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Case Report

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Philadelphia+ Chronic Myeloid Leukemia with *CALR* Mutation: A Case Report and Literature Review

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Myeloproliferative neoplasms (MPNs) are classified as chronic myeloid leukemia (CML) and Philadelphia chromosome-negative MPN. In MPN cases, the presence of a *BCR-ABL1* translocation with a coexisting mutation is exceptionally rare. Herein, we report the first documented patient with CML harboring *CALR* mutation in Korea. A 33-year-old woman was referred to our hospital in February 2015 with splenomegaly, leukocytosis, and thrombocytosis. She was diagnosed with CML and started receiving nilotinib. In October 2015, a major molecular response was observed, but thrombocytosis persisted. A repeat bone marrow (BM) examination revealed no specific findings. However, as thrombocytosis worsened, we changed nilotinib to dasatinib. In May 2019, owing to persistent thrombocytosis, we repeated the BM examination and found *CALR* mutation (15.97%) on the MPN–next generation sequencing (NGS) test. We then retrospectively performed repeat MPN-NGS testing using the BM aspirate sample obtained in 2015 and found *CALR* mutation (10.64%).

Key words
Myeloproliferative disorder, Philadelphia chromosome, Calreticulin

Introduction

Myeloproliferative neoplasms (MPNs) are a group of disorders in which bone marrow (BM) stem cells show abnormal growth and reproduction. In MPNs, abnormal stem cells produce excess numbers of one or more types of blood cells. These abnormal cells do not function properly and can cause serious health problems unless properly treated and controlled. Chronic myeloid leukemia (CML) is a type of MPN characterized by the translocation between chromosome 9 and 22, which is called Philadelphia chromosome, thereby resulting in the *BCR-ABL1* oncogene. According to 'The 2016 revision to the World Health Organization classification of myeloid neoplasm and acute leukemia', other classical MPNs can be diagnosed after CML is excluded. However, recent cases with the coexistence of *BCR-ABL1* and other MPN markers (i.e., *CALR*, *JAK2*, or *MPL* mutations) have been reported. Herein, we present a case of BCR-ABL1–positive CML with a *CALR* mutation in a patient with persistent

thrombocytosis who maintained a major molecular response after treatment with a tyrosine kinase inhibitor (TKI).

Case Report

A 33-year-old woman was referred to our hospital in February 2015 owing to an abdominal mass and abnormal laboratory values. Abdominal ultrasonography revealed splenomegaly (22 cm), and laboratory examinations revealed a white blood cell (WBC) count of 107,600/mm^3 and a platelet (PLT) count of 906,000/mm^3. BM examination revealed a marrow cellularity of 90%, with markedly increased megakaryocytes. Reticulin staining indicated grade 2-3 fibrosis. The karyotype was 45,XX,t(9;22)(q34;q11.2),der(13;14)(q10;q10)[15]. In case of der(13;14)(q10;q10), it was found in all cells continuously after treatment and presumed to be germline mutation. Fluorescence *in situ* hybridization analysis showed that 99.2% of 500 cells were positive for t(9;22), and real-time quantitative polymerase chain reaction showed that the transcript level for BCR-ABL1/ABL1^{IS} (international scale [IS]) was 25.603%. *JAK2* V617F was negative on conventional screening. Testing for *CALR* mutations was not yet available. Accordingly, the patient started receiving nilotinib. After 6 months of treatment with nilotinib, she achieved a major molecular response. In October 2015, she developed persist-

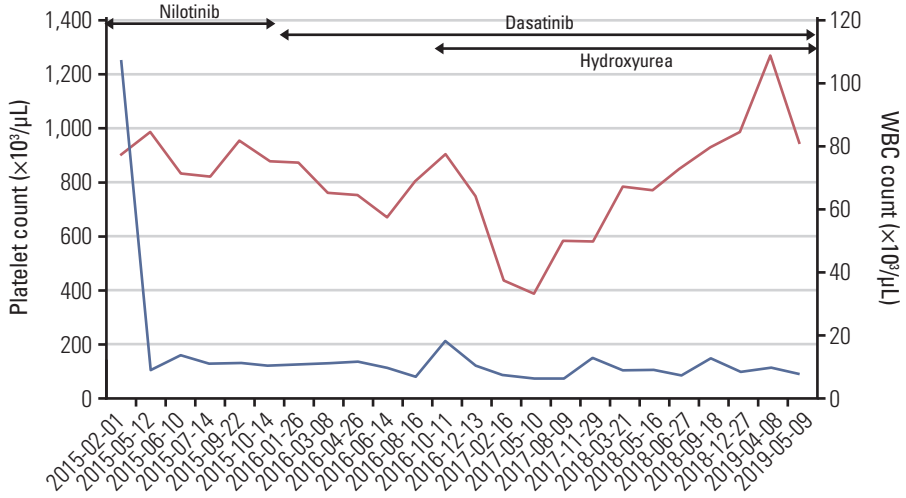

Fig. 1. White blood cell (WBC) and platelet counts during follow-up of a patient with chronic myeloid leukemia over the course of treatment.

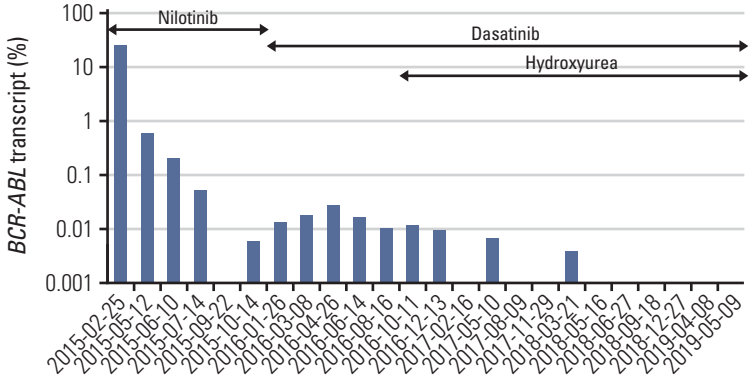

Fig. 2. *BCR-ABL1* transcript levels during follow-up of a patient with chronic myeloid leukemia over the course of treatment.

Table 1. Cases with *BCR-ABL1* translocation and *CALR* mutation

Patient No.	Study	Age (yr)/ Sex	Time of *BCR-ABL1* detection	Type of *CALR* /Time of detection	Treatment	
					Medication	Course
1	Cabagnols et al. [1]	73/F	At the time of diagnosis	Type 1/initial(R)	Ima → Dasa	Persistent thrombocytosis even though the *BCR-ABL1* transcript level decreased
2	Bonzheim et al. [2]	26/M	3 yr after diagnosis (no mutation initially)	Type 2/initial(R)	IFN →Nilo	Persistent thrombocytosis and no change in the *CALR* allele burden even though the *BCR-ABL1* transcript level decreased
3	Loghavi et al. [3]	67/M	At the time of diagnosis	Type 1/initial(R)	Dasa	Sustained PMF findings on bone marrow biopsy even though the *BCR-ABL1* transcript level decreased
4	Diamond et al. [4]	50/M	At the time of diagnosis (no initial sample)	Type 1/1 yr later	Ima	Persistent anemia and splenomegaly even though the *BCR-ABL1* transcript level decreased
5	Seghatoleslami et al. [5]	78/F	At the time of diagnosis (p190 type)	Type 1/initial	No data	Coexistence of *BCR-ABL1* transcript and *CALR* mutation in blast crisis in a patient with CML
6	Klairmont et al. [6]	90/F	4 yr after diagnosis (no mutation initially)	Type 1/11 yr later (no initial sample)	Ima	The initial presentation of thrombocytosis on a later bone marrow biopsy demonstrated a hypercellular marrow with megakaryocytic hyperplasia, reticulin fibrosis, and the absence of a *BCR-ABL1* translocation. We therefore hypothesized that the patient initially harbored *del52CALR* (testing for which was not yet available at the time of diagnosis), leading to the clinical and histologic manifestations of a Ph-MPN. The subsequent development of leukocytosis and acquisition of t(9;22)(q34;q11) suggests that CML evolved at a later point in time. *MPL V501A* mutation was also identified.
7	Dogliotti et al. [7]	61/F	At the time of diagnosis	Type 1/initial(R)	Ima	Persistent thrombocytosis even though the *BCR-ABL1* transcript level decreased
8	Xia et al. [8]	65/W	4 yr after diagnosis (no mutation initially)	Type 1/4 yr later (no initial sample)	Dasa	The initial presentation of thrombocytosis with a bone marrow aspiration demonstrated a normocellular-appearing marrow with atypical large megakaryocytes, consistent with ET. The karyotype and testing for JAK2p.V617F and *BCR-ABL1* showed normal results. Mild leukocytosis and thrombocytosis were observed even though the *BCR-ABL1* transcript level decreased

(Continued to the next page)

Table 1. Continued

Patient No.	Study	Age (yr)/ Sex	Time of *BCR-ABL1* detection	Type of *CALR* /Time of detection	Treatment	
					Medication	Course
9	Blouet et al. [9]	76/M	At the time of diagnosis	Type 1/initial(R)	Ima (+HU)	At diagnosis, the *CALR* mutation was present at a very low allele burden; it was not detectable on fragment analysis (sensitivity is ~5% of the allele burden) because of the higher representation of *BCR-ABL1* clones. Tyrosine kinase inhibitor (TKI) treatment led to a decrease of the *BCR-ABL1* clone and allowed the proliferation of the CALR clone that can explain the short time between the diagnosis of CML and ET.
10	Lewandowski et al. [10]	55/F	At the time of diagnosis	Type 1/initial(R)	Ima→ Nilo → Dasa HU/IFN	Persistent thrombocytosis even though the *BCR-ABL1* transcript level decreased

CALR, calreticulin; R, retrospectively; Ima, imatinib; Dasa, dasatinib; Nilo, nilotinib; ph-MPN, Ph-negative myeloproliferative neoplasm; CML, chronic myeloid leukemia; ET, essential thrombocythemia; HU, hydroxyurea; IFN, interferon.

ent thrombocytosis. A repeat BM examination did not show any specific findings. The karyotype was 46,XX,der(13;14)(q10;q10)[13]. However, as thrombocytosis worsened (PLT count, 1,006,000/mm^3) and splenomegaly persisted, we changed nilotinib to dasatinib. We then added hydroxyurea as part of the treatment, after which the thrombocytosis was controlled slightly but it worsened subsequently (Figs. 1 and 2). In May 2019, follow-up BM examination was performed, and we used the BM aspiration sample for MPN–next-generation sequencing (NGS): the patient had *CALR* mutation (c.1099_1136delinsAGGT), with an allele frequency of 15.97%, and *TET2* mutation (c.3409+1G>A), with an allele frequency of 7.83%. We then retrospectively performed repeat MPN-NGS testing using the BM aspiration sample obtained in 2015. We found that *CALR* mutation had been presence since 2015, with an allele frequency of 10.64%, but no *TET2* mutation was observed. We reported a case of CALR-positive CML with persistent thrombocytosis during TKI treatment.

The study protocol was approved by the Institutional Review Board of the Soonchunhyang University Seoul Hospital (No. 2020-01-012). The study patient was informed as the purpose of the study and provided her consent.

Discussion

CALR mutation was first recognized as a somatic mutation in patients with MPN who had no mutation in either *JAK2* or *MPL* in 2013 [11]. *CALR* mutation is observed in approximately 70% of patients with essential thrombocythemia (ET) or primary myelofibrosis who did not carry a mutation in either *JAK2* or *MPL* [11,12]. CALR-mutant MPNs are characterized by a gene signature associated with activating JAK2 signaling, and *CALR*-mutants activate the JAK/STAT pathway through MPL, leading to excessive PLT production [13]. More than 50 different mutations in *CALR* have been described, but a 52 base-pair deletion (type 1) or a 5 base-pair insertion (type 2) account for more than 80% of mutations [13]. Pietra et al. [14] showed an association between *CALR* type 1 mutations and a significant increase in the risk of myelofibrotic transformation among ET cases, whereas type 2 mutations were preferentially associated with an ET phenotype, low risk of thrombosis despite a very high PLT count, and an indolent clinical course.

The presence of a *BCR-ABL1* translocation with a coexisting mutation in *CALR* is exceptionally rare. A total of 10 cases have been reported since the first report was published in 2015, and the current report is the first in Korea (Table 1). In most cases, *CALR* mutation and *BCR-ABL1* translocation were synchronously identified or *BCR-ABL1* translocation

was detected after *CALR* mutation. Otherwise, the presence of *CALR* mutation was not confirmed, but there was a case where the diagnosis was changed to CML on the basis of the findings of ET. To the best of our knowledge, there has been no report of *CALR* mutation after CML diagnosis. TKIs were ineffective for *CALR*-mutated clones, as shown in previous studies. Accordingly, physicians should be careful while changing the treatment of patients with CML who achieve a molecular response but do not obtain a hematologic response. Additional molecular mutations may be identified in patients with CML with the recent widespread use of NGS testing. A recent study showed the presence of molecular aberrations other than *JAK2 V617F* and *BCR-ABL1* in 56% of patients with MPN, including mutations in *TET2*, *ASXL1*, *RUNX1*, *CBL*, *DNMT3A*, *PHF6*, *SF3B1*, and *TP53* [15]. In particular, *TET2* is a putative tumor suppressor gene located on chromosome 4q24. Only a few studies revealed *TET2* mutations in patients with MPN. However, there is no consensus regarding the exact role of TET2. In the current case, the relevance of identifying a new *TET2* mutation during the course of the disease is unclear. Although patients with CML with *BCR-ABL1* translocation have additional molecular aberrations, they are still diagnosed with CML on the basis of the current diagnostic criteria. However, further studies are needed regarding the diagnosis, treatment, and prognosis of these patients.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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