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Inhibition of Natural Type I IFN-Producing and Dendritic Cell Development by a Small Molecule Receptor Tyrosine Kinase Inhibitor with Flt3 Affinity

Roxane Tussiwand,* Nobuyuki Onai,* Luca Mazzucchelli,† and Markus G. Manz‡

In vivo steady-state type I natural IFN-producing and dendritic cell (DC) development is largely dependent on Flt3 signaling. Natural IFN-producing and DC progenitors and their respective downstream cell populations express the flt3 receptor, and Flt3 ligand (Flt3L)−/− mice have reduced while Flt3L-injected mice develop markedly increased numbers of both cell types. In the present study, we show that SU11657, a small multitargeted receptor tyrosine kinase inhibitor with Flt3 affinity, suppressed in vitro natural IFN-producing and DC development in Flt3L-supplemented mouse whole bone marrow cell cultures in a dose-dependent manner, while DC development in GM-CSF-supplemented cultures was not affected. In vivo SU11657 application led to a significant decrease of both natural IFN-producing and DCs, comparable to the reduction observed in Flt3L−/− mice. Conversely, Flt3L plasma levels increased massively in inhibitor-treated animals, likely via a regulatory feedback loop, without being able to compensate for pharmacological Flt3 inhibition. No obvious toxicity was observed, and hemopoietic progenitor cell and stem cell function remained intact as assessed by myeloid colony-forming unit activity and in vivo bone marrow repopulation assays. Furthermore, upon treatment discontinuation, IFN-producing and DCs recovered to normal levels, proving that treatment effects were transient. Given the importance of IFN-producing and DCs in regulation of immune responses, these findings might lead to new pharmacological strategies in prevention and treatment of autoimmune diseases and complications of organ or blood cell transplantation. The Journal of Immunology, 2005, 175: 3674–3680.

Natural type I IFN-producing cells (IPCs,3 also called plasmacytoid cells or plasmacytoid dendritic cells (pre-DCs)) and DCs are important regulators of innate and adaptive immune responses (1–5). IPCs and DCs are cells of the hemopoietic system and, with the exception of subpopulations as Langerhans cells (6), are continuously replenished by new input cells derived from bone marrow (BM) hemopoietic stem cells (HSCs) and progenitor cells (7).

Flt3, a receptor tyrosine kinase with homology to c-Kit (the receptor for stem cell factor [SCF]), and c-fms (the receptor for M-CSF) plays an important role in early hemopoietic development (reviewed in Ref. 8) and is a nonredundant cytokine for in vivo steady-state differentiation of IPCs and DCs: flt3 is expressed in mouse short-term hemopoietic stem cells (9, 10), in lymphoid and myeloid committed hemopoietic progenitor cell fractions (11–13), and, as we have shown recently, also on steady-state IPCs and DCs (12); Flt3 ligand (Flt3L) as a single cytokine is capable to induce differentiation of both IPCs and DCs in mouse whole BM cell cultures (14, 15); in vivo IPC and DC differentiation potential is confined to Flt3 expressing hemopoietic progenitor cells along both lymphoid and myeloid differentiation pathways (12, 13); Flt3L-deficient mice and mice with hemopoietic system confined deletions of Stat3, a transcription factor activated in the Flt3 signaling cascade, show massively reduced DCs in lymphoid organs (16, 17); and finally, injection of Flt3L dramatically increases IPCs and DCs in both mice (18–21) and humans (22, 23), with up to 30% of mouse spleen cells expressing CD11c and MHC class II (18, 24).

Given the above findings, we reasoned that targeted pharmacologic disruption of Flt3 tyrosine kinase signaling should lead to inhibition of both IPC and DC development. To test this, in the present study we evaluated in vitro and in vivo effects of SU11657, a small molecule multitargeted receptor tyrosine kinase inhibitor with Flt3 affinity that has been proven to be efficient in treatment of in vivo Flt3-driven experimental leukemia (25, 26) but also acts on other tyrosine kinases as vascular endothelial growth factor receptor (VEGF-R), platelet derived growth factor receptor (PDGF-R), and c-Kit (27).

Materials and Methods

Animals

C57BL/Ka-Thy1.1 (CD45.1 and CD45.2) and Flt3L−/− (16) mice were bred and maintained at the Institute for Research in Biomedicine animal facility, in accordance with institutional guidelines.

Abs and flow cytometry

Biotinylated, FITC-, PE-, or allophycocyanin-conjugated mAbs against the following mouse Ags were used: CD3ε (I45-2C11), CD11b (M1/70), CD19 (MB19-1), CD45RB/B20 (RA3-6B2), CD45.1 (A20), CD45.2 (104) CD90.1 (H11002), CD11b (M1/70), CD11c (HL3), and NK1.1 (PK136) (all BD Pharmingen). For visualization of biotinylated Abs, streptavidin-conjugated mAbs (Jackson ImmunoResearch) were used.

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3 Abbreviations used in this paper: IPC, IFN-producing cell; DC, dendritic cell; BM, bone marrow; HSC, hemopoietic stem cell; SCF, stem cell factor; Flt3L, Flt3 ligand; VEGF-R, vascular endothelial growth factor receptor; PDGF-R, platelet-derived growth factor receptor; CML, chronic myelogenous leukemia.
appropriate isotype-matched, irrelevant control mAbs were used to determine the level of background staining. Analysis was performed on a FACSCalibur and FACSCompantage System (BD Biosciences Immunocytometry Systems).

**DC and IPC cultures**

To generate IPCs and DCs, mouse whole BM cells (1 × 10^9/ml) were cultured in flat-bottom 48- or 96-well plates in RPMI 1640 medium, supplemented with 10% FCS, 10^{-4} M 2-ME, sodium pyruvate, antibiotics, and either 20 ng/ml murine GM-CSF (R&D Systems) or 100 ng/ml recombinant human Flt3L-Fc fusion protein. Flt3L-Fc fusion protein was produced in Drosophila cells as described previously (28). Increasing doses of SU11657, a small molecule multitargeted receptor tyrosine kinase inhibitor with demonstrated Flt3 tyrosine kinase affinity (IC_{50}, 0.05 mM to mutated, constitutively active Flt3) (25), VEGF-R (VEGF-R1 IC_{50}, 0.013 mM; VEGF-R2 IC_{50}, 0.017 mM), PDGF (PDGF-R IC_{50}, 0.005 mM), and c-Kit (IC_{50}, 0.04 mM) affinity (27) or SU10944, a small molecule receptor tyrosine kinase inhibitor with demonstrated VEGF-R affinity (28). No inhibition of IPC and DC differentiation was observed (Fig. 1, A and B). Also, to rule out that SU11657-induced inhibition of differentiation was due to effects independent of Flt3 signaling, we added SU10944, a tyrosine kinase inhibitor with VEGF-R affinity (29). No inhibition of IPC and DC differentiation was not affected (Fig. 1C). Thus, SU11657 inhibits IPC and DC development in Flt3L-supplemented whole BM cultures. DC differentiation likely via flt3 receptor tyrosine kinase inhibition in a dose-dependent manner.

In vivo SU11657 tyrosine kinase inhibitor application leads to IPC and DC reduction in spleen and lymph nodes, resembling IPC and DC levels in Flt3-deficient mice.

We first tested in vivo effects of SU11657 on cell numbers and composition of the hematolymphoid system. Six- to 8-week-old mice were lethally irradiated (2 × 6.5 Gy in a 4-h interval from a cesium 137 source, Biobeam 8000; STS) and were i.v. transplanted with 2.5 × 10^6 whole BM cells from vehicle- or SU11657-treated CD45.2 congenic mice. Animals were maintained on antibiotic water (ciprofloxacin) for 3 wk. Survival and engraftment was evaluated at 4 wk after transplantation.

For oral application via gavage, SU11657 was resolved in 100 mg/ml carboxymethyl-cellulose suspension. Six- to 8-wk-old mice received once daily 40 mg/kg SU11657, a dose previously determined to be sufficient for in vivo treatment of a murine experimental leukaemia carrying an activating Flt3 mutation (25). Control animals received vehicle (100 μl of carboxymethyl-cellulose suspension) alone. Because IPCs and DCs have a half-life of 4–11 days (30, 31) and early hemopoietic progenitor cells differentiate in 2 wk into IPCs and DCs in vivo (12, 32–34), mice were treated for 14 days, and organs were analyzed at day 15. Spleens and lymph nodes were cut in small fragments and digested under repeated agitation for 30 min at 37°C in RPMI 1640 medium supplemented with 10% FCS, 1 mg/ml collagenase (collagenase D; Roche Diagnostics System), and 50 μg/ml DNase (DNase I from bovine pancreas grade II; Roche Diagnostic System). Debris was removed by filtration, and red cells were lysed osmotically. Nucleated cells were stained and analyzed by FACSCalibur and CD11b mAbs. IPCs were identified as CD11b^-B220^-MHC class II’h lineage.’  

Flt3-ligand and SCF measurement

Serum concentrations of Flt3L and SCF were measured by ELISA, according to the manufacturer’s instructions (R&D Systems). RT-PCR

RNA was isolated from BM cells of SU11657 and vehicle-treated mice using TRIzol Reagent (Invitrogen Life Technologies), followed by DNase I (Invitrogen Life Technologies) treatment. cDNA was synthesized using random hexamers and Superscript II reverse transcriptase (Invitrogen Life Technologies). Five-fold dilutions of cDNA products were amplified by PCR using specific primers (GAPDH, 5' ACCACAGTCCATGCTACAC-3' and 5' TCCAC CACCTTGCCTGTA-3'; Flt3, 5' ATAGACCGGCTACACAA3' and 5' GCAAGACGCCTCAGAATT-3'; and c-Ki, 5' ACAGGACGAG CACAAAGGTTG-3' and 5' GCAGGACAAAGGACAAGAC-3'). PCR products were electrophoresed on an ethidium bromide-stained agarose gel.

**Statistical analysis**

Differences between groups were evaluated for statistical significance using the two-tailed paired Student’s t test, assuming equal variances.

**Results**

SU11657 inhibits both IPC and DC development in Flt3L-supplemented, whole BM cultures in a dose-dependent manner.

It was shown that both IPCs and DCs can be generated from Flt3L-supplemented mouse whole BM cultures (15). As expected, addition of increasing doses of SU11657- to Flt3L-supplemented whole BM cultures resulted in a dose-dependent suppression of both IPC and DC development (functional IC_{50}, 0.02 μM) (Fig. 1, A and B). Although tyrosine kinase inhibitors are designed to have high affinity to one selected target kinase, most inhibitors are not specific, and cross-reactivity to other tyrosine kinases will occur. To rule out that in vitro inhibition of IPC and DC development was due to SU11657-induced cross-inhibition of VEGF-R tyrosine kinase, we in parallel tested effects of SU10944, a tyrosine kinase inhibitor with VEGF-R affinity (29). No inhibition of IPC and DC differentiation was observed (Fig. 1, A and B). Also, to rule out that SU11657-induced inhibition of differentiation was due to effects independent of Flt3 signaling, we added SU10944, a tyrosine kinase inhibitor with VEGF-R affinity (29). No inhibition of IPC and DC differentiation was not affected (Fig. 1C). Thus, SU11657 inhibits IPC and DC development in Flt3L-supplemented whole BM cultures likely via flt3 receptor tyrosine kinase inhibition in a dose-dependent manner.

In vivo SU11657 tyrosine kinase inhibitor application leads to IPC and DC reduction in spleen and lymph nodes, resembling IPC and DC levels in Flt3-deficient mice.

We first tested in vivo effects of SU11657 on cell numbers and composition of the hematolymphoid system. Six- to 8-week-old mice were treated orally with 40 mg/kg body weight SU11657, a dose previously determined as optimal for in vivo application (25, 26), over 14 days. Control mice received vehicle treatment only. SU11657 treatment was well tolerated; mice showed no signs of sickness and did not lose weight (data not shown). Total cell numbers in BM and spleen were significantly reduced in SU11657-treated animals (Fig. 2, A and B). Reduction of BM and spleen cellular compartments was accompanied by a significant absolute and relative reduction of CD11b^-B220^-MHC class II' myeloid cells and also spleen NK1.1' NK cells, while relative CD19^-B220^-B cells and CD3^- T cells numbers were less, nonsignificantly affected in BM and spleen (Fig. 2, A and B). Thus, cell numbers and cellular composition in SU11657 tyrosine kinase inhibitor-treated animals were changed similar as in Flt3L-deficient and, in a reverse direction as compared with, Flt3L-injected mice, respectively (12, 16).

To evaluate if, similar to the in vitro findings, IPC and DC differentiation was inhibited in vivo by SU11657, spleen and lymph nodes of mice were analyzed for CD11c^-B220^- and CD11c^-MHC class II' cells. Indeed, DCs were significantly reduced in spleens and lymph nodes, and IPCs were significantly reduced in spleens, whereas lymph node IPC reductions did not reach statistical significance. IPC and DC percentages compared very closely to the respective cell populations in Flt3L^-/- mice (Fig. 3, A–C). DCs in treated animals did not differ from that in vehicle-treated animals in terms of CD80 and CD86 costimulatory marker expression, indicating that SU11657 treatment did not induce activation of DCs (data not shown). Upon discontinuation of SU11657 treatment, IPC and DC numbers returned to high normal levels within 10 days (Fig. 3, A–C). Thus, in vivo SU11657 tyrosine kinase inhibition led to reduction of both IPCs and DCs, comparable to IPC and DC levels observed in the absence of the cognate ligand (Flt3L^-/- mice), without irreversible blocking IPC and DC progenitors or HSCs.
FIGURE 1. SU11657 but not SU10944 inhibits both IPC (CD11c+B220+) and DC (CD11c+MHC class II+) development in Flt3L-supplemented, whole BM cultures in a dose-dependent manner. A, Graph shows percent reduction of CD11c+ cells in day 9 Flt3L whole BM cultures supplemented with increasing doses of SU11657 (■, solid line) or SU10944 (■, dashed line). Mean and SD of three independent experiments are shown. B, DC (CD11c+MHC class II+) and IPC (CD11c+B220+) reduction in 50 nM SU11657-supplemented cultures; no reduction is observed in 50 nM SU10944-supplemented cultures. C, Both SU11657 and SU10944 have no influence on DC (CD11c+MHC class II+) development in GM-CSF-supplemented whole BM cultures. B and C, Representative dot plots of three independent experiments, respectively.

FIGURE 2. Total BM and spleen cell numbers are reduced in 14 days SU11657-treated animals. A, Total BM cells are reduced in SU11657 (dark gray bar) compared with vehicle treated (light gray bar) animals (p = 0.01) with a preferential relative reduction of CD11b+ (Mac-1+) and/or Gr-1+ myeloid cells (p < 0.01). B, Total spleen cells are reduced in SU11657 (dark gray bar) compared with vehicle-treated (light gray bar) animals (p = 0.01), with a preferential relative reduction of CD11b+ (Mac-1+) and/or Gr-1+ myeloid cells (p < 0.01), and NK1.1+ cells (p = 0.02). Mean and SD of three independent experiments (two vehicle- and three SU11657-treated animals for each experiment) (A and B). Statistical significant differences are indicated.
In vivo SU11657 application leads to massive increase of serum Flt3L levels and undetectable surface Flt3 and c-Kit expression on lineage negative BM cells, while mRNA expression of both Flt3 and c-Kit are not altered.

Flt3 was shown to be expressed on short-term HSCs (9, 10) and in lymphoid and myeloid committed hematopoietic progenitor cell fractions (11–13), and stimulation of the receptor was demonstrated to expand early hematopoietic progenitors (8, 11, 12, 35). Therefore, we were interested to evaluate in vivo effects of SU11657 on short-term HSCs (9, 10) as well as lymphoid (36) and myeloid (37) progenitor cells. Surprisingly, within the lineage Ag-negative (CD3−, CD19−, B220−, CD11b−, Gr-1−, TER119−) BM cell fraction, both surface Flt3 (CD135) and c-Kit (CD117) receptors were not detectable by mAb staining in SU11657-treated animal.
BM (Fig. 4A). Therefore, HSCs and both lymphoid and myeloid progenitors could not be identified by phenotype. However, both Flt3 and c-kit mRNA transcripts were expressed at similar levels in lineage-negative cells of SU11657-treated animals and in non-treated controls (Fig. 4B). Thus, failure of surface detection was not due to down-regulation of gene transcription but possibly to receptor internalization upon ligation (38). Evaluation of Flt3L and SCF levels in sera of SU11657-treated animals revealed significantly ~10-fold elevated Flt3L and ~1.5-fold (nonsignificant) elevated SCF levels (Fig. 4C). Both surface Flt3 and c-Kit expression as well as elevated cytokine levels returned to normal values upon discontinuation of treatment (Fig. 4, A and C).

Thus, in vivo SU11657-mediated inhibition of tyrosine kinase activity led to a substantial increase of Flt3L and, to a somewhat lower extent, also of SCF, indicating that possibly regulatory feed-

back loops were activated that, however, were not able to overcome SU11657 induced effects on IPC and DC development.

**In vivo SU11657 application does not decrease myeloid colony-forming unit activity and in vivo repopulation potential of BM hematopoietic cells**

To determine whether in vivo SU11657 treatment would have a lasting negative effect on biological functions of myeloid progenitor cells and HSCs, we tested BM myeloid progenitor function in vitro and hematopoietic reconstitution potential in vivo. BM cells isolated from vehicle- or SU11657-treated animals were plated in myeloid colony-forming assays. As shown in Table I, no difference in colony formation was observed. Also, lethally irradiated animals, reconstituted with total BM cells from vehicle- or SU11657-treated mice were protected from radiation-induced death, and no differences in repopulation of BM and lymphoid organs at 4 wk after transplantation were observed (Table II).

Therefore, in vivo SU11657 treatment did not irreversibly impair myeloid progenitor cells and HSCs as assessed by myeloid colony forming, short-term in vivo repopulation, and radioprotective capacity.

**Discussion**

In vivo steady-state IPC and DC development largely depends on Flt3L as Flt3L⁻/⁻ mice have substantially decreased, and Flt3L-injected mice and humans develop increased absolute and relative IPC and DC numbers (16, 18, 19, 21–23). Both IPCs and DCs develop from mouse whole BM cells (14, 15) and from human CD34⁺ hematopoietic progenitors (39, 40) in Flt3L-supplemented cell cultures. Thus, inhibition of Flt3 signaling should lead to decreased differentiation of both cell populations. Following the finding that mutated, constitutively active Flt3 tyrosine kinase signaling is involved in human and experimental murine leukemia, small molecule tyrosine kinase inhibitors have been developed and are tested in preclinical and clinical settings (41–45). In the present study, we used SU11657, a multitargeted tyrosine kinase inhibitor with affinity to Flt3 (25) to evaluate the effects of inhibition on IPC development.

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<th>Table I. Myeloid colony formation is not inhibited in SU11657-treated animal bone marrow cells</th>
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*Table depicts mean colony-forming units granulocyte/macrophage/granulocyte-macrophage (CFU-G/M/GM), mixed colony-forming units (CFU-Mix), and colony-forming units erythrocyte/ megakaryocyte/erythrocyte-megakaryocyte-erythrocyte (CFU-E/Meg/MegE) at day 10 of cultures. A total of 10⁵ bone marrow cells was and plated each. Mean colony numbers of three independent experiments using two vehicle and two SU11657-treated mice each are shown. No significant differences were observed.

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<th>Table II. In vivo reconstitution potential is not inhibited in SU11657-treated animal bone marrow cells</th>
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*Table depicts mean percentages of CD45.2⁺ donor derived cells in lethally irradiated CD45.1⁺ recipient mice 4 wks after transplantation with 2.5 × 10⁵ whole bone marrow cells from vehicle or SU11657-treated animals. Mean values of two independent experiments are shown. In each experiment, three mice received combined bone marrow cells from two SU11657-treated animals, and two mice received combined bone marrow cells from two vehicle-treated mice.

**FIGURE 4.** SU11657 treatment leads to increased endogenous Flt3L levels and undetectable surface Flt3 and c-Kit receptors on lineage-negative progenitor cells, while mRNA expression of Flt3 and c-Kit are not altered. A, Contour plots show representative c-Kit (CD117) and Flt3 (CD135) expression on lin⁻ (CD3⁻ CD19⁻ B220 CD11b⁻ Gr-1⁻ TER119⁻ ) BM cells in vehicle- and SU11657-treated animals, as well as in mice 10 days after discontinuation of SU11657 treatment. Percentages of cells in respective gated areas are given. B, Flt3, c-Kit, and GAPDH mRNA expression in lin⁻ BM cells of vehicle- and SU11657-treated mice. Numbers on top indicate decreasing cell number equivalents of which RNA was used. Representative experiment of two. C, Bar graphs show serum Flt3L and SCF levels in vehicle (light gray bar) and SU11657 (dark gray bar)-treated mice, as well as in mice 10 days after discontinuation of SU11657 treatment. Mean and range of two independent experiments (two vehicle- and three SU11657-treated and two animals 10 days after discontinuation of SU11657 treatment per experiment). Statistically significant differences are indicated.
and DC development. Indeed, pharmacologic disruption of Flt3 tyrosine kinase signaling lead to inhibition of both IPC and DC development in a dose-dependent manner in vitro (Fig. 1). This effect was not due to cross-inhibition of VEGF-R tyrosine kinase signaling by SU11657 because SU10944, a tyrosine kinase inhibitor with VEGF-R affinity (29), showed no effect on development of both IPCs and DCs in vitro. Although the data presented here does not dissect to what extent the observed effects are mediated by SU11657 cross-reactivity to other receptor tyrosine kinases beyond VEGF-R, SU11657 effects were likely Flt3 mediated because 1) SU11657 addition to GM-CSF-supplemented BM cultures did not affect in vitro DC development, and 2) in vivo SU11657 treatment led to a significant decrease of relative and absolute IPC and DC numbers and a reduction of other hematopoietic cells as Mac-1+Gr-1+ cells and NK cells, closely resembling the cellular composition found in Flt3L−/− mice (Fig. 3). Thus, SU11657-mediated inhibition of the Flt3 tyrosine kinase is sufficient to explain the cellular alterations observed in vitro and in vivo.

Interestingly, SU11657 treatment led to a massive increase of endogenous Flt3L and moderate increase of SCF that, however, were not able to overcome the IPC and DC differentiation block induced by SU11657 (Fig. 4). Increased endogenous Flt3L levels were described during hemopoietic recovery following combined gamma irradiation and chemotherapy conditioning in humans, and these increases were positively correlated with DC recovery (46). Thus, these findings point to a remarkable Flt3/Flt3L regulatory-loop in hematopoiesis. It will be of interest to test how Flt3L expression is induced in SU11657 tyrosine kinase inhibitor-treated animals, if it is a direct result of decreased downstream Flt3 signaling, or if it is possibly caused by diminished hemopoietic stem and progenitor cell proliferation and differentiation. Similarly, it will be important to determine whether observed, nonsignificant alterations in SCF levels are due to SU11657 cross-reactivity to the c-Kit tyrosine kinase.

Of note, both 4-wk radiation protection and myeloid colony forming potential of in vivo SU11657-pretreated BM cells was not impaired, and IPC and DC numbers recovered within 10 days after discontinuation of treatment, about the time it takes for HSCs and early progenitors to differentiate to these cell populations upon in vivo transfer (32, 33, 47). Therefore, stem cell and myeloid progenitor function remained intact, at least as determined by these short-term assays (Tables I and II; Fig. 3).

Imatinib mesylate (also know as Glivec or STI571) is a small molecule protein tyrosine kinase inhibitor of Abl tyrosine kinases that also acts on other tyrosine kinases as the PDGF-R, c-Kit, and ARG (48–51). Imatinib is currently used with tremendous clinical success in the treatment of Bcr-Abl+ chronic myelogenous leukemia (CML) (52, 53). In two recent studies, Imatinib was described to inhibit DC development in human in vitro CD34+ stem and progenitor cell DC differentiation cultures and in vivo in Flt3L-injected mice (54, 55). Moreover, human DCs that developed in the presence of Imatinib mesylate were shown to be functionally impaired in their capacity to prime naïve T cells in vitro (54), and Flt3L-mediated antitumor effects were abrogated by consecutive application of Imatinib in vivo (55). Thus, concerns regarding the potential immunosuppressive effects of Imatinib have been raised (54, 55). However, in another report, human CML CD34+ cells were able to differentiate into IPCs only in the presence of Imatinib, and IPC levels were restored in responding but not in non-responding CML patients (56). In this study, it was suggested that Imatinib treatment was inducing up-regulation of surface Flt3 on hemopoietic progenitor cells, and successful treatment might lead to restoration of immune functions (56). Thus, results of current studies on Imatinib effects on IPC and DC development and function, as well as on Flt3 regulation, are at least controversial, and the molecular basis of these observations remains to be determined.

In contrast to this, it is known that Flt3L is a nonredundant cytokine for IPC and DC development (16, 21), and we demonstrated for the first time that application of a multtargeted receptor tyrosine kinase inhibitor with Flt3 affinity leads to decreased IPC and DC levels in vivo. Flt3 tyrosine kinase inhibitors used in clinical trials for the treatment of leukemia with mutated, constitutively active Flt3 (41–44) might therefore rather lead to suppression then restoration of immune functions. We would like to suggest that this effect might be exploited in other clinical settings where suppression of immune responses is a therapeutic goal, as in the treatment of autoimmune diseases or complications of organ or blood cell transplantation. For example, it has been shown by others and us that residual host DCs are involved in initiation of graft-vs-host disease after allogeneic cell transplantation in animal models (57–61). It is conceivable that implementation of a Flt3 tyrosine kinase inhibitor during the conditioning regimen before allogeneic hemopoietic cell transplantation would diminish host DC numbers, consecutively might prevent acute graft-vs-host disease, and thus could allow to reduce T cell immunosuppressive drugs in the posttransplantation period.

The data presented here provides the basis to evaluate Flt3 tyrosine kinase inhibitors as immunomodulators in autoimmune diseases and in immune system-mediated complications of organ or blood cell transplantation.

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Disclosures
The authors have no financial interest of interest.

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