Deep Molecular Response in Chronic Myeloid Leukemia: The New Goal of Therapy?

François-Xavier Mahon¹ and Gabriel Etienne²

Abstract

Chronic myeloid leukemia (CML) is caused by formation of the BCR–ABL1 fusion protein. Tyrosine kinase inhibitors (TKI) that target BCR–ABL1 are now the standard of care for patients with CML. Molecular monitoring of residual BCR–ABL1 mRNA transcripts, typically performed using real-time quantitative PCR, has improved treatment management, particularly for patients with CML in chronic phase. Major molecular response (MMR; i.e., a ≥3-log reduction in BCR–ABL1 transcript levels) is used in current treatment guidelines to assess prognosis. Recent evidence suggests that deeper molecular responses (≥4-log reductions in BCR–ABL1 transcript levels), particularly when attained early during treatment, may have even better correlation with long-term outcomes, including survival and disease progression. Furthermore, achieving deep molecular response is a requirement for entering trials evaluating treatment-free remission (TFR). In this review, we discuss the evolving definition of minimal residual disease and the various levels of molecular response under evaluation in current clinical studies. In addition, the available clinical data on achieving MMR and deeper levels of molecular response with TKI therapy, the prognostic value of deep molecular response, and factors that may predict a patient’s ability to achieve and sustain a deep molecular response on TKI therapy are also discussed. Available data from TFR studies are addressed. We discuss current knowledge of the ideal conditions for attempting treatment discontinuation, factors predictive of molecular relapse, when TKI therapy should be restarted, and which therapeutic strategies (when administered in the first-line setting and beyond) are expected to best enable successful TFR. Clin Cancer Res; 20(2); 1–13. ©2013 AACR.

Introduction

Dysregulated protein tyrosine kinase (PTK) activity is the hallmark of multiple neoplasms (1). Over the past decade, a broad array of drugs designed to selectively inhibit PTKs [i.e., tyrosine kinase inhibitors, (TKI)] have emerged as novel therapies for cancers with (2). Perhaps the most well-known PTK target to date is the BCR–ABL1 oncoprotein, which is critical to the pathogenesis of chronic myeloid leukemia (CML; ref. 3). The successful treatment of patients with CML with the BCR–ABL1 TKI imatinib has definitively validated this therapeutic strategy and established CML as a model disease for targeted cancer treatment (4).

With over a decade of imatinib use as first-line therapy in patients with CML, surrogate markers that strongly correlate with prognosis have been identified (5). The validation of surrogate endpoints for treatment effectiveness, such as complete hematologic response (CHR) and complete cytogenetic response (CCyR), has improved treatment management (6, 7). Most patients, particularly those with CML in chronic phase (CML-CP), will achieve these endpoints on a BCR–ABL1 TKI (8–10). The progress of molecular biology has presented the opportunity to look beyond CHR and CCyR and monitor residual disease on a molecular level (11, 12). Current clinical practice recommendations for CML advocate monitoring patients for hematologic response (i.e., blood counts returning to normal values), cytogenetic response (i.e., disappearance of the Philadelphia chromosome), and molecular response (i.e., reduction in BCR–ABL1 gene expression; refs. 6 and 7). Molecular monitoring is typically performed using real-time quantitative PCR (RQ-PCR), a simple technique that can be performed on peripheral blood samples and is both more sensitive and more convenient than conventional cytogenetics (13). The first level of response evaluated on the molecular scale, a major molecular response (MMR), corresponds to a 3-log reduction in BCR–ABL1 transcript levels from a standardized baseline (≤0.1% BCR–ABL1 on the International Scale [BCR–ABL1][IS]; refs. 11 and 14).

Authors’ Affiliations: ¹Laboratoire d’Hématologie, Centre Hospitalier Universitaire de Bordeaux and Laboratoire Hématologie Leucémique et Cible Thérapeutique, Biothérapies des maladies génétiques et cancers, Inserm U1035, Université Bordeaux Ségalen; and ²Centre Regional de Lutte contre le Cancer de Bordeaux et du Sud-Ouest, Institut Bergonié, Département d’Oncologie Médicale, Bordeaux, France

Corresponding Author: François-Xavier Mahon, Laboratoire d’Hématologie, CHU de Bordeaux and Laboratoire Hématologie Leucémique et Cible Thérapeutique, Biothérapies des maladies génétiques et cancers, Université Victor Ségalen Bordeaux 2, 146 rue Léo-Saignat, Inserm U1035, 33076 Bordeaux Cedex, France. Phone: 33-5-5757-1524; Fax: 33-5-5693-8883; E-mail: francois-xavier.mahon@u-bordeaux2.fr

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has been found to be associated with improved progression-
free survival (PFS; refs. 15 and 16) and event-free survival
(EFS; refs. 17–19), although its prognostic value is still
debated.

The European LeukemiaNet (ELN) defines response to
TKI therapy based on molecular and cytogenetic milestones
achieved at 3, 6, and 12 months, or beyond (6). The ELN
recommends evaluating molecular response using buffy
coat from peripheral blood every 3 months until MMR is
achieved, and then every 3 to 6 months. Cytogenetic
response should be assessed using bone marrow at 3, 6,
and 12 months, and then every 12 months once CCyR is
achieved. Some patients (e.g., patients with monosomy 7,
del[7q] or clonal chromosomal abnormalities) may require
additional long-term bone marrow follow-up.

Until recently, deep molecular responses beyond the level
of MMR have remained largely unexplored, given a lack of
standardized assay techniques (20). Several recent studies
have demonstrated that some patients with such responses
can achieve treatment-free remission (TFR), thereby chal-
 lenging the dogma that CML cannot be cured without
allogeneic bone marrow transplantation (21, 22). The
overall goals of therapy in CML—disease remission,
reduced risk of progression, and improved overall survival
(OS)—are clear; however, many questions remain about
the impact of molecular response on achieving these goals.
Recent data, to be discussed herein, suggest that in select
populations, obtaining a deep molecular response should
be considered a primary therapeutic goal.

The Challenge of Defining Deep Molecular
Response

The development of the International Scale for BCR–
ABL1 RQ-PCR assessment has enabled quantification of
molecular response in relation to a standardized baseline
(14, 23). The definition of MMR as $BCR-\text{ABL1 IS}_{\text{20}} \leq 0.1\%$
originates from the International Randomized Study of
Interferon Versus STI571 (IRIS; ref. 15). However, standard-
ization of deeper levels of molecular response has proven
less straightforward and is urgently needed to facilitate
improved interpretation of clinical results. Deeper levels
of molecular response may also be defined according to the
International Scale, wherein MR$^4$ indicates $\geq 4.0$-log
reduction ($BCR-\text{ABL1 IS}_{\text{20}} \leq 0.01\%$), MR$^{4.5}$ indicates $\geq 4.5$-log
reduction ($BCR-\text{ABL1 IS}_{\text{20}} \leq 0.0032\%$), and MR$^5$ indicates
$\geq 5.0$-log reduction ($BCR-\text{ABL1 IS}_{\text{20}} \leq 0.001\%$; Fig. 1; ref. 11).
However, the observation of undetectable $BCR-\text{ABL1}$ tran-
scripts is inherently linked to the sensitivity of the PCR
method, as well as the control gene, used. An ongoing
European Treatment and Outcome Study (EUTOS) collab-
oration aims to facilitate standardization of deep molecular
response across laboratories by establishing recommenda-
tions for response definitions and quality control (11).

Varying definitions of complete molecular response
(CMR) have been reported. For example, the National
Comprehensive Cancer Network (7) defines CMR as "no
detectable $BCR-\text{ABL1}$ chimeric mRNA as assessed by RQ-
PCR using the International Scale with a sensitivity of 4.5-

![Figure 1. Levels of molecular response in CML. MCyR, major
 cytogenetic response; MR, molecular response.](https://example.com/
figure1.png)
Deep Molecular Response in Chronic Myeloid Leukemia

Deep Molecular Response in Clinical Trials of CML-CP

Deep molecular responses have been assessed in multiple ongoing clinical trials of TKIs in patients with newly diagnosed CML-CP (Table 1). Given the current lack of standardization of PCR sensitivity, rates of CMR reported in these studies are not necessarily comparable. Discrepancies in rates of deep molecular response may also result from differences in assay techniques or study design. For example, PCR may be assessed using blood or bone marrow, and response may be defined by a PCR result at a single time point or confirmed by multiple samples. Other variables include the study population (e.g., the proportion of high-risk patients), median follow-up at time of analysis, and type and dose of TKI therapy received. Although several studies have shown that imatinib can elicit deep molecular responses in some patients, second-generation TKIs, such as nilotinib and dasatinib, have demonstrated higher rates of deep molecular response attained within shorter time periods (Table 1). The combination of TKI therapy with interferon (IFN), which may help drive leukemic stem cells (LSC) into the cell cycle (26), thereby inducing deep molecular response, has also been explored. Rates of deep molecular response were higher with this combination versus imatinib alone in one study using pegylated IFN (26), but similar in another study using IFN-α (Table 1; ref. 27).

The Clinical Relevance of Deep Molecular Response

Defining the value of achieving deep molecular response in patients with CML is an active area of ongoing research. There is conflicting evidence about whether achieving an MMR provides additional benefit beyond a CCyR (20). The leukemic cell burden is similar in patients with MMR and CCyR (only 1-log difference in BCR–ABL1 levels), and this difference may be too small to impact long-term outcomes such as PFS and OS. Events like progression or death are quite infrequent with frontline TKI therapy; therefore, long-term follow-up and large cohorts of patients would be required to definitively demonstrate any added benefit to achieving MMR.

However, deeper molecular responses provide more separation from CCyR in terms of residual disease burden; therefore, it may be easier to distinguish the unique benefits of such responses. Patients who achieve deep molecular response seem less likely to lose MMR, and several studies have shown that deep molecular responses correlate with better long-term clinical outcomes, such as EFS, PFS, and OS, and a low risk of progression and disease relapse (Table 2; refs. 28 and 29). For example, a study by Etienne and colleagues (30) found that EFS and PFS were longer in patients with CMR than those with CCyR, regardless of MMR status; OS was not significantly different between these groups. Another study by Falchi and colleagues (31) showed that patients with undetectable BCR–ABL1 levels by 18 or 24 months had 100% rates of OS, EFS, and transformation-free survival (TFS) at 6 years. In the German CML study IV, life expectancy in patients with MR3 or MR4.5 was the same as that in an age-matched population, and only 4 of 792 patients (0.5%) who achieved MR4 had disease progression (32).

These results provide encouraging evidence that achievement of deep molecular response is an important clinical goal and prompt the question: should patients with CCyR, who do not achieve these deep levels of molecular response, be switched to an alternate therapy? The ongoing Evaluating Nilotinib Efficacy and Safety in Clinical Trials–CMR (ENESTcmr) study aims to address this question by randomizing patients with detectable BCR–ABL1 transcripts after ≥2 years on imatinib to either continue on imatinib or switch to nilotinib (33). After 12 and 24 months of follow-up, more patients achieved MR4.5 and undetectable BCR–ABL1 (≥4.5-log RQ-PCR assay sensitivity) on nilotinib compared with continued imatinib (33). Yet perhaps the most convincing reason to strive for sustained deep molecular response in patients with CML-CP is the possibility that it may enable eventual achievement of TFR.

Deep Molecular Response and TFR

Reports of imatinib discontinuation in patients without sustained deep molecular responses have shown high and rapid rates of disease relapse (34–37). In contrast, a small pilot study of imatinib discontinuation with the stringent entry criteria of sustained CMR for ≥2 years on imatinib showed promising results, with 6 of 12 patients remaining in molecular remission after a median 18 months of follow-up (38). This proof of concept inspired numerous clinical studies of imatinib discontinuation in patients with stable, deep molecular responses on TKI therapy (Table 3). In all cases, many patients were able to remain relapse-free off therapy.

Importantly, molecular relapse, the trigger for reintroduction of TKI therapy in these studies, was often defined differently. For example, in the Stop Imatinib (STIM) study, molecular relapse was defined as positive RQ-PCR results on 2 consecutive assessments (39, 40), whereas in the STIM...
Table 1. Clinical trials of TKI therapy reporting deep molecular responses (MR₄ or deeper) occurring in patients with newly diagnosed CML-CP

<table>
<thead>
<tr>
<th>Trial</th>
<th>Samples, n^a</th>
<th>Assay sensitivity (IS standardized?)</th>
<th>Median f/u, mo</th>
<th>Treatment -- MR endpoints assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRIS (15, 24)</td>
<td>1</td>
<td>≥4.5 logs (established the IS)</td>
<td>25</td>
<td>IM 400 mg qd (n = 333) → CMR at 12 mo = 4%^b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>81</td>
<td>IM 400 mg qd (n = 297) → MR^4.5 by 81 mo = 70%/52%, MR^4.5 at 81 mo = 45%</td>
</tr>
<tr>
<td>ID-01_151 (66)</td>
<td>1</td>
<td>≥5 logs (No)</td>
<td>15</td>
<td>IM 400 mg b.i.d. (n = 112) → CMR = 28%</td>
</tr>
<tr>
<td>de Lavallade et al. (67)</td>
<td>≥2 consecutive</td>
<td>NR (Yes)</td>
<td>38</td>
<td>IM 400 mg qd (n = 204^b) → CMR = 5%</td>
</tr>
<tr>
<td>RIGHT (68)</td>
<td>1</td>
<td>≥4.5 logs (No^a)</td>
<td>17^f</td>
<td>IM 400 mg b.i.d. (n = 115) → CMR by 6, 12, 18 mo = 39%, 44%, 55%</td>
</tr>
<tr>
<td>Verma et al. (29)</td>
<td>1</td>
<td>≥4.5 logs (NR)</td>
<td>65</td>
<td>IM 400 mg qd (n = 73) or 800 mg qd (n = 208) → CMR = 44%</td>
</tr>
<tr>
<td>SPIRIT (26)</td>
<td>1</td>
<td>^9(Yes)</td>
<td>47^n</td>
<td>IM 400 mg qd (n = 159) → MR^4 at 12, 24 mo = 14%, 21%, CMR at 24 mo = 9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IM 400 mg qd + AraC (n = 158) → MR^4 at 12, 24 mo = 15%, 26%, CMR at 24 mo = 8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IM 400 mg qd + PEG-IFN (n = 159) → MR^4 at 12, 24 mo = 30%, 38%, CMR at 24 mo = 16%</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>IM 600 mg qd (n = 160) → MR^4 at 12, 24 mo = 17%, 26%, CMR at 24 mo = 8%</td>
</tr>
<tr>
<td>CML Study IV (27)</td>
<td>1</td>
<td>NR (Yes)</td>
<td>43</td>
<td>IM 400 mg qd (n = 324) → MR^4 by 12, 24, 36 mo = 8%, 31%, 46%</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>48</td>
<td>IM 400 mg qd + IFN-α (n = 350) → MR^4 by 12, 24, 36 mo = 12%, 30%, 41%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28</td>
<td>IM 800 mg qd (n = 338) → MR^4 by 12, 24, 36 mo = 20%, 43%, 57%</td>
</tr>
<tr>
<td>ENESTnd (10)</td>
<td>1</td>
<td>^1(Yes)</td>
<td>Min 36</td>
<td>NIL 300 mg b.i.d. (n = 282) → MR^4 by 1, 3 y = 20%, 50%. MR^4.5 by 1, 3 y = 11%, 32%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NIL 400 mg b.i.d. (n = 281) → MR^4 by 1, 3 y = 15%, 44%. MR^4.5 by 1, 3 y = 7%, 28%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IM 400 mg qd (n = 283) → MR^4 by 1, 3 y = 6%, 26%. MR^4.5 by 1, 3 y = 1%, 15%</td>
</tr>
<tr>
<td>ENEST1st (69)</td>
<td>1</td>
<td>NR (Yes)</td>
<td>6.5^f</td>
<td>NIL 300 mg b.i.d. (n = 205) → MR^4 by 3, 6 mo = 5%, 20%</td>
</tr>
<tr>
<td>GIMEMA (70, 71)</td>
<td>1; stable, 3 × 4 mo apart</td>
<td>≥4 logs (Yes)</td>
<td>48</td>
<td>NIL 400 mg b.i.d. (n = 73) → MR^4 at 12, 24, 36 mo = 12%, 27%, 25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51</td>
<td>NIL 400 mg b.i.d. (n = 73) → Stable MR^4 = 25%</td>
</tr>
<tr>
<td>MDACC NIL phase II (72)</td>
<td>1</td>
<td>≥5 logs (Yes)</td>
<td>17.3</td>
<td>NIL 400 mg b.i.d. (n = 51) → CMR by 6, 12, 30 mo = 4%, 11%, 15%</td>
</tr>
<tr>
<td>Nicolini et al. (73)</td>
<td>1</td>
<td>NR (Yes)</td>
<td>13.6</td>
<td>NIL 300 mg b.i.d. + PEG-IFN (n = 40) → MR^4 at 6, 12, 15 mo = 23%, 57%, 80%. MR^4.5 at 6, 12, 15 mo = 10%, 21%, 50%. MR^5 at 6, 12, 15 mo = 10%, 15%, 40%</td>
</tr>
<tr>
<td>DASISION (9)</td>
<td>1</td>
<td>NR (Yes)</td>
<td>Min 24</td>
<td>DAS 100 mg qd (n = 259) → MR^4.5 by 2 y = 17%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IM 400 mg qd (n = 260) → MR^4.5 by 2 y = 8%</td>
</tr>
</tbody>
</table>

(Continued on the following page)
<table>
<thead>
<tr>
<th>Trial</th>
<th>Samples, n</th>
<th>Assay sensitivity (IS standardized?)</th>
<th>Median f/u, mo</th>
<th>Treatment — MR endpoints assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0325 (74)</td>
<td>1</td>
<td>NR (1)</td>
<td>36</td>
<td>DAS 100 mg qd (n = 99) → MR4/MR4.5 at 1 y = 27%/21%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IM 400 mg qd (n = 91) → MR4/MR4.5 at 1 y = 21%/15%</td>
</tr>
<tr>
<td>MDACC DAS phase II (75)</td>
<td>1</td>
<td>≥5 logs (Yes)</td>
<td>24</td>
<td>DAS 100 mg qd or 50 mg b.i.d. (n = 50) → CMR at 6, 12, 30 mo = 0%, 7%, 0%</td>
</tr>
<tr>
<td>BELA (76)</td>
<td>1</td>
<td>≥4 logs (Yes)</td>
<td>13.8f</td>
<td>BOS 500 mg qd (n = 250) → MR4 at 12 mo = 12%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IM 400 mg qd (n = 252) → MR4 at 12 mo = 3%</td>
</tr>
</tbody>
</table>

Abbreviations: AraC, cytarabine; BELA, Bosutinib Efficacy and Safety in Chronic Myeloid Leukemia; b.i.d., twice daily; BOS, bosutinib; DAS, dasatinib; DASISION, Dasatinib Versus Imatinib Study in Treatment-Naive CML patients; ENEST1st, Evaluating Nilotinib Efficacy and Safety in Clinical Trials as First-Line Treatment; ENESTnd, Evaluating Nilotinib Efficacy and Safety in Clinical Trials—Newly Diagnosed Patients; f/u, follow-up; GIMEMA, Gruppo Italiano Malattie Ematologiche dell'Adulto; IM, imatinib; IRIS, International Randomized Interferon Versus STI571; IS, International Scale; MDACC, MD Anderson Cancer Center; min, minimum; MR, molecular response; NIL, nilotinib; NR, not reported; qd, daily; PEG-IFN, pegylated interferon α-2a; qd, once daily; RIGHT, Rationale and Insight for Gleevec High-Dose Therapy; SPIRIT, STI571 Prospective Randomized Trial; SWOG, Southwest Oncology Group.

*aNumber of samples required to meet criteria for response.

*bPatients with CCyR only.

*cSubgroup analysis of patients enrolled in the IRIS study in Australia and New Zealand from June 2000 to February 2007.

*dNote: 17 of these patients were also in the IRIS study.

*eBaseline BCR–ABL1/ABL1 ratio based on prestudy samples from participating laboratory.

*fMedian exposure.

*g≥25,000 copies of ABL were required for a sample to be considered adequate.

*hFor all patients; 48 mo for patients alive as of data cutoff.

*i≥3,000 copies of ABL were required for a sample to be considered adequate.

jStandardized to SWOG-specific BCR–ABL1 baseline level.
Table 2. Clinical outcomes of patients with newly diagnosed CML-CP who achieved deep molecular responses with TKI therapy

<table>
<thead>
<tr>
<th>Trial description</th>
<th>N</th>
<th>Median f/u, mo</th>
<th>Comparators</th>
<th>EFS rate</th>
<th>PFS rate</th>
<th>OS rate</th>
<th>TFS rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etienne et al. (30):</td>
<td>266</td>
<td>53.2</td>
<td>CCyR + MMR + CMR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95</td>
<td>98</td>
<td>OS was not</td>
<td>NR</td>
</tr>
<tr>
<td>First-line IM 400 mg</td>
<td></td>
<td></td>
<td>CCyR + MMR - CMR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65</td>
<td>82</td>
<td>different among</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CCyR - MMR</td>
<td>28</td>
<td>56</td>
<td>the 3 groups</td>
<td>NR</td>
</tr>
<tr>
<td>Falchi et al. (31):</td>
<td>495</td>
<td>73</td>
<td>No MR at 18 mo</td>
<td>78 (6 y)</td>
<td>NR</td>
<td>93 (6 y)</td>
<td>90 (6 y)</td>
</tr>
<tr>
<td>First-line IM (400 mg, n = 83; 800 mg, n = 204), NIL (n = 106), or DAS (n = 102)</td>
<td></td>
<td></td>
<td>MMR at 18 mo</td>
<td>94 (6 y)</td>
<td>NR</td>
<td>98 (6 y)</td>
<td>100 (6 y)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MR&lt;sup&gt;c&lt;/sup&gt; at 18 mo</td>
<td>97 (6 y)</td>
<td>NR</td>
<td>97 (6 y)</td>
<td>100 (6 y)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MR&lt;sup&gt;b&lt;/sup&gt; at 18 mo</td>
<td>93 (6 y)</td>
<td>NR</td>
<td>95 (6 y)</td>
<td>100 (6 y)</td>
</tr>
<tr>
<td>CML study IV (32):</td>
<td>1,525</td>
<td>67.5</td>
<td>Undetectable BCR-ABL&lt;sup&gt;b&lt;/sup&gt; at 18 mo</td>
<td>100 (6 y)</td>
<td>NR</td>
<td>100 (6 y)</td>
<td>100 (6 y)</td>
</tr>
<tr>
<td>First-line IM 400 mg, IM 400 mg + IFN-α, IM 400 mg + AraC, IM after IFN-α failure, or IM 800 mg</td>
<td></td>
<td></td>
<td>No MR at 24 mo</td>
<td>80 (6 y)</td>
<td>NR</td>
<td>92 (6 y)</td>
<td>90 (6 y)</td>
</tr>
<tr>
<td>Kantarjian et al. (28):</td>
<td>276</td>
<td>48</td>
<td>MMR at 24 mo</td>
<td>90 (6 y)</td>
<td>NR</td>
<td>97 (6 y)</td>
<td>100 (6 y)</td>
</tr>
<tr>
<td>Retrospective analysis. IM (400 mg, n = 71; 800 mg, n = 205)</td>
<td></td>
<td></td>
<td>MR&lt;sup&gt;b&lt;/sup&gt; at 24 mo</td>
<td>97 (6 y)</td>
<td>NR</td>
<td>100 (6 y)</td>
<td>100 (6 y)</td>
</tr>
<tr>
<td>Verma et al. (29):</td>
<td>281</td>
<td>65</td>
<td>Undetectable BCR-ABL&lt;sup&gt;b&lt;/sup&gt; at 24 mo</td>
<td>100 (6 y)</td>
<td>NR</td>
<td>100 (6 y)</td>
<td>100 (6 y)</td>
</tr>
<tr>
<td>Retrospective analysis. First-line IM (400 mg, n = 73; 800 mg, n = 208)</td>
<td></td>
<td></td>
<td>CCyR + MMR + CMR&lt;sup&gt;b&lt;/sup&gt; at 2 y</td>
<td>100 (5 y)</td>
<td>NR</td>
<td>NR</td>
<td>100 (5 y)</td>
</tr>
<tr>
<td>Press et al. (77):</td>
<td>90</td>
<td>49</td>
<td>CCyR + MMR - CMR&lt;sup&gt;b&lt;/sup&gt; at 2 y</td>
<td>96 (5 y)</td>
<td>NR</td>
<td>NR</td>
<td>96 (5 y)</td>
</tr>
<tr>
<td>All patients achieved CCyR on IM and had ≥2 measurements of BCR-ABL&lt;sup&gt;1&lt;/sup&gt; level after achieving CCyR</td>
<td></td>
<td></td>
<td>CCyR - MMR - CMR&lt;sup&gt;b&lt;/sup&gt; at 2 y</td>
<td>86 (5 y)</td>
<td>NR</td>
<td>NR</td>
<td>91 (5 y)</td>
</tr>
</tbody>
</table>

Abbreviations: AraC, cytarabine; DAS, dasatinib; f/u, follow-up; IM, imatinib; MR, molecular response; NIL, nilotinib; NR, not reported.

<sup>a</sup>Cytarabine was defined as undetectable BCR-ABL<sup>1</sup> transcripts with a sensitivity of ≥4.5 logs on 2 consecutive analyses ≥2 mo apart.

<sup>b</sup>Minimum sensitivity 4.5 logs.

<sup>c</sup>Minimum sensitivity 4 logs.

<sup>d</sup>Data shown are median relapse-free survival, defined as progression to accelerated phase/blast crisis, loss of complete hematologic response, or loss of CCyR.
<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment before d/c (response required for d/c)</th>
<th>Definition of relapse</th>
<th>Relapse-free pts, % (median f/u, mo)</th>
<th>Patients responding to TKI after relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trials of IM d/c</strong></td>
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<tr>
<td>STIM (ref. 40; N = 100)</td>
<td>IM for ≥36 mo (MR5 for ≥24 mo)</td>
<td>Confirmed loss of MR5</td>
<td>39% (30)</td>
<td>56/61 regained MR5</td>
</tr>
<tr>
<td>ALLG CML8/TWISTER</td>
<td>IM for ≥36 mo (MR4.5 for ≥24 mo)</td>
<td>Confirmed loss of MR4.5</td>
<td>45% (42)</td>
<td>22/22 regained MMR4</td>
</tr>
<tr>
<td>According to STIM (ref. 41; N = 66)</td>
<td>IM for ≥36 mo (MR4.5 for ≥24 mo)</td>
<td>Loss of MMR</td>
<td>64% (23)</td>
<td>24/24 regained MMR4</td>
</tr>
<tr>
<td>Korean study (ref. 78; N = 48)</td>
<td>IM for ≥36 mo (CMR for ≥24 mo)</td>
<td>Confirmed loss of MMR</td>
<td>81% (15.8)</td>
<td>8/9 regained MMR4</td>
</tr>
<tr>
<td>Stimm (ref. 40; N = 100)</td>
<td>IM for ≥36 mo (MR5 for ≥24 mo)</td>
<td>Loss of MMR and/or &gt;1-log increase in BCR-ABL1</td>
<td></td>
<td>Study ongoing</td>
</tr>
<tr>
<td><strong>Trials of NIL d/c</strong></td>
<td></td>
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</tr>
<tr>
<td>Nilo post-STIMc (ref. 57; N = 70)</td>
<td>NIL for ≥2 y in patients who failed TFR in STIM or STIM 2 (confirmed CMR after 2 y of NIL)</td>
<td>Loss of MMR on 2 consecutive assessments</td>
<td>Study ongoing</td>
<td>Study ongoing</td>
</tr>
<tr>
<td>ENESTFreedomc (ref. 57; N = 175)</td>
<td>First-line NIL for ≥2 y and 1 y NIL on study (MR4.5 for ≥1 y)</td>
<td>Loss of MMR</td>
<td>Study ongoing</td>
<td>Study ongoing</td>
</tr>
<tr>
<td>ENESTOp2 (ref. 57; N = 177)</td>
<td>TKI therapy for ≥3 y, including ≥2 y of second-line NIL and 1 y NIL on study (MR4.5 for ≥1 y)</td>
<td>Confirmed loss of MR4 or any loss of MMR</td>
<td>Study ongoing</td>
<td>Study ongoing</td>
</tr>
<tr>
<td>ENESTgoalc (ref. 57; N = 300)</td>
<td>IM for ≥1 y and NIL on study (MR4.5 for 1 or 2 y)</td>
<td>Confirmed loss of MR4</td>
<td>Study ongoing</td>
<td>Study ongoing</td>
</tr>
<tr>
<td>ENESTPathc (ref. 57; N = 1,058)</td>
<td>IM for ≥2 y and NIL on study (MR4 for 1 or 2 y)</td>
<td>Loss of MR4</td>
<td>Study ongoing</td>
<td>Study ongoing</td>
</tr>
<tr>
<td>TIGERc (ref. 57; N = 652)</td>
<td>First-line NIL + PEG-IFN-α vs. NIL for ≥2 y on study, then PEG-IFN-α vs. NIL maintenance therapy (stable MR4)</td>
<td>Loss of MMR</td>
<td>Study ongoing</td>
<td>Study ongoing</td>
</tr>
<tr>
<td><strong>Trials of DAS d/c</strong></td>
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<tr>
<td>DASFREEa (57) (N = 75)</td>
<td>DAS for ≥2 y (MR4.5 for ≥1 y)</td>
<td>Loss of MMR</td>
<td>Study ongoing</td>
<td>Study ongoing</td>
</tr>
<tr>
<td><strong>Trials of TKI d/c</strong></td>
<td></td>
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</tr>
<tr>
<td>STOP 2G-TKI (ref. 42; N = 34)</td>
<td>NIL or DAS for ≥36 mo (UMRD for ≥24 mo, with ≥20,000 ABL1 copies)</td>
<td>Loss of MMR</td>
<td>58.3% at 12 mo (14)</td>
<td>13/15 regained MMR</td>
</tr>
<tr>
<td>EURO-SKId (ref. 57; N = 500)</td>
<td>TKI therapy (first-line or second-line) for ≥3 y (MR4 for ≥1 y)</td>
<td>Loss of MMR</td>
<td>Study ongoing</td>
<td>Study ongoing</td>
</tr>
</tbody>
</table>

(Continued on the following page)
study, molecular relapse was less stringently defined as loss of MMR at any time (41). In According to STIM, all 24 patients who relapsed regained a response of MMR or better once imatinib was reintroduced (41); therefore, waiting until a patient loses MMR to reinitiate TKI therapy does not seem to be detrimental. This suggests that BCR–ABL1 positivity after TKI cessation does not necessarily equal disease relapse (22). However, the optimal molecular response threshold for triggering restart of TKI therapy remains to be established.

Data on discontinuation of second-generation TKIs are limited; however, the ongoing STOP 2G-TKI study has shown similar results to those of imatinib discontinuation trials (Table 3; ref. 42). Thus, available results suggest that stopping TKI therapy in patients with sustained deep molecular response can be safe and associated with prolonged TFR. Given the preliminary nature of available clinical data on TFR, both the ELN and the National Comprehensive Cancer Network currently recommend that patients remain on TKI therapy indefinitely and that TKI discontinuation only be attempted in controlled clinical trials (6, 7).

### Factors Affecting Achievement of Deep Molecular Response

Achievement of $B\!\!\!\!R$–$A\!\!\!\!B\!\!L\!\!\!I^{15} \leq 10\%$ at 3 or 6 months after initiating first-line imatinib has been shown to be predictive of long-term molecular response and improved outcomes (19, 43, 44), whereas delayed cytogenetic and molecular response on imatinib is associated with increased risk of progression (45). Landmark studies of second-generation TKIs have found $B\!\!\!\!R$–$A\!\!\!\!B\!\!\!L\!\!\!I$ levels at 3 months to be similarly predictive (46, 47). A retrospective analysis of patients treated with second-line nilotinib, dasatinib, or bosutinib found that $B\!\!\!\!R$–$A\!\!\!\!B\!\!\!L\!\!\!I^{15} \leq 10\%$ at 3 months from start of second-line treatment was associated with significantly higher cumulative incidence of MMR and CMR and improved OS, PFS, and EFS (48). An online database of patients with CML-CP treated with first-line imatinib in Japan found that patients with an MMR at 12 months were significantly more likely than those without to achieve undetectable $B\!\!\!\!R$–$A\!\!\!\!B\!\!\!L\!\!\!I$ transcripts by 72 months (49). Another study found a similar association in patients treated with first-line or second-line imatinib (24).

Other factors that may affect achievement of deep molecular response include risk score, sex, adherence to treatment, and dose intensity. For example, in the Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Patients (ENESTnd) phase III trial of first-line nilotinib versus imatinib, rates of deep molecular response were lowest among patients with high Sokal score, although more high-risk patients achieved MR$^{1.5}$ by 3 years on nilotinib versus imatinib (24%), 27%, and 9% in the nilotinib 300 mg b.i.d., nilotinib 400 mg b.i.d., and imatinib arms, respectively; ref. 10). Univariate analysis of a study in patients treated with first-line imatinib showed that females were more likely than males to achieve stable undetectable $B\!\!\!\!R$–$A\!\!\!\!B\!\!\!L\!\!\!I$ (with a RQ-PCR sensitivity of $\geq 4.5$ log) by 8 years (68% vs. 30%, respectively; ref. 50). Multivariate analysis of a study in Japanese patients found adherence to standard-dose imatinib to be predictive of CMR achievement (51). Another study reported adherence to standard-dose imatinib as the only independent predictor of CMR; poor adherence and failure to achieve MMR predicted treatment discontinuation and eventual loss of CCyR (52, 53).

### Table 3. Clinical trials of TKI discontinuation in patients with CML-CP and deep molecular response (Cont’d)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment before d/c (response required for d/c)</th>
<th>Definition of relapse</th>
<th>Relapse-free pts, % (median f/u, mo)</th>
<th>Patients responding to TKI after relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>DESTINY$a$ (ref. 57; $N = 168$)</td>
<td>IM, NIL, or DAS for $\geq 3$ y and half-standard dose of TKI for 1 y on study (MMR or MR$^4$ for $\geq 1$ y at therapeutic dose and 1 y at half-standard dose)</td>
<td>Loss of MMR</td>
<td>Study ongoing</td>
<td>Study ongoing</td>
</tr>
<tr>
<td>NCT00573378$c$ (ref. 57; $N = 40$)</td>
<td>NIL or IM for $\geq 3$ y, with stable dose for $\geq 1$ y and same TKI + PEG-IFN-α for 2 y on study (NR)</td>
<td>NR</td>
<td>Study ongoing</td>
<td>Study ongoing</td>
</tr>
</tbody>
</table>

Abbreviations: ALLG, Australasian Leukaemia and Lymphoma Group; DAS, dasatinib; d/c, discontinuation; f/u, follow-up; IM, imatinib; MR, molecular response; NIL, nilotinib; NR, not reported; PEG-IFN-α, pegylated interferon-α; RT-PCR, nested reverse transcriptase PCR; TMA, transcription-mediated amplification; UMRD, undetectable minimal residual disease.

$a$Patients achieved this level of response or better.

$b$UMRD defined as $< 100$ copies by TMA; thus, loss of UMRD was $> 100$ copies by TMA.

$c$Currently recruiting participants.

$d$Not yet recruiting participants.
Factors Affecting Durability of Deep Molecular Response Off Treatment

The high rates of and rapidity of disease relapse observed in patients who discontinue TKI therapy without deep molecular response (34–37) suggest that the main factor influencing relapse risk off therapy is the level of residual molecular disease present when TKI therapy is stopped. However, even among patients with sustained, undetectable disease on TKI therapy, a significant percentage of patients have experienced disease relapse (40), and several studies have evaluated factors potentially contributing to a patient’s ability to achieve successful TFR.

Studies of imatinib discontinuation have found an association between Sokal score and relapse risk (39, 40, 54, 55), suggesting that patients with high Sokal risk may have inherent biologic attributes that drive development of disease relapse once BCR–ABL1 inhibition is relieved. The duration of deep molecular response before TKI discontinuation and the overall duration of prior TKI treatment also seem to be important (39, 40, 56). Based on this, the majority of ongoing studies require patients to maintain a deep molecular response for ≥2 years and have had ≥3 years of prior TKI treatment before attempting discontinuation. More rapid achievement of deep molecular response may also be predictive of successful TFR (54, 55).

In the STIM study, the only factors associated with lower risk of relapse were low/intermediate Sokal score and duration of imatinib treatment >5 years (39, 40). Another study identified a subgroup of patients with increased risk of molecular relapse following imatinib discontinuation, characterized by the following: high Sokal score, >24 months to CMR, and <33 months of imatinib after achieving CMR; patients with any of these characteristics had a 0% probability of CMR persistence at 1 year, compared with 80% probability in patients without these characteristics (54). Several other ongoing TKI discontinuation studies aim to determine other factors that may play a role in relapse off treatment (Table 3). For example, the large, phase III ELIRO-SKI study (NCT01596114) will explore factors associated with molecular relapse in patients with stable MR4 who stop TKI therapy, and STIM 2 (NCT01343173) will evaluate factors predictive of sustained deep molecular response after imatinib discontinuation (57). Current data suggest that deep molecular response is heterogeneous, and different patients may have differing levels of residual disease burden despite having undetectable disease (22).

Most, if not all, patients with sustained CMR have persistent BCR–ABL1-positive cells (based on PCR at a DNA level; ref. 55 and 58); however, this persistence does not necessarily lead to relapse after treatment discontinuation. Furthermore, the kinetics of relapse after treatment discontinuation vary, with early and late molecular relapses observed (39). This discrepancy may reflect the heterogeneity of CML at the stem cell level. The development of highly sensitive techniques like ultra-deep sequencing (UDS) and massive parallel sequencing (MPS) may help clarify these differences. Indeed, both UDS and MPS detected more complex mutation dynamics in TKI-resistant patients than conventional Sanger sequencing (59, 60).

Mathematical modeling of the kinetics of molecular relapse in patients in the STIM study has led to a hypothesis that selective pressure exerted by imatinib treatment results in an increased frequency of LSC clones with slower growth and differentiation than the predominant clone at baseline (61). Imatinib therapy may affect the ability of LSCs to produce differentiated populations and may affect the cell division of progenitors and differentiated leukemic cells (61). The effects of imatinib on leukemia-initiating cells (LIC) may differ from patient to patient, and these differences may manifest in varying times to molecular relapse in patients with deep molecular response who stop imatinib treatment (Fig. 2).

It remains to be established whether the ultimate goal of treatment for patients with CML-CP will be a “clinical cure” (i.e., the absence of relapse) or a “biological cure” (i.e., absence of all leukemic cells, including LSCs; refs. 22, 62, 63). Multiple strategies for specifically targeting the LSC reservoir in patients with CML are under investigation, including combination of a BCR–ABL1 TKI with other targeted agents, such as inhibitors of the Hedgehog, Wnt/B-catenin, or Notch signaling pathways, or immunomodulatory agents, such as IFN (22, 62, 63). In one study, maintenance therapy with pegylated IFN following imatinib discontinuation led to retained or improved molecular responses in the majority of patients, most of whom did not have deep molecular responses (64). This may represent a viable strategy for patients who wish to stop TKI therapy in the absence of deep molecular response; the ongoing TIGER study is further investigating this approach (Table 3). Quantification of a patient’s residual LSC reservoir and the effect of such combination treatments on LSCs remains a challenge (22, 65). Given the potential diversity in disease burden and relapse risk in patients who discontinue TKI therapy, rigorous long-term follow-up is essential, and discontinuation should only be attempted in clinical trials.

Conclusions

Molecular monitoring affords a precise, convenient method for monitoring residual disease burden in patients with CML-CP. As TKIs have improved patient outcomes, clinical trial designs have begun to evaluate deeper levels of molecular response. Deep molecular responses are associated with improved rates of PFS, EFS, and OS, and reduced risk of progression to advanced disease. Furthermore, a sustained deep molecular response is an essential entry criterion for studies of TFR. The ability to achieve deep molecular response on therapy, and sustain this response off therapy, may be
affected by a variety of factors, including disease characteristics, risk score, and adherence to and time on treatment. As more is learned about the optimal criteria for successful TKI discontinuation, patients may have increased chances of achieving treatment-free disease control, a first step toward the elusive cure for CML.

**Disclosure of Potential Conflicts of Interest**
F.-X. Mahon has received commercial research grants from Bristol-Myers Squibb and Novartis Pharma. F.-X. Mahon is a consultant/advisory board member of Bristol-Myers Squibb, Novartis Pharma, and Ariad. G. Etienne is a consultant/advisory board member of Bristol-Myers Squibb, Novartis, Pfizer, and Ariad.

**Authors’ Contributions**
Conception and design: F.-X. Mahon, G. Etienne
Development of methodology: F.-X. Mahon
Writing, review, and/or revision of the manuscript: F.-X. Mahon, G. Etienne

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