16 Discontinuation or Cessation of Tyrosine Kinase Inhibitor Treatment in Chronic Myeloid Leukemia Patients with Deep Molecular Response

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16.1 Introduction

Chronic myeloid leukemia (CML) is more than ever the model of targeted therapy for human malignancies. The success of the first tyrosine kinase inhibitor (TKI) imatinib has profoundly changed the outcome for CML patients. Since TKI-treated CML patients have a near-normal life expectancy [1], important issues must be considered in the future: (a) long-term toxicities directly influencing the quality of life and ethical aspects of the treatment and (b) the economic impact of treating patients for their lifetime.

One of the best ways to consider these points is to ask the relevant question about stopping TKIs in good responding patients. Such a strategy has been proposed now as a result of several studies including more than 3000 patients in deep molecular remission (DMR, BCR-ABL1 (IS) < 0.01%) who have stopped a TKI. The main prognostic factors are the duration of DMR and the TKI treatment duration. However, many questions about the depth of molecular remission, other predictive factors including immunological factors, and safety are still open and unresolved. Based on recent data published to date, the recommendations of the National Comprehensive Cancer Network (NCCN) and the European LeukemiaNet (ELN) propose criteria when discontinuing TKI treatment safely in responding patients with CML is most appropriate [2, 3].

16.2 TFR Studies

The initiative was started with a pilot study in 12 patients with CML when it was proposed to discontinue imatinib (Rousselot et al. 2007). After a median follow-up of 18 months, 50% of patients remained off therapy without confirmed reappearance of peripheral blood BCR-ABL1 transcripts [4]. This pilot study provided a proof of concept that imatinib discontinuation could be achieved in selected CML patients. It was followed by a multicenter study entitled "Stop Imatinib" (STIM) trial [5]. Prospectively, 100 patients with chronic-phase CML receiving imatinib therapy in DMR were included. Fifty-one percent of the patients had been previously treated with IFN, and the other half were treated with imatinib only. Molecular relapse, which was arbitrarily defined as two positive RQ-PCR results over a period of 1 month showing a significant rise (1 log) in BCR-ABL1 transcripts, was a trigger for imatinib treatment

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again. Molecular recurrence-free survival rate at 65 months was 39%. For those patients who achieved the first 6 months without relapse (landmark analysis), the probability of relapse was 10% at 24 months [6]. Most patients who experienced molecular relapse did so within 6 months of imatinib cessation and remained responsive to re-treatment with imatinib as observed in the pilot study. Comparable results were reported in the Australasian Leukaemia and Lymphoma Group (ALLG) CML8 study (TWISTER) [7]. With a median follow-up of 8.6 years (range 5.7–11.2 years), 18 patients remained in continuous TFR (45.0%) [8]. Most relapses occurred within 6 months of stopping imatinib, and no relapses occurred beyond 27 months.

One of the important issues regards the definition DMR which was not uniform when the first trials started. New definitions were introduced in 2012 (see later). Other attempts at imatinib discontinuation, which did not meet the criterion of DMR, exhibited rapid molecular relapses [9–12].

Multiple TKI discontinuation studies have been published or are still ongoing confirming these results. In addition, registries on TFR outside clinical trials demonstrated the feasibility of TFR in routine care [13, 14].

Most trials confirmed that the duration of response, especially the duration of DMR, was important (). The validation of this criterion was reinforced using mathematical models confirming a biphasic dynamic of BCR-ABL1 transcript decline with a two-slope model of TKI the α slope corresponded to the rapid initial decrease in BCR-ABL1 transcript levels (cycling cells) after the start of treatment, and the β slope corresponded to the longer-term BCR-ABL1 dynamics (less proliferative cells) [15]. Another model based on the biphasic decline of BCR-ABL1 transcript levels suggested that 31% of the patients would remain in DMR after treatment cessation after a fixed period of 2 years in MR5, whereas 69% are expected to relapse [16]. Most recently, another mathematical model demonstrated an antileukemic immunologic effect in 21 patients with CML for whom BCR-ABL1/ABL1 time courses had been quantified before and after TKI cessation. Immunologic control was conceptually necessary to explain TFR as observed in about half of the patients [17].

Because the identification of patients who would benefit most from discontinuing TKIs remains a key issue, the question of the duration of molecular response before discontinuation is crucial.

It is also one of the objectives of the European Stop Kinase Inhibitor (EURO-SKI) trial from the European LeukemiaNet (ELN) that was running in 11 countries. The criteria for discontinuation were less strict than those in the STIM studies: the duration of TKI treatment prior to enrolment had to be at least 3 years and the PCR level below 0.01% within the previous year, i.e., a sustained DMR of 4 log had to be confirmed. Results of a planned interim analysis with the final analysis still pending [18] showed the following:

After a median follow-up of 27 months, molecular relapse-free survival of 755 evaluable patients was 61% (95% CI 57–64) at 6 months and 50% (46–54) at 24 months. No plateau was reached. Of these 755 patients, 371 (49%) lost MMR after TKI discontinuation, four (1%) died while in MMR for reasons unrelated to CML (myocardial infarction, lung cancer, renal cancer, and heart failure), and 13 (2%) restarted TKI therapy while in MMR. An additional six (1%) patients died in CP-CML after loss of MMR and re-initiation of TKI therapy for reasons unrelated to CML, and two (<1%) patients lost MMR despite restarting TKI therapy. In the prognostic analysis in 405 patients who received imatinib as first-line treatment, longer treatment-free duration and longer DMR duration were associated with increasing probability of MMR maintenance at 6 months with DMR duration being the most impotant factor.

These results were similar to another French trial of 218 patients [19].

The depth of response is an important factor in the decision to discontinue TKI treatment. The definition of molecular response and the standardization of BCR-ABL1 transcript measurement remain a concern. For this reason, the CML Working Group of the ELN has proposed revised definitions of MR taking into account the sensitivity of molecular tests, i.e., MR4 indicates ≥4-log reduction (BCR-ABL1 (IS) ≤ 0·01%),

MR4.5 indicates ≥4.5-log reduction (BCR-ABL1 (IS) ≤ 0·0032%), and MR5 indicates ≥5-log reduction (BCR-ABL1 (IS) ≤0.001%) [20, 21]. Different European laboratories working in a European molecular network validated this standardization and performed the molecular analyses of the EURO-SKI trial. Terms like complete molecular remission (CMR) or undetectable levels of minimal residual disease (UMRD) should not be used anymore. They indicate a negative RQ-PCR result and must be associated with a defined PCR assay sensitivity; however, it should be noted that leukemic cells may still be present even if RQ-PCR results are negative [22]. Current RQ-PCR methods can reliably detect up to a 5-log reduction in BCR-ABL1, but newer techniques, such as DNA-based PCR, RNA-based digital PCR, and replicated PCR, have demonstrated increased sensitivities and may enable the assessment of even deeper levels of molecular response [23].

The Imatinib Suspension and Validation (ISAV) trial is the first study using digital PCR in parallel with qRT-PCR [24]. The method seems to be more sensitive in this study as the prediction of relapse was more accurate. One hundred twelve patients with at least 2-year imatinib treatment and at least 18 months undetectable transcripts in qRT-PCR were followed for a median of 21.6 months. Cumulative incidence of relapses was 52% after 36 months. Relapse was defined as loss of MMR (two consecutive positive PCRs with one result at least above 0.1%).

However, it should be noted that after using an ultrasensitive PCR technique, a low level of BCR-ABL1 transcripts has been found in the blood of normal individuals, suggesting that a complete absence of transcripts may not be required to eradicate the disease [25, 26]. Most patients have detectable BCR-ABL1 DNA by highly sensitive methods (27). In the TWISTER study, nine patients in long-term TFR were monitored by highly sensitive individualized BCR-ABL1 DNA PCR. This technique provided more precise quantification and demonstrated a BCR-ABL1 DNA decrease from a median of MR5.0 in the first year of TFR to MR6.1 in the sixth year of TFR [8]. In the EURO-SKI trial, DNA and mRNA BCR-ABL1 measurements by qPCR were compared in 2189 samples (129 patients) and by digital PCR in 1279 sample (62 patients). A high correlation was found at levels of disease above MR4, but there was a poor correlation for samples during DMR. A combination of both methods resulted in a better prediction of molecular recurrence-free survival (MRFS). At 18 months after treatment cessation, patients with negative results for DNA- and RNA-based PCR had an MRFS of 80% and 100%, respectively, compared with those who were DNA positive/RNA negative (MRFS = 57% and 67%) or DNA positive/RNA positive (MRFS = 20% for both cohorts) [27].

Such a strategy should be prospectively validated and can improve TFR results.

In a lineage analysis (granulocytes, monocytes, B cells, T cells, and NK cells) of residual CML cells of 20 patients who were in TFR for >1 year, MRD was identified predominantly in the lymphoid compartment and not in granulocytes. B cells were more often BCR-ABL1 positive than T cells and at higher levels. These data suggest that MRD in the blood of TFR patients need not imply the persistence of multipotent CML cells [28].

We still do not know the threshold of residual disease which will allow us to safely stop TKI with the lowest rate of molecular recurrence. What is the definition of molecular relapse triggering re-treatment? It is also a very important question. It is absolutely necessary to use exactly the same criteria to compare studies to exclude misinterpreting the results. In the STIM studies, molecular relapse was defined by positivity of BCR-ABL1 transcript in qRT-PCR confirmed by a second analysis point indicating the increase of 1 log in relation to the first analysis point, at two successive assessments, or loss of MMR at one point. This definition leads to propose the term molecular recurrence instead of molecular relapse [29]. By comparison to the STIM study which was the first clinical trial proposing to stop TKI, the criteria triggering re-treatment after molecular relapse have now evolved. Since many studies pertaining to TKI cessation have been launched, we need to underline clearly the criteria for treatment re-challenge in future trials.

The French multicenter observational study (A-STIM [According to Stop Imatinib]) validated loss of MMR as a trigger for restarting TKI therapy in CP-CML patients who have stopped imatinib after achieving durable molecular response. In a first publication in 2014, 80 patients with CP-CML had stopped imatinib after sustained DMR of 2 years with the same definition as compared to STIM study [30]. Molecular relapse was less stringently defined as loss of MMR at any time for triggering re-treatment. The median follow-up after discontinuation was 31 months (range, 8–92 months). TFR was estimated 61% at 36 months, but it was estimated around 37%, i.e., similar to STIM or TWISTER results when STIM criteria were used.

Meanwhile, longer follow-up of the A-STIM study revealed very late loss of MMR. In total, 218 pts. were followed and the TFR rate was estimated to be 45.6% after 7 years. For 9/65 (14%) patients experiencing loss of MMR, molecular recurrence occurred after 2 years in TFR. The probability of remaining in TFR was 65.4% for patients having experienced fluctuations in minimal residual disease (MRD), at least two consecutive measurements BCR-ABL1 (IS) >0.0032% or loss of MR4, whereas it was 100% for those with stable DMR.

In addition, some studies reported cases where sudden BC after stopping occurred. A long-term molecular follow-up therefore remains mandatory for CML patients in TFR [31].

Another concept was followed in the DESTINY (De-Escalation and Stopping Treatment with Imatinib, Nilotinib, or sprYcel) study. TKI treatment was de-escalated to half the standard dose for 12 months before cessation. Analysis was performed according to MR level before study entry. Recurrence-free survival was 72% for DMR patients, 36% for the MMR group [32].

To address the feasibility of discontinuing nilotinib or dasatinib, academic- and pharma-sponsored studies were implemented.

Stopping after first-line therapy with either dasatinib or nilotinib was investigated in Dasfree and ENESTfreedom trial, respectively.

In the single-arm, phase 2 ENESTfreedom trial, patients ⩾2 years of frontline nilotinib therapy were enrolled. Patients with sustained DMR during the 1-year nilotinib consolidation phase were eligible for the TFR phase. In total, 215 patients entered the consolidation phase, of whom 190 entered the TFR phase. The median duration of nilotinib before stopping treatment was 43.5 months, the shortest of all TFR studies so far. At 48 weeks after stopping nilotinib, 98 patients (51.6%) remained in MMR or better [33].

In the Dasfree trial, a single-arm phase 2 trial, 84 patients were enrolled after first- or second-line therapy with dasatinib. At 2 years, TFR was 46%. Multivariate analyses revealed statistically significant associations between 2-year TFR and duration of prior dasatinib, line of therapy, and age (>65 years) [34].

Other studies confirmed these data such as the Japanese DADI trial (dasatinib discontinuation). Of 58 patients who discontinued dasatinib, 32 (55%) had TFR at 6 months with a median follow-up of 3·months. However, the definition of response and retreatment is not clearly described in the paper [35]. In a French trial, a first interim analysis reported outcomes of 60 patients with a minimum follow-up of 12 months. Twenty-six patients (43.3%) lost MMR. TFR rates at 12 and 48 months were 63.3% and 53.6%, respectively. In a univariate analysis, prior suboptimal response or TKI resistance was the only baseline factor associated with significantly worse outcome [36].

Other studies like ENESTPath and ENESTop focused on patients who switch to nilotinib in order to reach sustained DMR before entering a TFR phase. Whereas ENEStpath is still ongoing, in ENESTop, 163 patients who had switched from imatinib to nilotinib (for reasons including resistance, intolerance, and physician preference) entered the consolidation phase. One hundred twenty-six were eligible to stop the TKI. At 48 weeks and 96 weeks, 58% and 53% of patients maintained TFR, respectively [37].

16.3 Which Clinical and Biological Factors Might Predict TFR?

Besides the duration and depth of response, which other factors may be used to suggest the possibility of interrupting TKI treatment? In the

STIM study several potential factors for prediction of molecular recurrence were retrospectively assessed [5]. The probability of remaining in stable DMR after discontinuation was favorable in the low Sokal risk group when compared to the intermediate or high Sokal risk groups. Using multivariate analysis and logistic regression at 8 months, Sokal risk and imatinib therapy duration were confirmed as two independent prognostic factors for prediction of molecular relapse after imatinib cessation.

In EURO-SKI no prognostic score was found to be significantly associated with TFR. Despite this, longer treatment duration and longer DMR duration were associated with increasing probability of MMR maintenance at 6 months with DMR duration being the stronger factor. The final analysis is pending.

Other criteria such as age, sex, or depth of MR were significant in some smaller studies but not confirmed in others.

Using the criteria of the STIM and TWISTER studies, it should be possible to predict which patients are ideal for discontinuation of TKIs. Recently Branford and colleagues found in a study of 415 patients treated with imatinib for 8 years that the cumulative rate of stable $MR^{4.5}$ (for at least 2 years) was 43%. In these patients, the time to achieve MMR was correlated with the time to achieve stable $MR^{4.5}$.[38] In addition the only two independent factors, i.e., female sex and a low level of BCR-ABL1 value at 3 months, were statistically strongly linked to the prediction of sustained $MR^{4.5}$. Factors associated with sustained $MR^{4.5}$ and undetectable transcripts induced by TKI (imatinib, dasatinib, and nilotinib) were also analyzed in a multivariable analysis ($N = 495$) by Falchi et al. and showed that older age, higher baseline hemoglobin, higher baseline platelets, TKI modality, and response at 3 months were significant [39]. A long-term analysis has been performed in the German CML study IV. From more than 1500 patients the cumulative incidence of confirmed MR4.5 was 54% after 9 years [40]. The study demonstrated a link between MR4.5 achievement and better survival.

Most recently, the time for the BCR-ABL1 value to halve from the time of diagnosis ha been shown to be the strongest independent predictor of sustained TFR. Early molecular response dynamics were assessed from 115 patients attempting subsequent TFR after ≥12 months follow-up. The probability of sustained TFR at 12 months was 55%. TFR rate was 80% in patients with a BCR-ABL1 halving time of <9.35 days compared with only 4% if the halving time was >21.85 days ($P < 0.001$). The e14a2 BCR-ABL1 transcript type and duration of TKI exposure before attempting TFR were also independent predictors of sustained TFR [41].

In addition, immunological effects seem to play an important role in maintaining TFR. Several studies have reported that low NK cell numbers may predict early disease relapse after TKI discontinuation [42–45]. These studies suggest that NK cell-based immune surveillance may contribute to CML control after TKI cessation. In one of the studies, NK cell numbers were significantly different in early relapses (≤5 months after TKI stop) versus late relapses (>5 months after TKI stop) [44]. Thus, different mechanisms may be involved in return of the disease at different time points. It further remains to be determined if pharmacological use of agent(s) that stimulate NK cell function can increase the number of CML patients achieving deep molecular response and long-term TFR after TKI cessation. Whether NK cell number and function may also be used to predict disease relapse after TKI discontinuation needs to be investigated.

In EURO-SKI it was prospectively demonstrated in 122 patients that the expression of the T-cell inhibitory receptor (CTLA-4) ligand CD86 (B7.2) on plasmacytoid dendritic cells (pDC) affects relapse risk after TKI cessation. TFR rate was 30.1% for patients with >95 CD86 + pDC per 105 lymphocytes, but 70.0% for patients with <95 CD86 + pDC. Moreover, only patients with lower pDC derived a significant benefit from longer TKI exposure [46]. Other factors were retrospectively investigated like KIRs and pharmacogenetic factors influencing TKI uptake [47, 48].

16.4 Can we Cure CML?

The answer to this question depends on the definition of cure. If the definition of cure is "Absence of long-term leukemia relapse after treatment discontinuation," we have proven that it requires at least sustained DMR in TKI-treated patients. But we may never be able to prove that cure requires the eradication of residual leukemic cells. For instance, in the TWISTER study using PCR on DNA which is a non-routine technique increasing the sensitivity as compared to classical RQ-PCR to analyze patients who were considered in undetectable MR, leukemic cells were exhibited in all cases. In addition, as mentioned before using an ultrasensitive PCR technique, a low level of BCR-ABL1 transcripts has been found in the blood of normal individuals, suggesting that a complete absence of transcripts may not be required to eradicate the disease. However, newer research suggests that only lymphocytes may remain positive.

When patients still in MMR after TKI discontinuation were analyzed, clearly BCR-ABL1 fluctuations (defined by more than two consecutive positive values) were observed like, e.g., in A-STIM [26]. It means for those patients that leukemic cells persist, but the burden of the residual disease increases only in few patients even without treatment. These results are in agreement with the observation in patients who stopped interferon alpha in remission with clear evidence of residual disease without clinical relapse [49]. To speculate we could take the example from microbiology and infectious diseases where persistence of bacteria does not necessarily imply relapse. That is why John Goldman proposed some years ago the concept of "operational cure" [50]. This type of definition allows for the fact that, using an ultrasensitive PCR technique, low level of BCR-ABL1 transcripts can be found in the blood of normal individuals [22, 23].

In spite of these considerations if we want to decrease the rate of molecular recurrence after stopping TKI, we need to understand why quiescent leukemic stem cells (LSCs) are insensitive to TKIs, which is illustrated by the large number of publications focused on targeting the LSCs [51, 52]. Compared to normal stem cells, LSCs exhibit aberrant or nonregulated self-renewal, survival, and dormancy. Several strategies have been proposed including inhibiting survival/renewal pathways, sensitizing LSC (cycling or differentiating), immune targeting, or modifying the bone marrow niche; JAK/STAT, JAK2 kinase, the protein phosphatase 2A (PP2A), arachidonate 5-lipoxygenase gene (ALOX5), histone deacetylases (HDACs), sirtuin 1 (SIRT1), and BCL6 are among the most relevant targets for such a strategy [53–57]. Two of the most important pathways for self-renewal of CML-LSCs are the Wnt-catenin and the hedgehog (Hh) pathways [58, 59].

16.5 Side Effects after TKI Discontinuation

While imatinib and other TKIs can induce side effects in the musculoskeletal system, it has been assumed that such adverse events are reversible upon cessation of therapy. However, in all stopping trials, a substantial number of patients reported musculoskeletal pain starting or worsening 1–6 weeks after stopping TKI therapy. This was specifically investigated in a sub-cohort of the EURO-SKI trial where it occurred in 15 out of 50 patients [60]. The pain was localized to various parts of the body, including the shoulder and hip regions and/or extremities, sometimes resembling polymyalgia rheumatica. Symptoms were mild in most individuals, leading only to use of nonprescription drugs (paracetamol or NSAID), but some were more severely afflicted with manifestations interfering with everyday activities and requiring steroid therapy. Over time these symptoms seem to resolve. The rate of molecular recurrence in patients with musculoskeletal pain did not differ from those without these symptoms. These findings were confirmed in other studies [61].

This phenomenon is not restricted to imatinib pretreatment. Physicians should be aware of the possibility of adverse events appearing after stopping long-term TKI therapy. Further

investigations into underlying mechanisms are also warranted [61].

In conclusion, the subset of patients with DMR leading to cessation of treatment is heterogeneous. Around 40–60% of CML patients with stable DMR on TKI for at least 2 years are likely to remain in a prolonged TFR after treatment is stopped. Meanwhile international recommendations have included TFR as a treatment option to be considered in appropriate patients [2, 3].

Guidelines for TFR

NCCN[1]	ELN[2]
1. Age >18 2. Chronic phase disease, no history of AP, BC 3. TKI therapy ror >3 years 4. Quantifiable BCR-ABL1 transcripts 5. Stable MR (MR4) >2 years 6. Access to reliable PCR test 7. Monthly monitoring >1 year 8. TKI resumption within 4 weeks after MMR Loss 9. Consultation with CML Speciality center	1. Institutional criteria met and patient consent 2. Typical e13a2- or e14a2-BCR–ABL1 transcripts 3. Chronic phase disease 4. No prior treatment failure 5. First-line or second line (only intolerance) therapy Minimal criteria 1. Duration of TKI therapy >5 years (>4 years for 2nd gen. TKI) 2. Duration of DMR (MR4 or better) >2 years Optimal criteria: DMR >2 y for MR4.5, >3 y for MR4

TFR: Treatment-free remission
1. Radich Jp. et al. /Nat/Compr Canc Net 2018 2. Hochhaus A .et al Leukemics 2020.

Little is known about the possibility of stopping a second time. So far this seems to be successful in about 25% of patients [62]. Studies such as NAUT and DasStop2 which include IFN treatment are ongoing. A TFR approach as second stop should only be performed within clinical trials.

A long-term follow-up of different cessation studies will be necessary to affirm operational cure.

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