Clinical Cancer Research

# Evaluation of Residual Disease and TKI Duration Are Critical Predictive Factors for Molecular Recurrence after Stopping Imatinib First-line in Chronic Phase CML Patients

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## Abstract

**Purpose:** Tyrosine kinase inhibitor (TKI) discontinuation is an emerging goal in chronic myelogenous leukemia (CML) management and several studies have demonstrated the feasibility of safely stopping imatinib. A sustained deep molecular response on long-term TKI is critical prior to attempting treatment-free remission. Reproducible results from several studies reported recently, failed to identify robust and reproducible predictive factors for the selection of the best candidates for successful TKI cessation.

**Patients and Methods:** We conducted a prospective national phase II study evaluating the cessation of imatinib after at least 2 years of MR4.5 obtained on imatinib first-line in patients with chronic phase CML.

**Results:** A total of 218 patients with *de novo* chronic phase CML were involved in the study. The median follow-up after imatinib cessation was 23.5 (1–64) months, 2 patients died from unrelated causes, and 107 experienced a confirmed increase in *BCR-ABL1* levels defined as molecular recurrence. The molecular recurrence-free survival was 52% [95% confidence interval (CI), 45%–59%] at 6 months, and 50% (95% CI, 43%–57%) at 24 months. Droplet digital PCR (ddPCR) was used to evaluate more accurately low levels of *BCR-ABL1* in 175 of 218 patients at imatinib cessation. To apply positive *BCR-ABL1/ABL1* ratios on the international scale (IS), a conversion factor was calculated for ddPCR and the significant cut-off point was established at 0.0023%$^{IS}$. In a multivariate analysis, the duration of TKI ($\geq$74.8 months) and ddPCR ($\geq$0.0023%$^{IS}$) were the two identified predictive factors of molecular recurrence, with $P = 0.0366$ (HR, 0.635; 95% CI, 0.415–0.972] and $P = 0.008$ (HR, 0.556; 95% CI, 0.360–0.858), respectively.

**Conclusions:** We conclude that the duration of TKI and residual leukemic cell load as determined by ddPCR are key factors for predicting successful treatment-free remission for patients with *de novo* chronic phase CML.

*See related commentary by Yan et al., p. 6561*

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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## Translational Relevance

Imatinib duration does impact on recurrence-free survival after imatinib first-line cessation for treatment-free remission (TFR) in patients with chronic phase CML. Digital droplet PCR efficiently discriminates patients prone to molecular recurrence after imatinib first-line cessation for TFR.

## Introduction

Tyrosine kinase inhibitors (TKI) have revolutionized the paradigm of treatment in chronic myelogenous leukemia (CML), especially in chronic phase in nearly two decades. Despite eliminating the bulk of the tumor in a vast majority of patients, initial findings demonstrated that TKI would not be able to fully eradicate the disease (1), leaving the patients in a minimal residual state, implying the indefinite perpetuation of TKI for long-term disease control (2, 3). However, in some patients, the minimal residual disease becomes durably undetectable and this led to the first cessation attempts in limited pilot cohorts of patients (4), providing a rationale for the safety and the feasibility of such a strategy. We (5, 6) and others(7, 8) have initiated larger controlled trials of imatinib cessation in patients with durable deep molecular response (DMR) using stringent criteria for enrollment and retreatment, and demonstrated that this strategy remains safe, but successful in only approximately 50% of the patients (9). It is of note that more than half of these patients were heavily pretreated (with IFNα) and/or had a long-disease history (5, 6) prior to imatinib.

In this study we have addressed the same question in a controlled cohort of patients with chronic phase CML, on imatinib first-line since diagnosis, meeting stringent criteria for treatment-free remission (TFR) and retreatment, enrolled in a prospective national phase II trial, the STIM2 trial. Recently, a new promising and powerful molecular technique, the digital droplet PCR (ddPCR), has been introduced for improving quantification of BCR-ABL1 transcripts. The results obtained were included in a multivariate analysis to analyze the weight of such a factor in the prediction of successful TFR.

## Patients and Methods

### Patients

All patients over the age of 18 years, diagnosed with CML in chronic phase, harboring a major BCR-ABL transcript, and treated with imatinib as a first-line treatment at any dose for at least 3 years were included in 29 different centers belonging to French group of LMC. The STIM1 (5) criteria were used for the inclusion in the trial, briefly: sustained DMR defined as no detectable BCR-ABL1 transcript for 2 consecutive years with at least a sensitivity >4.5 logs according to the European Leukemia Net recommendations for minimal residual disease evaluation (5, 6, 10) on a minimum of five data points. The primary objective of this open-label phase II study was the assessment of the rate of major molecular response (MMR) loss on one data point or a molecular progression defined as at least 1-log increase between 2 consecutive assessments (see below). The ethics committee (Comité de Protection des Personnes CPP), in agreement with the French public health code, approved the protocol on the January 26, 2011. The investigations were approved by an institutional review board and in accordance with an assurance approved by the ANSM (Agence nationale de sécurité du medicament et des produits de santé, French Health Authority). Written informed consent was obtained from all patients. Enrolled patients were followed-up for 2 years after cessation. This study has been sponsored by the University Hospital of Bordeaux (Bordeaux, France) and is registered with ClinicalTrials.gov, under the NCT01343173 number. This study has been conducted in accordance to the last version of the declaration of Helsinki and followed the international ethical guidelines for biomedical research involving human subjects (Council for International Organizations of Medical Sciences). This study has been funded by the "Programme Hospitalier de Recherche Clinique 2010 of the French Ministry of Health (grant no. CHUBX 2010/25).

### Molecular assessments

***Quantitative real-time PCR.*** The molecular follow-up of major BCR-ABL1 transcripts was performed by quantitative real-time PCR (qRT-PCR) from peripheral blood, using *ABL1* as control gene. A total of at least 50,000 copies of ABL1 (with a least 10,000 copies ABL1 per replicate) was required for undetectable transcripts to reach a sensitivity of at least 4.7 log, as reported in the STIM1 study (5). A sixth data point was checked in the centralized laboratory (Molecular Biology and Hematology Laboratory, Bordeaux University Hospital, Pessac, France), at screening, just before imatinib cessation. Subsequent molecular follow-up was performed in the centralized laboratory or in one of the five French participating laboratories (Lyon, Nice, Paris, La Réunion island, and Toulouse), each laboratory having its own validated conversion factor. BCR-ABL1/ABL1 ratios were measured each month in the first year and then every 2 months during the second year after imatinib cessation.

Molecular recurrence was defined as the positivity of BCR-ABL1/ABL1 transcripts with at least 1-log increase between two consecutive assessments (in this case the date of relapse was the date of the first positive assessment) or as loss of MMR on a single assessment.

***Droplet digital PCR.*** BCR-ABL1/ABL1 levels were quantified by RT-ddPCR at screening, just before imatinib cessation, for each patient with available material. cDNA was synthesized with Invitrogen SuperScript IV Reverse Transcriptase according to the manufacturer's instructions from 1μg of total RNA and a final volume of 20 μL, except for final concentration of random hexamer for which we used 60 μM instead of 2.5 μM to improve sensitivity by increasing copy numbers of ABL1/replicates.

Duplex ddPCR using the same EAC (Europe Against Cancer) primers and probes used in qPCR was developed to amplify simultaneously BCR-ABL1 and ABL1 transcripts in the same well (11). Fluorescent Reporters FAM (6-fluorescein amidite) and HEX were used, respectively, for BCR-ABL1 and ABL1 probes and the quencher BHQ1 (Black hole quencher 1) was conjugated to the 3′ end of each hydrolysis probes (see Supplementary Table S1). For each sample, 20 μL reactions were prepared containing 1 × Bio-Rad ddPCR Super Mix (no dUTP), 0.9 μM each of forward and reverse primer, 0.6 μM of each probe, and 9 μL of cDNA. Then, 20 μL of premix and 75 μL of droplet generation oil (probe specific) were transferred in the

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corresponding wells of the DG8 cartridge (according to the manufacturer's instructions). Generation of droplets was made on the QX200 droplet generator. A minimum of 10,000 droplets were required to analyze the sample. Each emulsion was transferred into a well of 96-wells ddPCR plate and amplified on the Bio-Rad-C1000 Touch Thermocycler. Thermocycling conditions were 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds, and 62°C for 1 minute with final step at 98°C for 10 minutes and a hold step at 12°C. Number of positive and negative droplets for each gene with equivalent copies number was determined on the QX200 Droplet Reader using the QuantaSoft v7.0.1 software. Each patient was analyzed in duplicate. The positivity threshold value of ddPCR was determined by the analysis of 30 patients with negative non-CML to eliminate the noisy background. The BCR-ABL1/ABL1 percentage and SD was calculated for all these 30 values. Thereby, ddPCR was considered positive for patients with CML when the BCR-ABL1/ABL1 percentage was more than three SDs. To align positive BCR-ABL1/ABL1 ratios on the international scale (IS), a conversion factor was calculated for the RT-ddPCR as described previously (12). Briefly, a set of 30 patients with CML with BCR-ABL1/ABL1 levels between 0.01% and 10% obtained by RT-ddPCR were compared with percent obtained by the standardized IS reference qRT-PCR, for the same samples. Each value was $\log_{10}$-transformed and the mean of difference between each measurement was calculated. The antilog of the estimated mean bias provided the conversion factor to the IS. The concordance of the new IS RT-ddPCR values with the reference qRT-PCR values was assessed by the method of Bland and Altman (12). An acceptable concordance between the results of the qRT-PCR and the RT-ddPCR has been established according to the criteria of Müller and colleagues (13), that is, with more than 50% of the patients' samples within a 2-fold range, more than 75% of the patients' samples within a 3-fold range, and more than 50% of the patients' samples within a 5-fold range.

### Statistical analyses

Time to molecular relapse was measured from the date of imatinib discontinuation to the date of molecular relapse or the date of last molecular examination for patients who did not relapse at latest follow-up. We estimated the relapse-free survival and the TFR on the intention-to-stop population by the Kaplan–Meier method. To assess the cumulative incidence of molecular relapse and establish factors associated with DMR persistence, analyses were performed on the per-protocol population and the models of Fine and Gray, and the Gray's test accounting for competing events were used. We assessed age, sex, Sokal and European Treatment Outcome Study (EUTOS) long-term survival (ELTS) risk groups, time from CML diagnosis to initiation of imatinib and duration of imatinib therapy, time to DMR, DMR duration until discontinuation of imatinib, and values of ddPCR at screening as potential prognostic factors for molecular relapse. Quantitative factors were categorized into groups, with cut-off points set at the median apart for duration of DMR, which was categorized into two groups as follow: undetectable or lowest than the median value of positive cases, versus others. All variables were assessed by univariate analysis first. To summarize prognostic information, all variables associated with the outcome at the 0.10 level were then simultaneously entered in models accounting for competing events. Covariates were considered as additive of prognostic information to each other at an alpha level of 0.05. Estimates for HRs and corresponding 95% confidence interval (CI) were obtained for every significant outcome factor. Analyses were performed with the SAS version 94 Software (SAS Institute Cary).

## Results

Between April 2011 and March 2015, 224 patients were preincluded, 6 patients were finally excluded: 1 for consent removal, 1 for unconfirmed DMR, 2 for atypical transcripts, 1 for uncompliance, and 1 for inclusion criteria violation. Patients were analyzed according to the "intend-to-stop" (ITS) principle. Thus, 218 patients were enrolled in the study and 199 in "per-protocol" [19 additional exclusions in this principle: 11 patients had IFNα prior to enrolment, 4 had other treatments before imatinib (TKI2), and 4 patients had less than 2 years DMR]. The main characteristics of the patients studied in ITS are given in the Table 1.

### Survival without molecular recurrence

With a median follow-up of 23.5 months (range 1–64), 107 patients experienced a molecular recurrence and 2 died from an unrelated cause of disease. We gathered the last local molecular data available for all patients at the last monitoring turn around. One of the 2 patients was in molecular remission and the other experienced relapse but was in MMR at the time of death. Among the 107 relapses, 31 were due to 1-log increase of BCR-ABL1 transcript and 76 were loss of MMR. One occurred at the inclusion, 32 at month 1, 45 at month 2, 16 at month 3, 7 at month 4, 1 at month 5, 6, 9, and 3 at month 8. The 6-month probability of recurrence-free survival (RFS) was 52.2% (95% CI, 45–59); the 12-month probability of RFS was 50.3% (95% CI, 43–57), and the 24-month probability of RFS was 49.8% (95% CI, 43–56; Fig. 1). Five patients incorrectly rechallenged the treatment while they were in DMR so the probability of treatment-free survival was slightly different, that is, at 6 months 51% (95%

**Table 1.** General characteristics of the STIM2 patients ("intention-to-stop" population)

| Variables | | Value | % |
|---|---|---|---|
| Gender | Female | $n = 121$ | 55.5 |
| | Male | $n = 97$ | 44.5 |
| Sokal risk score | Low | $n = 89$ | 41.78 |
| | Intermediate | $n = 82$ | 38.50 |
| | High | $n = 42$ | 19.72 |
| ELTS risk score | Low | $n = 143$ | 80.34 |
| | Intermediate | $n = 26$ | 14.61 |
| | High | $n = 9$ | 5.06 |
| Median age at diagnosis | Years | 55.5 (15.3–84.8) | |
| Median age at imatinib cessation | Years | 61.9 (24.2–91.1) | |
| Interval diagnosis-imatinib initiation | Months | 0.9 (0[a]-58.5) | |
| Interval diagnosis-imatinib cessation | Months | 80 (39.1–188.3) | |
| imatinib duration | Months | 78.7 (38.5–150.2) | |
| Interval imatinib initiation-First CMR | Months | 28.2 (2.7–104) | |
| Undetectable MR duration prior to imatinib cessation | Months | 38.9 (24.1–124.6) | |

NOTE: General characteristics of patients ("intention-to-stop" population).
[a]One patient initiated imatinib the day of CML diagnosis.

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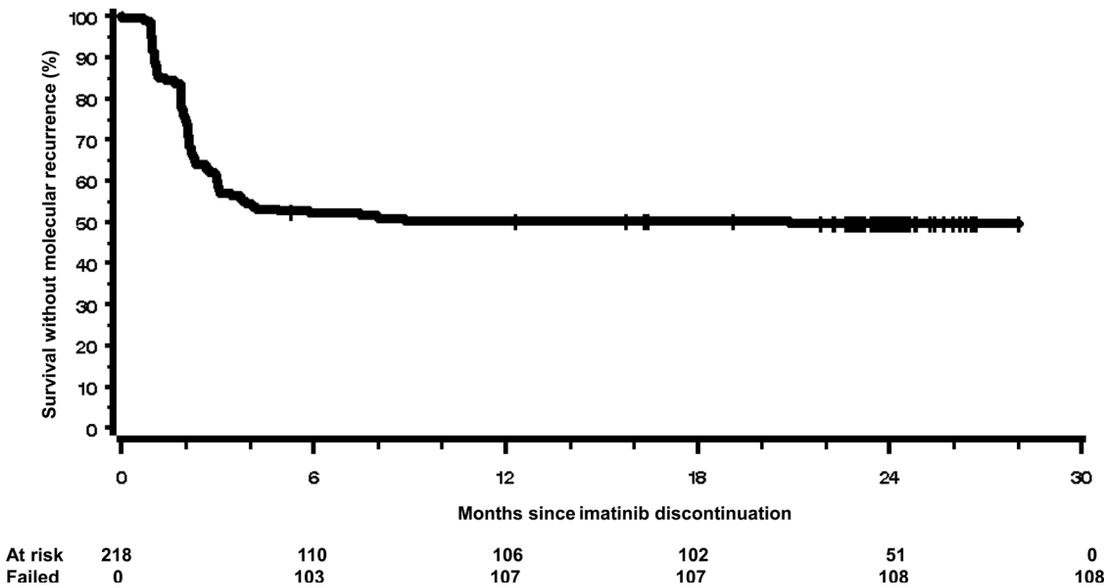


**Figure 1.**
MRFS after imatinib discontinuation ($N = 218$).

CI, 44–58), at 12 months 48% (95% CI, 42–55), and at 24 months 47% (95% CI, 41–54; Fig. 2).

### ddPCR

The conversion factor of RT-ddPCR, obtained from 30 patients with CML was 0.8899. The concordance between qRT-PCR and RT-ddPCR results are excellent, as shown in Fig. 3B. α, for reference method and β, for Bland and Altman method. A total of 97% of RT-ddPCR results were within a 2-, 3-, and 5-fold range as compared with $^{IS}$RT-qPCR results. BCR-ABL1/ABL1% by RT-ddPCR was obtained for 175 evaluable STIM2 patients before discontinuation. The median value of total copies of ABL1 was 164,600. Nevertheless, the positive threshold value obtained from 30 patients with CML was 0.0015%, that is, 0.0013%$^{IS}$, equivalent to 4.9-log of sensitivity for the ddPCR. A total of 75 STIM2 patients were found to be evaluated above this threshold value of 0.0013%$^{IS}$ at screening and considered "positive ddPCR" (ddPCR+), while they were undetectable by RT-qPCR. Half of them ($n = 38$) were above the median value of BCR-ABL1/ABL1 (0.0023%$^{IS}$; Fig. 3A.).

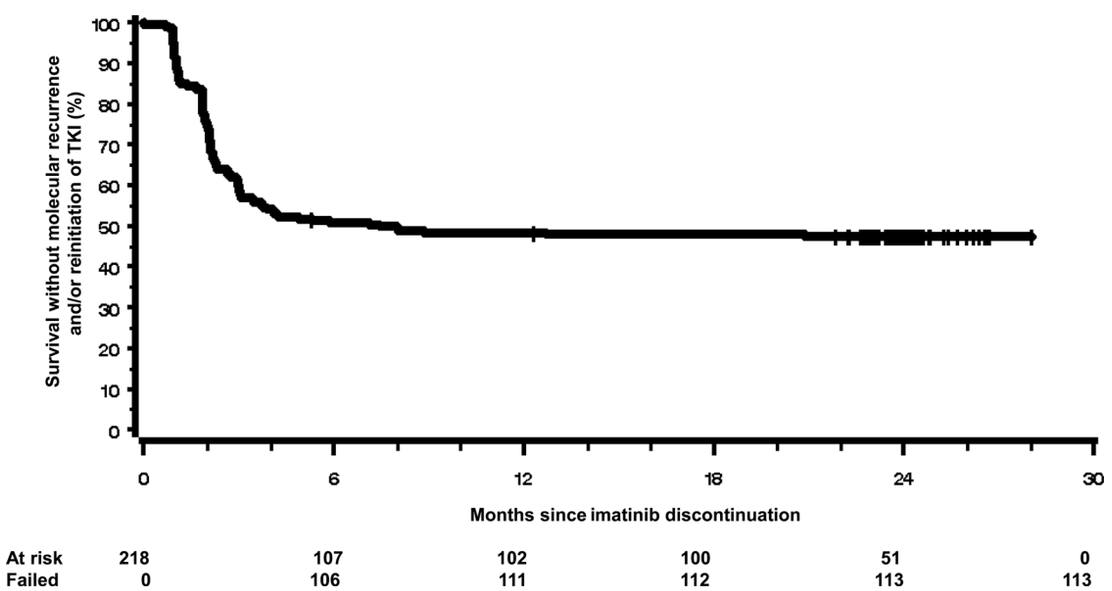


**Figure 2.**
Survival without molecular recurrence and treatment reinitiation ($N = 218$).

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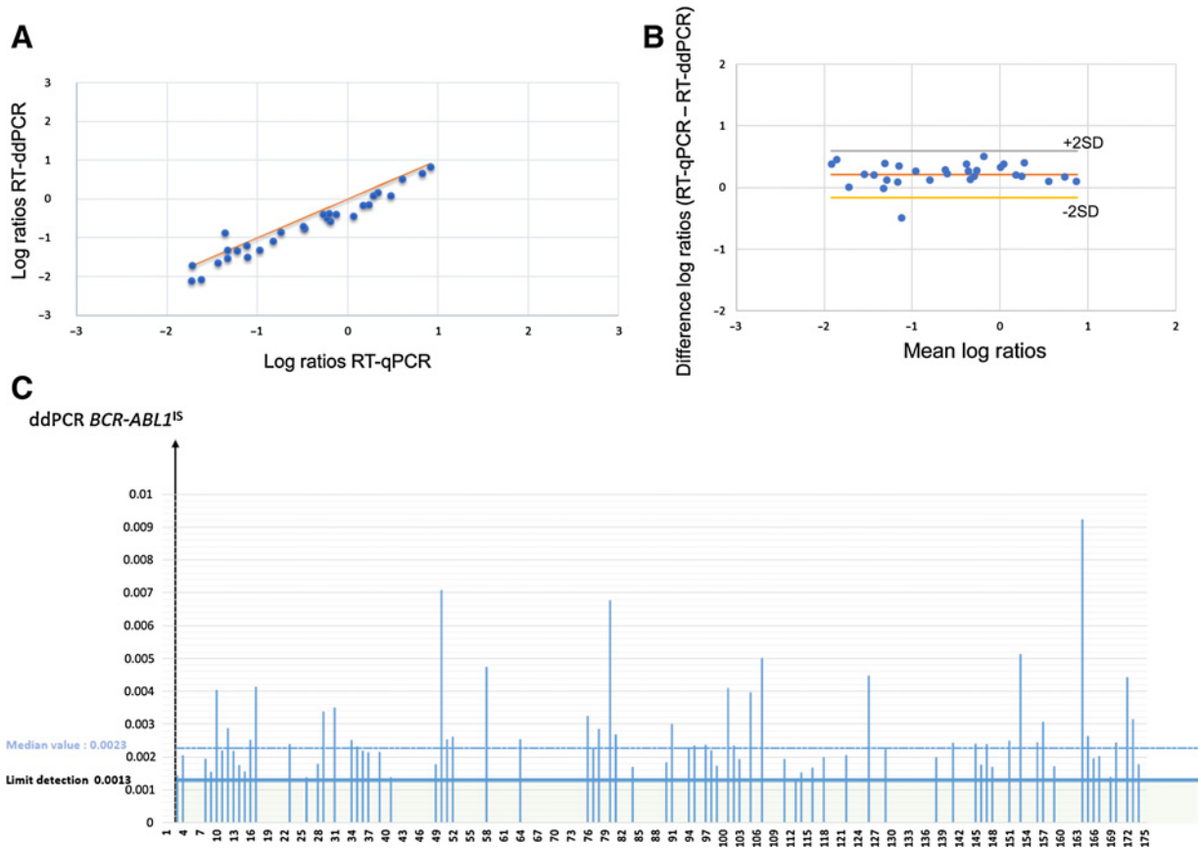


**Figure 3.**

**A,** Correlation between RT-ddPCR and qRT-PCR (reference method aligned on the IS) from 30 patients with chronic phase CML (outside of the STIM 2 study) with BCR-ABL1 ratios between 0.01%$^{IS}$ and 10%$^{IS}$. **B,** Band and Altman plot showing the dispersion around the mean of the log ratios difference (qRT-PCR − RT-ddPCR) according to the mean log ratios. **C,** RT-ddPCR *BCR-ABL1* values of 175 patients were undetectable using qRT-PCR. The limit of detection (LOD) of RT-ddPCR I was calculated by determination of SD of *BCR-ABL1* ratios from 30 no CML or healthy samples according to the formula: LOD = 3 × SD; 3 × 0.000499 = 0.0015, that is, 0.0013 using IS (conversion factor = 0.8889). Among the 175 samples the median value was 0.0026%, that is, 0.0023%$^{IS}$.

Patients with *BCR-ABL1/ABL1* ≥ 0.0023%$^{IS}$ were at higher risk of relapse at 6 and 12 months as compared with patients with *BCR-ABL1/ABL1* < 0.0023%$^{IS}$ [probability of relapse: 66% (55–79) vs. 44% (36–55) at 6 months and 68% (56–83) vs. 46% (38–56) at 12 months $P = 0.0053$; Fig. 4].

### Univariate and multivariate analyses

ddPCR was included in the univariate analysis and in the multivariate analysis. Imatinib duration, DMR duration, and quantitation by ddPCR were significant variables impacting the rate of molecular relapse as shown in Table 2. The Sokal and the ELTS score failed to discriminate patients but a trend was observed for ELTS score ($P = 0.0547$; Fig. 5). The other variables analyzed, such as gender ($P = 0.6246$), age at diagnosis ($P = 0.9662$), age at imatinib cessation ($P = 0.6197$), interval diagnosis-imatinib ($P = 0.9621$), interval imatinib initiation, and DMR ($P = 0.9978$) did not impact, and logically, the interval between CML diagnosis and imatinib cessation significantly impacted on the rate of relapse, as this interval is redundant with imatinib duration ($P = 0.0080$).

Multivariate analyses were performed adjusted on imatinib duration including patients with evaluable ELTS score ($n = 144$ patients). Two distinct parameters showed a significant impact on the rate of molecular recurrence: (i) ddPCR quantitation with a negative impact if the value was more than 0.0023%$^{IS}$, (ii) imatinib duration with a negative impact if <74.8 months. Of note, the ELTS score failed to show any impact. Using minimal *P* value approach, the best duration of treatment was 78 months, that is, 6.5 years ($P = 0.0094$), which was close to the median value. We performed four different multivariate analyses as shown in Table 3. The duration of imatinib treatment and ddPCR were the most significant factors. Patients with *BCR-ABL1/ABL1* < 0.0023%$^{IS}$ had twice less risk of relapse than those with *BCR-ABL1/ABL1* ≥ 0.0023%$^{IS}$ (HR, 0.556; 95% CI, 0.360–0.858; $P = 0.0081$). No association was observed between a ddPCR ≥ 0.0023%$^{IS}$ and sex, Sokal or ELTS score, age at diagnosis, age at imatinib discontinuation, duration of imatinib treatment, DMR duration, or the time-lapse for obtaining DMR.

## Discussion

In this study, we confirm the possibility of imatinib cessation in patients with *de novo* chronic phase CML using the same criteria as compared with the STIM1 initial study. In spite of these strict

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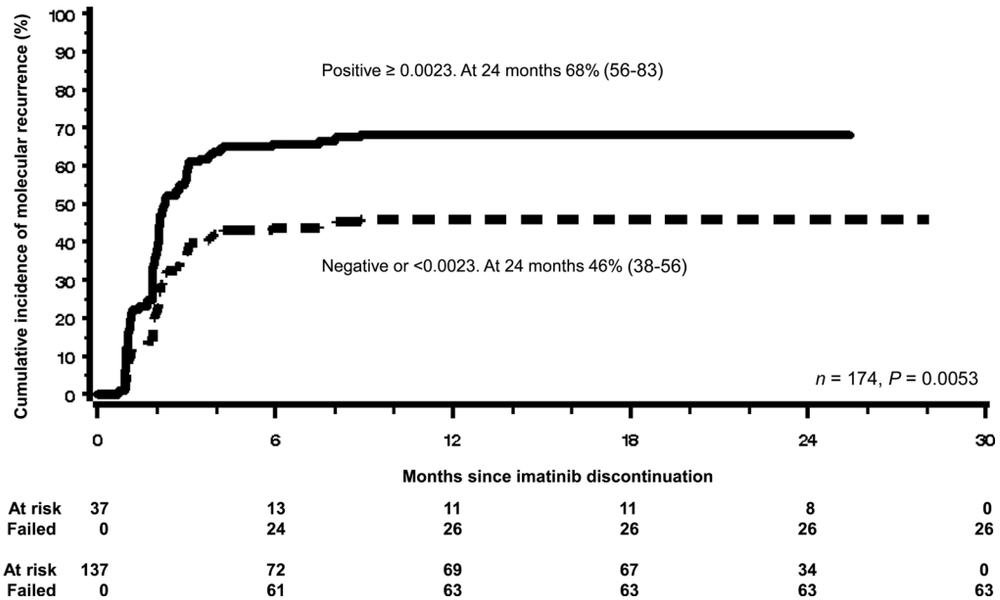


**Figure 4.**

Molecular recurrence-free survival estimate since imatinib first-line discontinuation in patients with a ddPCR $\geq$ or <0.0023%$^{IS}$ at imatinib cessation.

criteria of molecular recurrence definition, the molecular relapse-free survival (MRFS) was 50% at 24 months, slightly better than results obtained in STIM1. One of the aims of STIM2 study was to analyze the predictive factors on a homogeneous group of patients, that is, patient with *de novo* CML only treated with imatinib. Duration of treatment and duration of DMR are closely related in the statistical analysis, and it was difficult to separate them. So, we performed different models of multivariate analysis to keep only one that includes duration of treatment. The latter criterion has already been identified as an important factor in previous reports on TKI cessation. For instance, it is in agreement with what we found in the STIM1 study showing the impact of treatment duration (5) and with the recently reported EUROSKI study (14) showing that the total duration of TKI treatment and the duration of DMR were closely related. Clinical scoring such as Sokal scale may reflect indirectly the heterogeneity of chronic phase CML at diagnosis, and this kind of disease-inherent factor has also been reported in the STIM1 study. Here, in the STIM2 study, only a trend was observed with the ELTS scoring system, using univariate analysis but not confirmed in our four models of multivariate analysis. ddPCR has been reported to be more sensitive than qRT-PCR allowing detection of low BCR-ABL1 transcript copies that are not detected by qRT-PCR (15). In our study, 42% of patients in DMR with undetectable transcripts by qRT-PCR had ddPCR+. Among them, ddPCR identified a subgroup of patients (ddPCR $\geq$ 0.0023%$^{IS}$) with higher risk of relapse. This is, in part, in agreement with the results of the ISAV (16) and KID (17) studies, which described an impact of positive digital PCR (dPCR) on the risk of relapse, although in these two studies, no BCR-ABL1/ABL1 quantification of was performed for dPCR. In our study, the segregation of patients according to ddPCR (positive vs. negative) was not sufficient enough to predict disease recurrence, even if we consider patients for whom the molecular recurrence corresponds to a loss of MMR (data not shown). In other words, negative patients by RT-PCR and positive by ddPCR should not be rejected for a TFR strategy. This could be explained by the different PCR protocols, the preamplification steps (but not of BCR-ABL1 transcripts), and the digital platforms used (i.e., type of partitions). A positive

**Table 2.** Univariate analysis

| Variables | | 6-month probability (95% CI) | 12-month probability (95% CI) | *P* |
|---|---|---|---|---|
| All patients ($n = 199$) | Relapse (Competing events $n = 7$) | 47% (41–55) | 49% (44–56) | — |
| Sokal ($n = 194$) | Low | 46% (37–58) | 48% (39–60) | 0.8788 |
| | Other | 62% (39–57) | 49% (41–58) | |
| ELTS ($n = 161$) | Low | 46% (38–54) | 48% (41–56) | 0.0547 |
| | Other | 62% (50–77) | 65% (51–83) | |
| Imatinib duration ($n = 199$) | <74.8 months | 55% (46–66) | 57% (49–67) | 0.0166 |
| | ≥74.8 months | 39% (31–49) | 41% (32–51) | |
| CMR duration ($n = 201$) | <Median value | 56% (47–66) | 58% (51–67) | 0.0092 |
| | ≥Median value | 38% (30–49) | 40% (32–51) | |
| ddPCR quantitation ($n = 175$) | <0.0023%$^{IS}$ | 44% (36–53) | 46% (38–56) | 0.0053 |
| | ≥0.0023%$^{IS}$ | 66% (55–79) | 68% (56–83) | |

NOTE: Factors of molecular recurrence, univariate analysis. Competing events: TKI reinitiation without loss of molecular response ($n = 6$; 5 with CML only, 1 with intercurrent disease); unrelated CML death without loss of molecular disease ($n = 1$).

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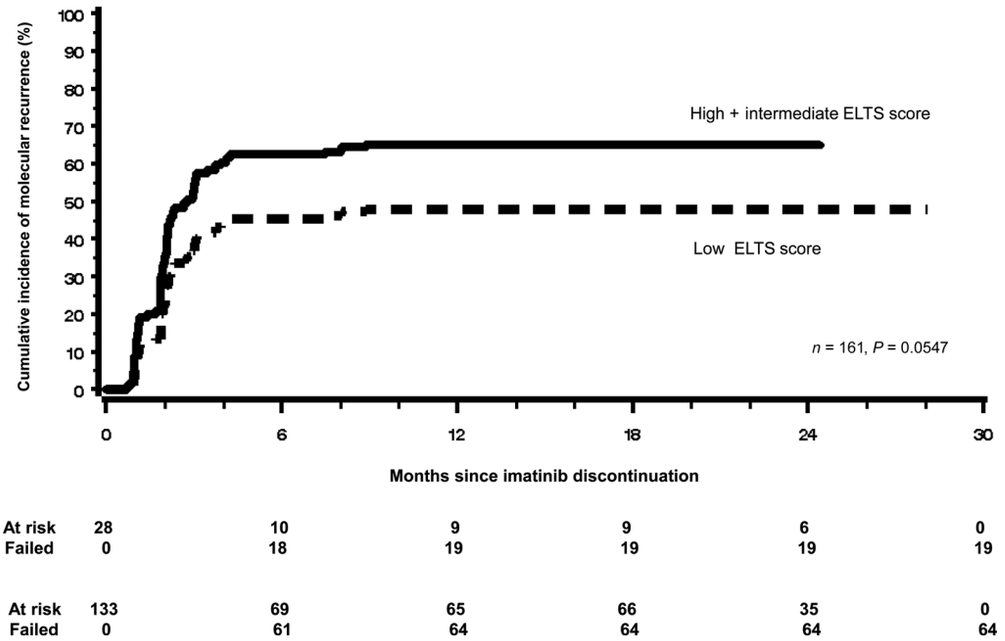


**Figure 5.**
Probability of molecular recurrence according to the ELTS score.

cut-off value of *BCR-ABL1* to predict relapse is however in agreement with the fact that some patients with detectable *BCR-ABL1/ABL1* in qRT-PCR before discontinuation can stop TKI without relapse (14). In other words, despite the persistence of detectable residual leukemic cells, the disease continues to be controlled after TKI cessation through unknown precise mechanisms. It is probably true when the disease is under the cut-off value (in our hands 0.0023%$^{IS}$), which could be better identified using the ddPCR technique. The EURO-SKI trial did not find any association between the depth of molecular response (MR4.5 vs. no MR4.5) and the MMR status at 6 months after imatinib cessation (18). Nevertheless the correlation between ddPCR and qPCR values is worse beyond MR4, if not MR3 too (15). So, the qualification of patients according to their molecular response level should be probably different if we use ddPCR instead of qPCR in discontinuation trials.

**Tables 3.** Different multivariate analyses performed including ELTS, imatinib duration, DMR duration, and ddPCR results

| Variables | | HR (95% CI) | *P* |
|---|---|---|---|
| **A** | | | |
| ELTS ($n = 144$) | Low Other | 0.661 (0.39–1.119) | 0.661 |
| Imatinib duration ($n = 144$) | <75 months ≥75 months | 0.574 (0.359–0.916) | 0.0201 |
| ddPCR quantitation ($n = 144$) | <0.0023% ≥0.0023% | 0.546 (0.337–0.885) | 0.0141 |
| **B** | | | |
| ELTS ($n = 144$) | Low Other | 0.695 (0.396–1.221) | 0.0216 |
| Median DMR duration ($n = 144$) | <75 months ≥75 months | 0.749 (0.460–1.219) | 0.2452 |
| ddPCR quantitation ($n = 144$) | <0.0023%$^{IS}$ ≥0.0023%$^{IS}$ | 0.560 (0.341–0.918) | 0.0216 |
| **C** | | | |
| Imatinib duration $n = 174$ | <75 months ≥75 months | 0.635 (0.415–0.972) | 0.0366 |
| ddPCR quantitation $n = 174$ | <0.0023%$^{IS}$ ≥0.0023%$^{IS}$ | 0.556 (0.3600–0.858) | 0.0081 |
| **D** | | | |
| Median DMR duration $n = 144$ | <75 months ≥75 months | 0.692 (0.451–1.063) | 0.0927 |
| ddPCR quantitation $n = 144$ | <0.0023%$^{IS}$ ≥0.0023%$^{IS}$ | 0.561 (0.360–0.876) | 0.0110 |

NOTE: Different models of multivariate analyses including ELTS, imatinib duration, DMR duration, and ddPCR.

The duration of imatinib treatment and the duration of DMR (as measured by standard qRT-PCR) are two confounding factors. The longer duration of treatment is, the more *BCR-ABL1* transcripts are likely to be undetectable by standard qRT-PCR. However, there is no association between the duration of treatment (or DMR) and ddPCR > 0.0023%$^{IS}$ suggesting that a treatment consolidation phase would not allow the full eradication of residual leukemic cells as detected by ddPCR in our cohort. In addition, other biological factors must be investigated to improve the success of TKI discontinuation trials. The 0.0023%$^{IS}$ cut-off value probably depends on the technique and particularly on preanalytic steps including the type of reverse transcriptase used, a technical hurdle, largely circumvented these last years, that also increases the sensitivity of qRT-PCR.

In conclusion, we believe that RT-ddPCR is a promising tool in discontinuation of TKI trials for patients with CML with undetectable transcripts by qRT-PCR, even if it probably requires a new effort of standardization between laboratories using RT-ddPCR, this standardization is currently in progress in Europe with the EUTOS program. RT-ddPCR could be used to better detect residual disease and predict molecular recurrence for patients who attempt to stop TKI treatment in the near future. RT-ddPCR should be included in the future algorithm of residual disease evaluation, probably in complement to qRT-PCR, particularly with the perspective of TFR.

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## Disclosure of Potential Conflicts of Interest

F.E. Nicolini reports receiving speakers bureau honoraria from Novartis, Incyte Biosciences, and Bristol-Myers Squibb, and is a consultant/advisory board member for Incyte Biosciences and Sun Pharma Ltd. F. Rigal-Huguet is a consultant/advisory board member for Bristol-Myers Squibb, Novartis, Pfizer, and Incyte. V. Dubruille reports receiving speakers bureau honoraria from Novartis, Pfizer, Bristol-Myers Squibb, and Ariad and is a consultant/advisory board member for Novartis and Ariad. P. Rousselot reports receiving commercial research grants from, and is a consultant/advisory board member for Pfizer and Incyte. D. Rea reports receiving speakers bureau honoraria from Bristol-Myers Squibb, Novartis, Incyte, and Pfizer, and is a consultant/advisory board member for Novartis. L. Legros is a consultant/advisory board member for Novartis, Pfizer, and Incyte. F. Guilhot is a consultant/advisory board member for Novartis and Celgene. No potential conflicts of interest were disclosed by the other authors.

## Disclaimer

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the article at all. The corresponding authors had full access to all data, had final responsibility for the decision to submit for publication, and fully agree in the content of the article.

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## References

1. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 2003; 348:994–1004.
2. Bower H, Bjorkholm M, Dickman PW, Hoglund M, Lambert PC, Andersson TM. Life expectancy of patients with chronic myeloid leukemia approaches the life expectancy of the general population. J Clin Oncol 2016;34: 2851–7.
3. Hochhaus A, Larson RA, Guilhot F, Radich JP, Branford S, Hughes TP, et al. Long-term outcomes of imatinib treatment for chronic myeloid leukemia. N Engl J Med 2017;376:917–27.
4. Rousselot P, Huguet F, Rea D, Legros L, Cayuela JM, Maarek O, et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. Blood 2007;109:58–60.
5. Mahon FX, Rea D, Guilhot J, Guilhot F, Huguet F, Nicolini F, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol 2010;11: 1029–35.
6. Etienne G, Guilhot J, Rea D, Rigal-Huguet F, Nicolini F, Charbonnier A, et al. Long-term follow-up of the French Stop Imatinib (STIM1) Study in patients with chronic myeloid leukemia. J Clin Oncol 2017;35:298–305.
7. Ross DM, Branford S, Seymour JF, Schwarer AP, Arthur C, Yeung DT, et al. Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER study. Blood 2013;122:515–22.
8. Takahashi N, Kyo T, Maeda Y, Sugihara T, Usuki K, Kawaguchi T, et al. Discontinuation of imatinib in Japanese patients with chronic myeloid leukemia. Haematologica 2012;97:903–6.
9. Rea D, Ame S, Berger M, Cayuela JM, Charbonnier A, Coiteux V, et al. Discontinuation of tyrosine kinase inhibitors in chronic myeloid leukemia: recommendations for clinical practice from the French Chronic Myeloid Leukemia Study Group. Cancer 2018;124:2956–63.
10. Cross NC, White HE, Colomer D, Ehrencrona H, Foroni L, Gottardi E, et al. Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. Leukemia 2015;29: 999–1003.
11. 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;17:2318–57.
12. Branford S, Fletcher L, Cross NC, Muller MC, Hochhaus A, Kim DW, et al. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. Blood 2008;112:3330–8.
13. Muller MC, Cross NC, Erben P, Schenk T, Hanfstein B, Ernst T, et al. Harmonization of molecular monitoring of CML therapy in Europe. Leukemia 2009;23:1957–63.
14. Saussele S, Richter J, Guilhot J, Gruber FX, Hjorth-Hansen H, Almeida A, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. Lancet Oncol 2018;19: 747–57.
15. Alikian M, Whale AS, Akiki S, Piechocki K, Torrado C, Myint T, et al. RT-qPCR and RT-digital PCR: a comparison of different platforms for the evaluation of residual disease in chronic myeloid leukemia. Clin Chem 2017;63:525–31.
16. Mori S, Vagge E, le Coutre P, Abruzzese E, Martino B, Pungolino E, et al. Age and dPCR can predict relapse in CML patients who discontinued imatinib: the ISAV study. Am J Hematol 2015;90:910–14.
17. Lee SE, Choi SY, Song HY, Kim SH, Choi MY, Park JS, et al. Imatinib withdrawal syndrome and longer duration of imatinib have a close association with a lower molecular relapse after treatment discontinuation: the KID study. Haematologica 2016;101:717–23.
18. Saussele S, Richter J, Guilhot J, Hjorth-Hansen H, Almeida A, Janssen JJWM, et al. Duration of deep molecular response" has most impact on the success of cessation of tyrosine kinase inhibitor treatment in chronic myeloid leukemia - results from the EURO-SKI trial. Blood 2017;130.

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