INTRODUCTION
Several studies have shown that CML patients with BCR-ABL1 transcript type e14a2 achieved a major molecular response (MMR) on tyrosine kinase inhibitor (TKI) therapy earlier than patients with e13a2. In contrast, transcript type had no effect on long-term survival. This raises the question whether the observed disparity in MMR achievement is driven by biological differences or technical aspects of BCR-ABL1 qPCR. The same primers and probe are currently used to quantify e13a2 and e14a2, however, the different length of amplicons may impact the PCR efficiency.

METHODS
Characterization of patient cohort (Table 1)
- 27 patients had e13a2 and 40 patients had e14a2.
- All patients were evaluated. Of these, 27 patients had e13a2 and 40 patients had e14a2.
- Quantification of gBCR-ABL1 (g-genomic)
  - Patient-specific genomic fusion was characterized by NGS.
  - gBCR-ABL1 was performed by patient-specific qPCR.
  - Albumin was used as the control gene to normalize results.
- Quantification of mRNA BCR-ABL1
  - Standardized real-time qPCR for BCR-ABL1 transcript quantification was performed using GUSB as control gene.
- BCR-ABL1 data evaluation
  - gBCR-ABL1 levels in follow-up samples were calculated relative to the diagnostic sample (gBCR-ABL1max) or sample at TKI start (gBCR-ABL1min).
  - Individual molecular responses at the mRNA level were calculated relative to the diagnostic sample (BCR-ABL1max) or sample at TKI start (BCR-ABL1min).
- Assessment of BCR-ABL1 cDNA amplification efficiency
  - 10-fold dilutions of plasmids containing either the e13a2 or e14a2 BCR-ABL1 transcript variants and an ABL1 reference sequence were distributed to laboratories across Europe (n = 19).
  - Data from 4 labs were excluded due to deviation in protocol or results outside 1 ± 2 SD of the data set. Results from 10 laboratories were analyzed in total.
  - The amplification efficiency of each transcript was determined for each laboratory by calculating standard curves from the plotted dilutions using the slope of the standard curve to determine efficiency, based on the EAC qPCR 2006 standard protocol.
  - The mean relative amplification ratios of BCR-ABL1 for both transcripts was calculated from plasmid Cq values, either with or without correction for BCR-ABL1 amplification efficiency.
- Statistical analysis
  - A bi-exponential mixed effect model was used to analyze differences in the leukemic decline in BCR-ABL1 levels, which is characterized by an initial steep decline (α slope) followed by a second moderate decline (β slope). The transcript type (e13a2 vs e14a2) was included as covariates.
  - Wald tests were applied to assess the statistical significance of the fixed-effect parameters.
  - Relative amplification efficiencies were compared by pairwise t-test, with Bonferroni correction for multiple comparisons.

RESULTS
- BCR-ABL1i5 vs individual molecular response (BCR-ABL1relDg; BCR-ABL1relIS)
  - 1 Differences between transcript type e13a2 vs e14a2 expressed on BCR-ABL1
    - Lower BCR-ABL1i5 levels were observed at the time of diagnosis (Figure 1A) in patients with e14a2 (median 36.6%, range 2.9-58.3) compared to e13a2 (median 41.6%, range 17.5-101.5). Similar results were found also at the time of TKI start (Figure 1B).
- Time to MMR according to the type of transcript
  - The time to MMR (BCR-ABL1i5 ≤ 35%) since diagnosis was significantly shorter for patients expressing e14a2 compared to e13a2 (p = 0.05).

CONCLUSION
- The observed differences in time to achieve MMR between e13a2 and e14a2 CML patients may be at least partially explained by differences in efficiency of amplification of the two transcript types by qPCR.
- A multicentre study is underway to assess how widespread this issue is, and how it may be addressed.