

## Major molecular response to imatinib in a patient with chronic myeloid leukemia expressing a novel form of e8a2 *BCR-ABL* transcript

A small proportion of patients with chronic myeloid leukemia (CML) express unusual types of *BCR-ABL* transcript. In a recent paper, Demehri et al<sup>1</sup> pointed out that a *BCR-ABL* transcript with an e8a2 junction may be more frequent than other *BCR-ABL* mRNA variants. Moreover, a review of eight so far described cases suggested that e8a2-positive CML might be more aggressive than CML with typical e13a2 and e14a2 *BCR-ABL* mRNAs. We report here a novel form of e8a2 *BCR-ABL* transcript in a CML patient who was treated with imatinib and monitored using real-time RT-PCR.

A 43-year-old man was diagnosed with CML in August 2004. At presentation, the spleen was palpable (3 cm below costal margin). The hematological parameters were the following: white blood cells,  $197 \times 10^9/l$  with a differential count of 7% blasts, 7% promyelocytes, 15% metamyelocytes, 23% myelocytes, 38% neutrophils, 3% eosinophils, 3% basophils, 1% monocytes, 3% lymphocytes; hemoglobin, 10.2 g/dl; platelet count,  $91 \times 10^9/l$ . The prognostic Sokal (1.09) and Hasford (862.6) scores were of intermediate risk. Cytogenetic and fluorescence *in situ* hybridization analyses showed the presence of t(9;22)(q34;q11) and *BCR-ABL* fusion gene in 100% of bone marrow metaphases.

RT-PCR analysis for *BCR-ABL* mRNA was performed using primers enabling the detection of transcripts with e13a2, e14a2 and e1a2 *BCR-ABL* junctions. RT-PCR with e13a2/e14a2 primers was negative, whereas with e1a2 primers, an aberrant band of high molecular weight (>1 kb) was observed (Figure 1a). Sequencing revealed that this band corresponded to a junction between *BCR* exon e8 and *ABL* exon a2 including a 46-nucleotide stretch from *BCR* intron 8 at the fusion point

(U07000 in GenBank, bases 108153 - 108198). This neo-exon in the *BCR* intron 8 is defined by cryptic donor and acceptor splice sites (Figure 1b). Using a new forward *BCR* e8 primer (5'-TCAATGAGGAGATCACACCCC-3') and the *ABL* a2 reverse primer,<sup>2</sup> we amplified a single fragment of expected size (173 bp) corresponding to this e8a2 transcript (Figure 1a).

The splicing of *BCR* exon 8 to *ABL* exon 2 would not produce an oncogenic BCR-ABL protein due to a generation of a premature stop codon.<sup>1</sup> In previously reported cases, the reading frame of e8a2 mRNA was correctly maintained by an insertion of intronic sequences generally derived from the *ABL* intron 1b or by breakpoints within the *BCR* exon 8. In the present case, a neo-exon from *BCR* intron 8 was spliced between *BCR* exon e8 and *ABL* exon 2, giving rise to an in-frame e8a2 *BCR-ABL* transcript. The recognition of these cryptic splicing sites, which are never used in the wild-type *BCR* gene, might simply result from new properties of the chimeric DNA fragment acquired during the re-organization of a neo-intron formed by *BCR* intron 8 and *ABL* intron 1. It is also possible that a second event such as a particular polymorphism or mutation activated one splicing enhancer in the vicinity of this neo-exon or altered the properties of a splicing factor that would be able to recognize these particular splice sites with good efficiency. Overall, translocation breakpoints generating e8a2 fusions may be very infrequent or, in contrast, may occur relatively frequently, but as they lead to a truncated BCR-ABL protein, only rare CML cases that express in-frame e8a2 transcripts are detected.

Clinically, e8a2-positive CML patients has no distinctive features at diagnosis.<sup>1</sup> In contrast to other reported patients with e8a2 *BCR-ABL*, who had a trend toward thrombocytosis, our patient presented with thrombocytopenia. Thrombocytopenia is occasionally found in untreated chronic phase CML. The mechanism is not clear, but

it might be presumed that leukemia cells do not differentiate into the megakaryocytic lineage while normal progenitors are inhibited.<sup>3</sup> Imatinib at standard dosage induced in the patient complete hematological and cytogenetical responses after 1 and 9 months of treatment, respectively. Molecular monitoring was performed using real-time RT-PCR with the *BCR* e8 primer, *ABL* primers and probe.<sup>4</sup> The *BCR-ABL/ABL* ratio in blood cells was 93.1% at diagnosis, 63.4% after 3 months, 0.037% after 9 months and 0.022% after 12 months of therapy. Thus, the patient has achieved a major molecular response (*BCR-ABL/ABL* ratio < 0.05%) within the first year of treatment, which is strongly predictive of a durable cytogenetic remission.<sup>5</sup> Previously reported patients with e8a2 transcripts were resistant to interferon-alpha treatment, suggesting a worse prognosis of CML with e8a2 *BCR-ABL*.<sup>1</sup> Our case provides evidence that e8a2 CML may be sensitive to the ABL tyrosine kinase inhibitor imatinib. More clinical observations and, perhaps, experimental studies of the protein translated from e8a2 *BCR-ABL* mRNA in a murine model may help to assess its leukemogenic activity and a possible influence on clinical course and outcome.

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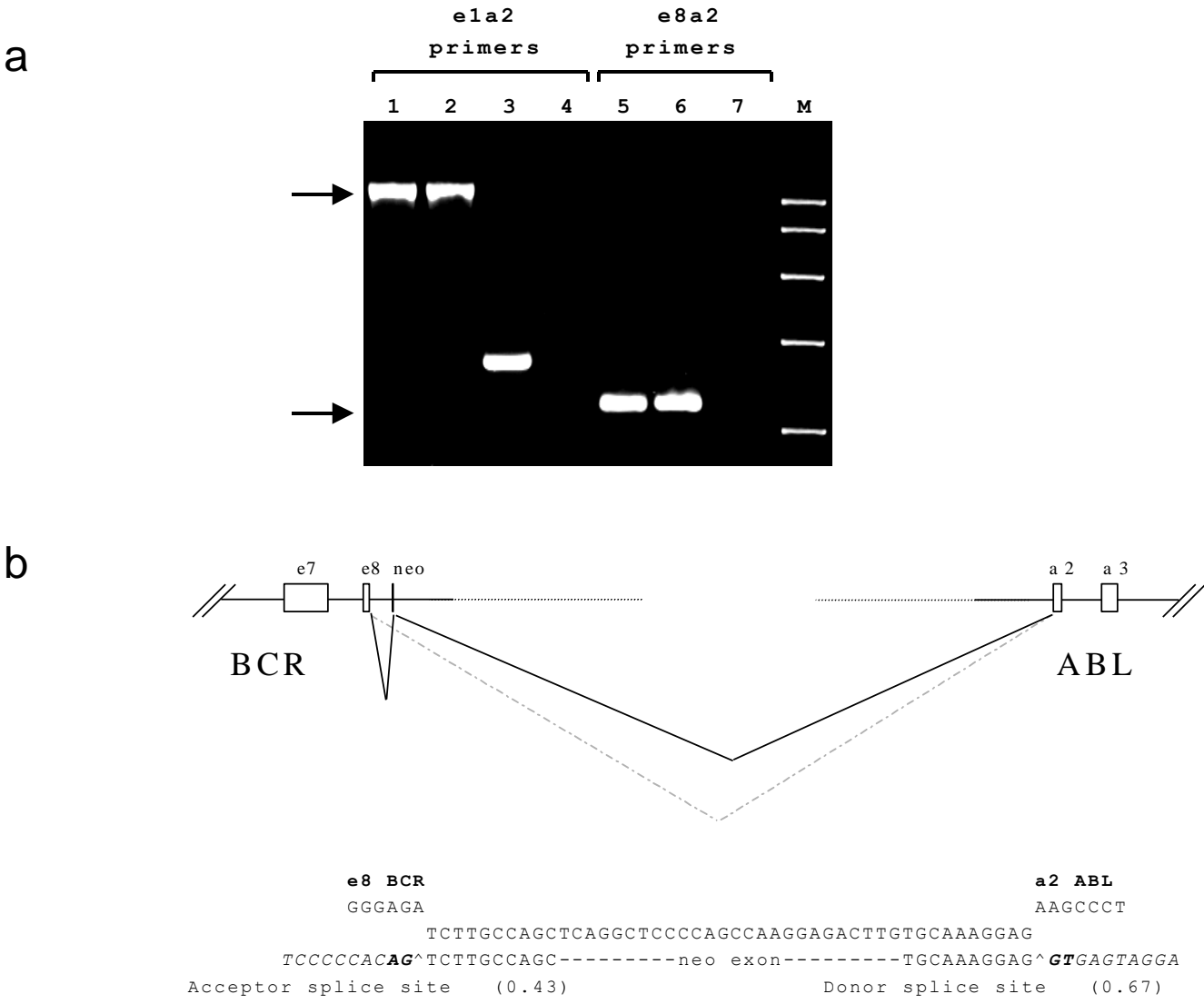
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## Figure legends

### Figure 1.

- (a) RT-PCR for *BCR-ABL* mRNA with e1a2 primers (lanes 1-4) and e8a2 primers (lanes 5-7). Patient's bone marrow (lanes 1 and 5) and peripheral blood samples (lanes 2 and 6). The arrow indicates large amplification products (> 1 kb) detected with e1a2 primers. Lane 3, positive control for e1a2 transcript (244 bp); lanes 4 and 7, blank controls; lane M, PCR markers (Promega).
- (b) Schematic representation and sequence of *BCR-ABL* junction in the mRNA of the patient. Solid lines indicate the splicing between BCR exon 8, neo-exon from *BCR* intron 8 and *ABL* exon 2, which occurred in the patient's mRNA. Dotted lines indicate an expected splicing between *BCR* exon 8 and *ABL* exon 2. Below, the first line is the sequence of the 3' end of BCR exon e8 and of the 5' end of *ABL* exon 2, the second line is the sequence of the neo-exon from BCR intron 8 and the third line is the sequence of acceptor and donor splice sites surrounding this new exon. Canonical dinucleotides GT and AG at the 5' and 3' intron boundaries are given in bold. The scores for the splice sites calculated using the splice site detector program NetGene2 are indicated.



Tchirkov et al, Figure 1