



A Novel e8a2*BCR-ABL1* Fusion Transcript without Insertion Sequence in a Patient with Chronic Myeloid Leukemia

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Dear Editor,

Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder characterized by a reciprocal translocation between the long arms of chromosomes 9 and 22, resulting in the formation of a Philadelphia (Ph) chromosome. Most chimeric *BCR-ABL1* fusion transcripts are e13a2(b2a2) or e14a2(b3a2), encoding a 210-kDa protein, p210, but some are a e1a2 transcript or e19a2 transcript encoding a 190-kDa protein or a 230-kDa protein, respectively [1]. Furthermore, some patients harbor rarer atypical transcript types such as e1a3, e6a2, e8a2, and e13a3. In addition, *BCR-ABL1* transcripts have been reported to correlate significantly with the prognosis of CML patients. We retrospectively studied a CML patient with a variant *BCR-ABL1* fusion transcript with no insertion between *BCR* exon 8 and *ABL* exon 2, to elucidate clinical characteristics of the rare e8a2 transcript CML.

The study was approved by the Human Research Ethics Committee of Wuhan Tongji hospital, Wuhan, China. Written informed consent was obtained from the patient. A 47-year-old woman was referred to Tongji Hospital in December 2013. Peripheral blood analysis revealed a white blood cell count of $124 \times 10^9/L$ (82.7% neutrophils, 7.2% lymphocytes, 5.9% monocytes, 3.0% eosinophils, and 1.2% basophils); platelet count of $613 \times 10^9/L$;

and hemoglobin level of 10.8 g/dL. The patient showed typical characteristics indicative of splenomegaly. The bone marrow smear indicated an abnormal cell composition including 4% myeloblasts. G-banding karyotype analysis showed 46, XX, t(9;22)(q34;q11) in all 10 analyzed metaphase cells (Fig. 1A). FISH analysis revealed Ph-positive cells with one green *BCR* signal, one red *ABL* signal, and two yellow *BCR-ABL* fusion signals, which were present in 95% of 200 interphase cells counted (Fig. 1B). Nevertheless, we failed to detect typical *BCR-ABL1* transcripts such as e13a2 or e14a2 by qualitative RT-PCR. Subsequently, Sanger sequencing of complementary DNA revealed the e8a2-type fusion transcript (Fig. 1C). Accordingly, the patient was diagnosed as having CML in the chronic phase. Therefore, imported imatinib (400 mg, po, qd) was administered. After two weeks, the imported imatinib was replaced by domestic imatinib (400 mg, po, qd) because of the high cost of the former. At the request of the patient, the cytogenetic response was measured irregularly using FISH rather than by performing molecular monitoring using real-time quantitative PCR (RTqPCR). Positive cells were undetectable after 17 months of treatment (Fig. 1D).

It is widely acknowledged that the direct junction between *BCR* exon 8 and *ABL* exon 2 fails to encode the chimeric oncogenic

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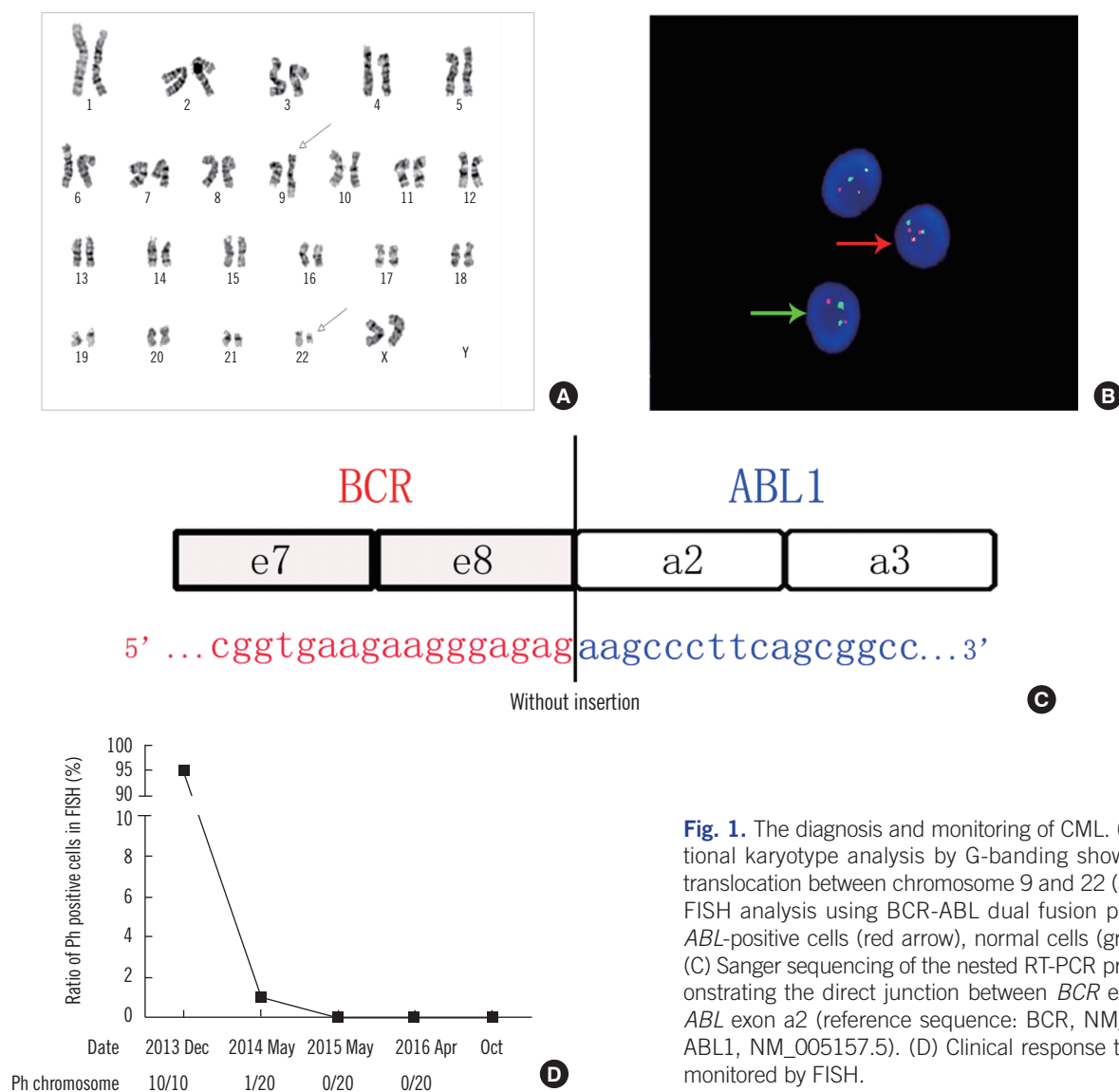


Fig. 1. The diagnosis and monitoring of CML. (A) Conventional karyotype analysis by G-banding showing typical translocation between chromosome 9 and 22 (arrows). (B) FISH analysis using BCR-ABL dual fusion probe. *BCR-ABL*-positive cells (red arrow), normal cells (green arrow). (C) Sanger sequencing of the nested RT-PCR product demonstrating the direct junction between *BCR* exon e8 and *ABL* exon a2 (reference sequence: *BCR*, NM_004327.3; *ABL1*, NM_005157.5). (D) Clinical response to treatment monitored by FISH.

protein owing to the presence of a premature stop codon (UAG) at position 7 after the fusion locus [2, 3]. Nevertheless, insertion of certain sequences could lead to the generation of an in-frame e8a2*BCR-ABL1* transcript. In the 14 cases reported previously, insertion sequences from *ABL* intron 1b were identified in five cases; those from *ABL* intron 1a in three cases; those from *BCR* intron 8 in two cases; and those from another definite gene such as *PRDM12*, *SPECC1L*, or *MAST2*, in four cases [4-6]. Interestingly, in our case, sequencing analysis revealed that the *BCR-ABL1* transcript was derived from a direct combination of *BCR* exon e8 to *ABL* exon e2, without any insertion between them. Moreover, this junction subtype could indeed encode the oncogenic protein resulting in occurrence of CML. Furthermore, the number of bases in this insertion sequence contained three

multiples of triplet bases [7]. Perhaps in simply considering the advent of the stop codon after the fusion contributing to the halt of normal translation of the *BCR-ABL1* transcript, the previous reports misinterpreted the accurate coding sequence in the open reading frame of the fusion transcript.

According to the analysis of all cases, thrombocytosis could not predict the prognosis of patients. The insertion segment derived from particular functional genes including *PRDM12*, *SPECC1L*, or *MAST2* demonstrated no association with poor molecular response. Additionally, tyrosine kinase inhibitors (TKIs) could effectively treat CML patients with the e8a2 transcript (Table 1). Just as in our case, the patient achieved complete cytogenetic response. Moreover, in an unique reported case where imatinib treatment was withdrawn after four years of constant

Table 1. Summary of CML cases with e8a2 *BCR/ABL1* fusion transcript

Reference	Sex	Age (yr)	Disease status	Thrombocytosis	Insertion fragment between BCR exon e8 and ABL exon a2	Therapeutic strategy	Best response during treatment	Clinical outcome in the last follow-up (month)
2	M	55	CP	NA	55 bp from an inverted ABL intron 1b	IFN	NA	Death (29)
8	M	40	CP	NO (normal)	55 bp from an inverted ABL intron 1b	IM	CCyR and MMR	MMR (33)
8	M	35	CP	NO (normal)	55 bp from an inverted ABL intron 1b	IM	CCyR and MMR	MMR (30)
8	M	47	CP	NO (normal)	55 bp from an inverted ABL intron 1b	IM	CCyR and MMR	MMR (39)
2	M	56	CP	NA	55 bp from an inverted ABL intron 1b	busulfan/Hu	CHR	Death (> 89)
2	F	75	CP	Yes	151 bp from ABL intron 1a	IM/cytarabine	CHR	Alive (> 4)
2	F	46	CP	Yes	91 bp from KIAA0376 gene	Hu/IFN	PHR	Alive (> 5)
3	M	43	CP	NO (thrombocytopenia)	46 bp from BCR intron 8	IM	CCyR and MMR	MMR (12)
10	M	46	CP	Yes	14 bp from ABL intron 1a	IM	CHR	CHR (3)
9	F	40	CP	Yes	16 bp from ABL intron 1a	IFN/Hu/IM	CHR and PMR	PMR (65)
6	M	67	CP	Yes	112 bp from PRDM12 gene	NI/IM	CMR	CMR (82)
6	F	58	CP	NO (normal)	154 bp from SPECC1L gene	IM	CCyR and MMR	MMR (66)
4	F	51	CP	Yes	148 bp from MAST2 gene	IM	CCyR and MMR	MMR (24)
7	M	25	CP	NO (thrombocytopenia)	27 bp from BCR intron 8	DA	CCyR AND EMR	CCyR (NA)

Abbreviations: M, male; F, female; CP, chronic phase; IFN, interferon-alpha; Hu, hydroxyurea; IM, imatinib; DA, dasatinib; CHR, complete hematological response; CCyR, complete cytogenetic response; PMR, partial molecular response; MMR, major molecular response; CMR, complete molecular response; NA, not available.

complete molecular remission, the e8a2 transcript became detectable after three months and reverted to an undetectable status again four months after resumption of imatinib treatment.

It is recommended that RT-qPCR be utilized to monitor the molecular level precisely. TKIs have been demonstrated to be effective in the treatment of e8a2-positive patients. However, a larger number of CML patients with the e8a2 transcript are needed to study the clinical features and prognosis.

Authors' Disclosures of Potential Conflicts of Interest

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

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