Dear Editor,

The development of the first target-specific tyrosine kinase inhibitor (TKI) and its introduction in the clinical practice radically changed chronic myeloid leukemia (CML) treatment. Monitoring therapeutic response to TKIs is a critical step in the management of CML.\(^1\)

Recently, several follow-up studies upon which the European Leukemia Net 2013 (ELN) recommendations were based, pointed to the importance of early clearance of leukemic cells as demonstrated by molecular methods. Attaining a BCR-ABL\(^1\) transcript level ≤10% three months after initial imatinib mesylate (IM) treatment was found to be associated with a favorable outcome, including longer progression-free (PFS) and overall survival (OS), and higher probability of achieving complete cytogenetic response (CCyR) and major molecular response (MMR).\(^1\)

Quantification of the BCR-ABL transcript level reflects leukemic burden, and is carried out by quantitative real-time polymerase chain reaction (RQ-PCR). Molecular response is based on the ratio of BCR-ABL transcript levels and a control gene. Results are expressed according to an international scale (IS) assigned to every patient at diagnosis, which is equal to 100% of BCR-ABL/control gene transcripts, regardless of the absolute amount of BCR-ABL transcripts. Thus, the actual leukemic burden of patients at diagnosis is not taken into account.\(^2\)

An ideal control gene would be expected to be uniformly expressed in different cell types regardless of its proliferative status as well as be unaffected by therapeutic regimens, constant between individuals and expressed at a level similar to BCR-ABL. In fact, this control gene does not exist, and BCR and ABL are the most widely used control genes for quantifying BCR-ABL transcripts, mainly due to historical reasons. However, both BCR and ABL control genes do not show linearity with BCR-ABL transcript levels above 10% contrary to the GUS gene that is not affected by high-level distortions which allow for better estimations of the BCR-ABL transcript level at diagnosis.\(^3\)

In this study, the BCR-ABL transcript levels of 31 CML patients under IM treatment were analyzed by RQ-PCR in respect to the ABL and GUS control genes at diagnosis and after three months of therapy. These patients were followed up for at least 24 months. The median BCR-ABL1/ABL and BCR-ABL1/GUS transcript levels at diagnosis were 87.84% (range: 16.24–184.4) and 29.8% (range: 5.76–216.9), respectively. At three months, the median BCR-ABL1/ABL transcript level was 7.14 (range: 0.053–307) whereas the median BCR-ABL/GUS transcript level was 21.94 (range: 0.19–85.16). Patients were classified as optimal responders or non-responders (failure of response) according to a BCR-ABL\(^1\) transcript level ≤0.1% and >0.1% at 12 months. In responders, the median BCR-ABL/ABL and BCR-ABL/GUS transcript levels at diagnosis were 68.13% (range: 26.8–99.29) and 23.77 (range: 8.2–62.97), respectively while in non-responders these levels were 86.10 (range: 30.87–96.11) and 40.92 (range: 17.21–96.85).

The median BCR-ABL/ABL of responders and non-responders at diagnosis was not significantly different (p-value = 0.89) while the median BCR-ABL/GUS between responders and non-responders at diagnosis was significantly different (p-value <0.001) indicating that, unlike ABL, GUS levels are capable of discriminating responders from non-responders (Figure 1). The median transcript level of BCR-ABL/GUS of responders at diagnosis was 28.38% which might be considered a threshold for early discrimination as patients with levels under 28.38% were less likely to achieve MMR at 12 months (p-value <0.05).

Comparisons of transcript levels three months after initiating IM treatment were also carried out considering a BCR-ABL/control gene\(^1\) threshold of ≤10% as discriminative of responders vs. non-responders. In patients considered to be optimal responders, the median of the transcripts estimated with any control gene was below 10%, as expected according to previous reports. We did not observe a statistically significant difference between ABL and GUS control genes (p-value = 0.19) in either responders or non-responders (p = 0.41), indicating that at the three month time point, both genes can be equally used as predictive biomarkers, showing significant differences between responders and non-responders (p-value = 0.003 for GUS and p-value = 0.01 for ABL; Figure 1).

The current availability of several therapeutic modalities for CML treatment requires early predictive parameters for
Figure 1 – The median levels of the transcripts considering both control genes (GUS and ABL) at diagnosis and after three months on imatinib (IM) as first line therapy. Patients are discriminated as responders or non-responders according to their response at 12 months according to the ELN guidelines. The medians of the transcripts were compared by Mann–Whitney unpaired two-tailed test. At diagnosis: ‘p-value < 0.001. At 3 months: ‘p-value = 0.01; “p-value = 0.003. Resp = responders; No Resp = non-responders; ns = non-significant.

future response. In this study, we used parameters recommended for identifying optimal response (with BCR-ABL ≤10 at three months and ≤0.1 at 12 months after initiating treatment). However, BCR-ABL/ABL transcript levels cannot be used as predictive estimates due to the lack of linearity of the ABL gene in assessing leukemic burden at levels above 10% and actual levels of BCR-ABL transcripts at diagnosis can be accurately estimated with GUS as the control gene. We found that high levels of BCR-ABL/GUS at diagnosis were associated with a lower probability of achieving optimal response (p-value <0.001) and low rates of CyCR after 12 months of IM therapy (p-value <0.001). These findings coincided with Vigneri et al. who showed that high rates of BCR-ABL/GUS were associated with a low probability of event-free survival (p-value <0.001) and PFS (p-value =0.01). As expected, the loss of ABL linearity resulting in transcript quantification with high levels of leukemic burden indicated that ABL, if used as the control gene at diagnosis, would not provide predictive estimates. Conversely, the use of GUS as the control gene allows for a reliable prediction of therapeutic response based on BCR-ABL transcript levels at diagnosis.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES


Simone Bonecker a, Marina Magnago b, Jaspal Kaeda c, Cristiana Solza b, Ilana Zalcberg Renault d,e

a Instituto Nacional do Câncer (INCA), Rio de Janeiro, RJ, Brazil
b Universidade do Estado do Rio de Janeiro (UERJ), Rio de Janeiro, RJ, Brazil
c Universitatsmedizin Berlin, Berlin, Germany
d Corresponding author at: Praça Cruz Vermelha, 23, 6° andar, Laboratórios CEMO, Centro, 20230-130 Rio de Janeiro, RJ, Brazil.
E-mail address: zalcberg@inca.gov.br (I. Zalcberg Renault).

Received 14 July 2014
Accepted 27 August 2014
Available online 31 January 2015

http://dx.doi.org/10.1016/j.bjhh.2014.08.003
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