Bosutinib as a fourth-line therapy for a patient with T315I-positive lymphoid blastic phase chronic myeloid leukemia: A case report

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Abstract. A 35-year-old male was diagnosed with chronic myeloid leukemia in the chronic phase and was prescribed 100 mg daily dasatinib. However, dasatinib was discontinued due to thrombocytopenia, and within six months, the disease progressed to the lymphoid blastic phase. Hyper-cyclophosphamide, vincristine, adriamycin and dexamethasone chemotherapy combined with 140 mg dasatinib or 600 mg imatinib was prescribed. The two inhibitors were soon discontinued due to severe thrombocytopenia and jaundice, respectively. Myelosuppression persisted subsequent to the nadir. Bone marrow (BM) aspiration and biopsy revealed hypercellular marrow filled with blasts. Sequencing of the leukemia cells revealed overlapping peaks for the wild-type sequence and the T315I mutant sequence. The patient was treated with 500 mg bosutinib (which was later reduced to 300 mg) for pretransplant cytoreduction. After 5 months, the patient's spleen exhibited a reduction in volume and the percentage of blasts in the BM decreased from 96.1 to 17.5%. The patient successfully underwent cord blood transplantation. The patient has been disease-free for 5 months subsequent to transplantation. This case suggests that bosutinib may be effective for cytoreduction prior to stem cell transplantation, unless the leukemia cells consistently harbor the T315I mutation.

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder that arises in hematopoietic stem cells (1). It is characterized by a reciprocal t(9;22) translocation that leads to the formation of the Philadelphia chromosome, which in turn produces the BCR-ABL1 fusion protein. BCR-ABL1 is a constitutively active tyrosine kinase, which transmits proliferation and survival signals through the Src family kinases, Lyn and Hck, to the downstream targets, STAT5 and Ras/ERK (2,3).

CML has a triphasic clinical course (1). In the chronic phase (CP), excessive proliferation of mature myeloid cells occurs (1). In the accelerated phase (AP), CML cells accumulate chromosomal and genetic abnormalities, and blasts in the peripheral blood (PB) or bone marrow (BM) increase to 10-20% (1). Eventually, the disease progresses to the blastic phase (BP), where blasts account for >20% of the cells in the PB or BM (1). In ~70% of BP cases the blast lineage is myeloid, whereas in the remaining 20-30% of BP cases the lineage is lymphoid (1).

Tyrosine kinase inhibitors (TKIs) have markedly improved the prognosis of patients with CML. Currently, five different TKIs are available for the treatment of CML: Imatinib, nilotinib, dasatinib, bosutinib and ponatinib (4). Imatinib, nilotinib and dasatinib are approved for first-line treatment of CML, and bosutinib and ponatinib are available for resistant and intolerant CML patients. As of July 2015, ponatinib is not yet available in Japan. Secondary resistance to TKIs occurs in 20-30% of patients with CML, and is mainly due to mutations in the *ABL1* kinase domain (5,6). The most intractable mutation is the T315I gatekeeper mutation, which potently interferes with the binding of TKIs to BCR-ABL1 (7). Of the five TKIs, only ponatinib is active against the T315I mutant (4,8-11). Stem cell transplantation is currently reserved for patients with CML-AP/BP and selected cases of CML-CP (4,12,13).

Bosutinib is a dual Src and ABL1 tyrosine kinase inhibitor (14-21). It is active against various BCR-ABL1 mutations, including those associated with imatinib, dasatinib and nilotinib resistance. However, bosutinib is not active against the T315I and V299L BCR-ABL1 mutations (4,10,11). Bosutinib inhibits Src family kinases, including Src, Lyn, Fgr and Hck (14). However, unlike other TKIs, bosutinib has exhibited minimal inhibitory activity against platelet-derived growth factor receptor and c-KIT (14). Therefore, bosutinib has a distinct

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toxicity profile compared to other TKIs (15-21). The present case study provides a report of a patient with T315I-positive CML-BP who was successfully treated with bosutinib as a fourth-line therapy prior to cord blood transplantation.

Materials and methods

Sequencing of the ABL1 kinase domain of BCR-ABL1. Total RNA was extracted from total BM cells using ISOGEN (Nippon Gene, Tokyo, Japan) and 1 μg from each sample was reverse transcribed using a Transcriptor 1st Strand cDNA Synthesis kit (Roche Diagnostics, Tokyo, Japan). The *ABL1* tyrosine kinase domain of *BCR-ABL1* was amplified with nested RT-PCR with the following primers: 1st forward, (designed on *BCR* gene), 5'-TTCAGAAGCTTCTCCCTGCAT-3' and reverse (located in *ABL* gene), 5'-CTTCGTCTGAGATAC TGGATTCCT-3'; 2nd forward (located in *ABL* gene), 5'-AAG CGCAACAAGCCCACTGTCTAT-3' and reverse (located in *ABL* gene), 5'-CTTCGTCTGAGATACTGGATTCCT-3'. Amplified fragments were directly sequenced using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Written informed consent was obtained from the patient in the present study, according to the Declaration of Helsinki.

Staining. BM aspirate smears were stained with May-Grünwald's and Giemsa's staining solution. Images were acquired at room temperature using a BX50 light microscope (Olympus Corporation, Tokyo, Japan) equipped with a DS-Fi2 camera, Digital Sight DS-U3 controller and NIS-Elements D software version 3.20 (all Nikon Corporation, Tokyo, Japan). BM biopsy samples were fixed in 10% neutral buffered formalin solution, decalcified in Osteosoft (Merck KGaA, Darmstadt, Germany), sectioned (3-μm thick), and stained with hematoxylin and eosin. Images were acquired at room temperature using a BX53 light microscope equipped with DP21 camera/controller (both Olympus Corporation).

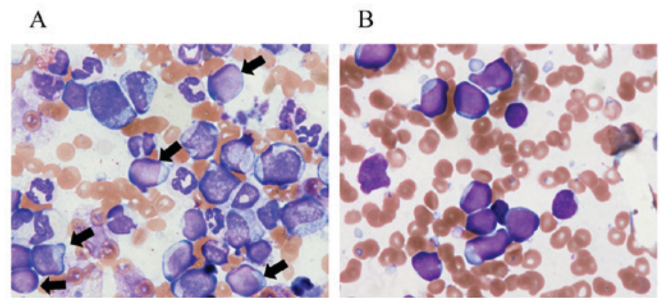

Figure 1. May-Grünwald Giemsa staining of BM aspirate smears. (A) BM at the initial diagnosis of CML-CP. Black arrows illustrate the blasts. (B) BM at the diagnosis of CML-BP. Original magnification, x400. BM, bone marrow; CML, chronic myeloid leukemia; CP, chronic phase; BP, blastic phase.

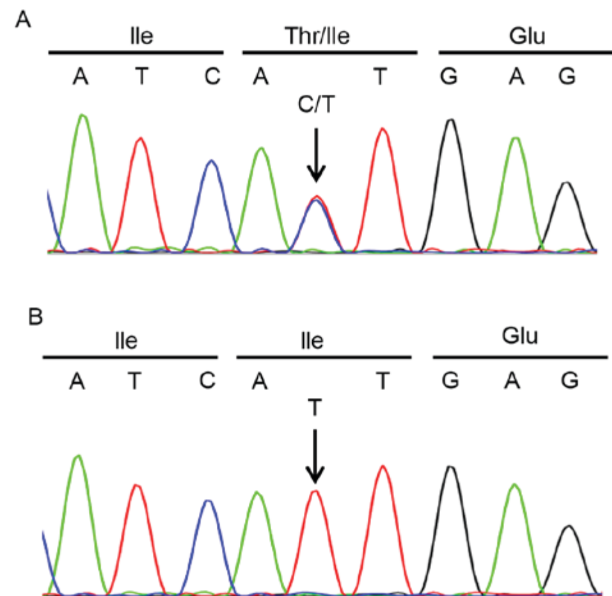

Figure 2. Sequencing chromatograms of the *BCR-ABL1* fusion gene, particularly the regions flanking amino acid T315 of *ABL1*. (A) Sequencing chromatogram that was obtained prior to bosutinib administration. The central codon, ACT, corresponds to wild-type T315. The overlapping peaks of wild-type and C >T (T315I) mutation *ABL1* are indicated with an arrow. (B) Sequencing chromatogram that was obtained four months subsequent to bosutinib administration. Only the T315I peak (ATT, indicated with an arrow) was detected.

Case report

A previously healthy 35-year-old Japanese male visited the Department of Surgery for a left inguinal hernia in March 2014. The patient had mild hepatomegaly and marked splenomegaly. His blood cell counts were: White blood cells (WBC) 345,820/μl (blasts 4.0%, promyelocytes 0.0%, myelocytes 24.5%, metamyelocytes 3.5%, band 16.5%, segmented 44.5%, monocytes 1.0%, lymphocytes 1.0%, eosinophils 2.0%, basophils 3.0%), hemoglobin 7.3 g/dl and platelets 113x10^3/μl. The following day, the patient was referred to the Department of Hematology. BM aspiration and biopsy revealed hypercellular marrow with 9.4% blasts (Fig. 1A) and the karyotype was 46,XY, t(9;22)(q34;q11.2)[20/20]. The patient was diagnosed with CML-CP. However, the disease rapidly progressed to AP (platelets 65x10^3/μl). The patient was hospitalized in April 2014, and 100 mg dasatinib was prescribed. WBC and platelet counts normalized, while hemoglobin levels increased. The splenomegaly was also reduced from 8 to 3.5 cm below the umbilicus. On the 9th day of hospitalization, the patient was discharged. The patient continued to take dasatinib until his platelet count lowered to 49x10^3/μl, when dasatinib was discontinued one month subsequent to the initial prescription. One month later, administration of dasatinib was resumed. Subsequent to this visit, no contact could be made with the patient.

In September 2014, the patient was hospitalized due to active nasal bleeding. Blood test results revealed levels of: WBC 3,790/μl, hemoglobin 7.6 g/dl and platelets 8x10^3/μl. BM aspiration and biopsy revealed hypercellular marrow with peroxidase-negative blasts (85.5%, Fig. 1B) characterized as $CD10^+/CD19^+/CD20^-/CD34^+/CD7^{dim}$. Cytogenetic analysis revealed a Philadelphia chromosome with additional aberrations: 46, XY, t(9;22)(q34;q11.2), del(15)(q?)[6/20], 47,idem, +der(22)t(9;22)[2/20], 50,idem,+X,del(13)(q?),+14,+21, +der(22)t(9;22)[3/20],46,XY[8/20]. A diagnosis of CML B-lymphoid BP was made. Hyper-CVAD (cyclophosphamide, vincristine sulfate, adriamycin, dexamethasone) chemotherapy

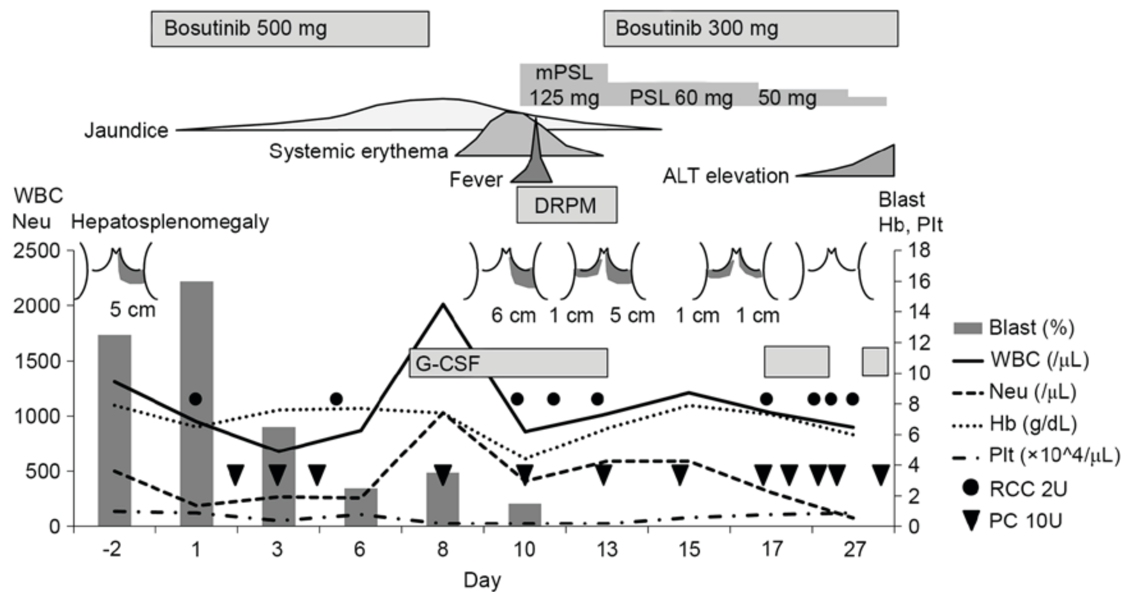

Figure 3. Summary of the clinical symptoms, blood analyses, and course of treatment for the present case report. Hepatosplenomegaly is indicated according to the size below the costal arches. mPSL, methylprednisolone; ALT, alanine transaminase; WBC, white blood cells; DRPM, doripenem; Neu, neutrophils; Hb, hemoglobin; Plt, platelets; G-CSF, granulocyte-colony stimulating factor (filgrastim); RCC 2U, 2 units of red cell concentrates; PC 10U, 10 units of platelet concentrates.

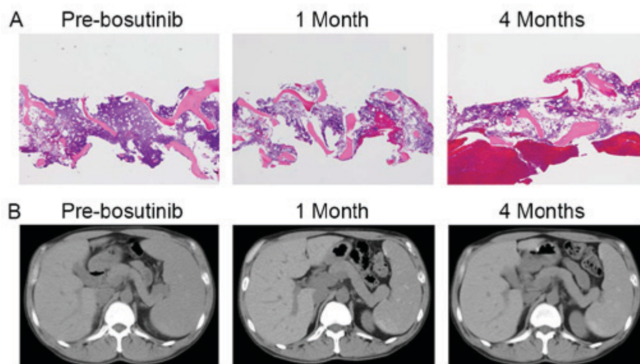

Figure 4. The patient's leukemia load was reduced with administration of bosutinib. (A) Hematoxylin and eosin staining of bone marrow biopsy samples that were collected prior to and 1 and 4 months subsequent to the administration of bosutinib. A reduction in cellularity was observed with time. Original magnification, x40. (B) Plain abdominal computed tomography scans were obtained prior to and 1 and 4 months subsequent to administration of bosutinib. Spleen volume was reduced following one month of bosutinib treatment. For the next three months, this reduced spleen volume was maintained.

was started in combination with 140 mg dasatinib (22). The patient's platelet counts remained as low as $10 \times 10^3/\mu l$, despite frequent transfusions. When dasatinib induced thrombocytopenia in the patient by day 7, it was discontinued and the patient was switched to 600 mg imatinib. Active nasal bleeding persisted even following cauterization. Therefore, dexamethasone was reduced from 8 doses to 5 doses to reduce mucosal toxicity. When the bilirubin and alkaline phosphatase levels reached 3.7 mg/dl and 1,012 IU/l, respectively, by day 16, imatinib was discontinued. However, pancytopenia persisted, and when BM aspiration and biopsy was performed on day 37, the blast level was 81.4%. Considering the high leukemia burden of the patient, allogeneic stem cell transplantation was considered to be a high risk treatment (4,12,13). Therefore, the patient underwent follow-up care in an outpatient setting, with transfusions performed 2-3 times per week.

In December 2014, the patient was re-admitted for leukemia debulking with bosutinib. BM aspiration and biopsy revealed hypercellular BM filled with blasts (96.1%). Cytogenetic analysis revealed 46,XY,t(9;22)(q34;q11.2),del(15)(q?)[18/19] and 50,idem,+X,add(1)(p11), add(13)(q12),+14,+21,+der(22) t(9;22)[1/19]. Quantitative PCR detected major *BCR-ABL1* at 3.1×10^5 copy/μg RNA. The T315I mutation was detected in the major *BCR-ABL1* sequencing. In the sequencing chromatogram, peaks of the wild type allele were revealed to overlap with the T315I mutant allele (Fig. 2), suggesting that almost half of the leukemia cells lacked the T315I mutation. Administration of bosutinib at 500 mg per day was prescribed (Fig. 3). Notably, blasts in the PB, as well as hepatosplenomegaly, disappeared within 2 and 3 weeks, respectively. Adverse effects experienced by the patient included diarrhea [CTCAE v4.0 Grade (G) 2], anorexia (G3), jaundice (G3, bilirubin 4.6 mg/dl), systemic erythema (G2) and papilloedema (G1). Bosutinib was discontinued on day 8 due to jaundice, yet was resumed at a dose of 300 mg on day 14. This lower dose was better tolerated, however the levels of alanine transaminase (ALT) became elevated (G3, 187 IU/l), which required temporary interruption. The patient was referred to a collaborating hospital for transplantation in February 2015. After two months of bosutinib prescription, the blast level was decreased to 19.4%, and major *BCR-ABL1* level was 3.5×10^4 copy/μg RNA. The patient continued to take bosutinib while waiting for the transplantation. After four months of bosutinib prescription, the patient's BM became more hypocellular (Fig. 4A) and his spleen did not show regrowth (Fig. 4B). Blasts in the BM were reduced to 17.5%, and a cytogenetic analysis revealed a normal karyotype (46, XY [20/20]). Sequencing of *ABL1* of the BM sample

only detected the T315I mutant allele (Fig. 2B). Stem cell transplantation was still considered necessary due to persistent pancytopenia and transfusion dependence, despite the absence of disease progression (based on the percentage of blasts in the PB or splenomegaly status). Subsequent to taking bosutinib for five months, the patient underwent cord blood transplantation at the end of April 2015 that was accompanied by high-dose cytarabine (2 g x 2/day for two days) followed by a conditioning regimen of fludarabine (180 mg/m^2), an intravenous injection of busulfan (12.8 mg/kg) and melphalan (80 mg/m^2). Engraftment was confirmed on day 25. BM aspiration was performed on day 36. The blast count was 0%, the karyotype was 46,XX [20/20] (as a result of a female donor), and *BCR-ABL1* was not detected by quantitative PCR. Subsequent to becoming transfusion-independent, the patient was discharged from the hospital on day 64. Acute graft-vs.-host disease (GVHD) was observed in skin (stage I), but not in the liver (stage 0) or gut (stage 0) (Grade I). Major *BCR-ABL1* level in PB was <0.0018% by International Scale at one month and two months subsequent to transplantation. Tacrolimus for GVHD treatment was discontinued at four months. At five months, the patient has no sign of relapse, and follow up was begun in an outpatient setting.

Discussion

Point mutations in the *ABL1* kinase domain have been associated with resistance to TKIs in patients with CML (4-6,10,11). In the present case report, a patient with T315I mutation-positive CML-BC demonstrated a response to bosutinib over five months, and successfully underwent cord blood transplantation. Initially, when exhibiting CML-CP, the patient was intolerant to treatment with dasatinib [due to thrombocytopenia and low compliance (23). In addition, the patients CML-BP disease was resistant to chemotherapy combined with the TKIs, dasatinib or imatinib (22). Correspondingly, the patient was revealed to carry the T315I mutation. It has been reported that bosutinib is ineffective against T315I-mutated BCR-ABL1 *in vitro* (4,10,11). In addition, in phase 1 and 2 clinical trials, CML-CP patients with the T315I mutation exhibited poor responses compared to those without the mutation (15-17). In Japan, bosutinib became available in December 2014 (21), whereas ponatinib (8,9) and the non-TKI, omacetaxine (24), which have been shown to be active against T315I-positive cases, were not available at this time. In the present case, the sequencing chromatogram of *ABL1* implied that at least half of the leukemia cells carried wild-type *BCR-ABL1* (Fig. 2A). Indeed, bosutinib was effective in debulking the leukemia cells in the patient. Furthermore, *ABL1* sequencing subsequent to the administration of bosutinib detected only the T315I peak (Fig. 2B). Thus, the present results demonstrate that when a patient carries cells with the T315I mutation, sequencing chromatograms of *ABL1* can potentially provide an estimate of the T315I-positive leukemia cell burden. Additionally, in cases where resistance/intolerance to multiple TKIs is a factor, bosutinib may potentially provide an effective pre-transplant therapy while promising new drugs including ponatinib (8,24), omacetaxine (24) and axitinib (25) that target the T315I mutation are becoming widely available. Furthermore, for individual situations, certain drugs may or may not be suitable due to preexisting comorbidities and/or pretreatments.

It has been reported that Src family kinases, including Lyn, Hck, and Fgr, perform critical roles in BCR-ABL1-independent TKI resistance (5,6) and disease progression in CML, particularly during the progression to B lymphoid BP (26-29). In leukemia cells from patients with CML who exhibited disease progression during imatinib therapy, Hck and Lyn were revealed to be strongly expressed and/or activated (26). Correspondingly, downregulation of *Lyn* by siRNA induced apoptosis in BCR-ABL1 positive blasts, particularly lymphoid blasts (27). In addition, mouse models have demonstrated that Lyn, Hck and Fgr are required for the transition from CML-CP to lymphoid BP (28,29). In the present case, the percentage of blasts in the BM decreased from 96.1 to 17.5% following treatment with Bosutinib. In addition, the spleen demonstrated sustained shrinkage over five months of the bosutinib treatment regimen. The reduction in leukemia load was more than would be expected just from the killing of T315I-negative blasts. Thus, it is possible that the dual Src/ABL1 kinase inhibitor, bosutinib, was able to effectively suppress Src, Lyn and Hck in the T315I-positive lymphoid BP cells, thereby additionally inducing anti-leukemia effects. It will be important for future studies to compare the sensitivity of CML cells from lymphoid BP vs. myeloid BP to bosutinib. To date, no such data are available (18,21).

The clinical course in this report suggested that the interruption of dasatinib for any reasons during CML treatment, particularly in late-CP or AP/BC, may induce a resistant clone against TKIs, even if the resistant mutated clone existed in BM prior to treatment with dasatinib. Indeed, poor adherence may be the predominant reason for failure to achieve adequate molecular responses in patients treated with imatinib for >2 years (23). A management of adverse effects and a continuation of TKI are essential for CML treatment. The selection of an appropriate TKI for each patient with CML with individual situations during TKI treatment as well as at a diagnosis of CML is the key to the best possible outcome (30).

In conclusion, the present case represents a patient with T315I-positive CML-BP. The patient responded to bosutinib as a fourth-line treatment. Bosutinib was also useful as a pre-transplant therapy for reducing leukemia cell load. Thus, patients who are resistant or intolerant to multiple TKIs, unless the leukemia cells are uniformly T315I-positive, may benefit from a bosutinib treatment regimen.

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