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CASE REPORT

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An Imatinib–non-responsive patient with an *ABL* Leu387Trp mutation achieves cytogenetic and molecular response under bosutinib: Case report and biological characterization

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Abstract

Leu387Trp mutation, aroused in an imatinib-non-responsive CML patient, was selected by imatinib treatment along with other unknown factors responsible for resistance, and then it was overcome by bosutinib. These results will be useful for treating patients with this rare mutation and will advise against automatically considering a new mutation as the cause of TKI resistance.

KEYWORDS

chronic myeloid leukemia, genetics, hematology, resistance, TKI

1 | INTRODUCTION

Here we report a case of a CML patient in whom a *ABL* point mutation (Leu387Trp or L387W) was identified during imatinib treatment. The in vitro characterization of the mutation response to TKIs does not suggest a significant role of the Leu387Trp in imatinib resistance acquisition. Nevertheless, cytogenetic and molecular responses were achieved only after switch to bosutinib.

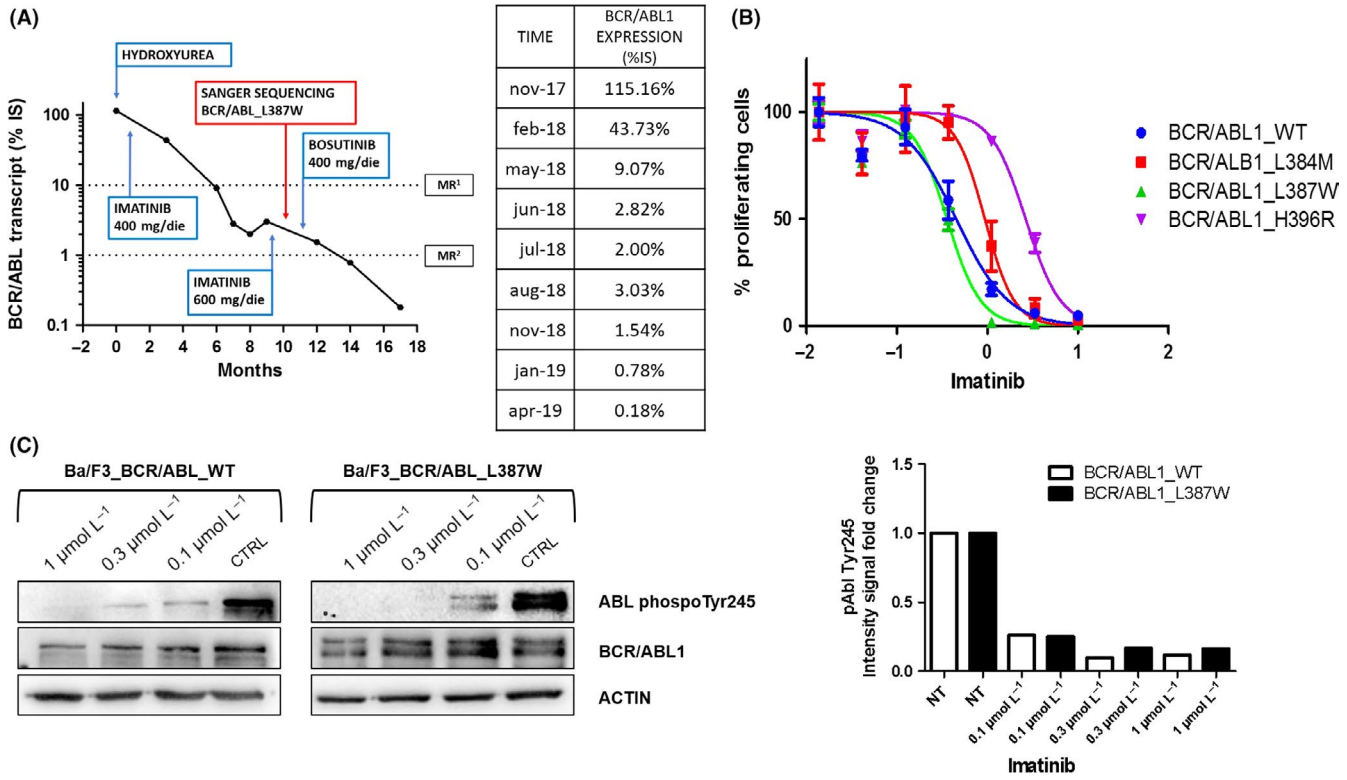
Chronic myeloid leukemia (CML) is a myeloproliferative disorder driven by the presence of the *BCR/ABL1* fusion gene on the Philadelphia chromosome, originated from the reciprocal translocation t(9;22)(q34.1;q11.2). BCR/ABL1 protein is characterized by enhanced and constitutive tyrosine kinase activity, which leads to the deregulation of downstream signaling pathways, mainly affecting cell cycle regulation, proliferation, and apoptosis.[1]

CML treatment is based on tyrosine kinase inhibitors (TKIs), giving patients a great improvement in survival and quality of life.[2] Nevertheless, some patients develop secondary resistance during treatment, frequently caused by appearance of point mutations in the kinase domain.[3] More than 100 *ABL* mutations have been associated with TKI resistance, but not all of them have been characterized in terms of sensitivity to TKIs.

Here we report a case of a young woman in whom a point mutation on *ABL* (Leu387Trp) was identified during imatinib treatment, with lack of cytogenetic response and the need to change TKI. This mutation was reported previously[4] but never characterized in terms of sensitivity to TKIs. We provide here an in vitro characterization of the mutation response to different TKIs, using Ba/F3 cells, stably expressing the mutated *BCR/ABL1* gene.

2 | CASE HISTORY

A 39-year-old woman was diagnosed with chronic phase CML in 2017 after cytogenetic analysis (46,XX t(9;22) 100%), confirmed by molecular analysis of t(9;22) BCR/



TIME	BCR/ABL1 EXPRESSION (%IS)
nov-17	115.16%
feb-18	43.73%
may-18	9.07%
jun-18	2.82%
jul-18	2.00%
aug-18	3.03%
nov-18	1.54%
jan-19	0.78%
apr-19	0.18%

FIGURE 1 A, Evolution of the molecular response based on the BCR/ABL transcript expression assessed by RT-qPCR and normalized by the International Scale (IS). Molecular response 1 (MR1) represents a BCR-ABL/ABL ratio ≤10%, molecular response 2 (MR2) represents a BCR-ABL/ABL ratio ≤1%. B, Dose-response curves of Ba/F3 cells carrying WT or mutated BCR/ABL, treated with increasing concentrations of imatinib for 72 h. Cell proliferation assay was performed with the CellTiter96 Aqueous One Solution Cell Proliferation Assay (Promega). C, Immunoblot analysis of total cell lysates from Ba/F3_BCR/ABL, WT, and L387W lines treated increasing concentrations of imatinib. Total BCR/ABL immunoblots were performed from the same lysates to show that the total protein levels are similar. Actin is shown as a further loading control. Western blot was performed using the following antibodies: αc-Abl (K-12) (sc131)(Santa Cruz Biotechnology), αp-AblT245 (2861)(Cell Signaling), αActin (A2066)(Sigma-Aldrich)

ABL1 transcript (115,16% IS; Figure 1A); the patient was assigned to intermediate risk by Sokal score (0.84) and low risk by Hasford score (398). The patient was initially treated with hydroxyurea, followed by 400 mg/d of imatinib, which was suspended for 4 weeks after 1 month of treatment because of severe neutropenia.

Analysis at 3 months showed lack of both cytogenetic (46,XX t(9;22) 100%) and molecular (BCR/ABL1 43,73% IS) responses. However, mutational analysis of the BCR/ABL1 gene was negative. After 6 months of treatment, the patient achieved a partial cytogenetic response (46,XX t(9;22) 33%) with an MR1 level of molecular response (BCR/ABL1 9.066% IS).

The patient was admitted in 2018 to our center, where MR1 molecular response was confirmed (BCR/ABL 2.82% IS). Thus, she continued on the same dose of imatinib, as it was globally well tolerated.

After 9 months of therapy, the bone marrow aspirate revealed the presence of an atypical translocation in 2 out of 25 analyzed metaphases, the t(9;22;10), and the cytogenetic response was still partial (8%). Therefore, imatinib dose was increased to 600 mg/d.

At the same time, sequencing of BCR/ABL1 gene revealed a point mutation in the BCR/ABL catalytic domain: Leucine 387 was replaced by tryptophan (Leu387Trp). Because of a further increase in PCR values (3.03% IS vs 2.00% IS), the patient was switched to bosutinib, 400 mg/d.

The bone marrow aspirate at 12 months from the diagnosis showed no atypical cells; cytogenetic analysis revealed a complete response with no evidence of t(9;22) or t(9;22;10) positive cells. Moreover, molecular response reached MR2 level at the last two follow-up (BCR/ABL1/ABL ratio = 0.52% IS and 0.18% IS). The patient is continuing bosutinib treatment (400 mg/d).

3 | DISCUSSION

In order to biologically characterize this mutation, we stably overexpressed BCR/ABL1, wild type (WT), and Leu387Trp, in the IL3-dependent murine pro-B cell line, Ba/F3. Expression of BCR/ABL1 fusion protein conferred IL3-independent growth to the cells. The presence of the

TABLE 1 Sensitivity of Ba/F3_BCR/ABL_WT and Ba/F3_BCR/ABL_387W lines to different tyrosine kinase inhibitors. After 72hours of treatment, cell proliferation was measured with the CellTiter96 Aqueous One Solution Cell Proliferation Assay (Promega). The inhibitors concentration range were:10μM-0,1μM for imatinib; 1μM-0.01μM for bosutinib, nilotinib and PF-114; 100nM-0.1nM for dasatinib and ponatinib

		IC50 [μmol/L]		RATIO
		BCR/ABL1 WT	BCR/ABL1 L387W	(BCR/ABL1 L387W)/(BCR/ABL1 WT)
INHIBITOR	Imatinib	0.4526	0.3572	0.789218
	Bosutinib	0.01702	0.02583	1.517626
	Dasatinib	0.0007529	0.001842	2.44654
	Nilotinib	0.009328	0.0355	3.805746
	Ponatinib	0.0009344	0.0018395	1.968643
	PF-114	0.01689	0.01809	1.071048

Sensitive ≤ 2	Moderately resistant 2.01-4	Resistant 4.01-10	Highly resistant > 10

Leu387Trp substitution was confirmed by Sanger sequencing (not shown). BCR/ABL1-Leu387Trp transcript levels were comparable to the WT as well as to two additional mutants previously described[5] (Leu384Met and His396Arg) that were used as comparators, since they hit residues in the same region of the kinase, that is, the activation loop.

The first aim of this study was to identify any sensitivity difference to imatinib exerted by the Leu387Trp mutation compared to the WT and to evaluate if it had the same sensitivity/resistance profile of other known mutations. Surprisingly, the Leu387Trp cell line did not show any significant difference in the response to imatinib treatment, as shown by comparable IC50 values (WT: 0.45 μmol/L; Leu387Trp: 0.36 μmol/L; Figure 1B and Table 1). This was further confirmed by Western blot analysis performed on total cell lysate from Ba/F3_BCR/ABL1_WT and Ba/F3_BCR/ABL1_Leu387Trp cell lines. BCR/ABL1 phosphorylation is only visible at low concentration treatments, thus confirming the efficacy of the treatment. Moreover, the inhibition pattern on BCR/ABL1_Leu387Trp is comparable to the WT one, again confirming the same sensitivity trait of the Leu387Trp mutant compared to the WT.(Figure 1C). As a comparison, Ba/F3_BCR/ABL1 cells carrying mutations in close vicinity (Leu384Met and His396Arg) did show a significant IC50 shift (Figure 1B).

Next, we evaluated the effects of other tyrosine kinase inhibitors (bosutinib,[6] dasatinib,[7] nilotinib,[8] ponatinib,[9] and PF-114[10]) on the Ba/F3_BCR/ABL1_ Leu387Trp cell line. Interestingly, the mutated cell line showed a moderate resistance to dasatinib and nilotinib, with ~2.4-fold and ~3.8-fold increase resistance compared to the WT, respectively. (Table 1).

Taken together, these results do not seem to suggest a significant role of the Leu387Trp mutation in the acquisition of imatinib resistance. Therefore, the Leu387Trp mutation can be considered as a mutation that was selected by imatinib treatment and gave the cells a certain advantage together with some other unknown factor. Moreover, we cannot rule out the in vivo existence of low-level clones with different BCR/ABL1 mutations. The advantage provided by the presence of the Leu387Trp mutation may be so subtle that it is not appreciable in in vitro models, but it may contribute to other complex mechanisms that eventually lead to imatinib resistance in vivo. Alternatively, it may represent a "passenger" mutation that was acquired together with other unknown alterations that caused resistance to imatinib. It is therefore hard to speculate which other factors are involved. Since the resistance to imatinib was successfully overcome by bosutinib, other kinases might be activated, which ultimately lead to the activation of by-pass pathways.

In conclusion, we present a case of Philadelphia-positive CML patient that responded poorly to imatinib, developed an activation loop mutation that, per sé, does not seem to confer significant resistance in vitro, and was successfully treated with second-line bosutinib, which achieved MR2 remission.

The importance of this mutation may not be huge from a biological point of view; however, we believe it is important for a physician who finds out that a patient is carrying such mutation during imatinib treatment, to know that bosutinib treatment will provide better outcomes. These results will provide useful insights to physician treating CML patients with this rare mutation in the future, and they should also advise against automatically considering a new mutation in a CML patient as the cause of TKI resistance.

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AUTHOR CONTRIBUTIONS

IC: made substantial contributions in conception and design, execution of experiments and analysis and interpretation of data, was involved in drafting of the manuscript and in revising it, and gave final approval for the version to be published. EB: made contribution by providing clinical data, was involved in the revision of the manuscript, and gave final approval for the version to be published. FB: made contribution by providing clinical data, was involved in the revision of the manuscript, and gave final approval for the version to be published. RP: made contribution by providing clinical data and made substantial contributions to the analysis and interpretation of data, was involved in the revision of the manuscript and gave final approval for the version to be published. LM: made substantial contributions in conception and design, analysis and interpretation of data, was involved in the revision of the manuscript, and gave final approval for the version to be published. CGP: provided funding for this work, made contribution by providing clinical data, was involved in the revision of the manuscript, and gave final approval for the version to be published.

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