Monitoring and Analysis of Chinese Chronic Myeloid Leukemia Patients Who Have Stopped Tyrosine Kinase Inhibitor Therapy*

Na SHEN[1], Yong YOU[1], Zhao-dong ZHONG[1], Li MENG[2], Jian-feng ZHOU[2], Ping ZOU[1], Xiao-jian ZHU[2], Hong-xiang WANG[3#], Fan-jun CHENG[1#]

[1]*Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China*

[2]*Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*

[3]*Department of Hematology, The Central Hospital of Wuhan, Wuhan 430014, China*

Summary: Discontinuation of tyrosine kinase inhibitor (TKI) therapy after achieving a persistent deep molecular response (DMR) is an urgently needed treatment goal for chronic myeloid leukemia (CML) patients and has been included in the National Comprehensive Cancer Network (NCCN) guidelines (version 2.2017) for CML. Indeed, various studies have confirmed the feasibility of discontinuing TKI therapy. In this study, we analyzed data from 45 CML patients who had discontinued TKI therapy. Univariate analysis was performed to predict factors that were potentially related to treatment-free remission (TFR) and identify the differences between early relapse and late relapse. Out of the 45 patients, 20 exhibited molecular relapse after a median follow-up of 18 months (range, 1–54 months), and the estimated TFR at 24 months was 40%. The univariate analysis revealed that a high Sokal score and interruptions or dose reductions during TKI treatment were the only baseline factors associated with poor outcomes. Our results indicate that TKI discontinuation could be successfully put into practice in China.

Key words: discontinuation; treatment-free remission; chronic myeloid leukemia; relapse

Chronic myeloid leukemia (CML) is a myeloproliferative disease caused by a reciprocal translocation t(9; 22) in hematopoietic stem cells that results in constitutive expression of the oncogenic tyrosine kinase *BCR-ABL1*[1, 2]. Tyrosine kinase inhibitor (TKI) that targets *BCR-ABL1* has revolutionized the prognosis of CML patients, turning this previously deadly hematopoietic malignancy into a chronic disease with low progression rates[3, 4]. Patients who remain on TKI for years can exhibit undetectable minimal residual disease (UMRD), maintaining long-term event-free survival with a very low tumor burden[4, 5]. However, lifelong TKI treatment is accompanied by several drawbacks, including expected and unexpected side effects, impaired quality of life and high drug costs[6, 7]. Although lifelong TKI therapy remains the consensus recommendation, TKI discontinuation has proven to be safe for some patients who sustain long-term treatment-free remission (TFR), which has become a new endpoint and an emerging goal in the management of CML[8, 9]. In the last ten years, numerous clinical trials have demonstrated that approximately 40%–50% of patients with long-term, stable deep molecular responses (DMRs) remain in TFR after discontinuing TKI[10, 11]. Recently, criteria for TKI discontinuation have been included in the NCCN guidelines (version 2.2017) for CML[12]. In this study, we report clinical observations of TKI cessation among Chinese CML patients.

1 PATIENTS AND METHODS

1.1 Patients

The study was approved by the Ethical Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology and conducted

Na SHEN, E-mail: hustshenna@126.com
#Corresponding authors, Fan-jun CHENG, E-mail: chengfanjun001@sina.com; Hong-xiang WANG, E-mail: ljwhx@21cn.com

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according to the principles of the Declaration of Helsinki. For this type of study formal consent is not required. Patients who were ≥12 years of age, had a confirmed diagnosis of CML in chronic or accelerated phase and were receiving ongoing imatinib treatment at the standard dose for at least five years or had switched to second generation TKI for at least one year were included in this study. In addition, a sustained molecular response 4.0 ($MR^{4.0}$) for more than 2 years was necessary for inclusion in the study. Patients with previous allogeneic hematopoietic stem cell transplantations were excluded. Patients treated with interferon-α (IFN-α) prior to being treated with TKI or TKI in combination with IFN-α as well as patients with additional chromosomal abnormalities were allowed in the study. A total of 45 patients from the Tongji Hospital and Union Hospital of Wuhan, China, were included in the study.

1.2 Monitoring and Definition of Molecular Response and Molecular Relapse

Molecular monitoring was performed using the international scale (IS) for *BCR-ABL1* level in peripheral blood. RT-PCR for *BCR-ABL1* mRNA was performed by Hubei Province Stem Cell Research and Application Center of Union Hospital, which was an authenticated RT-PCR detection center in Wuhan, China, using the European LeukemiaNet recommendations for minimal residual disease quantification[13]. The RT-PCR methods used in this study can detect at least a 4.5-log reduction in *BCR-ABL1* transcript levels. Molecular responses were assessed following the NCCN recommendations for *BCR-ABL1* mRNA quantification by RT-PCR[12]. $MR^{4.0}$ was defined as <0.01% *BCR-ABL1* IS, and $MR^{4.5}$ was defined as <0.0032% *BCR-ABL1* IS. Complete molecular response (CMR) was defined as *BCR-ABL1* mRNA being undetectable by RT-PCR. Molecular relapse was defined as the loss of major molecular response (MMR: <0.1% *BCR-ABL1* IS). In cases of relapse, patients' *BCR-ABL1* transcripts were measured every month in the first year, every two months after one year and every three months after two years.

1.3 Study Endpoints and Statistical Analysis

The primary endpoints were the rates of TFR at 6, 12 and 24 months, defined as the time interval between the discontinuation of TKI treatment and molecular relapse. The secondary endpoints were the predictive factors associated with TFR and the fold increase and doubling time of *BCR-ABL1* transcripts after molecular relapse between early and late relapse.

The rates of TFR were calculated by Kaplan-Meier analysis, and stratified groups were compared with the log-rank test. Univariate and multivariate Cox regression analyses were performed to identify factors that were potentially related to relapse after TKI discontinuation.

2 RESULTS

2.1 Patient Characteristics

The clinical features of the 45 patients who had discontinued TKI therapy are shown in table 1. The median age of these patients was 36 years (range, 13–76 years). At CML diagnosis, 2 patients were in the accelerated phase (AP) and the remaining patients were in the chronic phase (CP). Three patients had additional chromosomal abnormalities. In addition, 31, 12, and 2 patients had low, intermediate and high risk Sokal scores at CML diagnosis, respectively. Among these patients, 75.6% (34/45) continually took imatinib until discontinuation, and 11 patients switched from imatinib to nilotinib due to intolerance or resistance to imatinib during treatment. The primary causes of imatinib intolerance were superficial edema, skin eruptions, nausea, and muscle pain. However, 13 patients experienced interruptions or dose reductions during TKI treatment because of unsatisfactory compliance or pregnancy. Eight (17.8%) patients had undergone IFN-α treatment prior to TKI therapy, and none had received IFN-α in combination with TKI.

Table 1 Clinical characteristics of involved patients

Clinical characteristics	Patients, *n*=45 (till Sep. 2016)
Median age (range), years	36 (13–76)
Male, *n* (%)	23 (51.1)
Phase of disease, *n* (%)	
CP	43 (95.6)
AP	2 (4.4)
ACA	3 (7.3)
Prior exposure to IFN-α, *n* (%)	
No	37 (82.2)
≤ 12 months	4 (8.9)
> 12 months	4 (8.9)
Sokal score, *n* (%)	
Low	31 (68.9)
Intermediate	12 (26.7)
High	2 (4.4)
Prior TKI treatment, *n* (%)	
1 TKI: imatinib	34 (75.6)
2 TKI: imatinib+nilotinib	11 (24.4)
Indication for nilotinib, *n* (%)	
Intolerance to imatinib	9 (81.8)
Warning to imatinib	2 (18.2)
Duration of TKI therapy (range), months	77 (32–138)
Duration of imatinib therapy (range), months	32 (12–50)
Duration of nilotinib therapy (range), months	18 (1–54)

CP: chronic phase; AP: accelerated phase; ACA: additional chromosomal abnormality; TKI: tyrosine kinase inhibitor

2.2 Outcomes of TKI Discontinuation

Twenty-five of the 45 patients (55.5%) remained in TFR after a median follow-up time of 18 months

(range, 1–54 months). No events, such as the loss of hematologic response or a progression to the advanced phase, were noted in this trial. Among 20 patients who relapsed after TKI discontinuation, 17 had taken imatinib and 3 had taken nilotinib prior to discontinuation. Five of 8 patients who had been exposed to IFN-α prior to TKI therapy exhibited confirmed relapse after TKI discontinuation. Eight of 13 patients who experienced interruptions or dose reductions lost their MMRs after TKI discontinuation. Fifteen of 20 patients (75%) who met the definition of molecular relapse immediately resumed TKI treatment and regained the MMR with an average time of 3 months (range, 1–9 months). Three of these patients (2 with prior imatinib therapy) restarted nilotinib (300 mg twice daily), and 12 patients reinstated imatinib treatment (400 mg daily). The median *BCR-ABL1* halving time for these patient groups was 21.2 days (range, 16.3–26.4 days) and 25.7 days (range, 5.4–66.9 days), respectively. We were unable to contact with the remaining 5 patients who relapsed. The estimated TFRs were 67%, 51%, and 40% at 6, 12, and 24 months, respectively (fig. 1).

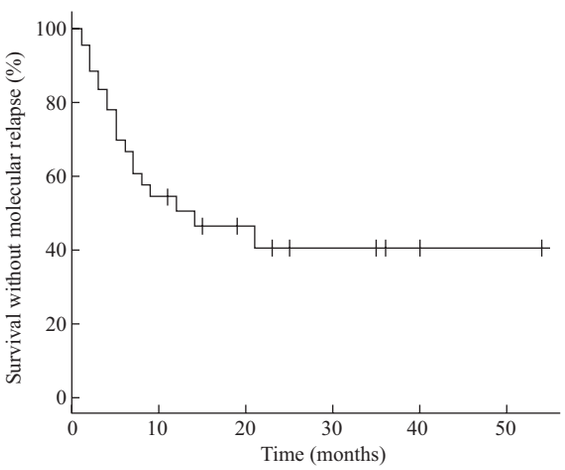

Fig. 1 The survival without molecular relapse of all patients who discontinued TKI treatment

2.3 Predictive Factors of TFRs

Univariate analysis was used to identify the factors that were potentially associated with TFR. Sex, age, prior exposure to IFN-α, time to complete cytogenetic response (CcyR), time to MMR, duration of MMR, duration of TKI therapy, switching to nilotinib, interruptions or dose reductions during TKI treatment, and the Sokal risk score were examined (table 2). At 24 months, 66.7% and 33.3% patients remained in TFR of low risk and non-low risk group, respectively (P=0.026, table 2). The low risk group exhibited longer survival without molecular relapse than the non-low risk group (P=0.018, fig. 2A). Similarly, the high risk patients exhibited a lower estimated TFR rate at 24 months (0 *vs.* 61.5%, P=0.023, table 2). Obviously, the non-low risk group at diagnosis had a greater risk of recurrence after discontinuation according to the Cox regression analysis (table 2). Moreover, the patients who experienced interruptions or dose reductions during TKI treatment exhibited lower survival rates than the patients who never experienced interruptions or reductions (P=0.057, fig. 2B). The results were also verified by multivariate Cox regression analyses.

2.4 The Difference between Early Relapse and Late Relapse

Thirteen of the 20 patients experienced molecular relapse within 6 months after stopping TKI therapy (median 3 months, here termed "early" relapse). At the time of TKI re-initiation after molecular relapse, the median time of *BCR-ABL1* fold increase and the doubling time was 74.3 (range, 9.6–1250 days) and 14.8 days (range, 3.1–46.9 days), respectively. Seven "late" relapses occurred after 6 months (median 9 months, range 7–21 months). The median time of *BCR-ABL1* fold increase and doubling at the time of TKI restart after molecular relapse was 62 (range, 12.2–10182 days) and 52 days (range, 20.7–177.6 days), respectively. Comparative analyses were

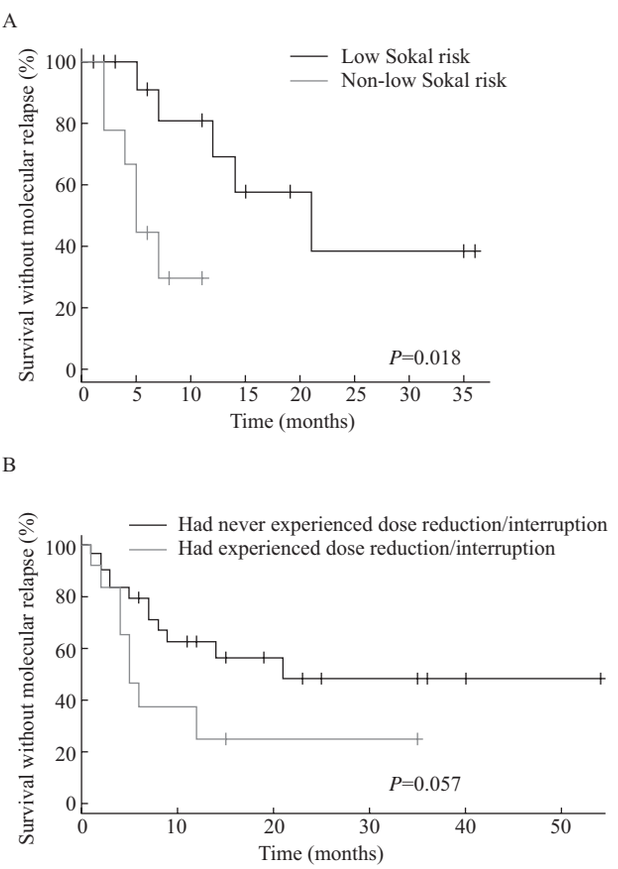

Fig. 2 A: The comparison of the survival without molecular relapse between patients in low Sokal risk and non-low Sokal risk. P=0.018; B: The comparison of the survival without molecular relapse between patients who had never experienced dose reduction/interruption and patients who had experienced dose reduction/interruption. P=0.057

Table 2 Potential factors for prediction of TFR by COX univariate analysis

Potential factors		Rate of TFR at 24 month	HR (95%CI)	*P* value
Sex	Male	69.6%	0.595 (0.237, 1.497)	0.270
	Female	40.9%		
Age	≤ 40 years	51.9%	1.009 (0.985, 1.033)	0.470
	> 40 years	55.6%		
Prior exposure to IFN-α	No	59.5%		
	≤ 12 months	25.2%	1.089 (0.387, 3.066)	0.328
	> 12 months	50.0%		
Time to CCyR	≤ 6 months	50.3%	1.785 (0.506, 6.294)	0.367
	> 6 months	72.7%		
Time to MMR	≤ 12 months	55.9%	0.778 (0.282, 2.152)	0.631
	> 12 months	54.5%		
Duration of MMR	≤ 36 months	66.7%	0.865 (0.357, 2.095)	0.748
	> 36 months	53.8%		
Duration of TKI therapy	≤ 50 months	60.1%	0.843 (0.348, 2.041)	0.705
	> 50 months	55.2%		
Switching to nilotinib	Yes	72.7%	0.522 (0.153, 1.784)	0.300
	No	50.0%		
Sokal risk	Low	66.7%	0.161 (0.032, 0.803)	0.026
	Non-low	33.3%		
Sokal risk	High	0%	25 (1.564, 399.681)	0.023
	Non-high	61.5%		
Experiencing interruptions or dose reduction during TKI treatment	Yes	38.5%	2.324 (0.938, 5.76)	0.069
	No	62.5%		

TFR: treatment-free remission; CCyR: complete cytogenetic response; MMR: major molecular response; TKI: tyrosine kinase inhibitor

performed to evaluate the potential factors that differed between early relapse group and late relapse group. No significant difference was observed with respect to prior exposure to IFN-α, time to CcyR, time to MMR, duration of MMR, duration of TKI therapy, Sokal score or interruptions or dose reductions during TKI treatment due to the limited sample size.

3 DISCUSSION

The idea that CML patients must have a lifelong TKI dependency has been challenged by evidence that TKI discontinuation is feasible in some patients with a DMR. A multicenter STIM study reported 100 CML-CP patients who sustained MR4.5 for more than 2 years after TKI cessation and that the probability of persistent MR5.0 at 24 months was 41% (95%CI 29–52), without evidence of CML recurrence[14]. The TWISTER study also reported that the estimated TFR at 24 months was 47.1% after imatinib discontinuation[15]. Our study analyzed the data from Chinese CML patients who discontinued TKI treatment and observed similar results to the above studies, with an estimated TFR of 40% at 24 months. Combining these data with our results suggests that it is possible to discontinue TKI treatment. In addition, these studies confirm that re-treatment with TKI after relapse is still effective and those relapsed patients can achieve CMR again. Discontinuation of TKI had been attempted by some patients who failed the first discontinuation in RE-STIM trials, demonstrating that a second discontinuation attempt was safe, and 42% patients exhibited sustained TFR at 24 months[16].

The factors that are potentially related to relapse after discontinuation remain uncertain. In general, longer durations of TKI therapy, longer durations of CMR before stopping TKI therapy and lower Sokal scores at diagnosis are associated with lower molecular relapse rates after discontinuation[15, 17, 18]. However, these factors are not absolute and may differ according to various factors in a given study. The number of patients included, racial heterogeneity, and differences in RT-PCR sensitivity across different laboratories, and significantly associated factors among studies with respect to TKI discontinuation are not always the same[5, 10]. Residual CML leukemia stem cells (CML-LSCs) are thought to be a major source of recurrence after cessation because TKI cannot effectively eradicate CML-LSCs[19, 20]. Our previous study and other studies have demonstrated that relapse after discontinuation was not associated with the number of LSCs[21, 22]. We speculated that the status of LSCs may be a key factor in CML relapse. A large number of studies have focused on the eradication of LSCs and achieved important results, but the key mechanism to eradicate these cells requires further studies[23, 24].

Similar to previous studies, our study observed that 65% (13/20) of the relapse occurred early (within 6 months after TKI cessation) and that these patients exhibited a greater fold increase and a shorter doubling

time of *BCR-ABL1* transcripts at the time of restarting TKI than patients who exhibited late relapse, revealing obvious differences in tumor growth kinetics according to clinical context. Ilander *et al*[25] reported that late relapses may have different biological characteristics from early relapses. The patients in the early relapse group had a lower relative proportion of NK cells than the non-relapse patients (median, 12.8% *vs*. 17.1%), whereas in the late relapse group, the median proportion of NK cells was similar to that observed in the non-relapse patients (20% of lymphocytes). Musjoki *et al*[26] observed that the proportion of NK cells was higher in STOP-IM and CMR groups than in fluctuating CMR and control groups. These results suggested that higher activation levels of effective NK cells may contribute to the maintenance of CMR and reflect the degree of *BCR-ABL1* reduction, which could be used to evaluate the imatinib treatment outcomes and withdrawal time. We speculate that the immune surveillance by NK cells plays an important role in CML patients after TKI withdrawal.

In conclusion, we confirmed that TKI may be safely discontinued in a portion of CML patients in China. However, future studies are needed to prevent relapse after discontinuing TKI. We will continue to collect relevant data using expanded sample size and prolonged observation period.

Conflict of Interest Statement

The authors declare that there is no conflict of interest with any financial organization or corporation or individual that can inappropriately influence this work.

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