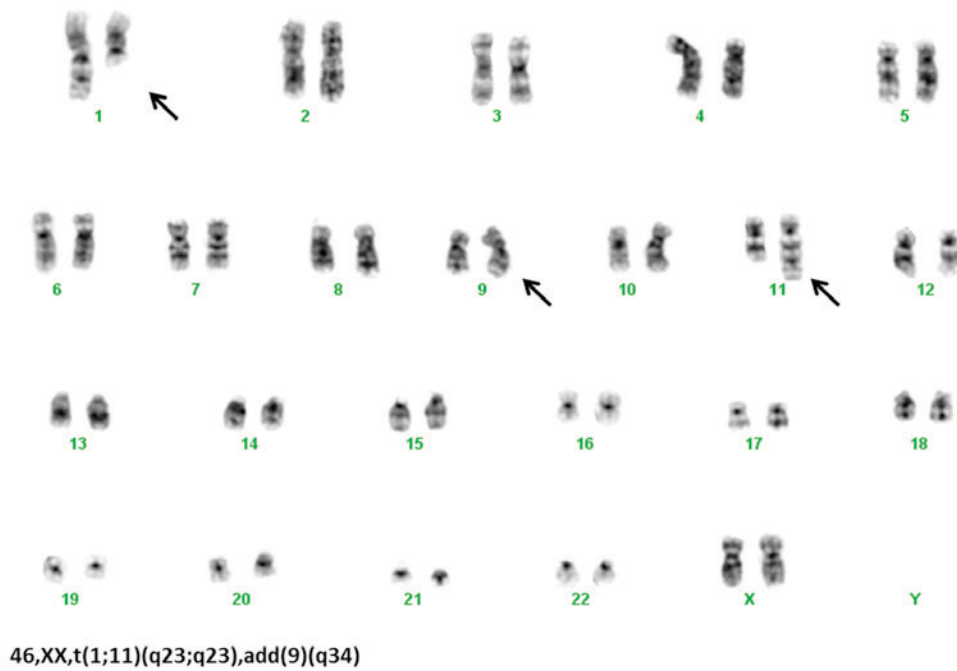


Figure 1. Metaphase showing translocation $t(1;11)(q21;q23)$ and the $add(9)(q34)$. The arrows indicate the chromosomes involved.

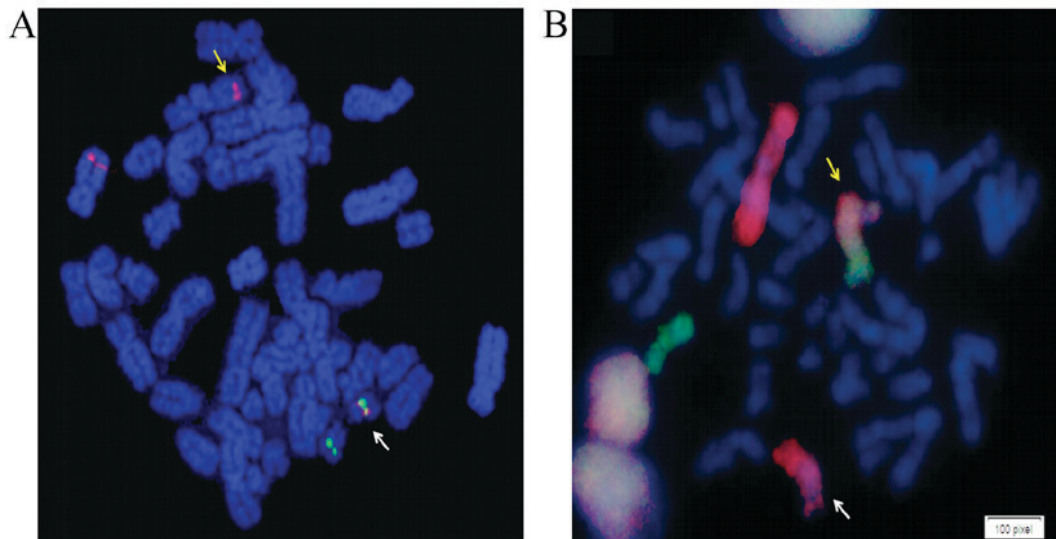


Figure 2. (A) FISH of the breakpoint cluster region-*ABL1* gene (dual fusion-dual color). The white arrow indicates the Ph chromosome and the yellow arrow shows the chromosome $der(9)$ with the residual *ABL* signal. (B) Painting FISH for chromosomes 1 and 11. The white arrow indicates the chromosome $der(1)$ and the yellow arrow shows $der(11)$. The absence of red or green signals on other chromosomes discounts a variant Ph, indicating an independent origin for the $t(1;11)(q21;q23)$. Ph, Philadelphia; FISH, fluorescence *in situ* hybridization; *ABL*, Abelson murine leukemia viral oncogene homolog 1.

chromosome at diagnosis. Genetic alterations in 11q23 are frequent in hematological malignancies, particularly in acute leukemia (6). These alterations affect the *MLL* gene in ~85% of cases and are associated with an adverse prognosis (14). In CML, alterations involving 11q23 and affecting *MLL* are rare, and have been reported only sporadically (15-19). However, this translocation could affect other genes such as *CBL*, generating a new chimeric gene that could deregulate the cell cycle and affect the treatment response (20,21).

Through use of *BCR-ABL1* dual fusion-dual color FISH probes (LIVE Probes; Lexel), as previously described (22), the present study found the absence of a second fusion signal

on $der(9)$, keeping only the residual signal of the *ABL1* gene, which is indicative of the deletion of the *BCR* sequence on $der(9)$ (Fig. 2A). This deletion was confirmed by molecular analysis of the reciprocal transcript *ABL1-BCR* (data not shown). Deletions on $der(9)$ have been reported in several studies in association with a bad response prior to the TKI era (6,11,23).

One of the most important mechanisms of resistance is via acquired mutations in the tyrosine kinase domain of *ABL1*. The T315I mutation was detected in the present patient during the last year of follow-up. This mutation is associated with the resistance to the majority of TKIs (24,25).

Although a long overall survival time (>15 years) was experienced in the present study, the CML patient has since progressed to a blast crisis. At present, the patient is receiving transfusions and supportive care for severe febrile neutropenia. Throughout the course of the disease, only a hematological response was achieved, with an occasional partial cytogenetic response and a lack of a molecular response, despite an increased dose of imatinib (from 400 to 600 to 800 mg/day) and a switch to second-generation TKIs (nilotinib and dasatinib). In the early stages, the t(1;11)(q21;q23) may have played a negative role in the outcome. Whether this atypical translocation may trigger the emergence of the T315I mutation CML is a matter for further investigation.

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