Complete Clearance of Ph+ Metaphases after 3 Months Is a Very Early Indicator of Good Response to Imatinib as Front-Line Treatment in Chronic Myelogenous Leukemia

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Key Words

Chronic myelogenous leukemia · Cytogenetics ·
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Abstract

Aim: To address the incidence and the prognostic role of a very early standard complete cytogenetic response (CCyR) or all Ph⁺ metaphases (MET⁺, when <20 cells were evaluable). **Methods:** We revised 182 chronic phase chronic myelogenous leukemia patients treated with frontline imatinib (IM) at two institutions from June 2002 to June 2011. **Results:** After 3 months of treatment, 138 patients (75.8%) achieved CCyR/MET⁺ while 44 patients (24.2%) still presented Ph⁺ metaphases (MET⁺) (<33%, 24 patients; ≥33%, 20 patients). On univariate analysis, palpable spleen enlargement ($p < 0.001$), WBC count $>100.0 \times 10^9/l$ at onset ($p < 0.001$), and male gender ($p = 0.019$) had a negative impact on achievement of CCyR/MET⁺ at 3 months. Among patients with CCyR/MET⁺ after 3 months, there were 15 failures (10.8%) com-

pared to 21 (47.7%) among patients with MET⁺ ($p < 0.001$). The 5-year overall survival was 97.0% in patients CCyR/MET⁺ at 3 months and 91.8% in patients MET⁺ at 3 months ($p = 0.277$); the 5-year progression-free survival was 88.2% in patients CCyR/MET⁺ at 3 months and 48.4% in patients MET⁺ at 3 months ($p < 0.001$). **Conclusions:** The achievement of CCyR/MET⁺ at 3 months seems to have prognostic relevance and could be a very early and useful indicator of an excellent response to IM beyond European LeukemiaNet guidelines.

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Introduction

The advent of imatinib (IM) completely changed the treatment and prognosis of patients with chronic myelogenous leukemia (CML) [1–3]. During IM treatment, achievement of a complete cytogenetic response (CCyR, Ph⁺ cells 0%) is a cornerstone towards optimal disease control and remains a crucial objective; it has been shown

in several studies (either randomized controlled trials or community-based evaluations) that CCyR is achievable in at least 70–80% of newly diagnosed CML patients receiving frontline IM treatment [4–6].

In addition, the time to CCyR achievement plays a major prognostic role in the follow-up of these patients; in the IRIS study, the faster the CCyR was the better the prognosis was [7], and this finding has been confirmed in several independent studies [8–10]. According to European LeukemiaNet (ELN) guidelines, CCyR should be achieved within the 12th month of IM treatment to define the response as ‘optimal’ [11].

It is worth noting that the worldwide accepted definition of CCyR requires the evaluation of at least 20 Ph⁺ metaphases; this threshold is used in clinical controlled trials, but in day-to-day clinical practice the evaluation is often performed on a lower number of metaphases, especially at the 3-month time point. The role and the prognostic value of Ph negativity evaluated on less than 20 metaphases (MET[−]) is still unclear and no comparison exists with the commonly defined CCyR.

The introduction of 2nd generation tyrosine kinase inhibitors (TKIs) (with a faster action than IM) in frontline CML therapy will probably lead to a revision of the ELN guidelines [12, 13]; it is conceivable that physicians will look for earlier and deeper objectives to achieve than those of ELN guidelines. Moving from this concept, Marin et al. [14] very recently emphasized the value of a single measurement of BCR-ABL transcript to discriminate, after 3 months of IM treatment, which patients would have an optimal or a poorer follow-up.

With a similar aim, we revised 182 chronic phase CML patients consecutively treated with frontline IM at two different institutions in Italy from June 2002 to June 2011, who had karyotype analysis after 3 months of treatment, to address the incidence and the prognostic role of a very early achievement of CCyR/MET[−].

Patients and Methods

We retrospectively evaluated all patients with Ph⁺ CML diagnosed at our two institutions with the following characteristics:

- early chronic phase (diagnosed less than 12 months prior);
- no prior treatment, apart from hydroxyurea (HU) given for less than 3 months to reduce the white blood cell (WBC) count;
- IM as first-line treatment.

Cytogenetic analyses were performed on bone marrow aspirates by chromosome standard G or Q banding techniques in all available metaphases from direct or short-term (24–48 h) cultures at diagnosis, after 3, 6, and 12 months of therapy with IM, and every 6 months thereafter.

Fluorescence in situ hybridization (FISH) on interphase cells was employed at 3 months if less than 5 metaphases were evaluable and it was performed with BCR-ABL extra-signal, dual-color, dual-fusion probes; the condition of MET[−] was defined when the number of positive marrow cell interphase nuclei was less than 2 of 200 (<1%).

Real-time quantitative polymerase chain reaction (RT-Q-PCR) to assess BCR-ABL transcript levels was performed according to suggested procedures and recommendations [15] and the results are expressed as the BCR-ABL/ABL ratio.

The condition of MET[−] at 3 months was defined when at least 5 metaphases were available and all were negative. In addition to the definition of MET[−], cytogenetic and molecular responses were categorized according to standard criteria: CCyR was defined as the presence of 100% Ph[−] metaphases in at least 20 metaphases, major cytogenetic response (MCyR) as the presence of >66% Ph[−] metaphases in at least 20 metaphases, and major molecular response (MMoR) as a BCR-ABL/ABL ratio <0.1.

Hematological and extra-hematological toxicities were graded according to the ECOG scale; for the purposes of present study, only severe toxicities (grade 3–4) were considered.

Any of the following events was considered as a failure: primary hematologic or cytogenetic resistance as defined by ELN guidelines, cytogenetic or molecular relapse, and evolution to the accelerated/blastic phase. The lack of MMoR after 18 months of IM treatment in a patient with stable CCyR was not considered a failure.

Data were expressed as means $\pm$ SD for normally distributed data, as medians and interquartile ranges (IR) for not-normally distributed data, or as percentage frequencies; comparisons between groups of patients were made by paired t test and χ^2 test, as appropriate; $p < 0.05$ was considered statistically significant. The survival curves were calculated using the Kaplan-Meier method. Overall survival (OS) was calculated from the date of diagnosis to death irrespective of the cause. Progression-free survival (PFS) was calculated from the date of diagnosis to any type of failure, as previously defined. The OS and PFS between different groups were compared using the log-rank test.

All calculations were made using a standard statistical package (SPSS for Windows, version 15.0; Chicago, Ill., USA).

Results

On the whole, 207 consecutive newly diagnosed CML patients in the chronic phase were treated with frontline IM at our two institutions from June 2002 to June 2011. Among these patients, 25 did not have evaluable cytogenetic data after 3 months of treatment: in particular, karyotype was not performed due to the physician's decision in 11 patients aged more than 75 years or due to patient refusal in 5 cases, while 9 additional patients failed both standard karyotype and FISH analysis. The remaining 182 patients (male/female ratio 92/90, median age 55.3 years, IR 41.3–67.4) had an evaluable karyotype/FISH analysis after 3 months of treatment and were considered in the present study.

Table 1. Clinical features at onset

	All patients	Rome	Pesaro	p
Male/female ratio	92/90	76/79	16/11	0.327
Median age at diagnosis, years	55.3	57.0	51.9	0.217
IR	41.3–67.4	41.7–68.4	36.4–62.1	
Sokal risk, %				
Low	50.5	49.0	59.2	
Intermediate	41.3	44.6	22.3	
High	8.2	6.4	18.5	0.035
Median WBC at diagnosis, $\times 10^9/l$	75.4	69.3	81.4	0.171
IR	35.4–125.5	35.0–121.2	38.4–150.0	
Median PLTs at diagnosis, $\times 10^9/l$	399	403	300	0.342
IR	262–553	280–558	205–438	

At onset, the median WBC and PLT counts were $75.4 \times 10^9/l$ (IR 35.4–125.5) and $399 \times 10^9/l$ (IR 262–553), respectively. The Sokal risk score was low in 92 patients (50.5%), intermediate in 75 (41.3%), and high in 15 (8.2%). The main clinical features of the patients diagnosed in the two hematological centers are shown and compared in table 1; the only significant difference was the excess of Sokal high-risk patients in the cohort from Pesaro.

A short pretreatment phase (<3 months) with HU was administered to 147 patients (80.7%). The starting daily dose of IM was 400 mg in 162 patients (89.0%), 800 mg in 14 patients (7.7%), and 300 mg in the remaining 6 patients (3.3%).

After 3 months of IM treatment, 138 patients (75.8%) achieved CCyR/MET– while 44 patients (24.2%) still presented Ph+ metaphases (<33% in 24 patients and $\geq 33\%$ in 20 patients) at cytogenetic analysis and were classified as MET+.

Among patients with CCyR/MET– after 3 months, 3 different groups were recognized according to the number of available metaphases as follows:

- 53 patients with at least 20 evaluable and all negative metaphases, thus in CCyR according to the standard criteria (group A);
- 67 patients with >5 and <20 evaluable and all negative metaphases (group B);
- 18 patients with <5 evaluable metaphases, in whom MET– was assessed with standard FISH analysis (group C).

The different clinical features at onset of patients who did or did not achieve CCyR/MET– at 3 months are reported in table 2. In univariate analysis, factors with a negative prognostic impact on achievement of CCyR/MET– at 3 months were: palpable spleen enlargement

($p < 0.001$), WBC count $>100.0 \times 10^9/l$ ($p < 0.001$), and male gender ($p = 0.019$). On the contrary, age >65 years, Sokal risk score, PLT count $>500 \times 10^9/l$, pretreatment with HU, and initial dose of IM did not appear to affect early CCyR/MET– achievement. In multivariate analysis, only WBC count $>100.0 \times 10^9/l$ ($p = 0.021$) and male gender ($p = 0.049$) retained their negative prognostic role on CCyR/MET– achievement at 3 months.

A score system to predict CCyR/MET– achievement at 3 months was built with the variables having prognostic significance at multivariate analysis: one point each was given for the presence at onset of a WBC count $>100.0 \times 10^9/l$ and male gender. One hundred eighteen out of 143 patients with score 0–1 (82.5%) achieved CCyR/MET– at 3 months compared to 20/39 patients with score 2 (51.3%) ($p < 0.001$).

The incidence of hematological toxicity grade 3–4 according to the WHO scale was significantly higher ($p = 0.003$) in patients who were MET+ at 3 months; on the contrary, no difference was observed between the two groups with regard to extra-hematological toxicity (table 3). The different types of hematological and extra-hematological toxicities are also reported in table 3.

RT-Q-PCR was not routinely tested after 3 months of IM treatment and was thus available in only 63/182 patients: its median value in the 49 patients who achieved CCyR/MET– was 0.307 (IR 0.12–1.51) compared to 4.65 (IR 1.34–25.0) in the 14 patients who were MET+ ($p < 0.001$).

Among the 138 patients with CCyR/MET– at 3 months, 108 (78.2%) achieved also an MMolR in the subsequent period of IM treatment; furthermore, a subsequent MMolR was achieved by 40/53 patients in CCyR (75.4%) compared to 57/67 patients with MET– (85.0%) and 11/18 patients with MET– by FISH-negative evaluation (61.1%)

Table 2. Clinical features at onset according to the cytogenetic response at 3 months

	CCyR/MET– at 3 months	MET+ at 3 months	p
Male/female ratio	63/75	29/15	0.019
Median age at diagnosis, years	56.6	51.1	0.303
IR	42.8–67.1	36.7–68.4	
Sokal risk, %			
Low	52.1	45.4	0.299
Intermediate	41.4	41.0	
High	6.5	13.6	
Median WBC at diagnosis, $\times 10^9/l$	61.2	114.0	0.001
IR	32.8–109.8	50.6–191.7	
Median PLTs at diagnosis, $\times 10^9/l$	403	356	0.309
IR	266–554	237–525	
Spleen enlargement (yes/no)	48/90	29/15	<0.001
HU pretreatment (yes/no)	110/28	37/7	0.521

Table 3. Toxicity according to the cytogenetic response at 3 months

	CCyR/MET– at 3 months	MET+ at 3 months	p
Hematological toxicity (WHO grade 3–4)	10 (7.2%)	11 (25.0%)	0.003
Anemia	3	4	
Neutropenia	7	7	
Thrombocytopenia	1	6	
Extra-hematological toxicity (WHO grade 3–4)	22 (18.6%)	5 (11.4%)	0.426
Cutaneous toxicity	5	1	
Pleural effusion	1	1	
Acute myocardial infarction	3	1	
Diarrhea	2	1	
Ocular toxicity	3	–	
Liver toxicity	3	1	

($p = 0.075$). In the whole CCyR/MET– group there were 15 failures (10.8%) during the subsequent follow-up (9 cytogenetic relapses, 5 molecular relapses, and 1 evolution to sudden blastic phase at 12 months). At the last follow-up, 4 patients had died (1 from disease progression and 3 from unrelated causes) and 134 were still alive (121 in IM treatment and 13 in 2nd generation TKI treatment).

Among the 44 patients with MET+ at 3 months, 32 (72.7%) achieved a CCyR and 19 (43.1%) also achieved an MMolR during the subsequent period of IM treatment; in this group, on the whole, there were 21 failures (47.7%) during the subsequent follow-up (12 primary cytogenetic resistances, 7 cytogenetic relapses, and 2 molecular relapses). At the last follow-up, 3 patients had died (1 from

disease progression and 2 from unrelated causes) and 41 were still alive (24 in IM treatment and 17 in 2nd generation TKI treatment).

The differences in the incidence of MMolR and failures between patients with CCyR/MET– and MET+ were highly significant ($p < 0.001$ for both MMolR and failures).

The 5-year PFS was 88.2% (95% CI 82.2–94.2) in patients achieving CCyR/MET– at 3 months and 48.4% (95% CI 32.2–64.6) in patients with MET+ at 3 months ($p < 0.001$) (fig. 1). Among patients with CCyR/MET– at 3 months, there was no statistically significant difference when comparing the PFS curves of the 3 subgroups previously described ($p = 0.834$) (fig. 2). In addition, there was no difference in 5-year PFS between patients with score

Fig. 1. PFS according to the cytogenetic response at 3 months.

Fig. 2. PFS in CCyR/MET- patients at 3 months according to the number of evaluable metaphases.

Fig. 3. PFS according to the score at baseline.

0–1 and patients with score 2 at baseline ($p = 0.108$) (fig. 3).

The 5-year OS was 97.0% (95% CI 93.7–100) in patients with CCyR/MET[−] at 3 months and 91.8% (95% CI 82.8–100) in patients with MET⁺ at 3 months ($p = 0.277$) (fig. 4).

Discussion

Up to now, the frontline therapy of CML patients in the chronic phase has been based on IM; the recent introduction of 2nd generation TKIs in the treatment of newly diagnosed patients makes it more difficult to choose what drug to employ and in what patient.

Two randomized studies comparing IM with 2nd generation TKIs in frontline treatment have shown that the latter have a more potent and faster effect which translates into a better PFS but not into a longer OS [12, 13]; on the other hand, 2nd generation TKIs are far more expensive than IM and this gap will grow in the next years. In addition, the significant advantage of 2nd generation TKIs versus IM in terms of cytogenetic response rates

slowly but progressively decreased during the treatment follow-up [16, 17]; last but not least, about two third of patients treated with frontline IM in all reported cohorts had optimal long-term disease control and a survival similar to that of matched healthy subjects.

Taken together, these considerations emphasize the role of prognostic factors at diagnosis for selecting patients who are at higher risk and thus require a frontline or early switch to 2nd generation TKIs; unfortunately, apart from the Sokal score, there is still a lack of other worldwide recognized factors at onset.

A possible option could be to identify very early indicators of an optimal response to frontline IM in order to switch as early as possible to 2nd generation TKIs only in patients with a high risk of adverse events during follow-up. The time checkpoints for optimal response to IM still used according to ELN guidelines seem too late for this new issue, and many earlier targets for IM treatment are currently under investigation.

The achievement of CCyR before 12 months is the first crucial target of therapy according ELN guidelines [11]. Up to now, the 3-month CCyR has been seldom evaluated for its prognostic significance during IM treatment; thus,

Fig. 4. OS according to the cytogenetic response at 3 months.

in the real-life setting, in many centers it is not routinely assessed. Furthermore, standard criteria for CCyR require at least 20 evaluable metaphases while in our real-life experience karyotypic analysis at 3 months did not result in a sufficient number of available metaphases in many patients. In our experience, on the contrary, MET- after 3 months, evaluated in at least 5 metaphases or detected by FISH, was a rather common finding; as a consequence, in this study we tested the role of MET- achievement after 3 months as a very early prognostic indicator in addition to standard CCyR.

The present data seem to confirm this hypothesis, as patients who achieved CCyR/MET- at 3 months had a better follow-up with a significantly higher rate of MMolR during the subsequent treatment, a significant reduction of failures, and a significantly longer PFS (fig. 1). It is worth noting that in our cohort of patients there was no difference with regard to the rate of MMolR, the number of failures, and PFS between patients with

standard CCyR and patients with MET- in <20 metaphases (fig. 2).

On the contrary, OS was similar irrespective of the cytogenetic response at 3 months; this finding is probably due to the high efficacy of 2nd generation TKIs in rescuing patients failing IM in both groups.

The occurrence of CCyR/MET- could be related to a different pattern of cell kinetics compared to patients with MET+. In particular, it could be explained by a higher sensitivity of Ph+ cells to IM and/or a higher number of residual normal stem cells capable of regaining a selective advantage and repopulating the marrow in MET- patients. This latter explanation is in agreement with our data showing a significant correlation between a smaller tumor burden at diagnosis (lower WBC count and spleen size) and achievement of CCyR/MET-; in addition, a small number of residual normal stem cells at baseline could also explain the higher incidence of early hematological toxicity observed in MET+ patients.

Furthermore, in multivariate analysis we identified 2 features at baseline predictive of the achievement at 3 months of CCyR/MET⁻ in our cohort of patients; based on these features, we built a score system capable of identifying patients at lower (score 0–1) and higher risk (score 2). This score, which warrants further confirmation in larger and independent cohorts of patients, could allow physicians to shift the treatment choice between IM and 2nd generation TKIs from the 3rd month to diagnosis, although the 5-year PFS between patients with different scores was not statistically different.

Marin et al. [14] very recently reported in a unicentric study the value of BCR-ABL measurement after 3 months of IM treatment and identified the threshold of 9.96 as the most powerful predictor of an optimal response; they also reported that the predictive power of BCR-ABL measurement at 6 and 12 months was superior to CCyR assessment at 6 and 12 months [14]. However, the prognostic value of BCR-ABL measurement at 3 months was not compared with the prognostic value

of cytogenetic response at the same time point. Thus, at present no conclusion on the relative value of very early molecular versus very early cytogenetic assessment can be drawn.

Finally, about 10% of patients achieving CCyR/MET⁻ at 3 months had a failure during follow-up; such a subgroup of patients was too small in our cohort to allow clinical insight, but it is clear that prognostication with CCyR/MET⁻ at 3 months should be further refined employing a molecular threshold at 6 months.

In conclusion, the achievement of CCyR/MET⁻ at 3 months with IM treatment seems a more common result than expected, and in our experience it appears to have an important prognostic role. As a matter of fact, the achievement of complete clearance of Ph⁺ cells at 3 months could be a very early and useful indicator of an optimal response to IM beyond ELN guidelines, allowing early and effective identification of patients in whom more potent 2nd generation TKIs are not needed versus patients in whom a prompt switch might be appropriate.

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