Effects of Signal Transduction Inhibitor 571 in Acute Myelogenous Leukemia Cells

Barbara Scappini, Francesco Onida, Hagop M. Kantarjian, Li Dong, Srdan Verstovsek,1 Michael J. Keating, and Miloslav Beran2

Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

ABSTRACT

STI571 is a 2-phenylaminopyrimidine derivative that inhibits c-abl, Bcr-Abl, and platelet-derived growth factor receptor tyrosine kinases. Recently, inhibition of stem cell factor (SCF)-induced c-kit phosphorylation and cell proliferation by STI571 was reported in the human myeloid cell line MO7e. Because ~70% of acute myelogenous leukemia (AML) cases are c-kit positive, we evaluated in vitro effects of STI571 on c-kit-positive cell lines and primary AML blast cells. At concentrations >5 μM, the drug marginally inhibited SCF-independent proliferation of cell lines and most of AML blasts. Treatment of AML cells with cytarabine and STI571 showed synergistic effect at low concentrations. Western blotting analysis documented a distinct band of STI571 showed synergistic effect at low concentrations. Western blotting analysis documented a distinct band of STI571 showed synergistic effect at low concentrations. Western blotting analysis documented a distinct band of STI571 showed synergistic effect at low concentrations.

INTRODUCTION

Cytokines, growth factors, or antibodies induce cellular responses, such as proliferation, maturation, apoptosis, or other pleiotropic functions, by means of signal transduction pathways, which involve different tyrosine kinases (1). Experimental models have shown how aberrations in tyrosine-kinase cascades are involved in the pathogenesis of diseases (2). Thus, protein kinases represent an ideal target for novel therapeutic strategies, especially in cancer treatment. One well-known example of a disease characterized by abnormalities in a tyrosine kinase is CML1. This pathological entity is characterized by a reciprocal translocation between chromosomes 9 and 22 (3); the resulting fusion gene, termed BCR-ABL, codes for a M, 210,000 chimeric protein with spontaneous tyrosine-kinase activity, which is responsible for transformation of CML clones (4, 5). STI571 (formerly CGP57148B) is a phenylaminopyrimidine derivative designed to be a potent, selective ATP-competitive inhibitor of the Abl tyrosine kinase. It specifically inhibits the c-abl, Bcr-Abl (6), and platelet-derived growth factor tyrosine kinases (7). In vitro, this drug blocked proliferation and induced apoptosis of BCR-ABL-positive cell lines (6) and of leukemic cells isolated from patients with CML (8, 9). In clinical trials in CML, STI571 effectively induced complete hematological and cytogenetic remissions in chronic-phase CML and temporarily controlled the disease in its accelerated and blastic phases (10–12).

Recently, STI571 has been reported to inhibit the phosphorylation of c-kit, the receptor for SCF (13). C-kit is a M, 145,000 transmembrane tyrosine kinase receptor that is structurally related to platelet-derived growth factor receptor and plays a fundamental role in hematopoiesis (14–16). The c-kit receptor has also been found in a number of other tissues (17–24) and in subsets of early human thymocytes (17) and may have a role in T-cell differentiation (18). Its activity is essential for normal cellular proliferation and maturation. Many human cancers have abnormal expression or activation of c-kit (25–29). Among the acute leukemias, 4% of early T lymphoblastic leukemia are c-kit positive (30), whereas c-kit expression is present in 65–90% of de novo AML cases (31–33) and represents a near-specific marker for myeloid lineage. Many human leukemic cell lines, especially myeloid, erythroid, and megakaryoblastic cell lines, are c-kit positive (34, 35). Nevertheless, the exact role and functional status of this receptor in these cells remain unknown. Heinrich et al. (13) demonstrated in MO7e c-kit-positive...
cell line that STI571 is able to inhibit SCF-dependent c-kit phosphorylation, with a consequent inhibition of cell proliferation.

The purpose of this study was to investigate the effects of STI571 in c-kit-positive cell lines and in AML-derived blast cells to verify whether this finding is a common phenomenon in c-kit-positive AML cells. Such information would be important for therapeutic exploration of STI571 in c-kit-positive malignancies. We found that STI571 was marginally effective in inhibiting cell growth and only at relatively high concentrations. In our experimental models, we did not observe SCF-independent c-kit phosphorylation. In a c-kit-positive cell line and in patient samples, c-kit phosphorylation was not induced by its specific ligand, suggesting deficient function of this pathway. A notable exception was the MO7e cell line, in which inhibition of SCF-induced c-kit phosphorylation by STI571 was also associated with the inhibition of cell growth. Our results suggest that in AML cells, c-kit is not spontaneously phosphorylated in the absence of SCF, and SCF seems to play only a marginal, if any, role in sustaining growth of leukemic cells.

MATERIALS AND METHODS

Drugs. STI571 was provided by Ciba-Geigy, now Novartis (Basel, Switzerland), and stored as a powder or as a 10 mM stock concentration in DMSO at −20°C. Cytarabine was initially dissolved in PBS. Fresh working solutions in PBS were prepared before each experiment.

Myeloid Human c-kit-positive Cell Lines. The MO7e cell line was kindly provided by Dr. Ralph B. Arlinghaus (M. D. Anderson Cancer Center). The in vitro growth of MO7e cells was growth factor dependent; either GM-CSF, IL-3, or SCF supported the continuous growth. MO7e cells were maintained in IMDM with 10% FCS and 10 units/ml IL-3 (Stem Cell, Vancouver, Canada). The percentage of c-kit-positive cells was 94% by flow cytometry. AML-4, an originally GM-CSF-dependent human myeloid leukemia cell line, was kindly provided by Dr. Emil J. Freireich (M. D. Anderson Cancer Center); these cells were cultured in IMDM supplemented with 10% FCS and 1 ng/ml GM-CSF (Stem Cell). The percentage of c-kit-positive cells was 76% by flow cytometry. As a negative control, we used human acute lymphoblastic leukemia-derived cell line Z119, kindly provided by Dr. Zeev Estrov (M. D. Anderson Cancer Center), which does not express c-kit receptor.

Primary Leukemic Cells. Primary leukemic cells were obtained from peripheral blood of 12 patients with AML, 2 of whom had chemotherapy-refractory AML. 3 had relapsed AML, 5 had newly diagnosed (untreated) AML, and 2 had newly diagnosed (untreated) APL (Table 1).

Leukemic cells from peripheral blood were isolated using Ficoll-Paque (density 1.077 grams/ml; Amersham, Arlington Heights, IL) and analyzed for expression and phosphorylation of c-kit immediately (patients 1–12) or after 30-min culture in IMDM, supplemented with 15% FCS and 5% PHA-LCM (Stem Cell); patients 1–10; or after 24 h in StemSpan SF expansion medium (Stem Cell), supplemented with 100 ng/ml SCF, 20 ng/ml IL-3, 20 ng/ml IL-6, and 50 ng/ml Flt-3 ligand (Stem Cell).

Flow Cytometric Analysis. As a part of patient work-up, the immunophenotype of leukemic cells was determined routinely with a panel of antibodies. The c-kit expression (CD 117) was assessed by YB5.B8 monoclonal antibody (BD PharMingen, San Diego, CA). The staining was performed according to the manufacturer’s recommendations. The percentage of c-kit-positive cells applies to the blast cell population gated by forward and side light scatter. No attempt was made to (semi) quantify the intensity of the immunofluorescence staining. Although this particular antibody is useful for in-flow cytometric analysis, it is not useful for Western blotting assays.

Proliferation Assays. Cells were plated in triplicate in 96-well plates at a concentration of 0.4 × 10^6/ml for cell lines and 1 × 10^6/ml for primary leukemic cells, in their respective media. Leukemic cell lines were grown in IMDM supplemented with 10% FCS. Leukemic cells from patients 1–10 were grown in IMDM supplemented with 15% FCS and 5% PHA-LCM (Stem Cell), whereas AML cells from patients 11 and 12 were grown in StemSpan SF expansion medium supplemented with SCF, IL-3, IL-6, and Flt-3 ligand as described above. Drugs were added at various concentrations (0.5, 2.5, 5, and 10 μM). After 72 h of exposure at 37°C in 5% CO2 in air, the impact of STI571 on cell proliferation was measured with the MTT (Sigma Chemical Co., St. Louis, MO) colorimetric dye reduction method. Inhibition of proliferation was evaluated as a percentage of control growth (no drug in the sample).

Alternatively, cells from two AML patients (11 and 12) were plated in triplicate into 24-well plates at a concentration of 1 × 10^6/ml and exposed at different concentrations of STI571 (0.5, 5, and 10 μM); viable cells were counted after staining with trypan blue at 24, 48, and 72 h. The results were expressed as the absolute numbers of viable cells.

Immunoprecipitation and Western Blotting Analysis. Cells were lysed in lysis buffer, suspended in SDS loading buffer, and loaded onto an 8% gel for 30 min. Alternatively, cells from two AML patients (11 and 12) were plated in triplicate into 24-well plates at a concentration of 1 × 10^6/ml and exposed at different concentrations of STI571 (0.5, 5, and 10 μM); viable cells were counted after staining with trypan blue at 24, 48, and 72 h. The results were expressed as the absolute numbers of viable cells.

Immunoprecipitation and Western Blotting Analysis. Cells were lysed in lysis buffer, supplemented with 15% FCS and 5% PHA-LCM (Stem Cell), supplemented with 100 ng/ml SCF, 20 ng/ml IL-3, 20 ng/ml IL-6, and 50 ng/ml Flt-3 ligand (Stem Cell).

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acrylamide gel (29:1). Alternatively, whole-cell lysates were suspended in an equal volume of 2 × SDS loading buffer and separated by SDS-PAGE on an 8% acrylamide gel. Each gel was electroblotted onto a polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA). The following antibodies were used for Western blotting: anti-c-kit (K69; Neomarkers), anti-α tubulin (Sigma Chemical Co.), and antiphosphotyrosine (RC20 monoclonal; Transduction Laboratories, Lexington, KY). Each membrane was incubated with primary antibody overnight at 4°C and then incubated again with secondary antibody (anti-mouse horseradish peroxidase-conjugated antibody; Bio-Rad, Hercules, CA) at room temperature for 2 h. Detection was performed by electrochemiluminescence (Amersham).

To additionally assure that band(s) identified in Western blots by monoclonal anti-c-kit antibody indeed represented c-kit protein, we used Z119 cells, which do not express c-kit receptor (see above) as negative control.

**Induction of c-kit Phosphorylation by Growth Factors and its Inhibition by STI571.** Exponentially growing leukemic cell lines were washed twice with PBS, starved overnight in SF medium, and restimulated for 15 min by the addition of SCF 200 ng/ml.

Primary AML cells were isolated with Ficoll-Paque, washed twice with PBS, and cultured in IMDM, supplemented with 15% FCS with or without 5% PHA-LCM for 30 min. Alternatively, the cells were starved for 4 h in SF IMDM and stimulated with 200 ng/ml SCF for 15 min.

In patients 11 and 12, cells were grown for 24 h in Stem-Span SF expansion medium supplemented with the above described cocktail of hematopoietic factors. After washing and starving for 6 h in StemSpan SF medium, the cells were restimulated with 200 ng/ml SCF for 15 min.

In all these different experimental conditions, cells were processed by Western blotting for the level of expression of c-kit and its phosphorylation status.

**Evaluation of Drug Combinations.** The activity of the drugs, used singly or in combination, was estimated by a computer-assisted analysis using the CalcuSyn software program (Biosoft, 1996). Briefly, this software calculates ED₅₀, performs isobologram analyses, and calculates the CI, a quantitative measure of the degree of drug interactions. For each given end point, a CI of 1 indicates an additive effect, a CI of <1 indicates a synergistic effect, and a CI of >1 indicates an antagonistic effect. Calculations of the CI were made under the assumption that the mechanisms of action of the two drugs were not mutually exclusive.

**RESULTS**

**Cell Proliferation.** The growth of MO7e leukemia cell line was completely dependent on the presence of IL-3, GM-CSF, or SCF. The growth rates were comparable with each of these factors. When grown for 3 days in the presence of 10% FCS, either alone or supplemented with SCF (200 ng/ml), IL-3 (10 units/ml), or GM-CSF (10 ng/ml), MO7e cells grew only in the presence of these growth factors. No differences in the cell yield were observed using alternative factors (Fig. 1A). In contrast, AML-4 cells grew in the presence of FCS alone and were independent of growth factors. In fact, the addition of SCF (200 ng/ml) or IL-3 (10 units/ml) failed to increase cell proliferation, whereas the presence of GM-CSF (10 ng/ml) had a marginally enhancing effect (Fig. 1B).

We then evaluated the effect of STI571 on IL-3-dependent and SCF-dependent MO7e proliferation. The addition of STI571 did not affect cell growth when cells were cultured with IL-3 (Fig. 2A) but suppressed proliferation significantly when cells were cultured with SCF (Fig. 2B). The addition of both IL-3 and SCF to the culture medium partially ameliorated the antiproliferative effects of STI571 (Fig. 2C).

In c-kit-positive AML-4 cells, the addition of increasing concentrations of STI571 had no significant effect on cell proliferation, both when they were grown in the presence of GM-CSF (10 ng/ml) and SCF (Fig. 3).

In primary AML-derived blast cells (patients 1–10) cultured in the presence of PHA-LCM, STI571 inhibited cell growth minimally at high concentrations (Fig. 4). No correlation was found between the extent of inhibition and percentage of c-kit-positive cells, detected by flow cytometry (P = 0.7, data not shown). The addition of cytarabine to STI571 at a ratio of 1:1 produced further growth inhibition, with a CI suggesting an enhancing effect of STI571 and a possible synergism between the two drugs in a range concentration between 0.5 and 5 μM (Fig. 5 and Table 2).

Finally, cells from the 2 patients (11 and 12) who had a high level of c-kit expression were cultured in a StemSpan SF media containing SCF alone (Fig. 6A) or in combination with IL-3, IL-6, and Flt-3 ligand (Fig. 6B). After trypan blue staining, the cell viability was assessed at 24, 48, and 72 h.
showed an active growth of leukemic cells with a 150–250% increase in the cell numbers after 72 h in the untreated cultures. In agreement with the extent of STI571 inhibition observed in cultures using IMDM supplemented with FCS and PHA-LCM, also in these culture conditions, STI571 inhibited cell proliferation only marginally at high concentrations. No differences in the inhibitory effects were noted between cell cultures in which the cell growth was sustained by SCF alone or by combination of SCF with additional hematopoietic factors.

C-kit Expression and Phosphorylation. C-kit expression was evaluated by Western blotting analysis in cell lines and in blast freshly derived from 12 AML patients. Results are shown in Fig. 7. A distinct band of Mr 145,000 corresponding to c-kit was detected in AML cell lines (Fig. 7 A) and in all AML-derived blast cells (Fig. 7 B). In some samples, two bands instead of one were observed, as a likely result of alternative splicing. Bands of comparable intensity were observed in AML samples with a low or high percentage of c-kit-positive cells identified by flow cytometry. The band(s) was absent in Z119 cells not expressing c-kit, both in the whole lysate and after immunoprecipitation (Fig. 7 C), confirming the specificity of the antibody against c-kit.

Western blotting with antiphosphotyrosine antibodies indicated that c-kit was not phosphorylated in MO7e cells cultured with IL-3 (Fig. 8 A) or in AML-4 cells grown in the presence of GM-CSF (Fig. 8 B). Findings were similar for the primary leukemia cells cultured for 30 min with 15% FCS, with or without 5% PHA-LCM and with or without 10 μM STI571, showing that c-kit was not phosphorylated in any of the standard culture conditions (Fig. 8 C). The addition of hematopoietic growth factors known to be present in the PHA-LCM failed to induce c-kit phosphorylation. The addition of PHA-LCM with or without STI571 had no effect on c-kit protein expression in leukemic cells (Fig. 8 D).

Incubation of MO7e cells for 15 min in the presence of 200 ng/ml SCF induced c-kit phosphorylation, as documented by Western blotting with antiphosphotyrosine. Pretreatment with STI571 for 90 min at concentrations as low as 1 μM effectively inhibited SCF-induced c-kit phosphorylation. Western blotting of the same membranes with anti-c-kit antibodies showed no effect of STI571 on the amount of c-kit protein (Fig. 9 A).

In contrast, in the AML-4 cell line, we were unable to detect any c-kit phosphorylation even after intense stimulation with SCF. Like in MO7e cells, c-kit protein expression in AML-4 cells was not affected by STI571 (Fig. 9 B).

After 4 h of starving in SF medium, we incubated c-kit-expressing, patient-derived leukemic blasts in the presence of various concentrations of STI571 (0–10 μM). After 90 min, the cells were stimulated with 200 ng/ml SCF for 15 min. Western blotting analysis with antiphosphotyrosine antibody revealed no induction of c-kit phosphorylation. No changes in the level of c-kit expression were observed in the presence of STI571 (Fig. 10 A).

To expand additionally on the results with primary leukemic cells, we conducted experiments in which AML cells were growing in presumably more optimal culture conditions in a
well-defined culture medium. Cells from 2 patients (11 and 12) were cultured for 24 h in a medium containing SCF, IL-3, IL-6, and Flt-3 ligand; washed; starved for 6 h in a StemSpan SF medium; exposed to STI571 10 μM for 90 min; and then stimulated with SCF for 15 min (Fig. 11B). In agreement with the above mentioned results, obtained in experiments with freshly derived AML cells from the same patients, we were unable to detect any c-kit activation after stimulation with its specific ligand.

**DISCUSSION**

Human AML samples (~65–90%) express c-kit, and this marker is used routinely to diagnose myeloid commitment. However, the exact role of c-kit in the pathophysiology of AML remains uncertain. Abnormalities in c-kit expression and function are assumed to play an important role in the pathogenesis of several human cancers, including small-cell lung cancer (23), neuroblastoma (24), breast cancer (26), melanoma (26), testicular cancer (27), and mast cell disorders (36). Two principal mechanisms of c-kit dysregulation in human malignancies have been described. In some cases, autocrine or paracrine production of SCF abnormally stimulates this receptor; in other cases, mutations that affected the c-kit polypeptide sequence result in continuous, SCF-independent phosphorylation of this kinase (23–26). The role of the mutated c-kit has been best defined in human isolated mastocytosis (36–44). Many, but not all, cases of mastocytosis are associated with mutations of c-kit leading to SCF-independent autophosphorylation. The most frequent mutation seems to involve codon 816 in the cytoplasmic domain of the receptor (37, 38), but mutations in codon 560 have been
been plotted using the average growth inhibition induced by the two drugs in 10 patient samples. According to this method, synergism is indicated by a CI < 1, additive effect is indicated by a CI = 1, and antagonism is indicated by a CI > 1. B, representation of the combined effect of STI571 and cytarabine in AML patient-derived cells. The graph has been plotted using the average growth inhibition induced by the two drugs in 10 patient samples.

Table 2  Effects of the combination of STI571 and cytarabine on the proliferation of AML-derived blast cells from 10 patients

<table>
<thead>
<tr>
<th>STI571 (µM)</th>
<th>Ara-C (µM)</th>
<th>Cell proliferation (% control ± SD)</th>
<th>Fraction affected</th>
<th>CIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>70.65 ± 22.88</td>
<td>0.29</td>
<td>0.7 + + +</td>
</tr>
<tr>
<td>2.5</td>
<td>5</td>
<td>44.73 ± 23.99</td>
<td>0.55</td>
<td>0.8 +</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>36.98 ± 24.94</td>
<td>0.63</td>
<td>1.1 ±</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>31.68 ± 20.96</td>
<td>0.68</td>
<td>1.7 ---</td>
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*a Computed with the use of CalcuSyn software program. <0.1 (+ + + +), very strong synergism; 0.1–0.3 (+ + +), strong synergism; 0.3–0.7 (+ + +), synergism; 0.7–0.85 (+ +), moderate synergism; 0.85–0.9 (+), slight synergism; 0.9–1.10 (+ +), nearly additive; 1.10–1.20 (+), slight antagonism; 1.20–1.45 (- --), moderate antagonism; 1.45–3.3 (-- --), antagonism; 3.3–10 (------), strong antagonism; >10 (------), very strong antagonism.

found (38). Mastocytosis has been associated occasionally with myelodysplastic or myeloproliferative disorders, as well as with acute myeloid leukemias (43–48). C-kit mutations have been described in cases of mastocytosis associated with AML (47–49), and mutations that activated c-kit have also been found in cases of AML not associated with mastocytosis as well (50–52). Gary et al. (51) found c-kit deletion-insertion mutations in 7 of 21 patients with the inversion 16 karyotype and a c-kit point mutation at codon 530 in 1 patient with translocation t(8;21). In another study of 33 patients with AML, one case of c-kit816V mutation was described (52). Nevertheless, many AML cases express c-kit without having any documented mutation of this receptor. Thus, the importance of this kinase in maintaining blast proliferation and survival remains uncertain.

C-kit tyrosine kinase was shown to be one of the sensitive targets for the tyrosine kinase inhibitor STI571 (8). Therefore, this drug may have a therapeutic potential in AML. STI571 was reported recently to inhibit proliferation of a c-kit-positive AML cell line, MO7e, and this effect on cell growth was associated with inhibition of c-kit phosphorylation (13). In this study, we examined the effects of STI571 on cell proliferation, c-kit expression, and c-kit phosphorylation in two c-kit-positive AML cell lines and primary leukemic cells from 12 patients with AML.

We used MO7e cell line as a model for c-kit-STI571 interactions. MO7e growth was reported to be dependent on the presence of c-kit ligand SCF (53). In our MO7e cells, cell growth was equally facilitated by two other hematopoietic growth factors, IL-3 and GM-CSF. This allowed us to investigate the effects of STI571 in situations when growth was mediated by alternative ligand receptor-dependent pathways. In this cell line, STI571 inhibited SCF-dependent cell growth in a dose-dependent fashion, as reported recently by Heinrich et al. (13), and this inhibition was associated with the suppression of c-kit phosphorylation. In contrast, IL-3-dependent proliferation of MO7e cells was not inhibited by STI571. Indeed, the effect of STI571-mediated inhibition of SCF-stimulated MO7e cell growth was diminished in the presence of IL-3. This observation suggests that in the presence of an inhibitor of c-kit tyrosine kinase, these cells may use alternative growth-regulating signal transduction pathways to escape the inhibitory effect of growth inhibitor(s).

In the autonomously growing AML-4 cell line, the addition of SCF failed to influence cellular proliferation or phosphorylation of c-kit. Consistent with these findings, STI571 was unable to inhibit cell growth.

As in the c-kit-positive, autonomously growing AML-4 cell line, STI571 was largely ineffective in inhibiting cell proliferation of blast cells, freshly derived from patients with AML, also when they were growing actively under optimal conditions in a StemSpan SF medium containing SCF alone or in combination of SCF with IL-3, IL-6, and Flt-3 ligand. In a few cases, high concentrations of STI571 reduced cell growth by ≤30% of the control, but this effect was not related to the frequency, in the leukemic population, of c-kit-positive cells identified by flow cytometry. The analysis performed by CalcuSyn, however, suggested that in this in vitro system, STI571 could enhance the antiproliferative effects of cytarabine at low doses of both drugs.

Western blotting with antiphosphotyrosine antibodies revealed no autophosphorylation of c-kit in primary AML cells analyzed in ≤4 h after obtaining the sample and isolating the cells or grown for a short period of time in standard cultures supplemented with FCS and PHA-LCM. Unexpectedly, no c-kit phosphorylation was found in either AML-4 cells or in primary leukemic cells expressing high levels of c-kit protein even after SCF stimulation, suggesting that under these conditions, the c-kit receptor was expressed but not functionally active. A
Fig. 6 Growth of leukemic blast cells derived from peripheral blood of 2 patients with c-kit-positive AML (#11 and #12) in the presence of increasing concentrations of STI571. AML cells were plated at 1 × 10^6 cells/well in a StemSpan SF medium containing 100 ng/ml SCF (A) or 100 ng/ml SCF (B), 20 ng/ml IL-3, 20 ng/ml IL-6, and 50 ng/ml Flt-3 ligand. Viable cells were counted at 24, 48, and 72 h after trypan blue staining. Each point is a mean of triplicate cultures.

Fig. 7 Expression of c-kit by Western blotting in two leukemic cell lines (A) and five AML-derived blast cells (B). Whole-cell lysates were immunoblotted with anti-c-kit (1) and anti-α-tubulin antibodies (2) as a control for protein loading. The numbers in parenthesis denote the percentage of c-kit-positive cells in respective populations and identified by flow cytometry. In C, Western blot against c-kit was performed in M07e and in Z119 after immunoprecipitation against c-kit.
A possible explanation coming to mind is that, under suboptimal culture conditions in media supplemented with FCS and no assured presence of the necessary hematopoietic factors in PHA-LCM, the primary leukemic cells fail to thrive/proliferate and are, thus, unable to phosphorylate c-kit. Such objection appears to be invalidated by experiments which documented active growth not only by the MTT assay but by an increase in the absolute numbers of viable leukemic cells in a defined SF medium developed for the human stem cell expansion. Such medium is likely to be the best available for the primary leukemic cells as well. Moreover, STI571 did affect the level of expression of c-kit protein neither in cell lines nor in AML-derived blast cells. C-kit was phosphorylated only in MO7e cells and only after intense SCF stimulation. This experimental system hence appears unique and not representative of the situation in primary, c-kit-positive leukemic cells.
Given the general lack of the effect of STI571 on AML cell growth in this study, we conclude that c-kit plays only a marginal role in sustaining proliferation of AML cells. Analysis of c-kit phosphorylation confirmed that this kinase was neither activated by SCF in freshly isolated AML cells nor in AML blast cells growing under standard growth conditions or in defined, SF expansion media. Such c-kit activation, associated with the inhibition of cell proliferation, was observed only in a unique experimental model represented by the MO7e cell line. The biological basis for the aberrant/deficient interaction between c-kit and its specific ligand is currently unknown and is the subject of ongoing investigation. Additional studies of c-kit-positive acute leukemias are necessary to identify specific situations in which presumably aberrant c-kit expression could be directly involved in the pathogenesis of the malignancy or in cell proliferation. Only in such cases could STI571 represent an effective new treatment.

REFERENCES


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