Treatment-Free Remission in Chronic Myeloid Leukemia

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Abstract: Tyrosine kinase inhibitors (TKIs) represent a major breakthrough in the treatment of chronic myeloid leukemia (CML). Thanks to these agents, CML has been transformed from a disease with limited treatment options and a dismal prognosis into a more indolent disease with survival comparable to that of the general population. The need for ongoing TKI therapy remains controversial for several reasons, including cost and toxicity. Studies in CML patients with a sustained deep molecular response have demonstrated that stopping TKI therapy is feasible and safe. Given the heterogeneity of results reported in clinical trials, practice guidelines for optimal patient selection and proper monitoring after discontinuation of TKIs are proposed outside of clinical trials. Current data available show that 40% to 60% of patients who stop therapy relapse; molecular relapses typically occur within 6 months, but nearly all relapsing patients regain response upon reinitiation of the TKI. Several factors that predict for relapse have been investigated. Duration of prior TKI therapy, achievement of deep molecular response, depth of molecular response, prior interferon treatment, and Sokal risk score have been shown to be potential predictors for relapse. Leukemia stem cells that are resistant to TKIs, and that persist despite undetectable BCR/ABL1 transcript levels, likely are responsible for disease relapse after discontinuation. Efforts geared toward better identification of low levels of BCR/ABL1 transcript using new techniques such as digital polymerase chain reaction, along with eradicating CML clones using combination therapies with agents such as pegylated interferon or venetoclax with TKIs, will hopefully lead to a functional cure of this disease.

Introduction

Chronic myeloid leukemia (CML), which is characterized by the Philadelphia chromosome and its molecular counterpart, the BCR-ABL1 fusion gene, has become a model for molecular targeted therapy. The treatment paradigm of CML has dramatically
changed with the introduction of tyrosine kinase inhibitors (TKIs). The life expectancy of patients with CML who have optimal responses to TKIs is now approaching that of the general population, particularly for younger patients. Although this disease remains rare, at approximately 1 case per 100,000 people, improved survival has led to an increased prevalence, and 400,000 patients with CML are expected in Europe by 2050. The US Food and Drug Administration (FDA) has approved 4 TKIs for the treatment of chronic phase CML (CP-CML) in the frontline setting: imatinib, dasatinib (Sprycel, Bristol-Myers Squibb), nilotinib (Tasigna, Novartis), and bosutinib (Bosulif, Pfizer). With TKI treatment, a substantial subset of patients with CP-CML achieve a deep molecular response (DMR), which is identified by measuring blood BCR-ABL1 transcript levels using real-time quantitative polymerase chain reaction (RQ-PCR). DMR represents a new cutoff of molecular residual disease: MR4.0 corresponds to a BCR/ABL1 ratio of less than 0.01%; MR4.5 corresponds to a ratio of less than 0.0032%; and MR5.0 corresponds to a 5-log reduction, or less than 0.001% according to the International Scale. Achievement of a sustained DMR is a goal of increasing relevance because it opens the possibility of treatment discontinuation. In 2006, Goldman and Gordon introduced the concept of “operational cure” into clinical practice. They observed that some patients who had received interferon (IFN) or an allogeneic stem cell transplant could stop treatment. In this subset of patients, RQ-PCR molecular monitoring showed the persistence of residual disease but did not require restarting treatment. These findings were then translated into the TKI era, leading to the concept of treatment-free remission (TFR) in select patients. To date, TFR is the most meaningful goal for patients treated with TKIs who have achieved sustained DMR.

This review analyzes evidence from both clinical trials and real-life experiences that relate to TKI discontinuation and the factors predicting successful TFR. It also describes potential mechanisms underlying the maintenance or failure of TFR, current practice guidelines outlining the criteria for TKI discontinuation, and the potential role of digital PCR in TFR.

The Concept of Treatment-Free Remission
The main rationales behind TFR are improving quality of life, reducing TKI-related adverse events, minimizing the pharmacologic and economic factors associated with lifelong therapy, and preventing toxicities in selected subsets of patients, such as adolescents.

Different studies have shown that prolonged TKI treatment may lead to adverse effects that negatively affect quality of life. For example, imatinib can lead to fatigue. Patients who are younger or female and receiving long-term therapy with imatinib have reported a worse quality of life compared with healthy individuals.

Long-term treatment in patients with CML can lead to TKI-related complications that may cause morbidity or mortality. An increased probability of cardiovascular events has been observed with several TKIs, especially nilotinib, and dasatinib has been associated with an increased risk for pleural effusion and pulmonary arterial hypertension. The ability to minimize the risk of developing new toxicities from long-term TKI therapy is a potential benefit of TFR.

Another paramount issue related to lifelong therapy is the annual cost of treatment per patient, which is currently estimated at €30,000 to €40,000 in most European countries and more than $100,000 in the United States. These costs may be somewhat offset by TFR. TKI discontinuation had a large economic impact in the EURO-SKI trial (European Stop Tyrosine Kinase Inhibitors), with an estimated savings of €22 million just for the 755 patients in this study.

Although CML is rare in the pediatric population, the most important concern in treating these patients is the safety of prolonged TKI treatment. Successful TFR in a small number of pediatric patients has been reported, but further studies are needed to clarify whether the TFR approach should be included in clinical practice for pediatric CML.

Trials With Imatinib
The French STIM trial (Stop Imatinib) enrolled CML patients on imatinib therapy for a minimum of 3 years with undetectable transcripts (with an RQ-PCR sensitivity of at least 5 logs) sustained for more than 2 consecutive years, and with at least 5 assessments during those 2 years. After a follow-up of 77 months after discontinuation of therapy, the molecular relapse–free survival rate was 43% at 6 months and 38% at 60 months, with the majority of relapses occurring within 6 months after imatinib cessation. In this trial, relapse was defined as detectable BCR/ABL1 transcript by RQ-PCR at 2 consecutive points, with a rise in the transcript level by 1 log at the second endpoint in relation to the first. However, with longer follow-up, it was found that some patients might experience reappearance of detectable transcripts at low levels with molecular fluctuations but not with a significant increase of the transcript.

Based on these findings, the A-STIM study (According to Stop Imatinib) proposed changing the criterion for treatment reinitiation to loss of major molecular response (MMR). In this trial, investigators found no difference in the 12-month molecular relapse rate between patients with stable undetectable BCR/ABL1 and those with occasional...
positivity. Furthermore, by 2 years the cumulative rate of molecular relapse was 36%, whereas the cumulative rate of loss of undetectable \( BCR/ABL1 \) (molecular relapse as defined in STIM) was 54%.14

Therefore, in the subsequent TFR trials, loss of MMR was considered the molecular threshold needed for a mandatory TKI reinitiation. Of the 31 patients in the A-STIM study who reinitiated treatment after loss of MMR, all regained MMR. Generally, all patients who relapsed in imatinib discontinuation trials remained sensitive to TKIs and regained molecular response upon reinitiation.14,17,19-21 One case of progression following TFR has been reported among more than 2000 patients attempting TFR in several trials; this occurred in a patient from the A-STIM study who progressed after responding to imatinib reinitiation following molecular relapse.22 Approximately one-half of patients in the trials experienced relapse within the first 6 to 12 months after stopping imatinib treatment, suggesting that in most cases relapses arise from residual leukemic cells with a fast proliferation (1 log per month).18

Data were recently published from the EURO-SKI study, the largest trial on TFR to date. This trial included 758 patients (94% treated with first-line imatinib) with at least 3 years of TKI treatment and MR4.0 sustained for at least 1 year. After a median follow-up of 27 months, molecular relapse–free survival was 61% at 6 months and 50% at 24 months. Of the 758 patients, 371 (49%) lost MMR after TKI discontinuation.14 Even though the criteria for TKI discontinuation were less stringent (4-log\(_{10}\) reduction or lower and duration of DMR < 1 year) than those in the STIM study (patients with negative PCR results and duration of DMR ≥ 1 year),17 the results of the 2 studies were similar, with most molecular recurrences occurring within the first 6 months after TKI discontinuation. Furthermore, the EURO-SKI study validated that loss of MMR is a safe criterion for TKI reinitiation, as was previously shown in the A-STIM study.18 The trial proposed a relationship between longer DMR and a more sustained MMR, which could help guide practitioners in better selecting patients for TFR. For each additional year a patient is in DMR before stopping imatinib, there is an increase of approximately 3% in the probability that the patient will remain in MMR 6 months after stopping therapy.

**Trials With Dasatinib or Nilotinib**

The DASFREE trial (Open-Label Study Evaluating Dasatinib Therapy Discontinuation in Patients With Chronic Phase Chronic Myeloid Leukemia With Stable Complete Molecular Response) reported results from a large population of patients discontinuing dasatinib as first-line therapy and beyond. Patients with CP-CML who were on dasatinib therapy for at least 2 years and in DMR (MR4.5) for at least 1 year were enrolled in the study. The TFR rate at 1 year was 49%, and 51% of patients lost MMR after a median of 4 months. All patients who lost MMR restarted dasatinib, and regained MMR after a median of 2 months.23

In the DADI study (Dasatinib Discontinuation), patients with CP-CML who received dasatinib following resistance to or intolerance of imatinib and had a \( BCR/ABL1 \) value of less than 0.0069% for at least 1 year were eligible to attempt TFR. Among 63 patients who discontinued dasatinib, TFR rates were 49% at 6 months and 44% at 36 months, but the criterion for molecular relapse (defined as \( BCR/ABL1 \geq 0.0069\%\)) was more stringent than in other studies.21

The D-STOP trial (Dasatinib Stop) evaluated TFR in patients with CP-CML who maintained DMR (\( BCR/ABL1 \leq 0.0069\%\)) for at least 2 years after receiving any TKI and dasatinib consolidation. Among patients who received consolidation therapy, 54 (83%) discontinued dasatinib treatment. After a median follow-up of 16 months, the estimated overall probability of TFR at 12 months was 63%. All relapsed patients responded to dasatinib reinitiation and achieved an MMR within 6 months of restarting dasatinib.24

The ENESTfreedom trial (Nilotinib Treatment-Free Remission Study in CML Patients) enrolled 215 patients with newly diagnosed CP-CML who were treated with nilotinib for at least 2 years and were in MR4.5 prior to study entry. Once enrolled, patients received 1 additional year of nilotinib consolidation; patients (n=190; 88.3%) who maintained a DMR during this time attempted TFR. The study reported that of the 190 patients who attempted TFR, 93 (49%) remained off treatment, without loss of MMR, at 96 weeks. Eighty-seven of the 88 patients who received remission regained MMR by the 96-week data cutoff (1 patient left the study).25

Nilotinib discontinuation was also evaluated as second-line treatment in the ENEStop trial (Treatment-Free Remission After Achieving Sustained MR4.5 on Nilotinib). The study enrolled patients treated with imatinib for more than 3 years with detectable molecular response who switched to nilotinib for more than 2 years (51 switched owing to intolerance, 30 switched owing to resistance, and 44 switched owing to physician preference) and discontinued when stable MR4.5 was reached. A total of 126 patients discontinued nilotinib in ENEStop; 58% remained off therapy at week 48 and 53% continued to remain off therapy at week 96 of the TFR phase.26 A subgroup analysis of ENEStop, based on reasons for switching to nilotinib prior to TFR, showed that the TFR rate at 48 weeks was similar between the 3 groups: 59% in the intolerance subgroup, 53% in the
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resistance subgroup, and 61% in the physician preference subgroup.27

The Stop Nilotinib trial (NILSt) enrolled 87 patients who had achieved MR4.5 with either imatinib or nilotinib and then received consolidation nilotinib for 1 additional year, maintaining the molecular response. Molecular relapse was defined as the loss of MR4.5. At 1 year after discontinuation, 53 (59%) of 87 patients maintained their response. Of 34 patients who lost MR4.5, 32 regained it after restarting nilotinib.28 Characteristics of the main trials, including TFR assessment after imatinib, nilotinib, and dasatinib discontinuation, are summarized in Table 1.

Predictors of Sustained TFR

A large number of clinical and biological predictive factors were assessed in order to better identify groups of patients with a high probability of maintaining a DMR after TKI discontinuation (Table 2), thereby decreasing the probability of requiring TKI reinitiation. Total duration of TKI therapy and DMR are the most consistently reported predictive factors for achieving a TFR. In the Stop Imatinib trial (STIM), long-term follow-up showed that patients with CML who received imatinib for more than 50 months experienced a lower rate of molecular relapses than those who received imatinib for less time.29 In the EURO-SKI trial, an interim analysis of 405 patients who received imatinib as first-line treatment documented that longer treatment duration (odds ratio per year, 1.14; P=.0010) and longer DMR durations (odds ratio per year, 1.13; P=.0032) were associated with increasing probability of MMR maintenance at 6 months.19 Several studies reported that the depth of molecular response may also be predictive of successful discontinuation.20,29,30 However, an interim analysis of the EURO-SKI study did not show

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Pts</th>
<th>TKI at Time of Discontinuation</th>
<th>Median Duration of TKI, y</th>
<th>Stable DMR at Discontinuation</th>
<th>Duration of s-DMR Before Discontinuation, y</th>
<th>TFR Time, y</th>
<th>TFR Rate, %</th>
<th>Retreatment Criteria</th>
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<tr>
<td>TWISTER33</td>
<td>40</td>
<td>Imatinib</td>
<td>5.9</td>
<td>UMRD</td>
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<td>&gt;UMRD × 2, &gt;MMR</td>
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<td>A-STIM18</td>
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<td>3.4</td>
<td>2</td>
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<tr>
<td>HOVONN62</td>
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<td>UMRD</td>
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<td>2</td>
<td>58.5</td>
<td>&gt;MMR × 2</td>
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<td>5</td>
<td>38</td>
<td>&gt;UMRD × 2, &gt;MMR</td>
</tr>
<tr>
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<td>9.1</td>
<td>MR4.5</td>
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<td>57.5</td>
<td>&gt;MR4.0 × 2, &gt;MMR</td>
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<td>Imatinib</td>
<td>8.1</td>
<td>MR4.5</td>
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<td>&gt;MMR</td>
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<td>Dasatinib</td>
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<td>48</td>
<td>≥0.0069%</td>
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<td>MR4.5</td>
<td>2</td>
<td>1</td>
<td>41</td>
<td>&gt;MMR</td>
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<td>ENESTfreedom35</td>
<td>190</td>
<td>Nilotinib</td>
<td>3.5</td>
<td>MR4.5</td>
<td>2.5</td>
<td>1.85</td>
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<td>Nilotinib</td>
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<td>MR4.5</td>
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<td>1.85</td>
<td>53.2</td>
<td>&gt;MR4.0 × 2, &gt;MMR × 1</td>
</tr>
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<td>NILSt28</td>
<td>87</td>
<td>Nilotinib</td>
<td>8.6</td>
<td>MR4.5</td>
<td>NR</td>
<td>1</td>
<td>58.9</td>
<td>&gt;MR4.5 × 2</td>
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<td>STAT265</td>
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<td>Nilotinib</td>
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<td>MR4.5</td>
<td>NR</td>
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<td>7.1</td>
<td>MR4.5</td>
<td>NR</td>
<td>1</td>
<td>41</td>
<td>&gt;MR4.5 × 2</td>
</tr>
</tbody>
</table>

DMR, deep molecular response; MMR, major molecular response; MR4.0, BCR/ABL1 ratio <0.01%; MR4.5, BCR/ABL1 ratio <0.0032%; NR, not reported; pts, patients; s-DMR, sustained deep molecular response; TFR, treatment-free remission; TKI, tyrosine kinase inhibitor; UMRD, undetectable minimal residual disease.
any significant difference in TFR rates when comparing patients with MR4.0, MR4.5, and MR5.0 before TKI discontinuation. An early loss of MR4.5 after discontinuation and prior TKI resistance defined by European LeukemiaNet criteria were also identified as potential predictors of TFR failure. In the STOP 2G-TKI study (Discontinuation of Dasatinib or Nilotinib in Chronic Myeloid Leukemia), the cumulative incidence of TFR rates at 48 months was 79.7% for patients maintaining MR4.5 at 3 months and 18.1% for those with less than MR4.5 but in MMR at 3 months (P<.00001). Furthermore, patients receiving first-line TKIs and patients

### Table 2. Predictors of Sustained TFR

<table>
<thead>
<tr>
<th>Significant Predictors</th>
<th>Study No. of Pts</th>
<th>TKI at Time of Discontinuation</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of TKI Treatment</td>
<td>STIM⁹⁹ 100</td>
<td>Imatinib</td>
<td>&gt;50 mo of imatinib associated with lower rate of molecular relapse (P=.49)</td>
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<tr>
<td></td>
<td>EURO-SKI¹⁹</td>
<td>772</td>
<td>Imatinib (94%)/dasatinib (2%)/nilotinib (4%)</td>
</tr>
<tr>
<td>Duration of DMR</td>
<td>US trial⁶⁷</td>
<td>100</td>
<td>Imatinib (46%)/nilotinib (14%)/dasatinib (35%)/ponatinib (2%)/bosutinib (2%)</td>
</tr>
<tr>
<td></td>
<td>TRAD⁵³</td>
<td>123</td>
<td>Imatinib</td>
</tr>
<tr>
<td>Early loss of MR4.5 after discontinuation</td>
<td>STOP 2G-TKI³²</td>
<td>60</td>
<td>Nilotinib (50%)/dasatinib (50%)</td>
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<tr>
<td>Prior TKI resistance</td>
<td>DADI⁶⁰</td>
<td>63</td>
<td>Dasatinib</td>
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<tr>
<td>Sokal score at baseline</td>
<td>TWISTER³³</td>
<td>40</td>
<td>Imatinib</td>
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<td>STIM⁹⁹</td>
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<td>ISAV³⁰</td>
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<td>Imatinib</td>
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<td>Previous IFN treatment</td>
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<td>Withdrawal syndrome</td>
<td>KID⁵⁰</td>
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<td>Transcript</td>
<td>Italian study⁶⁸</td>
<td>173</td>
<td>Imatinib (68.2%)/2G-TKI (31.8%)</td>
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<tr>
<td>Digital PCR</td>
<td>Italian study⁵⁹</td>
<td>142</td>
<td>Imatinib (76%)/nilotinib (20%)/dasatinib (3.5%)/bosutinib (0.5%)</td>
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<tr>
<td>NK cells</td>
<td>DADI⁶⁰</td>
<td>63</td>
<td>Dasatinib</td>
</tr>
</tbody>
</table>

2G-TKI, second-generation TKI; DMR, deep molecular response; IFN, interferon; MMR, major molecular response; mo, months; MR4.0, BCR/ABL1 ratio <0.01%; MR4.5, BCR/ABL1 ratio <0.0032%; NK, natural killer; PCR, polymerase chain reaction; pts, patients; TFR, treatment-free remission; TKI, tyrosine kinase inhibitor; y, years.
discontinuing their first-line TKI owing to intolerance had a higher probability of successful TFR than those previously experiencing resistance to their first-line TKI. In the DADI trial, previous imatinib resistance was associated with a higher molecular relapse rate; at 36 months, TFR in imatinib-resistant patients vs those who were intolerant was 8% vs 55.6%, respectively. Sokal risk score was also defined as a potential prognostic factor affecting TFR. Data from the TWISTER study (A Phase II Study of Withdrawal of Imatinib Therapy in Adult Patients With Chronic Phase Chronic Myeloid Leukemia in Stable Molecular Remission) suggested that a high Sokal score at diagnosis was associated with a higher rate of molecular relapse. In addition, the STIM trial showed an estimated survival rate at 18 months of 54% for patients with a low Sokal score, compared with 35% and 13% in those with intermediate and high scores, respectively. Other studies showed that presence of withdrawal syndrome, older age, and previous IFN treatment were associated with higher TFR after discontinuation. Having a higher number of circulating natural killer (NK) cells was correlated with improved rates of TFR. In the DADI study, univariate analysis revealed that a greater number of total NK cells (CD3–D56+), a greater number of cytolytic NK cells (CD16+D56+), a lower number of γδ+ T cells, and a lower number of CD4+ regulatory T cells were associated with successful maintenance of TFR. In a subanalysis of EURO-SKI, a higher proportion of NK cells was associated with better molecular relapse-free survival, but a similar association was not found when T cells or B cells were assessed. Furthermore, in patients with sustained TFR, the NK cell phenotype was mature and the tumor necrosis factor-α/IFN-γ cytokine secretion by NK cells correlated with successful drug discontinuation.

Mechanisms Underlying TFR Maintenance and Relapse

The exact mechanisms underlying successful TFR vs relapse are unclear. The identification of biomarkers directly linked to such mechanisms may be the only path to improving prediction of TFR in individual patients. Some patients with undetectable minimal residual disease become molecularly positive after stopping TKIs but continue to express BCR/ABL1 at low levels without further progression and loss of TFR. In the TWISTER study, highly sensitive patient-specific BCR/ABL1 DNA PCR was noted to be persistent even when patients had undetectable RQ-PCR. It was speculated that some type of immunologic phenomenon may be responsible for suppressing these leukemic clones. Circumstantial evidence exists to support this hypothesis, including the curative potential of donor lymphocyte infusion after relapse following allogeneic stem cell transplant and the immune effects of long-term treatment with IFN associated with successful TFR. Several studies have shown the persistence of quiescent leukemic stem cells (LSCs) in patients with CML after TKI discontinuation and after allogeneic stem cell transplant, which did not necessarily translate into relapse of disease. Mathematical modeling of the kinetics of molecular relapse in the STIM study has led to the hypothesis that imatinib may increase the frequency of LSC clones with slower growth and differentiation than the predominant clone at baseline. On the other hand, imatinib’s inhibitory effect on the leukemia initiating cells (LICs) might vary from patient to patient. In fact, in some patients, imatinib might successfully eradicate LICs, leaving only a small population of quiescent LSCs and enabling prolonged TFR, whereas populations of LICs with variable growth kinetics remain in other patients. Multiple strategies specifically targeting the LSC reservoir in patients with CML are ongoing; they include combination of a TKI with immunomodulatory agents, such as pegylated interferon (peg-IFN), or other targeted agents, such as inhibitors of B-cell lymphoma/leukemia 2 (BCL2), anti–programmed death 1 monoclonal antibodies, and dendritic cell vaccines. The addition of low-dose peg-IFN to imatinib and dasatinib induced deeper and faster molecular responses compared with those observed with either TKI alone. Moreover, the sequence of an imatinib/IFN-based first-line therapy followed by peg-IFN maintenance has resulted in sustained remissions in the majority of patients, irrespective of the prior achievement of molecular response. The ongoing TIGER study (TKI and Interferon Alpha Evaluation Initiated by the German Chronic Myeloid Leukemia Study Group) is further investigating this approach in newly diagnosed CP-CML patients; the trial compares the efficacy of nilotinib alone to nilotinib plus IFN-α as a first-line therapy. A stopping procedure is integrated in both arms to evaluate the role of IFN-α in the context of TFR. An interim analysis of the trial showed that the cumulative incidence of MMR after randomization at the 12-, 18-, and 24-month follow-up was 79.5%, 84.9%, and 89.4%, respectively, and that the probabilities of grade 3 to 5 adverse events after 3 years were 39.6% and 49.5% for the nilotinib and nilotinib plus peg-IFN-α2b induction treatment arms. This demonstrated favorable outcomes of nilotinib alone to nilotinib plus IFN-α as a first-line therapy. A stopping procedure is integrated in both arms to evaluate the role of IFN-α in the context of TFR.
marrow CML cells, and that a TKI and BH3 mimetic combination effectively eradicated CD34+CD38−, CD34+CD38+, and quiescent stem/progenitor CD34+ cells from blast crisis CML patient samples. A study combining dasatinib at 50 mg daily with venetoclax in frontline CP-CML is currently ongoing.

**Multiple TKIs and Second Discontinuation Attempt**

The STOP 2G-TKI study, which was conducted by the French Chronic Myeloid Leukemia Study Group, included patients with CP-CML who had received dasatinib or nilotinib for more than 3 years as second-line treatment after resistance and/or intolerance to imatinib, and who had MR4.5 for more than 24 months. TFR rates at 12 and 24 months were 63.3% and 53.3%, respectively, and the cumulative incidence of molecular relapse (defined as the loss of MMR) by 12 and 48 months was 35% and 45%, respectively. Prior suboptimal response and TKI resistance were the only baseline factors associated with a lower rate of TFR.

An observational Italian study assessed TFR rate in 295 patients with CP-CML enrolled in several institutions. A total of 72% of patients were on imatinib therapy and the remaining 28% were on nilotinib (n=58), dasatinib (n=23), or bosutinib (n=1) at the time of discontinuation. At 12 months, the estimated TFR was 68% for imatinib and 73% for second-generation TKIs, with no significant differences between the 2 groups. The authors assessed age, sex, Sokal score, type of transcript, previous IFN therapy, duration of TKI therapy, response at 3 months, time to DMR, DMR duration, line of therapy at stop, depth of molecular response, and reasons for stopping as potential prognostic factors for TFR. No significant association was identified, with the exception of age (a decreased risk of relapse in older vs younger patients).

RE-STIM (Second Tyrosine Kinase Inhibitor Discontinuation Attempt in Patients With Chronic Myeloid Leukemia), a French multicenter study, evaluated TFR in 70 patients who reattempted TKI discontinuation after a first unsuccessful attempt. All the patients had reached a sustained MR4.5 (>2 years) before attempting TFR the second time. TFR rates at 12, 24, and 36 months were 48%, 42%, and 35%, respectively. No progression toward advanced-phase CML occurred. In a univariate analysis, the speed of molecular relapse after the first TKI discontinuation attempt was the only factor significantly associated with outcome; the TFR rate at 24 months was 72% in patients who remained in DMR within the first 3 months after the first TKI discontinuation and 36% in those who did not.

A Canadian discontinuation trial called TRAD (Treatment-Free Remission Accomplished With Dasatinib in Patients With CML) is ongoing to determine whether using dasatinib can lead to a successful TFR among patients who require treatment after their first attempt to discontinue imatinib. Twenty-five of the 51 patients receiving dasatinib achieved MR4.5 for 12 months or longer and discontinued it for a second TFR attempt (TFR2). The estimated TFR2 rate was 21.5% (+8.5%) at 6 months. The 6-month TFR2 rate was 9% in the group that relapsed within 3 months of TFR1 and 30% in the group that relapsed within 3 to 6 months of TFR1 (P<.001).

These data suggest that more strict criteria should be considered for a TFR2 attempt, probably including achievement of deeper BCR/ABL1 levels (MR5.0) prior to the second TKI discontinuation.

**Clinical Practice Guidelines for TKI Discontinuation**

Given the heterogeneity of results reported in clinical trials and the difficulty of accurately predicting the success of TFR in individual patients, recommendations for patient criteria before stopping TKIs and for proper monitoring after discontinuation have been proposed. These practice guidelines include criteria for attempting TFR outside of clinical trials in order to help guide clinicians in safely and properly offering this option to selected patients. The concept of excluding patients with a history of advanced disease from possible discontinuation, as well as the molecular monitoring of the disease by a sensitive (≤MR4.5) reverse transcription PCR standardized to the International Scale monthly in the first 6 months after attempting TFR, is universally shared. Significant differences in the retreatment criteria exist, however. According to the NCCN guidelines,59 retreatment should occur when a patient loses MMR, has been treated with a previous TKI for 3 years, or has a depth of DMR that is MR4.0 or less for at least 2 years. In contrast, recommendations from ESMO55 and from Hughes and colleagues57 call for retreatment among patients who have an undefined MMR or who have a depth of DMR of MR4.5 or less for at least 2 years. Hughes and colleagues also call for retreatment among those who received a previous TKI for 8 years. The ESMO guidelines do not factor loss of MMR into retreatment criteria.

**Molecular Monitoring During TFR and Digital PCR**

The majority of molecular relapses were observed within 6 months of TKI cessation. Relapse after 6 months of TFR
TREATMENT-FREE REMISSION IN CHRONIC MYELOID LEUKEMIA

is rare, but it can still occur (no plateau of the TFR survival curves has been observed). Guidelines from the European Society for Medical Oncology recommend the need for frequent molecular monitoring, occurring monthly during the first half-year, every 6 weeks during the second half-year, and then every 3 months thereafter. However, in recent trials, monitoring was performed as infrequently as every 4 weeks initially, and after 6 months the interval was lengthened to every 3 months. A quantification of \( BCR/ABL1 \) messenger RNA, performed by RQ-PCR, is required before deciding on TFR. This method represents the most sensitive tool for the assessment of the disease status, particularly of measurable residual disease. However, in the TFR scenario, clinical evidence did not show a linear correlation between the depth of the DMR and the TFR maintenance rate. This may be explained by the intrinsic limitations of RQ-PCR, particularly concerning its lack of precision—especially in the quantification of the low levels of the target and the variation of its sensitivity from one test to another.

Digital PCR is reported to be 100 times more sensitive than RQ-PCR. Although it is not yet routinely applied for the standard analysis of molecular MRD in CML, preliminary data suggest that it could be more accurate for predicting TFR. In a recent Italian study, investigators reported that although RQ-PCR was not able to identify patients at elevated risk for molecular response loss after discontinuation (\( P=0.8100 \)), digital PCR had this ability (48% of patients with \( BCR/ABL1 \) values ≥0.468 vs 14% of patients with \( BCR/ABL1 \) values <0.468 lost DMR; \( P=0.0003 \)). Furthermore, the TFR rate of patients who discontinued TKI with a digital PCR of less than 0.468 was significantly higher compared with patients with a digital PCR of at least 0.468 (TFR at 2 years was 83% vs 52%, respectively; \( P=0.0017 \)). Finally, digital PCR may allow for better understanding of MRD.

### Table 3. Criteria for Discontinuing TKIs Based on Expert Recommendations and Guidelines

<table>
<thead>
<tr>
<th></th>
<th>ESMO(^{55} )</th>
<th>NCCN(^{54} )</th>
<th>French Chronic Myeloid Leukemia Study Group(^{56} )</th>
<th>Hughes(^{57} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCR-ABL1 transcript</strong></td>
<td>Typical, measurable</td>
<td>Typical, measurable</td>
<td>e13a2, e14a2, or e13a2 + e14a2</td>
<td>Typical, measurable</td>
</tr>
<tr>
<td><strong>CML history</strong></td>
<td>Chronic phase</td>
<td>Chronic phase</td>
<td>Chronic phase</td>
<td>Chronic phase</td>
</tr>
<tr>
<td><strong>Sokal score</strong></td>
<td>Not high</td>
<td>Not defined</td>
<td>Not defined</td>
<td>Not high</td>
</tr>
<tr>
<td><strong>Response to first-line TKI</strong></td>
<td>Optimal</td>
<td>No resistance</td>
<td>No allogeneic HSCT, progression, resistance, suboptimal response, or warning</td>
<td>Optimal</td>
</tr>
<tr>
<td><strong>DMR</strong></td>
<td>≤MR4.5</td>
<td>≤MR4.0</td>
<td>≤MR4.5</td>
<td>≤MR4.0</td>
</tr>
<tr>
<td><strong>Duration of DMR</strong></td>
<td>≤MR4.0 or ≥2 y</td>
<td>≥2 y</td>
<td>≥2 y</td>
<td>1-2 y</td>
</tr>
<tr>
<td><strong>Duration of TKI</strong></td>
<td>≥5 y</td>
<td>≥3 y</td>
<td>≥5 y</td>
<td>3-8 y</td>
</tr>
<tr>
<td><strong>Retreatment</strong></td>
<td>Not defined</td>
<td>Loss of MMR</td>
<td>Loss of MMR</td>
<td>Not defined</td>
</tr>
<tr>
<td><strong>Frequency of monitoring</strong></td>
<td>Monthly during the first half-year, every 6 weeks during the second half-year, and every 3 months later on</td>
<td>Monthly for the first 12 mo, every 6 wk during mo 13-24, and every 12 wk thereafter</td>
<td>Monthly during the first 6 mo, every 2 mo from 7-12 mo, quarterly during the second year, and then every 3-6 mo</td>
<td>Monthly during the first 6 mo, every 2-3 mo later on</td>
</tr>
</tbody>
</table>

CML, chronic myeloid leukemia; DMR, deep molecular response; ESMO, European Society for Medical Oncology; HSCT, hematopoietic stem cell transplant; MMR, major molecular response; mo, months; MR4.0, \( BCR/ABL1 \) ratio <0.01%; MR4.5, \( BCR/ABL1 \) ratio <0.0032%; TKI, tyrosine kinase inhibitor; wk, weeks; y, years.
and potentially permit earlier identification of patients who will experience molecular relapse.

**Future Directions**

Discontinuation studies in CML patients with sustained DMR have demonstrated that stopping TKI therapy is feasible and safe, and that some patients have long sustained TFR. The current data have shown that 40% to 60% of patients relapse while in TFR. Molecular relapses typically occur within 6 months, but nearly all relapsing patients regain response once TKI treatment is reinitiated. Although late relapses are rare, they do occur, and continuous monitoring is mandatory. Furthermore, some data have shown that attempting a second TKI discontinuation after molecular recurrence is possible, and this might be effective in approximately 30% of cases after an adequate duration of the re-achieved DMR.

TFR is an attractive strategy for CML patients owing to the potential freedom from lifelong therapy with TKIs, which are associated with adverse effects and high economic burden. Second-generation TKIs are known to achieve faster and deeper responses in comparison to imatinib but they also cost more, especially because imatinib is available as a generic. With the incorporation of TFR into the treatment strategy, the question remains as to whether second-generation TKIs are more cost-effective given that 40% to 60% of patients relapse after TFR, generally within the first 6 months. Because imatinib is still an effective therapy with decreased cost, it may be best to utilize it instead of second-generation TKIs as first-line treatment to help abrogate the economic burden in the era of TFR.

Several challenges remain regarding incorporation of TFR in CML. First, it may be successful only in a subset of patients. In fact, based on the stricter molecular criteria for discontinuation (MR4.5 sustained for ≥2 years), only 40% of patients receiving TKIs may attempt TFR. Among these, approximately 50% maintained molecular responses without reinitiating TKIs, and only 20% had successful TFR. Novel treatment approaches that include immunotherapy-based combination treatment and the combination of TKIs with venetoclax might be capable of eradicating the leukemic stem cell, thus increasing the rate of durable DMR. Therefore, a larger number of patients potentially will be eligible for discontinuation, with a lower rate of molecular relapse after stopping TKI therapy.

Another challenge in TFR is the proper identification of patients who have a high probability of relapsing versus those who are able to maintain TFR. Some of the most promising research addressing this question has been in the field of immunology regarding T-cell cytotoxicity and NK cell effects. In several studies, NK cell numbers or functional subsets of NK cells were shown to be higher in patients who maintain TFR than in those who lose molecular response during TKI discontinuation.

The improvement of techniques able to better quantify low levels of BCR/ABL1 transcript could also help select specific patients who would certainly benefit from a TFR attempt. In recent years, digital PCR has emerged to provide a more sensitive and accurate detection of very low levels of MRD. In the near future, digital PCR could become the main monitoring technique for selecting the best candidates for TFR.

**Conclusions**

TFR has become a standard part of CML care. Worldwide, more than 2000 patients with CML have attempted TFR, and no instances of disease transformation have been reported. Despite the remaining questions regarding which patients are the best candidates to attempt TFR and which factors may be considered predictive for TFR, treatment interruption is a safe option provided that adequate molecular monitoring is available, with prompt reinitiation of TKIs as soon as MMR has been lost.

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