

## BCR/ABL1 fluorescence *in situ* hybridization fusion signals on both copies of chromosome 22 in a Philadelphia-masked chronic myeloid leukemia case: implication for the therapy

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### Abstract

The cytogenetic hallmark of Chronic Myeloid Leukemia (CML) is the presence of Philadelphia (Ph) chromosome, which results from a reciprocal translocation t(9;22)(q34;q11). In this report, we describe a CML patient with no evidence of Ph chromosome but trisomy of chromosome 8 as single cytogenetic abnormality and a typical e14a2 (b3a2) BCR-ABL1 fusion transcript. Fluorescence In Situ Hybridization (FISH) analysis revealed an uncommon signal pattern: the fusion signals were located on both copies of chromosome 22. During the course of the disease the appearance of the p.(Tyr315Ile) mutation was recorded. To the best of our knowledge this is the first Ph chromosome-negative CML case with e14a2 (b3a2) BCR-ABL1 transcript and p.(Tyr315Ile) mutation.

### Introduction

Chronic Myeloid Leukemia (CML) is a myeloproliferative disorder characterized by neoplastic transformation of a hematopoietic stem cell leading to uncontrolled proliferation of myeloid cells and granulocytic leukocytosis of bone marrow (BM) and peripheral blood (PB). The clinical course of the disease is invariably characterized by progression to acute leukemia (blast crisis) if untreated, but this has been dramatically changed by the introduction of tyrosine kinase inhibitors (TKIs).<sup>1</sup> The cytogenetic hallmark of CML is the presence of Philadelphia (Ph) chromosome found in about 90-95% of patients, deriving from a reciprocal translocation t(9;22)(q34;q11).<sup>2</sup> The molecular consequence of this translocation is the production of a chimeric BCR/ABL1 fusion gene

which encodes an aberrant protein with prominent tyrosine kinase activity and is responsible for transformation to leukemia.<sup>3</sup> Ph chromosome variants are found in about 5-10% of CML patients and include complex translocations among three or more chromosomes, including 9 and 22.<sup>4</sup> Approximately 1% of patients with a clinical diagnosis of CML show no Ph chromosome and chromosomes 9 and 22 appear to be normal.<sup>5</sup> This subset of patients, classified as Ph chromosome-negative CML, nevertheless carries the BCR/ABL1 fusion gene but clinical features, response to therapy and prognosis are indistinguishable from Ph chromosome-positive CML patients.<sup>6</sup> In these “masked Ph” cases Fluorescence In Situ Hybridization (FISH) is a useful technique for determining both the presence of the translocation and otherwise undetectable cryptic structural chromosomal rearrangements.<sup>7</sup>

In this report we describe a Ph chromosome-negative CML patient with trisomy of chromosome 8 as the single cytogenetic abnormality and a typical e14a2 (b3a2) BCR-ABL1 fusion transcript, with an unusual FISH signal pattern due to the presence of fusion signals on both copies of chromosome 22. The p.(Tyr315Ile) mutation appeared during the course of the disease and the treatment approach adopted was successful.

### Case Report

In October 2018 a thirty-seven-year-old man presented in another Institution with hepatosplenomegaly and priapism. Hematological investigation revealed hyperleukocytosis (WBC > 500×10<sup>9</sup>/L) with 15% blast cells, and a diagnosis of CML with impending blast crisis (“accelerated phase” of CML) was made. After hydroxyurea debulking therapy, treatment with imatinib 600 mg/day was initiated but, due to the lack of a complete hematological response (CHR), it was replaced by dasatinib 80 mg/day in December 2018. The patient achieved a CHR, but subsequently experienced a loss of response, possibly due to the lack of compliance with the treatment. In September 2019 the patient was admitted to our hospital with sweats and weight loss. On examination the patient had hepatosplenomegaly. PB tests revealed anemia and thrombocytopenia, WBC count was around 100×10<sup>9</sup>/L, with >80% blasts with a B-lineage lymphoid phenotype, a clinical picture of B-cell lymphoblastic leukemia in overt blast crisis. A computed tomography scan revealed enlarged thoracic

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and abdominal lymph nodes. Initially, high dose chemotherapy was not deemed adequate due to serious concerns about his

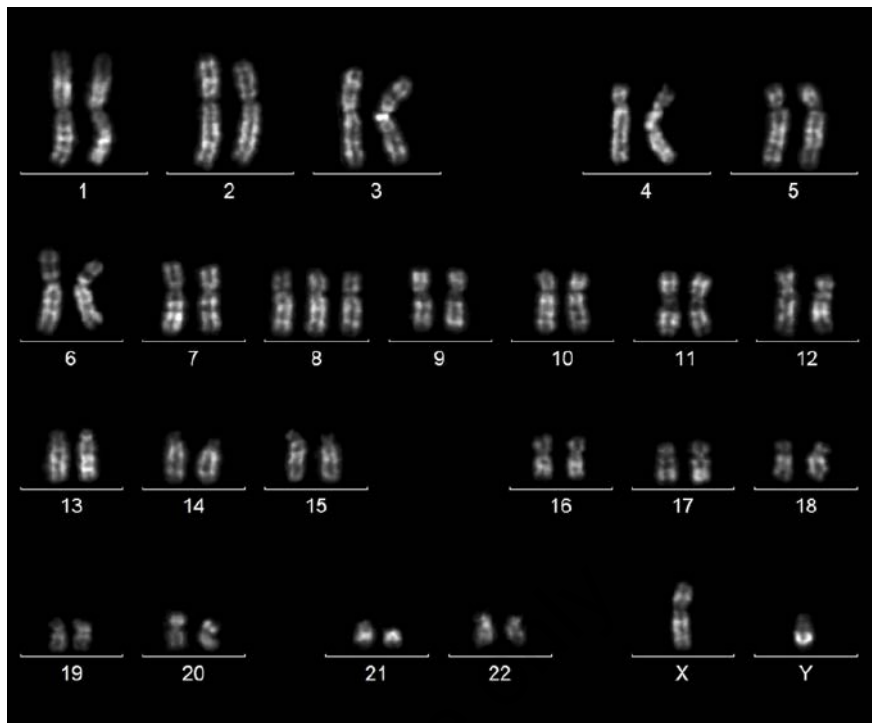
compliance with the treatments and behavioral disturbances. A therapy with methylprednisolone 1 mg/kg was started and dasatinib dose was optimized to 140 mg/day.

Flow cytometry analysis on PB showed 48% blasts CD45<sup>dim</sup>- CD10<sup>+</sup> CD13<sup>+</sup> CD19<sup>+</sup> CD20<sup>+</sup> CD22<sup>dim</sup> CD38<sup>+</sup> HLADR<sup>+</sup> cytCD79a<sup>+</sup> TdT<sup>+</sup> CD2<sup>-</sup> CD3<sup>-</sup> CD4<sup>-</sup> CD5<sup>-</sup> CD7<sup>-</sup> CD8<sup>-</sup> CD11b<sup>-</sup> CD11c<sup>-</sup> CD14<sup>-</sup> CD15<sup>-</sup> CD16<sup>-</sup> CD25<sup>-</sup> CD33<sup>-</sup> CD34<sup>-</sup> CD36<sup>-</sup> CD45RA<sup>-</sup> CD56<sup>-</sup> CD64<sup>-</sup> CD66c<sup>-</sup> CD117<sup>-</sup> CD235a<sup>-</sup> IgKappa<sup>-</sup> IgLamda<sup>-</sup> cytCD3<sup>-</sup> cytCD41<sup>-</sup> cytCD61<sup>-</sup> cytMPO<sup>-</sup>.

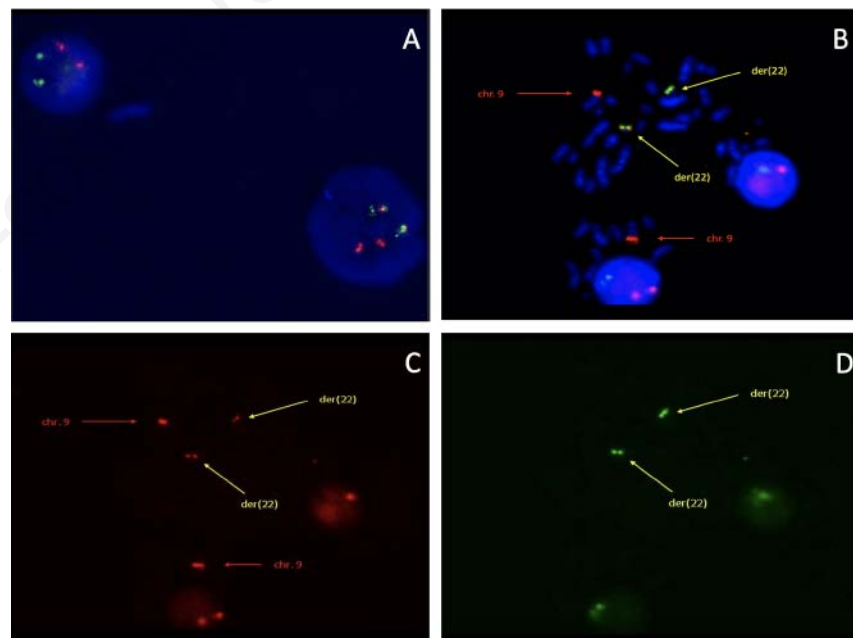
Cytogenetic analysis on PB revealed an abnormal male karyotype with trisomy of chromosome 8 in 8 over 22 metaphases. Chromosome 9 and 22 looked normal in all the analyzed metaphases (Figure 1). The karyotype was: 47,XY,+8[8]/46,XY[14]. *ABL1* and *BCR* genes rearrangements were observed in 30/100 interphase cells by FISH, showing an unusual signal hybridization pattern: two red signals and two fusion signals (Figure 2A). FISH analysis on DAPI counterstained metaphases revealed that the fusion signals were located on both copies of chromosome 22 (Figure 2B). The results from the FISH analysis enabled a better definition of the karyotype as follows:

47,XY,+8.ish der(22)  
ins(22;9)(q11;q34)(BCR+,ABL1+)x2[8]/  
46,XY[14].

Molecular analysis detected the presence of the BCR-ABL1 fusion transcript, with a typical e14a2 (b3a2) BCR-ABL1 isoform, and a BCR-ABL1 transcript level of 55.707% according to the International Scale (IS). Mutational status of ABL1 gene, by Sanger sequencing, did not show any variant. In November 2019 a new evaluation of ABL1 mutational status revealed the presence of p.(Tyr315Ile) mutation, related to resistance to imatinib and dasatinib. Due to the appearance of the p.(Tyr315Ile) mutation and the aggressive clinical presentation, including central nervous system (CNS) involvement, the patient was addressed to a therapeutic program including high dose chemotherapy (Hyper-CVAD scheme) and ponatinib (45 mg/day). After the first chemotherapy cycle (December 2019), BCR-ABL1 transcript level decreased to 0.176% IS and a CHR was obtained. Subsequently (January 2020) a major molecular response (MR3) was achieved with a BCR-ABL1 transcript level of 0.012% IS and blast clearance from the CNS. The same transcript level was observed in April 2020. The patient is still on treatment and under evaluation for allogeneic stem cell transplantation.



**Figure 1.** Quinacrine-banded metaphase cell from peripheral blood showing a 47 chromosomes male karyotype with trisomy of chromosome 8. Chromosomes 9 and 22 look normal.



**Figure 2.** A) FISH on interphase cells from peripheral blood hybridized with locus specific probe LSI BCR/ABL1 Dual Color/Dual Fusion (Vysis/Abbott, Illinois, USA), showing one normal nucleus with 2 red signals (ABL1 gene, 9q34.12) and two green signals (BCR gene, 22q11.23) and one abnormal nucleus with two red signals, and two fusion signals (red/green or yellow). B) FISH on metaphase cell, hybridized with locus specific probe XL BCR/ABL1 plus Translocation/Dual Fusion Probe (MetaSystems, Altlussheim, Germany) showing two red signals on chromosomes 9, two fusion signals on both chromosomes 22. C) Image B with spectrum orange filter. D) Image B with spectrum green filter.

Table 1. Clinical and laboratory features, treatments, and outcomes.

Author	Year	Sex	Age at diagnosis	Stage of the disease	Spleen enlarged	Peripheral blood			Bone marrow			Karyotype	BCR-ABL1 transcript	Therapy	Outcome
						WBC (10 <sup>9</sup> /L)	Hb (g/dL)	Plt (10 <sup>9</sup> /L)	Cellularity (%)	M:E ratio	Blast (%)				
Costa <i>et al.</i>	2003	F	58	AP	Yes	82.8	108	580	65	3:1	16	46,XX[20]	e13a2 (b2a2)	Imatinib (2001)	NMAloSCT (2002)
Virgili <i>et al.</i>	2008	F	NA	NA	NA	NA	NA	NA	NA	NA	NA	46,XX[20]	e13a2 (b2a2)	NA	NA
Our case	2020	M	37	AP	Yes	522	79	409	NA	22:1	15	47,XY,+8[8]/46,XY[14]	e14a2 (b3a2)	Dasatinib (2018) Ponatinib (2019)	MR3

AP, accelerated phase; F, female; Hb, haemoglobin; M, male; M:E, myeloid:erythroid; MR3, major molecular response; NA, not available; NMAloSCT, nonmyeloablative allogeneic stem cell transplantation; Plt, platelet count; WBC, white blood cell count.

## Discussion

Ph chromosome is one of the first described and best-characterized cytogenetic abnormality in cancer. It is a chromosomal aberration acquired at the hematopoietic stem cell level as a consequence of the reciprocal translocation t(9;22)(q34;q11). This rearrangement leads to the fusion between the 5' side of the *BCR* gene on chromosome 22 and the 3' side of the *ABL1* gene on chromosome 9. This fusion event produces the BCR-ABL1 oncoprotein whose aberrant activity induces a variety of signaling pathways regulating cell proliferation and survival.<sup>8,9</sup> Additional chromosomal abnormalities may be present at diagnosis in 10% of cases, or may appear during the course of the disease: they do not indicate an imminent blast crisis, although these additional anomalies do often arise at the time of acute transformation.<sup>10</sup> Although all chromosomes can be involved when additional aberrations occur, trisomy of chromosome 8 and double Ph chromosome are frequently reported in CML cases.<sup>11</sup> Ph chromosome cytogenetic variants are well known in CML, mostly deriving from a standard translocation t(9;22)(q34;q11) followed by further chromosomal rearrangements involving both the derivative chromosomes and, in the case of complex translocations, also other chromosomes.<sup>4</sup> As well as classical t(9;22)(q34;q11) these variants usually lead to the BCR/ABL1 fusion gene appearance.<sup>9</sup> One of these cytogenetic variant is called "masked Ph" (Ph-negative), characterized by normal chromosomes 9 and 22.<sup>12,13</sup> Approximately 1% of CML cases are Ph-negative but FISH or molecular analysis indicate the presence of the BCR/ABL1 fusion gene.<sup>14</sup> High genetic heterogeneity has been described in Ph-negative CML patients.<sup>15</sup>

In this report, we describe a CML

patient with no evidence of t(9;22)(q34;q11) but trisomy of chromosome 8 as a single cytogenetic abnormality, and BCR/ABL1 fusion gene detected by molecular and FISH analysis. Molecular analysis indicated a typical e14a2 (b3a2) BCR-ABL1 fusion transcript. The FISH results displayed a very unusual pattern with the fusion signals localized on both copies of chromosome 22 and the signals corresponding to the *ABL1* and *BCR* genes were retained in dimension (figure 2C-D). Since cryptic deletions have been reported in 21% of Ph-negative CML patients, frequently at the 9q34.1 chromosomal region,<sup>14</sup> we tested for the *ASS1* gene by FISH but it was not involved in the rearrangement (data not shown).

CML is well described in the literature but masked Ph and BCR/ABL1 positive cases are few. To the best of our knowledge this is the third Ph-negative CML case with the unusual FISH pattern described above, the first case was reported by Costa and the second by Virgili:<sup>7,16</sup> as with our patient both cases were in the accelerated phase of CML (Table 1). Nevertheless, our case differs slightly from the one reported by Costa *et al.* because in that case the two FISH signals corresponding to chromosome 9 were smaller in size, probably due to a deletion accompanying the rearrangement described. Conversely the two FISH signals corresponding to the chromosome 9, described in our case, are not reduced in both size and brightness.

We have formulated three different hypotheses, in order of complexity, to explain the rearrangement observed:

- i) a two-step mechanism, in which an insertion ins(22;9)(q34;q11) had occurred first, followed by a somatic recombination between the two homologous 22;
- ii) a three-steps mechanism, in which an insertion ins(22;9)(q34;q11) had occurred first, followed by the loss of

normal chromosome 22 and duplication of the der(22);

- iii) a multi-step mechanism in which two consequent translocations had occurred first, a standard t(9;22) translocation followed by a reverse translocation that reconstitutes the normal morphology of the partner chromosomes, as has already been suggested,<sup>13</sup> then followed by an insertion of ABL1/BCR from der(9) to the chromosome 22 not involved in the first translocation.

The second hypothesis is unlikely because the cytogenetic polymorphisms of chromosomes 22 are different. Conversely the first and the third are conceivable. Because the double fusion FISH signals on both copies of chromosome 22 have been considered to be equivalent to the double Ph chromosome,<sup>7</sup> it is likely that this particular gene rearrangement provides a proliferative advantage, further supported by the presence of chromosome 8 trisomy. Notably this case is the first CML Ph chromosome-negative patient with e14a2 (b3a2) BCR-ABL1 isoform, the more common transcript type described in CML, usually associated with earlier, deeper and higher molecular response rates.<sup>17</sup> Furthermore our patient is the first CML Ph chromosome-negative but p.(Tyr315Ile) positive case. The mutation appeared during the disease and it is well known that this variant provides resistance to all currently available TKIs except ponatinib. Indeed, the prompt change of therapy was successful.

## Conclusions

Therefore, our findings emphasize the importance of a detailed analysis of CML patients to characterize the molecular rearrangements present, not only to clarify their role in leukemogenesis, but to obtain a cor-

rect molecular genes assessment, their reorganization, their exact localization and the possible coexistence of multiple events. A multidisciplinary approach is mandatory for all hemato-oncological patients at onset of the disease to correlate clinical, morphologic, immunophenotypic, cytogenetic, molecular and FISH data to assist the therapeutic decisions.

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