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A 35-nucleotide insertion mutation in BCR-ABL1 in a patient with chronic myeloid leukemia in relapse after allogeneic stem cell transplantation.

Mutación de inserción de 35 nucleótidos en BCR-ABL1 en una paciente con leucemia mieloide crónica en recaída después de un trasplante alogénico de células madre

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Abstract

BACKGROUND: Tyrosine kinase inhibitors (TKI) are crucial when treating patients with chronic myeloid leukemia, as they are considered first-line therapy, replacing other potential curative strategies, such as stem cell transplantation (SCT). However, several patients in the chronic phase fail to achieve optimal response to initial therapy with imatinib, and alternatives should be considered.

CLINICAL CASE: A 53-year-old female patient diagnosed with Philadelphia chromosome-positive chronic phase chronic myeloid leukemia, initially treated with imatinib. She underwent a failed allogeneic SCT (allo-SCT), for which imatinib therapy was reinitiated and, after several years, drug resistance was documented. The BCR-ABL1^{35INS} mutation was found, and treatment with dasatinib, a second-generation TKI (2GTKI), was started. After twelve months on dasatinib, neither cytogenetic nor major molecular responses have been achieved.

CONCLUSIONS: Mutations in the *BCR-ABL1* gene causing alternative splicing variants are only one of the many proposed mechanisms of TKI resistance and should be taken into consideration when there is a failure to achieve response milestones to standard treatment.

KEYWORDS: Chronic myeloid leukemia; Tyrosine kinase inhibitors; Imatinib; Dasatinib.

Resumen

ANTECEDENTES: Los inhibidores de la tirosina cinasa son decisivos en el tratamiento de pacientes con leucemia mieloide crónica, ya que se consideran terapia de primera línea, reemplazando otras posibles estrategias curativas, como el trasplante de células madre. Sin embargo, varios pacientes en la fase crónica no logran una respuesta óptima a la terapia inicial con imatinib y deben considerarse alternativas.

CASO CLÍNICO: Paciente femenina de 53 años diagnosticada con leucemia mieloide crónica en fase crónica cromosoma Filadelfia positivo, tratada inicialmente con imatinib. Se sometió a un trasplante de células madre alogénico fallido (alo-SCT), por lo que se reinició la terapia con imatinib y, después de varios años, se documentó la resistencia a los medicamentos. Se encontró la mutación BCR-ABL1^{35INS} y se inició tratamiento con dasatinib, un TKI de segunda generación (2GTKI). Después de 12 meses con dasatinib, no se han logrado respuestas citogenéticas ni moleculares importantes.

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CONCLUSIONES: Las mutaciones en el gen BCR-ABL1 que causan variantes de corte y empalme alternativas son solo uno de los muchos mecanismos propuestos de resistencia a inhibidores de la tirosina cinasa y deben tenerse en cuenta cuando no se alcanzan los hitos de respuesta al tratamiento estándar.

PALABRAS CLAVE: Leucemia mieloide crónica; inhibidores de la tirosina cinasa; imatinib; dasatinib.

BACKGROUND

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm caused by a reciprocal translocation between the q arms of chromosomes 9 and 22: t(9; 22)(q34; q11), giving rise to an abnormal chromosome 22, also known as Philadelphia chromosome (Ph). This results in the *BCR-ABL1* fusion gene that is transcribed into BCR-ABL1 mRNA. Its protein product has an increased tyrosine kinase activity, and it is responsible for the malignant transformation of the disease.¹ Chronic myeloid leukemia has an incidence of 1 to 2 cases per 100,000 residents per year, representing approximately 15% of new cases of leukemia in adults.²

The development of target therapies, such as tyrosine kinase inhibitors (TKI), has revolutionized the management and course of the disease, leading to an increase in patient survival rates and CML prevalence. Nevertheless, rising rates of resistance to this drug group have been widely reported, and up to 10 to 15% of patients show resistance to imatinib, the first-generation TKI (1GTKI), which translates into treatment failure that requires more potent therapy options.³

CLINICAL CASE

A 53-year-old female, previously diagnosed with generalized anxiety disorder treated with

mirtazapine and clonazepam, presented in July 2007 due to a three-month history of spontaneous bruises, as well as profuse sweating usually in the afternoon and at night. Physical examination showed multiple ecchymoses in thoracic and pelvic limbs. Abdominal examination revealed mild splenomegaly, and there was no evidence of hepatomegaly. Lymphadenopathy was not found and the patient was afebrile.

Diagnostic assessment

Initial blood count showed pronounced neutrophilic leukocytosis with 108,600/mm³ leukocytes, hemoglobin and platelets were normal. An abdominal ultrasound was conducted, identifying mild splenomegaly. Bone marrow biopsy demonstrated morphology suggestive of a myeloproliferative neoplasm. Karyotyping was positive for t(9;22), and fluorescence *in situ* hybridization (FISH) analysis showed evidence of the fusion of ABL1 with BCR in 73% of bone marrow cells, supporting the diagnosis of CP-CML. **Table 1** shows the laboratory workup of the patient.

Therapeutic intervention

Waiting for BCR-ABL reports and given marked leukocytosis, she was started on hydroxyurea 500 mg and allopurinol 300 mg daily for two weeks. Subsequently, the 1GTKI was started (imatinib

Table 1. Relevant data in laboratory and imaging

Investigation	Findings
Complete blood count	Leukocytes 108 600/mm ³ , differential: neutrophils 92%, lymphocytes 4%, metamyelocytes 24%, blasts 0%. Hb 14.4 g/dL, platelets 225,000/mm ³
Serum LDH	376 UI/L
Peripheral blood smear	Normocytic normochromic erythrocytes marked neutrophilic leukocytosis, an increased number of myelocytes, and metamyelocytes
Bone marrow biopsy	Hypercellular marrow with morphology suggestive of a myeloproliferative neoplasm
Cytogenetics	46,XXt(9;22)(q34;q11.2) [20]
FISH analysis	Evidence of the 9;22 translocation resulting in the <i>BCR-ABL1</i> fusion in 146/200 cells (73%)
Abdominal ultrasound	Splenomegaly of 14 cm. The liver shows normal size and echogenicity

Hb: hemoglobin; LDH: lactate dehydrogenase; FISH: fluorescence *in situ* hybridization.

400 mg per day), and hydroxyurea was discontinued. Complete hematologic (CHR), complete cytogenetic (CCyR), and major molecular responses (MMR) were reached after four weeks, nine months, and five years respectively. Our patient had poor adherence to therapy for several weeks, leading to loss of all responses after seven years. The dose of imatinib was escalated up to 800 mg/day with no response.

Change to a 2GTKI was suggested, however, the patient did not have access to them. For this reason, she underwent allo-SCT in September 2014 without any adverse events. After four months, graft-versus-host-disease of the skin required the administration of prednisone 50 mg for a month, and imatinib at a daily dose of 200 mg was reinitiated.

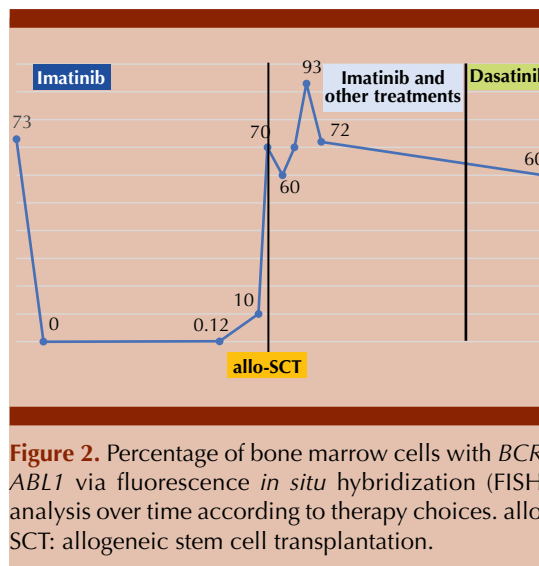
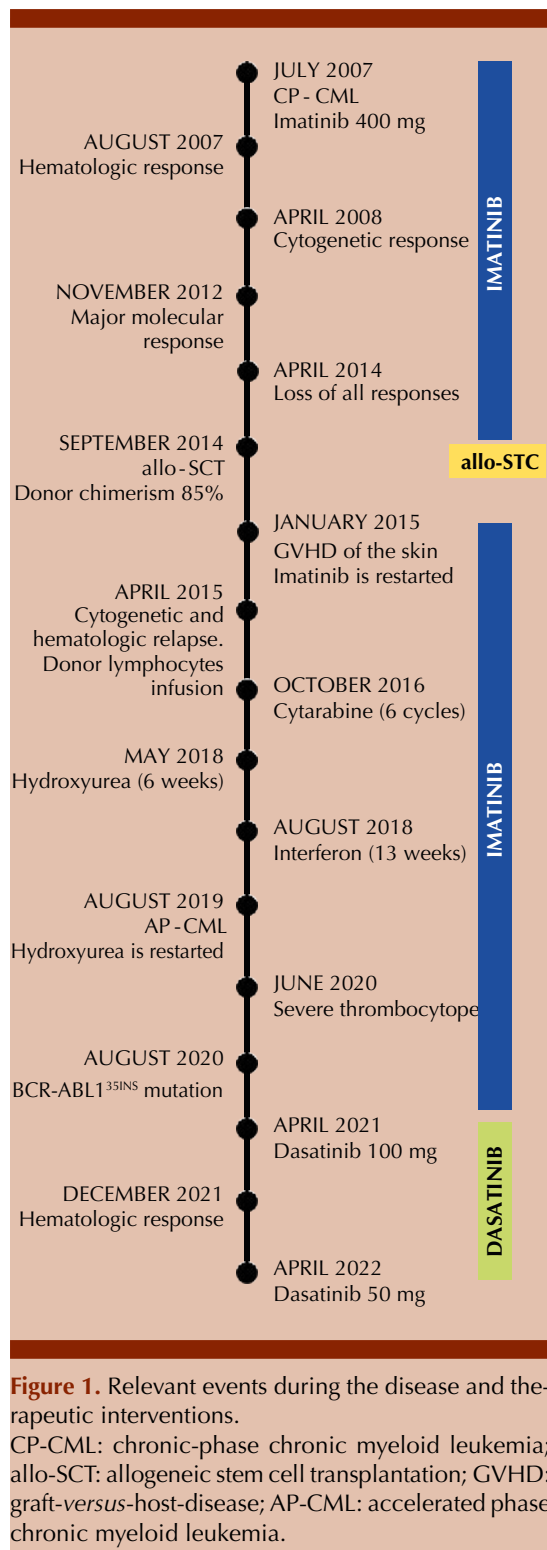
In April 2015, cytogenetic and hematologic relapses were detected, and donor CD3 lymphocytes were infused. During the following months, she complained about occasional mild to moderate bone pain. In addition, rising BCR-ABL1 transcript and decreasing donor chimerism (17%) were documented. Neither a 2GTKI nor another donor lymphocyte infusion was accepted by the patient. Thus, during the next four years, she alternatively received several therapies, such as subcutaneous cytarabine, hydroxyurea, and

interferon. Our patient did not show an optimal response.

In August 2019, the disease progressed to an accelerated phase, hence imatinib and hydroxyurea were started once again. During follow-up, she presented with severe thrombocytopenia (platelets 12 000/mm³) and no signs of hemorrhage, it was managed with one apheresis platelet transfusion and discontinuation of both drugs. Two weeks later, imatinib was restarted. Mutation analysis showed a mutation in the ABL kinase domain (KD), specifically the insertion of 35 nucleotides between exons 8 and 9. She was switched to a 2GTKI therapy with dasatinib 100 mg/day in April 2021, and since then, it has been intermittently suspended because of financial barriers. **Figure 1** summarizes the evolution of the disease and therapeutics our patient has received.

Follow-up and outcomes

Figure 2 shows the response to different therapies during the disease via FISH analysis. Until now, she remains asymptomatic and continues taking dasatinib 50 mg daily. She has acquired CHR, although CCyR has not been achieved yet after 12 months on dasatinib by the time of this report writing. However, further follow-up and quantification of the BCR-ABL1 transcript are needed.



DISCUSSION

Resistance to imatinib is categorized into primary and secondary. Primary resistance occurs when failure to achieve milestones is observed since the beginning of the treatment, whereas secondary or acquired resistance refers to relapse following an initial response.⁴ According to the European LeukemiaNet (ELN) 2020 recommendations for treating CML, failed response to TKI as first-line treatment is defined by the following criteria, expressed as BCR-ABL1 on the International Scale: (1) >10% if confirmed within 1-3 months, (2) >10% by 6 months, (3) >1% by 12 months, and (4) >1%, resistance mutations, or high-risk additional chromosome abnormalities in Ph+ cells at any time.⁵

Secondary resistance in patients in CP-CML is not an uncommon scenario. Up to 20 to 30% of patients show acquired resistance not only to 1GTKI but also to potent 2GTKI and third-generation TKI (3GTKI).⁶ When treatment failure is identified, many possible causes should be questioned, such as medication adherence, toxicity profile, and ingestion of other drugs or herbal supplements which may impair efficacy.⁷

Once these have been dismissed, a disease phase evaluation and assessment of any clonal evolution should be done, including physical examination, complete blood count with differential, bone marrow biopsy with cytogenetic testing, and mutation analysis of BCR-ABL1.⁸

Resistance to TKI can also be classified as either BCR-ABL1 dependent or independent. Acquired BCR-ABL1 KD mutations are present in approximately 60% of patients who experience relapse on imatinib, and point mutations are the most commonly described (ie, M244V, G250E, Y253F/H, E255K/V, T315I, F317L, M351T, E355G, F359V, and H396R/P).^{6,9} Second and third-generation TKI have been shown to be able to overcome several imatinib-resistant KD mutations. *Ergo*, it is recommended to change therapy to dasatinib if the patient has the Y253H, E255K/V, or F359V mutations, and to nilotinib when the F317L and V299L mutations are present. On the other hand, the T315I mutation is associated with resistance to all TKI except ponatinib. Therefore treatment with the 3GTKI should be started, not only when this mutant is identified, but also when there is resistance to at least one 2GTKI.^{2,5} Further mechanisms of resistance include overexpression of the BCR-ABL1 gene, abnormal activity in drug transport, activation of alternative signaling pathways, mutagenesis induced by reactive oxygen species activity, alteration in DNA damage repair mechanisms, genomic instability leading to the acquisition of additional genetic aberrations, epigenetic dysfunction, downregulation of the immune response, among others.^{8,10,11}

This case report identifies a 53-year-old female patient with the BCR-ABL1^{35INS} mutation between the 8 and 9 exons of ABL1, that was acquired several years after a failed allo-SCT, conferring resistance to imatinib. This splice junction mutation introduces a premature stop codon, resulting in the loss of the last 653

residues of BCR-ABL.¹² BCR-ABL truncation causes both: 1) conformational changes of the ABL kinase domain, leading to impairment of TKI binding, and 2) decreased kinase activity of the mutant ABL, giving rise to insufficient response to the drug, whilst disease progression may not be seen despite the persistence of cells harboring BCR-ABL1^{35INS}.¹³

It has been reported that BCR-ABL1^{35INS} is the most common BCR-ABL splice variant in patients that do not respond or lose response after long-term therapy with imatinib, and apparently, it is frequently seen following periods without treatment, just as seen in our patient.¹⁴ Still, there is no consensus whether the 35-bp insertion in the *BCR-ABL1* gene is associated or not with a worse prognosis or a higher risk of disease progression. Whilst some studies have demonstrated it does not contribute to TKI resistance *in vitro*, others suggest it leads to resistance not only to imatinib but also to dasatinib and nilotinib.¹⁵

CONCLUSIONS

Assessing patients with failure to achieve milestones when treated with TKI can be a true challenge. Point mutations in BCR-ABL KD are the leading cause of secondary resistance to imatinib. Hence, prompt mutation analysis is paramount to selecting the best therapeutic strategy, considering that certain TKIs are more effective for certain BCR-ABL1 mutations. However, other factors should be taken into account, for instance, the patient's comorbidities and treatment adherence. Splice mutations, such as BCR-ABL1^{35INS}, are not frequently described and, as a consequence, their clinical significance continues to be uncertain. Even though there is no consensus, clinicians should be aware of BCR-ABL variants in the setting of TKI failure. Nevertheless, further investigation is needed to define its role in the outcomes and prognosis of non-responding patients.

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