Imatinib Mesylate Resistance Through BCR-ABL Independence in Chronic Myelogenous Leukemia

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ABSTRACT

Imatinib mesylate (IM) binds to the BCR-ABL protein, inhibiting its kinase activity and effectively controlling diseases driven by this kinase. IM resistance has been associated with kinase mutations or increased BCR-ABL expression. However, disease progression may be mediated by other mechanisms that render tumor cells independent of BCR-ABL. To demonstrate this potential, IM-resistant cells were found in chronic myelogenous leukemia patients with continuous BCR-ABL gene expression but undetectable BCR-ABL protein expression. These cells were unresponsive to IM and acquired BCR-ABL-independent signaling characteristics. IM resistance in some patients may be mediated through loss of kinase target dependence.

INTRODUCTION

Reciprocal chromosomal translocation results in expression of a chimeric gene encoding the unregulated BCR-ABL tyrosine kinase (1, 2). BCR-ABL-mediated tyrosine phosphorylation promotes transformation of hematopoietic progenitor cells into chronic myeloid and acute lymphocytic leukemias that can be treated effectively with imatinib mesylate (IM), a BCR-ABL tyrosine kinase inhibitor (3, 4). However, despite the persistence of BCR-ABL expression throughout all phases of this disease, patients frequently relapse and progress on therapy (5–7). Clinical resistance has been associated with amplification or mutation of the BCR-ABL locus resulting in constitutive BCR-ABL signaling (8–11). Several mutations have been described that disrupt IM binding to the BCR-ABL kinase domain, resulting in the accumulation of an uninhibitable BCR-ABL kinase activity in chronic myelogenous leukemia (CML) cells (8). However, mutations are only distinguished by highly sensitive detection techniques and monoclonal expansion of mutant or amplified BCR-ABL-expressing cells are not consistently detected in IM-resistant patients (8–11). These observations suggest that other mechanisms of resistance and disease progression may exist. Cell line models of complete IM resistance suggest that BCR-ABL-independent signaling in CML cells may account for loss of sensitivity to BCR-ABL kinase inhibition (12). In this study, we describe isolation and characterization of cell lines established from CML patients that failed IM therapy. The results suggest that BCR-ABL independence accounts for IM failure in some patients.

MATERIALS AND METHODS

Drugs, Cell Lines, and CML Specimens. STI571 (active chemical component of IM) and CGP-76030 were kindly provided by Drs. E. Buchdunger and S. Spring (Novartis AG, Basel, Switzerland). BAY 11–7082 was obtained from Calbiochem (San Diego, CA). These agents were prepared as 10 mM stock solutions in DMSO. K562 and U-937 cells were obtained from Dr. Zeev Estrov (Department of Bioimmunotherapy, M. D. Anderson Cancer Center) and were maintained in RPMI 1640 with 10% FCS at a density of <10^5 cell/ml. K562 cells resistant to IM (K562-R) were described and characterized recently (12).

To analyze IM resistance in clinical samples, peripheral blood samples were taken from IM-treated blast crisis CML patients after disease progression (Tables 1 and 2). These specimens contained 15–84% blasts. The Internal Review Board of M. D. Anderson Cancer Center approved all studies involving human subjects, and informed consent was obtained from each patient before initiation of this procedure. Initially, specimens from CML patients that had measurable hematological responses to IM but subsequently progressed on therapy were analyzed for BCR-ABL expression, tyrosine phosphorylation, and cellular sensitivity to IM. Samples were also placed in culture with the intent of establishing continuous cell lines. As described below, three cell lines (WDT-1, -2, -3) have been established and completely characterized. Three additional cell lines (WDT-4, -5, -6) are currently being characterized.

Briefly, fresh peripheral blood (~18 ml) was overlaid onto Histopaque-1077 (Sigma, St. Louis, MO) and centrifuged at relative centrifugal force (RCF) 400 × g for 15 min. The cells at interphase were removed by aspiration and washed once with PBS. Cell preparations containing significant RBC contamination were subjected to treatment with ACK lysis buffer (0.154 M ammonium chloride, 0.01 M KHCO3, and 0.13 μM EDTA) for 30 min. Aliquots from each sample (representing 5 × 10^6 cells) were immediately lysed in 2× SDS sample buffer to examine BCR-ABL protein expression (immunoblot) or incubated for up to 24 h in the presence or absence of IM. These samples were lysed on ice in lysis buffer (13), and equal protein aliquots (50 μg) were subjected to phosphorylated (p)-Tyr immunoblotting. Cellular sensitivity to IM was estimated by plating 25,000 CML cells into individual wells of a 96-well plate and incubating with IM (at the indicated concentrations) for 48 h. Cell growth and survival in control and treated cells were estimated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as described previously (13).

Remaining cells from patients were used to establish WDT-1, -2 and -3 continuous cell lines. Mononuclear cells were initially cultured in RPMI 1640 with 10% fetal bovine serum for 7–10 days. Spent cell supernatant from these cultures was retained (frozen at −80°C) and used to propagate the serial culture. After the initial incubation interval, cells were subjected to centrifugation and resuspended in equal portions of fresh and spent initial culture media. This procedure was continued for 2 months or until spent media was depleted. Viability of these cultures declined through the first 4–5 passages but stabilized thereafter. Populations were considered stable if they were capable of sustained growth in the absence of additives or spent culture media for a minimum of 4 months and were characterized only after 4–6 months in culture. Cell surface markers were used to define cell lineage by flow cytometry as described previously (14).

Cytogenetic Analysis and Fluorescence in Situ Hybridization. For conventional cytogenetic evaluation, cells were cultured at 37°C for 24 h and harvested using Colcemid for 20 min followed by 3:1 methanol/acetic acid exposure for 10 min. Three methanol/acetic acid washes were used to clean and harden the cells. Twenty metaphases were then analyzed with GTG-banding.

Fluorescence in situ hybridization was performed using the Vysis LSI BCR/ABL ES Dual Color Translocation Probe. This probe is a mixture of the LSI ABL probe labeled with SpectrumOrange and the LSI BCR probe labeled with SpectrumGreen. The spanning ABL probe is approximately 650 kb extending from an area centromeric of the ASS gene to the telomeric of the last ABL exon. The SpectrumGreen BCR probe is approximately 300 kb, beginning between BCR exons 13 and 14 and extending well beyond the M-bcr region. Briefly, cells were pelleted on glass slides and denatured at 72°C for 5

Received 5/23/03; revised 10/6/03; accepted 11/6/03.

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min. The slide was subjected to dehydration by immediate transfer into cold (4°C) ethanol (70, 85, 100%) for 2 min each. The slide was washed with 0.4× SSC and 0.3% NP40 at 72°C for 2 min and incubated with 2× SSC/0.1% NP40 for 1 min at room temperature. Ten μl of probe was added to each slide and incubated in a humidified chamber for 24 h. Finally, the slide was washed and counterstained. Nuclei lacking (t9, 22) exhibit a two orange, two green signal pattern. In a nucleus possessing the (t9;22) involving the M-bcr, one green, one large orange, one smaller orange, and one fused orange/green signal is observed (15).

**Analysis of BCR-ABL Protein Expression and Signaling.** Protein levels of BCR-ABL were monitored by direct immunoblotting with anti-c-abl as noted or subjected to immunoprecipitation with anti-BCR (500 μg of cell lysate) and immunoblotting with anti-c-abl or anti-BCR. Activated BCR-ABL was detected by p-Tyr immunoblotting c-abl immunoprecipitates (from 500 μg of cell lysate). Downstream signaling intermediates [signal transducers and activators of transcription (Stat) 5, LYN, HCK, mitogen-activated protein kinase (MAPK), Akt, CrkL] were compared between cell samples by immunoblotting equal protein cell lysates. Antibodies against phosphorylated forms of signaling intermediates were used where available. Phosphorylated CrkL was detected in CrkL immunoprecipitates by p-Tyr immunoblotting. All immunoblots were developed with horseradish peroxidase-conjugated secondary antibodies (Bio-Rad Laboratories, Hercules, CA) and enhanced chemiluminescence reagent (Amersham Pharmacia Corp., Arlington Heights, IL).

**Reverse transcription (RT)-PCR Amplification of BCR-ABL and Sequencing of the ABL Kinase Domain.** For bcr-abl RT-PCR, mRNA was isolated as described below. RT-PCR reactions were performed in 50 μl using SuperScript One-Step RT-PCR with Platinum Taq from Invitrogen (Carlsbad, CA). Reagents were at the following final concentrations: 1× reaction mix; 1 μg of total RNA; 0.2 μM sense primer; 0.2 μM antisense primer; 4 mM MgSO4; and 2 units of RT/Platinum Taq mix. RT-PCR was performed on a MJ Research PTC-200 DNA Engine as follows: 30 min at 55°C followed by 2 min at 94°C; for PCR, 40 cycles at 94°C for 15 s, 59°C for 30 s, and 72°C for 80 s. Reactions were run on a 1% agarose gel, and the 1.3 kb bcr-abl bands were excised, purified, and eluted in 30 μl using a gel extraction kit from Qiagen (Valencia, CA). Platinum TaqDNA polymerase was used for nested PCR amplification of the abl kinase domain of the 1.3-kb bcr-abl PCR product (9). Reaction components were 1× PCR buffer, 0.2 mM each deoxynucleoside triphosphate, 1.5 mM MgCl2, 0.2 μM sense, 0.2 μM antisense, 5 μl of the eluted DNA from above, and 2.5 units of Platinum Taq. PCR was performed on a MJ Research PTC-200 DNA Engine as follows: 1 cycle of 94°C for 2 min and 30 cycles of 94°C for 15 s; 56°C for 30 s; and 72°C for 30 s. Reaction products were purified and, as above and sequenced on a Biombeck 3700 automated DNA sequencer. Primers were obtained from Sigma-Genosys (The Woodlands, TX), and the sequences were as follows: forward, TGTCTGCTCGCCATCGCCTG-3′; reverse, 5′-ggtaktgcagagtccg-3′; for amplification of a 1.3-kb bcr-abl product representing the BCR-ABL junction and kinase domain. The sequences used for the nested PCR of the 323-bp kinase domain were as follows: forward, 5′-ggcaacgg-acccgtctagtg-3′; and reverse, 5′-gtagtgccagcagttcggc-3′.

**BCR-ABL Northern Blot.** RNA was extracted with Trizol reagent as described previously (16). For Northern blot, 15 μg of total RNA were separated on a formaldehyde gel and transferred to a Schleicher and Schuell nylon membrane using standard protocols. The membrane was probed with 20 ng/μl of a biotinylated 1.3-kb bcr-abl PCR amplification product (as described above) using New England Biolabs' NEBlot Phototope kit according to the standard hybridization protocol. The probe was detected using the maximum sensitivity protocol from New England Biolabs' Phototope-Star detection kit (Beverly, MA). Ethidium bromide detection of 28S RNA was used as a loading control.

**Activated Nuclear Factor (NF)-κB Analysis.** To determine NF-B activation, we conducted electrophoretic mobility shift analysis essentially as described previously (17). Briefly, nuclear extracts prepared from CML cells or tumor necrosis factor-treated U937 cells (2×106/ml) were incubated with 100 nM end-labeled 45-mer double-stranded NF-B oligonucleotide (4 μg of protein with 16 fmol of DNA) from the HIV long-terminal repeat, 5′-TGTTA-CAAGGGACCTTCGCTGGGACCTTCTCCAGGGGCGTGG-3′ for 15 min at 37°C, and the DNA-protein complex formed was separated from free oligonucleotide on 6.6% native polyacrylamide gels. The dried gel was visualized by autoradiography.

**Antibodies.** Antibodies used in these studies include, phosphoMAPK, MAPK (Cell Signaling, Beverly, MA), phosphotyrosine, phosphoSTAT5, CrkL (Upstate Biotechnology Institute, Lake Placid, NY), c-abl (8E9; Oncogene Sciences, San Diego, CA), BCR, LYN, HCK, p-HCK (Santa Cruz Biotechnology Biologicals, Santa Cruz, CA) and actin (Sigma Chemical). Polyclonal anti-STAT5 (a/b) was kindly provided by Dr. Robert Kirken (University of Texas, Health Science Center, Houston, TX).

**RESULTS**

Clinical IM-resistant specimens from CML patients that progressed on therapy were analyzed for BCR-ABL protein expression and ex vivo sensitivity to IM. Heterogeneity in BCR-ABL protein expression and IM sensitivity was measured in two resistant patient samples (Fig. 1). Patient 1 had minimally detectable BCR-ABL protein expression and tyrosine phosphorylation that was unaffected by IM. However, these cells retained BCR-ABL gene expression when analyzed by competitive PCR analysis (~140,000 transcripts/cell; Ref. 18). Patient 3 expressed BCR-ABL protein and tyrosine phosphorylation that was inhibited in the presence of IM. Cells from patient 3, but not patient 1, were measurably sensitive to IM-mediated growth inhibition and apoptosis. Analysis of these specimens revealed an unexpected heterogeneity in BCR-ABL protein expression and ex vivo sensitivity to IM in CML cells. These results suggest that IM resistance in some patients may be associated with loss of BCR-ABL protein expression.

Signal transduction and protein expression in CML specimens is difficult to assess because of high proteolytic and phosphatase activity in neutrophils and granulocytes (19). To confirm BCR-ABL protein heterogeneity and to further assess IM resistance in CML patient samples, specimens obtained from these patients were placed in culture with the intent to develop representative IM-resistant CML cell lines from patients. Three cell lines (WDT-1, -2 and -3) were established by isolation of mononuclear cells and continued culturing in the presence of initially captured conditioned media as described (“Materials and Methods”; a fourth stable cell line is currently being characterized). Patient characteristics and previous treatment histories for these patients are reported in Table 1. Two patients (1 and 2) had similar treatment regimens before IM and similar duration of blast crisis phase but differed in their primary response to IM. Patient 1 had
a primary hematological response followed by progressive disease (after 10 months) that was unresponsive to IM dose escalation. Patient 2 was unresponsive to IM and rapidly progressed on therapy (2 months). A third patient had durable response to IM (14 months) before relapse. Relapse was not affected by dose-escalation.

All established cell lines derived from these CML patients (WDT-1, -2, -3) retained myeloid markers (CD31, CD33, CD34, CD38, CD45, c-kit) and formed colonies in methylcellulose in the absence of exogenous growth factors or cytokines. As described in the initial isolate, IM responsiveness was heterogeneous with WDT-1 cells expressing complete resistance to IM (Fig. 2A). To determine the nature of resistance in this patient-derived cell line, we examined cytogenetic and molecular characteristics. WDT-1 cells retained the 9;22 chromosomal translocation (93% by fluorescence in situ hybridization analysis and karyotyping; Fig. 2B; Table 2) and expressed BCR-ABL by PCR and Northern blot analysis (Fig. 2C). These characteristics did not distinguish WDT-1 cells from other isolated cell populations that expressed sensitivity to IM (WDT-2, -3). We did note reduced expression of the BCR-ABL gene in WDT-1 cells that was similar to expression detected in a K562 cell line selected for in vitro IM resistance (K562-R; Ref. 12). Analysis of BCR-ABL protein expression and activation by immunoprecipitation and immunoblot analysis failed to detect BCR-ABL protein or tyrosine phosphorylation in WDT-1 cells (Fig. 2D). Conversely, BCR-ABL was highly activated in WDT-3 cells whereas the WDT-2 cell line was similar to the IM-sensitive K562 cell line. Expression of c-abl was detected in all cell lines demonstrating that proteolysis was not responsible for loss of BCR-ABL detection in WDT-1 cells. These results suggest that the WDT-1 cell line mimics characteristics of the initial patient isolate with regard to BCR-ABL protein expression and resistance to IM.

Mutations in the kinase domain of BCR-ABL have been shown to exist in some IM-resistant CML patients. At least three BCR-ABL point mutations alter their inhibition by IM through binding pocket distortion without affecting ATP utilization or BCR-ABL tyrosine kinase activity. To address the role of BCR-ABL mutations in cells derived from resistant patients, WDT cells were incubated with IM, and BCR-ABL phosphorylation was examined after 30 min. IM reduced BCR-ABL tyrosine phosphorylation in both WDT-2 and -3 cells demonstrating that these cells retained IM-mediated kinase-inhibitory activity (Fig. 3A). WDT-1 cells do not express BCR-ABL, and BCR-ABL tyrosine phosphorylation was not detectable in control or treated cells. To examine the possible existence of other mutations in BCR-ABL from these cell lines, we sequenced the entire abl kinase domain from a nested PCR product derived from a bcr-abl 1.3-kb RT-PCR reaction as described previously (9). Mutations were not
detected in any of the cell lines. These results suggest that other resistance mechanisms predominate in the patients from which these cells were derived. At least one patient-derived cell line suggests that resistance is mediated by BCR-ABL protein deficiency and outgrowth of BCR-ABL signaling-independent cells (WDT-1).

Reactivation of BCR-ABL signaling has been suggested as a mechanism of IM resistance. To determine the role of kinase signaling in IM resistance, BCR-ABL downstream signaling was examined in WDT cells. Activated Stat5 was detected in BCR-ABL-positive WDT-2 and -3 cells but absent in WDT-1 cells (Fig. 3B). Although CrkL protein expression varied widely between these cell lines, p-CrkL was detected in WDT-2 and -3 but not WDT-1 cells. Both MAPK and Akt protein expression also varied and was not consistently associated with BCR-ABL activation in BCR-ABL (+) cells. For example, Akt and MAPK activation was detected in WDT-2 and WDT-3 cells, respectively, and was not associated with their level of expression or the state of BCR-ABL activation. Akt activation was measurable in the absence of BCR-ABL protein in WDT-1 cells, suggesting distinct signaling patterns in IM-resistant BCR-ABL-independent WDT-1 cells.

Src kinases have been shown to be activated by BCR-ABL and may play a role in CML disease progression and cytokine independence (20, 21). Overexpression or activation of src kinases mediates BCR-ABL independence and IM resistance in some CML cell lines (12). Two src kinases, LYN and HCK, were highly expressed (compared with K562 cells) or activated in all WDT cells and were independent of IM sensitivity. We have shown previously that LYN expression and activation was increased in IM-resistant K562 cells (K562-R) and was similar to that detected in IM-resistant WDT-1 cells (Fig. 4A). Src kinase inhibition with CGP-76030 (Novartis) reduced the proliferation and survival of BCR-ABL (+) WDT-2 and -3 cells (Fig. 4B). BCR-ABL-independent WDT-1 cells were also growth inhibited by src kinase inhibition, suggesting that src-family kinases play a role in the growth and/or survival of both BCR-ABL-dependent and -independent cells. Treatment with CGP-76030 suppressed Hck phosphorylation in all cell lines (Fig. 4C), whereas IM was effective in reducing Hck activation in BCR-ABL expressing cells (WDT-2, WDT-3). Inhibition of Hck phosphorylation correlated with the onset of caspase activation in all cell lines (Fig. 4C), suggesting a role for src kinases in apoptotic protection of both BCR-ABL-positive and -negative CML cells from IM-resistant patients.

Hematopoesis is controlled by the regulated expression and activation of protein kinases and transcription factors (22). Stat protein activation is associated with BCR-ABL signaling and transformation, but none of the previously described targets of BCR-ABL signaling (Stat1, 3, 5, or 6) were activated (by electrophoretic mobility shift analysis or phosphorylation analysis) in BCR-ABL protein-deficient WDT-1 cells (data not shown). NF-κB plays a central role in cytokine-regulated hematopoiesis (23), but its regulation by BCR-ABL has not been examined in clinical specimens. Activated NF-κB was detected in BCR-ABL-independent WDT-1 CML cells (Fig. 4D) but not other CML cell lines or models of IM resistance (K562). Treatment with NF-κB inhibitor (Fig. 4E) significantly reduced proliferation of WDT-1 cells (50% at 5 μM BAY 11–7082) but had limited effects on other WDT cell lines (24). BAY 11–7082 treatment suppressed NF-κB activation in both the original clinical specimen and the corresponding established WDT-1 cell line (Fig. 4F). These results demonstrate that BCR-ABL-independent activation of src-related kinases and NF-κB transcription factor are engaged in IM-resistant WDT-1 cells. These signaling cascades appear to contribute to BCR-ABL independence, are present in initial clinical specimens, and retained in established cell lines from IM-resistant patients.

**DISCUSSION**

Previous studies have shown that BCR-ABL mutations and amplification are associated with IM resistance and disease progression in some patients (8–11). These changes result in reactivation of BCR-ABL signaling as determined by phosphorylation of the adapter molecule, CrkL. In vitro models of BCR-ABL point mutations demonstrate variable degrees of interference in IM binding by these mutations, and only three of the mutations mapped have significant effects on IM-mediated kinase inhibition (>3-fold-reduced efficacy; Refs. 8 and 11). Amplification of the BCR-ABL gene may overwhelm the ability of a clinically achievable IM dose to suppress BCR-ABL signaling. However, mutations in resistant patients do not appear to represent monoclonal populations because both wild-type and mutant BCR-ABL genes are detected in resistant patients (8–11). In addition, cells isolated from resistant patients have not been recovered as continuous cell lines. Our original goal in this study was to more fully characterize resistance in clinical specimens by establishing stable cell lines with BCR-ABL gene mutations. We have detected BCR-ABL kinase domain mutations in some IM-resistant and -advanced phase CML patients, but their detection requires very sensitive techniques (nested PCR) and does not consistently correlate with reactivation of BCR-ABL signaling (data not shown; 8, 9). In contrast, we noted tremendous heterogeneity in BCR-ABL protein expression and established cell lines from three IM-resistant CML patients to further...
characterize IM resistance. Two cell lines expressed BCR-ABL kinase at levels similar to those of IM-sensitive cell lines (WDT-2, -3), and only one cell line (WDT-3) overexpressed BCR-ABL protein. However, these cells retained sensitivity to IM and were similar to other BCR-ABL(+) CML cell lines. Attempts to recover IM-resistant cells from these cell lines have not yet yielded any variants, suggesting that BCR-ABL-expressing cells from clinically resistant patients do not appear to be more susceptible to expression of an IM-resistant phenotype. We conclude that IM resistance in some patients is associated with phenotypic changes or pharmacological barriers that reduce IM responsiveness or availability. It has been noted that in vitro resistance can be reversed by growth in the absence of kinase inhibitor (25, 26), and similar mechanisms may be operant in WDT-2 and -3 cells. The presence of exogenous cytokines that activate signaling pathways shared by BCR-ABL activation (interleukin-3/ granulocyte macrophage colony-stimulating factor) do not appear to inhibit IM sensitivity in WDT cells as reported by recent in vitro studies (27, 28). However, recovery of BCR-ABL kinase-deficient and signal-independent cells from an IM-resistant patient (WDT-1; an additional BCR-ABL-independent cell line from another IM-resistant patient (WDT-4) is currently being characterized] suggests that clonal expansion of BCR-ABL-independent cells must be considered as an alternate mechanism of IM resistance in some CML patients. BCR-ABL independence may be mediated through accumulation of additional cytogenetic changes as noted in the WDT-1 cell line and other studies of advanced disease (12, 29–32). IM resistance in CML patients may help expose those elements and lead to a more complete understanding of this disease.

Although CrkL phosphorylation has been used as a surrogate marker of BCR-ABL kinase activity in clinical specimens, more recent analysis suggests that p-CrkL is not a reliable marker of disease remission or progression, especially in patients without detectable BCR-ABL mutations (11). This may be because of BCR-ABL-independent regulation of CrkL phosphorylation by other kinases and cytokines (33, 34). The observations described in this report suggest that a more complex assessment of BCR-ABL gene expression and function in IM-resistant CML patients may be needed. Recovery of BCR-ABL-independent cells from IM-resistant CML patients demonstrates that the current approach in assessing the role of BCR-ABL in IM resistance may be inadequate. More direct analysis of BCR-ABL protein expression and signaling as well as identification of secondary pathways that support CML cell growth and survival in IM-resistant patients (Fig. 4) are needed. This study demonstrates that activation of src kinases and NF-κB may play a role in IM resistance in some patients. Inhibition of activated Hck by IM or CGP-76030 engaged caspase cascades in both BCR-ABL (+) and (−) CML cells, suggesting additional tyrosine kinases can serve as therapeutic targets in CML (Fig. 4C). In WDT-2 and -3 cells, a combination of CGP-76030 with IM additively enhanced apoptosis of either agent alone, suggesting inhibition of common targets (data not shown). Tyrosine
kinase inhibitors that target both abl and src kinases may circumvent IM resistance and provide more effective therapy for CML patients (35).

Although uncommon in CML, NF-kB was constitutively activated in WDT-1 cells and the originating clinical specimen (Fig. 4F) and in at least one additional specimen (and established cell line) from another IM-resistant CML patient (WDT-5). The inhibitor of nuclear factor-kB (1xK) (BAY 11–7082) was effective in blocking NF-kB activation and in suppressing growth of WDT-1 cells (Fig. 4, D and E). These observations suggest a role for constitutive NF-kB activation in growth and survival of blasts from some IM-resistant CML patients (detected in two of six IM-resistant patients examined), and initial studies demonstrate a BCR-ABL-independent NF-kB activation mechanism.

In this report, the characteristics of newly established cell lines and specimens from IM-resistant patients were described. These studies suggest that BCR-ABL-independent signaling pathways may be activated and contribute to IM resistance in some patients. Established cell lines from these patients may be valuable in defining these elements and in describing novel mechanisms of clinical resistance to targeted therapy.

REFERENCES
Corrections

In the article by N. Donato et al., titled “Imatinib Mesylate Resistance Through BCR-ABL Independence in Chronic Myelogenous Leukemia,” which appeared in the January 15, 2004 issue of *Cancer Research* (pp. 672–677), Bharat B. Aggarwal’s middle initial was omitted and Shishir Shishodia’s name was misspelled. The correct author list is as follows: Nicholas J. Donato, Ji Y. Wu, Jonathan Stapley, Hui Lin, Ralph Arlinghaus, Bharat B. Aggarwal, Shishir Shishodia, Maher Albitar, Kimberly Hayes, Hagop Kantarjian, and Moshe Talpaz.

In the article by G. Akiri et al., titled “Lysyl Oxidase-Related Protein-1 Promotes Tumor Fibrosis and Tumor Progression in Vivo,” which appeared in the April 1, 2003 issue of *Cancer Research* (pp. 1657–1666), the nomenclature used for members of the lysyl oxidase gene family did not include the gene symbols currently recognized and approved by the HUGO Gene Nomenclature Committee and Mouse Genome Informatics database. As has been reported in recent reviews (2–4), the human lysyl oxidase gene family members are referred to as LOX or LO, LOXL or LOXL1, LOXL2, LOXL3, and LOXL4.

Akiri and colleagues acknowledge that when preparing their manuscript they were aware that there were two different nomenclatures for the lysyl oxidases, as discussed in their introduction, but they chose the one not approved, as they were unaware that an official nomenclature had been adopted by the HUGO Nomenclature Committee.

We would like to thank Drs. Katalin Csiszar and Sheri F. T. Fong, of the John A. Burns School of Medicine and the Cancer Research Center of Hawaii, University of Hawaii, Honolulu, HI, for bringing this nomenclature to the attention of *Cancer Research* readers.

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Cancer Res 2004;64:672-677.

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