Priority Report

BCR-ABL–Induced Deregulation of the IL-33/ST2 Pathway in CD34(+) Progenitors from Chronic Myeloid Leukemia Patients

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Abstract

Although it is generally acknowledged that cytokines regulate normal hematopoiesis in an autocrine/paracrine fashion, their possible role in chronic myelogenous leukemia (CML) and resistance to imatinib mesylate treatment remain poorly investigated. Here, we report that CD34(+) progenitors from patients with CML at diagnosis are selectively targeted by the cytokine/alarmin interleukin (IL)-33. Indeed, CML CD34(+) progenitors upregulate their cell surface expression of the IL-33–specific receptor chain ST2, proliferate and produce cytokines in response to IL-33, conversely to CD34(+) cells from healthy individuals. Moreover, ST2 overexpression is normalized following imatinib mesylate therapy, whereas IL-33 counteracts in vitro imatinib mesylate-induced growth arrest in CML CD34(+) progenitors via reactivation of the STAT5 pathway, thus supporting the notion that IL-33 may impede the antiproliferative effects of imatinib mesylate on CD34(+) progenitors in CML. Clinically, the levels of circulating soluble ST2, commonly considered a functional signature of IL-33 signaling in vivo, correlate with disease burden. Indeed, these elevated peripheral concentrations associated with a high Sokal score predictive of therapeutic outcome are normalized in patients in molecular remission. Finally, we evidenced a facilitating effect of IL-33 on in vivo maintenance of CD34(+) progenitors from patients with CML by using xenotransplant experiments in immunodeficient NOG mice, and we showed that engraftment of mouse BCR-ABL–transfected bone marrow progenitors was less efficient in IL-33–deficient mice compared with wild-type recipients. Taken together, our results provide evidence that IL-33/ST2 signaling may represent a novel cytokine-mediated mechanism contributing to CML progenitor growth and support a role for this pathway in CML maintenance and imatinib mesylate resistance. Cancer Res; 74(10): 2669–76. © 2014 AACR.

Introduction

Chronic myelogenous leukemia (CML) is a well-characterized myeloproliferative disorder, initiated by the presence of the Philadelphia chromosome generating the BCR-ABL oncogene, Poitiers;12CNRS, Institut de Pharmacologie et de Biologie Structurale; and13Université Paris-Sud 11, Orsay; 3INSERM U1082; 4Université de Poitiers; 5Service d’Immunologie et d’Inflammation; 6CHU de Poitiers; 7Etablissement Français du Sang Centre-Atlantique, site de Poitiers; 8Service d’Oncologie Hématologique et Thérapie Cellulaire; 9INSERM-CIC1402; 10Service de Cancérologie Biologique; 11Service d’Hémato logie et d’Oncologie Biologique, Poitiers; 12CNRS, Institut de Pharmacologie et de Biologie Structurale; and 13Université de Toulouse, Toulouse, France

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primary progenitors (11), constitute its natural targets, we hypothesized that IL-33 might enhance proliferation and/or mediate imatinib mesylate resistance in CML CD34(+) cells. We found that the IL-33 receptor ST2 was constitutively expressed and was functional in leukemic CD34(+) cells because it mediated the proliferation and cytokine production induced by IL-33, whereas CD34(+) cells from healthy donors (HD) neither expressed ST2 nor responded to IL-33. Based on these findings, we further investigated the relationship between BCR-ABL expression and responsiveness to IL-33 as well as its relevance to imatinib mesylate resistance.

Patients and Methods

Patient samples
Twenty-two patients with CML in chronic phase at diagnosis (CML-CP) and sixteen patients with CML currently treated with imatinib mesylate who have achieved major molecular remission [MMR-IM or BCR-ABL/ABL (IS) ≤0.1%; Oncology-Hematology Department, Poitiers, France] were included in this study. All patients gave informed consent in accordance with the Declaration of Helsinki for participation in the study that was approved by the scientific committee of the INSERM CIC1402 (Poitiers, France). The 15 healthy individuals were volunteers from the Pôle Biologie Santé (Poitiers, France). Peripheral blood mononuclear cells (PBMC) were isolated from blood samples collected on heparin from patients with CML or healthy donors mobilized with granulocyte colony-stimulating factor (G-CSF), and CD34(+) cells were further purified using an immunomagnetic cell sorting system (Miltenyi Biotech). Purity was generally above 95% upon reanalysis.

Mice
Six- to 9-week-old female wild-type (Janvier) and IL-33-deficient C57BL/6 mice (12), and NOG mice (IGR) were maintained in our animal facilities under specific pathogen-free conditions. All procedures were approved by the Animal Care Use Committee of Poitou-Charentes (CEEA, n° CE2012–018).

Engraftment of human cells in immunodeficient mice
Enriched primary CD34(+) cells (1 × 10^6) from patient with CML-CP were incubated in serum-free expansion medium (Stem Cell Technologies) supplemented with granulocyte macrophage colony-stimulating factor (GM-CSF: 200 pg/mL), stem cell factor (SCF: 200 pg/mL), G-CSF (1 ng/mL), and IL-6 (1 ng/mL) and with or without IL-33. After 3 days, recovered cells (5 × 10^5) were transplanted intravenously into NOG mice.

Retroviral mouse model of BCR-ABL CML-like syndrome
The retroviral vector MIGR-p210-BCR-ABL as well as the methodologic details of the murine bone marrow retroviral transduction and transplantation model of CML-like myeloproliferative neoplasm have been described in detail elsewhere (13).

Cell cultures
CD34(+) cell cultures were performed in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics. Enriched primary CD34(+) cells from patients with CML-CP or healthy donors were seeded into 96-well round (U)-bottom culture plates (2.5 × 10^4 per well) and incubated with the indicated compound(s).

In vitro treatment with cytokines and inhibitors
Human IL-33 (50 ng/mL), neutralizing anti-human GM-CSF monoclonal antibody (mAb; 1 µg/mL), and anti-human ST2 mAb (1 µg/mL), and SCF (50 ng/mL) were purchased from R&D Systems and Peprotech, respectively. AG490 (0.05 µmol/L) was purchased from Calbiochem. Imatinib mesylate (Glivec, Novartis) was used at 1 to 2.5 µmol/L depending on the time of exposure.

CFC assays
After a 3-day liquid-phase culture of CD34(+) cells with or without IL-33, remaining cells were harvested and seeded (1 × 10^7/25-mm dish) in methyl-cellulose supplemented with cytokines (MethoCult H4434, Stem Cell Technologies), and emerging colonies were counted 14 days later.

ELISA and Luminex
Luminex technology was used according to the manufacturer’s instructions to measure GM-CSF, IL-6, and IL-8 in culture supernatants (R&D Systems) and phosphoproteins in cell lysates (Millipore). Soluble ST2 levels were quantified in plasma by ELISA (R&D Systems).

Cell proliferation
For the quantification of CD34(+) cell proliferation, a bromodeoxyuridine (BrdUrd) colorimetric cell proliferation immunoassay was used (Roche). An 18-hour pulse with BrdUrd was performed, and BrdUrd incorporation was quantified by measuring the absorbance (O.D.), as recommended by the manufacturer.

FACS analysis
CD34(+) cells were stained with the following antibodies: anti-CD34-PE-Cy7, anti-CD38-FITC, and anti-STAT5-Alexa Fluor 647 (all from BD Biosciences), and anti-ST2-PE mAbs (R&D Systems). Membrane labeling as well as intracellular p-STAT5 staining were performed as previously described (3). A minimum of 1 × 10^5 viable cell events were acquired in the population of interest. For the quantification of apoptosis, CD34(+) cells were resuspended at room temperature for 15 minutes with Annexin V-FITC and 7-aminoactinomycin D (both from BD Biosciences) and then analyzed immediately. Cells were analyzed by six-color flow cytometry (FACSCanto II, FACSVerse, and FacsDivA software, BD Biosciences), and data were reanalyzed with FlowJo Software (Tree Star). Positive staining for each marker was determined by comparison with appropriate isotype-matched negative controls.

Statistical analysis
All statistical analyses used Prism 5 (GraphPad software Inc.). The statistical significance of differences in mean values was analyzed by the two-tailed Mann–Whitney nonparametric
Results and Discussion

The kinase activity of BCR-ABL upregulates ST2 cell surface expression on CD34(+) cells from patients with CML-CP and UT-7 cells

IL-33 mediates its biologic effects by stimulating the heterodimeric receptor ST2/IL-1RAcP (7). Knowing that IL-1RAcP is upregulated in primitive CML-CP CD34(+) cells (personal data; ref. 14), we first addressed the question whether this applied likewise to its coreceptor ST2. Flow-cytometric analysis revealed substantial ST2 expression on gated CD34(+) PBMCs from most patients with CML-CP examined, converse-ly to those recovered from healthy donors, which displayed this receptor at very low or undetectable levels (Fig. 1A and B). Importantly, both hematopoietic stem cell-enriched CD34(+) CD38(-) and CD34(+)CD38(+) subsets harbored ST2 contrasting to their counterparts from healthy donors (Fig. 1C). In accordance with a possible contribution of BCR-ABL TK activity to ST2 upregulation, we observed a remarkable reversal of ST2 overexpression, back to healthy donor levels, in patients having achieved MMR with imatinib mesylate therapy (Fig. 1A and B) as well as among gated CD34(+) PBMCs from patients with CML-CP after a 4-hour incubation with imatinib mesylate (Fig. 1D).

To confirm that ST2 could be induced as a corollary of oncogene expression, we transduced UT-7 cells with a retrovirus encoding BCR-ABL or with an empty control and found that ST2 was effectively upregulated on the surface of these transfected cells (Supplementary Fig. S1A). Conversely, exposure to imatinib mesylate led to a significant downregulation of ST2 on BCR-ABL–transduced UT-7 cells but not on their imatinib mesylate-resistant mutant T315I BCR-ABL–transduced controls (Supplementary Fig. S1B; ref. 15). Taken together, these findings show that CML-CP CD34(+) cells overexpress ST2 receptors and designate BCR-ABL TK activity as a plausible mechanism of upregulation.

CD34(+) cells from patients with CML-CP proliferate and produce cytokines in response to IL-33

The constitutive expression of ST2 and IL-1RAcP by CML-CP CD34(+) cells implied that IL-33 could exert a function in these cells. In support of this notion, the viable cell recovery after 3 days of liquid-phase culture with IL-33

![Image](cancerres.aacrjournals.org)
was higher among enriched primary CD34(+) cells from patients with CML-CP than from healthy donors (Supplementary Fig. S2A), raising the question whether IL-33 behaved like a survival and/or proliferative factor for CML-CP CD34(+) cells. Annexin V expression in CML-CP CD34(+) cells was not modified in these conditions, indicating that apoptosis was not controlled by IL-33 (Supplementary Fig. S3). In keeping with a growth-promoting function, CML-CP CD34(+) cells were significantly increased (Supplementary Fig. S2) and incorporated twice as much BrdUrd upon exposure to IL-33 than their normal counterparts (Fig. 2A). More than 90% of IL-33–induced proliferation of CML-CP CD34(+) cells was prevented by neutralizing antibodies against ST2, thereby confirming that IL-33 exerts its action in these cells via its specific receptor (Supplementary Fig. S2D). The cytokine/alarmin was as efficient as SCF, which is the only growth factor known so far for its capacity to induce, as a single agent, the selective proliferation of CML-CP CD34(+) cells (Supplementary Fig. S4; ref. 16). Taken together, our results demonstrate that CD34(+) cells from patients with CML-CP express ST2 and respond to IL-33, whereas their counterpart from healthy individuals does neither. Hence, it can be assumed that by targeting CD34(+) cells from patients with CML-CP, IL-33 behaves like a hematopoietic growth factor.

The human hematopoietic cell population defined by CD34 expression is heterogeneous, with only a small fraction of cells (1%-10%) containing virtually all the in vitro clonogenic potential, and a large percentage of more mature cells. Accordingly, even though hematopoietic stem cell-enriched CD34(+)CD38(−) and CD34(+)CD38(+) subsets both express ST2, the specific target of IL-33 in terms of expansion remains to be identified. However, the fact that the CML-CP CD34(+) cell population retained its overall in vitro clonogenic potential on a per cell basis, once it had proliferated in response to IL-33 (Fig. 2B), supports the conclusion that both clonogenic and more mature CD34(+) cells from patients with CML-CP respond to IL-33. The selective reactivity of CML-CP CD34(+) cells was further evidenced by cytokine production (Fig. 2C) because they secreted more GM-CSF, IL-6, and IL-8 in response to IL-33 than normal CD34(+) cells, for which cytokine production was almost undetectable.
IL-33 overcomes imatinib mesylate-induced inhibition of CML-CP CD34(+) cell proliferation by engaging the JAK2/STAT5 pathway

We next addressed the question whether IL-33–induced proliferation of CML-CP CD34(+) cells was maintained upon treatment with a therapeutic dose of 1 μmol/L (17) of imatinib mesylate, which induces a strong inhibition of cell proliferation. As illustrated in Fig. 3A and B, IL-33 counteracted the inhibitory effect of imatinib mesylate on both proliferation and cytokine production, revealing that in the presence of IL-33, even a marked inhibition of BCR-ABL kinase activity cannot totally block CML-CP CD34(+) progenitor functions. From these findings, it can be surmised that the residual BCR-ABL–dependent pathways in leukemic CD34(+) cells that persist despite therapeutically effective imatinib mesylate treatment (2) can be substantially amplified by IL-33. As to the molecular events involved, STAT5, a signaling component acknowledged as a common antiapoptotic and transforming target of BCR-ABL (18), would be a likely candidate. Evidence of its involvement was obtained from both a multiplex assay of phosphorylated signaling proteins and intracellular fluorescence-activated cell sorting (FACS) analysis (Fig. 3C) showing that IL-33 partially restored STAT5 activity in leukemic CD34(+) cells, thus overcoming the inhibitory effect of imatinib mesylate. That JAK2 is also involved in this recovery is suggested by the complete loss of IL-33–induced proliferation in the presence of the JAK2 inhibitor AG490 (Fig. 3D). In this regard, although GM-CSF is known to play an important role in TK inhibitor resistance by engaging the JAK2/STAT5 pathway (3, 4), only 10% to 15% of IL-33–induced proliferation of CML-CP CD34(+) cells was maintained upon imatinib mesylate plus IL-33 (50 ng/mL) and/or AG490 (0.05 mmol/mL). Each symbol represents one patient with CML-CP, CD34(+) cells after 2 days of culture with imatinib mesylate alone (untreated, light gray filled histogram), imatinib mesylate plus IL-33 (dark gray filled histogram), or without imatinib mesylate (black solid histogram). CD34(+) cells were analyzed for p-STAT5 staining after intracellular labeling with anti-p-STAT5-Alexa Fluor 647 or its isotype-matched negative control (gray dotted line). One representative experiment out of three is shown. D, the JAK2 inhibitor AG490 neutralizes the effect of IL-33 in restoring proliferation upon imatinib mesylate-induced inhibition. CD34(+) cell proliferation was determined by an 18-hour pulse of BrdUrd after 2 days of culture with or without IL-33 (50 ng/mL) and/or imatinib mesylate [1 μmol/L (17) and/or AG490 (0.05 mmol/L)]. Each symbol represents one patient with CML-CP, CD34(+) cells (2.5 × 10⁴ per well) from patients with CML-CP were incubated for 2 to 3 days with or without IL-33 (50 ng/mL) and/or imatinib mesylate (1 μmol/L). A, CD34(+) cell proliferation was determined by an 18-hour pulse of BrdUrd on day 2 and quantified by measuring the absorbance (O.D.). B, Luminex technology was used to simultaneously measure GM-CSF and IL-6 in supernatants from 2-day CD34(+) cell cultures. Data are mean ± SEM from three separate experiments. Each symbol represents one patient with CML-CP. Wilcoxon nonparametric test. C, IL-33 increases levels of phosphorylated STAT5 in imatinib mesylate-treated leukemic CD34(+) progenitors. Left, enriched primary CD34(+) cells (2.5 × 10⁴ per well) from patients with CML-CP (n = 3) were analyzed for p-STAT5 staining after intracellular labeling with anti-p-STAT5-Alexa Fluor 647 or its isotype-matched negative control (gray dotted line). One representative experiment out of three is shown. D, the JAK2 inhibitor AG490 neutralizes the effect of IL-33 in restoring proliferation upon imatinib mesylate-induced inhibition. CD34(+) cell proliferation was determined by an 18-hour pulse of BrdUrd after 2 days of culture with or without IL-33 (50 ng/mL) and/or imatinib mesylate [1 μmol/L (17) and/or AG490 (0.05 mmol/L)]. Each symbol represents one patient with CML-CP, CD34(+) cells (2.5 × 10⁴ per well) from patients with CML-CP were incubated for 2 to 3 days with or without IL-33 (50 ng/mL) and/or imatinib mesylate (1 μmol/L). A, CD34(+) cell proliferation was determined by an 18-hour pulse of BrdUrd on day 2 and quantified by measuring the absorbance (O.D.). B, Luminex technology was used to simultaneously measure GM-CSF and IL-6 in supernatants from 2-day CD34(+) cell cultures. Data are mean ± SEM from three separate experiments. Each symbol represents one patient with CML-CP. Wilcoxon nonparametric test. C, IL-33 increases levels of phosphorylated STAT5 in imatinib mesylate-treated leukemic CD34(+) progenitors. Left, enriched primary CD34(+) cells (2.5 × 10⁴ per well) from patients with CML-CP (n = 3) were incubated for 12 hours in the presence of imatinib (1 μmol/L) with or without IL-33 (50 ng/mL) and/or imatinib mesylate (1 μmol/L). A, CD34(+) cell proliferation was determined by an 18-hour pulse of BrdUrd on day 2 and quantified by measuring the absorbance (O.D.).
were cultured for 96 hours with or without IL-33 (50 ng/mL) and transplanted (5 × 10^6 cells/mouse) into sublethally irradiated (5 Gy) NOG mice. Mice were euthanized after 4 weeks, and marrow contents were obtained. A, graphs showing human cell engraftment in bone marrow assessed by labeling with anti-human CD45-PercP-Cy5.5 antibody (BD Biosciences) and analyzed by flow cytometry. Wilcoxon nonparametric test. B, specific cell subsets were detected by using antibodies to human CD34–PE, CD11b–PE, CD3–FITC, and CD19–APC (all from BD Biosciences). C and D, comparison of leukemic cell engraftment and disease-free survival between wild-type and IL-33–deficient mice receiving p210–BCR-ABL–transduced bone marrow cells. Donor wild-type mice were injected with 5-fluorouracil (150 mg/kg). After 4 days, mice were sacrificed, and enriched bone marrow cells were isolated and transduced with a retrovirus encoding GFP linked by an IRES sequence to BCR-ABL (MIGR-p210–BCR-ABL–GFP). After two rounds of infection, GFP(+)–transduced bone marrow cells (1.10^6) were transplanted into lethally irradiated (9.5 Gy) wild-type or IL-33–deficient mice. C, elevated counts (20%–80%) of circulating leukocytes expressing GFP (coexpressed from the BCR-ABL–carrying retrovirus) were evidenced in all wild-type recipients (n = 10) as early as 12 days posttransplantation, in clear contrast with the low counts (<2%) of GFP(+) leukocytes (P < 0.05, Fisher exact test) recovered in four out of 10 IL-33–deficient recipients on the same time point. A representative experiment out of two is shown. The dotted line separates engrafted from nonengrafted mice. D, Kaplan-Meier survival curves for wild-type (n = 16) or IL-33–deficient (n = 15) recipients of p210–BCR-ABL–transduced bone marrow cells. Disease-free survival was prolonged in IL-33–deficient animals, whereas all wild-type mice succumbed to a CML-like syndrome (see Supplementary Fig. S7) between 3 weeks and 4 weeks after transplantation. The Fisher exact test was used to compare the numbers of alive versus dead mice in the wild-type group with those in the IL-33–deficient group (P < 0.05). Combined data from two separate experiments out of three are shown.

Figure 4. Evidence for a facilitating effect of IL-33 on in vivo maintenance of CD34(+) progenitors from patients with CML and mouse BCR-ABL–transfected bone marrow progenitors. A and B, xenotransplant experiments in immunodeficient NOG mice. CD34(+) cells from patients with CML-CP (n = 5) were measured in healthy donors (mean ± SEM: 75.5 ± 15.2 pg/mL) and MMR-IM patients (mean ± SEM: 74.1 ± 17.4 pg/mL; Supplementary Fig. S6A). Interestingly, circulating sST2 levels were highest among patients with high Sokal risk score, which is predictive of therapeutic outcome (Supplementary Fig. S6B). This finding, together with the reduction of circulating sST2 in patients in molecular remission (Supplementary Fig. S6A), establishes a relationship with clinical stages and outcomes. Finally, even though high amounts of peripheral sST2 are probably in part a consequence of increased leukemic cell mass, we provide evidence that IL-33 contributes to the proliferation and maintenance of BCR-ABL cells. Support- ing this conclusion, we also evidenced an effect of IL-33 on in vivo maintenance of CD34(+) progenitors from patients with CML by using xenotransplant experiments in immunodeficient NOG mice. In comparison with their untreated counterparts, CML CD34(+) cells cultured with IL-33 demonstrated was prevented by neutralizing antibodies against GM-CSF (Supplementary Fig. S5). Together, these data are consistent with the involvement of the JAK2/STAT5 pathway in vivo independently from GM-CSF. They reveal a new mechanism of cytokine-mediated imatinib mesylate resistance through IL-33–induced leukemic CD34(+) cell proliferation.

ST2 upregulation correlates with disease burden

Clinically, high circulating levels of IL-33 were detected only in 3 patients out of 15 analyzed. However, it is commonly acknowledged that a reliable detection of the secreted form of this cytokine is difficult, we assessed instead the levels of soluble ST2 (sST2), which may act as a quencher of IL-33 and is regarded as a functional signature of IL-33 signaling in vivo (20). Indeed, high amounts of circulating sST2 were detected in the plasma from patients with CML-CP (mean ± SEM: 678.1 ± 121.4 pg/mL), whereas low/undetectable levels were measured in healthy donors (mean ± SEM: 75.5 ± 15.2 pg/mL) and MMR-IM patients (mean ± SEM: 74.1 ± 17.4 pg/mL; Supplementary Fig. S6A). Interestingly, circulating sST2 levels were highest among patients with high Sokal risk score, which is predictive of therapeutic outcome (Supplementary Fig. S6B). This finding, together with the reduction of circulating sST2 in patients in molecular remission (Supplementary Fig. S6A), establishes a relationship with clinical stages and outcomes. Finally, even though high amounts of peripheral sST2 are probably in part a consequence of increased leukemic cell mass, we provide evidence that IL-33 contributes to the proliferation and maintenance of BCR-ABL cells. Supporting this conclusion, we also evidenced an effect of IL-33 on in vivo maintenance of CD34(+) progenitors from patients with CML by using xenotransplant experiments in immunodeficient NOG mice. In comparison with their untreated counterparts, CML CD34(+) cells cultured with IL-33 demonstrated
significantly increased engraftment in bone marrow of NOG mice at 4 weeks after transplantation (Fig. 4A and Supplementary Fig. S7A), which was associated with an unmodified cell distribution, as revealed by the same proportion of CD33+ and/or CD11b-expressing myeloid cells in treated and untreated CD33(+) progenitors (Fig. 4B). These results extend our ex vivo data and demonstrate a facilitating effect of IL-33 on in vivo maintenance of CD33(+) progenitors from patients with CML. Finally, taking advantage of the availability of IL-33-deficient mice (12), which were used as recipients in a retroviral BCR-ABL transduction–transplantation model of CML-like syndrome (13), we were able to address the impact of endogenous IL-33 on CML development in vivo. As expected, 12 days after transplantation of a high dose of mouse BCR-ABL-GFP-transfected bone marrow progenitors, an elevated proportion (20%–80%) of circulating CML leukocytes was evidenced in all wild-type recipients (n = 10; Fig. 4C and Supplementary Fig. S7B). This clearly contrasted with the low proportion (<2%) of CML circulating leukocytes recovered in four out of 10 IL-33-deficient recipients analyzed in the same experimental setting. Accordingly, although all wild-type mice succumbed to BCR-ABL–induced CML-like syndrome after 3 to 4 weeks, overall survival was significantly prolonged in the IL-33-deficient mice (Fig. 4D and Supplementary Fig. S7C). Hence, we propose that IL-33 facilitates the development of leukemia by inducing and/or enhancing the proliferation of hematopoietic progenitors, including probably stem cells, in the bone marrow microenvironment from patients with CML-CP.

Recent studies provide definite evidence for the kinase activity of BCR-ABL in CML stem cells and for its inhibition by imatinib mesylate without affecting their survival (5). Here, we show that despite the addition of imatinib mesylate at a therapeutically effective dose of 1 μmol/L that blocks TK activity, CML progenitor cells maintain their proliferative response to IL-33. Hence, it might be argued that IL-33, as a component of the microenvironment, may also contribute to the loss of oncogene “addiction” of leukemic stem cells thereby rendering them resistant to imatinib mesylate treatment. Further experimental studies in mice are needed to determine whether in vivo IL-33 is capable of opposing the action of imatinib mesylate on BCR-ABL(+) progenitors in vivo.

In conclusion, our findings demonstrate that IL-33 is capable of sustaining constitutive proliferation as well as cytokine production in leukemic progenitors of patients with CML, and support the hypothesis that the IL-33/ST2 pathway is involved in the control of leukemic cell fate. They also provide an explanation for the selective targeting of CML-CP progenitor cells by IL-33, a feature that is not shared by other hematopoietic factors that cannot discriminate between normal and CML-CP progenitor cells. It remains to determine whether selective targeting of CML progenitors expressing ST2 would be a new useful therapeutic approach.

Disclosure of Potential Conflicts of Interest
I. Roy is consultant/advisory board member of Novartis and BMS. A. Turhan has received honoraria from the speakers’ bureau of Bristol Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

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References

7. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan T, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005;23:479–90.


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