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"Variant specific discrepancy when quantitating BCR-ABL1 e13a2 and e14a2 transcripts using the Europe Against Cancer qPCR assay". Is dPCR the key?

BCR-ABL1 variants quantitation improved by dPCR.

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Dear Editor

We read with great interest the article by Kjaer et al. [1] aiming at investigating the performance of a standardized qPCR assay in quantification of BCR-ABL1 transcripts in chronic myeloid leukaemia (CML) patients harbouring the e13a2 or e14a2 variant of the M-bcr (p210) fusion protein.

The Authors observed a significant difference in the slopes of qPCR amplification curves between the variants, and compared qPCR results with absolute quantitation of BCR-ABL1 by droplet digital PCR (ddPCR), demonstrating that mean qPCR values were consistently higher for e13a2 patients and lower for e14a2 patients as compared to ddPCR, while no significant difference was present considering the absolute quantification by ddPCR. Several groups, including ours, showed that e14a2 variant is associated to earlier, deeper and more stable molecular response than e13a2 [2-4], although no differences in long-term survival were found between the two groups in a

large cohort by the European Treatment and Outcome Study group [5]. Based on these observations the e14a2 variant has been considered biologically more favourable. The data presented by the Authors contrast with this interpretation and attribute the phenomenon at least partly to a technical aspect.

We recently studied 142 CML patients with deep molecular response (DMR ≥MR4.0) according to qPCR and comparatively monitored them for a median time of 24 months by quantitative and digital PCR. In this study, we found that the threshold of 0.468 BCR-ABL1 copies/μL allowed a better identification of the patients with "stable" DMR and, at TKI discontinuation, a better recognition of the patients with a higher probability to maintain the treatment-free remission (TFR). On the contrary, patients' stratification according to the standard qPCR classes (i.e. MR4.0, MR4.5, and MR5.0) failed at it [6].
We revised our 142 CML patients' samples in light of the transcript variants. Indeed, a difference was appreciable considering all the 512 samples collected throughout the duration of the study and evaluating the detectability of BCR-ABL1 by qPCR. e13a2 samples presented a higher rate (115/205) of detection compared to e14a2 ones (144/307) (p=0.0367). No difference was appreciable in terms of dPCR quantification, neither for fluorescence intensity, as considered by Kjaer and colleagues, nor for absolute level of transcript. (Figure 1).

Moreover, we confirmed that there were no differences in the mean of BCR-ABL1 values by dPCR between e13a2 and e14a2 patients at enrollment. Specifically, the proportion of patients with e13a2 or e14a2 was 45% and 55%, respectively, among patients with ≥0.468 BCR-ABL1 copies/μL, and 35% and 60%, respectively, among patients with <0.468 BCR-ABL1 copies/μL (p=0.573). In this last category, 5% presented both the transcripts. Therefore, in our cohort dPCR lasts the only variable able to identify patients with stable DMR and beneficiaries of TKI discontinuation.
These findings and the data reported by Kjaer et al. pose an important question about the reliability of BCR-ABL1 measurement by qPCR, especially in patients with the e13a2 variant. How many of them would not have been considered eligible for a treatment discontinuation or were incorrectly evaluated as molecularly relapsed? Is the reported higher probability of TFR maintenance in e14a2 patients [7,8] due to a technical issue rather than a biological phenomenon?

We totally agree with Kjaer et al. about the limitations of qPCR and strongly support the usefulness of dPCR as feasible alternative to provide a robust, sensitive and accurate quantitation of BCR-ABL1 in routine clinical practice. We also think that in the era of more potent TKI, precision medicine and personalized treatment programs, it is the time to introduce the dPCR in the CML management.

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Figure 1. BCR-ABL1 absolute quantification by dPCR. Samples are divided based on the transcript variant. dPCR values are reported as BCR-ABL1 copies/μl.

Figure 1