Predictive factors for successful imatinib cessation in chronic myeloid leukemia patients treated with imatinib

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Although recent studies have suggested that cessation of imatinib (IM) in chronic myeloid leukemia patients can be associated with sustained response, further validation is needed to explore predictive factors. In a prospective, multicenter study, chronic phase patients were eligible for cessation of IM therapy after more than 3 years if they had no detectable BCR-ABL1 transcript for at least 2 years. A total of 48 patients with a median age of 47 years (19–74 years) were enrolled. Twenty patients received IM for post-transplant relapse. After a median follow-up of 15.8 months (1.4–28.2 months) after IM discontinuation, nine of the non-transplant group lost undetectable molecular residual disease (UMRD) and major molecular response (MMR), whereas none of the 20 patients in the transplant group experienced UMRD loss. Probabilities for sustained MMR and UMRD were 64.4% and 66.3% in the non-transplant group, respectively. Of nine patients re-treated with IM, eight patients re-achieved MMR at a median of 1.7 months (0.9–2.8 months). Seven of these patients re-achieved UMRD at a median of 5.6 months (2.8–12.1 months). Previous transplantation, IM duration, and UMRD duration were significantly associated with sustained molecular responses. Our data strongly suggest that immunological control contributes to sustained suppression of residual leukemia cell expansion and that IM can be safely discontinued in patients with post-transplant relapse. Am. J. Hematol. 88:449–454, 2013. © 2013 Wiley Periodicals, Inc.

Introduction

Imatinib (IM) has been the standard of care for chronic phase (CP) chronic myeloid leukemia (CML) and 15–50% of CP CML patients who receive IM treatment achieve an undetectable molecular response (MR) at more than 5 years after initiation of treatment [1–3].

Although previous reports showed that the discontinuation of IM in patients with less than an undetectable MR has led to relapse [4–6], several studies have reported the possibility of successful IM discontinuation in CML patients with undetectable BCR-ABL1 transcripts [7–10]. Mahon et al. focused on the patients with undetectable molecular residual disease (UMRD) status of at least 2 years' duration and showed that the probability of persistent UMRD at 12 months after IM cessation was 41%, suggesting that cessation was plausible in some patients with sustained UMRD [11].

Hypotheses that may explain sustained UMRD after IM discontinuation include stem cell depletion, stem cell exhaustion, and immunological control [12]. Takahashi et al. observed that prior interferon treatment was significantly associated with a higher rate of sustained UMRD, which supported an immunological mechanism [13]. This result contrasts with that of Mahon who concluded that prior interferon treatment was not associated with sustained UMRD [11]. Precise identification of patients for IM cessation and the underlying mechanisms of sustained UMRD thus remain elusive.

This multicenter prospective Korean Imatinib Discontinuation Study (KIDS) was performed to identify predictors for safer, successful IM discontinuation and to explore additional contributing factors for sustained MRs in specific patient groups. Additionally, given the significant numbers of post-transplant cases, comparisons between transplant and non-transplant patient outcomes were analyzed.

Design and Methods

Study patients.

CP CML patients who were treated with IM for more than 3 years and who had undetectable levels of BCR-ABL1 transcripts as determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) for at

Additional Supporting Information may be found in the online version of this article.

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least 2 years were eligible for the KID study. Previous treatment with interferon was allowed. Patients who received IM for post-transplant relapse and sustained UMRD for more than 2 years (transplant group) were also eligible. For all transplant patients, repeated graft-versus-host-disease (GVHD) assessments were consecutively performed. Written, informed consent was obtained from each patient before participation in this trial. This study was approved by the Institutional Review Board of each participating institution and was conducted in accordance with the Declaration of Helsinki. The study protocol was registered with the National Institutes of Health clinical trial registry at www.cinicaltrials.gov as #NCT01564836.

Study objectives.

Main study objectives were (1) to evaluate the probability of sustained UMRD and major molecular response (MMR) at 12 months after discontinuation, (2) to measure the duration of the sustained MRs in individual patients, and (3) to identify contributing factors for successful sustained MRs. If loss of MMR occurred, IM was re-introduced based on 'molecular relapse (MolR)'. In patients who lost MMR, the probability of re-achieving MMR and UMRD, as well as the time until re-achievement of MMR/UMRD after IM resumption were evaluated.

Molecular monitoring.

To confirm undetectable BCR-ABL1 transcript levels by qRT-PCR for at least 2 years, duplicated qRT-PCR analyses were performed at more than six time-points. For all screening and subsequent follow-up samples, duplicated qRT-PCR and nested RT-PCR with at least 4.5-log sensitivity were tested in the central laboratory (Cancer Research Institute, The Catholic University of Korea, Seoul, Korea). The quality of RNA was assessed using Experion automated electrophoresis (Applied Bio-Rad, Hercules City, CA), and only qRT-PCR results with more than 50,000 ABL1 transcripts were analyzed. MMR was defined as a BCR-ABL1 transcript level of 0.1% or lower on the international scale (IS). UMRD was defined as negative PCR results in both duplicated qRT-PCR and nested RT-PCR assays.

After IM discontinuation, the MR was monitored using the following schedule: every month for the first 6 months, every 2 months up to 12 months, and every 3 months thereafter. The loss of MMR and UMRD were defined after confirmation by 2 consecutive tests within 4 weeks, and in cases of MMR loss, the MR was evaluated every month until MMR was re-achieved.

On the day after engraftment and at various time points of post-transplant, donor chimerism was tested using the AmpFlSTRIdentifiler PCR Amplification kit (Applied Biosystems, Foster City, CA). Chimerism results were reported as "percent donor chimerism" [14,15].

Statistical analysis.

Time to loss of UMRD and MMR were calculated starting from the date of IM discontinuation to the date of the first detection of BCR-ABL1 by 2 consecutive analyses and, for patients who did not lose MR, were assigned by default to the date of the last molecular examination. The probabilities of persistent UMRD and MMR after IM discontinuation were plotted using the Kaplan–Meier method. Potential prognostic factors for persistent UMRD and MMR were assessed using the two-tailed log-rank test and included age, sex, Sokal risk group, previous transplantation, time from CML diagnosis to initiation of IM therapy, duration of IM therapy, time from IM therapy to UMRD, and UMRD duration before IM discontinuation.

Receiver operating characteristic curve analysis was used for the categorization of quantitative factors. Covariates with a P value of <0.1 in the univariate analyses were added to Cox proportional hazards regression models. The association between categorical variables was assessed using either χ^2 or Fisher's exact tests. The Mann–Whitney U-test was utilized to compare continuous variables, and Spearman correlation coefficients were used to evaluate associations for continuous variables.

TABLE I. Patient characteristics

Parameters	Total
	($N = 48$)
Age (years), median (range)	47 (19–74)
Sex, Female, *N* (%)	28 (58)
Transcript type, *N* (%)	
b3a2	31 (65)
b2a2	12 (25)
other[a]	5 (10)
Previous allografting, *N* (%)	
No	28 (58)
Yes	20 (42)
Previous interferon therapy, *N* (%)	
No	45 (94)
Yes	3 (6)
Sokal risk, *N* (%)	
Low	20 (42)
INT	16 (33)
High	8 (17)
NA	4 (8)
Mean daily dose for first 1 year, (mg) median (range)	400 (200–600)
Time from Dx to IM therapy initiation (mos), median (range)	3.4 (0–113.9)
Time from IM therapy to UMRD (mos), median (range)	25.7 (0.5–63.5)
UMRD duration before IM cessation (mos), median (range)	56.8 (24.0–105.7)
IM therapy duration (mos), median (range)	85.3 (39.9–129.5)

Dx, diagnosis; IM, imatinib; INT, intermediate; mos, months; NA, not available.

[a] Other included one patient with b3a2 and b2a2; four patients, data not available.

Results

Patient characteristics

Patient characteristics are summarized in Table I. A total of 48 UMRD patients (including 20 men and 28 women) were enrolled at eight university hospitals. With a median age of 47 years (range, 19–74 years), the distribution of low, intermediate and high Sokal risk scores were 42%, 33% and 17%, respectively, with 8% unknown risk. Twenty-eight patients (58%) had IM without transplantation, and 20 patients (42%) received IM for post-transplant relapse. Three patients (including 1 in the non-transplant group and 2 in the transplant group) had a previous history of interferon treatment. Prior to discontinuation in all patients, the median time to UMRD was 25.7 months (range, 0.5–63.5 months) and the median IM duration was 85.3 months (range, 39.9–129.5 months) including 56.8 months (range, 24.0–105.7 months) of sustained UMRD. Detailed characteristics of the transplant group are summarized in Table II. All of 20 patients engrafted. Six developed grade 2 acute GVHD and completely resolved. Chronic GVHD of the limited type developed in five patients and resolved after a median of 12.4 months (range, 1.5–28.3 months). The median time to relapse was 25.7 months (range 5.7–83.8 months), and there were seven hematological relapses (HRel), five cytogenetic relapses (CyRel), and eight MMR losses (MRel) at the time of IM start. Four patients received donor lymphocyte infusion (DLI) containing 1–5 $\times$ 10^6 $CD3^+$ T cells/kg once ($n = 3$) or twice ($n = 1$). Among them, two patients received IM + DLI for MMR loss, and re-achieved MMR at 5.8 and 9.6 months, respectively, and UMRD at 11.0 and 9.6 months, respectively. Although the other two patients received DLI alone at the time of MMR loss, they progressed to cytogenetic (80%) and

hematological relapse (CP) at post-DLI 17.5 and 11.2 months, respectively, and then started IM. Therefore, the impact of DLI on UMRD achievement was minimal. The median time to UMRD was 18.5 months (range, 0.5–54.7 months), and IM was discontinued after a median UMRD duration of 70.1 months (range 29.0–105.7 months). There were no significant differences in IM duration, time to UMRD, or UMRD duration between patients grouped by post-transplant relapse.

TABLE II. The characteristics of patients with previous allografting

Parameters	$N = 20$ (%)
Sex of donor, Female	4 (20)
Sex pair (donor–recipient)	
Female to male/Others[a]	1 (5)/19 (95)
Disease phase at diagnosis	
CP/AP	20 (100)/0 (0)
Disease phase at transplant	
CP/AP	19 (95)/1 (5)
Treatment prior to allografting	
No TKI/IM	18 (90)/2 (10)
Donor type (HLA compatible)	
Related/unrelated	18 (90)/2 (10)
ABO match	
Match/mismatch	9 (45)/11 (55)
Sources of graft	
BM/PBSC	17 (85)/3 (15)
Conditioning intensity	
MAC/RIC	17 (85)/3 (15)
GVHD prophylaxis	
CS based/FK506 based	17 (85)/3 (15)
Acute GVHD, (%)	
None	14/20 (70)
Grade 2	6/20 (30)
Grade 3–4	0/20 (0)
Persistent duration (mos), median (range)	1.2 (0.6–15.4)
Chronic GVHD, (%)	
None	12/17[b] (71)
Limited	5/17 (29)
Extensive	0/17 (0)
Persistent duration (mos), median (range)	12.4 (1.5–28.3)
IST duration (mos), median (range)	10.5 (4.5–29.2)
Type of post-SCT relapse, (%)	
HRel	7/20 (35)
CyRel	5/20 (25)
MRel (loss of MMR)	8/20 (40)
DLI after post-SCT relapse	
Yes/No	4 (20)/16 (80)
Time from Dx to IM therapy initiation (mos), median (range)	36.5 (14.1–113.9)
Time from IM therapy to UMRD (mos), median (range)	18.5 (0.5–54.7)
UMRD duration before IM cessation (mos), median (range)	70.1 (29.0–105.7)
IM therapy duration (mos), median (range)	98.3 (39.9–127.0)

BM, bone marrow; CS, cyclosporine; CyRel, cytogenetic relapse;DLI, donor lymphocyte infusion; F, female; GVHD, graft-versus-host disease; HRel, hematological relapse; IST, immunosuppressant therapy; M, male; MAC, myeloablative conditioning; MRel, molecular relapse; PBSC, peripheral blood stem cell;RIC, reduced-intensity conditioning; TBI, total body irradiation.

[a] Others included male to male (n=6), female to female (n=3), and male to female (n=10).

[b] Three patients relapsed within 100 days from HSCT.

Outcomes after imatinib discontinuation

After a median follow-up of 15.8 (range, 1.4–28.2) months after IM discontinuation, nine patients (19%) lost UMRD and all of them subsequently lost MMR at a median time of 3.0 months (range, 1.9–7.6 months). All patients with MMR loss were in non-transplant group, whereas none of the 20 patients in the transplant group experienced MMR loss (Table III). Although one patient in the transplant group lost UMRD, he spontaneously re-achieved UMRD at subsequent analyses.

All nine patients who lost MMR were re-treated with IM for a median of 11.1 months (range, 2.8–17.0 months). Eight of these patients re-achieved MMR at a median of 1.7 months (range, 0.9–2.8 months) after resuming IM therapy and seven of these patients re-achieved UMRD at a median of 5.6 months (range, 2.8–12.1 months). The other one patient was re-treated with IM for 2.8 months and has shown gradually decreasing BCR-ABL1 transcripts.

The overall 12-month probability of sustained MMR and UMRD were 79.9% and 80.8%, respectively (Fig. 1). Probabilities for sustained MMR and UMRD were 64.4% and 66.3% in non-transplant group, respectively (Fig. 2). Probability of sustained UMRD is higher than that of MMR because one patient simultaneously lost UMRD and MMR, whereas eight patients lost UMRD and later lost MMR.

In the 37 patients with follow-up of more than 12 months (median 20.9 months, range 11.7–28.2), the overall 12-month probability of sustained MMR and UMRD were 78.4% and 78.4%, respectively. Probabilities for sustained MMR and UMRD were 63.6% and 63.6% in the non-transplant group ($n = 22$), respectively.

Predictive factors affecting sustained molecular responses

The results of the univariate analysis are summarized in Table IV. Of the 48 patients, the patients in transplant group had a higher probability of sustained MRs than patients in the non-transplant group (100% vs. 64.4% in MMR, $P = 0.004$; 100% vs. 66.3% in UMRD, $P = 0.005$). Longer duration of IM and duration of UMRD were also determined to be potential predictive factors for sustained MMR as well as UMRD (Supporting Information Fig. 1), whereas age, sex, transcript type, Sokal risk, first year dose intensity, and time from IM to UMRD were not associated with sustained MRs. In this study, the duration of IM therapy was correlated with the duration of UMRD ($r_s = 0.662$, $P < 0.001$). Therefore, considering this close relationship, these factors were not entered into the multivariate models.

Separate analysis in the non-transplant group revealed that longer duration of IM and duration of UMRD remained significant predictive factors for MMR loss as well as UMRD loss (Supporting Information Table SI).

TABLE III. Outcomes after imatinib cessation

	Total	Non-transplant group	Transplant group
	($N = 48$)	($N = 28$)	($N = 20$)
Follow-up duration (mos), median (range)	15.8 (1.4–28.2)	15.5 (1.4–27.8)	23.2 (3.7–28.2)
Loss of UMRD, *N* (%)	9 (19)	9 (32)	0 (0)
Loss of MMR, *N* (%)	9 (19)	9 (32)	0 (0)
Time to loss of MMR, mos (range)	3.0 (1.9–7.6)	3.0 (1.9–7.6)	–
Lossof MMR, re-treated with IM ($n = 9$)			
Duration of IM, mos (range)	11.1 (2.8–17.0)	11.1 (2.8–17.0)	–
MMR re-achievement, N(%)	8 (89)	8 (89)	–
UMRD re-achievement, N(%)	7 (78)	7 (78)	–
Time to MMR re-achievement, mos (range)	1.7 (0.9–2.8)	1.7 (0.9–2.8)	–
Time to UMRD re-achievement, mos (range)	5.6 (2.8–12.1)	5.6 (2.8–12.1)	–

IM, imatinib; mos, months; UMRD, undetectable molecular residual disease.

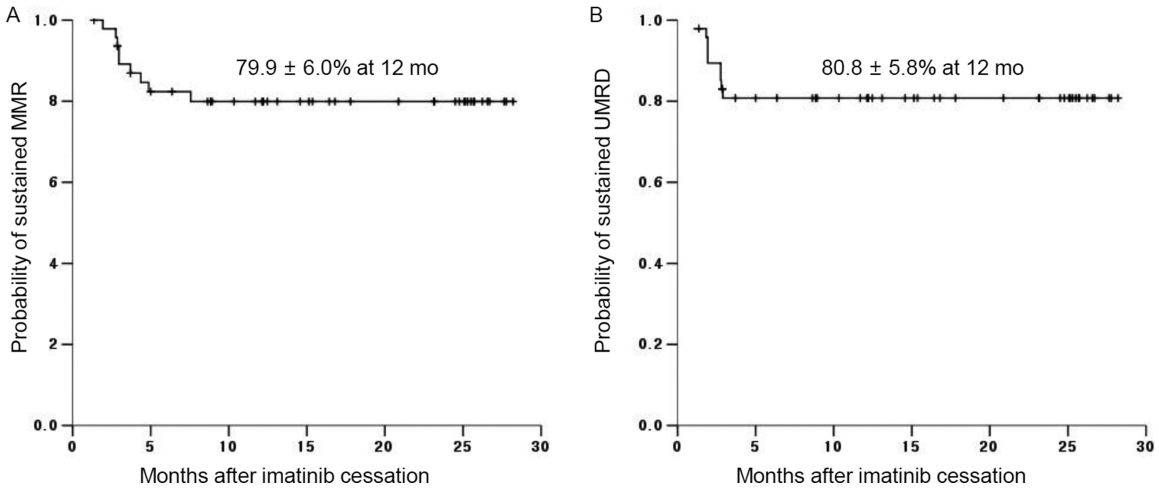

Figure 1. Kaplan–Meier estimates of outcomes after imatinib cessation relative in all patients. (A) probabilities for sustained MMR were 79.9% and (B) probabilities for UMRD were 80.8%.

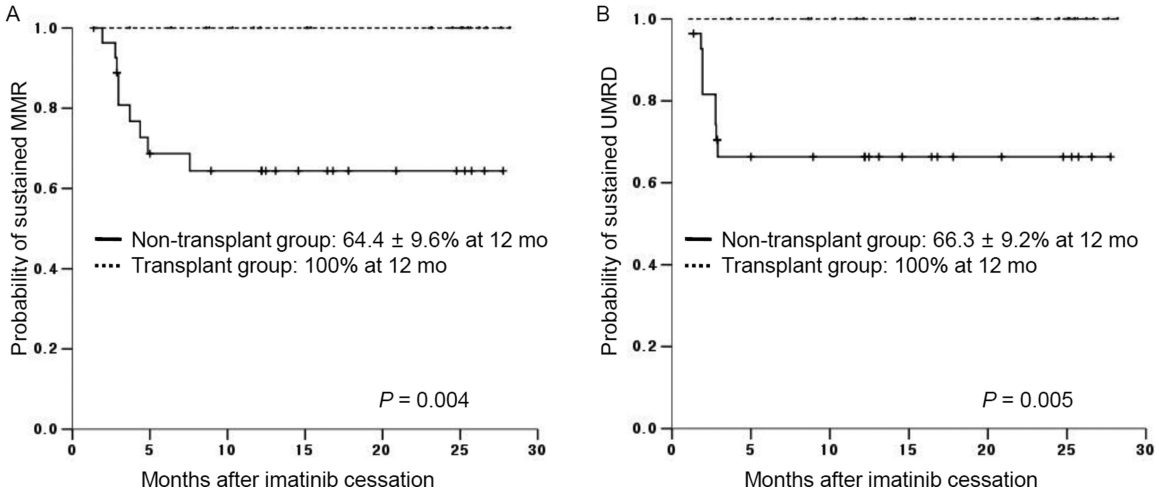

Figure 2. Kaplan–Meier estimates of outcomes after imatinib cessation relative to prior allografting (Yes/No). Relative to previous allografting (Yes/No), (A) probabilities for sustained MMR were 100% and 64.4%, respectively and (B) probabilities for UMRD were 100% and 66.3%, respectively.

Sequential assessment of chimerism in transplanted patients

The median donor chimerism at relapse was lower than the median donor chimerism on the day after engraftment (90.2% vs. 99.2% in eight patients; $P = 0.006$). Regarding donor chimerism at subsequent time points, the proportion increased to median percentages of 96.6 (range, 94.6–100 in 11 patients) and 97 (range, 93.6–100 in 13 patients) at UMRD on IM treatment and after IM cessation, respectively.

Discussion

The goal of this study was to evaluate additional prognostic factors predictive for sustained MMR and UMRD after IM discontinuation to better characterize and achieve successful IM discontinuation. In this study, the estimation of MR was assessed in detail by MMR and UMRD, and the 12-month probability of sustained UMRD of all patients was 80.8% (78.4% in 37 patients with at least 12 months follow-up), a higher rate than that reported by the 'STIM' (Stop imatinib) trial of Mahon et al [11] and others [7,10,13]. In a separate analysis excluding transplanted patients, the overall 12-month probability of sustained UMRD remained higher than that of the STIM trial. Possible explanations may different characteristics of enrolled patients due to the stringent PCR sensitivity criteria used to assess the accurate measurement of BCR-ABL1 transcript levels prior to discontinuation. Our study included the patients with allografting and had longer durations of IM therapy and UMRD before the discontinuation of IM than the aforementioned study. Moreover, a majority of enrolled patients (35/48; 72.9%) in our study were serially monitored with long-term serial molecular tests in a single standardized laboratory before entry into the study. A retrospective study by Takahashi et al. analyzed the clinical outcomes of CML patients who have been off IM therapy for at least 6 months and showed that the estimated 5-year relapse-free survival rate was 47% [13]. In another retrospective study of 14 CP CML patients treated with front-line IM therapy, the 1-year probability of sustained UMRD was 28.6%, which included patients who had fewer than 2 years of undetectable duration before IM discontinuation [7]. These results emphasize the importance of a more stringent PCR assay and the precise selection of patients who discontinued IM.

TABLE IV. Univariate analyses of variables affecting the probability of sustained MMR and UMRD

Variables	No	Probability of sustained MMR, % (95% CI)	*P*	Probability of sustained UMRD, % (95% CI)	*P*
Age of patient, years	48	(continuous)	0.270	(continuous)	0.309
Sex of patient			0.778		0.843
Male	20	78.0 (61.1–99.6)		78.6 (62.0–99.6)	
Female	28	81.2 (67.6–97.6)		82.1 (69.1–97.6)	
Transcript type			0.977		0.859
b3a2	31	79.9 (66.7–95.7)		80.1 (67.0–95.7)	
b2a2	12	82.5 (63.1–100)		83.3 (64.7–100)	
NA	5	66.7 (30.0–100)		66.7 (30.0–100)	
Previous allografting			0.004		0.005
No	28	64.4 (48.1–86.2)		66.3 (50.6–87.0)	
Yes	20	100		100	
Sokal risk			0.887		0.951
Low	20	77.4 (60.1–99.8)		79.7 (63.7–99.6)	
Intermediate	16	81.3 (64.2–100)		81.3 (64.2–100)	
High	8	85.7 (63.3–100)		85.7 (63.3–100)	
NA	4	75.0 (42.6–100)		75.0 (42.6–100)	
Mean daily dose for first 1 year	48	(continuous)	0.590	(continuous)	0.634
Time from IM therapy to UMRD			0.353		0.310
$<$25 months	23	85.0 (70.5–100)		87.0 (74.2–100)	
$\geq$25 months	25	75.0 (59.5–94.5)		75.1 (59.7–94.5)	
UMRD duration before IM cessation			$<$0.001		0.001
$<$42 months	13	41.7 (19.9–87.1)		50.3 (28.7–88.3)	
$\geq$42 months	35	91.1 (81.9–100)		91.4 (82.6–100)	
IM therapy duration			0.008		0.008
$<$78 months	19	59.6 (40.4–88.1)		63.2 (44.8–89.0)	
$\geq$78 months	29	92.7 (83.5–100)		92.9 (83.8–100)	

The confirmation of predictive factors for sustained MR and for conversion of stable remissions *on therapy* to stable remissions *off therapy*, i.e., cures rather than functional cure, is a key issue. Increased IM therapy duration was reported by previous studies to be strongly associated with a higher probability of sustained UMRD [7,11,13]. Sokal risk score [7,11], sex [11], prior interferon treatment [13], UMRD duration before IM discontinuation [13], and time to UMRD [7] were also reported as potential predictive factors, albeit with conflicting data. In our study, among patients with previous transplantation, there was no loss of MMR and UMRD. These data implicate that immunological control, as indicated by data from patients with allografting was the strongest factor for sustained MR. To exclude the influence of allografting, we performed separate analyses with a non-transplant group and found that IM duration and UMRD duration were significant predictors for sustained MR. From our results regarding predictors of sustained MR, IM duration was found to be consistent with findings from the STIM trial and others; however some variables, including the Sokal risk score and sex, were not consistent [11]. The significance of IM therapy duration supports the hypothesis that long-term IM therapy may induce leukemic stem cell exhaustion [12,16]. UMRD duration while on IM therapy, which partly overlapped with IM duration, was another significant predictive factor. However, we could not confirm Sokal risk score as a predictor, perhaps of the sensitivity of CML progenitors to IM therapy. This discrepancy may be caused by the relatively young age of our cohort. Interestingly, among the nine patients who lost MMR, six patients lost MMR within 3 months of discontinuing IM, which is consistent with a model of relapsing CML in which relapse is caused by residual leukemic cells rather than by slow-cycling stem cells [17,18]. These data are also similar to the findings of the STIM trial, in which 35 of 42 relapses occurred within 3 months [11]. Although two other patients lost MMR at approximately 4 months after discontinuing IM, one patient who received IM as an initial treatment and discontinued it after 77 months with a UMRD duration of 65 months relapsed at 8 months after IM discontinuation. This suggests that other factors may control the kinetics of relapse.

Notably, our study found an association between previous allografting and sustained MR. This implies that the immunological control exerted by the remaining, donor-derived cytotoxic T-cells (CTLs) may participate in the ongoing suppression of CML clones after IM discontinuation. The probability of post-transplant relapse is known to be reduced by the graft-versus-leukemia (GVL) effect in CML. Particularly, the effect of DLI on post-transplant relapse has highlighted the role of donor-derived T cells [19,20]. However, to our knowledge, there has been no evidence suggesting that immunological effects akin to those responsible for relapse-free survival after allografting or re-establishing remission after DLI occur in patients who receive IM treatment as post-relapse therapy and can translate into successful IM discontinuation. To quantify the changes in donor-derived cells, we performed a serial STR assay and found that the median value of donor cells at the time of post-transplant relapse was lower than that seen during engraftment and was restored to over 95% after achieving undetectable MR by IM therapy. Subsequently, complete donor chimerisms were sustained after IM discontinuation. This finding suggests that donor-derived CTLs can be re-expanded by continuing IM therapy, which may suppress the potential expansion of remaining CML clones, in particular with TKI cessation. The potentially significant effect on donor-derived cells of TKI cessation, in light of the potential immunomodulatory effects of other kinase targets aside from *bcr-abl,* merits investigation as the study of cessation of subsequent generation TKIs increases.

Interferon treatment may contribute to the elimination of leukemic cells via the induction of a tumor-specific cytotoxic T cell response [21–23], and can maintain the response induced by IM therapy [24–26]. Although the three patients with prior interferon treatment in our study did not relapse, it is difficult to make any conclusions because of the small number of patients, which included two patients with allografting.

Despite the limitations of a small number of patients and the inclusion of cases with incomplete long-term follow-up, the observed difference in outcome according to previous allografting in this study provides insight into the

importance of immunological control after IM discontinuation and suggests that further research is warranted in this phenomenon. The improved probability of sustained MR in patients who had longer IM therapy and UMRD durations prior to IM discontinuation underscores the necessity for utilizing increasingly sensitive PCR technology and more potent TKIs. Further clinical investigations in a larger patient population with longer follow-up are needed.

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Conflict of interest

There is no conflict of interest in connection with this article.

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