Chromosomal abnormalities in Philadelphia chromosome–negative metaphases appearing during imatinib mesylate therapy in patients with newly diagnosed chronic myeloid leukemia in chronic phase

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The development of chromosomal abnormalities (CAs) in the Philadelphia chromosome (Ph)–negative metaphases during imatinib (IM) therapy in patients with newly diagnosed chronic myeloid leukemia (CML) has been reported only anecdotally. We assessed the frequency and significance of this phenomenon among 258 patients with newly diagnosed CML in chronic phase receiving IM. After a median follow-up of 37 months, 21 (9%) patients developed 23 CAs in Ph-negative cells; excluding –Y, this incidence was 5%. Sixteen (70%) of all CAs were observed in 2 or more metaphases. The median time from start of IM to the appearance of CAs was 18 months. The most common CAs were –Y and +8 in 9 and 3 patients, respectively. CAs were less frequent in young patients (P = .02) and those treated with high-dose IM (P = .03). In all but 3 patients, CAs were transient and disappeared after a median of 5 months. One patient developed acute myeloid leukemia (associated with –7). At last follow-up, 3 patients died from transplantation-related complications, myocardial infarction, and progressive disease and 2 lost cytogenetic response. CAs occur in Ph-negative cells in a small percentage of patients with newly diagnosed CML treated with IM. In rare instances, these could reflect the emergence of a new malignant clone. (Blood. 2007;110:2991-2995)

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Introduction

Chronic myelogenous leukemia (CML) is characterized by the fusion of the Abelson oncogene (ABL) from chromosome 9q34 with the breakpoint cluster region (BCR) on chromosome 22q11.2 manifested as a translocation t(9;22)(q34;q11.2) known as the Philadelphia chromosome (Ph).1,2 Imatinib mesylate (IM) is a potent and selective tyrosine kinase inhibitor that has become standard therapy for patients with CML.2 High rates of cytogenetic response are achieved in patients treated with IM.3-6 Responses are durable particularly among those who achieve major molecular response (MMR).7,8

During the course of treatment with interferon or IM, a small subset of patients develop chromosomal abnormalities (CAs) in the Ph-negative metaphases as patients respond to therapy.9-11 This is to be distinguished from clonal evolution (CE) that is characterized by the appearance of additional cytogenetic abnormalities in the Ph-positive cells.12 CE is considered a criterion of accelerated phase, although when it represents the only criterion of transformation it is associated with a better prognosis compared with other criteria of accelerated phase.13-15

The development of CAs in Ph-negative metaphases has not been systematically evaluated among patients receiving IM as first-line therapy for CML in chronic phase (CP). Herein, we report our experience in such patients to determine the frequency and significance of these abnormalities.

Patients, materials, and methods

From March 2001 to July 2005, 258 patients with newly diagnosed CML in early CP were treated with IM as first line of therapy at M. D. Anderson Cancer Center and constitute the focus of this analysis. All patients were treated in clinical trials approved by the institutional review board and signed informed consent according to institutional guidelines and in accordance with the Declaration of Helsinki. The patients here reported were treated in 3 different clinical trials for patients with newly diagnosed CMA in chronic phase, registered at http://clinicaltrials.gov (study IDs: NCT00048672, NCT00038649, NCT00050531). Two hundred eight patients (81%) were treated with high-dose IM (800 mg/d) and 50 patients (19%) were treated with standard-dose IM (400 mg/d). Eligibility criteria and pretreatment and follow-up studies were as previously described.3,4,16

All patients had a pretreatment cytogenetic analysis, fluorescent in situ hybridization (FISH), and real-time polymerase chain reaction (PCR). Conventional cytogenetic analysis was done in bone marrow cells using standard G-banding technique. At least 20 metaphases were analyzed and marrow specimens were examined on direct or short-term (24 hours) cultures. Marrow cells were analyzed by FISH using the LSI BCR/ABL dual color extra-signal probe according to the manufacturer’s instructions (Vysis, Downers Grove, IL). Conventional cytogenetic analysis (FISH when routine cytogenetic analysis was not analyzable) and PCR were repeated every 3 months for the first year, and every 6 months thereafter. Patients were followed for survival every 3 months.

Response criteria were as previously described.4 Cytogenetic remission was judged by standard cytogenetic analysis; FISH was used only when routine cytogenetic analysis was not analyzable (ie, insufficient metaphases). For the purpose of this analysis, MMR was defined as a BCR-ABL/ABL ratio of less than .05%.17

Differences among variables were evaluated by the chi-square test and Mann-Whitney U test for categoric and continuous variables, respectively. Progression-free survival was measured from start of IM therapy until progression to the accelerated phase or blast crisis of CML, or death from any cause during treatment. Survival probabilities were estimated by the

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Results

Patients’ characteristics

After a median follow-up of 37 months (range, 4-61 months), 21 patients (8%) developed 23 CAs in Ph-negative metaphases during IM treatment. By definition, this phenomenon is evaluable only among patients who achieve a cytogenetic response. Thus, 21 of 246 evaluable patients (9%) developed these abnormalities at some time during the course of therapy with IM. Two patients had 2 different abnormalities present at different times. The median follow-up after the appearance of abnormalities in the Ph-negative metaphases was 21 months (range, 9-51 months). Fifteen of these abnormalities (65%) have been observed only once (ie, 800 mg) (P = .025). Older age and treatment with standard-dose IM (compared with high-dose IM) were the only features associated with development of CAs in Ph-negative metaphases, both by univariate and multivariate analysis.

Outcome

CAs in Ph-negative metaphases were frequently transient. The median follow-up after the appearance of abnormalities in the Ph-negative metaphases was 21 months (range, 6-45 months). Fifteen of these abnormalities (65%) have been observed only once (7 involving one metaphase). The remaining 8 abnormalities have been observed in a median of 3 analyses (range, 2-5) over a median of 9 months (range, 6-24 months). In 18 patients (86%), the abnormalities disappeared spontaneously in subsequent analyses; the median time from first occurrence to the disappearance was 5 months (range, 3-6 months).

Among the 2 patients who had only a mCyR at the time the CAs were first identified, one, with a del (7q) improved his cytogenetic response to a CCyR. The second one (with −Y) lost his mCyR after 9 months of therapy and died of progressive disease after 36 months. Two patients in PCyR at the time abnormalities were identified (trisomy 8 in both) improved their cytogenetic response to CCyR. The remaining 2 patients with PCyR at the time these abnormalities occurred maintained their response at the last follow-up, 3 and 45 months after the detection of the abnormalities. One of the patients in CCyR at the time the additional abnormalities were noted (del 20q) lost his response to a PCyR. All other patients who had achieved CCyR remained in CCyR: 10 achieved a MMR and 4 undetectable transcript levels. In 3 patients (14%), the additional abnormalities persisted after a median of 13 months (range, 3-24 months).

After a median follow-up of 36 months (range, 6-58 months) from the initiation of IM therapy and 21 months (range,
6-45 months) from the detection of the new chromosomal abnormal-
ity, 18 of the 21 patients were alive: 3 of them with undetectable
transcript levels, 7 in MMR, 6 in CCyR, and 2 in PCyR. Three
patients died, 3, 6, and 36 months after the additional abnormality
was noted. One patient (with both low Sokal and Hasford index
treated with high-dose IM), with \( /H_11002 \), died in PCyR from a
myocardial infarction; one (with both low Sokal and Hasford index
treated with standard-dose IM) with monosomy 7 developed a
secondary acute myeloid leukemia (AML) and died from multiple
organ failure after allogeneic stem cell transplantation from a one
antigen-mismatched unrelated donor; and one (with high Sokal and
intermediate Hasford scores treated with high-dose IM), with
\( /H_11002 \), died of disease progression. Apart from the second patient who
died, there was no evidence of dysplasia or secondary leukemias in
any other patient at the time of or after the detection of these
abnormalities. The estimated 4-year progression-free survival
(PFS) and overall survival (OS) were 63% (41%-98%) and 80%
(61%-100%), respectively, for patients with CAs, compared with
88% (84%-94%) (\( P = .04 \); 95% CI) and 96% (92%-99%) (\( P = .02 \);
95% CI), respectively, for those without CAs (Figure 1A,B).

Although CAs are frequently seen in only one cell, these
abnormalities are usually considered clonal if they are present in at
least 2 metaphases. Thus, we analyzed the outcome of patients with
CAs in at least 2 metaphases and those where it was present in only
1 metaphase. Patients were divided in 3 groups: patients without
CAs (\( n = 237 \)), patients with CAs detected in at least 2 metaphases
by the time of this analysis (\( n = 15 \)), and those with CAs detected
in only 1 metaphase (\( n = 6 \)). Both groups with CAs had an inferior
outcome for PFS (\( P = .16 \)).

Since most abnormalities occur within 36 months, we then
repeated the overall analysis considering only patients who had at
least 36 months of follow-up. Twelve of 140 such patients (8.6%)
developed CA. The development of CAs was associated with a
worse PFS but not OS. The estimated 4-year PFS and OS were 63%
(40%–100%) and 89% (71%–100%), respectively, for patients with CAs (n = 12), compared with 88% (82%–94%) (P = .05; 95% CI) and 99% (97%–100%) (P = .22; 95% CI), respectively, for those without CAs.

Discussion

To our knowledge, this is the first systematic analysis of the occurrence of CAs in patients receiving IM as first-line therapy for CML. In this study, after a median follow-up of 37 months, CAs in Ph-negative metaphases occurred in 9% of patients with newly diagnosed CML treated with IM that responded to IM (3% if −Y excluded and only those with ≥ 2 abnormal metaphases considered). The development of CAs in Ph-negative metaphases has been recognized after treatment with interferon-alpha (IFN-α) and other agents.19–21 It has also been described in patients treated with IM, usually after failure of prior therapies. There are only anecdotal reports in the literature of the development of CAs in Ph-negative cells in patients receiving IM as the first line of therapy for CML.9,11

These abnormalities were clonal in 70% of instances and persisted (ie, in more than one analysis) in 38% of patients. The majority of the abnormalities reported in the current study have been reported in previous studies in patients who received IM after failing other therapies.21 As reported in patients who developed CAs on IM after IFN failure, the abnormalities were frequently transient. Although the abnormalities observed are reminiscent of those seen in patients with myelodysplastic syndromes (MDSs), only rarely have they evolved to MDS or AML.9,10,22 In our series, one patient (with monosomy 7) developed AML, but no evidence of dysplasia or acute leukemia was seen in any other patient. This abnormality is frequently associated with secondary MDS/AML23 and was previously reported by Bumm et al in 8 patients treated with IM after IFN failure, representing 17% of all patients analyzed. Two patients in that series developed MDS with one having a chromosome 7 abnormality.11 We recently reported on 2 additional patients who developed AML after IM and IFN therapy, who had monosomy 7 (and one more with abnormalities of the long arm of chromosome 5).22 Few other patients with monosomy 7 who failed other therapies prior to IM have been reported to evolve to AML. Thus, it would appear that the emergence of this abnormality would merit closer follow-up. Whether the emergence of this abnormality by itself warrants a change in management (eg, stem cell transplantation) is controversial at the present time. However, the rate of development of AML or MDS is still very low, and the recognition of this abnormality by itself may not necessarily merit change of therapy in most patients. Interestingly, despite the rare occurrence of AML, the PFS and OS of patients with CAs in Ph-negative metaphases were significantly inferior, emphasizing the need for continued monitoring of patients with a full cytogenetic analysis.

Loss of chromosome Y was particularly common in this series, representing 39% of all abnormalities identified. The significance of −Y in this setting is unclear. It has been reported that this phenomenon is a common occurrence in male individuals with aging, where up to 6% may at some time develop −Y.24 However, Wiktor et al reported that this abnormality may be identified significantly more frequently among patients with hematologic malignancies, suggesting that there might be an element of genetic instability in these patients that may favor the loss of the Y chromosome.24 However, even if it is somehow associated with hematologic abnormalities, it may not have adverse clinical significance. Excluding this abnormality, trisomy 8 was the most frequent CA, occurring in 3 patients, and it was transient in all. This abnormality had no clear clinical significance in the current study. This is similar to what has been reported in patients treated with IM after interferon therapy.9 Feldman et al reported similar outcome in 5 patients developing trisomy 8 abnormalities.25 This appears to be in contrast to the poor outcome frequently reported for patients with blast-phase CML, de novo AML, and MDS with trisomy 8.26

The cause of these abnormalities is unclear. In prior studies, they have been reported in patients treated with IM after failure of prior therapy, suggesting these might be IM related.21 However, since these abnormalities have been observed also after therapy with IFN, it is unlikely that these are significantly linked to IM. Some reports also suggested that prior exposure to anthracyclines is a risk factor for the development of these abnormalities. However, our series shows that these changes can occur in patients who have never been exposed to other therapies. Another hypothesis is that this phenomenon could be a manifestation of multistep pathogenesis in CML,27 where the Ph-positive clone derived from an original Ph-negative stem cell clone, and is an additional “hit” for malignant transformation. Occasional cases of Ph-negative CML acquiring the Ph-positive clone later during the course of the disease have been reported.28

As IM specifically eradicates cells expressing the Ph chromosome and its tyrosine kinase, the appearance of abnormalities in Ph-negative cells may unmask some of these cells that express only the “first hit,” or a fragile normal stem cell with genetic instability that is susceptible to manifest clonal or nonclonal chromosomal abnormalities.

In conclusion, cytogenetic abnormalities occur in Ph-negative cells in a small fraction of patients with newly diagnosed CML who respond to IM therapy. Patients may require continued monitoring with cytogenetic analysis to identify these abnormalities, but in the absence of additional complications (eg, trilineage dysplasia) a change in treatment strategy is not necessarily indicated. Further studies in larger cohorts are required to confirm these observations and further define the optimal management of these patients.

Authorship

Contribution: E.J. analyzed the data and wrote the paper; H.M.K. designed the study, enrolled patients, and reviewed the paper; L.V.A. performed the cytogenetic analysis; S.O. enrolled patients and reviewed the paper; G.G.-M. enrolled patients and reviewed the paper; S.V. enrolled patients and reviewed the paper; J.S. analyzed data and performed statistical analysis; M.B.R. analyzed data; J.C. designed the study, analyzed the data, and wrote and reviewed the paper.

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