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Case Report

A Case of Tyrosine Kinase Inhibitor-Resistant Chronic Myeloid Leukemia, Chronic Phase with ASXL1 Mutation

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Keywords
Chronic myeloid leukemia · Tyrosine kinase inhibitor · Drug resistance · Leukemia oncogenesis · Clonal evolution

Abstract
Hematological malignancies, including chronic myeloid leukemia (CML), exhibit *ASXL1* mutations; however, the function and molecular mechanism of these mutations remain unclear. *ASXL1* was originally identified as tumor suppressor gene, in which loss of function causes myelodysplastic syndrome (MDS). *ASXL1* mutations are common and associated with disease progression in myeloid malignancies including MDS, acute myeloid leukemia, and similarly in CML. In MDS, *ASXL1* mutations have been associated with poor prognosis; however, the impact of *ASXL1* mutations in CML has not been well described. A 31-year-old male was diagnosed as CML-chronic phase (CP). Laboratory findings showed a white blood cell count of 187,200/μL, with asymptomatic splenomegaly. Blast count was 5.0% in peripheral blood and 7.3% in bone marrow. There was no additional chromosomal abnormality except for t(9;22)(q34;q11.2) by chromosomal analysis. At onset, the Sokal score was 1.4, indicating high risk. The patient received tyrosine kinase inhibitor (TKI) therapy, comprising nilotinib ~600 mg/day, bosutinib ~600 mg/day, ponatinib ~45 mg/day, and dasatinib ~100 mg/day. Nevertheless, after 1.5 years of continuous TKI therapy, the best outcome was a hematological response. Although additional chromosomal aberrations and *ABL1* kinase mutations were analyzed repeatedly before and during TKI therapy, known genetic abnormalities were not detected. Thereafter, the patient underwent bone marrow transplantation from an HLA 7/8 matched

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unrelated donor (HLA-Cw 1 locus mismatch, graft-versus-host direction). The patient achieved neutrophil engraftment, 18 days after transplantation, leading to complete remission with an undetectable level of *BCR-ABL1* mRNA. The patient, however, died from graft-versus-host disease and thrombotic microangiopathy after 121 days. Gene sequence analysis of his CML cell before stem cell transplantation revealed *ASXL1* mutations. Physiologically, *ASXL1* contributes to epigenetic regulation. In the CML-CP patient in this case report, *ASXL1* mutation conferred resistance to TKI through obscure resistance mechanisms. Even though a molecular mechanism for TKI resistance in *ASXL1* mutation in CML has remained obscure, epigenetic modulation is a plausible mode of CML disease progression. The clinical impact including prognosis of *ASXL1* for CML is underscored. And the treatment strategy of CML with *ASXL1* mutation has not been established. A discussion of this case was expected to facilitate treatment options.

Introduction

Chronic myeloid leukemia (CML) is a myeloid clonal disease driven by the *bcr-abl* fusion gene, which creates a constitutively active tyrosine kinase. This led to the development of *BCR-ABL1* tyrosine kinase inhibitors (TKIs), which provided long-term remission and improved life expectancy of TKI-treated CML patients [1]. The mechanism of TKI resistance in CML, caused by mutation of the *BCR-ABL1* kinase domain has been extensively investigated [2]. Importantly, the *BCR-ABL1* kinase-independent resistance mechanism due to newly acquired mutations or other genetic aberrations has been reported in a minority of TKI-resistant CML cases [3]. One such mutation was found in *ASXL1*, a histone-binding protein located on chromosome 20q11.2, that disrupts chromatin by enhancing or repressing gene transcription [4]. Then *ABL1* kinase-dependent resistance may promote a change of a patient's TKI treatment strategy. As a while, however, it is obscure how much impact the *BCR-ABL1* kinase-independent resistance brings on CML therapy plan.

Originally identified from sequence analysis of myelodysplastic syndrome (MDS) patients [5], mutations in *ASXL1* were a nonspecific genetic abnormality, associated with poor prognosis not only in MDS [6] but also in *bcr-abl*-negative myeloproliferative neoplasms [6]. Mutations in *ASXL1* have been found in the accelerated phase or blast phase [7] and in CML-chronic phase (CP) [8]. Surprisingly, several *ASXL1* mutations have also been reported in healthy people [9–11], indicating the pleiotropic nature of *ASXL1*. We present a case of CML-CP resistant to various TKIs and discuss the association between mutations in *ASXL1* and TKI resistance in CML.

Case Presentation

A 31-year-old male was diagnosed as CML-CP after an annual occupational health check-up revealed leukocytosis (WBC 187,200/μL), which was subjected to a further examination. The physical examination at his diagnosis revealed giant splenomegaly (palpable 15 cm below costal margin). Blast count was 5.0% in peripheral blood and 7.3% in bone marrow. There was no additional chromosomal abnormality except for t(9;22)(q34;q11.2) by chromosomal analysis. The patient's Sokal score was 1.5 indicating high risk, Hasford score was 1,332.4 indicating intermediate risk, EUTOS score was 134 indicating high risk, and ELTS score was 2.0877 indicating intermediate risk. A month after the diagnosis, the patient underwent TKI therapy comprising nilotinib up to 600 mg/day, followed by 600 mg bosu-

Table 1. Laboratory data before tyrosine kinase inhibitor treatment

WBC	125,200	/μL
Stab.	11.5	%
Seg.	31.0	%
Lym.	3.5	%
Mono.	0.0	%
Eos.	6.0	%
Baso.	6.5	%
Blast	6.5	%
Promyelo.	1.0	%
Myelo.	26.5	%
Metamyelo.	7.5	%
RBC	327×10^4	/μL
Hb	9.3	g/dL
Hct	30.4	%
MCV	93.0	fL
MCHC	30.6	%
PLT	51.0×10^4	/μL
CRP	1.20	mg/dL
TP	6.0	g/dL
Alb	4.1	g/dL
BUN	15.2	mg/dL
Cr	0.68	mg/dL
UA	6.2	mg/dL
T-Bil	0.6	mg/dL
GOT	14	U/L
GPT	17	U/L
ALP	235	U/L
γ-GTP	28	U/L
CPK	12	U/L
CHE	181	U/L
LDH	601	U/L
Na	140	mmol/L
K	4.5	mmol/L
Cl	105	mmol/L
PT	70	%
PT-INR	1.17	
APTT	46.5	s
FIB	311	mg/dL
ATIII	72	%
FDP	3	μg/mL
D-dimer	1.0	μg/mL

tinib, 45 mg ponatinib, and 100 mg dasatinib maximum daily dose. Laboratory data prior to TKI treatment are shown in Table 1. None of the TKIs exerted a clinical response, except for ponatinib, which yielded a hematological response (Fig. 1). No known mutations in the *ABL1* kinase domain were detected after TKI therapy, prompting sequencing analysis. We performed targeted panel sequencing, by using prior-stem cell transplantation sample, which includes 377 genes implicated in myeloid malignancies. This analysis revealed a frameshift mutation in *ASXL1* on chromosome 20q11. The patient underwent a stem cell transplantation with bone marrow donated by an unrelated HLA 7/8-matched (HLA-Cw1 locus mismatched, GVH direction) male from the Japan Marrow Donor Program. Tacrolimus and short-term methotrexate were used for graft-versus-host disease (GVHD) prophylaxis. On the 18th day after transplantation, the patient received neutrophil engraftment followed by reticulocyte engraftment 14 days later and platelet engraftment 22 days later. The patient achieved complete remission, with the bone marrow showing undetectable levels of *BCR-ABL1* mRNA

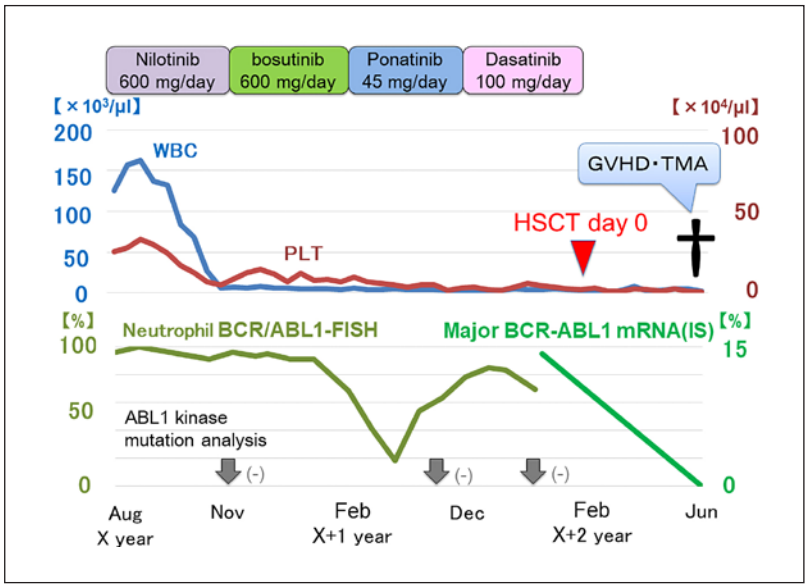

Fig. 1. The patient's clinical course. Nilotinib administration had an adverse effect on white blood cell and platelets, but did not diminish the *bcr-abl* fusion gene. The switch to bosutinib and dasatinib therapy did not change the response. The patient responded only to ponatinib treatment, which achieved a hematological response with 18% of *bcr-abl1* FISH. However, no durable response was available. Hematopoietic stem cell transplantation yielded a molecular response without detection of major *BCR-ABL1* mRNA by RT-PCR. The patient died of transplantation-related mortality, GVHD and TMA on the 121st day after transplantation. FISH, fluorescence in situ hybridization; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; PLT, platelet; TMA, thrombotic microangiopathy; WBC, white blood cells.

by RT-PCR (International Scale). Despite treatment, the patient died on the 121st day from GVHD and thrombotic microangiopathy, which developed after the patient presented with GVHD.

Discussion

Physiologically, ASXL1 encodes a chromatin-binding protein involved in epigenetic regulation [12] by recruiting the polycomb repressive complex 2 (PRC2), a histone methyltransferase, which regulates gene activity by trimethylation of lysine 27 on histone 3 (H3K27me) [13,14]. Mutations in *ASXL1* were originally reported and conferred poor prognosis in MDS [15] and chronic myelomonocytic leukemia (CMMoL) [5]. Frameshift mutations or nonsense mutations in exon 12 of *ASXL1* abrogate protein expression [12] and consequently disrupt its function as a tumor suppressor, often in a variety of hematological malignancies [8]. Mutations in *ASXL1* contribute to oncogenesis in hematopoietic cells, especially leukemogenesis, and promote myeloid transformation through loss of PRC2-mediated gene repression in MDS and CMMoL [12,16].

ASXL1 mutations are common and associated with disease progression in acute myeloid leukemia (AML) [17] and similarly in CML, where *ASXL1* mutations might be associated with poor prognosis and acute transformation [5]. Variation in prognosis and survival associated with *ASXL1* mutations is seen across studies [18]. *ASXL1* mutations are commonly associated with clonal hematopoiesis in healthy individuals [9–11], indicating that *ASXL1* mutation may

be a pre-leukemic event in hematopoiesis. Accumulating evidence points to a role for *ASXL1* mutations in leukemogenesis during early hematopoietic events in many hematological malignancies, as described in AML [18] and CML [19,20]. In the latter case, mutations in *ASXL1* occur early in CML stem cells, prior to *bcr-abl* translocation stage [19], as these cells clonally evolve [20]. This is considered an intrinsic event, rather than a *bcr-abl* fusion, which occurs after myeloid lineage differentiation [20].

In the two-hit model of AML development, class I mutation confers a proliferative or survival advantage, and class II mutations result in impaired myeloid differentiation as a secondary event [21]. Class II mutations in *ASXL1* lead to its loss of function, impairing granulomonocytic differentiation in early human hematopoietic progenitors and contributing to leukemogenesis. Indeed, silencing of *ASXL1* impairs the granulomonocytic lineage potential of human $CD34^+$ progenitor cells by altering its gene expression profile, but not proliferation and apoptosis [11,14]. Alternatively, *ASXL1* mutations might enhance other leukemogenesis pathways via other mechanisms, including epigenetic regulation.

ASXL1 plays a key role in epigenetic regulation of gene expression through methylation of histone H3K27, and disruption of *ASXL1* drives myeloid malignancies [22]. Also, *ASXL1* mutations may affect epigenetic regulation by inhibiting ubiquitination of lysine 119 at histone H2A (H2AK119). This may contribute to leukemogenesis though this remains to be proven [23]. Thus, even though a molecular mechanism for TKI resistance in *ASXL1* mutation in CML has remained obscure, epigenetic modulation is a plausible mode of CML disease progression.

ASXL1 mutations occur early in CML stem cells, prior to *bcr-abl* translocation stage, and therefore, there might not be a high risk of treatment failure following TKI therapy [20]. The patient received hematopoietic stem cell transplantation and went into remission. This optimal response could have been prolonged without TKI maintenance therapy. Taken together, *ASXL1* mutation might follow the HSCT trait as observed in this CML-CP case, which does not progress for years.

Conclusions

Whole exome sequence facilitated a clinical decision in this patient who went into remission. Though the clinical impact of *ASXL1* for CML is still under investigation, mutated *ASXL1* possibly explain the TKI resistance mechanism. This case illustrated the necessity of HSCT in *ASXL1* mutation-associated TKI-resistant CML-CP. In future, a discussion of CML with mutated *ASXL1* will facilitate treatment options.

Statement of Ethics

The Institutional Review Board approved the case report and submission of medical literature. We obtained written informed consent from the patient for participation in this study. We obtained consent to publish from the participants.

Disclosure Statement

The authors declare no competing interests. The authors declare no potential conflicts of interest.

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Author Contributions

O.I. and T.I. wrote the manuscript and made substantial contributions to concept and design; M.U., Y.N., K.K., and H.K. suggested important intellectual content and took part in the critical discussion; S.O. and N.K. managed the study and reviewed the manuscript; all authors read and approved the final version of the manuscript.

Availability of Data and Material

There are no other data analyzed in this study.

References

1 Cortes J, Rea D, Lipton JH. Treatment-free remission with first- and second-generation tyrosine kinase inhibitors. Am J Hematol. 2019 Mar;94(3):346–57.
2 Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. Nat Rev Cancer. 2005 Mar;5(3):172–83.
3 Patel AB, O'Hare T, Deininger MW. Mechanisms of resistance to ABL kinase inhibition in chronic myeloid leukemia and the development of next generation ABL Kinase inhibitors. Hematol Oncol Clin North Am. 2017 Aug;31(4):589–612.
4 Abdel-Wahab O, Figueroa ME. Interpreting new molecular genetics in myelodysplastic syndromes. Hematol Am Soc Hematol Educ Program. 2012;2012:56–64.
5 Gelsi-Boyer V, Trouplin V, Adélaïde J, Bonansea J, Cervera N, Carbuccia N, et al. Mutations of polycomb-associated gene ASXL1 in myelodysplastic syndromes and chronic myelomonocytic leukaemia. Br J Haematol. 2009 Jun;145(6):788–800.
6 Tefferi A, Pardanani A. Myeloproliferative neoplasms: a contemporary review. JAMA Oncol. 2015 Apr;1(1):97–105.
7 Grossmann V, Kohlmann A, Zenger M, Schindela S, Eder C, Weissmann S, et al. A deep-sequencing study of chronic myeloid leukemia patients in blast crisis (BC-CML) detects mutations in 76.9% of cases. Leukemia. 2011 Mar;25(3):557–60.
8 Togasaki E, Takeda J, Yoshida K, Shiozawa Y, Takeuchi M, Oshima M, et al. Frequent somatic mutations in epigenetic regulators in newly diagnosed chronic myeloid leukemia. Blood Cancer J. 2017 Apr 28;7(4):e559.
9 Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014 Dec 25;371(26):2477–87.
10 Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014 Dec 25;371(26):2488–98.
11 Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med. 2014 Dec;20(12):1472–8.
12 Pellagatti A, Boultwood J. The molecular pathogenesis of the myelodysplastic syndromes. Eur J Haematol. 2015 Jul;95(1):3–15.
13 Abdel-Wahab O, Adli M, LaFave LM, Gao J, Hricik T, Shih AH, et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. Cancer Cell. 2012 Aug 14;22(2):180–93.
14 Davies C, Yip BH, Fernandez-Mercado M, Woll PS, Agirre X, Prosper F, et al. Silencing of ASXL1 impairs the granulomonocytic lineage potential of human CD34$^+$ progenitor cells. Br J Haematol. 2013 Mar;160(6):842–50.
15 Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med. 2011 Jun 30;364(26):2496–506.
16 Gelsi-Boyer V, Brecqueville M, Devillier R, Murati A, Mozziconacci MJ, Birnbaum D. Mutations in ASXL1 are associated with poor prognosis across the spectrum of malignant myeloid diseases. J Hematol Oncol. 2012 Mar 21;5:12.

17 Boultwood J, Perry J, Pellagatti A, et al. Frequent mutation of the polycomb-associated gene ASXL1 in the myelodysplastic syndromes and in acute myeloid leukemia. Leukemia. 2010;24:1062–5.
18 Eriksson A, Lennartsson A, Lehmann S. Epigenetic aberrations in acute myeloid leukemia: early key events during leukemogenesis. Exp Hematol. 2015 Aug;43(8):609–24.
19 Boultwood J, Perry J, Zaman R, Fernandez-Santamaria C, Littlewood T, Kusec R, et al. High-density single nucleotide polymorphism array analysis and ASXL1 gene mutation screening in chronic myeloid leukemia during disease progression. Leukemia. 2010 Jun;24(6):1139–45.
20 Kim T, Tyndel MS, Kim HJ, Ahn JS, Choi SH, Park HJ, et al. Spectrum of somatic mutation dynamics in chronic myeloid leukemia following tyrosine kinase inhibitor therapy. Blood. 2017 Jan 5;129(1):38–47.
21 Gilliland DG. Molecular genetics of human leukemias: new insights into therapy. Semin Hematol. 2002 Oct; 39(4 Suppl 3):6–11.
22 Inoue D, Fujino T, Sheridan P, Zhang YZ, Nagase R, Horikawa S, et al. A novel ASXL1-OGT axis plays roles in H3K4 methylation and tumor suppression in myeloid malignancies. Leukemia. 2018 Jun;32(6):1327–37.
23 Inoue D, Fujino T, Kitamura T. ASXL1 as a critical regulator of epigenetic marks and therapeutic potential of mutated cells. Oncotarget. 2018 Oct 16;9(81):35203–4.