Brief report

Sustained complete hematologic remission after administration of the tyrosine kinase inhibitor imatinib mesylate in a patient with refractory, secondary AML

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Imatinib mesylate, a tyrosine kinase inhibitor targeting bcr-abl, platelet-derived growth factor receptor (PDGF-R), and c-Kit, effectively induces hematologic and cytogenetic remissions in bcr-abl$^+$ chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) with only mild to moderate side effects. Here, we describe the successful treatment of a 64-year-old man with c-Kit$^+$ secondary acute myeloid leukemia (AML) refractory to standard chemotherapy. Upon 2 weeks of imatinib mesylate administration, the patient achieved a complete hematologic remission in peripheral blood. In addition, complete clearance of leukemic blasts in bone marrow and a significant cytogenetic response lasting for more than 5 months was observed. Sequence analysis of exons 2, 8, 10, 11, and 17 of the c-Kit receptor did not reveal structural alterations as previously described in a subset of AML cases. This is the first report of complete remission achieved upon administration of imatinib mesylate in a patient with highly refractory, secondary AML. (Blood. 2003;101:2960-2962)

Introduction

C-Kit (stem cell factor [SCF] receptor), a member of the class III family of receptor tyrosine kinases, is expressed on mast cells, melanocytes, germ cells, intestinal cells of Cajal (ICCs), and hematopoietic progenitor cells.[1-4] Binding of SCF results in c-Kit dimerization and subsequent activation of the Janus kinase–signal transducer and activator of transcription (JAK-STAT) pathway, the phosphatidylinositol 3-kinase (PI 3-kinase), and the mitogen-activated protein (MAP) kinase promoting cell growth and differentiation.[5]

In addition to its presence in normal cells, c-Kit expression has been observed in a variety of malignant states, including mast cell tumors, small-cell lung cancer, and gastrointestinal stromal tumors (GIST).[6-8] In acute myeloid leukemia (AML), c-Kit expression can be detected in 65% to 90% of de novo cases.[9] SCF stimulation of leukemic blasts results in increased c-Kit tyrosine phosphorylation and induction of proliferation, indicating a functional role of c-Kit in AML.[9-10] In addition, some AML blasts show c-Kit activation without SCF stimulation, implicating c-Kit-activating mutations.[11]

Imatinib mesylate, a 2-phenyloaminopyrimidine derivative, has been demonstrated to specifically inhibit bcr-abl, the platelet-derived growth factor receptor (PDGF-R), and c-Kit tyrosine kinases.[12-13] Imatinib mesylate effectively induced clinical remissions in bcr-abl$^+$ chronic myeloid leukemia (CML)[14] as well as significant responses in patients suffering from GIST.[15] It has been demonstrated that imatinib mesylate inhibits SCF-induced c-Kit activation and c-Kit-dependent proliferation and abrogates the antiapoptotic effects of c-Kit activation in M-07e cells.[16] Recently, inhibition of c-Kit has been shown to result in increased expression of active caspase-3 and increased cleavage of poly (adenosine diphosphate–ribose) polymerase (PARP) in acute myeloid leukemia blasts.[17]

Here, we describe a complete hematologic remission upon imatinib mesylate administration in a patient with c-Kit$^+$ AML refractory to standard chemotherapy. Sequence analysis of exons 2, 8, 10, 11, and 17 of the c-Kit receptor from leukemic blasts of this patient did not exhibit activating mutations as described previously in a subset of AML cases.[18]

Study design

Case report

In July 2000, myelodysplastic syndrome was diagnosed in a 64-year-old white man. Bone marrow cytology revealed subtype refractory anemia with ringed sideroblasts (RARS) according to the World Health Organization (WHO) criteria. Cytogenetic analysis showed a normal karyotype. In October 2000, owing to progression to refractory anemia with excess blasts (RAEB) the patient was treated with cytosine arabinoside (Ara-C; 200 mg/m^2/d, days 1 through 5) and daunorubicin (45 mg/m^2/d, days 1 through 2). However, postchemotherapy serial bone marrow aspirates still revealed persistent RAEB. In May 2001, progression to secondary AML, French-American-British (FAB) subtype M2, was diagnosed. Fluorescence-activated cell sorter (FACS) analysis revealed 70% of bone marrow mononuclear cells CD34$^+$CD117$^+$ (c-Kit$^+$) and myeloperoxidase-positive

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(MPO+). Cytogenetic analysis showed clonogenic evolution featuring trisomy 8 (8 of 30 metaphases). The patient was treated with standard chemotherapy consisting of Ara-C (200 mg/m^2/d, days 1 through 7) and daunorubicin (45 mg/m^2/d, days 1 through 3). Bone marrow analysis on day 18 after initiation of chemotherapy showed refractory AML with 76% blasts. Therefore, a second course of chemotherapy with high-dose Ara-C (2000 mg/m^2/d, days 1 through 6) was started. Again, bone marrow analysis on day 16 revealed refractory AML with persistence of 83% blasts. On the basis of strong expression of c-Kit on approximately 40% to 50% of leukemic blasts, we decided to administer the tyrosine kinase inhibitor imatinib mesylate after written informed consent. Safety of imatinib mesylate administration was assessed by physical examinations, vital signs, and laboratory testing. Oral treatment with imatinib mesylate at a daily dose of 600 mg was started on day 21 after high-dose Ara-C. At 5 weeks after initiation of this treatment, the patient achieved a complete hematologic remission. Side effects of imatinib mesylate with a grade II skin rash were minimal. At 24 weeks after start of imatinib mesylate treatment, the patient experienced a relapse with 11% blasts in peripheral blood. Despite dose escalation up to 800 mg per day, further progression could not be prevented.

Methods

Cytochemical assays, FACS analysis, nested reverse-transcriptase–polymerase chain reaction (RT-PCR) for *BCR-ABL*, and cytogenetic studies were performed on bone marrow aspirates by means of standard methods. Immunohistochemical staining was performed on slides of paraffin-embedded bone marrow biopsies with the use of the monoclonal CD34 antibody (Immunotech, Hamburg, Germany) by the alkaline phophatase/anti-alkaline phosphatase (APAAP) method and the polyclonal p145 antihuman c-Kit (CD117) antibody, the anti-Fas (CD95) antibody (both Dako, Hamburg, Germany), the PDGF-R alpha and beta antibodies (R&D Systems, Wiesbaden, Germany), and the antiphospho-c-Kit (Tyr719) antibody (Cell Signaling Technology, Frankfurt, Germany) by the peroxidase/diaminobenzidine (DAB) method. For double staining, the APAAP and DAB method were used sequentially on the same sections. For mutation analysis, genomic DNA from bone marrow smears was extracted.[19] The *C-KIT* exons 2, 8, 10, 11, and 17 were amplified by standard PCR, as described,[20] and sequenced.

Results and discussion

Here, we present the case of a 64-year-old patient with secondary AML refractory to standard chemotherapy. Immunohistochemical analysis (Figure 1A) and FACS analysis (data not shown) revealed strong expression of c-Kit. In addition, positive staining by means of an antiphospho–c-Kit (Tyr719) antibody indicated activation of c-Kit in the majority of leukemic blasts (Figure 1C).

For this reason together with the preclinical evidence of a pathogenetic role of c-Kit signaling in AML, imatinib mesylate therapy was initiated. At this time, bone marrow blast infiltration was 83% despite completion of 2 cycles of Ara-C treatment (Figure 2).

Imatinib mesylate monotherapy resulted in a rapid increase of peripheral blood neutrophiles and platelets. Upon 2 weeks of treatment, the patient achieved a complete remission in peripheral blood (absolute neutrophil count [ANC], greater than 1.5×10^9/L [greater than 1500/μL]; platelets, greater than 150×10^9/L). At 5 weeks after the start of imatinib mesylate, a bone marrow aspirate showed complete clearance of leukemic blasts (blast cell count, 2.5%; Figure 2) with minimal myelodysplastic features. Immunohistochemical staining of bone marrow biopsies (Figure 1B) as well as FACS analysis displayed clearance of blasts positive

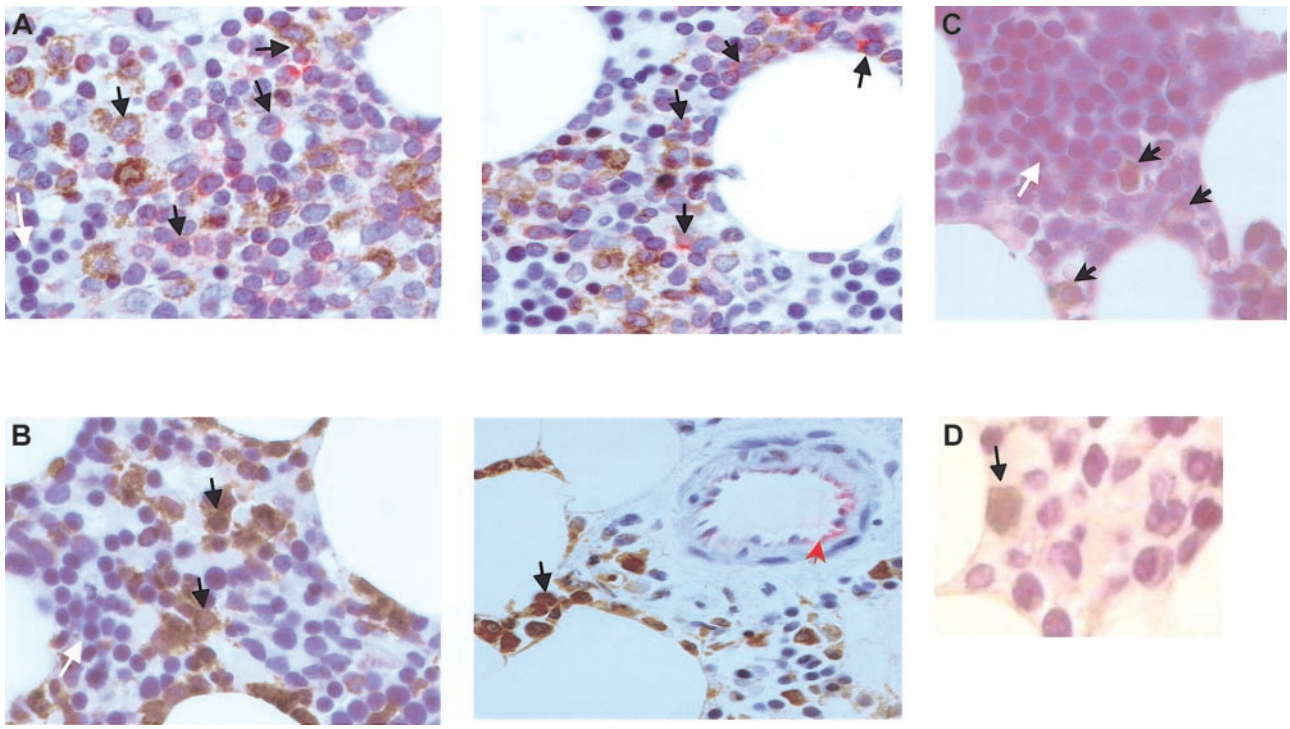

Figure 1. Immunohistochemical analysis of bone marrow biopsies. Immunohistochemistry was performed as described in detail in "Methods." (A) Approximately 40% to 50% of leukemic blasts (black arrows) showed coexpression of CD34 and CD117. Erythropoietic cells (white arrow) are negative for CD34 and CD117. (B) Day 118 after imatinib mesylate therapy: leukemic blasts with coexpression of CD34 and CD117 are not present. Some regenerating immature myeloid precursor cells (black arrows) show CD117 positivity. Endothelial cells of marrow arterioles show reactivity for CD34 (red arrow), serving as an internal positive control. Erythropoietic cells (white arrow) are negative for CD34 and CD117. (C) Positive staining by means of an antiphospho–c-Kit (Tyr719) antibody indicated activation of c-Kit. Black arrows show phospho–c-Kit+ blasts. Erythropoietic cells (white arrow) serve as internal negative control. (D) Prior to imatinib mesylate, approximately 10% of leukemic blasts showed expression of Fas (black arrow). Upon complete remission, fewer than 5% of blasts were left for analysis. In this situation, only an occasional Fas+ blast was found (data not shown). Original magnifications × 100 (A-C) and × 150 (D).

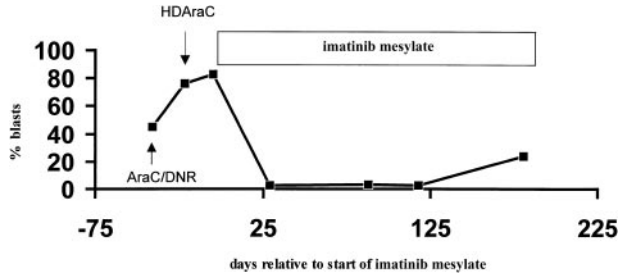

Figure 2. Bone marrow blast cell infiltration during different treatment courses. Leukemic blast cell infiltration as determined by bone marrow cytology. Duration of treatment with antileukemic drugs and imatinib mesylate is indicated at the top of the figure.

for c-Kit (2% of bone marrow mononuclear cells [MNCs] $CD34^+CD117^+$). In addition to c-Kit, immunohistochemical staining of PDGF receptors α/β and Fas was performed. Only low expression of PDGF-Rs was detected in the majority of leukemic blasts (data not shown). This result is in line with a previous report that expression of the PDGF-R does not contribute to malignant proliferation of AML.[21] At the start of imatinib mesylate treatment, 10% of blasts were $CD95^+$ (Figure 1D). Imatinib mesylate therapy did not result in up-regulation of Fas-expression (data not shown). This is in agreement with a recent study demonstrating no effects of imatinib mesylate on Fas or Fas-ligand expression.[22]

Cytogenetic analysis revealed significant reduction in trisomy 8^+ metaphases (from 8 of 30 at initial diagnosis to 1 of 30). Cytogenetics and nested RT-PCR for *BCR-ABL* have been performed at various time points during complete remission and in relapse. At no time point were leukemic clones positive for *BCR-ABL* or positive for rearrangement of PDGF receptors found.

Activation of c-Kit may be due to autocrine/paracrine signaling or to structural alterations, which confer factor-independent proliferation. In AML, activating "regulatory-type" mutations have been described in exon 8, associated with inv(16), and rarely in exon 10.[6,23] Additional activating mutations have been described in exons 2, 11, and 17.[18] Recently, a novel *C-KIT*–activating mutation Asn822Lys in exon 17 was identified in AML cells.[24] Genomic PCR testing followed by sequence analysis (data not shown) failed to detect such alterations in bone marrow samples of the patient. Together, these findings suggest that the imatinib mesylate–induced response seen in this patient is most likely due to inhibition of c-Kit signaling. However, whether c-Kit is activated because of an unidentified mutation, autocrine production of Kit-ligand, or exposure to Kit-ligand on stromal cells cannot be determined.

In conclusion, this is the first report of a sustained complete hematologic remission upon administration of the tyrosine kinase inhibitor imatinib mesylate in a patient with $bcr\text{-}abl^-$, $c\text{-}Kit^+$ AML. Targeting c-kit might be a promising therapeutic option for a subset of c-Kit–dependent AMLs. To test these hypothesis, we recently initiated a clinical phase 2 trial.

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