Negative $MR^{4.0}$ chronic myeloid leukaemia and its possible implications for treatment-free remission

ABL1 tyrosine kinase inhibitors (TKI) have dramatically improved the outcome for chronic myeloid leukaemia (CML) patients, resulting in a life expectancy that approaches that of the general population. Nevertheless, lifelong TKI therapy may have consequences, including chronic adverse events that can substantially impact patients' quality of life, adherence to therapy and treatment success. Recently, several clinical discontinuation trials have demonstrated that 40–60% of chronic phase CML patients (CP-CML) who have achieved a stable deep molecular response (DMR) can stop therapy without relapsing (Breccia & Foà, 2018). Laboratory recommendations for scoring DMR were previously defined as $MR^{4.0}$ [either detectable disease ⩽0·01% *BCR-ABL*^{IS} ($MR^{4.0}$ positive) or undetectable disease in cDNA with 10 000–31 999 *ABL1* transcripts or 24 000–76 999 *GUSB* transcripts ($MR^{4.0}$ negative)], $MR^{4.5}$ [either detectable disease ⩽0·0032% *BCR-ABL*^{IS} ($MR^{4.5}$ positive) or undetectable disease in cDNA with 32 000–99 999 *ABL1* transcripts or

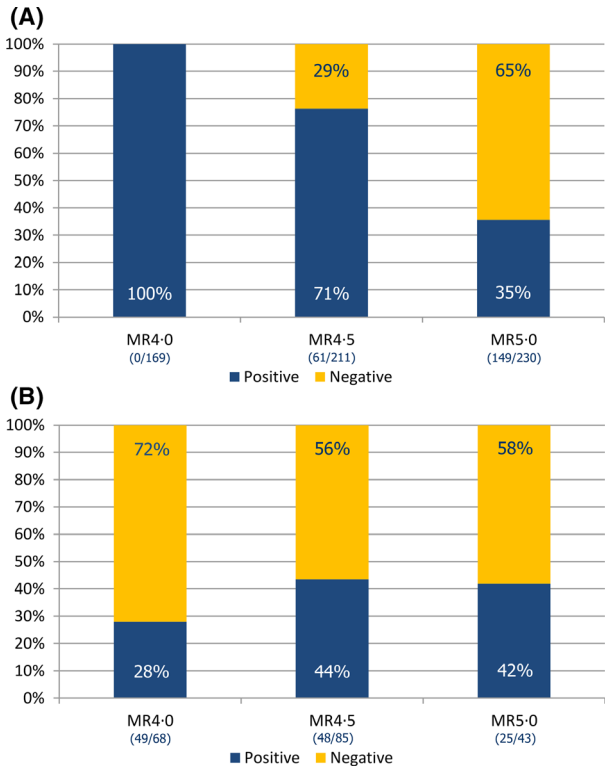

Fig 1. Stratification of chronic myeloid leukaemia patients in deep molecular response by level of molecular response. (A) Deep molecular response data from 610 Portuguese chronic myeloid leukaemia patients (B) Deep molecular response data from 196 chronic myeloid leukaemia patients included in the EURO-SKI trial (Saussele *et al*, 2014).

77 000–239 999 *GUSB* transcripts ($MR^{4·5}$ negative)], and $MR^{5·0}$ [either detectable disease ⩽0·001% $BCR\text{-}ABL^{IS}$ ($MR^{5·0}$ positive) or undetectable disease in cDNA with ⩾100 000 *ABL1* transcripts or ⩾240 000 *GUSB* transcripts ($MR^{5·0}$ negative)] (Cross *et al*, 2015).

Most trials enrolled only patients with a molecular response of 4·5 or deeper, sustained for at least two years (Breccia & Foà, 2018). Recently, the EURO-SKI study evaluated the feasibility of treatment interruption in patients in confirmed $MR^{4·0}$ for at least 1 year (Saussele *et al*, 2018). Treatment duration and molecular response duration emerged as variables that had the largest effect on treatment cessation success (Saussele *et al*, 2018). A sub-analysis of the EURO-SKI study detected no differences in molecular relapse-free survival (RFS) between patients in $MR^{4·0}$ who never achieved a $MR^{4·5}$ (61%) compared to detectable $MR^{4·5}$ (51%) or undetectable $MR^{4·5}$ (62%) (Pfirmann *et al*, 2016). A subsequent analysis reported no differences in molecular relapse after stopping therapy in $MR^{4·0}$, whether residual disease was detected or not (Pfirmann *et al*, 2017). These observations seem to suggest that there is no difference between $MR^{4·0}$ and $MR^{4·5}$ in terms of success of treatment-free remission (TFR). Despite these results, there is no consensus as to whether or not the depth of the molecular response is a prognostic factor for TFR, which is reflected in the discrepancy between the various guidelines and opinions of experts in the field regarding the optimal molecular response required, $MR^{4·0}$ or $MR^{4·5}$, for a TFR attempt (Hughes & Ross, 2016; Mahon, 2017; Hochhaus *et al*, 2018; National Comprehensive Cancer Network, 2018). In addition, despite the efforts made in recent years to standardize the quantification and reporting of *BCR-ABL1* levels in CML patients (Cross *et al*, 2015), the degree of sensitivity variation of the different methodologies among different laboratories remains unknown.

We hypothesized that a discrepancy in the classification of the level of molecular response between laboratories involved in clinical trials could introduce a bias in the evaluation of the prognostic impact of TFR. In an attempt to clarify this issue, we evaluated a cohort of 1087 CML patients treated with TKIs in Portugal monitored by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). The patients were monitored in two European Treatment and Outcome Study (EUTOS)-certificated laboratories in Porto and Lisbon and in three additional centres that have a validated conversion factor. All laboratories reported the results in the international scale according to Cross *et al* (2015). *ABL1* and *GUSB* reference genes were used for normalization and classification of depth of molecular response in *BCR-ABL1* negative cases in 4 and 1 laboratories, respectively. Only patients with typical *BCR-ABL1* transcripts (e13a2 and e14a2) were included. At the last evaluation, 610 patients (56%) were in DMR: 169 (28%) in $MR^{4·0}$, 211 (35%) in $MR^{4·5}$ and 230 (38%) in $MR^{5·0}$ (Fig 1A). Interestingly, none of the 169 patients classified as $MR^{4·0}$ had undetectable disease. In addition, as expected, the frequency of undetectable disease-negative cases was higher in the $MR^{4·5}$ (29%, 61/211) and $MR^{5·0}$ (65%, 149/230) groups (Fig 1A), as the threshold of sensitivity of the current qRT-PCR methodology was reached. We compared our results to those of the EURO-SKI trial, which used the molecular monitoring data of patients from 61 centres in 11 European countries (Saussele *et al*, 2018). As no detailed stratification of CML patients by molecular response was published in the last analysis of the EURO-SKI trial (Saussele *et al*, 2018), we used the data of the first interim analysis, which included 200 patients (Saussele *et al*, 2014). Of these, 196 were evaluable for molecular response: 68 (35%) were in $MR^{4·0}$, 85 (43%) in $MR^{4·5}$ and 43 (22%) in $MR^{5·0}$ (Fig 1B). The frequency of $MR^{4·0}$ with undetectable disease was 72% (49/68), decreasing to 56% (48/85) and 58% (25/43) in the $MR^{4·5}$ and $MR^{5·0}$ groups, respectively. The unexpected distribution of the EUROSKI data could potentially be caused by a small number of laboratories with poor performing assays or poorly calibrated assays that overestimate the number of reference gene transcripts. Supporting this hypothesis is the absence of detectable disease in our $MR^{4·0}$ patients using optimized qRT-PCR methodologies not available at the time in most laboratories, before $MR^{4·5}$ standardization within the EUTOS project. However, this does not explain the observed lower

proportion of undetectable disease observed in the $MR^{4·5}$ and $MR^{5·0}$ groups when compared with the $MR^{4·0}$ group in the EURO-SKI trial. In addition, the higher proportion of $MR^{5·0}$ with detectable disease observed in the EURO-SKI when compared with our series is also difficult to explain. Nevertheless, if this data sampling of the EURO-SKI trial is representative of the final series, there may be a bias in patient stratification by molecular response, making it impractical to attempt any association between molecular response depth and TFR success, at least for patients with undetectable disease. Thus, more studies are needed in order to clarify whether TFR can be accomplished successfully in CML patients with responses less than $MR^{4·5}$. Moreover, our data also suggests that: (i) the molecular response criteria used to select patients for TFR trials should be modified, so that all patients with $MR^{4·0}$ and undetectable disease should be excluded and (ii) the results of the distribution of patients by molecular response level should be published with the TFR data. Finally, considering the limitations of qRT-PCR, the implementation of even more sensitive and accurate methods, such as digital PCR, may provide more robust estimates of molecular response levels, improving the selection of patients for a TFR trial.

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Conflict of Interests

JEG: Speakers bureau for AbbVie, Janssen, Pfizer and Roche; AA: Speakers bureau for Celgene and Novartis; NC JD, SM, MLA, MC, SB, ATS, FP, ML, LR, MCF and MRT declare no competing financial interests.

Author Contributions

NC and MRT designed the study. JD, SM, MLA, MC, SB, ATS, FP, ML, LR, MCF, JEG and AA provided molecular data. NC was responsible for the collection, analysis and interpretation of data. NC and MRT were involved in drafting the manuscript. All authors contributed to revisions, read, and approved the final version of this manuscript.

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References

Breccia, M. & Foà, R. (2018) Current information and recommendations on the discontinuation of TKI inhibitors in chronic myeloid leukemia. *Current Oncology Reports*, **20**, 23.

Cross, N.C., White, H.E., Colomer, D., Ehrencrona, H., Foroni, L., Gottardi, E., Lange, T., Lion, T., Machova Polakova, K., Dulucq, S., Martinelli, G., Oppliger Leibundgut, E., Pallisgaard, N., Barbany, G., Sacha, T., Talmaci, R., Izzo, B., Saglio, G., Pane, F., Müller, M.C. & Hochhaus, A. (2015) Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. *Leukemia*, **29**, 999–1003.

Hochhaus, A., Saussele, S., Rosti, G., Mahon, F.-x, Janssen, J.j w m, Hjorth-Hansen, H., Richter, J. & Buske, C. (2018) Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, **29**, iv261–iv261.

Hughes, T.P. & Ross, D.M. (2016) Moving treatment-free remission into mainstream clinical practice in CML. *Blood*, **128**, 17–23.

Mahon, F.X. (2017) Treatment-free remission in CML: who, how, and why? *Hematology*, **2017**, 102–109.

National Comprehensive Cancer Network. (2018) Chronic Myeloid Leukemia (Version 2.2019). Available at: https://www.nccn.org/professionals/physician_gls/default.aspx#site.

Pfirmann, M., Mahon, F.X., Guilhot, J., Richter, J., Almeida, A., Janssen, J.J.W.M., Mayer, J., Porkka, K., Panayiotidis, P., Olsson-Stromberg, U., Berger, M.G., Diamond, J., Ehrencrona, H., Kairisto, V., Machova Polakova, K., Müller, M.C., Mustjoki, S., Hochhaus, A., Saussele, S. & Hjorth-Hansen, H. (2016) No differences in molecular relapse-free survival after stopping imatinib treatment of chronic myeloid leukemia between patients with prior 4·5 log reduction

(MR4·5) but detectable and patients with undetectable disease in the EURO-SKI trial. *Blood*, **128**, 789–789.

Pfirmann, M., Mahon, F.X., Guilhot, J., Richter, J., Almeida, A., Janssen, J.J.W.M., Mayer, J., Koskenvesa, P., Panayiotidis, P., Olsson-Stromberg, U., Berger, M.G., Diamond, J., Ehrencrona, H., Kairisto, V., Machova Polakova, K., Müller, M.C., Mustjoki, S., Hochhaus, A., Saussele, S. & Hjorth-Hansen, H. (2017) Chronic myeloid leukemia patients were not different in molecular relapse after stopping imatinib in MR4 whether residual disease was detected or not-when adjusting for number of control transcripts. *Haematologica*, **102**, 153–153.

Saussele, S., Richter, J., Guilhot, J., Müller, M.C., Dietz, C., Porkka, K., Hjort-Hansen, H., Gruber, F., Panayiotidis, P., Ossenkoppele, G.J., Mayer, J., Medina Almeida, A., Machová Poláková, K., Ehrencrona, H., Kairisto, V., Diamond, J., Mustjoki, S., Hochhaus, A., Pfirrmann, M. & Mahon, F.-X.(2014) First interim analysis of a pan-european stop trial using standardized molecular criteria: results of the EURO-SKI trial. *Haematologica*, **99**, 792–792.

Saussele, S., Richter, J., Guilhot, J., Gruber, F.X., Hjorth-Hansen, H., Almeida, A., Janssen, J.J.W.M., Mayer, J., Koskenvesa, P., Panayiotidis, P., Olsson-Strömberg, U., Martinez-Lopez, J., Rousselot, P., Vestergaard, H., Ehrencrona, H., Kairisto, V., Machová Poláková, K., Müller, M.C., Mustjoki, S., Berger, M.G., Fabarius, A., Hofmann, W.K., Hochhaus, A., Pfirrmann, M., Mahon, F.X. & EURO-SKI Investigators. (2018) Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. *Lancet Oncology*, **19**, 747–757.