Letter to the Editor
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Chronic Myeloid Leukemia With Rare Variant b2a3 (e13a3) *BCR-ABL1* Fusion

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Dear Editor,

CML is characterized by reciprocal t(9;22)(q34;q11) translocation, which generates the BCR/ABL1 protein; that protein plays a critical role in the pathogenesis of CML. CML patients commonly harbor *BCR-ABL1* fusion transcripts of types b3a2 (e14a2) or b2a2 (e13a2), while types e1a2 or e19a2 are less common. CML cases with b2a3-type fusion, in which the *ABL1* exon 3 (a3) rather than exon 2 (a2) is fused to *BCR*, are rare. To date, only eight CML cases with solely b2a3-type fusion have been reported [1-6]. Here, we report a CML case with this rare b2a3-type *BCR-ABL1* fusion and review the literature.

A 57-yr-old man with marked leukocytosis was referred to a tertiary-care hospital and diagnosed as having CML in the chronic phase. Peripheral blood analysis showed a white blood cell (WBC) count of 384.7×10^9/L with 1% blasts, as well as a hemoglobin concentration of 7.3 g/dL, and a platelet count of 424×10^9/L. The bone marrow aspirate revealed a hypercellular marrow with left-shifted myeloid series and an increased number of megakaryocytes, with the occasional dwarf form. G-banded karyotyping of the bone marrow cells demonstrated t(9;22)(q34;q11.2) in all of the 17 metaphase cells analyzed. A *BCR/ABL1* gene rearrangement test by using reverse transcription PCR (RT-PCR) with home-brewed primers complementary to the *ABL1* exon 2 (a2) failed to detect the *BCR-ABL1* fusion transcript (reference sequence: *BCR*, NM_004327.3; *ABL1*, NM_005157.5). Negative results were also obtained with quantitative real-time RT-PCR analysis using the ipsogen *BCR-ABL1* Mbcr IS-MMR Kit (Qiagen, Hilden, Germany). However, multiplex RT-PCR using the HemaVision kit (DNA Technology, Aarhus, Denmark) showed an atypical band of approximately 220 base pairs, suggesting the presence of the b2a3-type *BCR-ABL1* fusion transcript (Fig. 1A). Sanger sequencing of the RT-PCR product revealed fusion between the *BCR* exon 13 (b2) and the *ABL1* exon 3 (a3) (Fig. 1B). The patient was started on nilotinib, which has been continued to the present.

The clinical significance of b2a3-type fusion in CML has not been determined owing to its rarity. Therefore, we reviewed the literature for CML cases with this type of fusion in order to understand its clinical characteristics (Table 1). Cases with concomitant expression of another type of fusion transcript were eliminated to exclude the clinical effect of the other fusion transcript. All the eight patients underwent favorable clinical courses; none progressed to the transformed stages of accelerated or blast phases during the follow-up period. Additionally,

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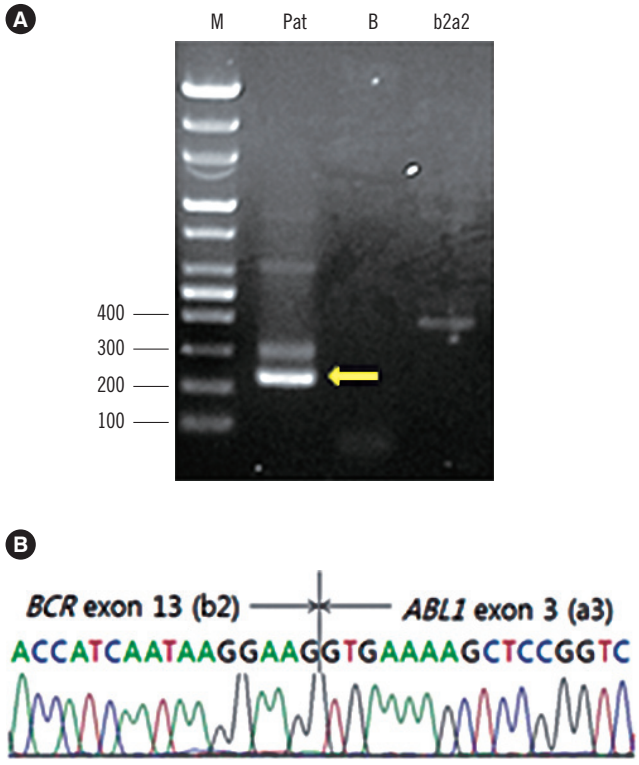

Fig. 1. (A) Agarose gel electrophoresis of multiplex reverse transcription (RT)-PCR product showing an atypical band of approximately 220 base pairs, suggesting the presence of the b2a3-type *BCR-ABL1* fusion transcript. Lane M, bp markers; Lane Pat, reported patient with variant b2a3-type fusion transcript; Lane B, blank; Lane b2a2, positive control with b2a2 fusion transcript. (B) cDNA sequence results of the RT-PCR product demonstrating the b2a3-type fusion transcript (reference sequence: *BCR*, NM_004327.3; *ABL1*, NM_005157.5).

b2a3-type fusion CML seems to be sensitive to tyrosine kinase inhibitor (TKI) therapy. All five cases using TKI achieved complete cytogenetic or major molecular responses. In contrast, two cases that used interferon α (IFN-α) as initial treatment modality showed only partial hematologic responses.

ABL1 exon 2 (a2), which is missing in the b2a3-type fusion, codes part of an Src homology (SH) 3 domain, known as a negative regulator of ABL1 tyrosine kinase [7]. Deletions and mutations of the SH3 domain can solely elevate tyrosine kinase activity [7, 8]. Therefore, it can be postulated that absence of the SH3 domain in the BCR/ABL1 fusion protein would result in aggressive clinical outcomes. However, several reports have suggested that the SH3 domain induces STAT5 expression, which in turn contributes to BCR/ABL1-dependent leukemogenesis *in vivo* [9]. In a murine model, *BCR/ABL1* with SH3 deletion retains the ability to induce CML but shows delayed disease onset and increased survival compared with b2a2 mice [10]. One possible explanation for the delayed disease onset and increased survival of these mice is the effect of the SH3 deletion on adhesion, invasion, and homing [9]. These findings are consistent with favorable clinical outcomes for b2a3-type CML patients, but further studies are needed to clarify the phenotypic characteristics of b2a3-type CML.

Rare fusion transcripts may escape detection when methods that are optimized to detect typical fusion transcripts are used. Initially, we failed to detect b2a3-type *BCR/ABL1* transcripts using the home-brewed PCR kit, which is designed only for typical fusion type CML. Since the b2a3-type fusion transcript lacks exon 2

Table 1. Summary of CML cases with the b2a3 *BCR/ABL1* fusion transcript

Reference	Sex/Age (yr)	Initial CBC (WBC/Hb/Platelet)*	Diagnostic phase	Karyotype	Treatment	Status at last F/U (month)
Liu, *et al*. (2003) [1]	M/59	254/9.6/180	CP	Ph	Hy	Myelofibrosis (24)
Liu, *et al*. (2003) [1]	NA	NA	CP	Ph	NA	CP (96)
Liu, et al. (2003) [1]	M/49	87/11.5/331	CP	Ph	IFN/Hy>Imatinib	CCR (24)
Moravcova, *et al*. (2005) [2]	M/42	27.8/14.3/233	CP	Ph	IFN>Imatinib	CCR (29)
Pienkowska-Grela, *et al*. (2007) [3]	M/39	163/NA/NA	CP	Complex with Ph†	Hy>Imatinib	CCR (22)
Masuko, *et al*. (2009) [4]	M/37	8.67/11.5/2,016	CP	Complex with Ph‡	Imatinib	MMR (24)
Achkar, *et al*. (2010) [5]	F/25	122/10.3/156	CP	Complex with Ph§	Hy	CP (NA)
McCarron, *et al*. (2015) [6]	M/66	NA	CP	Ph	Imatinib	CCR (14)
Present case	M/57	384.7/7.3/424	CP	Ph	Nilotinib	NA (3)

*Values are presented in the International System of Units (WBC, $\times 10^9$/L; Hb, g/dL; Platelet, $\times 10^9$/L); †51,XY,+8,+8,+8,+8,t(9;22)(q34;q11.2),+der(22)t(9;22)[6]/52,XY,idem,+der(22)x2[6]/46,XY,t(9;22)(q34;q11.2); ‡46,XX,der(9) (9pter →9q13: :9q34→9qter),der(22) (22pter→22q11: :9q34: :9q34: :9q13→9pter); §48,XX,+8,+der(12)t(12;19)(p11.2;q13.3),t(9;12;19;22)(q34.1;p11.2;q13.3;q11.2)[8]/47,XX,+8,t(9;12;19;22)(q34.1;p11.2;q13.3;q11.2)[2]/47,XX,+der(12)t(12;19)(p11.2;q13.3),t(9;12;19;22)(q34.1;p11.2;q13.3;q11.2)[2]/46,XX,t(9;12;19;22)(q34.1;p11.2;q13.3;q11.2)[8].
Abbreviations: CBC, complete blood count; WBC, white blood cell; F/U, follow-up; CP, chronic phase; Ph, t(9;22)(q34;q11); Hy, hydroxyurea; IFN, interferon; NA, not available; CCR, complete cytogenetic response; MMR, major molecular response.

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of the *ABL1* gene, PCR using primers that bind to sequences in the *ABL1* exon 2 may fail to amplify target fusion transcripts.

We report a case of CML with the b2a3-type *BCR/ABL1* fusion transcript. The literature review shows that the majority of b2a3-type CML cases have a benign prognosis and good sensitivity to TKI therapy. Clinical laboratories should be aware that rare fusion transcripts such as b2a3 may not be detected when using primers complementary to *ABL1* exon 2.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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