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COMMENTARY

BCR::ABL1 transcripts and clinical outcome - interrogating the technique

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Monitoring treatment response for patients with chronic myeloid leukaemia (CML) is largely reliant on standardised molecular monitoring that measures peripheral blood *BCR*::*ABL1* transcript levels. An international reporting scale (IS) is applied globally to align data derived from diverse methods to a common scale and its use is mandated by international guidelines and recommendations.[1,2] Standardisation of molecular methods that measure transcript values over 4 to 5 orders of magnitude was a major achievement. Importantly, it has allowed treatment intervention decisions based on milestone molecular response values achieved over the first year of tyrosine kinase inhibitor therapy. Global adoption of standardised molecular monitoring was facilitated by the development of a World Health Organisation certified reference panel for quantification of *BCR*::*ABL1* transcripts,[3] and the availability of commercial products for *BCR*::*ABL1* transcript quantification.

Most of the real-time quantitative PCR methods rely on amplification of the two common *BCR*::*ABL1* transcript types (termed e13a2 and e14a2) in a single assay and using a common set of primers. These transcripts differ by a single exon of 75 base pairs. Moreover, methods that use a calibration standard to generate a standard curve to measure the quantitative values rely on a single calibrator that invariably contains the e14a2 transcript. The standard curve is used to determine the e14a2 transcript level as well as the shorter e13a2 transcript, when present. PCR has a tendency to amplify shorter fragments more efficiently than longer fragments, which theoretically means the quantitative signal for the shorter e13a2 transcript could be detected earlier than the longer e14a2 transcript. This would generate higher transcript values for e13a2 containing products.

A study by Dominy et al.[4] published in this issue, demonstrates that e13a2 transcript levels were indeed overestimated using their molecular assay. *BCR*::*ABL1* transcript values for e13a2 containing samples were on average 1.38 to 1.95-fold overestimated. The systematic bias of e13a2 transcripts was demonstrated by the generation of an e13a2 specific standard curve. Transcript values using the specific standard curve were lower than those calculated using the e14a2 standard curve. The data suggest there may be a benefit for measuring e13a2 transcripts with a specific standard curve. However, the most relevant evidence for whether separate assays for measuring the different transcripts is necessary relies on the demonstration of a clinical impact with over-estimation of *BCR*::*ABL1* for patients bearing the e13a2 transcript. There is currently no consensus for an association between *BCR*::*ABL1* transcript type and outcome, and there are no guidelines or recommendations for a different treatment approach for patients with e13a2.[1,2] Nevertheless, overestimation of e13a2 transcript levels could negatively impact the interpretation of the milestone molecular response levels upon which treatment decisions are made. Furthermore, overestimation could delay the achievement of a deep molecular response and hence, qualification for a trial of treatment cessation with the aim of achieving treatment-free remission.

Commentary on: Dominy et al. Assessment of quantitative polymerase chain reaction for BCR–ABL1 transcripts in chronic myeloid leukaemia: Are improved outcomes in patients with e14a2 transcripts an artefact of technology? *Br J Haematol*, 2022; 197:52-62

Fusion Gene Nomenclature - the Human Genome Organisation (HUGO) Gene Nomenclature committee has implemented use of the double colon (::) for the description of fusion genes (e.g. BCR::ABL1) to replace the historical nomenclature (e.g. BCR-ABL1).

The conclusion from Dominy et al.[4] was that the systematic overestimation of the e13a2 transcript level using their assay did not sufficiently impact treatment outcome to warrant a redesign of their assay. Guidelines and recommendations rely on a static *BCR*::*ABL1* value at milestone timepoints for treatment decisions: 10% IS; 1% IS; and 0.1% IS. Critical timepoints are 3 and 6 months of therapy where a *BCR*::*ABL1* value of >10% IS can mandate therapeutic intervention. Dominy et al.[4] found that 24% and 25% of patients with the e13a2 transcript and *BCR*::*ABL1* values >10% at 3 and 6 months, respectively, were reclassified as less than 10% after bias correction of the e13a2 transcript value. This occurred for patients with values close to the decision level of 10% IS. However, as has been stressed over a number of years and highlighted in international guidelines and recommendations,[1,2] assessing the trend of response over time is essential for the appropriate interpretation of response.[5,6] Patients with a value close to the cut-off at milestone timepoints may continue on a downward trajectory. In this case repeat molecular analysis is recommended to resolve whether intervention is indeed warranted. The study by Dominy et al.[4] suggests that *BCR*::*ABL1* values above the milestone *BCR*::*ABL1* values for patients with the e13a2 transcript may be overestimated and close scrutiny of subsequent tests is required to ensure the trend in transcript values is declining. This is already the currently recommended monitoring strategy. Constantly declining values is a positive signal and a constant rise indicates the potential for loss of response, drug resistance or drug cessation. These assessments can be made independently of accurate conversion to the international reporting scale.

The method used by Dominy et al.[4] was a laboratory developed method rather than one of the widely used commercially available assays. However, the laboratory method used a common set of PCR primers and probes that were developed for method harmonisation by a Europe Against Cancer (EAC) initiative.[7] These primers and probes are extensively used across Europe and more broadly. Just how frequently do these methods generate e13a2 transcript values that are overestimated? This question was addressed in a study by Kjaer et al.[8] that compared *BCR*::*ABL1* values generated by 3 laboratories that used the EAC protocol. Samples bearing the e13a2 fusion consistently demonstrated enhanced amplification efficiency compared with e14a2. Absolute quantification using digital droplet PCR compared with the EAC real-time PCR protocol demonstrated a statistically significant bias in the e13a2 values compared with e14a2 of 1.8-fold, 4.6-fold and 6.5-fold. The discrepancy was attributed to a technical issue related to the real-time PCR method. Importantly, a discrepancy of 6.5-fold for patients with the e13a2 transcript could alter the interpretation of the molecular response and is therefore potentially clinically relevant.

There are clinical differences observed for patients according to the *BCR*::*ABL1* transcript type. A recent interesting observation is the association between the e14a2 transcript and treatment-free remission.[9,10] Nevertheless, there is growing evidence of overestimation of e13a2 transcripts by some real-time PCR methods. Furthermore, the degree of overestimation may vary between individual laboratories and methods. Therefore, the clinical relevance of this observation will vary. The potential for discrepant results due to technical issues should be appreciated by molecular laboratories and ideally the degree of difference investigated to determine whether interpretation of the molecular response could be impacted. The technical bias, if it exists, will affect a minority of patients since 37.9% of patients express the e13a2 fusion.[11]

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CONFLICT OF INTEREST

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REFERENCES

1. Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;34(4):966–84.
2. NCCN clinical practice guidelines in oncology: Chronic myeloid leukemia. Version 2. 2022.
3. White HE, Matejtschuk P, Rigsby P, Gabert J, Lin F, Lynn Wang Y, et al. Establishment of the first World Health Organization international genetic reference panel for quantitation of BCR-ABL mRNA. Blood. 2010;116(22):e111–7.
4. Dominy KM, Claudiani S, O'Hare M, Szydlo R, Gerrard G, Foskett P, et al. Assessment of quantitative PCR for BCR-ABL1 transcripts in CML: Are improved outcomes in patients with e14a2 transcripts an artefact of technology? Br J Haematol. 2022;197(1):52–62.
5. Hanfstein B, Shlyakhto V, Lauseker M, Hehlmann R, Saussele S, Dietz C, et al. Velocity of early BCR-ABL transcript elimination as an optimized predictor of outcome in chronic myeloid leukemia (CML) patients in chronic phase on treatment with imatinib. Leukemia. 2014;28(10):1988–92.
6. Branford S, Yeung DT, Parker WT, Roberts ND, Purins L, Braley JA, et al. Prognosis for patients with CML and >10% BCR-ABL1 after 3 months of imatinib depends on the rate of BCR-ABL1 decline. Blood. 2014;124(4):511–8.
7. Gabert J, Beillard E, van der Velden VH, Bi W, Grimwade D, Pallisgaard N, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe against cancer program. Leukemia. 2003;17(12):2318–57.
8. Kjaer L, Skov V, Andersen MT, Aggerholm A, Clair P, Gniot M, et al. Variant-specific discrepancy when quantitating BCR-ABL1 e13a2 and e14a2 transcripts using the Europe against cancer qPCR assay. Eur J Haematol. 2019;103(1):26–34.
9. Claudiani S, Apperley JF, Gale RP, Clark R, Szydlo R, Deplano S, et al. e14a2 BCR-ABL1 transcript is associated with a higher rate of treatment-free remission in individuals with chronic myeloid leukemia after stopping tyrosine kinase inhibitor therapy. Haematologica. 2017;102(8):e297–9.
10. Shanmuganathan N, Pagani IS, Ross DM, Park S, Yong ASM, Braley JA, et al. Early BCR-ABL1 kinetics are predictive of subsequent achievement of treatment-free remission in chronic myeloid leukemia. Blood. 2021;137(9):1196–207.
11. Baccarani M, Castagnetti F, Gugliotta G, Rosti G, Soverini S, Albeer A, et al. The proportion of different BCR-ABL1 transcript types in chronic myeloid leukemia: An international overview. Leukemia. 2019;33(5):1173–83.