Treatment of Philadelphia-Positive Chronic Myeloid Leukemia with Imatinib: Importance of a Stable Molecular Response

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Abstract

Purpose: The achievement of a major molecular response (MMoR) at 12 months is a surrogate marker of progression-free survival in chronic myeloid leukemia patients treated with imatinib.

Experimental Design: We evaluated the prognostic value of the long-term evolution of the molecular response based on a retrospective analysis of 130 late chronic phase chronic myeloid leukemia patients who achieved a complete cytogenetic response (CCgR) with 400 mg/d imatinib and have now a median follow-up of 72 months (range, 48-77).

Results: In 71 (55%) patients, molecular response was consistently major (stable MMoR); in 19 (15%) patients, molecular response was occasionally less than major (unstable MMoR); in 40 (30%) patients, MMoR was never achieved (never MMoR) during all the course of CCgR. Patients with stable MMoR had a longer CCgR duration and a significantly better progression-free survival compared with patients with absent or unstable MMoR. The achievement of a MMoR, if maintained continuously, conferred a marked long-term stability to the CCgR: patients with a stable MMoR have a significantly lower risk of losing the CCgR than patients with unstable and never MMoR (4% versus 21%, P = 0.03, and 4% versus 33%, P < 0.0001, respectively). Finally, if a MMoR is not maintained consistently, the risk of losing the CCgR is higher but not significantly than if it is never achieved (33% versus 21%, P = 0.5).

Conclusions: These data confirm that achieving a MMoR is prognostically important but point out that the prognostic value of achieving a MMoR is greater if the response is confirmed and stable.

Imatinib mesylate (Glivec; Novartis Pharma) is the standard frontline treatment of Philadelphia-positive chronic myeloid leukemia (CML; refs. 1, 2). The complete cytogenetic response (CCgR) rate ranges between 70% and 90% in early chronic phase (3–5) and between 40% and 60% in late chronic phase (6–9); complete cytogenetic responders have a significantly greater benefit in terms of overall survival (OS) and progression-free survival (PFS) if compared with patients failing to achieve a CCgR. The quantification of residual Bcr-Abl transcripts in patients in CCgR by quantitative reverse transcription-PCR (QRT-PCR) is a sensitive tool to determine minimal residual disease and to monitor the response (10–12). Better molecular responses correlate with improved outcome and an early major molecular response (MMoR) may have additional prognostic significance. Achievement of a MMoR within 12 months of imatinib therapy was associated with the best PFS (13, 14). However, other studies have not confirmed a significant difference in OS and PFS in complete cytogenetic responders in function of the level of molecular response achieved (15). Moreover, besides the prognostic value of an early MMoR, the significance of the fluctuations of the MMoR during the course of a stable CCgR is yet to be fully defined, even due to the variability still innate in the QRT-PCR evaluation of minimal residual disease in CML (11). For these reasons, the European LeukemiaNet guidelines (16) do not consider as a failure but a simple “warning” the detection of “any increase” in the Bcr-Abl transcript levels.

The endpoints of the present work are to clarify (a) whether the long-term stability of the MMoR, besides its early achievement, might help to identify, among complete cytogenetic responders, those eventually candidates to lose more frequently the CCgR and (b) if according to the level and the trajectory of the MMoR (stable, unstable, or never achieved) is

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Translational Relevance

In chronic myeloid leukemia patients who achieve a complete cytogenetic response (CCgR) to imatinib, monitoring the molecular response is a sensitive tool to determine minimal residual disease and an early achievement of a major molecular response correlates with prolonged survival.

However, in clinical practice, molecular monitoring is continuous and Bcr-Abl transcript levels are frequently subjected to fluctuations, whose clinical effect is difficult to determine.

We have analyzed the long-term evolution of the molecular response of 130 chronic myeloid leukemia patients who achieved a CCgR with 400 mg/d imatinib. According to the trajectory of the molecular response during a 6-year follow-up, patients were divided in three categories (stable, unstable, and never major molecular response) characterized by significantly different risks of CCgR loss and by different long-term outcomes.

This may be a useful tool helping the clinician decide how to manage the patient in CCgR with unstable or unsatisfactory molecular response.

Materials and Methods

The patients on study have been enrolled in a phase II multicentric prospective trial (CML/002/STI571), which was promoted by the Italian Cooperative Study Group on CML, between July 2000 and June 2001. The outline of the study, inclusion criteria, and response definitions have been reported previously (17). Two hundred seventy-two patients were eligible for this analysis. Patients were equally distributed by age, sex, and duration of the chronic phase before imatinib treatment.

NOTE: A total of 277 patients were treated with imatinib after failure of IFN-α. One hundred fifty-three patients obtained a CCgR. One hundred thirty patients were excluded because three molecular tests were not available.

Cytogenetic analysis was done by standard banding techniques before treatment, at 3-month interval for the first 12 months, and at 6- to 12-month intervals thereafter; the CCgR was defined as absence of Philadelphia-positive metaphases (at least 20 metaphases analyzed).

Molecular analysis was done in three laboratories (Bologna, Turin, and Naples) during the first year of imatinib treatment (17) at 3-month intervals, analyzing bone marrow and peripheral blood samples; afterwards, peripheral blood samples were collected from all participating centers and sent to the laboratory of Bologna University Hospital at 6- to 12-month intervals. Molecular response was assessed by a standardized QRT-PCR method on an ABI PRISM 7700 Sequence Detector (Perkin Elmer). The three laboratories have been involved previously in the European Union concerted action aimed at standardizing the protocols for QRT-PCR, and the interlaboratory reproducibility was high, with a coefficient of variation of 11% for BCR/ABL mRNA (17). Results were expressed as a ratio of BCR-ABL to the housekeeping gene (%; ref. 18), which was β2-microglobulin from 2000 to December 2003. In January 2004, β2-microglobulin was substituted with ABL. To transform BCR-ABL/β2-microglobulin data into BCR-ABL:ABL data, from January to April 2004, 50 samples were assessed in duplicate; the BCR-ABL/β2-microglobulin ratio was plotted against the BCR-ABL:ABL ratio, and the slope of the linear regression equation was used to derivate the estimated BCR-ABL:ABL ratios, applying the formula: BCR-ABL:ABL ratio = 57.74 × BCR-ABL/β2-microglobulin ratio (19).

MMoIR was defined as a ratio Bcr-Abl:Ab < 0.05%, which corresponds to a 3-log reduction from the median baseline value calculated in our laboratory. Undetectable Bcr-Abl transcript levels were defined as a ratio Bcr-Abl:Ab < 0.001%, corresponding to the lowest level of detectability of the method (10^−9), whereas a complete molecular response required an undetectable BCR-ABL transcript level confirmed by negative nested PCR, whose lowest limit of detectability is 10^−7.

Statistics. The duration of the CCgR was calculated by the product-limits method of Kaplan-Meier (20), with 95% confidence interval (95% CI), from the date of the first CCgR to the date of CCgR loss or of last cytogenetic evaluation, whichever came first. The Kaplan-Meier (20) method was also used to calculate the OS from the date of first imatinib dose to the date of death or last contact, whichever came first. PPS was calculated by the same method from the time of first imatinib intake to the first documentation of accelerated phase/blast crisis. For patients without progression to accelerated phase or blast crisis, time to progression to accelerated phase/blast crisis was censored at last contact. Differences were measured using the log-rank test (21). The

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NOTE: A total of 277 patients were treated with imatinib after failure of IFN-α. One hundred fifty-three patients obtained a CCgR. One hundred thirty patients were eligible for this analysis. Patients were equally distributed by age, sex, and duration of the chronic phase before imatinib therapy among the three groups.
Fisher's exact test and the χ² test were employed for comparisons between groups, as appropriate. All P values were two-sided and the significance level for all statistical tests was 0.05.

Results and Discussion

The 130 patients evaluated (Table 1) were 59% males; median age at starting imatinib was 49 years (extremes, 18-78 years). The median follow-up is 72 months (range 48-77 months). Three of these patients had achieved a CCgR to IFN-α and received imatinib after CCgR loss. All patients received 400 mg imatinib, with the exception of 5 (4%) patients who escalated to 800 mg, because the CCgR had not yet been achieved, at very different time points ranging from 24 to 60 months. Compliance to 400 mg imatinib was good (ranging between 90% and 100%). Overall, 20 of 130 (15%) patients have lost the CCgR; 9 (7%) patients died and 6 (5%) progressed to the accelerated phase/blast crisis. Patients were considered altogether and divided, based on the trajectory of the molecular response, into three categories (Table 1):

**Category 1: Stable MMolR.** In 71 (55%) patients, the molecular response was always at least major. Bcr-Abl transcript levels resulted always <0.05% after the achievement of the CCgR and always fluctuated below this limit. MMolR was the stable and best result obtained in 42 of 71 (59%) patients but became occasionally undetectable in 28 of 71 (39%) patients. One patient had a stable undetectable molecular response throughout the follow-up. Patients with progressively decreasing levels of Bcr-Abl transcript were included in this group if the MMolR, once obtained, was confirmed in at least two consecutive tests and was never lost in the subsequent evaluations. The 3 patients who had previously achieved a CCgR to IFN-α belong to this group.

**Category 2: Unstable MMolR.** Nineteen (15%) patients achieved a MMolR (Bcr-Abl:Abl < 0.05%) in at least two evaluations, but in some occasions the Bcr-Abl transcript levels were >0.05%. Bcr-Abl transcript levels fluctuated below and above the MMolR limit. However, the increase in transcript levels ranged between 0.5 and 1.0 log with respect to the MMolR (the molecular response fluctuated between 0.05% and 0.05% in one patient; in another patient, molecular response fluctuated between 0.00% and 0.01%).

**Category 3: Never MMolR.** In 40 (30%) patients, the molecular response was always less than major (Bcr-Abl transcript levels resulted always >0.05%). In these patients, Bcr-Abl transcript levels were very variable (ranging from 0.08% to 1.6%) but always >0.05%. Patients with a MMolR not confirmed in at least two evaluations were included in this category.

For each category, it was possible to derive different probabilities of maintaining the CCgR and different long-term outcomes. Compared with the categories of patients with unstable and never MMolR, patients with stable MMolR showed a better outcome in terms of duration of the CCgR, OS, and PFS. Figure 1A shows the Kaplan-Meier estimated probability of maintaining the CCgR after stratification according to QRT-PCR categories. Patients who achieved a stable MMolR had a significantly longer CCgR duration than patients with unstable (95.5%; 95% CI, 91-100% versus 77%; 95% CI, 56-96%; P = 0.008) or never MMolR (62%; 95% CI, 51-73%; P < 0.0001). A sustained MMolR was also predictive for a better OS (97%; 95% CI, 96-100% versus 84%; 95% CI, 73-95% in
patients with never MMolR; \( P = 0.014 \); and a better PFS (100% versus 90%; 95% CI, 83-100% in patients with unstable or never MMolR; \( P = 0.006 \); Fig. 1C).

The outcomes of patients belonging to categories 2 and 3 resulted not significantly different in terms of risk of losing the CCgR (Table 2): the CCgR was maintained by 79% of patients with unstable MMolR and by 67% of patients without a MMolR (\( P = 0.2 \)), and both groups showed comparable PFS rates (89%; 95% CI, 75-100% in patients with unstable MMolR versus 90%; 95% CI, 85-100% in patients who never achieved a MMolR; \( P = 0.9 \); Fig. 1C).

Mutational analysis was available at the time of CCgR loss in 5 of 20 patients, who resulted to be wild-type, except for 1 patient, positive for the T315I mutation.

To date, the majority of patients treated with imatinib in early chronic phase achieve a CCgR and most of the complete cytogenetic responders achieve also a MMolR. In our experience, a relevant proportion (30%) of late chronic phase patients achieved a durable CCgR without obtaining also a MMolR until relapse or last follow-up. In the largest published experience, dealing with patients treated in early chronic phase (4), the level of the molecular response obtained during the first 12 months on imatinib is used as surrogate marker of long-term outcome: complete cytogenetic responders obtaining a >3-log reduction of BCR-ABL transcripts (corresponding to a MMolR) have the best long-term outcome (100% PFS), whereas those patients not achieving a MMolR have a 95% long-term PFS, a difference statistically significant but probably clinically less relevant. Early surrogate markers of long-term outcome are by sure important. However, once the MMolR has been achieved, the long-term evolution of such result is also relevant, because it may give useful information for the practical management of these patients. Little data are currently available on the durability of the molecular response and the clinical effect of the fluctuations of the response. Hughes et al. (11) reported a frequency of confirmed MMolR of 72% in a subset of early and late chronic phase patients, but follow-up was short and no analysis of the association between the stability of the molecular response and the duration of the CCgR was done. However, the absence of a MMolR or a significant (at least 10-fold, 1 log) increase of transcript levels resulted associated with a higher incidence of cytogenetic relapse.

We show that late chronic phase patients, who obtained a stable CCgR during imatinib treatment, could be stratified, after a prolonged period of observation, into three categories based on the trajectory of the molecular response. Fifty-five percent of them obtained a stable MMolR, whereas 15% showed an unstable MMolR and 30% never achieved a MMolR. Even in this setting of late chronic phase patients, a sustained MMolR conferred to the CCgR a high level of stability, with a negligible risk of losing the response (4%). On the other hand, a “fluctuating” or a constantly absent MMolR translated into a significantly higher probability of CCgR loss [17 of 59 (29%) patients]. The long-term observation of these two categories of patients with unstable or unsatisfactory molecular response do not show yet a marked outcome difference, as expected, being all these patients highly sensitive to imatinib with a slow propensity to progression to accelerated phase/blast crisis even after having lost the CCgR. However, the PFS curve (Fig. 1C) was significantly better for the “sustained MMolR” category versus the “unstable and never MMolR” (100% versus 90%; \( P = 0.006 \)). All these differences were not due to a lead-time bias, as patients in all groups were followed for a similar period (Table 1). Iacobucci et al. (7) reported the long-term outcome of 454 late chronic phase patients, treated with 400 mg imatinib after IFN-\( \alpha \) failure, 57% of which obtained a CCgR. The 6-year PFS (12-month landmark analysis) of complete cytogenetic responders was 88%. No data concerning the long-term QRT-PCR-based follow-up were provided. In our experience (8), the PFS rate of all 153 complete cytogenetic responders (not a 12-month landmark but patients with confirmed CCgR) resulted to 90%, similarly to the results reported by Hochhaus et al.

However, thanks to the prospective molecular monitoring implemented in our trial, it was possible to further stratify the patients according to the level and the evolution of their molecular response between patients with stable and durable responses (100% long-term PFS) and those with less stable responses (90% PFS). Most of these molecular results have been generated in the past 6 years and we cannot rule out that some of the “fluctuations” observed were originated by the variability of the results of the molecular evaluation. However, the not different long-term outcomes of complete cytogenetic responders who showed molecular fluctuations and those with consistently high levels of Bcr-Abl transcript may suggest that their residual leukemic burdens are comparable in size and in propensity to progression. It should be highlighted that most of the fluctuations observed were increments of 0.5 or 1 log of Bcr-Abl transcript levels. The reproducibility of the molecular response investigated by QRT-PCR in CML is not optimized across different laboratories around the world and the risk of therapeutic overreactions following an increment of Bcr-Abl transcript levels is real and recently underscored (13). These “fluctuations” must be evaluated on a patient basis and the decision-making process must be supported by (conventional and fluorescence in situ hybridization) cytogenetic data, imatinib blood level testing, study of the mutational status, and, more importantly, an early confirmation of the “suboptimal” level of molecular response (no clinical decision should be based on a single QRT-PCR determination). On the other

| Table 2. Proportion of patients who lost the CCgR according to QRT-PCR categories |
|----------------------------------|------------------|------------------|------------------|------------------|
| All patients \((n = 130)\)       | Patients with stable MMolR \((n = 71)\) | Patients with unstable MMolR \((n = 19)\) | Patients with never MMolR \((n = 40)\) |
| No. (%) patients who lost the CCgR | 20 (15)          | 3 (4)            | 4 (21)           | 13 (33)          |

NOTE: Four percent of patients with stable MMolR lost the CCgR (versus 21% with unstable MMolR; \( P = 0.01 \) and versus 33% of patients with never MMolR; \( P = 0.001 \), Fisher’s exact test).
hand, a steady MMoR is a reliable tool to individuate patients with a persistent long-term stability of their response, even if treated in late chronic phase.

In conclusion, this observation of the molecular data of complete cytogenetic responders in the long-term suggests that patients who achieve a stable MMoR have a higher probability of maintaining a stable CCgR, which correlates with better PFS and OS rates. On the other hand, a patient whose CCgR is not supported by a stable MMoR deserves a close monitoring and, in all probability, a different imatinib schedule (dose escalation) or a second-generation TKI treatment.

References


Disclosure of Potential Conflicts of Interest

M. Baccarani and G. Saglio have received commercial research grants from Novartis. F. Pane has received commercial research grants from Novartis, Bristol-Myers Squibb, and Roche. G. Saglio is a member of an advisory board for Novartis and Bristol-Myers Squibb. R. Gianantonia and G. Rege-Cambrin have received honoraria from Bristol-Myers Squibb and Novartis.

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