Chronic Myeloid Leukemia Following Therapy With Imatinib Mesylate (Gleevec)

Bone Marrow Histopathology and Correlation With Genetic Status

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Abstract

We evaluated bone marrow pathologic features and cytogenetic and molecular genetic status of 13 patients with interferon-resistant, chronic-phase chronic myeloid leukemia (CML), treated with imatinib mesylate (Gleevec). All had morphologic evidence of CML in the blood and bone marrow and were positive for bcr-abl by reverse transcriptase–polymerase chain reaction, fluorescence in situ hybridization (FISH), or both. Follow-up marrow biopsies, interphase FISH for bcr-abl, and conventional cytogenetics were performed at 3-month intervals (up to 24 months) after therapy initiation. All patients exhibited a reduction in bone marrow cellularity with decreases in myeloid/erythroid ratios at 3 to 6 months after therapy. The percentage of bcr-abl-positive cells by FISH decreased in all patients (pretherapy median, 73%; 3 months median, 47%). Cytogenetic and FISH data defined 2 groups after 6 months of follow-up: 5 patients became negative for bcr-abl by FISH; 8 remained positive, 4 of whom developed signs of clonal cytogenetic evolution. Patients who became negative for bcr-abl had no morphologic evidence of CML at 15 to 24 months of follow-up, whereas patients who remained positive redeveloped morphologic features of CML as cellularity increased. Some bcr-abl-positive patients showed signs of progression, including 2 patients who developed myeloid blast phase. Although all patients demonstrated an initial decrease in bone marrow cellularity after imatinib mesylate therapy, continued follow-up showed that histopathologic findings correlated with genetic response.

Chronic myeloid leukemia (CML) is a clonal stem cell disorder characterized by excessive proliferation of cells of the myeloid series. The hallmark of CML is the Philadelphia chromosome that arises from a reciprocal translocation between chromosomes 9 and 22.1,2 The molecular consequence of this translocation is a bcr-abl chimeric gene whose protein product (bcr-abl) exhibits enhanced tyrosine kinase activity. This constitutively activated enzyme activity is essential to the transforming function of bcr-abl and is a major factor in the pathophysiology of CML.3 The presence of bcr-abl in all patients with CML and the requirement of kinase activity for bcr-abl function make this an attractive target for a selective kinase inhibitor.

In the 1990s, Druker and colleagues4 discovered a molecule, imatinib mesylate, that inhibited bcr-abl kinase activity.4,5 This novel inhibitor was shown to be highly effective in blocking abl tyrosine kinase activity by binding and inactivating the adenosine triphosphate–binding pocket of abl6 in the leukemic cells in CML. It also inhibited the stem cell factor receptor c-kit (CD117)7 and the platelet-derived growth factor receptor8 but had little effect on other tyrosine kinases.

These encouraging laboratory findings led to clinical trials of imatinib mesylate, which included patients with chronic-phase CML in whom treatment with interferon had failed9 and patients with CML in blast crisis or Philadelphia chromosome–positive acute lymphoblastic leukemia.10 Treatment with this agent reduced the leukocyte count to less than 10,000/µL (10.0 × 109/L) within weeks in patients with chronic-phase CML. In many cases, cytogenetic analysis showed a reduction of the proportion of metaphases positive for the Philadelphia chromosome to low levels or to zero.
Imatinib mesylate (Gleevec, Novartis, Basel, Switzerland) also showed substantial activity against blast crisis of CML and Philadelphia chromosome–positive acute lymphoblastic leukemia.10 The patients tolerated the agent well and experienced few side effects.

In May 2001, STI571 (imatinib mesylate) was licensed for use in the United States.11 Follow-up of patients treated with imatinib mesylate is still short, but it has emerged as the treatment of choice in CML, particularly in patients without a suitable donor for hematopoietic stem cell transplantation.12 Imatinib mesylate is effective against gastrointestinal stromal tumor,13-15 nearly all cases of which are positive for CD117/c-kit. Therefore, it is important that clinicians and pathologists be familiar with the hematologic changes that occur after therapy with this agent.

The purpose of this article is to report the bone marrow biopsy characteristics of patients with CML who were treated with imatinib mesylate. Bone marrow findings were correlated with the genetic status of the patients at the time of the biopsies.

Materials and Methods

Patients

The patient population consisted of 13 adults with interferon-resistant CML in chronic phase who were treated with a 400-mg daily oral dose of imatinib mesylate as part of a multi-institutional phase 2 study.16 Patients 1 through 12 were sequential enrollees from Northwestern Memorial Hospital, Chicago, IL. Patient 13 was enrolled at Oregon Health and Science University, Portland, and followed up at Northwestern Memorial Hospital. Before therapy, all patients had morphologic evidence of CML and were positive for bcr-abl by reverse transcriptase–polymerase chain reaction (RT-PCR) and interphase fluorescence in situ hybridization (FISH).

Bone Marrow Examinations

Bone marrow biopsies from the posterior iliac crest were performed at 3, 6, 9, 12, and 15 months in all 13 patients after the initiation of imatinib mesylate therapy. In addition, 9 patients underwent biopsies at 18 months, 8 at 21 months, and 6 at 24 months. The biopsy specimens were fixed in B-5 and stained with H&E. In selected cases, the biopsy specimens also were evaluated for reticulin (Wilder reticulin) and stained with periodic acid–Schiff. Bone marrow aspirate smears and blood smears obtained at the time of the bone marrow biopsies were stained with Wright-Giemsa and evaluated.

Cytogenetic and FISH Analysis

Cytogenetic analysis by conventional procedures was performed on bone marrow aspirates each time a biopsy was done. Interphase FISH on bone marrow aspirates to detect bcr-abl gene fusions also was performed at the time of each subsequent bone marrow biopsy. The translocation probe (Vysis, Downers Grove, IL) consisted of differently colored, directly labeled bcr and abl probes. The abl probe begins between exons 4 and 5 and continues for approximately 200 kilobases (kb) toward the telomere on chromosome 9. The bcr probe begins between bcr exons 13 and 14 (M-bcr exons 2 and 3) on chromosome 22 and extends centromeric about 300 kb beyond the m-bcr regions. Analysis was performed using smears of the bone marrow aspirates that were fixed and denatured according to procedure. The probe mixture was prepared, denatured, and applied immediately to the smear. The smears then were hybridized overnight in a moist environment at 37°C, washed using a rapid wash procedure, and coverslipped with DAPI II counterstain (Vysis). Cells were viewed with a fluorescence microscope using a triple band pass filter containing aqua, rhodamine, and fluorescein filters. Depending on the quantity of hematopoietic cells present in the sample, 300 to 400 intact cells were counted on each case and scored as negative (2 green [bcr]/2 red [abl] signals) or positive (1 green/1 red/1 yellow or 2 yellow signals). The results were reported as a percentage of positive or negative signals. The reference range for the laboratory is fewer than 10% fusion signals.

RT-PCR for bcr-abl

RT-PCR for bcr-abl was performed, before initiation of treatment with imatinib mesylate, using a commercially available kit (IVS Technologies, Carlsbad, CA) and a modification of a previously described method.17

Results

Peripheral Blood Cell Counts

Before starting treatment with imatinib mesylate, the leukocyte counts ranged from 8,900 to 70,900/µL (8.9-70.9 × 10^9/L) and were more than 10,000/µL (10.0 × 10^9/L) in 11 of 13 patients Figure 1. At 3 months, all 13 patients had normal or decreased leukocyte counts. The decreases in leukocyte counts were sustained in all 13 patients for 6 months, and counts remained normal to the end of the follow-up period with the following exceptions: In 4 patients (1, 6, 9, and 12), the leukocyte count increased. Two of these patients (9 and 12) had features of chronic-phase CML with leukocytosis developing at 15 and 21 months, respectively. Patient 6 had leukocytosis including neutrophilia, shift to
immaturity, and basophilia that persisted to the last follow-up, from month 12 to month 15. Patient 1 developed a transient elevation of the leukocyte count at 21 months that was unaccompanied by features of CML.

Interphase FISH for bcr-abl

Specimens from 12 of 13 patients analyzed before imatinib mesylate therapy was initiated were positive for the bcr-abl fusion gene Figure 2. The bcr-abl positivity was confirmed in these patients by RT-PCR analysis. The percentage of positive cells ranged from 36% to 89% (mean, 73%). All 12 patients exhibited a decrease in the percentage of cells positive for bcr-abl at 3 months, the time of the first biopsy after initiation of imatinib mesylate therapy, with the percentage of positive cells ranging from 13% to 70% (mean, 42%). Of the 13 patients, 5 (patients 1-5) became negative for bcr-abl. At 3 months after the initiation of therapy, the percentage of bcr-abl-positive cells in these 5 patients ranged from 13% to 29%. At 6 months, 4 of the 5 patients were negative for bcr-abl, and at 9 months, all 5 patients were negative for bcr-abl. The negativity for the bcr-abl fusion gene by FISH persisted in all 5 patients to 21 to 24 months. In the remaining 8 patients (patients 6-13), the percentage of bcr-abl-positive cells decreased at 3 months, but all had a higher percentage of positive cells (35%-70%; mean, 55%) than any of the patients who became negative for bcr-abl. The percentage of bcr-abl-positive cells was not less than 24% in any of the 8 patients at the 9-, 12-, and 15-month analyses. At 15 months, the percentage of bcr-abl-positive cells by FISH in the 8 patients ranged from 29% to 85%, with a mean of 45%, which was lower than the mean of 67% before initiation of imatinib mesylate therapy. The mean percentage of positive cells in the 4 cases analyzed at 21 to 24 months was 79% (range, 51%-91%). Three patients developed double bcr-abl signals; patient 6 at 3 months, patient 7 at 6 months, and patient 12 at 6 months.

Conventional Cytogenetic Analysis

Conventional cytogenetic analysis of the aspirate showed 100% of the metaphases to be positive for t(9;22) in all 13 patients before therapy. In 5 patients (patients 1-5), the karyotype normalized with loss of t(9;22); this first occurred at 3 months in 1 patient (patient 5), at 6 months in 1 patient (patient 4), at 9 months in 2 patients (patients 1 and 3), and at 15 months in 1 patient (patient 2). Patient 2 had only 1 of 25 metaphases positive for t(9;22) in the 6-, 9-, and 12-month cytogenetic studies before becoming negative at 15 months. All 5 patients were cytogenetically normal at 15 months. These 5 patients are the same 5 patients who became negative for bcr-abl by FISH and remained negative through the duration of follow-up.

In 8 of 13 patients (patients 6-13), decreases in the percentage of bcr-abl-positive cells were not noted by FISH, with 100% of the cells being positive for t(9;22) each time the aspirate was studied, from 0 to 24 months. Of the 8 patients, 4 underwent clonal evolution and developed chromosomal abnormalities in addition to the single t(9;22) that was present before therapy Table 1.

Bone Marrow Biopsy Findings Before Imatinib Mesylate Therapy

Before imatinib mesylate therapy, the bone marrow biopsies of the 13 patients exhibited morphologic features typical of CML in chronic phase. Bone marrow biopsies were performed on 12 patients before initiation of imatinib mesylate therapy, and samples were sufficient to determine cellularity; all 12 were hypercellular, with cellularity ranging from 60% to 100% (median, 95%). In 11 of 12 patients, the hyper-
cellularity was secondary to myeloid hyperplasia with myeloid/erythroid (M/E) ratios ranging from 3.5 to 16. The other patient (patient 1) had 100% cellularity with both myeloid and erythroid hyperplasia (M/E ratio, 1.7:1). Bone marrow blasts ranged from 0.6% to 5.6%. Basophils ranged from 0% to 15%. Megakaryocytes were normal to moderately increased in number and smaller than normal. Reticulin stain showed moderately increased fibrosis in 3 patients (patients 10-12). Sea-blue histiocytes were identified in many patients but were prominent, occurring in clusters, in only 1 (patient 7).

Bone Marrow Findings for Patients Who Became Negative for bcr-abl

Before therapy, the bone marrow specimens were 90% to 95% cellular. All 5 patients exhibited a decrease in cellularity in the 3-month biopsy specimens, with cellularity ranging from 30% to 80% (mean, 49%). The reduction in cellularity corresponded to prominent decreases in myeloid proliferation with M/E ratios of 2 or less at 3 months. The number of megakaryocytes at 3 months remained unchanged from before therapy. As the imatinib mesylate therapy continued, the cellularity continued to drop, with nadir cellularity reached at 9 months in 4 patients and at 15 months in 1 patient (nadir cellularity ranged from <5% to 50%; mean, 19%). In 2 patients (patients 2 and 3), the cellularity in the bone marrow samples never dropped below normocellular levels, while in 2 other patients (patients 1 and 5), markedly hypocellular bone marrow (≤5%) developed at 9 months that corrected on follow-up studies. Moderate reticulin fibrosis not present at the initiation of therapy accompanied the transient hypocellularity in 1 patient (patient 5).

The bone marrow cellularity for all 5 patients increased after the nadir, with 3 patients developing mild hypercellularity and 2 continuing to be hypocellular. At 18 to 24 months, the biopsy specimens from the 5 patients ranged from 15% to 60% cellularity. None of the patients had morphologic evidence of CML in the bone marrow. Myeloid hyperplasia and basophilia were absent, and megakaryocytes appeared normal. The corresponding leukocyte counts for the 5 patients were less than 10,000/µL (<10 × 10⁹/L) at the latest follow-up (6-24 months), with absence of immature neutrophils and no basophilia. The platelet counts were normal or slightly decreased.

Bone Marrow Findings for Patients Who Remained Positive for bcr-abl

All 8 patients who retained bcr-abl exhibited a decrease in cellularity after therapy with imatinib mesylate that was first evident at 3 months in 3 patients and 6 months in 1 patient. The nadir cellularity in 5 patients was normocellular to moderately hypocellular (20%-40%), while 3 patients (patients 8, 10, and 12) developed marked hypocellularity (<5%). The severe hypocellularity in patient 12 persisted from 9 to 18 months, and then the cellularity increased sharply at 21 months. The reductions in cellularity corresponded to prominent decreases in myeloid proliferation in all patients, with the most severe reductions in cellularity characterized by decreases in both myeloid and erythroid production. The number of megakaryocytes remained normal to slightly increased in all except the 3 patients with marked hypocellularity, in whom the number was decreased.

Table 1

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All patients who developed increased marrow cellularity during treatment had bone marrow biopsies that revealed overt morphologic evidence of CML with myeloid hyperplasia and frequent basophilia. Four patients developed marked megakaryocytic hyperplasia (patients 6-8 and 11), with megakaryocytes arranged in clusters or sheets throughout the biopsy sections. In 2 patients (patients 7 and 8), the megakaryocytes remained small, as in chronic-phase CML; they subsequently decreased to normal levels as the cellularity later decreased or stabilized. However, the megakaryocytes in the other 2 patients (patients 6 and 11) became morphologically dysplastic and the number remained increased. Both of these patients exhibited cytogenetic clonal evolution. Patient 11 developed myeloid blast phase at 15 months, and patient 12 developed myelomonocytic blast phase at 24 months.

**Image 1** Histopathologic findings in a patient who became negative for t(9;22) after treatment with imatinib mesylate. A, The bone marrow from patient 1, who became negative for t(9;22) by 9 months of follow-up, initially was hypercellular and showed typical features of chronic-phase chronic myeloid leukemia (CML) (H&E, original magnification ×40). B, The aspirate had findings characteristic of CML, including a marked myeloid predominance and basophilia (Wright-Giemsa, original magnification ×600). C, After initiation of treatment with imatinib mesylate, the marrow cellularity dropped to a nadir of <5% at 3 months (H&E, original magnification ×40). D, The bone marrow was moderately hypocellular by 9 months (H&E, original magnification ×40). E, By 21 months of follow-up, the bone marrow showed no morphologic evidence of CML. The cellularity was mildly decreased (H&E, original magnification ×100). F, The bone marrow aspirate at 21 months of follow-up demonstrated normal hematopoiesis (Wright-Giemsa, original magnification ×600).
Sea-blue histiocytes were prominent in samples from 5 patients (patients 6-8, 12, and 13) who retained *bcr-abl*. These cells were highlighted by periodic acid–Schiff stain and occasionally occurred in clusters or sheets throughout the biopsy specimen. In patient 12, the sea-blue histiocytes occurred in markedly hypocellular bone marrow; they were the primary cell type and were accompanied by marked fibrosis with reticulin fibers surrounding individual histiocytes.

Three patients in this group (patients 10-12) had moderately increased reticulin fibrosis before initiation of imatinib mesylate therapy. After treatment, the reticulin decreased to normal. In one of these patients (patient 10), the reticulin returned to normal within 12 months after initiation of therapy and fibrosis did not recur. In 2 patients (patients 11 and 12) the reticulin transiently decreased but fibrosis reappeared in later biopsy specimens. In contrast with the biopsy specimens obtained before initiation of imatinib mesylate therapy, the reticulin deposition was now focally deposited rather than diffuse. In both of these cases, fibrosis was associated with a return of the morphologic features of CML with a marked increase in the number of megakaryocytes.

**Discussion**

We report the changes in bone marrow histopathologic features, cytogenetics, and interphase FISH that occurred in patients with interferon-resistant, chronic-phase CML after treatment with imatinib mesylate, a specific inhibitor of the *bcr-abl*-encoded tyrosine kinase. The first genetic analysis 3 months after initiation of imatinib mesylate therapy revealed a decreased percentage of *bcr-abl* FISH fusion signals from pretherapeutic levels in all patients. During treatment, 2 patient groups emerged. By 3 to 9 months of follow-up, a group of 5 patients became negative for the *bcr-abl* fusion signal by FISH and remained negative through the 15- to 24-month follow-up. Conventional cytogenetic analysis confirmed the negativity for t(9;22) in the 5 patients negative

**Figure 4** Cellularity in bone marrow specimens from 8 patients who remained positive for *bcr-abl* after treatment with imatinib mesylate.

**Image 4** Histopathologic findings in a patient who remained positive for t(9;22) after treatment with imatinib mesylate. **A**, Patient 6, who retained t(9;22) throughout the course of treatment, originally had typical features of chronic myeloid leukemia, including marked marrow hypercellularity (H&E, original magnification ×40). **B**, By 6 months, the marrow was normocellular (H&E, original magnification ×40). **C**, At 12 months, the marrow hypercellularity had returned and focal clusters of megakaryocytes were seen (H&E, original magnification ×40). **D**, There was marked peripheral thrombocytosis and basophilia at 12 months. No bone marrow aspirate was obtained (Wright-Giemsa, original magnification ×1,000).
by FISH. The remaining 8 patients remained positive for the t(9;22), with 100% of the observed metaphases containing the translocation throughout the observed period. Those who remained positive had lower percentages of positive signals at early follow-up periods than before imatinib mesylate therapy, with an increasing percentage of fusion signals seen in a subgroup of patients over time.

Both groups initially responded favorably to therapy, with a decrease in bone marrow cellularity and decreased myelopoiesis; the decrease in marrow cellularity corresponded to a decrease in FISH fusion signals. Since all patients lost morphologic signs of CML after initiation of therapy, it was difficult to predict outcome based on histopathologic findings alone. There were no morphologic findings to definitively separate these 2 groups. However, longer analysis time of the histopathologic features of bone marrow biopsy specimens showed striking differences between the 2 groups. Patients in the group that retained evidence of the bcr-abl fusion ultimately regained morphologic evidence of CML as cellularity increased following the nadir biopsy. Some showed signs of progression, including myeloid blast phase. A subset of the patients in this group developed clonal cytogenetic abnormalities in addition to the Philadelphia chromosome, most commonly a double Philadelphia chromosome. In contrast, the biopsy specimens of the patients who remained negative returned to morphologic features that were indistinguishable from normal and that persisted to the end of the follow-up period. Even though the cellularity in this group was variable, including in biopsy specimens that were mildly to moderately hypercellular, there was no definitive morphologic evidence of CML.

These observations seem to differ from those reported previously, perhaps owing to differences in methods and length of follow-up. Hasserjian et al\(^1\) concluded that reduction in marrow cellularity in response to imatinib mesylate was independent of cytogenetic response. However, genetic data in that report were limited to conventional analysis, and the median follow-up was approximately 9 months, whereas our report includes FISH analysis and a longer follow-up. Braziel et al\(^2\) reached similar conclusions regarding bcr-abl–positive and bcr-abl–negative cases. In their study FISH analysis and assessment of bcr-abl by RT-PCR were performed in addition to conventional analysis, and the longest follow-up period was 14 months. In our patient population, it was difficult to detect a difference in the histopathologic findings between the bcr-abl–positive and bcr-abl–negative groups before 12 months of follow-up; the differences became increasingly apparent with longer follow-up, with overt features of CML noted between 12 and 24 months.

In our series, FISH was more sensitive than conventional cytogenetic analysis for detecting initial decreases in the percentage of Philadelphia chromosome–positive cells. A drop in the percentage of Philadelphia chromosome–positive cells was not detected by conventional cytogenetic analysis until the FISH percentage of positive cells dropped below
approximately 20% to 30%. The reasons for this are unknown but may reflect the fact that more cells are screened in FISH than in conventional cytogenetics. Therefore, FISH would detect smaller changes in positivity than would conventional cytogenetics. Second, dividing cells are selected preferentially for analysis by conventional analysis; cells in any phase of the cell cycle can be analyzed by FISH. If a higher percentage of CML cells are in metaphase compared with the other bone marrow constituent cells, a selection bias may increase the percentage of Philadelphia chromosome-positive cells detected by conventional cytogenetics. Neither the FISH technique that we used nor conventional cytogenetic analysis are sufficiently sensitive for the detection of minimal residual disease. PCR-based testing may be more appropriate for this purpose.

The use of conventional cytogenetics in our series as a complement to FISH was advantageous in permitting us to confirm the emergence of a second Philadelphia chromosome in 3 patients and to detect other signs of clonal evolution. Amplification of the \textit{bcr-abl} gene is one postulated mechanism of imatinib mesylate resistance.\textsuperscript{20} Of note, the patients who underwent clonal evolution demonstrated histopathologic evidence of progression of CML, including 2 patients who developed blast phase after initial response to imatinib mesylate therapy.

Several histopathologic findings can be explained by the CML disease process. Sea-blue histiocytes were prominent in many of the patients receiving imatinib mesylate, particularly those who continued to exhibit \(t(9;22)\). They frequently are encountered in CML and contain phagocytosed cellular debris related to the rapid cell turnover characteristic of the disease.\textsuperscript{21} Because \textit{bcr-abl} inhibitors such as imatinib mesylate interfere with the proliferative capacity of the cells, it follows that the death of these cells would be followed by phagocytosis by macrophages.

Another phenomenon noted was a change in marrow fibrosis after treatment with imatinib mesylate. In 3 patients, all from the group that remained positive for \textit{bcr-abl}, there was a moderately increased level of reticulin deposition present diffusely throughout the biopsy specimens before initiation of imatinib mesylate therapy. In one of these patients (patient 10) the fibrosis resolved within 12 months after initiation of therapy. However, in 2 patients, focal rather than diffuse fibrosis was detected in subsequent biopsy specimens within 3 to 9 months. In both of these cases, the fibrosis was associated with morphologic features of CML with a marked increase in the number of megakaryocytes. A reduction in marrow fibrosis in CML cases treated with imatinib mesylate has been reported, and the \textit{bcr-abl} status in patients with reduced fibrosis was not noted.\textsuperscript{22} Since 3 patients in our series had a persistence of \textit{bcr-abl}, it is possible that imatinib mesylate reduces marrow fibrosis even in cases in which the malignant clone is not eradicated. However, it is difficult to evaluate these findings, since fibrosis may be focal in CML, and subsequent apparent decreases in marrow fibrosis may be due to biopsy of a less fibrotic area of the marrow and unrelated to the direct effects of imatinib mesylate or the persistence or eradication of the malignant clone.

We report an initial favorable bone marrow histopathologic response that correlates with a decreased percentage of \textit{bcr-abl}-positive cells in all patients with interferon-refractory CML treated with imatinib mesylate. Assessment of genetic status to evaluate persistence or loss of \textit{bcr-abl} is important for predicting later outcome. Continued follow-up will be important to determine the durability of morphologic and cytogenetic remission in \textit{bcr-abl}-negative patients.

\textbf{References}


