T315I Mutation in Ph-positive Acute Lymphoblastic Leukemia is Associated with a Highly Aggressive Disease Phenotype: Three Case Reports

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Abstract. *T315I mutation in BCR-ABL causes resistance to therapy with tyrosine kinase inhibitors (TKIs) in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph$^+$ ALL) cases. We report three cases of relapse accompanied by T315I mutation during rapid disease progression. Case 1 was a 64-year-old male. During chemotherapy, qPCR detected a decrease of BCR-ABL to 190 copies once, but this suddenly increased to 22,000 copies. The patient received dasatinib, but the disease relapsed hematologically when the T315I mutation was detected. Retrospective analysis revealed that the T315I mutated clone already existed at the molecular relapse occurrence. Case 2 was a 25-year-old male. The patient underwent bone marrow transplantation (BMT) at the first molecular complete remission (CR), but 102 days after BMT, the ALL relapsed at the molecular level. Although he received imatinib, ALL immediately fully relapsed; the T315I mutation was detected. Case 3 was a 40-year-old female. Molecular CR was achieved by induction therapy, but ALL relapsed at the molecular level (9,200 copies). The patient received dasatinib, but relapsed hematologically, and the T315I mutation was observed. She underwent umbilical cord blood transplantation, but relapsed. In these three cases, survival from the time of the T315I mutation detection was 4, 2, and 6 months, respectively. The T315I mutation in Ph$^+$ ALL was associated with a highly aggressive disease phenotype. In order to make appropriate therapeutic decisions, it is important to analyze the mutations immediately at the time of molecular relapse.*

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A high complete remission (CR) rate has been reported in newly diagnosed Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph$^+$ ALL) following (tyrosine kinase inhibitor) TKI combined chemotherapy (1). However, resistance and relapse pose a problem. ABL-kinase domain mutations account for resistance in over 80% of cases of Ph$^+$ ALL. In particular, T315I is quite commonly detected at the time of relapse and currently remains the most troublesome mutation (2). We analyzed the mutations in three patients with relapse, after treatment with TKI combined chemotherapy or hematopoietic stem cell transplantation (HSCT), and detected T315I mutations in each one of them. As recently revealed for multiple genetically distinct leukemia-initiating cell sub-clones in Ph$^+$ ALL, our cases also suggest the importance of developing therapies that eradicate all intratumoral sub-clones efficiently.

Case Reports

Case 1. A 64-year-old male was diagnosed with Ph$^+$ ALL in March 2009. A blood test revealed the following: white blood cell (WBC)=74,900/μl (blast cells 55%), Hb=13.4 g/dl, platelets (Plt)=28,000/μl, and lactate dehydrogenase (LDH)=2,084 IU/l. Upon G-banding examination, there were additional chromosomal abnormalities other than t(9:22)(q34;q11.2). Cytogenetical complete remission (CR) was achieved by induction therapy according to the Japan Adult Leukemia Study Group (JALSG) Ph$^+$ ALL 208 protocol (3), but minimal residual disease (MRD) was detected (minor-*BCR-ABL* mRNA=23,000 copies/μg RNA). As the patient did not have a human leukocyte antigen (HLA) -identical sibling or a fully-matched unrelated bone marrow donor, the consolidation therapy was continued. The MRD favorably decreased to 190 copies/μg RNA on June 16 (after consolidation therapy 1-1), but increased to 22,000 copies/μg RNA on July 14 (after consolidation therapy 1-2) (Table I). The patient consequently received dasatinib, but the ALL hematologically relapsed on August 18th, and the T315I mutation was detected. The patient

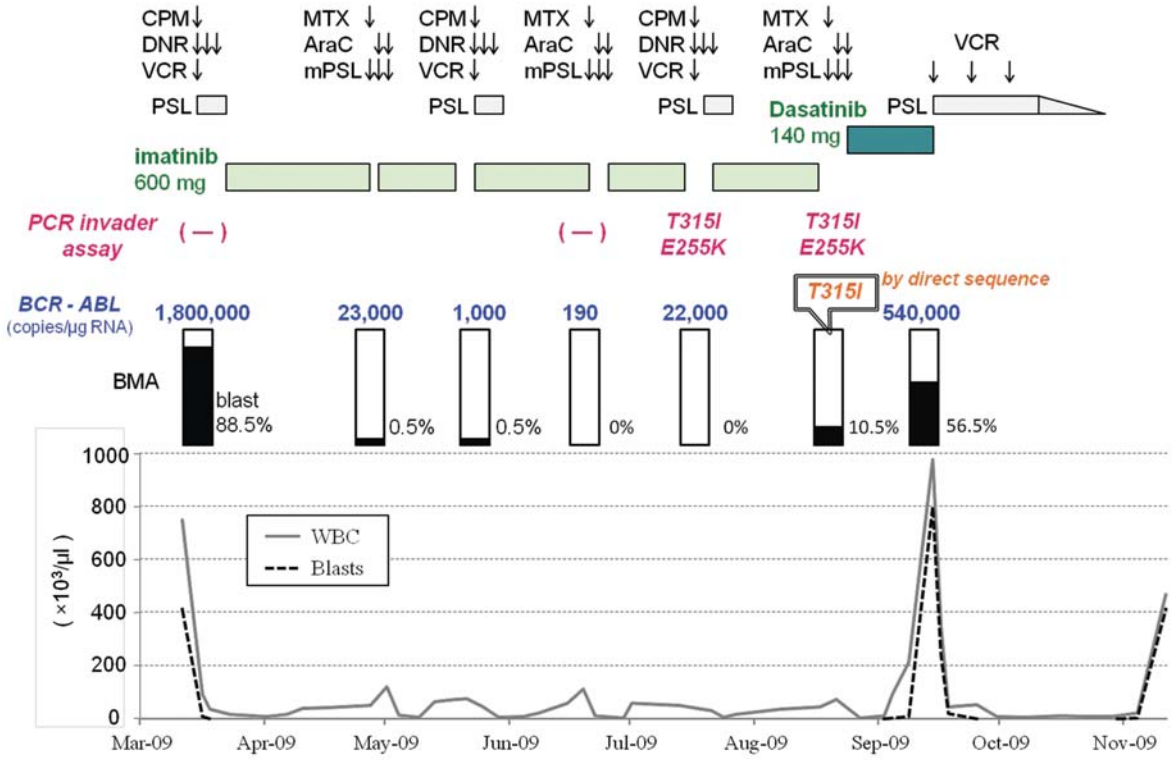

Figure 1. *Clinical course of Case 1 after induction therapy. CPM, Cyclophosphamide; DNR, daunorubicine; VCR, vincristine; PSL, prednisolone; mPSL, methyl prednisolone; MTX, methotrexate; Ara-C, cytarabine; BMA, bone marrow aspiration.*

underwent salvage chemotherapy, but died of disease progression on November 11th. Earlier samples were then analyzed and mutations (T315I and E255K) were also detected in the sample of July 14th (Figure 1).

Case 2. A 26-year-old male was diagnosed with Ph+ ALL in July 2009. A blood test revealed the following: WBC=81,600/μl (blast cells 69%), Hb=15.8 g/dl, Plt=76,000/μl, and LDH=814 IU/l. Molecular CR was favorably achieved by imatinib (IMA) combined chemotherapy, and he underwent HLA-matched allogenic bone marrow transplantation (BMT), from his brother, with a myeloablative conditioning regimen consisting of 30 mg/kg of etoposide, 120 mg/kg of cyclophosphamide, and 12 Gy of total body irradiation in the first CR on December 8, 2009. On January 18, 2010, only 102 days after BMT, hematological CR was sustained, but Ph+ ALL relapsed at the molecular level (minor-*BCR-ABL* mRNA=180,000 copies/μg RNA). Thus, imatinib was resumed, and cyclosporine was tapered, but the ALL immediately fully relapsed. T315I mutation was detected on February 1st. The patient underwent re-induction therapy but died of disease progression and multiple organ failure on November 11st (Figure 2).

Case 3. A 40-year-old female was diagnosed with Ph+ ALL in December 2009. A blood test revealed the following: WBC=241,500/μl (blast cells 98%), Hb=8.6 g/dl, Plt=22,000/μl, LDH=771 IU/l. Upon G-banding examination, there were no additional chromosomal abnormalities. Molecular CR was achieved by the induction therapy according to the JALSG Ph+ ALL 202 protocol (3). As the patient did not desire to undergo allo-HSCT in the first CR, consolidation therapy was completed, and maintenance therapy with imatinib and prednisolone was continued. But pancytopenia was observed, and the ALL relapsed at the molecular level (minor-*BCR-ABL* mRNA: 9,200 copies/μg RNA) in November 2010. Hence, she received dasatinib instead of imatinib, but the ALL hematologically relapsed in December 2010. Although she underwent re-induction therapy, the residual leukemia and the T315I mutation were observed. Subsequent salvage therapy was also not effective. We performed umbilical cord blood transplantation with a myeloablative conditioning regimen consisting of 120 mg/kg of cyclophosphamide, and 12 Gy of total body irradiation on April 15, 2011. Although cytogenetical CR was achieved, MRD was detected on day 28 after transplantation (minor-*BCR-ABL* mRNA=610 copies/μg RNA). Tacrolimus was tapered, but the ALL

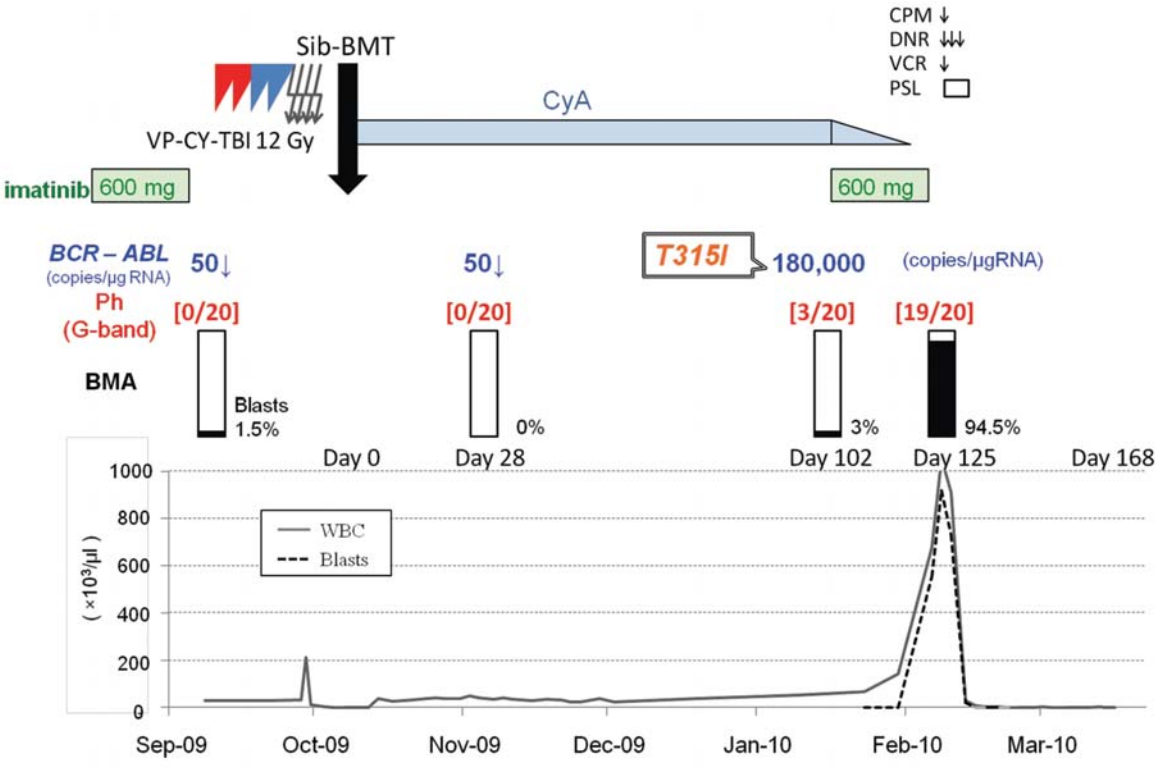

Figure 2. *Clinical course of Case 2 after sibling-bone marrow transplantation (sib-BMT). VP, Etoposide; CY, cyclophosphamide; TBI, total body irradiation; CyA, cyclosporine A; Ph, Philadelphia chromosome; BMA, bone marrow aspiration.*

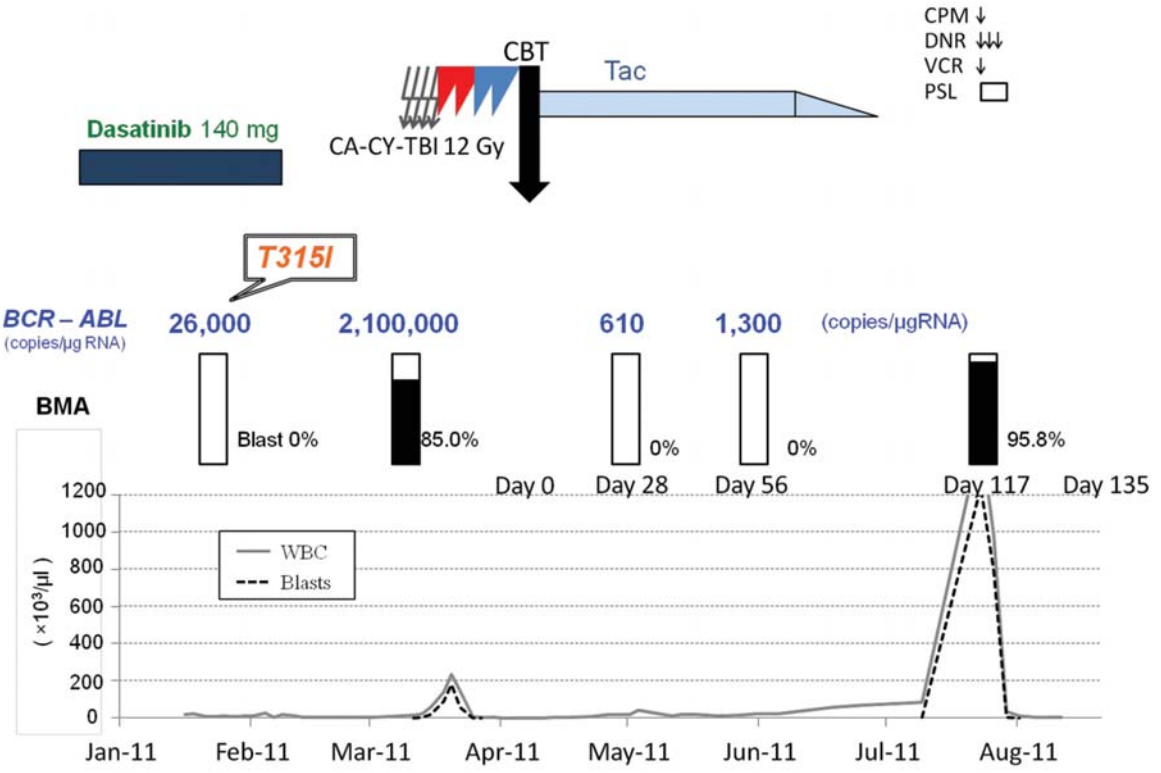

Figure 3. *Clinical course of Case 3 after T315I detection and cord blood transplantation (CBT). CA, Cytarabine; CY, cyclophosphamide; TBI, total body irradiation; Tac, tacrolimus; CPM, cyclophosphamide; DNR, daunorubicine; VCR, vincristine; PSL, prednisolone.*

Table I. *Patient characteristics and outcomes of the three cases of Philadelphia chromosome-positive acute lymphoblastic leukemia with T315I mutation.*

Patient	Case-1	Case-2	Case-3
Age years/Gender	64/Male	26/Male	40/Female
WBC at diagnosis	74,900/μl	81,600/μl	241,500/μl
BCR-ABL (copies/μg RNA)	Major (1,800,000)	Minor (2,300,000)	Minor (2,600,000)
Best response (copies/μg RNA)	190	<50	<50
Therapy before T315I detection	JALSG Ph+ ALL 208* (Induction~C1-2)	JALSG Ph+ ALL 202‡ (Induction~C2-1) →sib-BMT	JALSG Ph+ ALL 208* (Induction~C2-4)
TKI	IMA	IMA	IMA/DAS
Mutations	T315I, E255K	T315I	T315I
Diagnosis to T315I detection	4 months	5 months	9.5 months
T315I detection to outcome	4 months	2 months	6 months
Outcome (cause)	Died (relapse)	Died (relapse)	Died (relapse)

*JALSG Ph+ ALL 208 induction: cyclophosphamide (CPM), daunorubicine (DNR), vincristine (VCR), prednisolone (PSL), imatinib (IMA), and triple-IT (methotrexate (MTX), cytarabine (Ara-C), dexamethasone (DEX)), consolidation 1: MTX, Ara-C, methyl prednisolone (mPSL), IMA, and triple-IT, consolidation 2 : CPM, DNR, VCR, PSL, IMA, and triple-IT. ‡JALSG Ph+ ALL 202 induction : CPM, DNR, VCR, PSL, IMA, and triple-IT, consolidation 1: MTX, Ara-C, mPSL, and triple-IT, consolidation 2: IMA and triple-IT.

immediately fully relapsed, and she died of disease progression on June 22, 2011 (Figure 3).

Discussion

This report covers the precise history and clinical course of three Ph+ ALL patients with T315I mutation. In the three cases under study (Table I), disease progression was very rapid and the survival from the time of the T315I mutation detection was 4, 2, and 6 months, respectively. Nicolini *et al.* (4) reported that median overall survival from the time of T315I detection was 2.5 months for Ph+ ALL patients in retrospective observational study. The finding of T315I mutation in Ph+ ALL is associated with a highly aggressive disease phenotype, not only with resistance to TKIs.

On the other hand, the reason why the mutation is associated with such an aggressive course is presently unclear. Although there is some debate concerning the biological activity of this mutation (5, 6), Miething *et al.* (7) reported that in the absence of imatinib, there is no growth advantage for cells carrying *BCR-ABL* with T315I mutation, compared to the wild type, neither *in vitro* nor in a murine transplantation model. Therefore, cellular adaptive changes, such as additional mutations, or *BCR-ABL* amplifications, caused by instability of the leukemia cells might account for the proliferative advantage of the leukemia clone.

In case 2, even after the best available therapy including the HLA- matched allogenic BMT, the disease reocurred. Recently, by using xenografting and DNA copy number alteration (CNA) profiling of Ph+ ALL, Notta *et al.* (8) demonstrated that genetic diversity occurs in functionally-defined leukemia-initiating cells and that many diagnostic patient samples contain multiple genetically distinct leukemia-initiating cell sub-clones. This finding also suggested the importance for developing therapies that eradicate all intratumoral sub-clones efficiently.

In case 3, we performed umbilical cord blood transplantation after relapse accompanied with T315I mutation. Nicolini *et al.* (9) reported 64 cases (67 transplants) of Ph+ leukemia with T315I mutations, treated with HSCT (33 in CML-CP, 9 in AP, 17 in BP, 4 with Ph+ ALL, and 4 in unknown phase at transplantation). Survival probabilities 24 months after HSCT were 59%, 67%, 30%, and 25% for CP, AP, BP and Ph+ ALL, respectively after median follow-up of 26 months (range =1.8-154.5 months). The occurrence of chronic graft- *versus* host-disease had a positive impact on overall survival (p=0.047). They concluded that HSCT is a valuable therapeutic tool for eligible patients with T315I mutation. Thus, in the absence of effective agents for Ph+ ALL with T315I, allo-HSCT might be an option.

Some novel targeting agents with potential activity against *BCR-ABL* T315I are currently in clinical and preclinical development (10, 11). Ponatinib (AP24534) is a pan-*BCR-ABL* inhibitor developed for treatment-refractory CML and has significant activity in patients who fail second-line TKI (dasatinib or nilotinib) therapy (10). A pivotal phase II trial is underway (12). It is also hoped that these drugs will provide an optional therapy for Ph+ ALL with T315I, but their efficacy is still under investigation.

In conclusion, it is important to analyze mutations when disease progression is suspected and to immediately make appropriate therapeutic decisions, such as HSCT or third-generation TKI.

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