Kinetics of early and late molecular recurrences after first-line imatinib cessation in chronic myeloid leukemia: updated results from the STIM2 trial

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Abstract

Discontinuation of tyrosine kinase inhibitors in chronic phase chronic myeloid leukemia is feasible in clinical practice based on recently published international recommendations. Nevertheless, factors predictive of molecular recurrence have not been fully elucidated and long-term follow-up of patients enrolled in clinical studies are required in order to update knowledge on discontinuation attempts particularly in terms of the safety and durability of treatment-free remission (TFR). In the current study, we updated results from the STIM2 study in the light of the consensual criterion of molecular recurrence reported in different international recommendations. Among the 199 patients included in the per-protocol study, 108 patients lost a major molecular response. With a median follow-up of 40.8 months (5.5-111 months), the probability of treatment-free remission was 43.4% [36.3-50.4] at 5 years, 40.9% [32.8-47.3] at 7 years and 34.5% [25.6-43.3] at 9 years. Molecular recurrence occurred between 0 to 6 months, 6 to 24 months and after 24 months in 75 patients (69%), 15 patients (14%) and 18 patients (17%), respectively. Notably, the kinetics of molecular recurrence differed significantly between these three subgroups with a median time from loss of MR4 (*BCR::ABL1* ^{IS}≤0.01%) to loss of major molecular response of 1, 7 and 22 months, respectively. Predictive factors of molecular recurrence differed according to the time of occurrence of the molecular recurrence. Durations of imatinib treatment and deep molecular response as well as *BCR::ABL1/ABL1* levels at cessation of tyrosine kinase inhibitor treatment, as quantified by reverse transcriptase droplet digital polymerase chain reaction, are involved in molecular recurrence occurring up to 24 months but not beyond. (*ClinicalTrial.gov Identifier NCT#0134373*).

Introduction

Treatment of chronic myeloid leukemia (CML) has been revolutionized by tyrosine kinase inhibitors (TKI) and the life expectancy of CML patients is now close to that of the general population in countries with access to healthcare.[1] Improving patients' quality of life, by successful discontinuation of TKI treatment in patients in deep molecular response (DMR), is now the goal to reach. Besides the two independent preliminary studies, i.e. the STIM1[2] and TWISTER[3] trials, several TKI discontinuation studies have been performed worldwide[4,5] and have confirmed the proof of concept of a possible prolonged treatment-free remission (TFR) in some chronic phase CML patients. Based on these large studies, international recommendations for TKI discontinuation have been proposed and define the optimal selection of the patients before cessation as well as adequate molecular follow-up after cessation and molecular recurrence leading to TKI resumption.[6,7]

However, the probability of remaining free from TKI treatment after discontinuation has been assessed differently depending on patients' characteristics before discontinuation, the definition of molecular recurrence leading to TKI resumption, and the follow-up duration after TKI discontinuation. This statistical point is important and the occurrence of other events, as compared to molecular recurrence, may influence TFR results. These competing events include CML-unrelated deaths without molecular recurrence and TKI resumption before molecular recurrence. The latter may be partly explained by the evolution of the definition of molecular recurrence over time, which has evolved from loss of undetectable residual molecular disease with a 1-log *BCR::ABL1*^{IS} increase to loss of major molecular response (MMR) at any time.[7,8] With the benefit of hindsight, it has been observed that the detection and quantification of low levels of *BCR::ABL1* transcripts after discontinuation of TKI treatment does not necessarily lead to loss of MMR and that TKI resumption after MMR loss is associated with the achievement of a second DMR in most, if not all, patients.[8] Thus, molecular recurrence after TKI discontinuation can be defined as a *BCR::ABL1*^{IS} higher than 0.1%^{IS} (MMR loss).

Given that molecular recurrence may occur late, the frequency, kinetics and potential predictive factors of such recurrences remain to be clarified, and updates of TKI discontinuation studies with a prolonged follow-up are necessary.

We previously reported the preliminary results of the STIM2 study (NCT#01343173)[9] and confirmed the importance of the duration of treatment with imatinib before it was stopped and the depth of molecular remission at cessation on molecular recurrence-free survival (MRFS). In the current work, we updated and re-analyzed the outcome of the STIM2 cohort of patients with a longer median follow-up (40.8 *vs*. 23.5 months) taking into account the updated recommended criterion of molecular recurrence i.e., MMR loss instead of a 1-log increase between two consecutive assessments or MMR loss on a single assessment, as initially defined.[8]

The objectives of this study were to re-evaluate TFR strategies according to different indicators, allowing a comparison of the results with other discontinuation studies and to determine the incidence, kinetics and potential predictive factors of molecular recurrence according to their time of occurrence.

Methods

Population

Patients at least 18 years old, diagnosed with chronic phase CML, who had undetectable major *BCR::ABL1* for 2 consecutive years and had been treated with imatinib as first-line treatment at any dose for at least 3 years were included in 29 different centers belonging to the France Intergroupe Leucemie Myeloide Chronique (Fi-LMC) group. This study (NTC#01343173) was started in 2011 and was conducted in accordance with the Declaration of Helsinki. The ethics committee, in concordance with the French public health code, approved the protocol (EudraCT number: 2011-000068-91). Written informed consent was obtained from all patients.

Molecular follow-up

Molecular follow-up was performed until 24 months as described previously,[9] and in the local laboratory beyond 24 months, every 3 to 6 months. All local laboratories belong to the French "Groupe des Biologistes Moléculaires des Hémopathies Malignes" with regular rounds of validation of the European Treatment Outcome Study (EUTOS) conversion factor and programs of external quality control. *BCR::ABL1/ABL1* ratios were aligned on the international scale. Each molecular response was scored according to the European LeukemiaNet recommendations.[10]

Endpoints and statistical analysis

All the analyses were conducted in the per-protocol population.

Molecular recurrence-free remission (MRFR) was measured as the time from imatinib cessation to molecular recurrence and TFR as the time from imatinib cessation to molecular recurrence or TKI resumption (whichever came first). MRFS was measured as the time from imatinib cessation to molecular recurrence or death and treatment-free survival (TFS) as the time from imatinib cessation to molecular recurrence, TKI resumption or death. Molecular recurrence was defined as the first oc-

currence of MMR loss.

Depending on each type of survival, the other events which were not taken into account were considered as competing events: resumption of treatment before molecular recurrence for MRFR/MRFS and/or death before molecular recurrence for MRFR/TFR (Table 1).

The cumulative incidence of events for TFR, MRFS and MRFR were estimated by a Fine and Gray model taking into account potential competing events. TFS was estimated by Kaplan-Meier and Fine and Gray models to assess the cumulative incidence of events. Patients with no event were censored at the date of the last available molecular follow-up. TFS and TFR were also analyzed by landmark analysis at 6 and 24 months for patients without molecular recurrence at these time points.

In order to analyze the kinetics of molecular recurrence, time from the first assessment of detectable *BCR::ABL1* transcripts to a 1-log increase of the transcripts, and time from MR4 loss to MMR loss (DMR4-MMR loss) were compared using a *t*-test between patients who experienced molecular recurrence ≤6 months *versus* 6-24 months *versus* >24 months after imatinib discontinuation.

We analyzed factors associated with loss of MMR before and after 6 months and before and after 24 months as compared with patients with no molecular recurrence at the last follow-up.

We assessed age, sex, type of transcript, Sokal and EUTOS long-term survival (ELTS) scores, quantity of transcript (determined by reverse transcriptase droplet digital polymerase chain reaction, RT-ddPCR) at TKI cessatioin,[9] duration of undetectable MR4.5 and duration of treatment. Quantitative factors were categorized into groups, with cut-offs set at the median. All variables were assessed by univariate analysis using usual tests: the Pearson χ^2 and Kruskal-Wallis tests. *P*-values were corrected using the Holm-Bonferroni method after pairwise analyses with the Dunn test for continuous variables and χ^2 tests for qualitative variables. Patients in whom competing events occurred (death or resumption of treatment before MMR loss) or who were lost to follow-up were considered not to have relapsed in the univariate analysis. Analyses were performed with R software.

Results

Population

One hundred and ninety-nine patients were included. The baseline characteristics of the patients are summarized in *Online Supplementary Table S1*. With a median follow-up of 40.8 months (range, 5.5- 111) from imatinib discontinuation, 108 (54%) patients lost a MMR. Ten patients resumed their treatment before MMR loss. Twelve patients were lost to follow-up after 24 months. Four patients died from a cause unrelated cause to their disease before the loss of MMR (n=3) or after loss of MMR but before resumption of treatment (n=1). Two additional patients died from an cause unrelated to their disease after MMR loss and resumption of treatment while they were in DMR.

Table 1. Cumulative incidence of events according to the definition of the event.

	TFS	TFR	MRFS	MRFR
Cumulative incidence % (95% CI) at 5 years	58.32 (51.29-65.34)	56.64 (49.6-63.68)	53.29 (46.19-60.40)	51.62 (44.52-58.71)
Cumulative incidence % (95% CI) at 9 years	67.2 (58.4-76)	65.52 (56.67-74.37)	62.17 (53.21-71.13)	60.50 (51.50-69.49)
N of events	121	118	111	108
Molecular recurrence	108	108	108	108
Death	3	0	3	0
TKI resumption without molecular recurrence	10	10	0	0
N of competing events	0	3	10	13
Death	0	3	0	3
TKI resumption without molecular recurrence	0	0	10	10
Median time to the event (range) months	4.37 (1.08; 98.92)	4.31 (1.08; 98.92)	4.53 (1.08; 98.92)	4.26 (1.08; 98.92)

TFS: treatment-free survival; TFR: treatment-free remission; MRFS: molecular recurrence-free survival; MRFR: molecular recurrence-free remission; TKI: tyrosine kinase inhibitor.

Treatment-free survival and remission and molecular recurrence-free survival and remission

The TFS and TFR probabilities were, respectively, 41.7% [34.7-48.7] and 43.4% [36.3-50.4] at 5 years, 38.4% [31.2-45.6] and 40.9% [32.8-47.3] at 7 years and 32.8% [24.0-41.6] and 34.5% [25.6-43.3] at 9 years. Depending on the event taken into account (molecular recurrence and/or TKI resumption, molecular recurrence, death and/or TKI resumption), the cumulative incidence of events varied from 51.6% to 58.32% at 5 years and from 60.5% to 67.2% at 9 years (Figure 1, Table 1).

Among patients who experienced molecular recurrence and resumed TKI (n=106), 103 (97%) regained MR4 within 3 months (median; min-max: 1-22 months) and MR4.5 within 5.5 months (median; min-max: 2-61 months). Three patients were lost to follow-up after the MMR was re-obtained.

Imatinib, dasatinib, nilotinib or bosutinib were resumed in 90, six, nine and one patients, respectively.

When considering patients without molecular recurrence at 6 months or 24 months, the cumulative incidences of molecular recurrence or TKI resumption were then 28.1% [19.7-36.5] and 12.97% [5.8-20.2] at 5 years after TKI discontinuation respectively (Figure 2) and the TFS rates at 5 years after TKI discontinuation were 69.1% [61.05- 78.3] and 84.7% [77.3- 92.7], respectively (*Online Supplementary Figure S1*).

Time to loss of major molecular response and kinetics of molecular recurrence

Among the 108 patients who lost a MMR, 25 were previously reported as “relapsers” with 1-log increase in transcripts. Among them three had lost their MMR beyond 24 months (at 25, 38 and 90 months after the 1-log increase in transcripts, which had occurred at 11, 4 and 6 months, respectively).

Seventy-five patients lost their MMR before 6 months

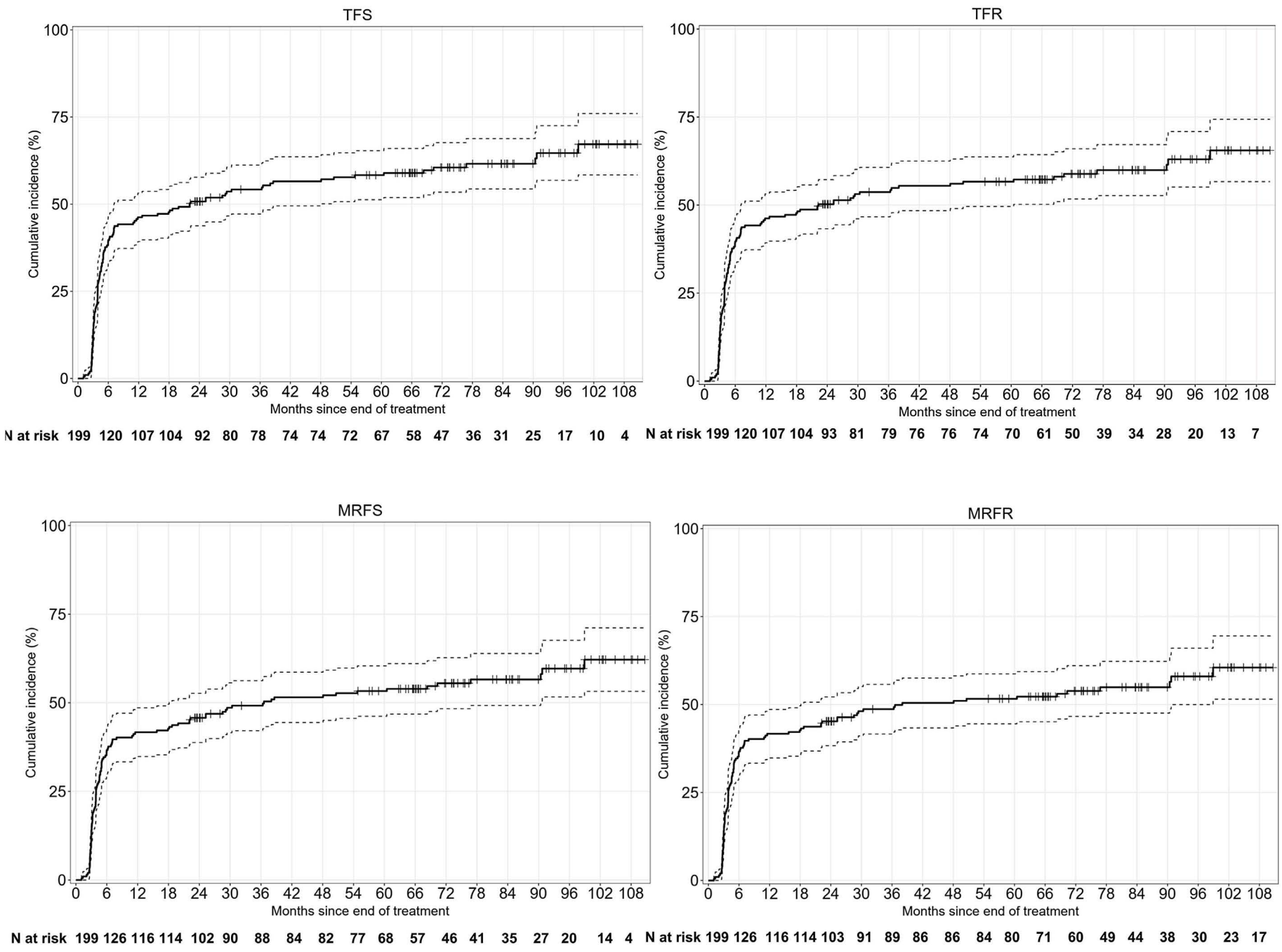

Figure 1. Cumulative incidences of events according to treatment-free and molecular recurrence-free status. TFS: treatment-free survival; TFR: treatment-free remission; MRFS: molecular recurrence-free survival; MRFR: molecular recurrence-free remission.

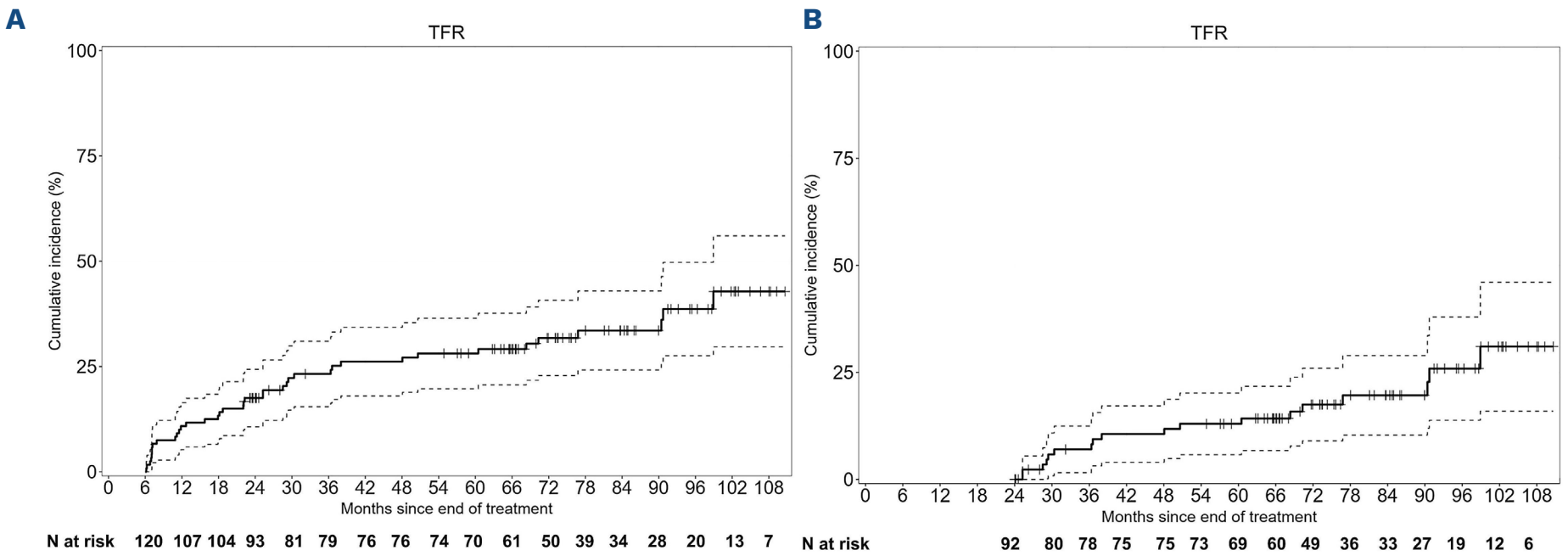

Figure 2. Cumulative incidences of molecular recurrence or tyrosine kinase inhibitor resumption for patients without molecular recurrence at (A) 6 months and (B) 24 months. TFR: treatment-free remission.

Table 2. Kinetics of molecular recurrences according to the time of losing the major molecular response.

	≤6 months (N=75)	6-24 months (N=15)	>24 months (N=18)
Time from imatinib stop to MMR loss, months, mean	3.7	13.7	51.8
DMR4-MMR loss ≤1 months (N=60) Mean: 0.7 months	59 (79%)	1 (7%)	0 (0%)
DMR4-MMR loss 1-3 months (N=18) Mean: 2.3 months	15 (20%)	2 (13%)	1 (5%)
DMR4-MMR loss 3-6 months (N=10) Mean: 5.6 months	1 (1%)	6 (40%)	3 (17%)
DMR4-MMR loss >6 months (N=20) Mean: 22.5 months	0 (0%)	6 (40%)	14 (78%)

MMR: major molecular response; DMR4-MMR loss: time from loss of deep molecular response (MR4) to loss of major molecular response; MR4: molecular response 4-log.

whereas 15 molecular recurrences occurred between 6 and 24 months and 18 occurred beyond 24 months. The kinetics of the molecular recurrences differed between these three groups. Molecular recurrences occurring before 6 months showed fast kinetics with a 1-log increase within a median time from stopping imatinib to the molecular recurrence of 2 months (range, 1 to 5 months) as compared to 5 months (range, 2 to 10 months) for molecular recurrences between 6 and 24 months and 19.5 months (range, 3 to 77 months) for molecular recurrences after 24 months.

To illustrate the different kinetics of molecular recurrence, the time between MR4 and MMR loss were determined in these three groups (Table 2).

Thus, 60 (55.5%) patients had DMR4-MMR loss within 1 month, 18 (16.7%) patients had DMR4-MMR loss between 1 and ≤3 months, 10 (9.3%) patients had DMR4-MMR loss between 3 and ≤6 months and 20 (18.5%) patients had DMR4-MMR loss beyond 6 months. Almost all the patients who lost a MMR before 6 months had a DMR4-MMR loss in ≤3 months (74/75) and 79% (59/75) had a DMR4-MMR loss in ≤1 month. Among patients who lost a MMR between 6 and 24 months only one had a DMR4-MMR loss in ≤1 month, 3/15 (20%) showed DMR4-MMR loss in ≤3 months, and 6/15 (40%) had a DMR4-MMR loss beyond 6 months. Among patients who experienced MMR loss beyond 24 months, overall the DMR4-MMR loss occurred after 6 months in 77.8% (14/18). Only one late molecular recurrence occurred beyond 24 months in a patient with fast kinetics (DMR4-MMR loss: 3.3 months) (Table 2). The mean DMR4-MMR loss was significantly different between patients who lost their MMR before 6 months and those

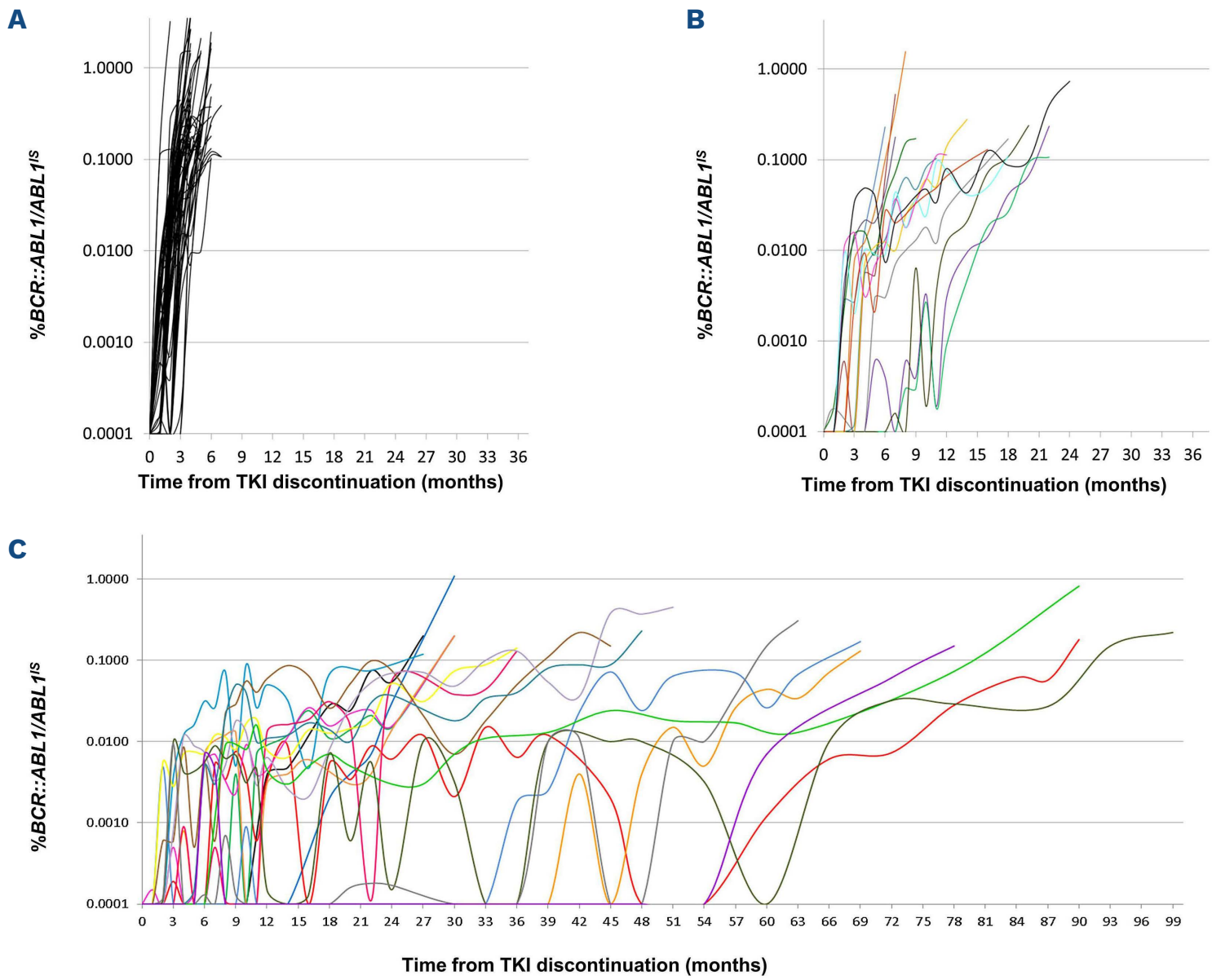

Figure 3. Kinetics of the 108 molecular recurrences in the per-protocol population. (A) Loss of major molecular response (MMR) before 6 months (n=75). (B) Loss of MMR between 6 and 24 months (n=15). (C) Loss of MMR beyond 24 months (n=18). IS: International Scale; TKI: tyrosine kinase inhibitor.

who lost the MMR between 6 and 24 months or beyond 24 months (P<0.0001) with a median time from loss of MR4 to MMR loss of 1, 7 and 22 months, respectively. The kinetics of the *BCR::ABL1/ABL1* transcripts of the 108 molecular recurrences are shown in Figure 3.

Of note, among the 78 patients alive who did not experience molecular recurrence and/or TKI resumption, 12 (15%) patients lost a MR4.5 on at least one assessment within 24 months after TKI discontinuation. Three of them also lost MR4 and two among 12 showed a 1-log increase between two assessments. These 12 patients who had fluctuating levels of *BCR::ABL1* maintained their MMR with a median follow-up from TKI discontinuation of 83 months (range, 54-114 months).

Conversely, among the 18 patients with molecular recurrences beyond 24 months, only one patient (5%) had undetectable *BCR::ABL1* (only one positive assessment in MR5) within 24 months after TKI discontinuation and lost the MMR at 69 months after TKI discontinuation.

So, if we exclude patients with molecular recurrence or resumption of treatment before molecular recurrence within 24 months after TKI discontinuation or who died before molecular recurrence, 12/29 (41%) patients who had fluctuating *BCR::ABL1* within 24 months did not lose their MMR and 17/29 (59%) lost the MMR.

Factors predictive of loss of major molecular response according to the time of the molecular recurrence

Using univariate analysis, predictive factors associated with molecular recurrence were analyzed in patients in whom the molecular recurrence occurred before or after 6 months and before or after 24 months from discontinuing imatinib and compared to patients without molecular recurrence at the last follow-up (Tables 3 and 4). The median duration of DMR, the median duration of imatinib treatment and *BCR::ABL1* ratio (assessed by RT-ddPCR) ≥0.0023% (IS) at imatinib cessation were significantly associated with a higher probability of molecular recurrence within 24 months following treatment discontinuation.

These differences were not observed when we compared patients who lost their MMR beyond 24 months and those who did not lose the MMR.

It should be noted that when we removed patients with potential competing events (death, resumption of treat-

Table 3. Characteristics of the STIM2 patients (per-protocol population) according to the patients' outcome (with late molecular recurrence >24 months).

Variables	No MRec (N=91)	MRec ≤24 m (N=90)	MRec >24 m (N=18)	*P*-value	*P*-value no *vs.* MRec ≤24 m	*P*-value no *vs.* MRec >24 m	*P*-value MRec ≤24 m *vs.* >24 m
Age, years; median (min; max)	52 (15; 78)	55 (24; 84)	58 (32; 77)	0.310			
Gender, N (%)							
Female	56 (61.5)	49 (54.4)	5 (27.8)	**0.030**	0.414	**0.030**	0.178
Male	35 (38.5)	41 (45.6)	13 (72.2)				
Sokal score, N (%)				0.314			
Low	33 (36.3)	43(47.8)	6 (33.3)				
Intermediate	40 (39.9)	27(30)	7(38.9)				
High	12 (13.2)	16 (17.8)	2 (11.1)				
Unknown	6 (6.6)	4 (4.4)	3 (16.7)				
ELTS score, N (%)				0.123			
Low	63 (69.2)	61 (67.8)	11 (61.1)				
Intermediate	8 (8.8)	10 (11.1)	0 (0)				
High	1 (1.1)	7 (7.8)	0 (0)				
Unknown	19 (20.9)	12(13.3)	7 (38.9)				
BCR::ABL1 transcript type, N (%)				0.065			
e14a2	43 (47)	42 (46.7)	4 (22.2)				
e13a2	13 (14.3)	34 (37.8)	5 (27.8)				
e14a2 + e13a2	13 (14.3)	13 (14.4)	3 (16.7)				
Unknown	22 (24.2)	1 (1.1)	6 (33.3)				
DMR duration, mth; median (min; max)	41.3 (23.0; 124.3)	33.9 (24.5; 117.5)	43.3 (24.1; 65.7)	**0.017**	**0.015**	0.624	0.452
Imatinib duration, mth; median (min; max)	82.7 (38.5; 149.9)	71.5 (38.4; 143.5)	79.5 (56.7; 110.5)	**0.020**	**0.021**	0.775	0.392
BCR::ABL1[a] at TKI stop N (%)							
<0.0023%[IS]	68 (74.7)	56(62.2)	13 (72.2)				
≥0.0023%[IS]	8 (8.8)	24 (26.7)	5 (27.8)	**0.010**	**0.015**	0.144	1.000
Unknown	15(16.5)	10 (11.1)	-				

[a]Transcript levels determined by droplet digital polymerase chain reaction. *P* values corrected by the Holm-Bonferroni method. Statistically significant values are shown in bold. MRec: molecular recurrence; ELTS: EUTOS long-term survival; DMR: deep molecular response; mth: months; TKI: tyrosine kinase inhibitor; IS: International Scale.

ment before MMR loss or loss of follow-up beyond 24 months) from the group of patients without molecular recurrence within 24 months, durations of imatinib treatment and DMR were the only significant variables with an impact on the rate of molecular recurrence occurring before 24 months (*data not shown*).

Interestingly, proportions of males were higher in the groups of patients relapsing beyond 6 or 24 months than in the group who did not lose the MMR (66.7% or 72.2% *vs*. 38.5%; *P*=0.030). Nevertheless when we restricted the analysis to non-relapse patients without competing events, only a trend was observed (*data not shown*).

Discussion

In the current work we updated the follow-up of the STIM2 study using loss of MMR at any time after imatinib discontinuation as the single criterion for molecular recurrence. In previous analyses, molecular recurrence was

defined as a 1-log *BCR::ABL1* transcript increase in two consecutive assessments or MMR loss at any time. This new analysis was feasible because most of the patients who experienced a 1-log increase and were previously considered as "relapsers" did not resume TKI treatment until they lost their MMR. Based on this STIM2 update, and with a median follow-up after imatinib discontinuation of 40.8 months, the 5- and 9-year cumulative incidences of molecular recurrence, TKI resumption and death were estimated at 58.32% and 67.2%, respectively. Taking into ac-

Table 4. Characteristics of the STIM2 patients (per-protocol population) according to the patients' outcome (with late molecular recurrence >6 months).

Variables	No MRec (N=91)	MRec ≤6 m (N=75)	MRec >6 m (N=33)	*P*-value	*P*-value no *vs.* MRec ≤6 m	*P*-value no *vs.* MRec >6 m	*P*-value MRec ≤6 m *vs.* >6 m
Age, years, median (min; max)	52 (15; 78)	56 (24; 84)	58 (32; 77)	0.450			
Gender, N (%)							
Female	56 (61.5)	43 (57.3)	11 (33.3)	**0.018**	0.696	**0.030**	0.101
Male	35 (38.5)	32 (42.7)	22 (66.7)				
Sokal score, N (%)				0.414			
Low	33 (36.3)	36 (48)	13 (39.4)				
Intermediate	40 (39.9)	23 (30.7)	11 (33.3)				
High	12 (13.2)	12 (16)	6 (18.2)				
Unknown	6 (6.6)	4 (5.3)	3 (9)				
ELTS score, N (%)				0.065			
Low	63 (69.2)	49 (65.3)	23 (69.7)				
Intermediate	8 (8.8)	8 (10.7)	2 (6.1)				
High	1 (1.1)	7 (9.3)	0 (0)				
Unknown	19 (20.9)	11 (14.7)	8 (24.2)				
BCR::ABL1 transcript type N(%)				0.064			
e14a2	43 (47.3)	34 (45.3)	12 (36.4)				
e132a2	13 (14.3)	30 (40)	9 (27.3)				
e14a2 + e13a2	13 (14.3)	10 (13.3)	6 (18.2)				
Unknown	22 (24.2)	1 (1.3)	6 (18.2)				
DMR duration, mth; median (min;max)	41.3 (23.0; 124.3)	34.6 (24.5; 117.5)	34.7 (24.1; 65.7)	**0.032**	**0.048**	0.158	0.731
Imatinib duration, mth; median (min;max)	82.7 (38.5; 149.9)	75.8 (38.4; 143.5)	71.3 (56.7; 84.9)	**0.033**	0.112	0.059	0.399
BCR::ABL1[a] at TKI stop, N (%)				**0.006**	**0.042**	**0.015**	0.528
<0.0023%[IS]	68 (89.5)	49 (65.3)	20 (60.6)				
≥0.0023%[IS]	8 (10.5)	18 (24)	11 (33.3)				
Unknown	15 (16.5)	8 (10.7)	2 (6.1)				

[a]Transcript levels determined by droplet digital polymerase chain reaction. *P* values corrected by the Holm-Bonferroni method. Statistically significant values are shown in bold. MRec: molecular recurrence; ELTS: EUTOS long-term survival; DMR: deep molecular response; mth: months; TKI: tyrosine kinase inhibitor; IS: International Scale.

count the reported competing events (10 patients resumed TKI without MMR loss according to the initial definition of molecular recurrence and protocol recommendations and 3 patients died before MMR loss), the 5- and 9-year cumulative incidences of molecular recurrence and resumption of treatment, molecular recurrence and death, or molecular recurrence alone were estimated at 56.64%, 53.29%, and 51.62% and 65.52%, 62.17% and 60.5%, respectively.

The differences are slight but considering the median age at imatinib discontinuation (61.9 years) and a longer follow-up, the incidence of CML-unrelated deaths would increase over time and emphasize these differences. Furthermore, more deaths reported in large cohorts of patients treated with imatinib front-line are now not related to CML.[11,12] Among these, deaths related to second malignancies are not unusual and raise another question concerning additional potential competing events in the evaluation of a TFR strategy. Indeed, we do not know the effect of various chemotherapeutic regimens on leukemic CML stem cells and their possible role in the maintenance of DMR after TKI discontinuation. Although such treatment was not reported in this update, we believe that chemotherapy for another malignancy after TKI discontinuation should also be considered and reported as a competing event.

The second objective of this update of the STIM2 study was to identify and characterize potential late molecular recurrences. Indeed, updates with a longer follow-up of the two pivotal studies (STIM1 and TWISTER) did not report any molecular recurrence beyond 27 months after imatinib discontinuation, with a median follow-up of 6.4 and 8.6 years, respectively.[13,14] Since these reports we have identified one molecular recurrence 7 years after imatinib discontinuation in the STIM1 study (update currently ongoing) and preliminary results of the EURO-SKI discontinuation trial based on a larger cohort of patients show that the cumulative incidence of molecular recurrences continues to increase slowly 24 months after TKI discontinuation,[15] with late molecular recurrences beyond 36 months estimated to occur in approximately 10% of patients in the AFTER-SKI study.[16] Rousselot *et al.*[17] recently reported the occurrence of late molecular recurrences, defined as loss of MMR after 2 years of TKI cessation, in nine out of 65 patients for an estimated incidence of 13.8% and a median time from stopping TKI treatment to molecular recurrence of 3.6 years. With respect to the first report published 2 years ago and in order to identify potential predictive factor(s) of late molecular recurrences, we performed this analysis in the “per-protocol” population (199 patients).[9] The long-term follow-up of the STIM2 study confirms that late molecular recurrence can occur beyond 24 months.

In this study, molecular recurrences occurred between 0 to 6 months, 6 to 24 months and after 24 months in 75 (69%), 15 (14%) and 18 (17%) patients, respectively. Notably, the kinetics of the molecular recurrence, based on DMR4-MMR loss, were significantly different between these three subgroups with a median time from loss of MR4 to MMR loss of 1, 7 and 22 months, respectively.

As previously reported,[4] most molecular recurrences occur during the first 6 months following imatinib discontinuation and although molecular recurrences continue to occur beyond 6 months, the probability of remaining treatment-free increases over time. Taking into account the late molecular recurrences reported in this study, the TFR at 5 years among patients without molecular recurrence at 6 months or 24 months were 71.9% [61.03- 80.3] and 87.03% [79.8- 94.2], respectively.

We analyzed predictive factors for the loss of MMR before and after 6 months or 24 months. While DMR duration as well as duration of imatinib treatment and *BCR::ABL1* levels at TKI cessation, quantified by RT-ddPCR, are associated with the risk of molecular recurrence as previously reported, these factors were unable to predict molecular recurrence after 24 months.

We therefore hypothesize that these late molecular recurrences may be explained by mechanisms that are intrinsic to the host rather than to the disease. It has been suggested by several studies[18–23] that these mechanisms include the involvement of the immune system in the control of molecular residual disease after TKI cessation. Indeed, quantitative changes in several immune effectors (such as natural killer cells,[18,20,22,23] innate $CD8^+$ T cells,[21] $CD86^+$ plasmacytoid dendritic cells,[19] $\gamma\delta^+$ T cells, $CD4^+$ regulatory T cells[22,23] and myeloid-derived suppressor cells[23]) have been associated with a higher likelihood of achieving TFR. Interestingly, patients with a molecular recurrence occurring before 6 months had higher natural killer-cell levels compared to patients with no molecular recurrence whereas patients relapsing beyond 6 months had similar natural killer-cell counts to those of non-relapsing patients.[20] It would be interesting to see whether, with a longer follow-up and using the 24-month threshold, these differences are maintained or not. It is possible that other elements of the immune system are also involved in the occurrence of molecular recurrences beyond 24 months. Unfortunately, no immunological data were collected in STIM2 and only a prospective study with a long-term follow-up evaluating all the potential factors would allow these hypotheses to be investigated.

In conclusion, despite a decrease in the risk of molecular recurrence over time, late events are possible and warrant the need for sustained, long-term molecular monitoring. However, based on the kinetics of late molecular recurrences observed in this study with a median time from MR4 loss to MMR loss of 22 months, *BCR::ABL1*^{IS} assessment can be safely performed every 6 months in patients

without molecular recurrence 2 years after imatinib cessation.

Disclosures

SD is a speaker for Novartis and Incyte. FEN is a consultant for BMS, Incyte Biosciences, Sun Pharma Ltd and Novartis, and a speaker for Novartis, Incyte Biosciences and BMS. DR is a consultant for Novartis, and a speaker for Novartis, BMS, Incyte Biosciences and Pfizer. PC-M. is a speaker for Novartis, BMS and Pfizer. AC is a speaker for BMS, Incyte Biosciences and Novartis. VC is a speaker for Incyte Biosciences. FR-H is a speaker for Novartis, BMS, Incyte Biosciences and Pfizer; AG-B is a speaker for Novartis, BMS, Incyte Biosciences and Pfizer. LL is a speaker for Novartis, Incyte Biosciences, BMS and Pfizer. PR is a consultant for Pfizer, and a speaker for BMS, Novartis, Incyte Biosciences and Pfizer. MB is a speaker for Novartis and BMS. GE is a speaker for BMS, Incyte Biosciences, Novartis and Pfizer. FXM is a consultant for Novartis and a speaker for Incyte Biosciences, BMS, Novartis and Pfizer. VD, PL, JL, MEB, MG, JCI, PT, HJ-A, JM, HJ, BJ, PZ, TH, BV, EC, FL , FR and SM have nothing to disclose.

Contributions

SD, FXM, GE, FEN and DR designed the study. SD, GE and FXM wrote the manuscript. SM performed the statistical analyses. FR monitored all the centers and helped in collecting the data. FEN, PCM, AC, ME-B, FR-H, VC, BV, VD, PL, PR, D, AG-B, LL, JL, MG, J-CI, PT, HJ-A, JM, HJ, BJ, PZ, TH, BV, MB, EC, FG, FL, GE and FXM enrolled patients in the study, followed them up, and provided clinical data. All authors proof-read the manuscript and agree on its content.

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Data-sharing statement

All the data are available on request to the corresponding author.

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