**Supplementary data to:**

***The recurrent atypical e8a2 BCR::ABL1 transcript with insertion of an inverted 55 base pair ABL1 intron 1b sequence: a detailed molecular analysis***

Thomas Burmeister, Lars Bullinger, Philipp le Coutre

(References cited in the text refer to the main manuscript.)

**Materials and Methods**

**Nucleic acid isolation**

Total RNA was isolated using *TRIzol* (Thermo Fisher Scientific, Darmstadt, Germany) and DNA was isolated using the *QIAamp DNA Mini Kit* (QIAGEN, Hilden, Germany).

**Oligonucleotides**

Oligonucleotides were obtained from *TIB Molbiol* (Berlin, Germany).

**Long-range PCR for the characterization of the *BCR::ABL1* break on der(9)**

Five microliters genomic DNA was used in the long-range PCR with the *Expand Long Template PCR System* kit (Roche, Mannheim, Germany) with buffer 2 and the following cycler program: 95 °C 2 min, 15 cycles (94 °C 30 s, 65 °C 30 s, 68°C 6 min), 20 cycles (94 °C 30 s, 65 °C 30 s, 68 °C 8 min with 20 s increment/cycle), and 68 °C 10 min, 4 °C hold. The following PCR oligonucleotides were used (5'-3'): BCR-8-F1 GAGGGAAGGGCGAGCAGGACCCTTGA, iABL1b-R AGGAGTAATCTCTTCCTCGTTGATGAAGCT.

**Long-range inverse PCR**

Three restriction enzymes were tested: *Hind*III, *Kpn*I and *Taq*I. *FastDigest* enzymes were used according to the manufacturer’s recommendations (Thermo Fisher Scientific, Darmstadt, Germany). The conditions for the inverse long-range PCR were partially adopted from previous work.[19] Five hundred nanograms of genomic DNA were digested in a 50 µl volume, the reaction mix was inactivated, purified using the *MaXtract High Density* kit (QIAGEN, Hilden, Germany), ethanol-precipitated and dissolved in a final volume of 30 µl. The entire volume was used in the ligation procedure (50 µl final volume, 5 U T4 ligase, 16 °C overnight). After purification and ethanol precipitation as described above, the ligation mix was dissolved in 30 µl H2O. Five microliters was used in the long-range PCR with the *Expand Long Template PCR System* kit (Roche, Mannheim, Germany) as described above but with an annealing temperature of 63 °C. One enzyme-specific reverse (R) PCR primer was combined with a forward (F) primer. For the enzyme *Taq*I: TaqI-iR 5'-AGCAACGTGCCCACGCTCCTGGA-3' in combination with ABL1-iF 5'-AGCTTCATCAACGAGGAAGAGATTACTCCT-3'.

**RT–PCR for *BCR::ABL1* and *ABL1::BCR* transcripts**

The *BCR::ABL1* mRNA transcript was detected by multiplex RT–PCR, as previously described.[8] The following oligonucleotides were used to investigate the expression of an *ABL1::BCR* mRNA transcript: 5'-TGCTGACTTGTGGAGATGCAGCGAAT-3' (in *ABL1* exon 1b) and 5'-TCTCTCTCTGGATGTCATTCTTGATCTGG-3' (in *BCR* exon 10). PCR conditions were otherwise the same as for the *BCR::ABL1* multiplex RT–PCR.

**Real-time quantitative RT–PCR for the e8a2 *BCR::ABL1* transcript.**

Real-time quantitative RT–PCR was performed using a forward primer in *BCR* exon 8 (5'-TCCATGACGGTGAAGAAGGGAG-3'), the EAC primer ENR561 and probe ENP541 (both located in *ABL1* exon 2), and *ABL1* as a reference housekeeping gene as previously described.[9, 10]

**Sanger sequencing**

Apart from the specified oligonucleotides, several ad hoc designed oligonucleotides were used for Sanger sequencing of PCR products. Technical Sanger sequencing was performed by *Microsync SeqLab* (Göttingen, Germany). Analysis of chromatograms and sequence data assembly was performed at the author's laboratory in Berlin.