CASE REPORT

Clinical activity of ponatinib in one patient with chronic myeloid leukemia in chronic phase with e19a2 transcript and T315I mutation

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Abstract

Background: Chronic myeloid leukemia (CML) is a hematological disorder that in rare cases, mainly in CML neutrophilic, presents the e19a2 rearrangement. The encoded product is a 230-KDa protein. Despite the remarkable responses to treatment of most patients, a small but significant fraction of them develop clinical resistance to the tyrosine kinase inhibitors (TKIs). The most common mechanism of resistance is point mutations in the *ABL1* kinase domain. The recently approved third-generation TKI ponatinib demonstrated remarkable activity in patients with multi-TKI-resistant disease. Particularly impressive was its efficacy in patients with T315I mutation that is resistant to all other TKIs. *Methods:* Qualitative PCR was carried out by multiplex approach. Relative transcripts quantification was performed by one-step real-time PCR, with a specific Taqman probe and primers for the e19a2 rearrangement. We carried out a mutational screening by high-resolution melting, and the mutation was identified by Sanger method. The mutation burden was quantified by quantitative PCR using allele-specific primers. *Results:* In a patient with CML, we identified a PCR product corresponding to e19a2 rearrangement harboring T315I mutation. At the time of mutational analysis, during dasatinib treatment, the T315I clone was 100% and the quantification of *BCR-ABL1* was 18%. After ponatinib therapy, the T315I mutation burden decreased down to undetectable levels and the *BCR-ABL1* transcripts showed a very low value (0.011%). *Conclusions:* Here, we report the hematological, cytogenetic, and molecular response of a patient with refractory CML in chronic phase with e19a2 transcripts, carrying T315I mutation that was successfully treated with ponatinib.

Key words chronic myeloid leukemia; T315I; e19a2; ponatinib

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Chronic myeloid leukemia (CML) is characterized by t(9;22)(q34;q11) that generates the Philadelphia (Ph) chromosome (1). The *BCR-ABL1* fusion gene encodes for three oncoproteins that vary in size depending on the break-point site in the *BCR* gene: major (M-BCR, encodes for p210), minor (m-BCR, encodes for p190), and micro (μ-BCR, encodes for p230) (2). The μ-BCR breakpoint connects exon 19 of *BCR* with exon 2 of *ABL1* giving rise to the e19a2 transcript, which has been associated with a mild CML phenotype (3), defined as Ph^+ chronic neutrophilic leukemia (Ph^+-CNL). Ph^+-CNL is an uncommon myeloproliferative disorder, which shows a more indolent course than classical CML (4, 5).

Three tyrosine kinase inhibitors (TKI) targeting the BCR-ABL1 protein (imatinib, nilotinib, and dasatinib) have been approved for the treatment of patients with newly diagnosed chronic-phase CML. Despite the remarkable responses of most patients, a small but significant fraction of patients

develops clinical resistance to the TKIs, some of which is attributed to the BCR-ABL1 kinase domain mutations. The recently approved third-generation TKI ponatinib (AP24534) demonstrated remarkable activity in patients with multi-TKI-resistant disease. Particularly impressive was its efficacy in patients with T315I mutation that is resistant to all other TKIs (6, 7). This report describes the hematological, cytogenetic, and molecular response of a patient with refractory CML in chronic phase with e19a2 transcripts, carrying T315I mutation and treated with ponatinib.

Patients and method

In July 2004, a 58-yr-old woman presented splenomegaly, showing hematological values as indicated in Table 1 (Diagnosis column). Karyotype analysis performed at diagnosis showed 46,XX,t(9;22)(q34;q11) in 100% of metaphases analyzed with no additional cytogenetic abnormalities. RT-PCR analysis using primers for M-BCR gave a specific band corresponding to e14a2; thus, other rearrangements were not sought. The patient was diagnosed with chronic-phase CML with high-risk sokal and imatinib 400 mg/d was started. Six months later complete hematological response and major cytogenetic response were achieved, and no specific band for M-BCR was observed by RT-PCR. In August 2007, the analysis by dual-color FISH showed the *BCR-ABL1* fusion signals in a 10% of nuclei analyzed; consequently, the dose of imatinib was increased to 800 mg/d. In December 2008, the patient showed a loss of cytogenetic response (63% Ph^+); therefore, the therapy was changed to nilotinib 800 mg/d, but only a partial hematologic response was achieved. Thus, 6 months later, she started treatment with dasatinib, initially at a dose of 100 mg/d then increased to 140 mg/d twice; however, due to cytotoxicity, the dose must be decreased to 100 mg/d. In November 2012, her blood analysis showed the values indicated in Table 1 (Mutation detection column), while cytogenetic study identified >95% Ph^+ cells. Because of her very low responsiveness to all three TKIs, a more exhaustive molecular study was carried out. Surprisingly, the RT-PCR for e13/e14-a2 transcripts resulted negative; then, by multiplex PCR assay that enables to identify all possible rearrangements (2), we identified a PCR product corresponding to e19a2 (Data not shown). With the aim to quantify the abundance of Ph^+ cells with e19a2, we cloned a 1263-bp DNA fragment, overlapping the e19a2 breakpoint region, in a plasmid, that upon spectrometric quantification was properly diluted and used to construct a calibration curve. Relative transcripts quantification was performed by one-step real-time PCR, with a specific Taqman probe and primers for the e19a2 rearrangement (2, 8). At this time point, as indicated in Fig. 1, the *BCR-ABL1/ABL1* ratio was 18%. Due to the lack of molecular response, we proceed to search for mutations in the *ABL1* kinase domain. As previously reported by us (9), we carried out a mutational screening by high-resolution melting on Rotor-Gene (Qiagen, Hilden, Germany). Melting profile was compatible with the presence of mutations in ATP-binding site (Fig. 1); then, with the aim to identify this mutation, a fragment of 586 bp (9) corresponding to exons 4–7 was sequenced by Sanger method and the T315I mutation was identified. This mutation was quantified by quantitative PCR using allele-specific primers (ARMS-qPCR) (9) and all Ph^+ cells (100%) resulted mutated. Moreover, using ARMS-qPCR, we were able to track mutation back over time and follow up this mutation forward during the treatment (Fig. 1). After the discovery of the T315I mutation, dasatinib treatment was discontinued and the therapy was rotated to hydroxyurea for 9 months and then to ponatinib 45 mg daily, until the present. As shown in Fig. 1, both mutation burden and *BCR-ABL1* levels constantly

Table 1 Patient's hematologic profile

Peripheral blood	Diagnosis	Mutation identification
HGB (g/dL)	14.4	12.1
HCT (%)	45	35
WBC ($\times 10^9$/L)	24	68
PLT ($\times 10^9$/L)	103.6	92
Myeloblasts (%)	0	0
Myelocytes	2	0
Band (%)	2	1
Neutrophil (%)	77	88
Eosinophil (%)	3	3
Basophil (%)	3	2
Lymphocyte (%)	13	6
Monocyte (%)	0	0
Bone marrow Myeloblasts (%)	3	NA

HGB, hemoglobin; HCT, hematocrit; PLT, platelets; WBC, white blood cell count; NA, not apply.

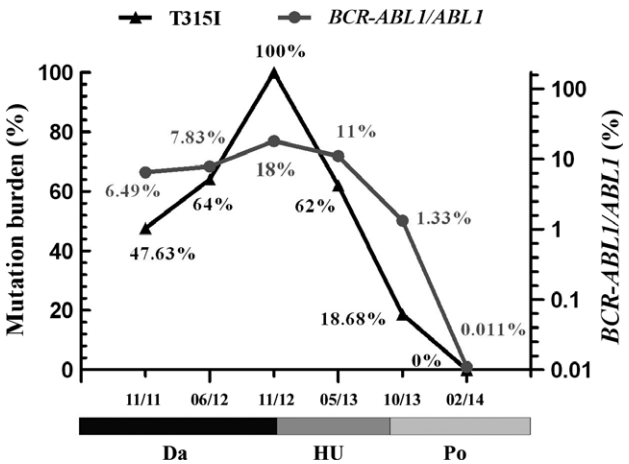

Figure 1 Molecular analysis studies of the patient. Graph indicating serial measurements of both *BCR–ABL1* transcript levels (right log-*Y*-axis) and T315I mutation levels (left linear-*Y*-axis). This graph shows the dynamics of both $BCR\text{–}ABL1^{T315I}$ (by ARMS-qPCR) and *BCR–ABL1* transcripts (by qRT-PCR) of the patient during the last 26 months. Da, dasatinib; Hu, hydroxyurea; Po, ponatinib.

decreased down to undetectable level for T315I mutation and very low values for *BCR-ABL1/ABL1* ratio (0.011%).

Discussion

Mutations in the kinase domain of *BCR-ABL1* are the most prevalent mechanism of acquired TKIs resistance in patients with CML. One of the most common mutations, which is present in up to 20% of patients with resistance to TKI, is the so-called gatekeeper T315I substitution, which blocks access of the drug to the enzyme's ATP-binding site and confers a high degree of resistance to first- and second-generation TKIs. Ponatinib is a novel potent oral tyrosine kinase inhibitor that blocks native and mutated *BCR-ABL1*, including the mutant T315I in Ph^+ leukemia (6, 7). To our knowledge, this is the first report in the literature about the use of ponatinib in a CML patient carrying the e19a2 transcript and the T315I mutation. Although a CML patient with e19a2 rearrangement and T315I mutation has been reported, dasatinib treatment could only prevent the progression of the disease to accelerated phase; (10) nevertheless, molecular levels seem to maintain high values. This is similar to what occurred with our patient, under dasatinib, for whom not only e19a2 transcripts persisted similarly high but in addition the T315I clone increased up to 100% (Fig. 1). The efficacy of ponatinib in CML patients with e1a2 or e19a2 is unclear because of the rarity of these entities in CML. A current report considers that ponatinib might not be very effective in CML patients with e1a2 transcripts (11). Our experience suggests that ponatinib would be an excellent inhibitor in treatment of CML patients with e19a2 harboring T315I mutation. More studies with ponatinib treatment on patients with rare *BCR-ABL1* transcripts are necessary to draw conclusions.

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Competing interest

The authors declare no conflict of interest.

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