A new aberrantly spliced BCR-ABL1 transcript variant (e13a1) identified in routine monitoring using different quantitative reverse transcription polymerase chain reaction techniques in a patient with chronic myeloid leukemia

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Abstract
Quantitative reverse transcription polymerase chain reaction (qRT-PCR) of BCR-ABL1 transcript level is an essential part of routine disease monitoring in patients with chronic myeloid leukemia. One patient sample (e13a2 transcript detected by nested PCR) attracted attention by revealing an aberrantly spliced BCR-ABL1 transcript variant e13a1. The last 38 base pairs (bp) of BCR exon 13 were replaced by a 37 bp insertion of the ABL1 intron 1–2/exon 1 sequence. The rare aberrant BCR-ABL1 fusion transcript can cause discrepancies in molecular diagnostics. This scenario highlights the importance of an individual characterization of the BCR-ABL1 fusion sequence in case of unclear qRT-PCR results.

KEYWORDS
BCR-ABL1, CML, qRT-PCR, rare transcript, splice variant

1 | INTRODUCTION

The reciprocal translocation between chromosomes 9 and 22 (t(9;22)(q34;q11)) resulting in the Philadelphia chromosome (Ph) and the BCR-ABL1 fusion gene is causal to the development of chronic myeloid leukemia (CML). The occurrence of different BCR-ABL1 mRNA fusion variants (most commonly e13-a2, e14-a2, and e1-a2) results in the expression of an abnormal BCR-ABL1 fusion tyrosine kinase in the majority of the patients. In most cases, the breakpoints occur within the major breakpoint cluster region (M-bcr) within the BCR gene. The breakpoints are less often located in two other breakpoint cluster regions, termed minor (m-bcr) and micro (µ-bcr). Rare atypical BCR breakpoints outside these cluster regions, novel BCR-ABL1 transcripts with insertion and/or deletion of different BCR and ABL sequence sections, and atypical splicing events were detected [1–12]. Here, we report on the occurrence of a novel BCR-ABL1 transcript generating most likely a functional BCR-ABL1 tyrosine kinase in a Ph-positive CML patient where standard diagnostic quantitative reverse transcription polymerase chain reaction (qRT-PCR) procedure showed no amplification of the typical BCR-ABL1 transcripts.

2 | MATERIALS AND METHODS

A 69-year-old female patient was diagnosed with CML in 2005. At diagnosis, the translocation t(9;22)(q34;q11) and the transcript type e13a2 were determined extramurally. The patient samples were obtained with written informed consent in accordance with the declaration of
for LightCycler PCR systems is shown in red bold letters. The sense primer binding site for multiplex PCR is shown in blue bold letters and the sense primer for the TaqMan PCR system is underlined. Since the primer binding sites for multiplex and TaqMan PCRs are deleted, no PCR amplification products could be generated. However, the primer binding sites for nested and LightCycler PCR systems remained unaffected and, therefore, PCR products were amplified.

Helsinki, and the analysis was approved by the institutional review board of the Medical Faculty of Mannheim, Heidelberg University (Heidelberg, Germany). The first monitoring sample was investigated in our laboratory 6 months after the start of imatinib therapy. At this time, a qualitative multiplex PCR assay [13] for detection of the regular \( BCR-ABL1 \) transcript types (e13a2 and e14a2) was also performed in our laboratory in Mannheim. The molecular monitoring of the \( BCR-ABL1 \) fusion transcript was performed using two standard qRT-PCR methods (LightCycler [LC] and TaqMan [TM]) for molecular monitoring of usual fusion transcripts [14, 15]. Both methods differ in PCR amplicon length (LC: 596 bp; TM: 228 bp) and primer/probe combinations. In addition, the samples were retrospectively investigated with our nested PCR assay for regular transcripts and our one-step PCR assay for irregular transcripts. PCR amplicons were characterized by Sanger DNA sequencing.

3 RESULTS AND DISCUSSION

For evaluation of the externally transmitted (e13a2) transcript type, we performed our qualitative in-house multiplex PCR assay as previously described [13].

Since the patient received imatinib therapy over 6 months, the negative multiplex PCR result for any transcript variant could be explainable by a low \( BCR-ABL1 \) quotient or a binding failure of primer(s) or probe. Therefore, we performed a nested-PCR assay [16] and we succeeded in the detection of amplicons with typical e13a2 transcript length. To exclude target limiting effects in the nested PCR, we used a one-step PCR assay for atypical transcripts (in-house unpublished) with different primer combinations compared to multiplex PCR. These experiments confirmed the positivity of e13a2 transcript length.

Sanger DNA sequencing of one-step PCR products revealed an atypical \( BCR-ABL1 \) transcript variant (e13a1) as shown in Figure 1. The primer binding site for the \( BCR \) sense primer of the TM qRT-PCR method [15] and the qualitative multiplex PCR assay [13] was deleted (38 bp deletion) and replaced by a 37 bp insertion of \( ABL1 \) intron 1–2/exon 1 sequence. This sequence exchange resulted in missing PCR amplicons. Using the primer/probe combination of the LC qRT-PCR method [14], PCR amplicons were detectable (Figure 2). The binding site of the \( BCR \) sense primer was located 73 base pairs further upstream and was, therefore, not affected by the deletion/insertion.

In addition, the fusion of the 37 bp intron sequence (instead of the missing 38 bp \( BCR \) exon 13 fragment), resulted in the in-frame fusion \( \text{NM}_004327.4:\text{c.2670}_2707\text{delinsAACAGCTCTGCCTGGGAGCGGAGAGGACTGGGATAGAAA} \) of \( BCR \) and \( ABL1 \) (Figure 3). Because no frameshift occurred, it is assumed that the resulting fusion gene leads to functional \( BCR-ABL1 \) protein synthesis and is capable of propagating CML. To what extent the genetic rearrangement has an influence on the patient’s response to imatinib therapy is unclear since the patient ranged from MR4.5 to MMR during monitoring. Furthermore, a point mutation in the \( ABL1 \) exon 1 sequence leads to the exchange of glutamic acid against lysine (E27K). The potential impact of this amino acid exchange on protein folding and activity requires further investigations. For future monitoring, the qRT-PCR method for this patient has to be performed by LC instead of the TM PCR system.
FIGURE 3  Fusion sequence of e13a1 on cDNA basis with the respective amino acid code. The deletion/insertion event resulted in an in-frame fusion of the BCR and ABL1 genes. In red bold letters, the G>A mutation in the ABL1 exon 1 sequence insertion is shown resulting in an amino acid change from glutamine to lysine (E27K).

4  | CONCLUSION

Our scenario highlights the importance of an individual characterization of the BCR-ABL1 fusion sequence in case of unclear qRT-PCR results. It is of high advantage if various validated detection methods are available in parallel in diagnostic laboratories so that the occurrence of BCR-ABL1 transcript variants and changed primer binding sites do not have a negative influence on therapy decisions.

AUTHOR CONTRIBUTIONS
Naumann N contributed to manuscript drafting, data curation, and evaluation and reviewed the literature. Spiess B contributed to manuscript drafting, reviewed the literature, and was involved in supervision. Bross-Bach U provided patient material and reviewed the manuscript. Seifarth W, Fabarius A, Hofmann W-K, and Saußele S contributed to manuscript drafting, reviewing, and supervision. All authors issued final approval for the version to be submitted.

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CONFLICT OF INTEREST
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DATA AVAILABILITY STATEMENT
All data that support the findings of this study are included in the manuscript.

ETHICS STATEMENT
The study design adhered to the tenets of the Declaration of Helsinki and was approved by the relevant institutional review board (Medical Faculty Mannheim, University of Heidelberg, 2013-509N-MA and 2020-593N). The patient gave written informed consent.

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