treatment cessation is considered. Currently, many laboratories standardize to the IS by exchanging patient samples with reference laboratories, an expensive and time-consuming process. A *BCR-ABL1* reference panel accredited by the World Health Organization (WHO) has been developed as a primary standard for IS calibration (MR^1-MR^4), but access to the material is restricted.
Aims: (1) To develop a secondary reference panel that is traceable to and faithfully replicates the WHO panel in manufacturing materials and methods, with an additional $MR^{4.5}$ level. (2) To assess the secondary panel in an international multi-center study.
Methods: As with the WHO panel, the secondary panel was manufactured by lyophilizing K-562 and HL-60 cell mixtures. Reverse-transcription droplet digital polymerase chain reaction (RT-ddPCR) was used to quality assess and calibrate the secondary panel to the WHO panel. The secondary panel was subsequently evaluated by 44 laboratories to determine the optimal sample input for each assay, and to assess assay performance including PCR efficiency, IS accuracy, sensitivity, linearity and precision.
Results: A secondary panel with *BCR-ABL1* levels of MR^1, MR^2, MR^3, MR^4 and $MR^{4.5}$ was successfully developed and IS calibrated to the WHO panel using RT-ddPCR against *ABL1*, *BCR* and *GUSB*. Quality control assessments indicated that the secondary panel had minimal residual moisture, excellent vial-to-vial homogeneity and >2.5 years real-time stability. The multi-center evaluation of the panel demonstrated compatibility with >44 *BCR-ABL1* assays of different configurations. Interestingly, in a standard curve experiment, we found that >40% of *BCR-ABL1* assays showed signs of inadequate optimization such as poor linearity and suboptimal PCR efficiency. When optimized sample inputs were used, 60% of tests achieved mean%BCR-ABL1 values within 2-fold of the panel's assigned values. Furthermore, 84% achieved good precision (≤0.25 log standard deviation) from MR^1 to MR^4, and 76% achieved a 100% detection rate at $MR^{4.5}$. Finally, 58% obtained IS conversion factors from the secondary panel equivalent to their current ones, most of which were obtained via sample exchange. Correlation analysis indicated that better PCR optimization was associated with better assay performance, and increased sample input improved detection rate and precision at $MR^{4.5}$. Different assay configurations were not found to be correlated with alterations in%BCR-ABL1 results.
Summary/Conclusions: We successfully developed the first secondary panel that is traceable to and fully replicates the WHO primary standards, with an additional $MR^{4.5}$ level. The panel was shown to be compatible with *BCR-ABL1* assays of different configurations in an international multi-center study. Importantly, once assay conditions were optimized, a high degree of precision and $MR^{4.5}$ sensitivity were achievable, yet IS accuracy was demonstrated in only 60% of cases. These findings indicate that there remains an unmet need for a simple and broadly available calibration mechanism, such as this secondary panel, to ensure IS accuracy is maintained in laboratories over time. The secondary panel and its derivatives can also be used as reference samples for assay analytical validation, optimization and quality assessment.

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TYROSINE KINASE INHIBITORS DISCONTINUATION IN CHRONIC MYELOID LEUKEMIA: A RETROSPECTIVE ANALYSIS OF 208 ITALIAN PATIENTS

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Background: In the last 10 years different studies analyzed the outcome of patients (pts) with sustained complete molecular remission (CMR) who discontinued imatinib, reporting rates of treatment-free remission (TFR) ranging from 33 to 66% depending on the definition of molecular relapse. On these bases it is judged safe to discontinue treatment with tyrosine-kinase inhibitors (TKIs) in experimental contexts.
Aims: To evaluate TFR in the setting of clinical practice according to the Italian experience where most of the pts who discontinued TKIs were not included in prospective protocols.
Methods: We retrospectively analyzed the outcome of pts treated in 23 Divisions of Hematology in Italy, who discontinued TKIs in deep molecular response (MR). General and clinical information such as TKI at discontinuation, line of treatment, type of MR at discontinuation, reasons for discontinuation were collected. We estimated TFR with the Kaplan-Meier method. Prognostic factors for TFR were assessed by univariate Cox regression model analysis.
Results: We analyzed a total of 208 pts who discontinued TKIs from June 2003 to October 2015. Median age was 59 years (Interquartile Range, IQR, 46-72). 102 were male, 106 female; 52%, 35% and 13% were low, intermediate and high Sokal score respectively. 168 pts (81%) discontinued in first line; 38 pts in second line (63% for intolerance to prior TKIs) and 2 pts in third line. 153 pts (74%) were on treatment with imatinib (all frontline), 26% with either nilotinib or dasatinib. Median duration of treatment with the last TKI was 75.2 mos (IQR 50-114); median time to CMR (undetectable transcript or MR4/MR4.5/MR5) with the last TKI was 23.3 mos (IQR 11.1-45.2). Median duration of CMR was 23 mos (IQR 11-45) before stop. At 3 mos of last TKI 28% of pts were in MR3, 26% were in PCyR and/or had a transcript <10%, 45% were in CCyR and/or had a transcript <1%, and 1% had no response. 184 pts had a response defined according to molecular standardization: 8% were MR3, 30% were MR4, 36% were MR4.5, 26% were MR5. Reasons for discontinuation were: toxicity for 28% of pts, pregnancy for 10%, pt request for 56%, enrollment in ISAV protocol for 10 pts. After a median follow-up of 11 mos (IQR 1-149), estimated TFR was 71% (95%CI 64.6%>78.1%) (figure 1). 69 pts restarted treatment. Reasons for restarting were: loss of MR4 for 20% of pts, loss of MR3 for 55%, loss of CCyR for 12%, other reasons for 13%. Median time to restart treatment was 6 mos (IQR 4-11). We assessed age, sex, Sokal score, type of transcript, previous IFN therapy, duration of TKI therapy, response at 3 mos, time to CMR, CMR duration, line of therapy at stop, depth of MR, reasons for stop as potential prognostic factors for TFR, but no statistically significant association were found, with the exception of response MR5 at stop (MR5 *vs* MR4, HR: 0.43, 95%CI 0.21-0.87, p=0.02) and age [HR 0.62 (95%CI 0.42-0.93, p=0.02), *i.e.* a decreased risk in older *vs* younger pts (difference of age=26 years)]. Pts who had to restart therapy were treated with imatinib (57), nilotinib (10), dasatinib (2). All of them regained at least MR3. No pts progressed. All pts were alive at the last follow up with the exception of 7 who died for reasons unrelated to CML.

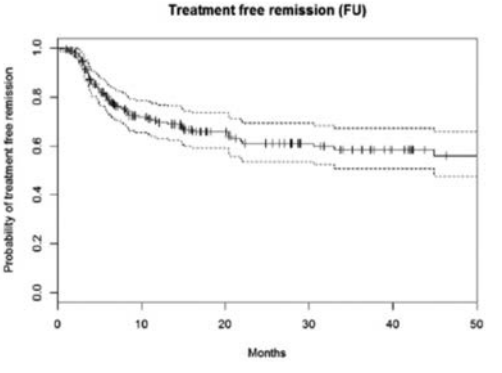

Figure 1. TFR of patients who discontinued TKIs in Italy.

Summary/Conclusions: Although our population is heterogeneous and worthy of specific sub-analysis, our experience confirms that discontinuation of imatinib, nilotinib and dasatinib is feasible and safe in the clinical practice. No progressions occurred, considering that 1/4 of our population had a follow-up longer than 12 years.

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MANAGING CHILDREN AND ADOLESCENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH HIGH-DOSE IMATINIB. THE ITALIAN EXPERIENCE

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