

Significance of deeper molecular responses in patients with chronic myeloid leukemia in early chronic phase treated with tyrosine kinase inhibitors

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Most patients with chronic myeloid leukemia (CML) in chronic phase (CP) treated with tyrosine kinase inhibitors (TKI) achieve complete cytogenetic response (CCyR). An increasing number of patients also achieve deep molecular responses (MR). We determined the frequency and significance of deep MR after TKI therapy for CML in CP. MR included: major molecular response (MMR), MR4, MR4.5, and undetectable transcripts (UND), i.e., BCR-ABL/ABL of ≤ 0.1 , ≤ 0.01 , $\leq 0.0032\%$, and undetectable transcripts, respectively. Four hundred eighty-three patients received imatinib 400 mg/day (IM400, 71, July 2000 to April 2001), imatinib 800 mg/day (IM800, 204, June 2001 to July 2005), nilotinib (106, July 2005 to date), or dasatinib (102, November 2005 to date). UND rates at 36 months were 18.1, 30.6, 29.2, and 28.6%, respectively. Patients achieving UND have superior transformation-free survival (TFS) and overall survival (OS) versus those obtaining \leq MMR, but not other MR levels. At the 18- and 24-month landmark analysis, patients achieving UND have no advantage in TFS and OS compared to those achieving a lesser degree of MR. Among patients achieving MR4.5, those who maintain it for ≥ 2 years (*susMR4.5*) have no additional benefit in TFS or OS. Most patients with early CP CML receiving TKI achieve MMR. BCR-ABL transcripts become undetectable in a significant fraction of them. Deeper MR at 18 or 24 months are not associated with a benefit in TFS or OS. Furthermore, achieving *susMR4.5* does not appear to further reduce the risk of transformation or death. Am. J. Hematol. 88:1024–1029, 2013. © 2013 Wiley Periodicals, Inc.

Introduction

Tyrosine kinase inhibitors (TKI) have dramatically changed the treatment landscape of chronic myeloid leukemia (CML) improving the rates of complete cytogenetic response (CCyR), minimizing transformation to accelerated phase (AP) or blastic phase (BP), and prolonging patient survival [1,2]. Most patients with CML in early chronic phase (CP) achieve CCyR with imatinib at standard or higher doses [3,4], or with second-generation TKIs (nilotinib [5,6] or dasatinib [7,8]). The achievement of CCyR correlates with improved event-free survival (EFS), transformation-free survival (TFS), and overall survival (OS) across all Sokal risk categories [9].

Molecular testing with reverse-transcriptase (RT)-polymerase chain reaction (PCR) for the BCR-ABL fusion transcript shows persistent CML in the majority of patients at the time of CCyR [10,11]. By molecular analysis, a CCyR corresponds approximately to a 2-log reduction in the BCR-ABL transcript levels, or BCR-ABL transcripts of 1% in the International Scale (IS). Major molecular response (MMR) was initially defined in the International Randomized Study of Interferon versus STI571 (IRIS) as a 3-log reduction of the transcript levels from baseline [12] and subsequently standardized by the IS as transcripts of $\leq 0.1\%$ [13]. MMR achieved at 18 months has been associated with improved EFS [12,14–18]. However, no benefit in OS has been demonstrated of achieving MMR among patients that achieve CCyR.

With longer follow-up and the use of more potent TKI modalities, an increasing number of patients achieve and maintain deeper levels of molecular response (MR), including undetectable BCR-ABL transcripts (UND). Moreover, a fraction of patients who maintain a BCR-ABL/ABL ratio (%BCR-ABL) of ≤ 0.0032 , also referred to as MR4.5, for at least 2 years on imatinib, may not relapse upon treatment

discontinuation [19,20]. However, whether deeper responses confer long-term survival or other clinical benefit, beyond the potential for treatment discontinuation, is not fully defined.

We analyzed patients with early CP CML treated with four different TKI modalities to assess the frequency and prognostic impact of different levels of MR. We also studied the clinical significance and predictive factors for the achievement of a MR4.5 maintained for ≥ 2 years.

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This research has been supported in part by MD Anderson Cancer Center Support (Grant CA016672) and the National Institute of Health (Grant P01 CA049639).

Conflict of interest: H.K.M. has received research funding from Bristol-Myers Squibb, Ariad, Pfizer, and Novartis. E.J.J. has received honoraria from Pfizer, Novartis, and Bristol-Myers Squibb. F.R. has received research funding from Bristol-Myers-Squibb and has received honoraria from Bristol-Myers Squibb, Novartis, and Pfizer. J.E.C.: is consultant for Ariad, Pfizer, and Teva and has received research funding from Ariad, Bristol-Myers Squibb, Novartis, Chemgenex, and Pfizer. L.F., X.W., D.V., S.O., G.B., A.Q., G.G., S.V., and J.A.B. declare no conflicts of interest.

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Contract grant sponsor: MD Anderson Cancer Center; Contract grant number: CA016672; Contract grant sponsor: National Institute of Health; Contract grant number: P01 CA049639.

Received for publication 15 July 2013; Revised 24 July 2013; Accepted 25 July 2013

Am. J. Hematol. 88:1024–1029, 2013.

Published online 1 August 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/ajh.23560

TABLE I. Patient and Disease Characteristics at the Time of Treatment Start

Characteristic	All patients (N = 483)	IM400 (N = 71)	IM800 (N = 204)	NILO (N = 106)	DASA (N = 102)
Median age (years) [range]	48 [15–86]	48 [15–79]	48 [17–85]	50 [17–86]	48 [19–83]
Male:female (%)	283 (59):200 (41)	38 (53):33 (46)	124 (61):80 (39)	64 (60):42 (40)	57 (56):45 (44)
Median WBC ($\times 10^9/l$) [range]	28.3 [0.8–342.5]	20.7 [1.6–277.0]	27.5 [2.2–283.0]	41.5 [1.4–342.5]	27.1 [0.8–294.7]
Median hemoglobin (g/dl) [range]	12.3 [6.2–16.7]	12.7 [7.9–15.7]	12.4 [6.2–16.7]	12.3 [8.0–15.8]	11.9 [6.7–16.2]
Median platelets ($\times 10^9/l$) [range]	343 [52–2,954]	386 [126–1,425]	342 [96–1,458]	319 [52–2,954]	347 [108–1,782]
Median PB blasts (%) [range]	0 [0–12]	0 [0–2]	0 [0–12]	0 [0–7]	0 [0–5]
Median PB basophils (%) [range]	3 [0–19]	3 [0–16]	3 [0–19]	3 [0–13]	3 [0–19]
Median BM blasts (%) [range]	2 [0–14]	1 [0–6]	2 [0–14]	2 [0–8]	2 [0–8]
Median BM basophils (%) [range]	2 [0–15]	2 [0–9]	3 [0–15]	2 [0–13]	2 [0–12]
Median spleen size ^a (cm) [range]	0 [0–21]	0 [0–17]	0 [0–21]	0 [0–20]	0 [0–20]
Clonal evolution, n (%)	14 (3)	1 (1)	5 (2)	2 (2)	6 (6)
Sokal risk group, n (%)					
Low	297 (61)	46 (65)	112 (55)	67 (63)	72 (71)
Intermediate	138 (29)	20 (28)	66 (32)	29 (27)	23 (22)
High	48 (10)	5 (7)	26 (13)	10 (10)	7 (7)

^a From the costal arch.

IM400, imatinib 400 mg daily; IM800, imatinib 800 mg daily; NILO, nilotinib 400 mg twice daily; DASA, dasatinib 100 mg daily; WBC, white blood cell; PB, peripheral blood; BM, bone marrow.

Materials and Methods

Patients

Patients with CML in early CP enrolled in consecutive or parallel clinical studies of TKI therapy, all conducted at MD Anderson Cancer Center (MDACC), were included in this analysis. Treatments and study periods were: imatinib 400 mg/day (IM400) from July 2000 until April 2001 [21], imatinib 800 mg/day (IM800) from June 2001 to July 2005 [3,4], nilotinib 400 mg twice daily (NILO) from July 2005 to date [5] and dasatinib 100 mg/day (DASA) from November 2005 to date [7]. We included only patients enrolled on or before November 2011 to allow enough follow-up for this analysis. All studies were approved by the MDACC institutional review board and conducted in accordance with the declaration of Helsinki. Eligibility criteria for each study have been previously described [3,5,7,21].

Molecular testing and outcome measures

The level of MR was assessed by real-time quantitative (RQ)-PCR. A qPCR assay developed in the clinical molecular diagnostic laboratory at MDACC was used to monitor patients for the presence of BCR-ABL fusion transcripts as described previously [22]. Briefly, 2.85 μ g of total RNA at 100 ng/ μ l concentration was reverse transcribed in a 60- μ l final volume using Superscript II reverse-transcriptase enzyme (Life Technologies, Carlsbad, CA). A multiplex qPCR was then performed using 5 μ l of cDNA in a single tube to detect BCR-ABL transcripts e1a2, e13a2 (b2a2), and e14a2 (b3a2) along with ABL for normalization. BCR-ABL and ABL transcript levels are detected simultaneously and quantitative results are expressed as the percent ratio of BCR-ABL to ABL transcript levels. The BCR-ABL transcript type(s) were determined by subsequent capillary electrophoretic separation of the fluorochrome-labeled products [22]. Test characteristics, including sensitivity, were established according to Clinical Laboratory Improvement Amendments and College of American Pathologists guidelines and the assay can detect 1 BCR-ABL fusion transcript in 100,000 ABL copies. The assay was standardized to the IS and all samples were tested in the diagnostic laboratory as part of routine clinical work up of patients. The following levels of MR were studied: MMR, MR4, MR4.5, and UND corresponding, respectively, to transcript levels of ≤ 0.1 , ≤ 0.01 , $\leq 0.0032\%$, and undetectable. To compare the frequencies of and outcomes associated with different levels of MR, patients were divided in groups according to their best level of %BCR-ABL as follows: no-MR: $> 0.1\%$; MMR: $0.01\% < \text{MMR} \leq 0.1\%$; MR4: $0.0032\% < \text{MR4} \leq 0.01\%$; MR4.5: $\text{UND} < \text{MR4.5} \leq 0.0032\%$; UND: undetectable transcripts. TFS was defined as the time interval between treatment start and transformation to AP/BP or death. OS was defined as time to death from any cause at any time or last follow-up.

Statistical analysis

Patient characteristics were summarized using median (range) for continuous and frequency (percentage) for categorical variables. Kaplan–Meier method was used to estimate the probability of TFS and OS, and log-rank tests were performed to compare the differences among different MR groups. Landmark analyses were conducted to

assess the association between MR and survival outcomes. Univariate and multiple logistic regression models were fit to assess the association between patient characteristics or treatment received and the achievement of sustained MR4.5 for ≥ 2 years (i.e., *susMR4.5*). All analyses were conducted in SAS 9.3 and Splius 8.2.

Results

Patient populations

From March 2000 to November 2011, 483 consecutive patients with CML in early CP were treated on TKI clinical trials. Of these, 71 received IM400, 204 IM800, 106 NILO, and 102 DASA. Median age was 48 years (15–86). Approximately 10% of patients had a high-risk Sokal score. The distribution of baseline patient- and CML-related characteristics was similar among the four treatment groups. Median time from diagnosis to TKI initiation was 26 days (0–365) (Table I).

Response to TKI therapy

Follow-up time for the entire population was 75.1 months (2.5–140.3 months). Since clinical trials of second-generation TKI were initiated more recently, follow-up of patients treated with NILO or DASA is significantly shorter [30.4 (2.5–77.5) and 36.4 (2.5–72.5) months, respectively] compared to that of patients receiving standard or high-dose imatinib [128.7 (16.4–140.3) and 103.5 (3.7–132.2) months, respectively] ($P < 0.0001$).

The cumulative rate of CCyR was 89% for the entire cohort and 84.5, 89.7, 89.6, and 90.2% for IM400, IM800, NILO, and DASA, respectively. Among patients that achieved CCyR, the rates of best MR were no-MR in 7.7% of patients, MMR in 14.2%, MR4 in 5.6%, MR4.5 in 22.4%, and UND in 50.1%. As the cumulative rate of deeper MR is time-dependent, in order to compare MR rates across treatment groups with different follow-up times, we analyzed the frequency of the various levels of MR at 36 months. The percentages of patients achieving UND at 36 months were similar among patients treated with IM800, NILO, or DASA (30.6, 29.2, and 28.6%, respectively) but lower in those receiving IM400 (18.1%). Also, 27.3% of patients treated with IM400 who achieved CCyR failed to achieve at least a MMR at this time point, a rare occurrence in the other groups (Table II). Median time to reach MMR was 9.3, 5.9, 5.5, and 5.8 months for patients treated with IM400, IM800, NILO, and DASA, respectively, while time to achieve UND was 32.8, 24.3, 17.4, and 27.1 months, respectively. The cumulative rates of different MR in the four treatment groups are shown in Fig. 1.

Clinical outcome

Because the prognostic significance of CCyR is well established in patients receiving frontline TKI therapy for CP CML, we analyzed the clinical outcome according to the depth of MR only in patients who achieved CCyR. The resulting population included 430 patients, 429 of whom were molecularly evaluable. The depth of MR inversely correlated with the risk of losing CCyR (39, 21, 21, 15, and 3% in patients with no-MR, MMR, MR4, MR4.5, and UND, respectively) or MMR (25, 33, 19, and 3%, respectively). Very few patients that had achieved CCyR progressed to

AP or BP. That notwithstanding, a small advantage in TFS was observed in the UND cohort compared to the no-MR or MMR cohorts (Fig. 2A), with most transformations occurring within the first 24 months of therapy. The 6-year OS of patients achieving UND was superior compared to that of patients in the no-MR or MMR group, but not to that of patients achieving MR4 or MR4.5 (Fig. 2B). Notably, transformation accounted for a minority of deaths in all categories.

Landmark analysis

The median time to achieve UND was ~24 months for the entire population. To account for this lead-time, we performed landmark analyses in patients who achieved CCyR and were still on study at 18 or 24 months. Patients who achieved UND at 18 or 24 months had no advantage in terms of TFS compared to those with a lesser degree of MR at both landmarks (Fig. 3A,B). Similarly, OS was not different among patients with different levels of MR at these same landmarks (Fig. 3C,D). A total of 15 deaths were recorded in the 18 months landmark population and 12 deaths in the 24 months one. Only one death was due to transformation on or outside the study period.

TABLE II. Rates of Different Levels of MR Among 430 Patients Who Achieved CCyR at Any Time

Treatment group	Evaluable N	No MR		MMR		MR4		MR4.5		UND	
		N	%	N	%	N	%	N	%	N	%
Best response (at any time)											
IM400	60	7	12	8	13	7	12	10	16	28	47
IM800	182	9	5	16	9	9	5	38	21	110	60
NILO	95	9	10	20	21	7	7	24	25	35	37
DASA	92	8	9	17	18	1	1	24	26	42	46
All patients	429 ^a	33	8	61	14	24	6	96	22	215	50
At 36 months											
IM400	44	12	27	8	18	5	12	11	25	8	18
IM800	147	8	5	26	18	20	14	48	33	45	30
NILO	48	4	8	8	17	5	10	17	36	14	29
DASA	56	5	9	15	27	1	2	19	34	16	28
All patients	295	29	10	57	19	31	11	95	32	83	28

^a One patient who achieved CCyR did not have follow-up RQ-PCR.

MR, molecular response; CCyR, complete cytogenetic response; MMR, major molecular response; UND, undetectable BCR-ABL transcripts; IM400, imatinib 400 mg daily (N = 71); IM800, imatinib 800 mg daily (N = 204); NILO, nilotinib 400 mg twice daily (N = 106); DASA, dasatinib 100 mg daily (N = 102).

susMR4.5

We analyzed the frequency and prognostic consequences of achieving a *susMR4.5*. In this analysis, we included patients who achieved MR4.5 at any time and identified within such group those with *susMR4.5*. To allow sufficient time to achieve and maintain MR4.5, we only included the 348 patients with minimum follow-up of 40 months. Among them, 261 patients achieved MR4.5, 70 (26.8%) unsustained and 191 (73.2%) *susMR4.5*. We observed no

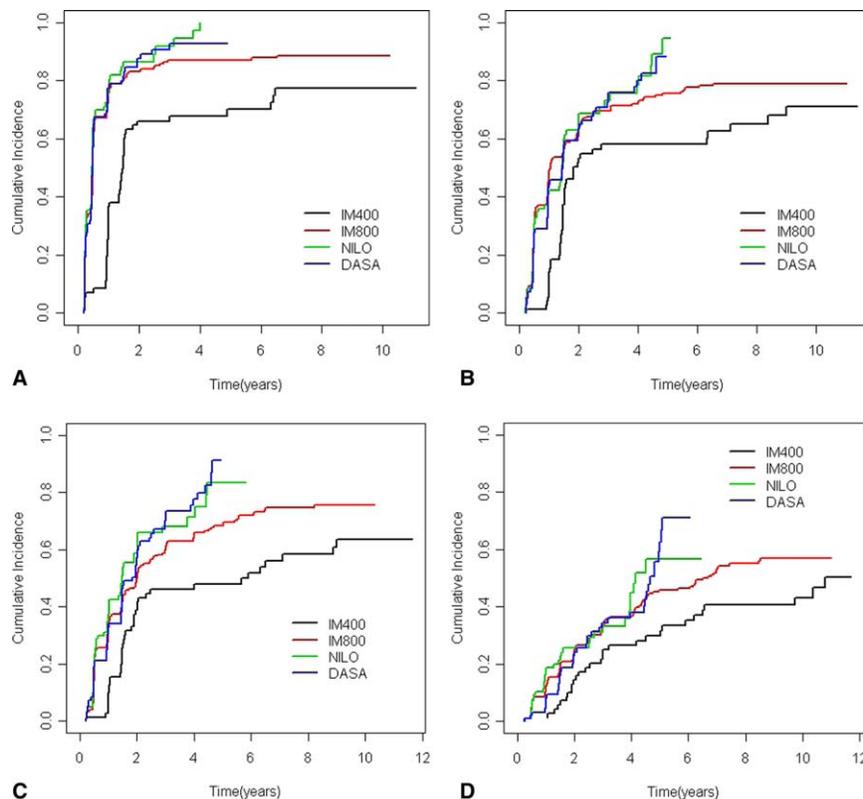


Figure 1. Cumulative incidence of MMR (A), MR4 (B), MR4.5 (C), and UND (D) according to treatment group. In each plot, for a given treatment group, the cumulative incidence step-function curves increase with each event, with the height of the curve representing the actual proportion of patients who had achieved response by that time point, and the curves continuing until the last response was achieved. IM400, imatinib 400 mg daily, n = 71; IM800, imatinib 800 mg daily, n = 204; NILO, nilotinib 400 mg twice daily, n = 106; DASA, dasatinib 100 mg daily, n = 102. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

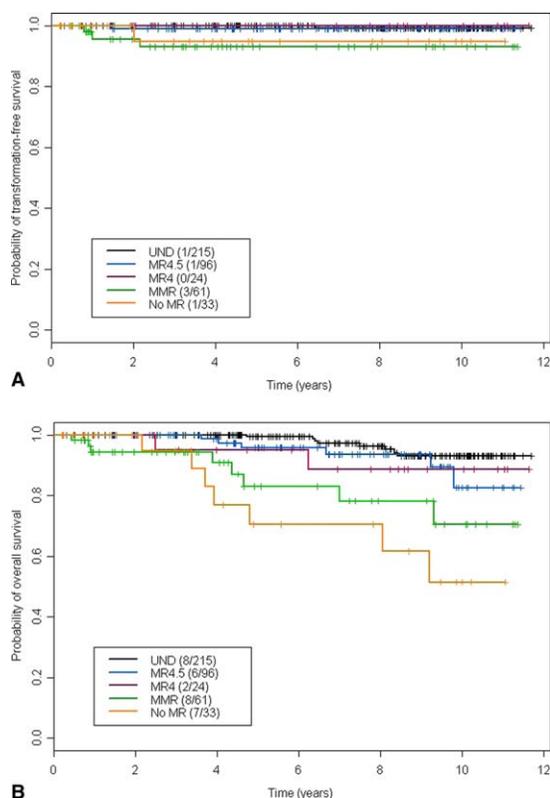


Figure 2. Transformation-free survival (A), and overall survival (B) according to different levels of molecular response in patients achieving complete cytogenetic remission at any time. *P* values for the comparison MR4.5, MR4, MMR, and no-MR versus UND are 0.49, 0.74, 0.001, and 0.02, respectively (A), and 0.16, 0.28, <0.0001, and <0.0001, respectively (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

significant difference in terms of TFS or OS (Figs. 4A and B) between the two groups. A total of 14 deaths were recorded, 5 (7.1%) in patients with unsustained MR4.5 (Parkinson's disease, 1, post-transplant complications, 1, cardiovascular, 1, other cancers, 1, unknown, 1) and 9 (4.7%) in those with *sus*MR4.5 (transformation, 1, cardio/cerebrovascular, 3, other cancers, 2, bowel obstruction, 1, suicide, 1, unknown in remission, 1). Factors associated with the probability of achieving a *sus*MR4.5 in univariate analysis included baseline age, hemoglobin level, platelet counts, PB basophils, spleen size, IS %BCR-ABL, TKI modality, and 3- and 6-months CCyR. Of these, older age, higher platelet counts, treatment with second-generation TKIs (vs. imatinib), and achievement of CCyR at 3 or 6 months were independently predictive in the multivariable analysis (Table III).

Discussion

In the present analysis, we showed that BCR-ABL transcripts may become undetectable in a substantial proportion of patients with early CP CML treated with TKI. The cumulative incidence of deep MR and the fraction of patients achieving UND is similar with IM800 or second-generation TKIs, but lower with standard-dose imatinib. We and others reported high rates of deep MR with the use of IM800 [3,4]. While a formal comparison between high-dose imatinib and second-generation TKIs in terms of quality of MR was beyond the scope of our study, our data suggest that high-dose imatinib may be a valid treatment option if the aim is to achieve deep MR.

We observed a lower risk of losing CCyR, progression to AP or BP, or death in patients with deeper MR. The achievement of lower levels of BCR-ABL transcripts with interferon therapy [23] or imatinib [12,14–18] has been associated with durable cytogenetic responses. However,

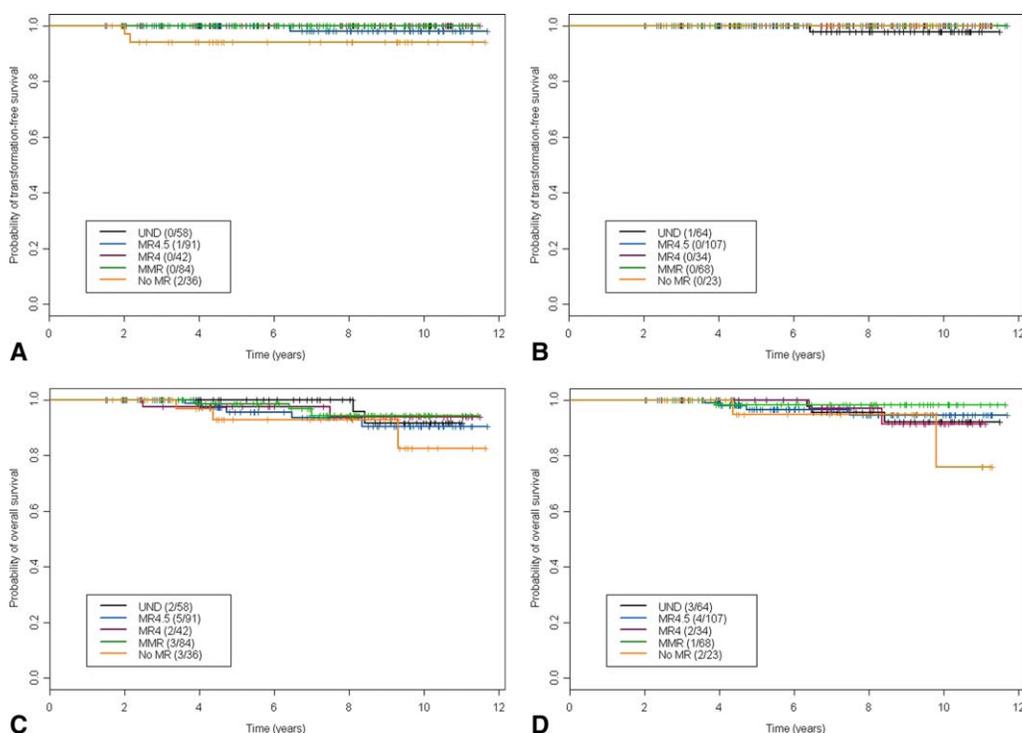


Figure 3. Landmark analysis at 18 and 24 months for transformation-free survival (A and B) and overall survival (C and D) according to different levels of molecular response in patients with complete cytogenetic response. *P* values for the comparison MR4.5, MR4, MMR, and no-MR versus UND are 0.03, 0.25, 0.37, and 0.15, respectively (A), 0.16, 0.30, 0.61, and 0.16, respectively (B), 0.48, 0.86, 0.89, and 0.24, respectively (C), and 0.94, 0.99, 0.38, and 0.44, respectively (D). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

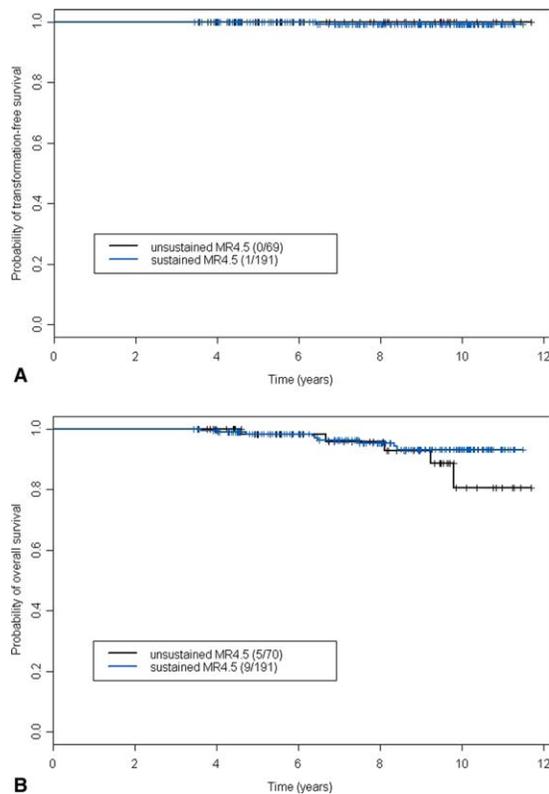


Figure 4. TFS (A) and OS (B) of patients with susMR4.5 compared to those with unsustained MR4.5 after a minimum follow-up of 40 months. *P* values for the comparison are 0.58 (A) and 0.30 (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE III. Multivariable Logistic Regression Model for the Achievement of susMR4.5

Variables	Odds ratio	(95% CI)	<i>P</i>
TKI modality			
IM800 (vs. IM400)	1.68	(0.86–3.28)	0.13
NILO (vs. IM400)	0.39	(0.18–0.86)	0.02
DASA (vs. IM400)	0.45	(0.21–0.98)	0.04
Age ^a	1.02	(1.01–1.04)	0.008
Platelet count ^a	1.73	(1.20–2.49)	0.004
Response to TKI therapy			
CCyR at 3 months	2.14	(1.28–3.59)	0.004
CCyR at 6 months	3.70	(1.76–7.75)	0.0005

^a Age and platelet count are included as continuous variables. IM400, imatinib 400 mg daily; IM800, imatinib 800 mg daily; NILO, nilotinib 400 mg twice daily; DASA, dasatinib 100 mg daily; TKI, tyrosin kinase inhibitor; CCyR, complete cytogenetic response.

the probability of loss of CCyR was not different between patients with MMR or CMR in prior studies [15]. Achieving MMR by 18 months in patients with early CP CML in CCyR on imatinib has also been shown to confer an advantage in EFS and, to a lesser extent, TFS. However, OS was not shown to be significantly different [18]. Other studies have shown different results. In a series of 269 CP CML patients treated upfront with imatinib at our institution, MR at various time points predicted for survival (mainly PFS), but not independently of the degree of cytogenetic response [24]. Similarly, among 224 patients with CP CML investigators at Hammersmith found that in patients in CCyR the achievement of MMR at 12 or 18 months did not translate into a 5-year PFS or OS benefit [17]. More recently, we reproduced these observations in patients treated upfront with second-generation TKIs. In that analysis, there was no difference

in CCyR duration or EFS among patients achieving CCyR with or without MMR at all landmarks between 3 and 18 months [25].

In this analysis, among patients that achieved CCyR, those achieving UND at any time showed superior TFS and OS compared to patients obtaining no better than MMR. This is undoubtedly influenced by the longer time needed to achieve UND resulting in a lead-time bias as patients who achieve UND by definition have not had earlier events. When adjusting for the time to achieve the best MR, the clinical benefit of achieving UND vanished, with no differences observed in TFS or OS even between the best (UND) and the worst (no-MR) MR category. This likely reflects the fact that most events (or failures) represent loss of response, which can be frequently salvaged with subsequent TKIs or other options, as opposed to AP and, particularly, BP that are more frequently irreversible but fortunately occur rarely once patients achieve CCyR. A frequent clinical question is whether a patient that achieves CCyR but not MMR or deeper MR should be considered for a change in therapy. Based on these observations, the risk of transformation or death is minimal for such patients and a change of therapy cannot be justified at the present time in the absence of a prospective study demonstrating not only the achievement of deeper responses, but, more importantly, an improved long-term outcome after such change. Interestingly, the 33 (7%) patients who never achieved a MMR in our population were equally distributed among the four treatment groups and only one of them belonged to the high-risk Sokal category.

Given the excellent clinical outcome of CML patients achieving and maintaining deep MR, TKI discontinuation has become a pressing clinical question. A consensus requirement for considering discontinuation is the achievement of MR4.5 for two consecutive years [19,20]. In the Australian CML8 study [19] and the French STop IMatinib (STIM) trial [20] patients with undetectable transcripts for ≥ 2 years on imatinib were discontinued and closely followed with RQ-PCR. Forty-four percent of patients in the former and 38% of patients with >12 months follow-up in the latter relapsed molecularly. It is unclear how often the criteria for sustained UND are met in clinical practice. In the present analysis around 75% of patients treated for ≥ 40 months ($n = 348$) achieved MR4.5. Three quarters of them maintain such response for ≥ 2 years. Assuming that $\sim 40\%$ of patients will not relapse after treatment discontinuation, only about 15% of all patients in our series could potentially maintain their response after stopping TKI therapy. Approaches to improve these ratios should be pursued. Marin et al. [26] suggested that achievement of the deepest responses is strongly correlated with adherence to therapy. Adherence was not assessed in our studies other than by patient diaries, which are known to be inaccurate. Also, further studies should investigate whether a different, perhaps longer, period of susMR4.5 should be considered as the minimum requirement for TKI discontinuation. Mathematical models can be applied to define patient-specific optimal MR4.5 durations before discontinuing therapy [27].

An important practical question is how to identify patients who are likely to achieve a susMR4.5 early after therapy initiation. Recently, Branford et al. reported a cumulative MR4.5 rate of 43% at 8 years and found female sex and lower %BCR-ABL at 3 months to predict for the achievement of stable MR4.5 for ≥ 2 years [28]. In our analysis, we confirmed the predictive value of early response to TKI therapy, but also found older age, higher platelet count, and initial treatment with second-generation TKI to be independently associated with the achievement of susMR4.5. Early response to TKI has been shown to correlate with

the achievement of deeper MR and excellent long-term survival [18]. Furthermore, we previously observed that adolescent/young adult CML patients have lower rates of CCyR and MMR compared to older patients, perhaps due to poorer adherence to TKI therapy [29]. Finally, the use of second-generation TKI has resulted in faster and higher rates of high-quality MR [6,8]. The relationship between higher platelet counts and the achievement of *susMR4.5* needs further confirmation and study.

Our analysis has two possible limitations. First, the four treatment groups considered were part of separate studies in different time periods, and not a single, randomized trial. However, all patients were consecutively enrolled and eligibility criteria for all studies were similar. Characteristics at study entry were not different across groups and timing of surveillance and patient management policies have been homogeneous throughout the study periods. Thus, we believe these results are representative of what can be expected in routine clinical practice. Second, in the landmark analyses, only a small number of transformations or deaths were recorded overall. Therefore, the present analysis may be underpowered to detect significant differences in outcome between MR categories. Whether, and to what extent, such potential statistical difference may also have a clinical significance is even less clear.

In conclusion, with TKI therapy, MR4.5 can be achieved in a significant fraction of patients with CML CP. The achievement of deeper MR can minimize the risk of loss of CCyR or MMR. However, no additional benefit is observed in terms of TFS or OS in patients already with stable CCyR. Sustained deep MR were not associated with an additional benefit in TFS and OS, suggesting that patients with CCyR and residual detectable disease still have an excellent long-term outcome with minimal risk for transformation. The clinical value of treatment changes to aim for deeper MR needs to be tested prospectively.

Author Contributions

L.F., J.E.C., and H.M.K. designed research. H.K.M., A.Q., S.O., E.J.J., F.R., G.B., G.G., S.V., J.A.B., and J.E.C. treated patients, collected, analyzed, and interpreted data. L.F. and D.V. collected analyzed and interpreted data. X.W. performed statistical analysis, analyzed, and interpreted data. R.L. analyzed and interpreted data. All authors wrote and approved the final version of the manuscript.

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