

[CASE REPORT]

Acute Megakaryoblastic Leukemia Harboring a Subclone Expressing *BCR-ABL1* Fusion Gene Product

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Abstract:

Acute myeloid leukemia (AML) with *BCR-ABL1*, also termed Philadelphia chromosome-positive AML (Ph+ AML), is a rare leukemia subtype classified by the World Health Organization in 2016. The characteristics of Ph+ AML have not been fully identified yet. We herein report a patient with Ph+ AML who phenotypically exhibited megakaryoblastic characteristics, FAB:M7 and harbored a subclone expressing *BCR-ABL1* gene fusion products. This case suggests that *BCR-ABL1* was acquired as a subclone due to a secondary event that might have occurred late during leukemia evolution. Our findings may aid in deciphering the mechanism underlying Ph+ AML development in future studies.

Key words: Ph+ AML, acute megakaryoblastic leukemia, *BCR-ABL1* fusion gene

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Introduction

Acute myeloid leukemia (AML) with *BCR-ABL1*, also termed Philadelphia chromosome-positive AML (Ph+ AML), is a rare subtype of leukemia. It contributes to approximately 0.48-3% of newly diagnosed cases of AML (1, 2). Ph+ AML should be distinguished from chronic myeloid leukemia in myeloid blast crisis (CML-MBC) (1) or mixed phenotype acute leukemia (MPAL) with t(9;22)(q34;q11); *BCR-ABL1* (3). However, the disease characteristics of Ph+ AML have not yet been fully identified (4).

Ph+ AML was classified as a high-risk disease in the recently updated guidelines of European Leukemia Net (ELN) (5) and current National Comprehensive Cancer Network (NCCN) (6). Its treatment has not been established yet and remains controversial due to the absence of systematic clinical data (1). Furthermore, although the *BCR-ABL1* oncoprotein has been shown to be a selective target in the treatment of CML, positioning of a targeted therapy using tyrosine kinase inhibitors (TKIs) in Ph+ AML is controversial (2). Neuendorff et al. suggested that the co-incidence of spontaneously occurring *BCR-ABL1* within clonal hematopoiesis may explain the development of Ph+ AML and

that *BCR-ABL1* is an additional modification that emerges as a *BCR-ABL1*-positive subclone (1). We experienced a patient presenting acute megakaryoblastic leukemia with a *BCR-ABL1*-expressing subclone who was diagnosed with Ph+ AML based on the morphological, phenotypical, and cytogenetic properties according to the World Health Organization (WHO) classification (7).

We herein report a patient with Ph+ AML who might have acquired *BCR-ABL1* as a subclone due to a secondary event during leukemia evolution, as proposed previously (1).

Case Report

A 69-year-old woman presented at Suzuka Kaisei Hospital for general malaise that had persisted for 1 month. She did not have any remarkable history or mention any antecedent hematological abnormality.

A physical examination revealed anemia in the conjunctiva. No other abnormalities, such as splenomegaly, were observed. The laboratory findings were as follows: white blood cell count, $2.2 \times 10^9/L$; blasts, 4%; myelocytes, 2%; neutrophils, 41%; lymphocytes, 46%; monocytes, 4%; basophils, 2%; eosinophils, 1%; hemoglobin, 61 g/L; and platelet count, $20 \times 10^9/L$. The blasts were lymphoblast-like with a

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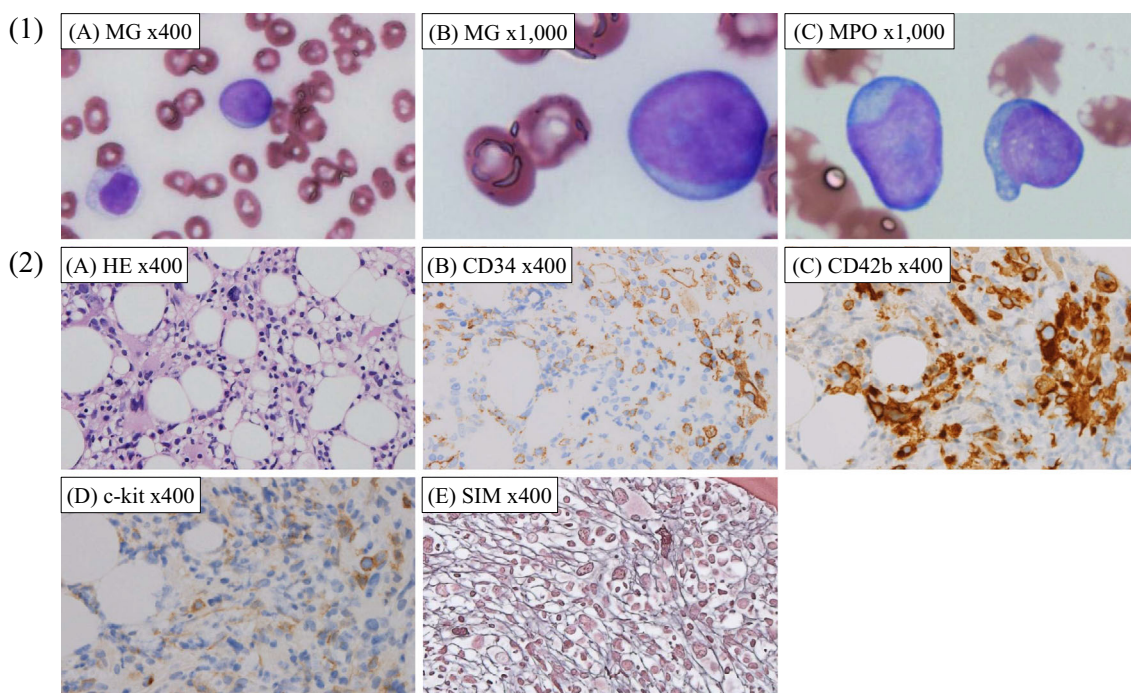


Figure 1. (1) Peripheral blood smear findings using May-Giemsa stain at (A) 400, (B) 1,000 magnification, and (C) MPO at 1,000 magnification. (2) Histological findings of bone marrow biopsy using (A) HE, (B) CD34, (C) CD42b, (D) c-kit, and (E) silver impregnation staining at 400x magnification. HE: Hematoxylin and Eosin staining, MG: May-Giemsa staining, MPO: myeloperoxidase, SIM: silver impregnation method

high nuclear-cytoplasmic ratio and negative for myeloperoxidase (MPO) staining, and some exhibited cytoplasmic blebs [Fig. 1-(1)].

Bone marrow aspiration could not be performed due to 'dry tap.' Instead, a bone marrow biopsy was performed, which revealed that the megakaryoblasts were immunophenotypically positive for CD34, CD42b, and c-kit and negative for MPO [Fig. 1-(2) A to D]. The immunophenotypes by flow cytometry of the peripheral blood revealed that the blasts were positive for CD13, CD34, CD117(c-kit), and HLA-DR (Fig. 2). Bone marrow fibrosis was categorized as grade 2 according to the European consensus (8) [Fig. 1-(2) E].

Real-time polymerase chain reaction (RT-PCR) screening of the peripheral blood detected 4,000 copies of the minor *BCR-ABL1* gene and 40,000 copies of the *WT1* gene. Interphase fluorescence *in situ* hybridization (FISH) of *BCR-ABL1* revealed the presence of a *BCR-ABL1* fusion signal. Interphase FISH for *BCR-ABL1* of peripheral blood neutrophils showed findings of 0% in segmented cells and 4% in mononuclear cells. A G-banding karyotype analysis did not exhibit t(9;22), and complex karyotype abnormalities were observed [Fig. 3-(1)]. In addition, a gene-mutation analysis was negative for *NPM1*, *FLT3-ITD*, *JAK2V617F*, *MPLW515 L/K*, and *CALR* types 1 and 2.

Based on these findings, she was diagnosed with acute megakaryoblastic leukemia expressing the *BCR-ABL1* gene [AML with *BCR-ABL1* based on the WHO 2016 classification (7)]. FISH of the bone marrow biopsy sample revealed

the presence of one *BCR-ABL1*-positive cell among 200 blasts (Fig. 4). We considered the *BCR-ABL1*-positive clone to be a subclone.

The patient was hospitalized and treated with cytarabine/idarubicin as induction therapy without administering a TKI. She successfully achieved hematological complete remission. The number of minor *BCR-ABL1* gene copies decreased to 260, and interphase FISH was not detected in the bone marrow.

She was subjected to two additional courses of consolidation therapy. Four months later, the disease recurred with re-growth of blasts in both peripheral blood and bone marrow. At the relapse, 39,000 copies of *WT1* mRNA were detected using RT-PCR in the bone marrow; however, minor *BCR-ABL1* was undetectable. A G-banding karyotype analysis did not exhibit t(9;22), and complex karyotype abnormalities were observed at the relapse [Fig. 3-(2)]. FISH of the bone marrow biopsy sample revealed no fusion signal corresponding to *BCR-ABL1*. The disease was uncontrollable even through the treatment with gemtuzumab/ozogamicin, and the regimen of low-dose cytarabine, aclarubicin and granulocyte colony-stimulating factor priming (CAG) (Fig. 5).

She refused further treatment and switched to best supportive care.

Discussion

AML with *BCR-ABL1* is a rare subtype of leukemia and was considered a provisional entity of myeloid neoplasm

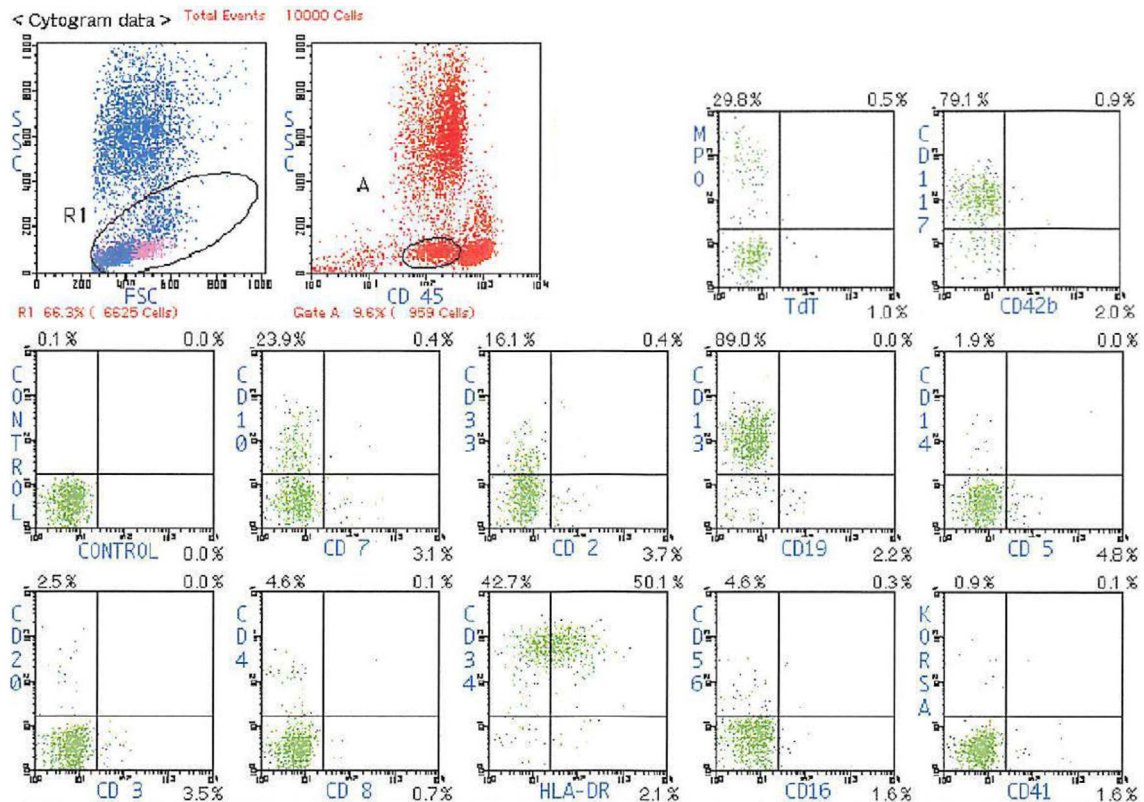


Figure 2. Flow cytometry of peripheral blood at the diagnosis.

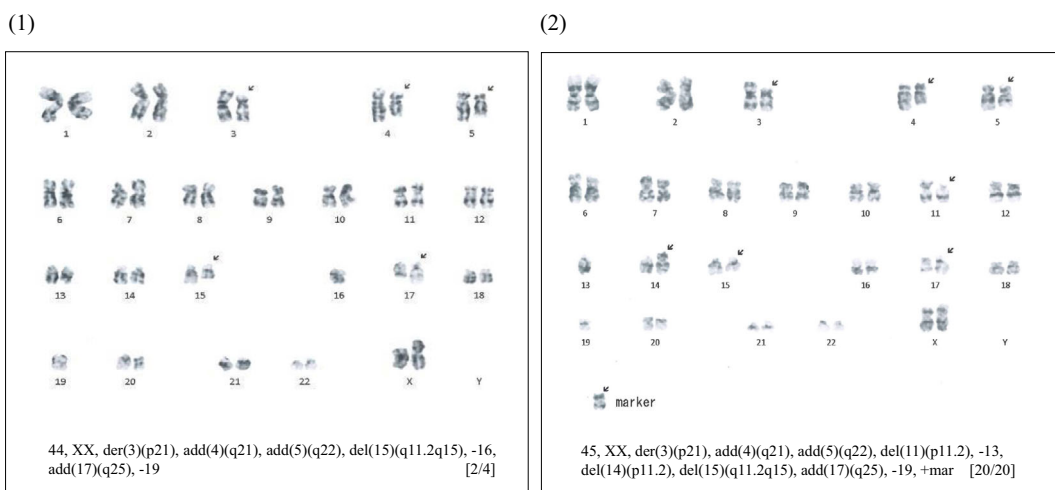


Figure 3. A chromosomal analysis (G-banding) of peripheral blood at the diagnosis (1) and bone marrow at the relapse (2).

and acute leukemia (1). It was newly included in the revised WHO classification in 2016 (3). However, the disease characteristics of Ph+ AML have not been fully identified yet (4). It is important to distinguish Ph+ AML from CML-MBC (1). It was reported that the following characteristics supported the diagnosis of AML rather than CML: no antecedent hematological anomaly, no basophilia or significant splenomegaly at the diagnosis, the detection of p190-transcript, the occurrence of *BCR-ABL1* transcripts in < 100% of metaphases, and a persistent complete cytogenetic response after conventional chemotherapy (1).

We encountered a case of Ph+ AML expressing a subclone of *BCR-ABL1* fusion gene product and a majority of non-*BCR-ABL1* clones. The leukemic blasts exhibited morphologically homogeneous megakaryoleukemic characteristics. These findings suggest that the Ph+ AML subclone expressing *BCR-ABL1* might have developed as a secondary clone during leukemogenesis.

Ph+ AML cells often aberrantly express lymphoid markers (9). These should be distinguished from MPAL with t(9;22)(q34;q11); *BCR-ABL1*, which express two types of key lineage-defining markers according to the 2016 WHO classi-

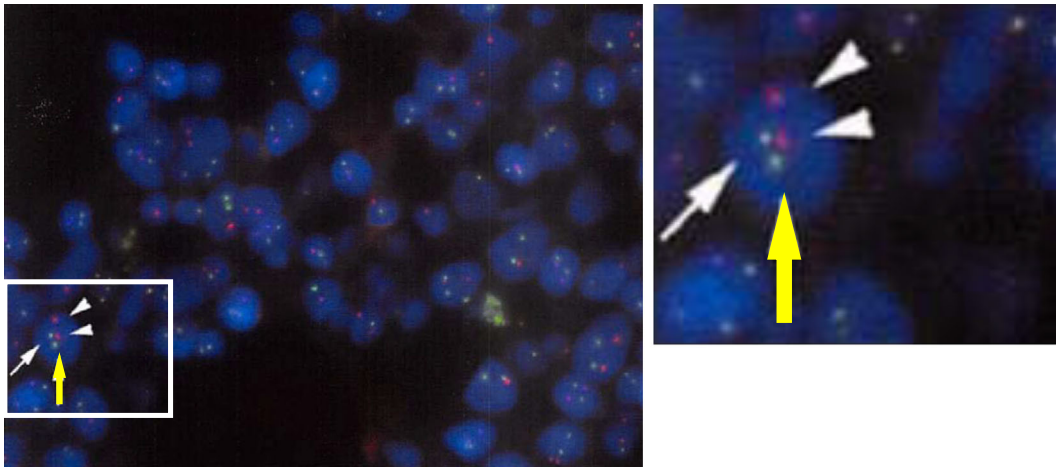


Figure 4. A FISH analysis of the bone marrow biopsy sample. The white arrow indicates a native *BCR* gene and a native *ABL1* gene in a nucleus. The green signal shows a native *BCR* gene, and the red one shows a native *ABL1* gene. The yellow arrow indicates a fusion signal corresponding to a *BCR-ABL1* gene. FISH: fluorescence *in situ* hybridization

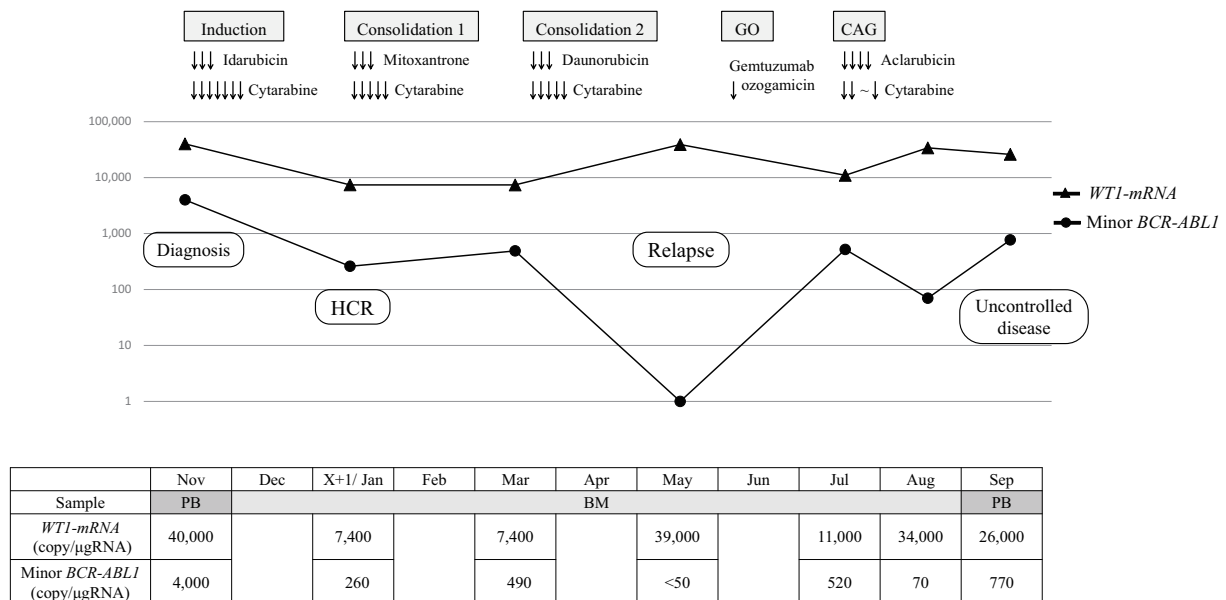


Figure 5. Temporal changes in the expression of *BCR-ABL1* and *WT1* genes assessed through an RT-PCR analysis of bone marrow or peripheral blood. At the relapse, 39,000 copies of *WT1* mRNA were detected, and the copy number of *BCR-ABL1* gene was below the detection level. HCR: hematological complete remission, RT-PCR: real-time polymerase chain reaction

fication (3). Ph+ AML consists of a morphologically single blast population, whereas most MPAL cases exhibit a dimorphic blast population with some blasts resembling lymphoblasts and others myeloblasts (7). In our case, the morphological analysis indicated the presence of a single blast population, which represented a typical immunophenotype of acute megakaryoblastic leukemia (M7) according to the French-American-British (FAB) classification (7). Considering these morphological, phenotypical, and cytogenetic properties, our patient was diagnosed with Ph+ AML.

BCR-ABL1 transcripts may temporarily be expressed at low levels in the blood of healthy individuals. However, the

consequences associated with leukemogenesis are unknown (10). The higher frequency of *BCR-ABL1*-positive carriers in healthy populations than the incidence of Ph+ leukemia indicates that the *BCR-ABL1* aberration is not sufficient for the malignant transformation of hematopoietic cells (11). Neuendorff et al. showed that Ph+ AML develops with the co-occurrence of spontaneously occurring *BCR-ABL1* during clonal hematopoiesis on an underlying 'mutational background' and displays a small and/or temporary fraction as a *BCR-ABL1*-positive subclone (1). Bacher et al. reported that several AML cases harbored *BCR-ABL1* as subclones, with other chromosomal abnormalities in most of

the cells in metaphase in addition to t(9;22) (12). These additional *BCR-ABL1* alterations were supposedly acquired as secondary changes occurring in the late phase of leukemia evolution, so it is not the primary driver mutation (13).

Furthermore, the prognosis of Ph+ AML depends on the genetic background (concurrent aberrations) compared to *BCR-ABL1* (13), whereas Ph+ AML is classified as a high-risk disease according to the recently updated guidelines of ELN (5) and current NCCN (6). In our case, the expression of a minor *BCR-ABL1* gene in RT-PCR was low, and a *BCR-ABL1* fusion signal was detected using FISH in only 1 out of 200 blasts in the bone marrow biopsy sample. Therefore, we suspected that *BCR-ABL1*-positive clone might be a subclone that emerged due to a secondary event during the disease development and not as the founding clone of AML. Considering the temporal changes in the expression of minor *BCR-ABL1* and *WT1* genes in the bone marrow or peripheral blood using RT-PCR, it is conceivable that the *BCR-ABL1*-positive subclone was present in a minor population at the early phase of the disease, and *BCR-ABL1*-negative founding clones survived, thereby leading to relapse after two additional courses of consolidation therapy. At the time of relapse, RT-PCR revealed 39,000 copies of *WT1* mRNA with the minor *BCR-ABL1* below the detection level. A FISH analysis of the bone marrow biopsy sample revealed the absence of positive cells among 200 blasts. The results of RT-PCR and FISH and the clinical course of our case support the proposed mechanism underlying the development of Ph+ AML (1).

Treatment of Ph+ AML has not been established yet; it remains controversial due to the absence of systematic clinical data (1). In particular, targeted therapy using TKIs has not been established (14). Thus far, several case reports have described implementing TKI treatments either alone or in combination with chemotherapy for Ph+ AML (1, 2, 4, 12-17). Some cases achieved hematologic responses to TKI treatment, but the response durations were limited (2). The reason for the limited response duration of TKIs against Ph+ AML is due to the clonal diversity of Ph+ AML, as opposed to CML in which the TKIs respond for a longer duration (1). A previous case report described how TKI treatment led to the eradication of *BCR-ABL1*-positive clones in AML, despite the overall growth of refractory AML cells (15). This suggests that TKI treatment carries a risk of clonal selection of *BCR-ABL1*-negative clone (1), leading to mutational evolution of clones other than *BCR-ABL1*-positive clones (15). Therefore, treatment with a single molecule-targeting agent, such as a TKI, may not be sufficient for achieving disease control of Ph+ AML, possibly because *BCR-ABL1* is not a driver mutation present in the founding clone. However, such treatment might temporarily inhibit the proliferation of the *BCR-ABL1*-positive subclone (1). Therefore, while TKI treatment cannot be routinely recommended as part of first-line therapy (1), it might be a reasonable approach as a part of salvage therapy or may be able to be implemented in combination with allogeneic stem cell transplantation (4).

neic stem cell transplantation (4).

TKIs might be a safe and effective option for treating patients with Ph+ AML who are in poor general condition and cannot tolerate chemotherapy (18). Furthermore, combination therapy using venetoclax with TKI-based regimens showed encouraging activity in heavily treated patients, and the patients who achieved hematological responses showed higher number of Ph+ cells in metaphase or more *BCR-ABL1* PCR copies at baseline than non-responders (18). Therefore, TKIs may be useful as an option for salvage therapies, especially in heavily treated patients with a poor performance status and high baseline Ph+ cells in metaphase (1, 4, 18). Our patient was newly diagnosed with AML, and her general appearance was good (performance status 0). At the time of the diagnosis, a low level of minor *BCR-ABL1* expression was detectable in the peripheral blood, and AML cells exhibited complexed karyotypes without t(9;22) in metaphase. Therefore, TKI treatment was not administered. At the relapse, TKIs were unsuitable as salvage therapy because the *BCR-ABL1*-positive clone had already disappeared.

In conclusion, Ph+ AML cells are cytologically heterogeneous with clonal diversity (14), and the *BCR-ABL1* fusion gene may emerge as a subclone due to a secondary event possibly occurring at the late phase of leukemia evolution (13). These findings support the concept of the previously proposed mechanism underlying the development of Ph+ AML (1).

The authors state that they have no Conflict of Interest (COI).

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