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 a 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...
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.

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**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-
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

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        | 3.27×10⁴ /µL|
| Hb         | 9.3 g/dL    |
| Hct        | 30.4 %      |
| MCV        | 93.0 fl     |
| MCHC       | 30.6 %      |
| PLT        | 5.10×10⁴ /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   |
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 \textit{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 \textit{ASXL1} occur early in CML stem cells, prior to \textit{bcr-abl} translocation stage [19], as these cells clonally evolve [20]. This is considered an intrinsic event, rather than a \textit{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 \textit{ASXL1} lead to its loss of function, impairing granulomonocytic differentiation in early human hematopoietic progenitors and contributing to leukemogenesis. Indeed, silencing of \textit{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, \textit{ASXL1} mutations might enhance other leukemogenesis pathways via other mechanisms, including epigenetic regulation.

\textit{ASXL1} plays a key role in epigenetic regulation of gene expression through methylation of histone H3K27, and disruption of \textit{ASXL1} drives myeloid malignancies [22]. Also, \textit{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 \textit{ASXL1} mutation in CML has remained obscure, epigenetic modulation is a plausible mode of CML disease progression.

\textit{ASXL1} mutations occur early in CML stem cells, prior to \textit{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, \textit{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 \textit{ASXL1} for CML is still under investigation, mutated \textit{ASXL1} possibly explain the TKI resistance mechanism. This case illustrated the necessity of HSCT in \textit{ASXL1} mutation-associated TKI-resistant CML-CP. In future, a discussion of CML with mutated \textit{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.

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