BCR-ABL Messenger RNA Levels Continue to Decline in Patients with Chronic Phase Chronic Myeloid Leukemia Treated with Imatinib for More Than 5 Years and Approximately Half of All First-Line Treated Patients Have Stable Undetectable BCR-ABL Using Strict Sensitivity Criteria

Susan Branford,1 John F. Seymour,2 Andrew Grigg,3 Chris Arthur,4 Zbigniew Rudzki,1 Kevin Lynch,5 and Timothy Hughes3

Abstract

Purpose: In the first years of imatinib treatment, BCR-ABL remained detectable in all but a small minority of patients with chronic myeloid leukemia. We determined whether BCR-ABL continues to decline with longer imatinib exposure and the incidence and consequence of undetectable BCR-ABL.

Experimental Design: BCR-ABL levels were measured in a subset of 53 imatinib-treated IRIS trial patients for up to 7 years (29 first-line, 24 second-line). Levels were deemed undetectable using strict PCR sensitivity criteria.

Results: By 18 months, the majority achieved a 3-log reduction [major molecular response (MMR)]. BCR-ABL continued to decline but at a slower rate (median time to 4-log reduction and undetectable BCR-ABL of 45 and 66 months for first-line). The probability of undetectable BCR-ABL increased considerably from 36 to 81 months of first-line imatinib treatment (95% confidence interval, 0.07% to 0.36%). Undetectable BCR-ABL was achieved in 18 of 53 patients and none of these 18 lost MMR after a median follow-up of 33 months. Conversely, MMR was lost in 6 of 22 (27%) patients with sustained detectable BCR-ABL and was associated with BCR-ABL mutations in 3 of 6. Loss of MMR was recently defined as suboptimal imatinib response. There was no difference in the probability of achieving molecular responses between first- and second-line patients but first-line had a significantly higher probability of maintaining MMR ($P = 0.03$; 96% (95% CI, 88-100%) versus 71% (95% CI, 48-93%)).

Conclusions: With prolonged therapy, BCR-ABL continued to decline in most patients and undetectable BCR-ABL was no longer a rare event. Loss of MMR was only observed in patients with sustained detectable BCR-ABL.

Treatment for patients with chronic myeloid leukemia (CML) has been transformed by the use of the BCR-ABL tyrosine kinase inhibitor imatinib mesylate (1–6). Response is most effectively determined by measuring BCR-ABL mRNA levels with real-time quantitative PCR (7–9), and it has recently been proposed that real-time quantitative PCR be done at regular intervals of 3 months (10). The International Randomized Study of IFN versus STI571 (IRIS) commenced in 2000 and randomly assigned patients with newly diagnosed chronic-phase CML to receive imatinib 400 mg/d ($n = 553$) or IFN-α and low-dose cytarabine ($n = 553$; ref. 3). The study established for the first time a level of molecular response that correlated with exceptional progression-free survival for patients treated with imatinib (7). The patients with a 3-log reduction of BCR-ABL transcripts from a standardized baseline value for untreated patients, termed a major molecular response (MMR), had an estimated 97% probability of remaining progression-free at 54 months of imatinib treatment (11). Although 40% of patients treated with first-line imatinib achieved a MMR at 12 months, only 4% had undetectable BCR-ABL using strict real-time quantitative PCR sensitivity criteria at a median of 19 months (7).

It is unknown if BCR-ABL levels stabilize and remain detectable with long-term imatinib treatment or whether BCR-ABL continues to decline below the level of detection.
Undetectable BCR-ABL may not equate to eradication of minimal residual disease because the sensitivity of real-time quantitative PCR analysis is limited to 4 to 5 log below the standardized baseline and significant numbers of residual leukemic cells may still be present (12). For this reason, we prefer not to use the term “complete molecular response.” Nevertheless, whereas BCR-ABL remains detectable, it is an absolute indication of the persistence of leukemia and therefore the potential for resistance and disease progression. BCR-ABL kinase domain mutations are the major mechanism of acquired imatinib resistance (13–15). Although mutations are most commonly detected in patients without a major cytogenetic response (14, 15), we have previously reported mutations and relapse in a minority of patients with minimal residual disease (16).

In the early phase of the IRIS study, molecular analysis was only done for patients who achieved a complete cytogenetic response (Philadelphia chromosome negative) and the analysis was interrupted after the first 24 months of study. In the patients treated in the IRIS study in Australia and New Zealand, molecular analysis was done for all patients from the time of enrollment, which commenced in 2000, and analysis was not dependent on the cytogenetic response. The first analysis of these patients in 2003 reported that imatinib-treated patients had initial profound reductions of BCR-ABL and early measurements were predictive of subsequent response (9). With an additional 4 years of follow-up in this patient cohort, we aimed to determine if BCR-ABL levels continued to decline with prolonged imatinib treatment and whether the frequency of undetectable BCR-ABL remained a rare event. We also determined whether there was a difference in the molecular response between patients who commenced imatinib as first-line therapy or on crossover to imatinib after IFN treatment.

**Patients and Methods**

Patients. Patients with chronic-phase CML ($n = 53$) treated with imatinib in the IRIS study from June 2000 to February 2007 in Australia and New Zealand were studied. Patients provided written informed consent according to the Declaration of Helsinki and the study was approved by Ethics Committees at all participating sites.

The patients were divided into two cohorts. Cohort 1 was composed of 29 newly diagnosed patients treated with imatinib 400 mg/d (first-line imatinib). These patients had a median follow-up of 81 months (25th-75th percentile, 76-84 months). Cohort 2 was composed of 24 patients treated with IFN-α plus cytarabine who crossed over to imatinib therapy at 400 mg/d from 3.5 to 22 months of study (second-line imatinib; ref. 9). The median follow-up after commencing imatinib in these patients was 66 months (25th-75th percentile, 44-72 months). Details on the study design, conduct, and treatment plan for the IRIS trial were previously reported (3).

**Molecular response.** Molecular response was determined at our institution by real-time quantitative PCR analysis using RNA extracted from 20-mL peripheral blood at 3- to 6-month intervals as previously reported (17, 18). The **BCR** gene was quantitated to control for variation in the quality of the RNA and for differences in the efficiency of the reverse transcription reaction. The result was reported as the percentage ratio BCR-ABL/BCR. The testing laboratory was one of three laboratories that carried out the molecular analysis in the IRIS trial and, as such, has a defined BCR-ABL value representing MMR (BCR-ABL/BCR, 0.08%; ref. 7). Loss of MMR in patients who maintained their imatinib dose was defined as a $2\times$-fold increase from nadir to a level above 0.08%, which was confirmed on subsequent analysis. In total, 821 real-time quantitative PCR analyses were undertaken for the imatinib-treated patients.

For the purposes of this study, BCR-ABL was only deemed undetectable if a sensitivity of at least 4.5 log below the standardized baseline was achieved and confirmed on subsequent analysis. The BCR control transcript level determined sensitivity according to a formula that was derived from the Europe Against Cancer Program (19): sensitivity = log$_{10}$ [0.8 / (10/BCR transcripts)], where 0.8 is the standardized baseline BCR-ABL/BCR and 10 is the BCR-ABL detection limit. The sensitivity of each RNA sample varied and was dependent on the RNA quality and reverse transcription efficiency.

We evaluated molecular response by the achievement of three categories of BCR-ABL reduction from the standardized baseline: 1, MMR (≥3-log reduction); 2, ≥4-log reduction; and 3, undetectable BCR-ABL (sensitivity of ≥4.5-log reduction).

BCR-ABL kinase domain mutation analysis was done using direct sequencing (20). Patients were initially assessed for mutations – 6 monthly and after 2004 upon an increase of BCR-ABL of ≥2-fold (16) or on notification of disease progression. In total, 223 mutation analyses were done. Disease progression was as defined previously for the IRIS trial and included the following events: death from any cause, the development of accelerated phase or blast crisis, loss of a complete hematologic response, and loss of a major cytogenetic response (3).
Statistics. The χ² statistic was used to test for differences in the frequency of undetectable BCR-ABL over time. Time to event analyses were compared using the log-rank test with SPSS software (SPSS, Inc.).

Results

Patients remaining on trial. Of 29 patients treated with first-line imatinib, 24 (83%) remained on imatinib treatment at a median follow-up of 81 months. Of the 5 patients who discontinued the study, 3 had disease progression (accelerated phase in 2 and loss of complete hematologic response in 1). Of 24 patients treated with second-line imatinib, 17 (71%) remained on imatinib treatment at a median follow-up of 66 months since commencing imatinib. Of the 7 patients who discontinued the study, 4 had disease progression [blast crisis in 1, loss of major cytogenetic response in 2, and 1 patient died with acute myeloid leukemia (Philadelphia chromosome negative) following myelodysplastic syndrome].

Molecular response of first-line imatinib treated patients. By 81 months of imatinib treatment, the cumulative probability of MMR for the 29 first-line patients was 87% [95% confidence interval (95% CI), 74-100%], of ≥4-log reduction of BCR-ABL was 70% (95% CI, 52-89%), and of undetectable BCR-ABL was 52% (95% CI, 32-72%). There was a relatively rapid 3-log reduction of BCR-ABL with the median time to MMR of 18 months. BCR-ABL continued to decline in the majority of patients although at a considerably slower rate. The estimated median time to a 4-log reduction of BCR-ABL was 45 months of imatinib treatment and to undetectable BCR-ABL was 66 months. The actual incidence of undetectable BCR-ABL increased every year for the first 6 years of imatinib treatment. At a median follow-up of 81 months, 13 of the 29 (45%) patients who commenced first-line imatinib had undetectable BCR-ABL, a frequency significantly higher than occurred in these patients at 36 months [2 of 29 (7%) patients; P = 0.003].

Molecular response of second-line imatinib treated patients. For the 24 patients treated with second-line imatinib, the cumulative probability of MMR by 66 months was 90% (95% CI, 73-100%), of ≥4-log reduction was 40% (95% CI, 18-62%), and of undetectable BCR-ABL was 27% (95% CI, 6-48%). Similar to the first-line patients, there was a relatively rapid 3-log reduction of BCR-ABL with a median time to MMR of 18 months. However, the median time for BCR-ABL to reduce a further 1-log from the 12-month time point was not reached, again indicating that the rate of BCR-ABL reduction slowed considerably. Statistically, there was no difference in the probability of a molecular response between the patients treated with first- or second-line imatinib (Fig. 1).

Durability of undetectable BCR-ABL. Eighteen of the 53 patients achieved undetectable BCR-ABL. After a median follow-up of 33 months (25th-75th percentile, 26-42 months) from attainment of undetectable BCR-ABL, 16 of the 18 patients had shown undetectable BCR-ABL on every subsequent assessment. Two patients (one first-line and one second-line) had intermittently detectable BCR-ABL over 30 and 27 months, respectively. However, BCR-ABL was undetectable in both patients at the last analysis and none of the positive values were greater than BCR-ABL/BCR 0.02%, which indicates that MMR was maintained. Intermittently detectable BCR-ABL may reflect the varying sensitivity achieved with each RNA sample. Evidence of another clonal abnormality was investigated and was not found in any of the patients with undetectable BCR-ABL.

Prediction of undetectable BCR-ABL. Because there was no significant difference in the probability of achieving the molecular responses between the first- and second-line patients, the groups were combined to evaluate whether early reduction of BCR-ABL was predictive for the subsequent achievement of undetectable BCR-ABL. The 53 patients were divided into groups dependent on their molecular response at 3-monthly...
intervals up to 12 months. The achievement of MMR at 3, 6, 9, or 12 months was highly predictive for the subsequent achievement of undetectable BCR-ABL (Table 1). The best discriminator at 6 years of imatinib treatment was a MMR at the 12-month time point. The probability of achieving undetectable BCR-ABL for patients with a MMR at 12 months was 72% (95% CI, 50-94%) compared with only 5% (95% CI, 0-15%) for those without a MMR ([P] < 0.0001; Fig. 2).

**Durability of MMR for patients with sustained detectable BCR-ABL.** Twenty-two of 53 patients achieved MMR and BCR-ABL remained detectable. These patients had a median follow-up of 47 months (25th-75th percentile, 33-60 months) from attainment of MMR. Six of the 22 (27%) patients lost MMR (1 first-line and 5 second-line patients). The median duration from attainment of MMR to loss was 15 months (range, 6-39 months). The median increase of BCR-ABL in the six patients was 325-fold (range, 4-to 1,900-fold) with BCR-ABL mutations detected in three. Loss of response included loss of complete cytogenetic response in two patients, loss of complete hematologic response in one, and blast crisis in one. Loss of MMR in the remaining two patients triggered early therapeutic intervention before loss of response could be further assessed. Both patients regained MMR after an imatinib dose increase to 800 mg/d. Of the 41 patients who remained on trial at the last assessment, only 3 had not achieved a MMR (2 first-line patients and 1 second-line). These patients maintain a complete cytogenetic response.

**Probability of maintaining MMR.** Although there was no significant difference in the probability of achieving MMR between the patients treated with first- or second-line imatinib, there was a significant difference in the probability of maintaining MMR once it was attained. Those who commenced first-line imatinib had a significantly higher probability of maintaining MMR compared with those who commenced second-line imatinib ([P] = 0.03; 96% (95% CI, 88-100%) versus 71% (95% CI, 48-93%); Fig. 3).

**Mutation analysis.** Six patients acquired mutations during imatinib treatment; two treated with first-line imatinib and four with second-line imatinib (Table 2). All patients with a mutation subsequently lost their best response. Three of the six patients had achieved a MMR before the mutation was detected. Mutations were detected between 9 and 30 months of commencing imatinib treatment. There was no significant difference in the probability of acquiring mutations between patients who commenced first- or second-line imatinib. The estimated probability of detecting a mutation for first-line patients was 7% (95% CI, 0-17%) and for second-line patients was 18% (95% CI, 2-34%; [P] = 0.27; Fig. 4). Of the seven patients with disease progression, mutations were detected in three.

**Discussion**

This report is the longest molecular follow-up of patients treated in the IRIS trial. Mathematical modeling of the kinetics of CML over the first 12 months of imatinib treatment suggested a biphasic exponential decline of leukemic cells with an initial rapid depletion of differentiated cells followed by a slower clearance of leukemic progenitors (21). Our results show that the slow reduction of BCR-ABL continues for more than 5 years in the majority of patients. There was no difference in the probability of achieving molecular responses between the patients treated with first- or second-line imatinib. However, the follow-up for patients treated with second-line

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**Table 2.** Patients who acquired BCR-ABL kinase domain mutations

<table>
<thead>
<tr>
<th>Patient identifier</th>
<th>Months of imatinib when mutation was detected</th>
<th>Mutation</th>
<th>Best molecular response</th>
<th>Response after mutation was detected</th>
<th>Current status</th>
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<tbody>
<tr>
<td>First-line, 9</td>
<td>9</td>
<td>F359V</td>
<td>0 to 1-log reduction MMR</td>
<td>AP</td>
<td>Deceased at 12 mo after commencing imatinib</td>
</tr>
<tr>
<td>First-line, 29</td>
<td>27</td>
<td>E255K</td>
<td>Loss of CCR</td>
<td>Undetectable BCR-ABL after allogeneic transplant</td>
<td></td>
</tr>
<tr>
<td>Second-line, 1</td>
<td>24</td>
<td>F359V</td>
<td>2- to 3-log reduction MMR</td>
<td>Loss of CCR and clonal evolution</td>
<td>Undetectable BCR-ABL on dasatinib</td>
</tr>
<tr>
<td>Second-line, 10</td>
<td>30</td>
<td>E453G</td>
<td>Loss of MMR</td>
<td>Undetectable BCR-ABL after dasatinib</td>
<td></td>
</tr>
<tr>
<td>Second-line, 14</td>
<td>24</td>
<td>T315I</td>
<td>2- to 3-log reduction MMR</td>
<td>Loss of MCR</td>
<td>Deceased after allogeneic transplant</td>
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<tr>
<td>Second-line, 24</td>
<td>23</td>
<td>D276G</td>
<td>BC</td>
<td>Deceased</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AP, accelerated phase; CCR, complete cytogenetic response; MCR, major cytogenetic response; BC, blast crisis.
therapy was shorter, and longer follow-up may discern a difference. Previous molecular studies of chronic-phase patients with CML reported a low incidence of PCR negativity within the first few years of imatinib treatment (7, 22). Our study now identifies a substantial increase in the number of patients with undetectable BCR-ABL beyond 3 years of imatinib treatment. The probability of undetectable BCR-ABL was 52% for first-line imatinib-treated patients at a median of 81 months.

The ability to detect BCR-ABL is a function of the quality of the RNA sample, the efficiency of the reverse transcription reaction, and the sensitivity of the quantitative assay. Our study used strict PCR sensitivity criteria to determine undetectable BCR-ABL as did the original IRIS trial molecular report (7). Whether achieving undetectable BCR-ABL is superior to a major cytogenetic response and the incidence of progression-free survival remains unknown. The minimum requirement to satisfy the criteria for progression in the IRIS trial was loss of major cytogenetic response and the incidence of progression decreased beyond 2 years of imatinib treatment (6). Therefore, discriminating a difference in the progression-free survival of patients who achieve a MMR may be difficult, particularly if patients receive early therapeutic intervention on loss of MMR.

Loss of MMR has recently been defined as a suboptimal response, meaning that the patient may still have a substantial benefit from continuation of imatinib, but the long-term outcome of the treatment would not likely be as favorable. In this situation, the patient becomes eligible for other treatments (10). Among the patients in our study with undetectable BCR-ABL using strict PCR sensitivity criteria, none lost MMR after a median follow-up of 33 months from first attainment of undetectable levels. In contrast, among the patients with a MMR wherein BCR-ABL remained detectable, 27% lost MMR. Our results are similar to a recent report of patients predominantly treated with imatinib in late chronic phase in which those with PCR negativity did not relapse (23). Achieving undetectable levels of BCR-ABL using strict sensitivity criteria may identify patients likely to maintain optimal imatinib response. Furthermore, having a MMR at 12 months of imatinib therapy was highly predictive of subsequent undetectable BCR-ABL. (72% versus 5%).

The increasing frequency of occurrence of undetectable BCR-ABL in patients with long follow-up and its apparent stability in this study may be consistent with a model of gradual depletion of leukemic stem cells (24, 25). The model proposed by Roeder et al. (25) predicts that leukemic stem cells may be completely eliminated with long-term imatinib treatment if resistance does not occur. However, based on the currently available data, the model of Dingli and Michor (26) predicts that imatinib cannot cure patients with CML because leukemic stem cells are intrinsically resistant to imatinib. Whereas leukemic stem cell eradication in CML may not be directly measurable, an undisputed prerequisite would be undetectable BCR-ABL using strict sensitivity criteria. In a recent report of 12 patients who ceased imatinib after a period of at least 2 years of undetectable BCR-ABL, 6 patients relapsed within 6 months whereas the remainder maintained response (27). The authors hypothesized that these relapses may reflect the kinetics of proliferating CML cells, which may be eradicated or controlled in patients who maintain response. In our cohort, we did not have the opportunity to follow patients who ceased imatinib after a period of undetectable BCR-ABL.

BCR-ABL kinase domain mutations were detected in a small minority of patients, and those treated with first-line imatinib had only a 7% probability of a mutation at a median of 81 months of treatment. This compares favorably to patients treated with imatinib in advanced disease where an estimated 61% of patients in accelerated phase had a detectable mutation by 24 months (28). There was no significant difference in the probability of acquiring a mutation during imatinib treatment for patients treated with first- or second-line imatinib. The six patients with mutations had received up to 30 months of imatinib treatment at the time of mutation detection. All lost their best response and three of the patients with mutations were among the seven with disease progression as defined in the IRIS trial.

In conclusion, with extended follow-up, the BCR-ABL levels continued to decline in the majority of patients and the number attaining undetectable BCR-ABL increased substantially between 3 and 6 years of imatinib treatment. For patients with a MMR, suboptimal response in terms of loss of MMR was only observed in patients with consistently detectable BCR-ABL and was more frequent in patients treated with second-line imatinib. Sensitive long-term molecular analysis may identify patients likely to maintain MMR and optimal response but this observation needs to be verified on a larger data set.

Acknowledgments

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References

Continuing Decline of BCR-ABL with Imatinib Therapy


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