Individual molecular response evaluation on both DNA and mRNA BCR-ABL1 level diminished differences in time to molecular response achievement between CML patients with e13a2 vs e14a2 transcript type

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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 standardly used to quantify e13a2 and e14a2, however, the different length of amplicons may impact the PCR efficiency.

OBJECTIVE

This EUTOS study aimed to investigate differences in molecular response between CML patients with e13a2 and e14a2 based on quantification of BCR-ABL1 at both genomic DNA and mRNA levels.

METHODS

Characterization of patient cohort (Table 1)

- DNA patient-specific assays were successfully applied in 71 of 81 newly diagnosed patients.
- Four patients from 71 were excluded due to a quick TKI change after the start of first-line TKI treatment (1 patient), combination therapy with interferon alpha (2 patients) or higher than normal TKI doses (1 patient).
- Altogether, data from 67 patients were evaluated. Of these, 27 patients had e13a2 and 40 patients had e14a2.

Scheme 1 Schedule of sample collection in months (M)

Table 1 Characterization of 81 prospectively analyzed CML patients

Number of patients	n=81*
Median age at the time of diagnosis (range)	55 (19-79)
Sex	Male =43; Female =38
EUTOS score	Low =76; High =4; ND =1
Sokal score	Low =35 ; Intermediate =35; High =10; ND =1
Hasford score	Low =36 ; Intermediate =41 ; High =3 ; ND =1
First line treatment (dose)	imatinib = 65 nilotinib = 14 nilotinib + IFN = 2
Median months of 1st line treatment	25.7 (0.2-45.3) months
Change therapy due to intolerance or therapy failure Median month since 1st line treatment	n=17 Median=6.8 (0.2-38.5)
CML non-related death	4

Quantification of gBCR-ABL1 (g=genomic)

- Patient-specific genomic fusion were characterized by NGS.
- gBCR-ABL1 was performed by patient-specific qPCR.
- Albumin was used as the control gene to normalise 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-ABL1$_{RelDg}$) or sample at TKI start (gBCR-ABL1$_{RelTKI}$)

$$\%\ \text{gBCR-ABL1}_{RelDg} = (\%\ \text{gBCR-ABL1}_{sample})/(\%\ \text{gBCR-ABL1}_{Dg})*100$$

- Individual molecular responses at the mRNA level were calculated relative to the diagnostic sample (BCR-ABL1$_{RelDg}$) or sample at TKI start (BCR-ABL1$_{RelTKI}$)

$$\%\ \text{BCR-ABL1}_{RelDg} = (\%\ \text{BCR-ABL1}_{sample})/(\%\ \text{BCR-ABL1}_{Dg})*100$$

Assessment of BCR-ABL1 cDNA amplification efficiency

- 10-fold dilution series of plasmids containing either the e13a2 or e14a2 BCR-ABL1 transcript variants and an ABL1 reference sequence were distributed to laboratories across Europe (n = 14). Data from 4 labs were excluded due to deviations in protocol or results outside 1.5 x IQR of the data set. Results from 10 laboratories were analysed in total.
- The amplification efficiency of each transcript was determined for each laboratory by constructing standard curves from the plasmid dilutions using local BCR-ABL1 monitoring protocols, based on the EAC BCR-ABL1 RT-qPCR assay.
- The mean relative amplification ratio of BCR-ABL1: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 biphasic 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 types (e13a2 vs e14a2) were included as covariates.
- Wald tests were applied to assess the statistical significance of the fixed-effect group effects.
- Relative amplification efficiencies were compared by pairwise t-test, with Bonferroni correction for multiple comparisons.

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RESULTS

BCR-ABL1^{IS} vs individual molecular response (BCR-ABL1$_{RelDg;}$ gBCR-ABL1$_{RelDg}$)

1) Differences between transcript type e13a2 vs e14a2 expressed on BCR-ABL1^{IS}

Initial level of BCR-ABL^{IS} according to the type of transcript

Figure 1A BCR-ABL1^{IS} level at diagnosis

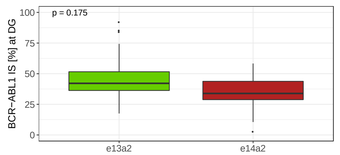
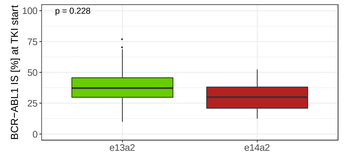


Figure 1B BCR-ABL1^{IS} level at TKI start



- Lower **BCR-ABL1^{IS}** 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

Figure 2A Time to MMR (BCR-ABL1^{IS}) since diagnosis

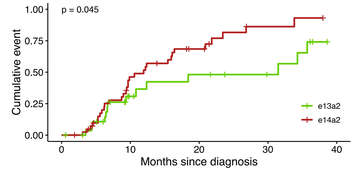
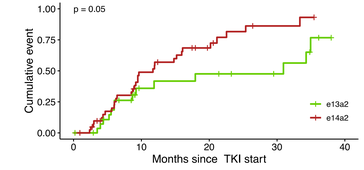


Figure 2B Time to MMR (BCR-ABL1^{IS}) since TKI start



- The time to MMR (**BCR-ABL1^{IS} ≤0.1%**) since diagnosis or TKI start was significantly shorter for patients expressing e14a2 compared to e13a2 (p≤0.05).

2) Individual molecular response (IMR)

Bi-exponential mixed effects model

Figure 4A Differences between transcript types on the α and β slopes of BCR-ABL1$_{RelDg}$

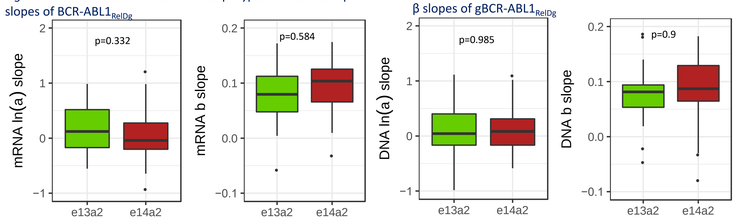


Figure 4B Differences between transcript types on the α and β slopes of gBCR-ABL1$_{RelDg}$

- No differences were found between e14a2 vs e13a2 **BCR-ABL1$_{RelDg}$ and gBCR-ABL1$_{RelDg}$** on the α and β slopes during TKI therapy (Figure 4A and 4B).

Relative amplification of transcript types using plasmid dilutions in 10 EUTOS labs

Figure 6A Difference in relative amplification between transcript types before correction for efficiency. Amplicon lengths indicated.

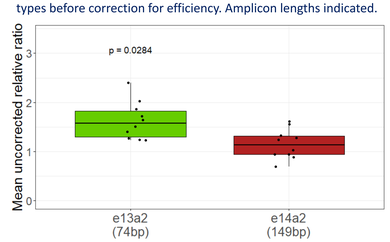
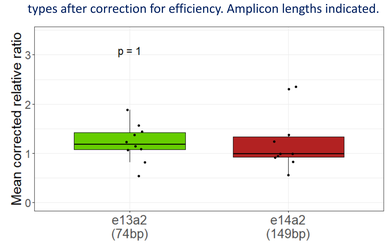


Figure 6B Difference in relative amplification between transcript types after correction for efficiency. Amplicon lengths indicated.



- The relative amplification of e13a2 (1.63) is significantly higher than e14a2 (1.15, Figure 6A). After correction for BCR-ABL1 amplification efficiency, there was no difference in relative amplification (e13a2 = 1.22, e14a2 = 1.25, Figure 6B).

Time to 3 log reduction of IMR according to the type of transcript

Time to 3 log reduction of *BCR-ABL1$_{RelDg}$* since diagnosis (e13a2=27; e14a2= 39)

*Figure 5A Time to 3 log reduction of **mRNA BCR-ABL1$_{RelDg}$** since diagnosis*

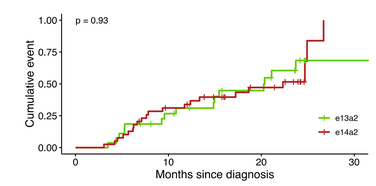
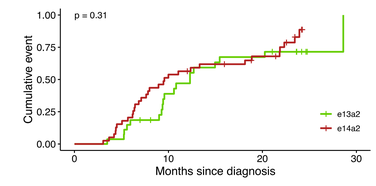


*Figure 5B Time to 3 log reduction of **gBCR-ABL1$_{RelDg}$** since diagnosis*



Time to 3 log reduction of *BCR-ABL1$_{RelTKI}$* since TKI start (e13a2=29; e14a2= 40)

*Figure 5C Time to 3 log reduction of **mRNA BCR-ABL1$_{RelTKI}$** since TKI start*

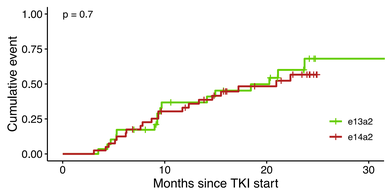
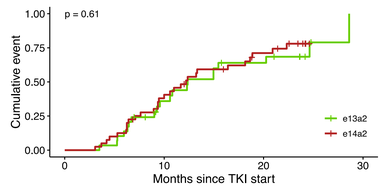


*Figure 5D Time to 3 log reduction of **gBCR-ABL1$_{RelTKI}$** since TKI start*



- No differences were found between e14a2 vs e13a2 in time to 3 log reduction since diagnosis (Figure 5A and 5B) or start of TKI therapy (Figure 5C and 5D), respectively, based on IMR at both mRNA and DNA level.

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.