Title
Final 3-year Results of the Dasatinib Discontinuation Trial in Patients With Chronic Myeloid Leukemia Who Received Dasatinib as a Second-line Treatment

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Final 3-year Results of the Dasatinib Discontinuation Trial in Patients With Chronic Myeloid Leukemia Who Received Dasatinib as a Second-line Treatment

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Original Study

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https://doi.org/10.1016/j.clml.2018.03.004
We describe the results of a prospective trial of the discontinuation of second-line dasatinib treatment in chronic myeloid leukemia patients who maintained a deep molecular response for >1 year. The treatment-free remission rate at 36 months was 44.4%. High natural killer cell counts before discontinuation correlated significantly with successful therapy discontinuation.

Introduction: We previously reported an interim analysis of the DADI (dasatinib discontinuation) trial. The results showed that 48% of patients with chronic myeloid leukemia in the chronic phase who maintained a deep molecular response (DMR) for ≥1 year could discontinue second- or subsequent-line dasatinib treatment safely at a median follow-up of 20 months. However, the results from longer follow-up periods would be much more useful from a clinical perspective. Patients and Methods: The DADI trial was a prospective, multicenter trial conducted in Japan. After confirming a stable DMR for ≥1 year, dasatinib treatment subsequent to imatinib or nilotinib was discontinued. After discontinuation, the loss of DMR (even of 1 point) was defined as stringent molecular relapse, thereby triggering therapy resumption. The predictive factors of treatment-free remission (TFR) were analyzed. Results: The median follow-up period was 44.0 months (interquartile range, 40.5–48.0 months). The estimated overall TFR rate at 36 months was 44.4% (95% confidence interval, 32.0%–56.2%). Only 2 patients developed a molecular relapse after the 1-year cutoff point. The presence of imatinib resistance was a significant risk factor for molecular relapse. Moreover, high natural killer cell and low γδ T-cell and CD4+ regulatory T-cell (CD25+CD127low) counts before discontinuation correlated significantly with successful therapy discontinuation. Conclusion: These findings suggest that discontinuation of second- or subsequent-line dasatinib after a sustained DMR of ≥1 year is feasible, especially for patients with no history of imatinib resistance. In addition, the natural killer cell count was associated with the TFR.

Patients and Methods

Study Patients

Patients with CML in the chronic phase who were undergoing second- or subsequent-line dasatinib therapy after receiving imatinib were eligible if they had achieved a DMR (before registration). Patients were also required to be aged ≥15 years, with adequate...
performance status (World Health Organization performance score, 0-2) and no severe dysfunction of the primary organs (eg, liver, kidney, and lung).

Previous treatments in addition to imatinib and apart from allogeneic hematopoietic stem cell transplantation were allowed. Patients harboring additional chromosomal abnormalities in Philadelphia chromosome-positive cells at the diagnosis and patients with a history of BCR-ABL1 mutations associated with dasatinib resistance (T315I, F317L, and V299L) were excluded from the present study because they were presumed to have a high risk of relapse after therapy discontinuation. Patients undergoing treatment for other malignancies were also excluded. Imatinib resistance and intolerance were defined according to the judgment of the treating doctor and in accordance with European LeukemiaNet 2006 definition.

Molecular Monitoring

Real-time quantitative reverse transcription polymerase chain reaction (RQ-PCR) analyses were performed at a single central laboratory: Bio Medical Laboratories (BML Inc, Tokyo, Japan). A molecular response was standardized to the BCR-ABL1 IS using the laboratory’s conversion factor (0.87).ABL1 was used as an internal control to validate amplification of BCR-ABL1. The number of stable ABL1 copies amplified was checked using a good quality RNA sample; this equated to 87,000 copies of ABL1 per RQ-PCR reaction (200 ng of total RNA). The quality and quantity of sample RNA used for BCR-ABL1 amplification was validated as 87,000 copies of ABL1.

Definition of DMR

The study cutoff point for RQ-PCR analysis was set such that the decision regarding a DMR or molecular relapse after discontinuation would be reliable. The lowest concentration of BCR-ABL1 cDNA required to ensure ~100% detection sensitivity and reliability was measured in-house. Thus, a minimum of 10 copies of BCR-ABL1 per assay (200 ng of total RNA), corresponding to 0.0069% BCR-ABL1, was selected. DMR was defined as a CML sample harboring < 0.0069% BCR-ABL1, corresponding to molecular response 4 (MR4.0).

Study Design

The DADI trial was a prospective, multicenter, single-arm phase II study conducted in Japan. CML patients with a confirmed DMR were preregistered. To confirm a sustained DMR, RQ-PCR analysis was performed every 3 months throughout the preregistration period (ie, a consolidation phase comprising 1 year of dasatinib treatment after preregistration). Only patients who achieved a sustained DMR at 4 subsequent consecutive data points (during the 1 year) were enrolled in the DADI study. After drug discontinuation, patients were monitored by RQ-PCR every month for the first year, every 3 months during the second year, and every 6 months during the third year. During molecular monitoring, a BCR-ABL1 of ≥ 0.0069% detected at a minimum of 1 analysis point was defined as stringent molecular relapse, thereby triggering therapy resumption. The total follow-up duration was set at 36 months after drug discontinuation. Patients with molecular relapse resumed dasatinib immediately and at the previously effective dose. These patients were then tested using RQ-PCR at 1, 3, 6, and 12 months after reintroduction of dasatinib. Routine cytogenetic analysis during molecular monitoring after discontinuation is not common in Japan; therefore, it was not performed in the DADI trial. During the study period, dasatinib dose reductions were permitted to manage AEs. The ethics committees of the participating hospitals approved the clinical trial. All participants provided written informed consent in accordance with the Declaration of Helsinki.

Peripheral Immunoprofiles of Patients Receiving Dasatinib

The T- and natural killer (NK)-cell profiles in the peripheral blood were evaluated throughout the prereregistration period. The blood cell counts and differentials were measured using an automated blood cell count analyzer, and the lymphocyte fraction was determined by flow cytometry using forward scatter versus side scatter gating. The average number of lymphocyte subsets was calculated across the 5 data points. Immunophenotypic examination was performed using 2- or 3-color flow cytometry with a FACS-Calibur system running CellQuest, version 3.3, software (BD Biosciences, Franklin Lakes, NJ). Flow cytometry was performed at a single central laboratory (BML Inc). All antibodies used in the study were purchased from BD Biosciences. The lymphocyte subsets detected by flow cytometry were as follows: CD8+ T cells (CD3+ CD8+), NK cells (CD3+ CD56+ and CD16+ CD56+), T-large granular lymphocytes (CD57+ CD3-), NK-large granular lymphocytes (CD57+ CD56+), γδ+ T cells (CD8+ TCRγδ+ and CD8+ TCRγδ-), and CD4+ regulatory T cells (Treg; CD4+ CD25+ CD127low). The CD4/8 ratio was determined by 2-color analysis of CD4 and CD8 cell populations.

Study Endpoints

The primary endpoint was the proportion of patients who maintained TFR at 6 months. TFR was defined as the interval from initiation of dasatinib discontinuation to the date of stringent molecular relapse. The main secondary endpoints were the TFR at 12 months and the clinical effect of dasatinib resumption on patients with relapse. Age, gender, the Sokal score at the diagnosis, the reasons patients switched from imatinib to dasatinib (clinical response to previous imatinib), a history of interferon (IFN)-α therapy, total duration of both imatinib and dasatinib treatment, total dose of dasatinib from the time of drug initiation, total dose of dasatinib during the 1 year before drug discontinuation, and the mean absolute number of T and NK cell subsets in the peripheral blood during dasatinib treatment were used to identify factors predictive of TFR.

Statistical Analysis

To determine whether dasatinib discontinuation corresponded to the TFR in CML patients compared with data from the previous study, a sample size of ≥ 50 patients was selected to provide a power of > 80%. Using the cutoff points for each continuous variable determined by the concordance index, patients were dichotomized into high and low count groups. The TFR rates were calculated using Kaplan-Meier analysis, and the stratified groups were compared using the log-rank test. P < .05 was considered to indicate statistical significance. Statistical analysis was performed using SAS software, version 9.3. The study is registered with the Japan Primary Registries Network (registry no. UMIN000005130).

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Role of the Funding Source

The funder of the study collected the data but played no role in the study design, data analysis, or data interpretation. The funder reviewed the manuscript before journal submission. Four of us (J.I., H.T., S.M., J.S.) were able to access raw data, and 1 of us (S.K.) had full access to all the data and bears the ultimate responsibility for the decision to publish.

Results

Patient Characteristics

From April 1, 2011 to March 31, 2012, 88 patients who had achieved a DMR with second- or subsequent-line dasatinib treatment were enrolled in the consolidation phase. Of these 88 patients, 25 were excluded from the discontinuation phase: 24 patients with fluctuating BCR-ABL1 transcript levels and 1 in whom minor BCR-ABL1 transcripts were detected. Thus, 63 patients were included in the dasatinib discontinuation phase. Of the 63 patients, 13 (21%) had switched from imatinib because of the development of resistance, 36 (57%) had switched because of imatinib intolerance, and 14 (22%) had switched at the patient’s request because they believed dasatinib would have a more robust clinical effect. The Sokal risk score was low in 65%, intermediate in 14%, high in 14% of patients, and missing for 5%. The best responses to previous imatinib treatment were complete a hematologic response in 10%, a complete cytogenetic response in 8%, a major molecular response (MMR) in 78%, and missing for 5%. The median duration of imatinib treatment was 62 months (range, 1-122 months). Previous treatments other than imatinib included IFN-α in 12 patients, nilotinib in 4, nilotinib after IFN-α in 1, bosutinib in 1, and hydroxyurea in 1. The median duration of dasatinib treatment at enrollment was 17 months (range, 12-72 months), and the median total duration of imatinib and dasatinib treatment was 82 months (range, 23-142 months).

TFR After Dasatinib Discontinuation

The previous interim analysis was performed at a median follow-up point after dasatinib discontinuation of 20.0 months. At that time, 30 patients had TFR and 33 had developed molecular relapse, which had occurred within the first 7 months after dasatinib discontinuation. After the interim analysis, 2 patients (both of whom had switched to dasatinib because to imatinib intolerance) experienced molecular relapse at 18 and 21 months after dasatinib discontinuation. Of the 35 patients who had developed molecular relapse, only 1 patient experienced an IS > 1%. The overall probability of TFR at 6, 12, and 36 months was 49% (95% confidence interval [CI], 31%-61%), 48% (95% CI, 35%-59%), and 44% (95% CI, 32.0%-56.2%), respectively (Figure 1A).

Factors Associated with TFR or Molecular Relapse

In the final analysis, the most significant risk factor associated with loss of TFR (ie, loss of a DMR, even at a single analysis point) was the presence of imatinib resistance before second- or subsequent-line dasatinib therapy. Although the TFR at 36 months was only 8% (95% CI, 0%-29%) in imatinib-resistant patients, it was 55.6% (95% CI, 38.05%-69.94%) and 50.0% (95% CI, 22.86%-72.21%) in imatinib-intolerant patients and patients who had decided to switch to dasatinib, respectively (Figure 1B).

Interestingly, 3 of the 12 relapse events in the imatinib-resistant patients (25%) and 1 of the 23 relapse events in the nonresistant patients (0.23%) occurred as loss of a MMR (IS > 0.1%). To confirm the differences in the kinetics of relapse according to the presence or absence of imatinib resistance, we calculated the BCR-ABL1 doubling time by comparing the BCR-ABL1 levels between the last confirmed DMR and molecular relapse. The median BCR-ABL1 doubling time in imatinib-resistant patients was significantly shorter than that in non-resistant patients (16.3 days, range, 7.0-98.7 days; vs. 52.3 days, range, 7.3-341.4 days; P = .0493). Neither the Sokal score nor the duration of preceding TKI treatment, both of which have been associated with an increased risk of molecular relapse in previous imatinib discontinuation trials, were identified as risk factors in the present analysis. Of the 5 patients who had received nilotinib as a previous treatment and switched to dasatinib because of intolerance, 4 achieved a sustained TFR.
<table>
<thead>
<tr>
<th>Risk Factors for Molecular Relapse</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TFR at 36 mo (%), 95% CI</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥45</td>
<td>50.0 (34.9-63.3)</td>
<td>0.60 (0.30-1.20)</td>
</tr>
<tr>
<td>&lt;45</td>
<td>29.4 (10.7-51.1)</td>
<td>1</td>
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<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>63.6 (40.2-79.8)</td>
<td>0.51 (0.23-1.13)</td>
</tr>
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<td>Male</td>
<td>34.1 (20.2-48.5)</td>
<td>1</td>
</tr>
<tr>
<td>Sokal risk score</td>
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<tr>
<td>Low</td>
<td>46.3 (30.7-60.5)</td>
<td>0.90 (0.34-2.37)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>22.2 (3.37-51.3)</td>
<td>1.93 (0.61-6.11)</td>
</tr>
<tr>
<td>High</td>
<td>44.4 (13.5-71.9)</td>
<td>1</td>
</tr>
<tr>
<td>Reasons for switch from imatinib to dasatinib</td>
<td></td>
<td></td>
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<tr>
<td>Imatinib resistant</td>
<td>7.7 (0.48-29.2)</td>
<td>3.05 (1.17-7.96)</td>
</tr>
<tr>
<td>Imatinib intolerant</td>
<td>55.6 (38.1-69.9)</td>
<td>0.83 (0.34-2.02)</td>
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<tr>
<td>Patient choice</td>
<td>50.0 (22.9-72.2)</td>
<td>1</td>
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<tr>
<td>Imatinib resistant</td>
<td>7.7 (0.48-29.2)</td>
<td>3.48 (1.66-7.29)</td>
</tr>
<tr>
<td>Not imatinib resistant</td>
<td>54.0 (39.3-66.6)</td>
<td>1</td>
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<tr>
<td>History of IFN-α therapy</td>
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<tr>
<td>Yes</td>
<td>53.8 (24.8-76.0)</td>
<td>0.75 (0.31-1.79)</td>
</tr>
<tr>
<td>No</td>
<td>42.0 (28.3-55.1)</td>
<td>1</td>
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<tr>
<td>Total TKI duration* treatment, mo</td>
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<tr>
<td>≥50</td>
<td>48.9 (33.7-62.4)</td>
<td>0.61 (0.30-1.23)</td>
</tr>
<tr>
<td>&lt;50</td>
<td>33.3 (13.7-54.5)</td>
<td>1</td>
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<tr>
<td>CD4/8 ratio</td>
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<td>≥1.03</td>
<td>33.3 (13.7-54.5)</td>
<td>1.39 (0.69-2.81)</td>
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<td>&lt;1.03</td>
<td>46.3 (30.7-60.6)</td>
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<td>Mean CD8+ T (CD3+CD8+) cell cutoff</td>
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<tr>
<td>≥664/μL</td>
<td>33.3 (13.7-54.5)</td>
<td>1.48 (0.73-2.99)</td>
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<tr>
<td>&lt;664/μL</td>
<td>46.3 (30.7-60.6)</td>
<td>1</td>
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<tr>
<td>Total NK (CD3+CD5+) cell cutoff</td>
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<tr>
<td>≥539/μL</td>
<td>51.3 (34.8-65.6)</td>
<td>0.50 (0.25-0.99)</td>
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<tr>
<td>&lt;539/μL</td>
<td>25.0 (9.10-44.9)</td>
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<tr>
<td>Cytolytic NK (CD16+CD56+) cell cutoff</td>
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<td></td>
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<tr>
<td>≥506/μL</td>
<td>54.1 (36.9-68.4)</td>
<td>0.45 (0.23-0.88)</td>
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<tr>
<td>&lt;506/μL</td>
<td>22.7 (8.2-41.5)</td>
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<tr>
<td>Mean T-LGLs (CD57+CD3+) cutoff</td>
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<tr>
<td>≥509/μL</td>
<td>35.0 (15.7-55.2)</td>
<td>1.37 (0.68-2.73)</td>
</tr>
<tr>
<td>&lt;509/μL</td>
<td>46.2 (30.2-60.7)</td>
<td>1</td>
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<tr>
<td>NK-LGL (CD57+CD56+) cutoff</td>
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<tr>
<td>≥528/μL</td>
<td>52.8 (35.5-67.4)</td>
<td>0.52 (0.27-1.03)</td>
</tr>
<tr>
<td>&lt;528/μL</td>
<td>26.1 (10.6-44.7)</td>
<td>1</td>
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<tr>
<td>γδ+ T cell cutoff</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥120/μL</td>
<td>16.7 (4.12-36.5)</td>
<td>2.51 (1.26-5.03)</td>
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<tr>
<td>&lt;120/μL</td>
<td>53.7 (37.4-67.4)</td>
<td>1</td>
</tr>
<tr>
<td>Regulatory T (CD4+CD25+CD127dim) cell cutoff</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥88/μL</td>
<td>16.7 (2.65-41.3)</td>
<td>2.30 (1.09-4.84)</td>
</tr>
<tr>
<td>&lt;88/μL</td>
<td>48.9 (34.1-62.2)</td>
<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval; HR = hazard ratio; IFN-α = interferon-α; LGL = T-large granular lymphocytes; NK = natural killer; Ref = reference; TFR = treatment-free remission; TKI = tyrosine kinase inhibitor.

*Both imatinib and dasatinib.
To examine the relationship between the immune response to dasatinib treatment and subsequent TFR, we assessed the immune profiles in 59 patients (for whom complete data sets were available) during the dasatinib consolidation phase. In general, these patients had high lymphocyte counts during dasatinib treatment, with some fluctuations. After discontinuation of dasatinib, the lymphocyte counts decreased rapidly (within ~3 months).

We found it interesting that, in line with the data from the interim analysis, the present univariate analysis revealed that a greater number of total NK cells (CD3⁺CD56⁺) and cytolytic NK cells (CD16⁺CD56⁺) and a lower number of γδ⁺ T cells (Supplemental Figure 1; available in the online version) and CD4⁺Tregs were associated with successful maintenance of TFR (Table 1, Figure 2). Multivariate analysis revealed that only the absence of imatinib resistance and a greater number of cytolytic NK cells (CD16⁺CD56⁺) were independently associated with maintenance of TFR. When the number of total NK cells (CD3⁺CD56⁺) rather than the number of cytolytic NK cells was entered into multivariate analysis, the number of total NK cells was no longer a significant factor.

**Outcomes of Patients With Molecular Relapse**

Overall, 33 of the 35 patients with relapse restarted dasatinib. Of the 2 patients who did not restart dasatinib, 1 preferred treatment with nilotinib and 1 did not wish to restart dasatinib. Subsequently, the latter experienced fluctuations. After discontinuation of dasatinib, the lymphocyte counts decreased rapidly (within 6 months after dasatinib discontinuation, with only 2 (6%) showing that dasatinib induces persistent expansion of clonal resistant patients had already gained aggressive characteristics before the patients had started second-line dasatinib therapy. This was likely due to alterations in gene expression patterns, although additional chromosomal abnormalities or BCR-ABL1 mutations in CML cells, which have been associated with TKI resistance, were absent.

Third and, more interestingly from a clinical perspective, we have confirmed the results of the interim analysis in that the immunologic response during dasatinib consolidation was significantly associated with TFR. CML has long been known to be susceptible to an immunologic antileukemic response. In contrast, CML patients will have quantitative and functional defects within the NK cell compartment at the diagnosis. Moreover, Kreutzman et al showed that dasatinib induces persistent expansion of clonal...
cytotoxic T and NK cells, which has been associated with a favorable response. Furthermore, patients who maintained TFR after imatinib discontinuation have persistent leukemia cells, suggesting that the immunologic response plays a role in disease control.18

In the present study, univariate analysis revealed that a greater number of total NK cells (CD3+CD56+) and cytolytic NK cells (CD16+CD56+) and a lower number of γδ+ T cells and CD4+ Tregs were associated with successful maintenance of TFR. Multivariate analysis identified the absence of imatinib resistance and a greater number of cytolytic NK cells (CD16+CD56+) as the only factors independently associated with maintenance of TFR. When the number of total NK cells (CD3+CD56+) rather than the cytolytic NK cells was entered in the multivariate analysis, the number of total NK cells did not remain a significant factor. These results are in line with those from a recent report, which showed that a greater proportion of naive NK cells (CD56bright cells) within the NK cell population is associated with an increased risk of molecular relapse.19 Hughes et al20 conducted a detailed analysis of the effector and suppressor immune responses in patients at each phase (diagnosis, TKI treatment, and sustained TFR) and suggested that achievement of TFR is associated with both restoration of effector responses and reduced immune suppression.20

The present study had several limitations. First, when considering the optimal duration of molecular remission before dasatinib discontinuation, the data suggest that 1 year is sufficient for patients without imatinib resistance. In contrast, the data from the present study were insufficient to examine the correlation between DMR duration and TFR rate. The present trial was not a prospective trial of patients who were taking dasatinib as first-line treatment but a prospective trial of patients who were in DMR and receiving dasatinib as second-line treatment. The present trial started to enroll patients, the methods of BCR-ABL1 gene transcript quantification had not yet been standardized. Thus, it was difficult to determine the exact DMR duration. Nonetheless, we believe the findings of the present trial will have significance in offering most patients who receive TKIs outside of clinical trials the opportunity to experience TFR.

In the first-line imatinib discontinuation trials, imatinib was discontinued after 2 years of ≥ MR4.5.6,7 To examine whether the use of dasatinib enables a shorter consolidation period, we have initiated another study of dasatinib discontinuation (registry no. UMIN000011099) in which we will investigate the safety and efficacy of discontinuing first-line dasatinib in CML patients after ≥ 3 years of dasatinib treatment, including ≥ 1 year of DMR (the final year).

In addition, the optimal level of BCR-ABL1 transcript that triggers the restart of dasatinib remains unestablished. Although we defined the trigger as the loss of DMR, a recently reported imatinib discontinuation study (A-STIM trial) defined the trigger as the loss of a MMR and reported the comparable DMR regaining rate.23 It might be more reasonable to define the trigger as the “loss of a MMR” in future trials.

Conclusion

We have confirmed the feasibility of dasatinib discontinuation in patients without imatinib-resistant disease who maintain MR4.0 for ≥ 1 year. Moreover, we have demonstrated that both the characteristics of leukemia cells (an intrinsic factor) and the host immunologic responses (an extrinsic factor) are involved in maintenance of TFR. These findings could be useful for identifying patients who will derive the greatest benefit from TKI discontinuation and enable optimization of the algorithm used for treatment of CML patients taking TKIs. In particular, the use of the cytolytic NK cell numbers during TKI treatment as a determinant of eligibility for TKI discontinuation is an attractive option, although further investigations are needed. In contrast, the patients with imatinib-resistant disease probably require novel strategies to improve TFR. These could include concurrent use of IFN-α during the consolidation phase or after TKI discontinuation.

Clinical Practice Points

- Previous studies have shown that ~ 40% of CML patients who have maintained an MR4.5 for ≥ 2 years with first-line imatinib treatment could successfully discontinue imatinib treatment.
- The present study has shown that 44.4% of CML patients who maintained a DMR for ≥ 1 year with second- or subsequent-line dasatinib treatment maintained long-term TFR at 36 months.
- The presence of imatinib resistance was identified as a significant risk factor for molecular relapse.
- The high NK-cell and low γδ+ T-cell and CD4+ regulatory T-cell (CD25+CD127low) counts before discontinuation correlated significantly with successful therapy discontinuation.
- These findings could be useful in identifying patients likely to derive the greatest benefit from TKI discontinuation.
- In particular, the use of cytolytic NK cell numbers during TKI treatment as a determinant of eligibility for TKI discontinuation is an attractive option, although further investigations are needed.

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Disclosure


Supplemental Data

The supplemental data accompanying this article can be found in the online version at https://doi.org/10.1016/j.clml.2018.03.004.
References

Supplemental Figure 1 Kaplan-Meier Estimates of Treatment-free Remission After Discontinuation of Dasatinib in Patients With Chronic Myeloid Leukaemia Stratified by T-cell Immunoprofile During Dasatinib Treatment. T-cell Profiles in the Peripheral Blood Were Evaluated Throughout the Preregistration Period. The Average Number of Lymphocyte Subsets Was Calculated Across the 5 Data Points. Graphs Showing Treatment-free Remission Period Stratified by (A) CD4/CD8 Ratio and Number of (B) CD8+ Cells, (C) T-large Granular Lymphocyte (LGL) cells, and (D) γδ T cells. LGL Phenotypes Were Defined as CD57+ CD3− (T-LGL) Cells or CD57− CD56− (NK-LGL) Cells