



Case Report

Dasatinib Monotherapy to Maintain Complete Remission before Allogeneic Bone Marrow Transplantation under Monitoring of the Blastic Chronic Myelogenous Leukemia Biphenotypic Cells and *Bcr-Abl* Transcripts

Shinichiro Kawamoto^{1,3*}, Noboru Yonetani^{1,4}, Ryota Masutani², Toshiyuki Ikemoto² and Takayuki Takubo¹

¹Department of Clinical and Laboratory Medicine, Osaka Medical College, Osaka, Japan

²Department of Central Clinical Laboratory, Osaka Medical College Hospital, Osaka, Japan

³Department of Medical Oncology and Hematology, Kobe University Hospital, Hyogo, Japan

⁴Department of Hematology, Kobe City Medical Center General Hospital, Hyogo, Japan

Abstract

A 49-year-old male with blastic phase chronic myelogenous leukemia (CML-BP) with CD13/CD19 double-positive markers was treated with a combination of tyrosine kinase inhibitors and chemotherapy, followed by allogeneic bone marrow transplantation (allo-BMT). The biphenotypic cells and *bcr-abl* transcripts were monitored during the treatment course, and the efficacy of dasatinib for the treatment of the allo-BMT eligible CML-BP patient was evaluated. Following a single course of hyper-CVAD/MA with imatinib mesylate, the biphenotypic cells and *bcr-abl* mRNA became undetectable by nested real-time polymerase chain reaction (PCR) one month after the allo-BMT. Thus, dasatinib monotherapy is feasible for maintaining the remission of CML-BP for at least a short duration before allo-BMT.

Keywords: Tyrosine kinase inhibitors; Monotherapy; Leukocytosis; Dasatinib

Introduction

Tyrosine kinase inhibitors (TKIs) have the potential to cure chronic myelogenous leukemia (CML). This outstanding effect in

the treatment of CML is proven by the fact that withdrawing TKIs is possible in some optimal responders. Because of the emergence of TKI-resistant clones, possibly due to mutations in the *bcr-abl* fusion gene, such as the T315I mutation, it has been thought that TKIs monotherapy is not sufficient to treat CML-BP and Philadelphia chromosome positive acute lymphoblastic lymphoma (Ph+ALL) [1]. Therefore, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is recommended for eligible CML-BP and Ph+ALL patients hoping for anti-leukemic immune response.

However, the best strategy for the treatment of CML-BP and Ph+ALL patients after the first CR until allo-HSCT is unclear, and the number of reports that have evaluated the efficacy of TKI monotherapy for the treatment of CML-BP is limited.

In this case report, we traced the minimal residual disease by detecting biphenotypic cells and *bcr-abl* transcripts using flow cytometry (FCM) and real-time PCR, respectively, during the treatment course, including the dasatinib monotherapy periods and the periods before and after the allo-BMT in a CML-BP patient. We also discussed the role of dasatinib in CML-BP treatment.

Case Report

A 49-year-old male had a past history of leukocytosis and an elevated lactate dehydrogenase level of 13,500/ μ l and 625 IU in 2009 and 12,700/ μ l and 566 IU/l in early 2010, respectively. In May 2010, dyspnea and low back pain developed, and the patient consulted a general physician. A peripheral blood examination revealed leukocytosis of 32,430/ μ l with 74% blast cells and anemia. The patient was then referred to our hospital. The results of the physical examination were almost normal, except for the anemia of the conjunctiva, and splenomegaly was not observed. Bone marrow aspiration showed that nucleated cells consisted of 95% blasts, although no increasing of eosinophils or basophils was observed (Table 1). The blast cells showed both myeloid and lymphoid morphology and a mixture of both (Figure 1). The blast cells expressed the myeloid markers CD13 and CD33 in

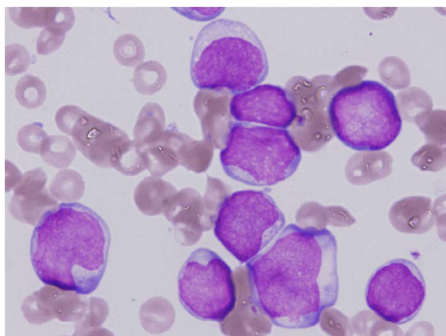
Table 1: Laboratory findings at diagnosis

RBC	256 \times 10 ⁴ / μ l	AST	25 IU/L	CD2	0.5%
Hb	7.0g/dl	ALT	28 IU/L	CD3	0.1%
Ht	22.5%	γ-GTP	35 IU/L	CD5	2.5%
WBC	31,230/ μ l	LDH	601 IU/L	CD7	21.5%
Blast	74.0%	Cr	1.02 mg/dl	CD10	88.6%
Pro.	2.0%	UA	6.5 mg/dl	CD13	71.9%
Myelo	3.5%	Glu	105 mg/dl	CD11b	4.2%
Met.		CRP	4.01 mg/dl	CD11c	2.3%
Band	1.5%			CD14	0.3%
Seg.	2.0%	Bone Marrow		CD15	0.5%
Eos.		NCC	12.15 \times 10 ⁴ / μ l	CD19	97.5%
Baso.		Meg	(-)	CD20	8.2%
Mono	0.5%	Blast	94.5%	CD22	52.8%
Lymph	16.5%			CD33	35.4%
Ebl				CD79a	98.3%
Pit.	18.5 \times 10 ⁴ / μ l			cy MPO	0.5%
				HLA-DR	99.1%
				KOR-SA3544	26.3%

Abbreviation: NCC-Nucleated cell count; RNA- Ribonucleic acid

*Corresponding author: Shinichiro Kawamoto, Chuoh-ku Kusunokicho 7-5-2, Kobe City, 650-0017, Hhyogo, Japan, Tel: +81-078-382-5111; Fax: +81-078-382-5821; E-mail: skawamo@med.kobe-u.ac.jp

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(Original magnification $\times 1000$) The blast cells had a phenotype of either myeloblast or lymphoblast cells, or a mixture of these phenotypes

Figure 1: May-Grunwald Giemsa staining of leukemia cells.

addition to the lymphoid markers CD19, CD22 and 79a, although other myeloid markers, including cytoplasmic myeloperoxidase, CD14, CD15, CD11b and CD11c, were not expressed. According to the scoring system for the definition of acute biphenotypic leukemia, the blast cells were defined as myeloid-marker positive ALL cells [2]. Real-time PCR detected both transcripts for major and minor *bcr-abl* mRNA, although the *bcr-abl* fluorescence *in situ* hybridization of peripheral neutrophils performed after induction chemotherapy was negative. G-banding identified an unusual cytogenetic abnormality of 46, XY, t(12;22)(q15;11.2), add(21)(q22) in 20 of 20 cells without the Philadelphia chromosome.

The blast cells did not increase for one week without treatment. A single course of hyper-CVAD/methotrexate with cytarabine plus a 400 mg daily dose of imatinib mesylate was administered as induction therapy (Figure 2). Although the patient was complicated by bacteremia and liver-renal dysfunction during the induction therapy, hematological complete remission was obtained. Then, an unrelated BMT was planned. For the consolidation and maintenance of the first CR, 140 mg/day of dasatinib was administered until the u-BMT, instead of sequential chemotherapy, to avoid organopathy.

Therefore, the minimal residual disease (MRD) was monitored carefully by a combination of FCM and quantitative real-time reverse transcriptase PCR (qRT-PCR) to detect CD33/CD19 and CD13/CD19 double-positive cells and *bcr-abl* mRNA, respectively. After the administration of dasatinib, the MRD decreased to below the limits of quantitation of FCM and qRT-PCR. Large granular lymphocytosis appeared at the same time. The MRD was suppressed at a low level for four months until the u-BMT. The conditioning regimen used for allo-BMT consisted of 60mg/kg cyclophosphamide administered in two doses and 12 Gy of total body irradiation administered in six fractions. A number of 1.78×10^6 /kg CD34-positive cells enriched from the HLA DRB1 1 locus-mismatched unrelated bone marrow were transplanted. Tacrolimus and short-term methotrexate were used for immunosuppression. Major *bcr-abl* mRNA was not detected by qRT-PCR after myeloablative BMT conditioning, whereas nested PCR showed positive results, and flow cytometry detected a temporal increase in the CD33/19 and CD13/19 double-positive cells. The allo-BMT was conducted successfully, and nested PCR turned negative two months later. To prevent a relapse, dasatinib was reinitiated for two weeks four months after the allo-BMT. However, it was soon discontinued because of edema, dyspnea and stomach pain. Tacrolimus was tapered gradually, and was discontinued at six months after allo-

BMT. Then, dasatinib was reinitiated eight months after the allo-BMT. Despite hematological complete remission confirmed by microscopic examination, elevated numbers of CD33/19 and CD13/19 double-positive cells were observed and dasatinib was discontinued. The condition of the patient was quite stable three years after the diagnosis and 30 months after u-BMT without relapse.

Discussion

We diagnosed the patient as having lymphoid crisis of CML with an atypical phenotype due to his past history and lack of increases in the levels of eosinophils/basophils or splenomegaly, and similar to a previous report, *bcr-abl* FISH was not detected from the peripheral blood after induction therapy with imatinib mesylate and hyper-CVAD [3].

Bcr-abl positive acute leukemia including CML-BP continues to have a poor prognosis with a high relapse rate after conventional chemotherapy. CML-BP patients treated with imatinib exhibit a hematologic remission rate of 33% to 61% (lymphoid BC, 36%-80%), a major cytogenetic remission rate of 35% to 56%, a 2-year survival rate of 20% to 30% and a median survival of eight to 11 months [4]. Imatinib mesylate with hyper-CVAD improves the response rate and overall survival of Ph+ALL patients with subsequent allo-BMT [5]. Although sequential chemotherapy using dasatinib with hyper-CVAD was effective in newly-diagnosed Ph+ALL patients without allogeneic stem cell transplantation, grade3/4 treatment-related toxicities were observed during consolidation therapy, with a rate as high rate as that following induction therapy [6].

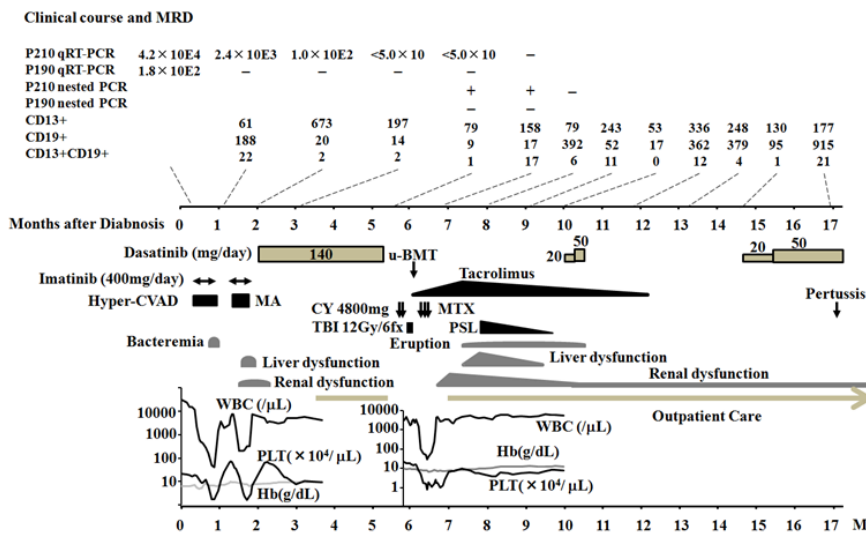
Src family kinases (SFKS) also play critical roles in the development of CML-BP as well as Ph+ALL, independent of *bcr-abl* [7]. Although TKIs have a variety of targets besides *bcr-abl* tyrosine kinase, the activation of SFKS induces resistance to imatinib. Dasatinib is a dual SFK/*bcr-abl* inhibitor that is effective in CML-BP and Ph+ALL. TKIs often induce side effects requiring discontinuation or a change to another drug due to their multi-kinase activity. However, most of the side effects are tolerable, and many CML patients are treated with TKIs indefinitely. Therefore, consolidation and maintenance by TKI monotherapy after the first remission, instead of sequential chemotherapy, is expected to reduce the complications until allo-HSCT and improve its outcome in patients with *bcr-abl* positive acute leukemia.

In the present case report of CML-BP for which allo-BMT was conducted during the first complete remission, the feasibility of dasatinib monotherapy for maintenance after induction therapy was evaluated with quantitative monitoring of the minimal residual disease (MRD) by FCM and reverse transcriptase polymerase chain reaction (RT-PCR) to detect biphenotypic markers and *bcr-abl* mRNA, respectively.

The number of CD13/CD19 double-positive cells decreased soon after the induction therapy. However, although there is a possibility that were false positive results, they were still detected after allo-BMT at a low frequency.

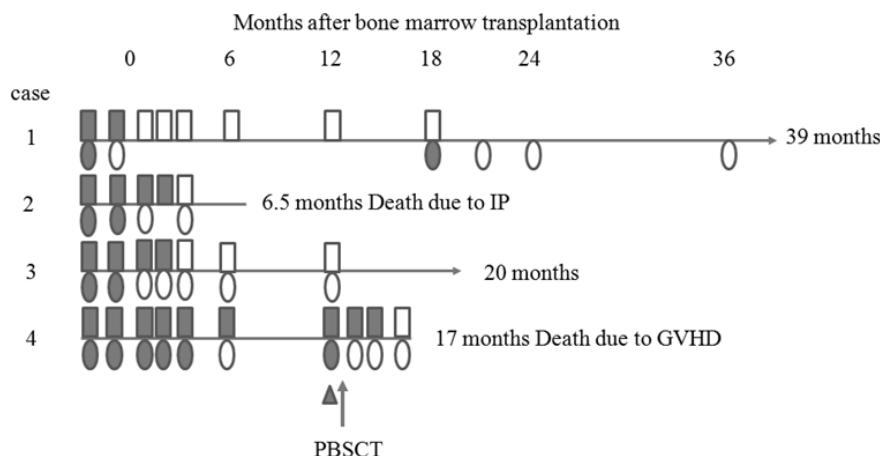
Similarly, *bcr-abl* mRNA was suppressed by dasatinib monotherapy, and p190 mRNA first became negative by quantitative RT-PCR. Then the p210 mRNA decreased gradually until it was below the limit of detection. Then, the p210 *bcr-abl* mRNA became negative by nested PCR two months after the allo-BMT.

In a previous report, Fujimaki et al. analyzed the expression



Abbreviations: CY-Cyclophosphamide; TBI-Total body irradiation; MTX- Methotrexate; PSL-Prednisolone

Figure 2: Clinical course of the patient and the MRD.



The boxes reflect the major *bcr-abl* and the circles reflect minor *bcr-abl*. (open=negative, closed=positive) The closed triangle indicates bone marrow relapse.

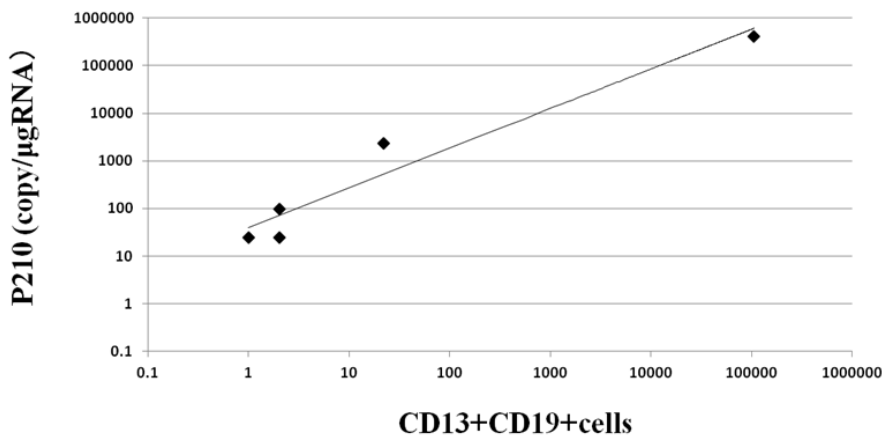
Figure 3: Detection of *bcr-abl* by RT-PCR.

of both p210 and p190 *bcr-abl* mRNA in double-positive acute lymphoblastic leukemia patients before and after allogeneic bone marrow transplantation (Figure 3) [8]. In their four cases, p190 mRNA negativity was documented after chemotherapy in one case and soon after allo-BMT in three cases. P210 *bcr-abl* mRNA became negative one to three months after allo-BMT in three cases. In one case, the p210 *bcr-abl* did not become negative even after allo-BMT, and the patient relapsed.

Although there were differences in the diagnosis between their cases and our present case, dasatinib seems to be able to decrease the *bcr-abl* positive leukemia cells as effectively as myeloablative conditioning for allo-HSCT, and allo-immunity is required for complete eradication of MRD.

Kikuchi et al. analyzed the relationship between the MRD level after induction therapy, and the overall survival. The lower MRD level (detected by PCR on day100) showed much higher overall survival rate [9]. In their study, the concordance between the FCM and PCR results was also confirmed.

Using the results of dual monitoring, we further analyzed the relationship between the number of biphenotypic cells and the copy number of p210 *bcr-abl* mRNA. As shown in Figure 4, although the sample number was not sufficient for a statistical analysis, there was an apparent correlation between the biphenotypic cell numbers and p210 *bcr-abl* mRNA copy number. Based on this result, it appears that leukemia cells existed at a frequency of one in 10^5 to 10^6 in the bone marrow nucleated cells.



The FCM method. One million nucleated bone marrow cells were stained with the indicated antibodies and combined with 100,000 of calibration beads after being washed. The CD13/CD19 double-positive cells were counted until fifteen thousand calibration beads were detected. t

Figure 4: Correlation of the biphenotypic cell numbers and P210 bcr-abl mRNA copy numbers.

In case 4 in Fujimaki's report, BMT was conducted from a HLA 1 locus mismatched sibling donor for ALL during the first CR. However, hematological relapse was observed one year later without negative conversion of the p210 mRNA. Munoz et al. reported that there was a higher possibility of false negative PCR results in comparison with the results of flow cytometry [10]. Therefore, as MRD was detected on PCR in our case, although the value was low, in addition to flow cytometry, we concluded that performing allo-BMT was appropriate at that point. As mentioned above, CD13/CD19 double-positive cells continued to be observed after allo-BMT. Hence, we attempted to re-administer dasatinib twice intermittently after the allo-BMT in order to inhibit a relapse. However, the number of double-positive cells slightly increased after the administration of dasatinib. In addition, a pertussis infection was observed under dasatinib treatment. We suspect that immune suppression is induced by dasatinib, as described in a previous report [11].

Further investigations with a large number of patients and that analyze how long TKI monotherapy can maintain CML-BP and Ph+ALL are required. Then, a randomized study that compares sequential chemotherapy with dasatinib monotherapy before allo-HSCT should be performed for confirmation of the favorable outcomes of the treatment.

In conclusion, dasatinib effectively maintains the first CR of CML-BP patients, and is therefore expected to improve the outcome of allo-HSCT. However, due to its immune-suppressive effects, the use of dasatinib after allo-HSCT requires precise follow-up.

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