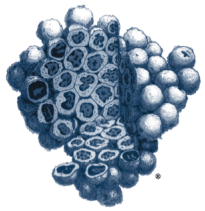

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Despite Inhibition of Hematopoietic Progenitor Cell Growth In Vitro, the Tyrosine Kinase Inhibitor Imatinib Does Not Impair Engraftment of Human CD133 + Cells into NOD/SCIDß2m Null Mice

Laurence Pirson, Frédéric Baron, Nathalie Meuris, Olivier Giet, Emilie Castermans, Roland Greimers, Ivano Di Stefano, André Gothot and Yves Beguin

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TRANSLATIONAL AND CLINICAL RESEARCH

Despite Inhibition of Hematopoietic Progenitor Cell Growth In Vitro, the Tyrosine Kinase Inhibitor Imatinib Does Not Impair Engraftment of Human CD133$^+$ Cells into NOD/SCIDβ2m^{Null} Mice

LAURENCE PIRSON,[a,b] FRÉDÉRIC BARON,[a,b] NATHALIE MEURIS,[a,b] OLIVIER GIET,[a,b] EMILIE CASTERMANS,[a,b] ROLAND GREIMERS,[c] IVANO DI STEFANO,[a,b] ANDRÉ GOTHOT,[a,d] YVES BEGUIN[a,b]

[a]Center for Cellular and Molecular Therapy, University of Liège, Liège, Belgium; [b]Department of Medicine, Division of Hematology, University of Liège, Liège, Belgium; [c]Laboratory of Pathological Anatomy and Cytology, University of Liège, Liège, Belgium; [d]Department of Clinical Biology, Division of Laboratory Hematology, University of Liège, Liège, Belgium

Key Words. Allogeneic stem cell transplantation • Chronic myeloid leukemia • Imatinib

ABSTRACT

There is potential interest for combining allogeneic hematopoietic cell transplantation (HCT), and particularly allogeneic HCT with a nonmyeloablative regimen, to the tyrosine kinase inhibitor imatinib (Glivec; Novartis, Basel, Switzerland, http://www.novartis.com) in order to maximize anti-leukemic activity against Philadelphia chromosome-positive leukemias. However, because imatinib inhibits c-kit, the stem cell factor receptor, it could interfere with bone marrow engraftment. In this study, we examined the impact of imatinib on normal progenitor cell function. Imatinib decreased the colony-forming capacity of mobilized peripheral blood human CD133$^+$ cells but not that of long-term culture-initiating cells. Imatinib also decreased the proliferation of cytokine-stimulated CD133$^+$ cells but did not induce apoptosis of these cells. Expression of very late antigen (VLA)-4, VLA-5, and CXCR4 of CD133$^+$ cells was not modified by imatinib, but imatinib decreased the ability of CD133$^+$ cells to migrate. Finally, imatinib did not decrease engraftment of CD133$^+$ cells into irradiated nonobese diabetic/severe combined immunodeficient/β2m^{null} mice conditioned with 3 or 1 Gy total body irradiation. In summary, our results suggest that, despite inhibition of hematopoietic progenitor cell growth in vitro, imatinib does not interfere with hematopoietic stem cell engraftment. STEM CELLS *2006;24:1814–1821*

INTRODUCTION

The Philadelphia chromosome results from the balanced t(9;22) chromosomal translocation, which leads to an aberrant *BCR/ABL* fusion gene [1, 2]. The Philadelphia chromosome is present in more than 90% of the cases of chronic myeloid leukemia (CML), and a *BCR/ABL* chimeric gene is expressed in approximately half of Philadelphia chromosome-negative CML patients [1, 2]. In addition, a *BCR/ABL* fusion gene is present in 5% of children and 20%–30% of adults with acute lymphoblastic leukemia (ALL) [2]. Imatinib mesylate (Glivec) is a tyrosine kinase inhibitor that prevents signaling through the chimeric BCR/ABL tyrosine kinase and blocks the proliferation of Philadelphia chromosome-positive cell lines and clonogenic CML progenitor cells [3]. Tyrosine kinases inhibited by imatinib include ABL, the platelet-derived growth factor receptor (PDGFR), and c-kit (the signal transductor of the stem cell factor [SCF] receptor) [4, 5].

SCF is constitutively expressed by bone marrow endothelial cells and fibroblasts. It suppresses apoptosis and induces cell cycle entry [6]. Treatment of mice with neutralizing anti-c-kit receptor antibody ACK-2 induces bone marrow aplasia [7]. In vitro, SCF promotes colony growth in synergy with erytropoietin, interleukin (IL)-3, and GM-CSF among others [8]. SCF also mediates stem cell self-renewal in vivo [9]. Interestingly, SCF has been shown to regulate CD34$^+$ cell adhesion to fibronectin [10] and to mediate both chemokinetic and chemotactic signals [11]. As such, SCF is

Correspondence: Yves Beguin, M.D., University of Liège, Department of Hematology, CHU Sart Tilman, 4000 Liège, Belgium. Telephone: +32-4-366 72 01; Fax +32-4-366 88 55; e-mail: yves.beguin@chu.ulg.ac.be Submitted June 29, 2005; accepted for publication March 31, 2006; first published online in STEM CELLS *EXPRESS* April 13, 2006. 1066-5099/2006/$20.00/0 doi: 10.1634/stemcells.2005-0290

likely to be involved in controlling stem cell homing and engraftment. In the post-transplantation period, treatment with SCF reduces radiation-induced death [12]. Conversely, SCF neutralization increases sensitivity to radiation therapy [13].

It is generally accepted that, after allogeneic hematopoietic cell transplantation (HCT), cure of CML and Philadelphia chromosome-positive ALL depends on the contribution of the graft-versus-leukemia (GVL) effect [14–17]. This notion prompted the development of allogeneic transplantation after reduced-intensity [18–20] or truly nonmyeloablative conditioning [21–27]. In these approaches, the conditioning regimen is substantially reduced, and reliance is placed on GVL effects for eradicating host leukemic cells [28]. However, although this strategy seems very promising for more indolent diseases, results in advanced-phase CML and in Ph-positive ALL remain disappointing. These observations provide a rationale for combining allogeneic HCT, and particularly allogeneic HCT with a nonmyeloablative regimen, to imatinib in order to maximize antileukemic activity against Philadelphia chromosome-positive leukemias. However, because SCF plays a major role in homing and proliferation of hematopoietic stem cells (HSCs), preclinical studies are needed to assess the impact of c-kit and PDGFR inhibition on hematopoiesis and on hematological reconstitution after allogeneic HCT. These are the aims of this report.

Materials and Methods

Cells

After informed consent was obtained, G-CSF-mobilized peripheral blood (PB) mononuclear cells were obtained from normal adult volunteers according to the guidelines established by the Ethical Committee of the University of Liège (Liège, Belgium). Mobilization was achieved by daily G-CSF administration at 10 μg/kg for 5 consecutive days. Cells were collected by apheresis on day 5. HSCs were subsequently isolated by CD133 selection with the Clinimacs device (Miltenyi Biotec, Bergisch Gladbach, Germany, http://www.miltenyibiotec.com) according to the manufacturer's instructions. Mean $CD133^+$ cell positivity in the final product was more than 95%. Cells were cryopreserved in small aliquots in 90% fetal calf serum (Invitrogen, Carlsbad, CA, http://www.invitrogen.com) and 10% dimethyl sulfoxide (Sigma, Bornem, Belgium, http://www.sigmaaldrich.com).

Cord blood (CB) samples were collected in acid citrate dextrose solution in accordance with standard procedures for CB banking. All samples were processed within 24 hours of delivery. All material was obtained after informed consent and used according to the guidelines established by the Ethical Committee of the University of Liège. Mononuclear low-density cells were isolated by centrifugation over Ficoll-Paque (GE Healthcare, Little Chalfont, Buckinghamshire, U.K., http://www.gehealthcare.com).

The murine stromal MS-5 cells were cultivated in α-minimal essential medium (Cambrex Bio Science Verviers S.p.r.l., Verviers, Belgium, http://www.cambrex.com) supplemented with 10% fetal bovine serum (FBS; Invitrogen) at 37°C.

Short-Term Suspension Cultures

$CD133^+$ cells were plated in Iscove's medium supplemented with 20% BIT (bovine serum albumin [BSA], insulin, and human transferrin) serum substitute (StemCell Technologies, Meylan, France, http://www.stemcell.com), 100 μg/ml penicillin, and 100 μg/ml streptomycin (Cambrex Bio Science Verviers S.p.r.l.). Cells were stimulated by a combination of 100 ng/ml SCF (Amgen, Thousand Oaks, CA, http://www.amgen.com), 50 ng/ml thrombopoietin (Amgen), and 100 ng/ml flt3-ligand (FL; R&D Systems, Abingdon, U.K., http://www.rndsystems.com) and maintained at 37°C in a 100% humidified atmosphere with 5% CO_2.

Colony-Forming Cell and Long-Term Culture-Initiating Cell Assays

Colony-forming unit-granulocyte/macrophages (CFU-GM) and BFU-E assays were performed in Methocult H4435 (StemCell Technologies). In a first set of experiments, imatinib mesylate was added to Methocult medium at 0, 0.5, 1, and 2 μM on day 0. In a second set of experiments, $CD133^+$ cells were first cultured in vitro with 0, 0.5, 1, and 2 μM imatinib (see Short-Term Suspension Cultures) for 48 hours and then washed and cultured in Methocult without imatinib.

Colony-forming unit-megakaryocyte (CFU-Mk) assays were performed with the Mega Cult-C kit (StemCell Technologies) as recommended by the manufacturer.

Absolute frequencies of long-term culture-initiating cells (LTC-ICs) were determined by limiting dilution analysis as previously described [29, 30]. In a first set of experiments, imatinib at 0, 1, 2, and 5 μM was added on the day of addition of $CD133^+$ cells as well as with each weekly half medium change ($n = 3$). In a second set of experiments, $CD133^+$ cells were first cultivated in vitro with 0, 1, 2, and 5 μM imatinib (see Short-Term Suspension Cultures) for 48 hours and then washed and assayed for LTC-IC without any imatinib ($n = 3$). The percentage of LTC-IC was calculated using L-calc software (StemCell Technologies).

FACS Analysis

For cell cycle analysis, $CD133^+$ cells recovered after 48 hours of culture with or without imatinib were incubated in phosphate-buffered saline (PBS) 0.6% IGEPAL CA-630 (Sigma) containing 100 μg/ml propidium iodide (PI). After a 30-minute incubation in the dark, cells were run on a FACSort flow cytometer (Becton, Dickinson and Company, Franklin Lakes, NJ, http://www.bd.com), collecting FL-2 channel values. The percentage of cells in the different phases of the cell cycle was determined with Modfit software (Becton, Dickinson and Company). The percentage of cells in cycle was calculated as follows: percentage = $[(S + G_2/M \text{ cells})/\text{total cells}] \times 100$.

For analysis of integrin (very late antigen [VLA]-4 and VLA-5) and CXCR4 expression, fluorescein isothiocyanate (FITC)-conjugated anti-VLA-4 (CD49d; Coulter Immunotech, Marseille, France, http://www.immunotech.fr) or FITC-conjugated anti-VLA-5 (CD49e; BD Biosciences PharMingen, San Diego, http://www.bdbiosciences.com/pharmingen) were used in combination with phycoerythrin-conjugated anti-CXCR4 (BD Biosciences PharMingen). $CD133^+$ cells recovered after 48 hours of culture with or without imatinib were incubated with antibodies or isotype-matched control immunoglobulin G for 20 minutes on ice in the dark. Cells were washed in PBS 1% calf serum (Cambrex Bio Science Verviers S.p.r.l.) and fixed in PBS 1% paraformaldehyde (Sigma).

Data were acquired on a FACSort flow cytometer. CXCR4 and integrin density were expressed as the mean channel fluorescence ratio (MCFR) defined as MCF of CXCR4 or integrin expression divided by MCF of fluorescence-matched isotypic control.

For quantification of apoptosis and necrosis, flow cytometric analysis using annexin V fluorescein and PI (Annexin-V-Fluos Staining kit; Roche, Basel, Switzerland, http://www.roche.com) was used as described by the manufacturer.

Flow Cytometric Cell Sorting

Cultured $CD133^+$ cells were sorted on the basis of cell cycle status as previously reported [29]. Briefly, cells were incubated in serum-free medium supplemented with 5 mg/ml Hoechst 33,342 (Molecular Probes, Eugene, OR, http://probes.invitrogen.com) during 30 minutes at 37°C. Cells in G_0/G_1 or in $S/G_2/M$ were sorted by a FACS Vantage (Becton, Dickinson and Company). The purity of sorted cells was confirmed by DNA staining of sorted cells and always exceeded 90%.

Migration Assays

Migration assays were performed in 6.5-mm diameter 5-μm-pore transwells (Corning Life Sciences, Acton, MA, http://www.corning.com/lifesciences). Two million $CD133^+$ cells were plated in 100 μl of Myelocult (StemCell Technologies) in the upper chamber of the transwell. The bottom compartment was filled with Iscove's modified Dulbecco's medium (Cambrex Bio Science Verviers S.p.r.l.) supplemented with 10% FBS and 100 ng/ml stromal derived factor-1 (SDF-1) (PeproTech EC Ltd., London, http://www.peprotechec.com). After incubation at 37°C during 3 hours, nonmigrating and migrating cells were harvested by two standardized washes using warm PBS containing 1% BSA. Nonmigrating and migrating cells were counted using Trypan blue exclusion. The percentage of nonmigrating and migrating cells was calculated relative to the total number of cells recovered (recovery >95%).

Adhesion Assays

Adhesion assays were carried out on fibronectin-coated plates as previously described [29]. Adherent and nonadherent cells were counted using Trypan blue exclusion. The percentage of adherent and nonadherent cells was calculated relative to the total number of cells recovered (recovery >95%). Adhesion on BSA-coated control plates was less than 2%.

Transplantation into Nonobese Diabetic/Severe Combined Immunodeficient/$\beta 2m^{null}$ Mice

Nonobese diabetic (NOD)/severe combined immunodeficient (SCID)/$\beta 2m^{null}$ mice were sublethally irradiated on day 0. Four hours later, 6×10^5 human $CD133^+$ HSCs were inoculated intravenously. Mice were started on day 0 on a placebo (sterilized water, first group) or on imatinib (50 mg/kg every morning and 100 mg/kg every evening, second group) by gavage. Blood was collected 1, 2, and 4 weeks after the transplant with a heparined capillary (Hirschmann Laborgerate, Mannheim, Germany, http://www.hirschmann-laborgeraete.de) after cutting the mouse tail. Mice were sacrificed 6 weeks after xenotransplantation. Blood was collected on anticoagulant citrate dextrose by vena cava puncture. Cells were counted with an ABX automatic cell counter (ABX Hematology, Montpellier, France, http://www.horiba-abx.com) with appropriate corrections for dilution by ACD. Bone marrow cells were harvested from the two femurs, suspended into single-cell suspension, counted, and stained with anti-human CD45 (BD Biosciences PharMingen) to determine the overall level of human chimerism. Engraftment was compared between the two groups of mice.

Statistical Analyses

One-way analysis of variance tests were used to assess the impact of imatinib on colony and LTC-IC formation and migration/adhesion abilities of $CD133^+$ cells. Unpaired and paired Student's *t* tests were used to compare the effects of imatinib on the percentage of $CD133^+$ cells in cycle and the engraftment of $CD133^+$ cells into NOD/SCID$\beta 2m^{null}$ mice. Welsh's correction was used in case of unequal variance. The probability of survival was studied by Kaplan-Meier methods. Statistical analyses were carried out with GraphPad Prism (GraphPad Software, San Diego, http://www.graphpad.com).

RESULTS

Imatinib Decreased Generation of Colony-Forming Units from CB and PB

Results of 10 consecutive CB cultures were analyzed for CFU-GM and BFU-E generation. Imatinib added in CFU culture assays on day 0 at 0.5, 1, and 2 μM decreased CFU-GM formation by a mean ± SD of 11% ± 16%, 20% ± 21%, and 44% ± 19%, respectively ($p < .0001$), and BFU-E formation by 12% ± 22%, 41% ± 19%, and 55% ± 28%, respectively ($p < .0001$) (Fig. 1A).

In another series of experiments, CFU-GM and BFU-E generation by PB $CD133^+$ cells was analyzed in the presence of imatinib. Imatinib added in the culture on day 0 at 1, 2, and 5 μM decreased CFU-GM formation by a mean of 67% ± 13%, 62% ± 29%, and 80% ± 16%, respectively ($p < .0001$), BFU-E formation by 62% ± 24%, 70% ± 21%, and 85% ± 8%, respectively ($p < .0001$), and CFU-Mk formation by a mean of 59% ± 30%, 29% ± 28%, and 79% ± 8%, respectively ($p < .0001$) (Fig. 1B).

Finally, PB $CD133^+$ cells were first plated in cytokine-supplemented suspension culture for 48 hours with imatinib at the same concentrations and then washed and cultured in CFU assays without imatinib ($n = 3$). Imatinib at 1, 2, and 5 μM decreased CFU-GM formation by a mean of 17% ± 9%, 11% ± 5%, and 38% ± 6%, respectively ($p = .0003$), and BFU-E formation by −8%± 42%, 10% ± 24%, and 51% ± 12% ($p = .08$), respectively (Fig. 1C).

Imatinib Did Not Decrease the Development of Primitive Progenitor Cells in Long-Term Cultures

In a first set of experiments ($n = 3$), imatinib was added to long-term cultures at 1, 2, and 5 μM on day 0 and at each weekly medium change for 5 weeks. Imatinib treatment had no significant impact on LTC-IC frequency (Fig. 1D). In a second set of experiments, cells were first plated in cytokine-supplemented suspension cultures for 48 hours with imatinib at the same concentrations and then washed and replated in LTC-IC assays without imatinib. Again, the frequency of LTC-IC relative to the number of plated cells was similar with or without imatinib pre-treatment at any dosage (Fig. 1D).

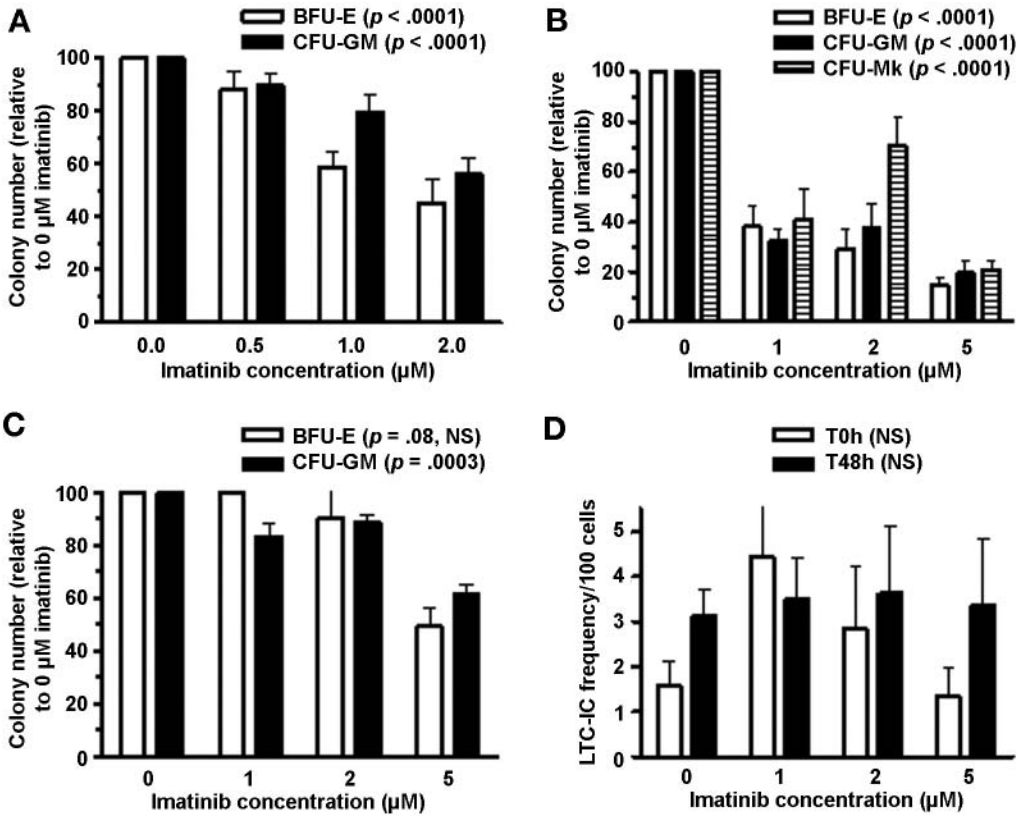

Figure 1. Impact of imatinib in vitro (1). **(A):** Effect of imatinib on CFU-GM and BFU-E growth from unmanipulated cord blood cells. Results are expressed as percentages relative to experiments without imatinib (see text). **(B):** Effect of imatinib on CFU-GM, BFU-E, and CFU-Mk growth from PB CD133-selected cells. Results are expressed as percentages relative to experiments without imatinib (see text). **(C):** Effect of 48-hour preculture with or without imatinib on subsequent CFU-GM and BFU-E growth from PB CD133-selected cells. Results are expressed as percentages relative to experiments without imatinib (see text). **(D):** Influence of imatinib on LTC-IC generation from PB CD133-selected cells. In a first set of experiments, imatinib (when present) was maintained throughout the assay (with columns). In a second set of experiments, $CD133^+$ cells were cultured for 48 hours with or without imatinib and then washed and assayed for LTC-IC without imatinib (black columns). *P* values were calculated with one-way analysis of variance tests. Abbreviations: CFU-GM, colony-forming unit-granulocyte/macrophage; CFU-Mk, colony-forming unit-megakaryocyte; LTC-IC, long-term culture-initiating cell; NS, not significant; PB, peripheral blood.

Imatinib Decreased the Number of PB $CD133^+$ Cells in Cycle but Did Not Induce Apoptosis

To determine whether the reduction of colony generation by imatinib was due to reduced proliferation, $CD133^+$ cells were analyzed by FACS after 48 hours of culture with various concentrations of imatinib in SCF-containing medium. The percentage of cells in cycle (G_2/M + S) was 28% ± 12% for control cells (without imatinib) and 18% ± 8% ($p = .01$), 19% ± 15% ($p = .01$), and 16% ± 11% ($p = .003$) for cells treated with 0.5, 1, and 2 μM imatinib, respectively (Fig. 2A–2C). When the same experiment was performed in a medium containing IL-3 instead of SCF, the percentage of cells in cycle was not decreased by imatinib (data not shown).

To determine whether the reduction of colony generation by imatinib was due to apoptosis of $CD133^+$ cells, $CD133^+$ cells after 0, 2, 4, 6, 24, 32, and 48 hours of culture with 0–2 μM imatinib were labeled with annexin V and PI and then analyzed by FACS. As shown in Figure 2D, imatinib did not induce apoptosis of $CD133^+$ cells. In addition, percentages of necrotic cells after 48 hours were similar without or with imatinib at 1, 2, and 5 μM in the culture (11%, 10%, 16%, and 12%, respectively) (data not shown).

Imatinib Did Not Modify VLA-4, VLA-5, and CXCR4 Expression by PB $CD133^+$ Cells

Because VLA-4, VLA-5, and CXCR4 play a major role in the homing of HSCs, we investigated the effect of imatinib on the expression of these surface receptors by FACS after 48 hours of suspension culture in three different experiments. MCFR values of VLA-4, VLA-5, and CXCR4 were not modified by imatinib (Fig. 3A).

We have previously shown an increased expression of VLA-4 and VLA-5 in cycling $CD34^+$ cells, resulting in increased binding and defective migration across fibronectin [29, 30]. To analyze whether this observation was also encountered in imatinib-treated $CD133^+$ cells, we analyzed VLA-5 and VLA-4 expression in cycling and noncycling cells in three different experiments. We observed a parallel upregulation of VLA-4 and VLA-5 expression in cycling cells, which was similar in imatinib-treated and control cells (Fig. 3B).

Imatinib Increased Adhesion to Fibronectin and Decreased Migration of $CD133^+$ Cells

$CD133^+$ cells cultured in the presence of 0, 1, 2, and 5 μM imatinib were analyzed in adhesion assays ($n = 3$). As shown in Figure 3C, imatinib increased the ability of $CD133^+$ cells

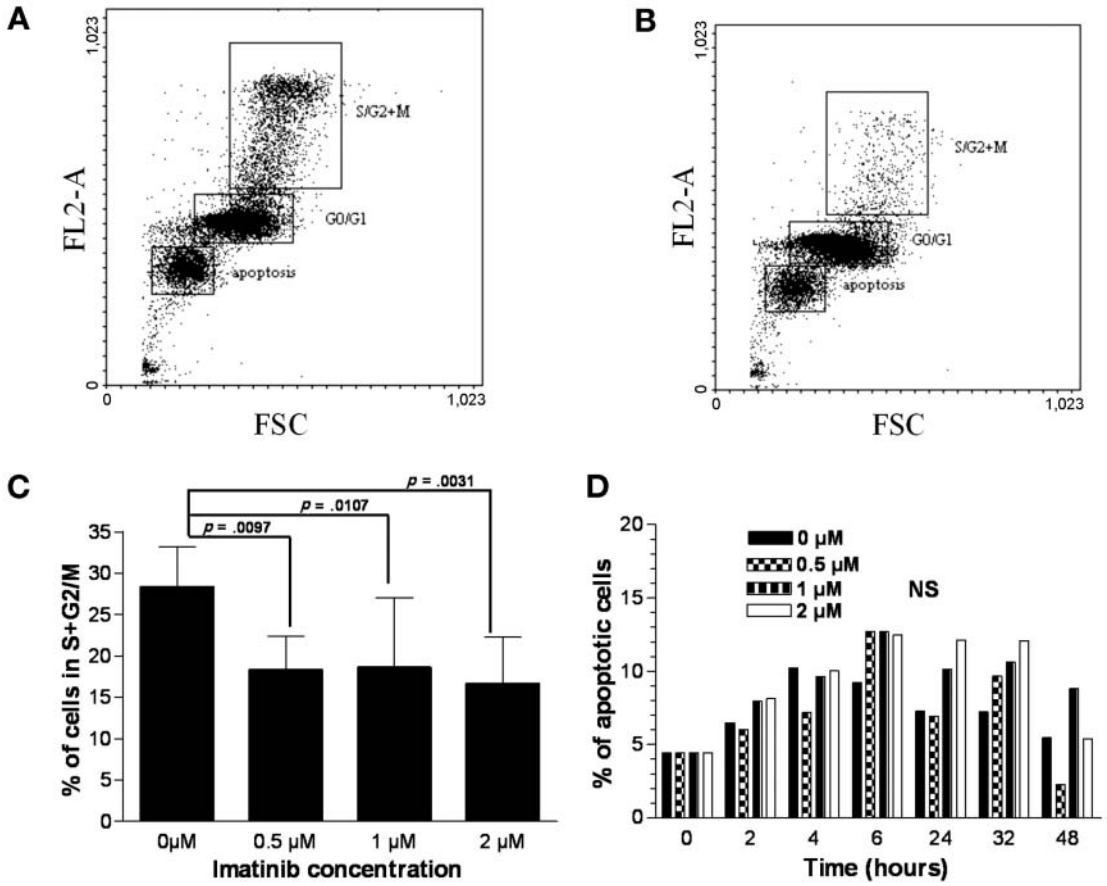

Figure 2. Imatinib decreased the number of peripheral blood CD133$^+$ cells in cycle but did not induce apoptosis. Cells were cultured in the absence **(A)** or in the presence **(B)** of 2 μM imatinib and then stained with Hoechst 3342 and separated into noncycling (G_0/G_1) and cycling ($S + G_2/M$) fractions. A representative experiment is shown. The plots are presented in FL2-A (DNA content) and FSC. **(C):** Effect of various imatinib concentrations on the percentage of cycling cells. **(D):** Effect of various imatinib concentrations on the percentage of apoptotic cells. Abbreviations: FSC, forward scatter; NS, not significant.

to adhere to fibronectin. The mean percentages of adhesion with 0, 1, 2, and 5 μM imatinib were 18.1% ± 3.6%, 39.6% ± 14.5%, 41.3% ± 6.9%, and 33.2% ± 18.3%, respectively.

CD133$^+$ cells cultured in the presence of 0, 1, 2, and 5 μM imatinib were also analyzed in migration assays ($n = 4$). The mean percentages of migration through filters toward SDF-1 were 54.9% ± 9.8%, 42.2% ± 14.1%, 31.1% ± 4.9%, and 22.3% ± 6.9%, respectively (Fig. 3D).

Imatinib Did Not Impair Engraftment of Human CD133$^+$ Cells into NOD/SCIDβ2m^{null} Mice

In a first set of experiments, 15 mice (six controls and nine treated with imatinib) received 3 Gy total body irradiation (TBI) before transplantation. Six of the nine mice in the imatinib group died 7, 8, 10, 10, 11, and 39 days after the transplant, from irradiation toxicity, including persistent marrow aplasia. The other nine mice were sacrificed on day 42. Among surviving animals, bone marrow cellularity was 107,600 ± 7,584 cells for control mice versus 105,000 ± 7,506 cells for imatinib-treated mice (not significant [NS]) (Fig. 4A). Bone marrow human chimerism was 21.0% ± 9.4% for control mice versus 37.9% ± 2.8% for imatinib-treated mice ($p = .021$) (Fig. 4C).

In a second set of experiments, 11 mice (five controls and six treated with imatinib) received only 1 Gy TBI before transplantation. There was a trend for lower WBC count ($p = .06$), platelet count ($p = .2$), and Hb level ($p = .6$) in the imatinib group (Fig. 4B). All 11 mice were sacrificed on day 42, and human cells were detected in the bone marrow of all mice. Bone marrow human chimerism was 4.6% ± 5.4% for control mice versus 3.9% ± 2.9% for imatinib-treated mice (NS) (Fig. 4D).

In a third experiment, mice were transplanted with CD133$^+$ cells pretreated during 48 hours with (15 mice) or without (15 mice) imatinib 2 μM after 2 Gy TBI. One mouse in the imatinib group died 37 days after the transplant. On day 42, the level of human chimerism was assessed. Bone marrow cellularity was similar in the two groups. Bone marrow human chimerism was 1.5% ± 0.2% for control mice versus 2.0% ± 0.4% for imatinib-treated mice (NS) (Fig. 5).

DISCUSSION

Myelosuppression is common in CML patients treated with imatinib [31]. Because the BCR/ABL clone is responsible for the major part of hematopoiesis in CML patients,

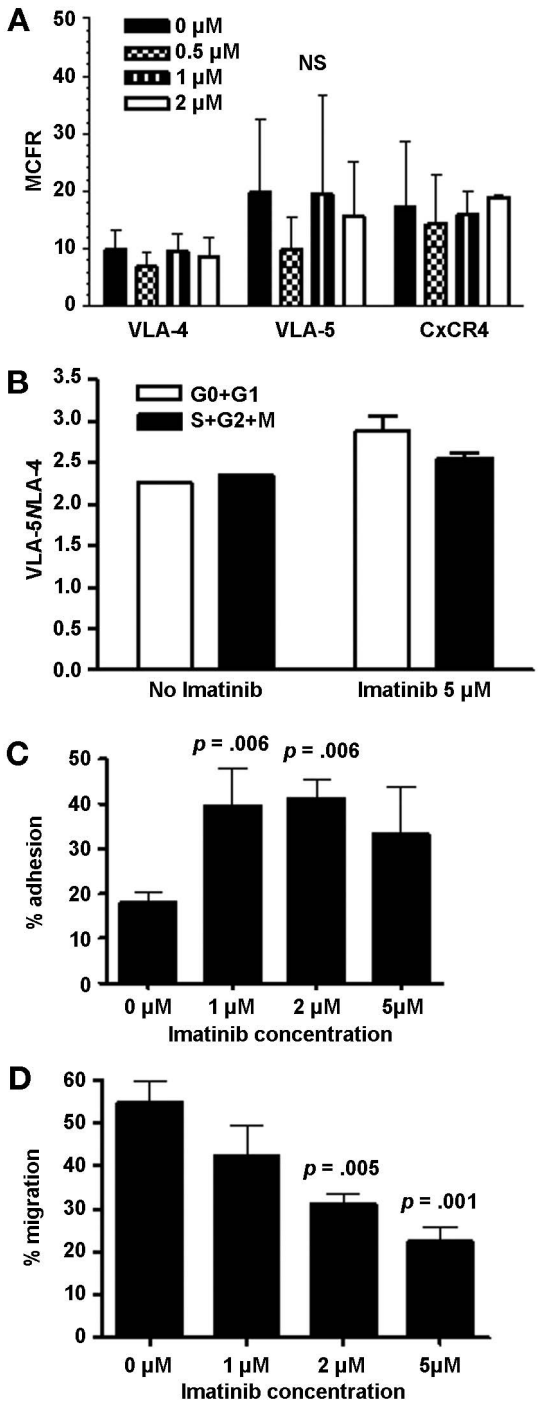

Figure 3. Impact of imatinib in vitro (2). **(A):** Impact of imatinib on VLA-4, VLA-5, and CXCR4 expression by PB CD133$^+$ cells. **(B):** Impact of cell cycle on expression of VLA-5 relative to VLA-4 (VLA-5/VLA-4 ratio) in cells cultured 48 hours with or without imatinib. **(C):** Impact of imatinib on adhesion to fibronectin of cultured PB CD133$^+$ cells. **(D):** Impact of imatinib on migration across fibronectin of cultured PB CD133$^+$ cells. Abbreviations: MCFR, mean channel fluorescence ratio; NS, not significant; PB, peripheral blood; VLA, very late antigen.

myelosuppression is an expected therapeutic effect. On the other hand, because imatinib also inhibits c-kit (the receptor for SCF), myelosuppression in CML patients may also be due, in part, to the inhibition of this tyrosine kinase. In this article, we investigated the impact of imatinib on normal hematopoiesis. Several observations have been made.

First, as reported by Bartolovic et al. [32], imatinib at therapeutic doses (1 μM is achieved in patients using imatinib at a daily dose of 300–400 mg) inhibited CFU-GM and BFU-E formation by a mean of 20% and 40%, respectively. These results agree with previous observations reporting a 10%–20% inhibition of CFU-GM formation with 1 μM imatinib [33, 34]. CFU inhibition was even greater when PB CD133$^+$-selected cells were used instead of unmanipulated CB cells. In addition, our study showed that imatinib also inhibited the formation of CFU-Mk colonies. Two factors may explain this inhibition of colony formation: a reduction of cell proliferation and/or a toxic (apoptotic or necrotic) effect of imatinib on CD133$^+$ cells. Our results show that imatinib significantly decreased (by $\pm$ 30% at 1 μM) the number of CD133$^+$ cells in cycle after 48 hours of culture in a medium containing SCF. We observed that this anti-proliferative effect resulted mostly from specific inhibition of c-kit because it was not observed when IL-3 was substituted by SCF. On the other hand, our data also showed that imatinib is not toxic for CD133$^+$ cells, because we observed a similar level of apoptosis and necrosis in CD133$^+$ cells treated or not treated with imatinib, in agreement with previous reports [32, 34].

Second, when cell proliferation is not artificially stimulated by exogenous cytokines, imatinib treatment did not significantly inhibit LTC-IC formation, providing evidence (as previously reported) that the immature stem cell compartment is less/not sensitive to the inhibitory effect of imatinib in these conditions [32, 34].

Third, we did not observe any impact of imatinib on VLA-4, VLA-5, or CXCR-4 expression. However, imatinib increased adhesion and decreased migration of CD133$^+$ cells across fibronectin. Our group [35, 36] reported that the activation state of integrins in ex vivo cultured progenitor cells is not related to their expression level. Also, the inverse relationship between adhesion strength and migration capacity has been observed in various cell systems. In hematopoietic cells, higher cell cycle activity is associated with stronger binding and reduced motility [30, 37]. However, because imatinib inhibits CD133$^+$ cell proliferation, its effect on hematopoietic cell adhesion and migration appears to be independent of cell cycle activity. Further work will be required to analyze the action of imatinib on tyrosine kinases implicated in adhesion and migration, such as the focal adhesion kinase or the related kinase PYK2 that is expressed in CD34$^+$ cells [38].

Finally, we showed that continuous in vivo imatinib administration did not alter engraftment of human CD133$^+$ cells into NOD/SCIDβ2m^{null} mice. This was true even when the pre-transplant irradiation dose was reduced to 1 Gy. Also, prior incubation in imatinib-supplemented culture medium did not impair PB CD133$^+$ cell-repopulating capacity. These results suggest that imatinib has no negative impact on engraftment abilities of human CD133$^+$ cells and might support the results of Hoepfl et al., who demonstrated that imatinib, given at 25 mg/kg twice daily, has no significant influence on hematopoietic engraftment in a mice syngeneic bone marrow transplantation model [39]. On the other hand, the persistent marrow aplasia leading to early death in six of nine mice given imatinib after 3 Gy TBI in the current study, might be explained by an inhibitory effect of high-dose (150 mg/kg per day)

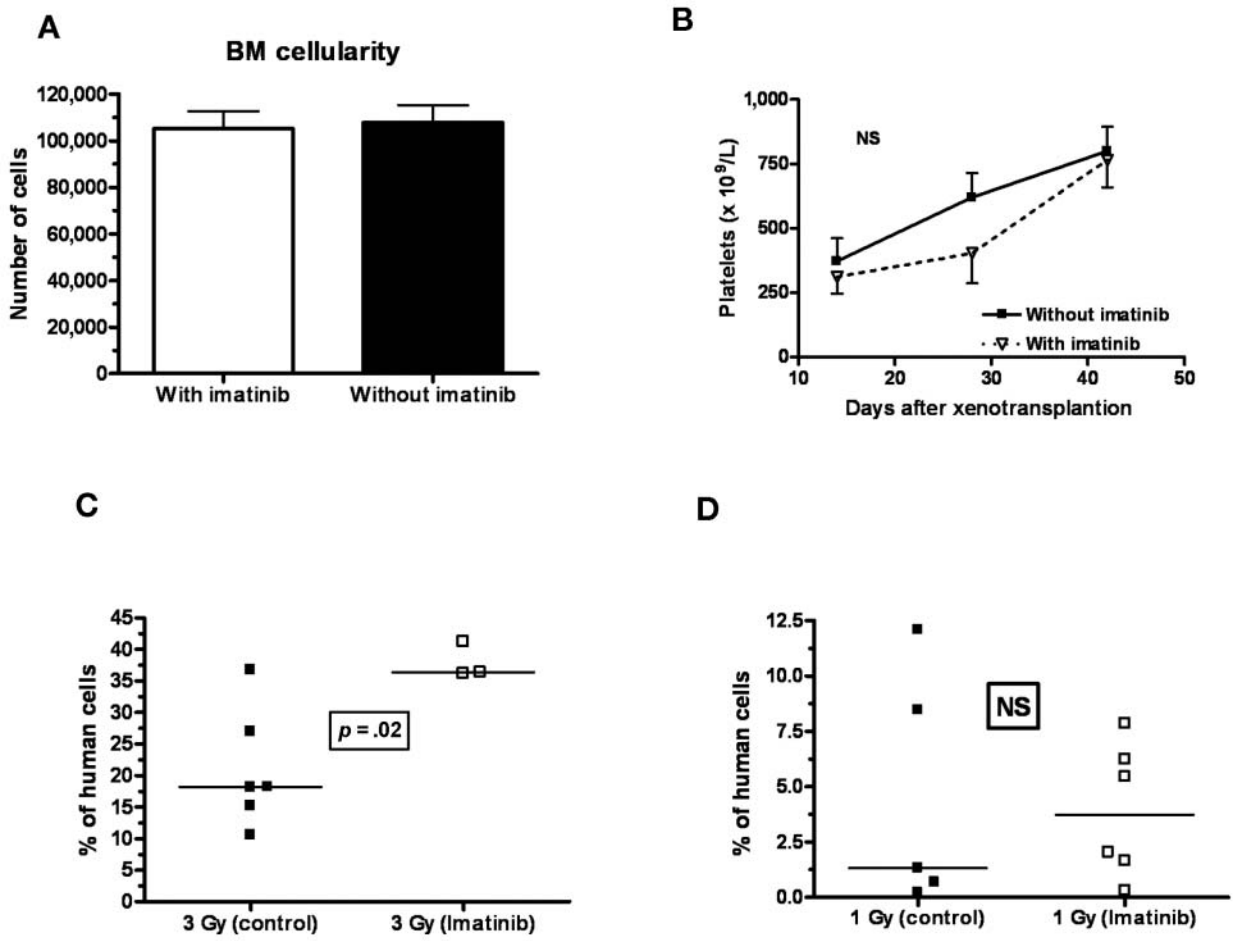

Figure 4. Impact of imatinib in vivo. **(A):** Impact of continuous imatinib administration on BM cellularity 6 weeks after xenotransplantation of peripheral blood CD133$^+$ cells in mice conditioned with 3 Gy TBI. **(B):** Impact of imatinib on platelet recovery in mice conditioned with 1 Gy TBI. **(C):** Impact of continuous imatinib administration on BM human chimerism 6 weeks after xenotransplantation in mice conditioned with 3 Gy TBI. Continuous lines show the medians. **(D):** Impact of imatinib administration on BM human chimerism 6 weeks after xenotransplantation in mice conditioned with 1 Gy TBI. Continuous lines show the medians. Abbreviations: BM, bone marrow; NS, not significant; TBI, total body irradiation.

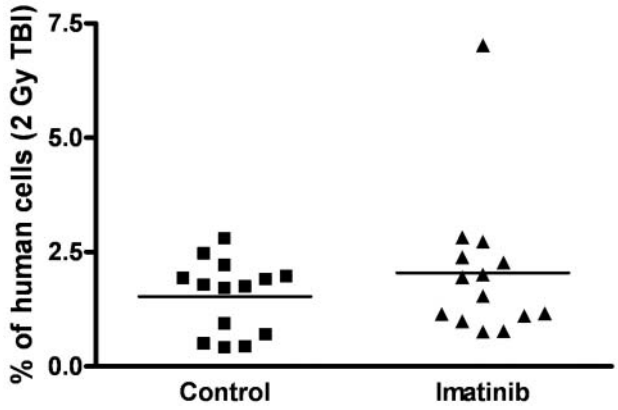

Figure 5. NOD/SCID repopulating activity of PB CD133$^+$ cells pre-incubated in 2 μM imatinib-supplemented medium. Mice were conditioned with 2 Gy TBI but did not receive imatinib. Human chimerism was assessed 6 weeks after transplantation. Abbreviations: NOD/SCID, nonobese diabetic/severe combined immunodeficient; PB, peripheral blood; TBI, total body irradiation.

imatinib on mouse hematopoiesis. Furthermore, because human hematopoietic cells do not give rise to differentiated progeny in the xenotransplantation model, this study did not assess a negative effect of imatinib on the generation of human differentiated progeny, which is likely to be present given the inhibition of CFU-formation in vitro by imatinib and given a previous report from Neta et al. [13], who observed that SCF neutralization increased sensitivity to radiation therapy.

SUMMARY

Our results suggest that, despite inhibition of hematopoietic progenitor cell growth in vitro, imatinib does not interfere with hematopoietic stem cell engraftment. Further studies are needed to analyze the impact of imatinib on graft-versus-tumor effects and on post-transplant immune reconstitution [40].

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DISCLOSURES

The authors indicate no potential conflicts of interest.

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Despite Inhibition of Hematopoietic Progenitor Cell Growth In Vitro, the Tyrosine Kinase Inhibitor Imatinib Does Not Impair Engraftment of Human CD133 + Cells into NOD/SCIDß2m Null Mice

Laurence Pirson, Frédéric Baron, Nathalie Meuris, Olivier Giet, Emilie Castermans, Roland Greimers, Ivano Di Stefano, André Gothot and Yves Beguin

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