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Footnote

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TO THE EDITOR:

Increased tumor burden in patients with chronic myeloid leukemia after 36 months of imatinib discontinuation

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The Imatinib Suspension and Validation (ISAV) study[1] is a multicenter trial of imatinib discontinuation (ID) among patients with chronic myeloid leukemia (CML) in undetectable deep molecular remission (U-DMR). After 12 months of follow-up, 48% of patients relapsed (total n = 107), with the majority of relapses occurring within the first 9 months. An inverse relationship between patient age and risk of relapse was also observed at this timepoint. Here we report the final results of ISAV after a median follow-up of 49 months, as well as the dynamics of leukemic tumor load as determined by digital polymerase chain reaction (dPCR) in nonrelapsed patients. This trial is registered at www.clinicaltrials.gov (NCT01578213).

Eligible patients were 18 years and older and had CML, either in chronic or accelerated phase, with U-DMR of at least 18 months' duration and at least 3 consecutive negative quantitative real-time PCR (Q-RT-PCR) just before study entry (supplemental Figure 1, available on the *Blood* Web site). A total of 107 patients were enrolled at 15 centers worldwide between 2011 and 2013. U-DMR was defined as an undetectable BCR/ABL1 by Q-RT-PCR as determined by local laboratories. Between 10 000 and 32 000 copies of the ABL1 control gene molecules were amplified, corresponding to a sensitivity of MR4 and MR4.5, respectively. All but 1 laboratory used the International Scale.

Within 7 days of providing informed consent, 20 mL of blood were collected from the patient in PAX gene tubes (PreAnalytiX GmbH, Switzerland) for dPCR analysis and the patient discontinued imatinib therapy. Q-RT-PCR tests were performed[1] monthly for the first 6 months, then every 2 months until up to 36 months from ID to assess for the maintenance of the major molecular remission (MMR) (BCR-ABL/ABL <0.1%). Patients still in MMR at 36 months entered the follow-up phase, during which Q-RT-PCR monitoring was performed every 6 months for an additional 2 years. Loss of MMR was defined as at least 1 BCR-ABL1/ABL1 value above 0.1% among 2 consecutive positive Q-RT-PCR tests. Patients with a loss of MMR resumed imatinib at the same dose used before treatment interruption and were monitored by standard Q-RT-PCR for 2 additional years.[1]

dPCR.RNA (3 μg) was reverse transcribed to complementary DNA as previously described.[2] The final concentration of the reagents were: $MgCl_2$ (5 mM); PCR Buffer (1X); DTT (100 nM); dNTPs (10 nM each); M-MLV reverse transcriptase (16 U); RNAse Inhibitor (0.2 U); and Random Hexamers Primers (12.5 mM) (Thermo Fisher Scientific). Droplet dPCR experiments were performed by the QX200 system (BioRad). The BCR-ABL1 fusion and ABL1 transcripts were quantified using DigiDropP210 MasterMix and DigiDropP210 Positive Control (Bioclarma), according to the manufacturer's protocol and using appropriate negative controls (no template). The target concentration in each sample was expressed as percentage of BCR-ABL1/ABL1, and represents the mean of 3 replicates; negative wells were counted as "0."

Epidemiological rate and Kaplan-Meier method with log-rank test were used to analyze time-to-event end points. Regression analysis was performed by the exponential model. Time to molecular relapse was measured from the date of ID to the date of molecular relapse. The follow-up of imatinib-free patients was estimated using "reverse Kaplan-Meier" method. All the analyses were performed using Stata software, version 16.

Patient median age at ID was 60 years (range, 18-84), 2 patients had accelerated phase CML and 52% had a low Sokal score. The median duration of imatinib treatment was 103 months (range, 44-155), with a median duration of U-DMR of 25.8 months (range, 18-99). Median time from CML diagnosis to ID was 108.2 months. Median follow-up from ID was 49 months (range, 3-68). At the end of the study (3 + 2 years), 56 patients (52.3%; 95% confidence interval [CI], 43-61.5) lost MMR and resumed imatinib. The median observed time to relapse after ID was 5.0 months (range, 1.4-55.6). Two patients experienced late relapses at 45.5 and 55.6 months. The first patient was a 66-year-old woman who had received imatinib for 103 months before study enrollment. She maintained U-DMR until 33 months after ID when her Q-RT-PCR became positive. She then lost MMR in 12 months. The second patient was a 64-year-old woman who received imatinib for 125.4 months before enrollment. She had an isolated positive Q-RT-PCR result 36 months after ID, without losing DMR. Then, a new positivity was seen at the 43rd month and MMR was lost in 12 months. Among patients who lost MMR, 95.9% achieved MMR again after resuming imatinib. The median time to response was 1.9 months (95% CI, 1.2-2.2). No patient showed disease progression or developed resistance after treatment was resumed. The cumulative incidence of relapse was 52.6% with a 5-year relapse-free survival (RFS) of 47.4% (95% CI, 37.6-56.5; Figure 1A). A significant association with age was observed (Figure 1B; supplemental Figure 2), with patients aged 50 years and older having a significantly better RFS. Patients with undetectable dPCR at ID also had a higher RFS (Figure 1C).[1] A multivariate analysis confirmed the significant age effect on RFS; there was no relationship between RFS and the duration of imatinib treatment or U-DMR and RFS (Table 1). At the end of the follow-up phase, 37/51 nonrelapsed patients (73%) tested positive for Q-RT-PCR. Among these patients, 7 had only 1 positive Q-RT-PCR, whereas 30 had an average of 4.3 positive results (range, 2-19). A further evaluation was conducted by dPCR in 34 nonrelapsed patients for whom complementary DNA was available at both ID and 36 months: dPCR was positive in 7/34 patients (22%) at ID, a value similar to that already reported for the ISAV cohort.[1] This value increased to 88% at 36 months. Mean BCR-ABL1/ABL1% values at discontinuation were 0.00143% ± 0.00060 (standard error). After 36 months, they increased to 0.0115% ± 0.0020 ($P < .001$), with a change of approximately 1 logarithm (supplemental Figure 3A). This change corresponds to an increase of 2×10^7 to 2×10^8 leukemia cells.[3] Regarding Q-RT-PCR, 9/34 patients (26.5%) tested positive at month 36, with mean BCR-ABL1/ABL1% values of 0.0015 ± 0.0035 (standard error). Q-RT-PCR trends during the study phase are shown in supplemental Figure 3B. There was no correlation between the results of Q-RT-PCR performed during the study and the dPCR status at 36 months: patients who consistently tested negative by Q-RT-PCR were uniformly negative by dPCR at ID but by 36 months, 83.3% had a positive dPCR; among patients with positive Q-RT-PCRs during the study, 25% had a positive dPCR at ID, and 89.3% were positive at 36 months. This finding suggests that very few

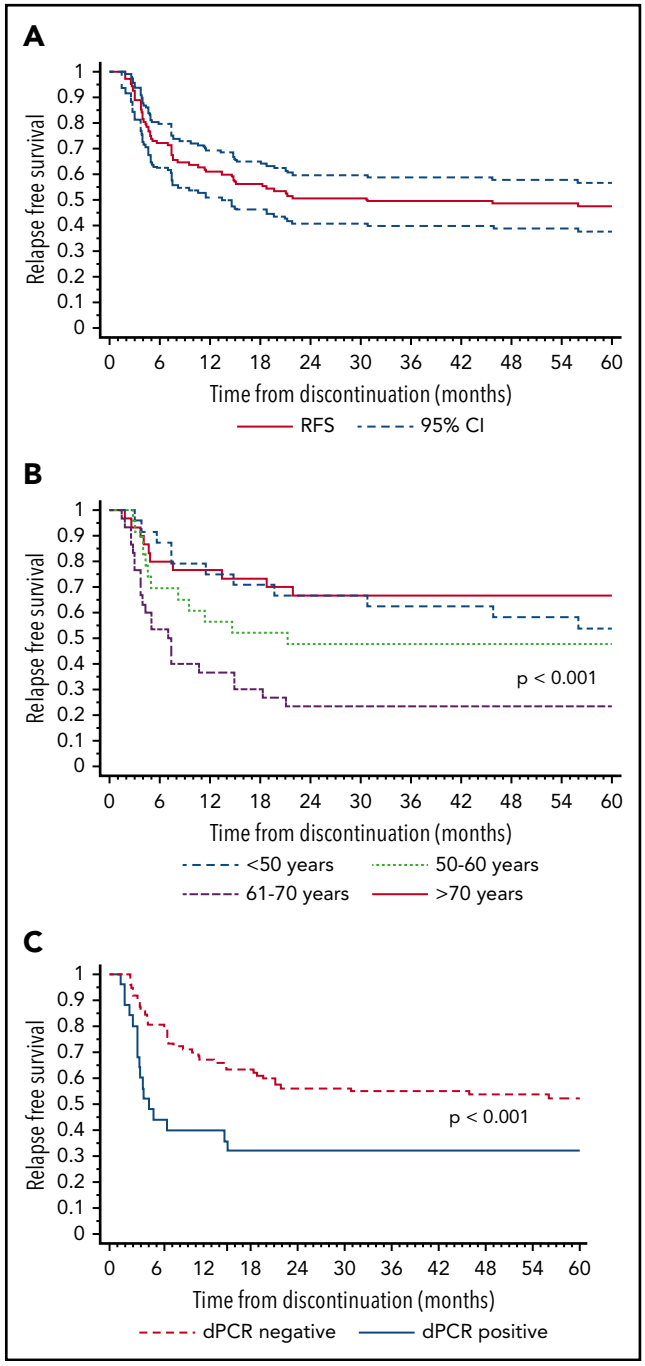

Figure 1. Disease relapse in patients who entered the ISAV study. (A) Relapse-free survival (RFS) from imatinib discontinuation (months). (B) RFS according to different age groups. (a) <50 years (n = 30); (b) 50-59 years (n = 22); (c) 60-70 years (n = 25); (d) >70 years (n = 30). Log-rank test $P < .001$. (C) RFS according to dPCR results at the time of imatinib discontinuation.

if any patients were really free of BCR-ABL$^+$ cells at ID, and that the increase in these cells occurred regardless of the Q-RT-PCR values after ID. Finally, 2/4 patients who tested negative by dPCR at 36 months showed dPCR positivity at ID.

Treatment discontinuation for CML patients with stable U-DMR is part of routine clinical practice. Trials such as Stop Imatinib (STIM),[4] CML8 TWISTER study,[5] European Stop Tyrosine Kinase Inhibitor Study (EURO-SKI),[6] Open-Label Study Evaluating Dasatinib

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Table 1. Multivariate exponential regression models on the rate of relapse

	Rate ratio	95% CI		P
Age 50-60 y	0.41	0.20	0.86	**.02**
Age ≥60 y	0.27	0.15	0.49	**<.001**
dPCR positivity	2.13	1.15	3.94	**.02**
Time from imatinib start to first U-DMR evaluation	0.99	0.98	1.00	.11
Duration of previous imatinib treatment	1.00	0.99	1.02	.78

Statistically significant parameters are indicated in boldface type.

Therapy Discontinuation in Patients With Chronic Phase Chronic Myeloid Leukemia With Stable Complete Molecular Response (DASFREE),[7] and others[8-12] consistently show that imatinib can be safely interrupted, with 2-year RFS reproducibly around 50%. The ISAV study, which was conducted in both academic and non-academic institutions, like EURO-SKI, reported a similar 5-year RFS, and an inverse relationship between age and the risk of relapse. This latter finding was confirmed in the DASFREE study,[7] in an Italian registry,[13] and recently by Claudiani et al[14] and could be explained by an age-related exhaustion of the clonogenic capabilities of Ph^+ quiescent CML cells. It is not clear why other studies did not detect this relationship, but it may be explained by differences in the age structure of the various populations or by differences in analysis methods.

Finally, a new molecular remission was achieved in >95% of patients with the resumption of imatinib, thus reiterating the general safety of treatment discontinuation.

However, the majority of nonrelapsed patients showed at least 1 positive Q-RT-PCR, indicating that ID results in some leukemic regrowth, or at least some increase in the BCR/ABL1 transcript, albeit in a self-limited manner. By using dPCR, the number of leukemic cells could be estimated to have increased by approximately 1 log over 3 years, even in patients still in molecular remission and off treatment. This raises concern about the increased possibility that 1 cell could undergo further genetic changes and progress. There are now reports of evolution to blast phase among patients who stop therapy.[15,16] However, despite the increased leukemic burden, patients in ISAV maintained MMR. Understanding the molecular mechanisms underlying this lack of continuous growth of apparently leukemic cells in the absence of treatment, would be important to assure a safe long-term outcome for patients. Even if numbers are low, there were patients who tested positive by dPCR at ID and became negative 3 years later, suggesting a self-exhaustion of $BCR\text{-}ABL^+$ cells over time in some cases. In conclusion, these results suggest that:

1. Both the number of CML cells at ID (estimated by dPCR results) and their functional status (of which age can be considered a surrogate marker) are likely relevant predictors of RFS.
2. The overall increase in $BCR\text{-}ABL^+$ cells over time, though apparently self-limited, should be studied further.
3. It is necessary to perform long-term monitoring in patients who discontinue imatinib, to rapidly resume therapy in the event of loss of MMR, and thus prevent disease progression.

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Authorship

Contribution: C.G.-P. conceived the study and wrote the manuscript with E.D. and S.A.; E.A., P.L.C., B.M., E.P., C.E., M.B., S.A., E.D.B., A.G., M.A.-C., F.S., and A.I. enrolled the patients; C.F., J.P., and D.F. performed the experiments; S.M., L.A., M.L.B., and P.C. performed the statistical analysis; and all the authors critically reviewed and approved the manuscript.

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Footnotes

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The online version of this article contains a data supplement.

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