Prediction of response to imatinib by prospective quantitation of BCR-ABL transcript in late chronic phase chronic myeloid leukemia patients


(Study writing committee for the GiMEMA Working Party on Chronic Myeloid Leukemia)

1Institute of Hematology and Medical Oncology L. and A. Sera`gnoli, University of Bologna; 2CEINGE Biotecnologie Avanzate and Department of Biochemistry and Medical Biotechnology, University of Naples Federico II; 3Division of Hematology and Internal Medicine, Department of Clinical and Biological Science, University of Turin; 4Hematology Section, DIMMP Department, University of Bari; 5Division of Hematology, IRCCS Poli`clinico San Matteo, University of Pavia; 6Hematology Unit, Santa Maria delle Croci Hospital, Ravenna; 7Department of Internal Medicine and Department of Haematology and Oncology, University of Genova

Received 22 August 2005; revised 3 November 2005; accepted 7 November 2005

Imatinib mesylate (STI571), a specific Bcr-Abl inhibitor, has shown a potent antileukemic activity in clinical studies of chronic myeloid leukemia (CML) patients. Early prediction of response to imatinib cannot be anticipated. We used a standardized quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR) for BCR-ABL transcripts on 191 out of 200 late-chronic phase CML patients enrolled in a phase II clinical trial with imatinib 400 mg/day. Bone marrow samples were collected before treatment, after 12, 20 and at the end of study treatment (52 weeks) while peripheral blood samples were obtained after 2, 3, 6, 10, 14, 20 and 52 weeks of therapy. The amount of BCR-ABL transcript was expressed as the ratio of BCR-ABL to β2-microglobulin (β2M). We show that, following initiation of imatinib, the early BCR-ABL level trends in both bone marrow and peripheral blood samples made it possible to predict the subsequent cytogenetic outcome and response. We propose this method as the method of choice for monitoring patients on imatinib therapy. QRT-PCR studies may be able to identify degrees of molecular response that predict both complete cytogenetic response and long term stability, as well as patterns of response that provide an early indication of relapse and imatinib resistance.

Key words: Imatinib mesylate, late-chronic phase, chronic myeloid leukemia.

introduction

The hybrid BCR-ABL gene is associated with the development and progression of Philadelphia chromosome-positive (Ph+) chronic myeloid leukemia (CML) [1–6]. Imatinib (imatinib mesylate; formerly STI571; Gleevec in Europe; Glivec in the United States; Novartis Pharmaceuticals, Basel, Switzerland), a specific BCR-ABL tyrosine-kinase inhibitor, has shown a potent antileukemic activity in recent phase I and II clinical studies of CML [6–8]. Imatinib induces a very rapid hematologic response in almost all Ph+ CML patients who are in chronic phase, in about 50% of those who are in accelerated phase [8] and in a smaller but interesting proportion of those who are in blastic phase [6] or have Ph+ acute leukemia [8]. More importantly, it has been shown that a major cytogenetic response (CgR) may be achieved in more than 50% of the patients who begin the treatment in late chronic phase [3, 8, 9] and in more than 80% of the patients who are treated front-line [10]. Most of these CgRs are complete and stable and are likely to have a beneficial effect on survival. Despite the fact that the introduction of imatinib mesylate may lead to a substantial revolution in the management of Ph+ CML, prediction of the response to imatinib cannot be anticipated with certainty [11]. Also the extent to which the leukemic cell burden is reduced and the duration of the response cannot yet be determined.

For CML patients, methods for predicting and monitoring response to treatment have changed considerably in recent years from the repeated examination of bone marrow...
metaphases for the presence of the Ph+ chromosome in patients treated with interferon-α (IFNα) or allogenic stem cell transplantation (SCT), to quantitative assays based on reverse transcriptase polymerase chain reaction (QRT-PCR). QRT-PCR has provided extremely valuable for assessing and monitoring minimal residual disease in patients who achieve Ph negativity after treatment with IFNα or with imatinib mesylate or after allogenic SCT [12]. The application of this method requires patients to be monitored serially in complete cytogenetic remission with repeated bone marrow examinations. Decreasing or rising amounts of BCR-ABL transcripts detected by QRT-PCR may indicate the need for reduced or further therapy, respectively.

To assess the role of standardized QRT-PCR in predicting the response to imatinib therapy and CCGr, serial examinations were carried out on peripheral blood or bone marrow samples collected at early time points in 191 out of 200 CML patients entered into a phase II clinical trial conducted by the GIMEMA Working Party on CML. We show that, following initiation of imatinib, the early BCR-ABL level trends made it possible to predict the subsequent cytogenetic outcome after 6 and 12 months of treatment, and that these early trends are also predictive of progression-free survival.

**materials and methods**

**patients and clinical study**

The treatment and study protocol (CML/002/STI571) was designed and sponsored by the GIMEMA Working Party on CML [14] according to Good Clinical Practice and the principles of the Helsinki Declaration. The study was approved by the Independent Ethical Committee of the University of Bologna S. Orsola Hospital and subsequently by the independent ethical committees of all the participating institutions. Novartis Pharma supplied the drug free of charge and provided partial support for data and sample collection and adverse event monitoring. The design of the treatment protocol was the same as that of a previous phase II international study of imatinib in late chronic phase Ph+ CML [8, 9] and has been described in a previous report [15].

Briefly, male or female patients were eligible for inclusion in this study if they were at least 18 years of age and had Ph+ CML. Patients were required to be in the first chronic phase (less than 15% blast cells, less than 30% blast cells and promyelocytes and less than 15% basophils in peripheral blood (PB) and in bone marrow (BM), with a platelet count of more than 100 × 10⁹/l), and to have already been treated with IFNα and proving either intolerant of, or resistant to it [15, 16]. These patients have a median time from diagnosis of three years were thus defined as ‘late’ chronic phase [15]. Patients who were resistant to IFNα were divided into two groups, namely those showing hematologic resistance (i.e. failing to achieve or to maintain a complete hematologic response (CHR) to IFNα) and those showing cytogenetic resistance (i.e. failing to achieve or to maintain a complete or partial cytogenetic response to IFNα). Intolerance and resistance were defined exactly as in previous studies [13]. Treatment consisted of imatinib alone, 400 mg once daily. Criteria for dose reductions and treatment discontinuation have been described [15]. The hematologic response was defined as complete (CHR) if the leukocyte count was below 10 × 10⁹/L, if no immature cells were recorded in the differential count, if the platelet count was below 450 × 10⁹/l and if the spleen was not palpable. The accelerated or blast phase (ABP) was defined by any one of the following: more than 15% blast cells or more than 30% blast cells and promyelocytes or more than 15% basophils in PB or BM. BM cells were sampled for cytology, cytogenetics and molecular biology at baseline, after 3, 6, 9, and 12 months, and every 6 months thereafter.

One hundred and ninety-one (191) patients out of 200 are reported on here, of whom 32 (17%) were hematologic failures, 103 (54%) cytogenetic failures and 56 (29%) intolerant of IFNα.

These three groups presented no appreciable differences as regards age (median 47, 48 and 52 years), Hb concentration (median 120, 127 and 131 g/l), white cell count (median 12, 10 and 11 × 10⁹/l) and platelet count (median 299, 291 and 283 × 10⁹/l). The spleen was enlarged and palpable in 60% of the hematologic failures, in 27% of the cytogenetic failures and in 39% of those presenting IFNα intolerance. The time elapsing from diagnosis of CML to treatment ranged from 1 to 160 months (median: 38 months) and was longer for cytogenetic failures (median: 50 months) than for hematologic failures (median: 34 months) and those with IFNα intolerance (median: 28 months). All patients were observed for a minimum of 12 months (range: 12–36 months; median: 26 months). All patients gave written informed consent for participation in the study.

**samples for cytogenetic and molecular analyses**

BM samples (5 ml) were collected at baseline, after 3 and 6 months, and at the end of study treatment (12 months). Subsequent samples were obtained every six months only from the patients who were in CCgR at 12 months. Cytogenetic analysis was performed locally as reported [15]. PB samples were collected at baseline, after 29 days, 57 days, 3, 6 months and at the end of study treatment (12 months). All the BM and PB samples were sent at room temperature within 24 h by express delivery to the central laboratory in Bologna, where they were processed as described below, stored and distributed to the three reference laboratories in Bologna, Turin and Naples (referenced centers) for RT-PCR analysis. Total leukocytes were extracted from 5 ml of BM aspirate or 20 ml of PB after separation on a Ficoll Hypaque gradient according to a previously reported method [12]. Mononuclear cells (about 50 000 per aliquot) were re-suspended in 600 μL of GITC, as reported [17] and a portion of the aliquot (200 μL) of the GITC solution was sent to the reference laboratories. RNA extraction was performed with phenol/chloroform or with commercially available extraction kits, as reported [1, 12]. The reverse transcription (RT) reaction conditions were the same for BCR-ABL, Ab and β2-microglobulin (β2M) mRNA amplifications [15, 18]. Qualitative RT-PCR for BCR-ABL transcript was routinely performed at enrollment and during follow-up, as previously described [12, 19, 20]. All tests were done twice to confirm the results.

**real-time quantitative RT-PCR**

Real-time quantitative RT-PCR was performed on an ABI PRISM 7700 Sequence Detector (Perkin Elmer, Foster City, CA) [1]. To compensate for differences in RNA quality or RT efficacy, parallel TaqMan assays were run on each sample for β2M and ABL as housekeeping genes, and the absolute levels of BCR-ABL mRNAs were normalized both with respect to β2M and ABL mRNA content [18]. Primers and probes for BCR-ABL, ABL and β2M were established in the context of a collaborative effort which involved 25 different centers from 10 European countries (European Concerted Action, SANCOSO) as reported [12, 18]. Primers, probes and plasmids containing the amplified sequences for BCR-ABL, ABL and β2M, respectively, were selected using Primer Express Software (Perkin Elmer), were purchased from IPSOGEN Inc. (Marseille, France) and used according to manufacturer’s guidelines. The RT-PCR assay was performed in triplicate as reported [17]. Preliminary experiments were done to optimize the real time RT-PCR set-up [18]. Integrity and quality of RNA were checked by gel electrophoresis. Furthermore, RNA samples that repeatedly gave β2M Cts higher than 25.7 were operationally considered degraded and eliminated from further evaluation. Finally, in the case of
BCR-ABL, when Cts were higher than the intercept value of the relative standard curve of the run (Ct corresponding to one copy), the samples were considered negative (MRD below the detection limit of the technique).

statistics

Comparisons of frequencies were made with a \( \chi^2 \)-squared test or the Fisher exact test, as appropriate. Comparisons between groups were performed using the log-rank test as reported [15]. Overall survival and time to progression to ABP were calculated by the product limit method according to Kaplan and Meier. All statistical calculations were done using SPSS.9 software (Chicago Inc.)

results

half of the patients treated with imatinib for two or more years obtained complete, stable cytogenetic responses

The hematologic and cytogenetic response rates during the first and second year of the study period are reported in Table 1. Eighty-nine percent of patients achieved a CHR [15]. Eighty percent of patients remained in continuous CHR after one year, and 78% were in continuous CHR throughout the subsequent years of therapy and follow-up. Eighty-five patients (44%) achieved a CCgR at least once and another 33 patients achieved a PCgR, making a major CgR rate of 61% after 1 year. The rate of major CgRs did not consistently increase from 3 months (41%) to 12 months (48%) or 24 months (51%). The incidence of CgRs improved significantly over time, the CCgR rate doubling from 16% at 3 months to 33% at 12 months, and then rising further to 36% at 24 months. Eighty-five patients achieved a CCgR at least once during the one-year study period. In nine cases the response was recorded only once, was unstable, and rapidly reverted to partial, minor, minimal or none. One of these nine patients progressed to ABP. In the remaining 76 patients the CCgR was stable throughout the study period. In the subsequent year of follow-up, one patient committed suicide. Loss of CCgR occurred in eight patients. Therefore 67 out of 85 cases (79%) who achieved a CCgR at least once were still in continuous CCgR after 2 years of treatment, corresponding to 33% of the entire study population (Table 1). CCgR, prior disease duration and treatment dose have been reported previously [15]. For those patients who achieved stable CCgRs the mean daily dose was 94% of the scheduled dose (375 mg; SD 52).

The type of transcript was identified in 186/191 cases (97%) and was b3a2 (or b3a2/b2a2) in 101 cases, b2a2 in 82 cases and e19a2 (coding for P230) in three cases. Major and complete CgR rates were similar (56% and 40% for b3a2 cases versus 49% and 34 % for a2b2 cases). None of the three e19a2 (P230) cases achieved a CCgR. The three P230 cases were kept separate and the other cases were analyzed for possible correlation between the amount of transcript and CgR (Table 3).

On BM samples, the amount of transcript expressed as the ratio of BCR-ABL to \( β2M \) per cent was 0.246, 0.253 and 0.383 in complete cytogenetic responders, partial to minimal responders and non responders, respectively (Table 2a). In contrast, the respective amounts of transcript expressed as the ratio of BCR-ABL to \( β2M \) per cent were 0.072, 0.066 and 0.103 in PB of the same groups of patients (Table 2b). The relationship between BCR-ABL transcript and clinical and cytogenetic response has been reported [11, 15, 21, 22].

On BM samples, the amount of transcript expressed as the ratio of BCR-ABL to \( β2M \) per cent was 0.246, 0.253 and 0.383 in complete cytogenetic responders, partial to minimal responders and non responders, respectively (Table 2a). In contrast, the respective amounts of transcript expressed as the ratio of BCR-ABL to \( β2M \) per cent were 0.072, 0.066 and 0.103 in PB of the same groups of patients (Table 2b). The relationship between BCR-ABL transcript and clinical and cytogenetic response has been reported [11, 15, 21, 22].

There was no difference between the patients who achieved a ‘stable’ CCgR (i.e. patients in CCgR at 3 consecutive cytogenetic analysis carried out every three months of therapy) and the cases who had a less stable CCgR, whether the response was partial, minor or even minimal either in BM or in PB (Table 2a and b). However, in the 34 patients who achieved no CCgR at all the transcript level prior to treatment was significantly higher than in any other response category, both in BM and in PB (0.583 and 0.103, respectively) \( (P < 0.001) \) (Table 2a and b).

Table 1. Hematologic and cytogenetic response during the first and second year study period. All calculations are based on all 191 cases (intention-to-treat). All cases but two were evaluable for response at least once (overall), while several cases (14 to 20%) were not evaluable for cytogenetic response at each time point

<table>
<thead>
<tr>
<th>Hematologic response</th>
<th>Cyogenetic response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NE Complete</td>
</tr>
<tr>
<td>3 months</td>
<td>0</td>
</tr>
<tr>
<td>6 months</td>
<td>0</td>
</tr>
<tr>
<td>9 months</td>
<td>5</td>
</tr>
<tr>
<td>12 months</td>
<td>6</td>
</tr>
<tr>
<td>Overall</td>
<td>0</td>
</tr>
</tbody>
</table>

NE, not evaluable.
the quantity of neoplastic transcript after two months of imatinib therapy was predictive of the subsequent cytogenetic unresponsiveness

We sought to establish whether the quantity of neoplastic transcript was able to predict the subsequent cytogenetic response and, in this case, when the molecular assay of BCR-ABL transcript could prove predictive. The amount of BCR-ABL transcript in BM cells was measured prior to treatment (baseline), at intervals during treatment (12 and 24 weeks), and at the end of the one-year study period (52 weeks or 12 months). In addition, in order to detect rapid decreases in BCR-ABL transcript and possibly achieve early prediction of response to therapy, blood was collected and studied at 29 and 57 days after the start of therapy (Table 3). In the patients who had achieved a CCGR, the degree of the MR was also assessed after 18 to 24 months both in BM and in PB. Table 3 and Figure 1a and b show the MR patterns according to the degree of CgR in BM and PB, respectively.

In the BM samples, in the non responders (‘None’ in Table 3, left side), the transcript level remained substantially steady during the treatment, with a small and non-significant decrease from 0.583 at baseline to 0.215 at 12 months (median values). In the BM samples, in the non responders (‘None’ in Table 3, right side), the transcript level again remained substantially steady during the treatment, with a small, non significant decrease from 0.253 at baseline to 0.044 at 12 months (median values). In the complete cytogenetic responders (CgR in Table 3) the amount of transcript was already significantly (P<0.01) decreased by more than 2 log, from 0.246 baseline to 0.0062 after 3 months, and decreased further to 3 log, (i.e. 0.0009 after 6 months) and to 0.0008 after 12 months (median values). In the partial CgRs the decrease in transcript level was also significant, but the maximum decrease was less than two logs (from 0.253 at baseline to 0.044 at 12 months) (Table 3). This observation, which was confirmed in the PB samples (see below), possibly reflects the fact that, after the initial response to imatinib, this group of patients progressively loses its responsiveness or reduces its drug compliance.

As far as PB samples were concerned, in the non responders (‘None’ in Table 3, right side), the transcript level again remained substantially stable during treatment, with a small, non significant decrease from 0.103 baseline to 0.089 after 29 days of therapy and to 0.083 after 57 days of imatinib. In this group of cytogenetic non responders the BCR-ABL/β2M ratio percent was 0.117 (median values) at 12 months. In the complete, stable Cg responders the amount of transcript was significantly reduced by more than 1 log, from 0.072 baseline to 0.030 and 0.008 at 29 and 57 days of therapy, respectively.

After 3 months, in the PB samples it was 0.002 and decreased further to 0.0004 after 6 months and to 0.0005 after 12 months (median values) (three log reduction). In the partial CgRs the decrease in transcript level was also significant, but the maximum decrease was less than two logs (from 0.066 at baseline to 0.013 at 12 months) (Table 3). The differences in the reduction of neoplastic transcript between non versus complete responders were statistically significant both at 2 months (+57 days) of imatinib therapy in PB or at 3 months of therapy in BM samples (P < 0.01) (Fig. 1). Furthermore, the differences in the reduction of neoplastic transcript between non versus partial responders were not statistically significant at 2 months (P = 0.09) but turn into significant a 3 months of therapy either in BM or PB samples (P < 0.05) (Fig. 1). This means that, in ‘late’ chronic phase CML patients at least, it may be possible to predict the complete and ‘stable’ cytogenetic response to imatinib in blood after only 2 months of therapy. It is worth noting that in the group of partial responders the (median) amount of transcript was lower and there was a greater reduction (two logs) at 6 months (0.0054) than at 12 months (0.044).

**Table 2.** Transcript level baseline and CgR in: (a) bone marrow

<table>
<thead>
<tr>
<th>Cytogenetic response</th>
<th>No. of cases/samples</th>
<th>Transcript level in BM</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>64/61</td>
<td>0.246</td>
<td>0.397</td>
<td>0.466</td>
<td></td>
</tr>
<tr>
<td>Partial to minimal</td>
<td>67/66</td>
<td>0.253</td>
<td>0.385</td>
<td>0.377</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>34/31</td>
<td>0.583</td>
<td>0.551</td>
<td>0.517</td>
<td></td>
</tr>
</tbody>
</table>

(b) peripheral blood

<table>
<thead>
<tr>
<th>Cytogenetic Response</th>
<th>No. of cases/samples</th>
<th>Transcript level in PB</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>64/56</td>
<td>0.072</td>
<td>0.164</td>
<td>0.279</td>
<td></td>
</tr>
<tr>
<td>Partial to minimal</td>
<td>67/55</td>
<td>0.066</td>
<td>0.170</td>
<td>0.334</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>34/32</td>
<td>0.103</td>
<td>0.111</td>
<td>0.095</td>
<td></td>
</tr>
</tbody>
</table>

The transcript level is expressed as the ratio of Bcr-Abl to β2-microglobulin×100.

**Table 3.** Bcr-Abl transcript level in bone marrow (left) and in peripheral blood (right) in patients who failed to achieved any cytogenetic response (None), or who achieved a partial to minimal, or complete, stable cytogenetic response (CgR)

<table>
<thead>
<tr>
<th>Transcript level in bone marrow(median)</th>
<th>None</th>
<th>Partial to minimal</th>
<th>Complete CgR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.583</td>
<td>0.253</td>
<td>0.246</td>
</tr>
<tr>
<td>429 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>457 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>0.143</td>
<td>0.068</td>
<td>0.0062</td>
</tr>
<tr>
<td>6 months</td>
<td>0.129</td>
<td>0.0054</td>
<td>0.0009</td>
</tr>
<tr>
<td>12 months</td>
<td>0.215</td>
<td>0.044</td>
<td>0.0008</td>
</tr>
<tr>
<td>18–24 months</td>
<td></td>
<td></td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transcript level in peripheral blood(median)</th>
<th>None</th>
<th>Partial to minimal</th>
<th>Complete CgR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>429 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>457 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>0.103</td>
<td>0.066</td>
<td>0.072</td>
</tr>
<tr>
<td>6 months</td>
<td>0.089</td>
<td>0.032</td>
<td>0.030</td>
</tr>
<tr>
<td>12 months</td>
<td>0.083</td>
<td>0.014</td>
<td>0.008</td>
</tr>
<tr>
<td>18–24 months</td>
<td>0.027</td>
<td>0.012</td>
<td>0.002</td>
</tr>
<tr>
<td>45 days</td>
<td>0.035</td>
<td>0.009</td>
<td>0.0004</td>
</tr>
<tr>
<td>12 months</td>
<td>0.117</td>
<td>0.013</td>
<td>0.0005</td>
</tr>
<tr>
<td>18–24 months</td>
<td></td>
<td></td>
<td>0.0002</td>
</tr>
</tbody>
</table>
the proportion of complete molecular responders among patients with CCgR progressively increases during imatinib therapy and the amount of BCR-ABL transcript continues to decrease in CCgR patients.

In addition, we investigated whether the rate of patients with ‘molecular responses’ to imatinib continues during treatment. We confirmed our previously reported data on the molecular response patterns of the group of patients who achieved CCgRs in 3 or 6 months (early) (54 pts) and of those who achieved CCgRs in 9 or 12 months (late) (22 pts).

We further sought if the ‘quality’ of the response, i.e. progressive decrease in BCR-ABL/β2M ratios, could be assessed by our assay. Interestingly, the amount of BCR-ABL transcript in BM (61 samples at baseline; 46 samples at 18–24 months) versus PB (56 samples at baseline; 46 samples at 18–24 months) of the 76 patients obtaining CCgRs (Tables 3 and Figure 2) showed the same response pattern.

We recorded the results as BCR-ABL/β2M ratio and, in this case, the limit of the detection power of the method is $10^{-5}$ (four log reduction). In some cases this limit was reached after only 3 months. After 12 months eight patients out of 55 had an amount of transcript $\leq 0.00001$ in BM, while at the same time 16/55 patients presented the same results in PB ($P < 0.01$). These data suggest that detection of minimal residual disease in BM is more sensitive than in PB, at least in our assay. At 18 to 24 months of treatment the upper limit of detection (i.e. $\leq 0.00001$) was reached in 16/46 cases in BM and in 18/46 in PB ($P = n.s.$). There was no detectable difference between early responders (30%) and late responders (27%).
We also listed the results as cycle threshold (Ct) of BCR-ABL, and, in this case, the most sensitive detection limit of our assay was 38°Ct, as reported in the European Concerted Action standardization for fusion transcripts [18] (Figure 2). In other words, if expressed as Ct value, value over the 38°Ct has to be considered ‘negative’ for BCR-ABL transcript. The limit of this manner to express quantitative results is that it is related to the quality of the RNA analyzed. The proportion of patients who obtained a Ct above 38 cycles after 12 months of therapy (i.e. low amount or undetectable amount of BCR-ABL transcript) was almost twice as much in PB than in BM (19 versus 10 pts.) (P<0.01), but similar (22 versus 26 pts; 49% versus 56%) during further follow-up (P = n.s.) (Figure 2). By means of nested PCR, we confirmed that all but one of these patients were qualitatively PCR negative. These results suggest that the proportion of complete molecular responders among patients with CCgR progressively increases during imatinib therapy and the amount of BCR-ABL transcript continues to decrease in CCgR patients.

The proportion of CCgR patients losing the complete cytogenetic response is about 10% per year but only 3% in those who obtain a four log reduction in amount of BCR-ABL transcript in BM

During the second year of treatment the CCgR was lost in eight cases. In one patient no further samples were mailed to the molecular biology center in Bologna and therefore no analysis was done. In two patients the MB was not assessed. In five of the remaining six patients the amount of BCR-ABL transcript was always detectable, but was still undetectable in one patient 5 months before CCgR loss. As a matter of fact, only one of 28 cases where the BCR-ABL amount was found at least once to be ≤0.00001 lost the CCgR as against five of 45 cases where the BCR-ABL level never reached a level of 0.00001.

We also analyzed the distribution of complete, stable CgRs in relation to the maximum reduction in BCR-ABL level. This could be measured in 60 of 76 cases. It was less than two logs in nine cases, three of which (33%) lost the CCgR. It was between two and four logs in 33 cases, two of which (6%) lost the CCgR. Also one of the 18 cases (5%) with a maximum log reduction of more than four lost the CCgR. No statistical significance can be attributed to these relationships due to the small number of cases and events, but they are nevertheless worthy of note.

Follow-up

During the trial period, which was one year, 21/191 patients abandoned the study treatment, one for protocol violation, 11 for progression to APB, and nine for adverse events. Two of the latter progressed to APB after discontinuation of treatment, with the result that the total progression rate at one year was 13/191 or 7%. With a median follow-up of 26 months (range, 12–36), the overall rate of progression to APB was 17/191 or 9%, with a small and slightly significant difference in favor of MCgRs as compared to the others (4% versus 13%, log-rank P = 0.037). The overall survival was 97% for major CgRs versus 92% for the others (log-rank P = 0.122).

Discussion

Despite the extensive use of imatinib in CML, it is still too early to identify, prior to the start of therapy, which patients may have a chance to obtain a complete cytogenetic response, the latter being a conventional surrogate marker of long-term disease control. Our work on 191 CML patients in late chronic phase and in relation to imatinib therapy for more than one year has explored the significance of prospective molecular monitoring of BCR-ABL transcript levels in both BM and PB in predicting cytogenetic response and clinical outcome. Major and complete cytogenetic responses have been verified as reliable indicators of long-term survival in patients treated with IFNα [13, 23]. In the imatinib era, a certain amount of information is available on the long term clinical significance of complete cytogenetic responses, but little information is available regarding the clinical significance of a reduction in the amount of BCR-ABL transcript during the first months of the therapy. If chronic phase CML patients achieving MCR on imatinib have a significantly lower risk of progression in the subsequent 24 months than patients not achieving MCR, [8] our molecular results suggest that significant reductions in the BCR-ABL/β2M ratio below 0.0001 are predictive of patients obtaining a major or complete cytogenetic response. Our results further demonstrate that such a reduction in the molecular level of BCR-ABL transcript is predictive both of the probability of obtaining a complete or major cytogenetic response and of progression-free survival, at least in the short term.

Results similar to ours, though obtained with a different molecular assay and with a less regular monitoring schedule, are reported by a prospective randomised trial called ‘IRIS’, which enrolled 1106 newly diagnosed CML patients [24]. Previously, smaller-scale studies [11, 21, 22, 25] on patients treated with imatinib showed a strong correlation between the percentage of Ph+ metaphases in BM and PB BCR-ABL levels as measured by Q-PCR. When the QRT-PCR values in imatinib-treated patients were grouped according to the cytogenetic response categories, very little overlap was observed in the BCR-ABL levels within the cytogenetic categories among patients who had simultaneous blood Q-PCR and bone marrow cytogenetics. In all these studies it is too early to understand what level of molecular response can be used as an indication of long term disease control and more research is needed before we can conclude whether ‘molecular responses’ are of long term prognostic value in terms of cytogenetic response in imatinib-treated patients.

In this study we have conducted a quantitative analysis and serial comparison between peripheral blood and bone marrow sample. We demonstrate that the Q-PCR BCR-ABL assay on blood samples provides information of similar quality and value to that obtained by the assay performed on bone marrow samples. The results obtained in blood are informative both at the level of the cytogenetic response that could be obtained and in their predictive clinical value. The difference in amounts of neoplastic transcript before therapy in BM versus PB was statistically significant (P <0.001), reflecting either the higher percentage of myeloid precursor in BM than in PB or the smaller amount of lymphocytes in BM, essentially after
separation of blood on Ficoll-Hypaque gradient. We found that BM precursors (CD34+ and CD33+ cells) have a larger amount of BCR-ABL transcript compared to more mature myeloid cells (data not shown). Furthermore, it has been demonstrated that PB has a larger amount of β2M compared to BM after Ficoll-Hypaque gradient separation [18].

An important issue is how these CML patients can be clinically observed and monitored during therapy. Other authors have reported that early reduction of BCR-ABL transcript levels is predictive of the subsequent cytogenetic response in chronic phase patients [11, 22, 25]. We demonstrate that the patients who obtained a more than three log reduction in BCR-ABL transcript after 26 or 57 days of imatinib therapy are more likely to obtain a major cytogenetic response.

Finally, the frequency of molecular responses was assessed in our study; The terms 'PCR negative' and 'complete molecular response' should be used with caution. They imply an absolute lack of measurable leukemia, which may be misleading. There is inherent variability in the sensitivity of QRT-PCR and nested PCR assays between laboratories and between samples. Using the current technology, a sensitivity of more than 4.5 logs below baseline can usually be achieved. As in the IRIS study, a BCR-ABL level 4.5 logs below baseline was defined as the maximum measurable response in our study and the cycle threshold of 38 cycles for BCR-ABL is the maximum measurable level of disease.

In our study major molecular response, defined as a three or more log decrease below baseline value, was achieved in 39% of late chronic phase CML patients after 12 months of imatinib therapy. There is a stable downward trend in median BCR-ABL levels for patients in CCgR and the number of patients achieving a 4.5 log response increased during the second year of imatinib therapy, as reported also by other authors [26]. The follow-up results of patients with chronic-phase CML after IFN-α failure treated with imatinib mesylate remain very encouraging. We analyzed the proportion of CCgR patients losing the complete cytogenetic response and we observed that it was about 10% per year but only 3% in those who obtain a four log reduction in amount of BCR-ABL transcript in BM. Our results suggest that two consecutive assay with five-fold increase predict the loss of CCgR. This information is very important in monitoring strategies. While it may be reasonable to maintain imatinib therapy at the standard dose for patients in CCgR, it would be useful to formally assess higher doses of imatinib or combination therapy in patients where leukemia remains detectable at the molecular level and is not progressively declining. In conclusion, on the basis of the accuracy and sensitivity of QT PCR for measuring BCR-ABL levels in peripheral blood and in bone marrow, we propose this method as the method of choice for monitoring patients on imatinib therapy. QRT-PCR studies may be able to identify degrees of molecular response that predict both complete cytogenetic response and long term stability, as well as patterns of response that provide an early indication of relapse and imatinib resistance.

working party members

The following members of the GIMEMA Working Party on CML actively participated in this study: G. Lucarelli and G. Polimeno (Acquaviva delle Fonti); P. Galieni and C. Bigazzi (Ascoli Piceno); V. Liso (Bari); V. Zampaglione (Biella); P. Coser, and R. Quaini (Bolzano); E. Abruzzese (Roma); M. Gobbii and M. Miglino (Genova); E. Pogliani, C. Gambacorti Passerini and M. Micolis (Monza); M. Lazzarino and E. Orlandi (Pavia); P. Bernasconi and R. Invernizzi (Pavia); R. Fanin and M. Tiribelli ( Udine); D. Russo and M. Malagola (Brescia); G. Alimena and M. Breccia (Roma); G. Rossi and A. Capucci (Brescia); F. Nobile, M. Martino and E. Oliva (Reggio Calabria); L. Gugliotta and P. Avanzini (Reggio Emilia); P. Fattori (Rimini); G. Leone and S. Sica (Roma); L. Annino (Roma); M. C. Petti (Roma); E. Montefusco and E. Conte (Roma); A. M. Carella (Genova and San Giovanni Rotondo); M. Longinotti and S. Pardini (Sassari); E. Gottardi, M. Fava (Orbassano); L. Cavanna, D. Vallisa and E. Trabacchi (Piacenza); A. Bacigalupo (Genova); B. Rotoli, B. Izzo and L. Luciano (Napoli); F. Ferrara and E. Schiavone (Napoli); V. Mettivier (Napoli); A. Tabilio, C. Mecucci and D. Falzetti (Perugia); G. Visani and G. Nicolini (Pesaro); T. Barbui, U. Giussani and R. Bassan (Bergamo); V. Rizzoli and L. Mangoni (Parma); M. Bocchia and F. Lauria (Siena); E. Volpe and F. Palmieri and N. Cantore (Avellino); M.C. Michieli (Aviano); S. Amadori and A. Cantonetti (Roma); A. Levis, and M. Pini (Alessandria); E. Angelucci and E. Usala (Cagliari); A. Cuneo and G.L. Scarafani (Ferrara); E. Curioni and F. Radaelli (Milano); R. Marasca and G. Leonardi (Modena); E. Morra and E. Pungolino (Milano); V. Montefusco (Milano); A. Peta and F. Iuliano (Catanzaro); P. Leonzi and S. Rupoli (Ancona); A. Bosi and S. Santini (Firenze); R. Giustolisi and F. Stagno and P. Guglielmo (Catania); F. Porretto (Palermo); A. Liberati and E. Donti (Perugia); E. Zuffa and B. Giannini (Ravenna); P. Mazza and M. Cervellera (Taranto); D. Ferrero and C. Della Casa (Torino); M. Candela and G. Danieli (Ancona); S. Morandi and C. Bergonzi (Cremona); A. Gabbas and D. Noli (Nuoro); G. Semanzato and L. Trentin ( Padova); S. Mirto, S. Tringali and D. Turri (Palermo); V. Abbadessa, G. Marini and Caracciolo (Palermo); M. Musso and F. Porretto (Palermo); A D‘Emilio ( Vicenza); A. Bonati ( Parma); M. Petrini, F. Parineschi and R. Fazzi (Pisa); F. Ricciuti and M. Pizzuti (Potenza); E. Callo and P. Pregno ( Torino); F. Ghelrizonzi and C. Tecchio (Treviso); A. Ambrosotti and V. Meneghini (Verona); R. Di Lorenzo and G. Fioritoni (Pescara); G. Quarta, and M. Girasoli (Brindisi); E. De Biasi (Castelfranco Veneto); M. Monaco and E. Capussela (Foggia); A. Gallamini and M. A. Pistone (Cuneo); A. De Blasio (Latina); C. Musolino (Messina); S. Bassi, A. Poirer, S. Luatti, C. Nicci, E. Montanari, G. Marzocchi, F. Buontempo, T. Grafone, S. Colarossi and M. Renzulli (Bologna).

acknowledgements

Supported by COFIN 2003 (Molecular therapy of Ph-positive leukemias), by FIRB 2001, by the University of Bologna (60% grants), by the Italian Association for Cancer Research (AIRC), by the Italian National Research Council (CNR), and by grants from the Campania Region, Fondazione del Monte di Bologna e Ravenna and AIL.
references

20. Martinei G, Amabile M, Giannini B et al. Novel types of bcr-abl transcript with breakpoints in BCR exon 8 found in Philadelphia positive patients with typical chronic myeloid leukemia retain the sequence encoding for the DBL- and CDC24 homology domains but not the pleckstrin homology one. Haematologica 2002; 87: 688–694.