Ki11502, a novel multitargeted receptor tyrosine kinase inhibitor, induces growth arrest and apoptosis of human leukemia cells in vitro and in vivo

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Ki11502 is a novel multitargeted receptor tyrosine kinase (RTK) inhibitor with selectivity against *platelet-derived growth factor receptor alpha/beta (PDGFRα/β).* Ki11502 (0.1-1 nM, 2 days) profoundly caused growth arrest, G_0/G_1 cell-cycle arrest, and apoptosis associated with down-regulation of Bcl-2 family proteins in the eosinophilic leukemia EOL-1 cells having the activated *FIP1-like 1/PDGFRα* fusion gene. Ki11502 decreased levels of p-PDGFRα and its downstream signals, including p-Akt, p-ERK, and p-STAT5, in EOL-1 cells. Of note, Ki11502 was also active against imatinib-resistant PDGFRαT674I mutant. In addition, Ki11502 inhibited proliferation of biphenotipic leukemia MV4-11 and acute myelogenous leukemia MOLM13 and freshly isolated leukemia cells having activating mutations in *FMS-like tyrosine kinase* 3 (*FLT3).* This occurred in parallel with the drug inhibiting FLT3 and its downstream signal pathways, as measured by fluorescence-activated cell sorting using the phospho-specific antibodies. In addition, Ki11502 totally inhibited proliferation of EOL-1 cells growing as tumor xenografts in SCID mice without any noticeable adverse effects. Taken together, Ki11502 has profound antiproliferative effects on select subsets of leukemia including those possessing imatinib-resistant mutation. (Blood. 2008;111:5086-5092)

Introduction

The class III/V receptor tyrosine kinases (RTKs), including FMS-like tyrosine kinase 3 (FLT3), c-KIT, platelet-derived growth factor receptor (PDGFR), and vascular endothelial growth factor receptor (VEGFR), are implicated in the pathophysiology of several cancers. These RTKs all share the same topology, consisting of 5 extracellular immunoglobulin-like domains, a juxtamembrane (JM) domain, a kinase domain interrupted by a kinase insertion domain, and an intracellular C-terminal domain.[1] Ligand binding to RTKs results in the activation of downstream effectors, including protein kinase B/Akt, signal transducers and activators of transcription (STATs), and extracellular signal-regulated kinases (ERKs) 1/2, leading to cell proliferation, differentiation, and/or survival.[2,3] Recent studies have revealed that activating mutations of RTKs frequently occur in acute myelogenous leukemia (AML) patients. For example, internal tandem duplications (ITDs) of the JM domain of *FLT3* (*FLT3-ITD*) are present in 20% to 30% of de novo AML cases[4]; and approximately 7% of AML patients possess the D835 mutation, a point mutation in the activation loop of the second kinase domain of *FLT3*.[4] These mutations result in constitutively activated FLT3 and are associated with elevated blast counts, increased relapse rates, and poor overall survival in AML.[5-8] Mutations of *c-KIT* and *PDGFR* are also associated with subsets of AML[9-12] and gastrointestinal stromal tumors (GISTs).[13,14] Therefore, inhibition of the kinase activity of these receptors may be a useful approach to selected AML patients.

The hypereosinophilic syndrome (HES) is a rare hematologic disorder characterized by the sustained overproduction of eosinophils (> 1500 eosinophils/μL) in the absence of other causes of eosinophilia and organ damage caused by tissue infiltration of eosinophils.[15] HES is reclassified as chronic eosinophilic leukemia (CEL) when clonality is demonstrated. *FIP1L1/PDGFRα* generated by an interstitial deletion on chromosome 4q12 was found in a subset of CEL as well as HES.[12,16,17] The individuals with *FIP1L1/PDGFRα* were successfully treated with low dose of imatinib; however, imatinib resistance mediated by T674I, which is analogous to T315I mutation found in chronic myeloid leukemia, was observed in one patient[16,17] This mutation has potential to become a serious problem in individuals with HES or CEL treated with imatinib.

Ki11502 is a novel multikinase inhibitor with selectivity against PDGFR, which was initially identified through screening a compound library.[18] This study explored the effect of Ki11502 against a variety of leukemia cells.

Methods

Reagents

Ki11502 (Figure 1) was dissolved in 100% dimethyl sulfoxide to a stock concentration of 10 mM and stored at −80°C. Imatinib was provided by Novartis (Basel, Switzerland) and dissolved in 100% dimethyl sulfoxide to a stock concentration 10 mM.

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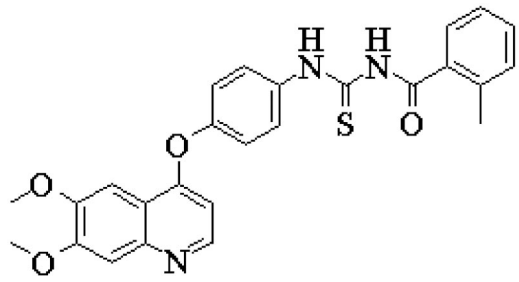

Figure 1. Chemical structure of Ki11502 (molecular weight, 473.6).

Cells

Characteristics of cell lines were described previously.[19] Leukemia cells from patients were freshly isolated with informed consent and Kochi University institutional review board approval. The informed consent was obtained in accordance with the Declaration of Helsinki.

$CD34^+$ hematopoietic stem cells were isolated from healthy volunteers by magnetic cell sorting using CD34 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) as previously described.[20] Cells (1×10^6 cells/mL) were added 1:10 to methylcellulose medium H4534 (StemCell Technologies, Vancouver, BC) to yield a final concentration of 1% methylcellulose, 30% fetal calf serum, 1% bovine serum albumin, 0.1 mM of mercaptoethanol, 2 mM L-glutamine, 50 ng/mL stem cell factor, 10 ng/mL granulocyte-macrophage colony-stimulating factors, and 10 ng/mL interleukin-3 (IL-3). Cells were plated in 24-well plates in the presence or absence of Ki11502 (0.01-2 μM), incubated at 37°C in a humidified atmosphere containing 5% CO_2, and the resulting colonies were counted 2 weeks later. All experiments were done in triplicate plates per experimental point.

FLT3 genotyping

FLT3-ITD and *FLT3-D835* mutations were examined as previously described.[19]

In vitro kinase assays

A panel of kinases was purchased from Invitrogen (Carlsbad, CA). The ability of Ki11502 and imatinib to inhibit these kinases was measured by ELISA-based assay according to the manufacturer's instruction.

Thymidine uptake studies

DNA synthesis was measured by tritiated thymidine uptake [^{3}H-TdR] (PerkinElmer, Waltham, MA) as previously described.[19] All experiments were performed in triplicate and repeated at least 3 times.

Cell-cycle analysis by flow cytometry

Cell-cycle analysis was performed as previously described using the CellQuest software package (BD Biosciences, San Diego, CA).[21]

Apoptosis assays

The ability of Ki11502 to induce apoptosis of leukemia cells was measured using the annexin V–FITC apoptosis detection kit (BD PharMingen, San Diego, CA), according to the manufacturer's instructions.

Immunoblotting

Immunoblotting was performed as previously described.[19] Anti-Bcl-2, anti-Bcl-xL (Cell Signaling Technology, Beverly, MA), anti-Mcl-1, and anti-β-actin antibodies were used. All antibodies except for anti-Bcl-xL antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Phosphorylation analysis of PDGFRα

Lysates from EOL-1 or MV4-11 cells were prepared as previously described and were immunoprecipitated with anti-PDGFRα (C-20, Santa Cruz Biotechnology) or anti-FLT3 (C-18, Santa Cruz Biotechnology) antibody and protein G Sepharose (Pierce, Rockford, IL).[19] The precipitated samples were subjected to Western blot analysis. The membrane was sequentially probed with antiphosphotyrosine (Cell Signaling Technology), and anti-PDGFRα or FLT3 antibodies.

FACS

The impact of Ki11502 on RTK and its downstream signal pathways was assessed by fluorescence-activated cell sorting (FACS) using the phosphor-specific antibodies. Anti-Flt3/CD135 (ab23895, Abcam, Cambridge, United Kingdom), -phospho-FLT3 (Tyr591) (Cell Signaling Technology, 3461), -phospho-p44/42 MAPK (T202/Y204)(E10) (Alexa Fluor 488 Conjugate, Cell Signaling Technology, 4374), -p44/42 MAPK (Cell Signaling Technology, 9102), -Akt (Cell Signaling Technology, 9272), -phospho-Akt (Ser473) (Cell Signaling Technology, 9271), -STAT5 (C-17) (Santa Cruz Biotechnology, sc-835), and -phospho-STAT5 (Tyr694) (Cell Signaling Technology, 9351) antibodies were used.

Xenotransplantation of human leukemia EOL-1 cells into SCID mice

Female SCID mice (Charles River Japan, Tsukuba, Japan) had free access to sterilized commercial rodent chow and filtered tap water. Animals were subcutaneously injected with 2×10^6 EOL-1 cells/tumor in 0.1 mL Matrigel (BD Biosciences). When EOL-1 cells formed palpable tumors, mice were divided randomly into control (n = 3) and treatment groups (n = 3), and treatment was begun. Ki11502 (50 mg/kg) was given to mice by gavage twice a day for 5 days. The dose of these agents was determined by our preliminary studies (data not shown). Control diluent was given to the untreated control mice. Body weight and tumors were measured twice a week. All animal experiments were approved by the Institutional Review Board.

Results

Ki11502 is a potent multitargeted receptor tyrosine kinase inhibitor

The in vitro kinase assay found that Ki11502 was a potent inhibitor of PDGFRα and β with additional activities against FLT3, KIT, and KDR (Table 1). The activity of Ki11502 against PDGFRα/β was as potent as that of imatinib (Table 1). Of note, Ki11502 was able to inhibit imatinib-resistant PDGFRαT674I, found in CEL.[15-17] In

Table 1. Activity of Ki11502 against a panel of kinases

Kinase	Ki11502, IC_{50} (nM)	Imatinib, IC_{50} (nM)
PDGFRα	<10	<10
PDGFRβ	<10	<10
PDGFRαT674II	129.6	>1000
ABL	>1000	25
KIT	86.81	250
KITT670I	427.8	>1000
FLT3	37.54	>1000
FLT3D835Y	541.6	>1000
KDR	210	>1000
IGF1-R	>1000	>1000
EGFR	>1000	>1000
FGF-R2	>1000	>1000

PDGFR indicates platelet-derived growth factor receptor; FLT3, fms-related tyrosine kinae; IGF1-R, insulin-like growth factor 1 receptor; EGFR, epidermal growth factor receptor; and FGF-R2, fibroblast growth factor receptor.

Concentrations that cause 50% inhibition (IC_{50}) are given.

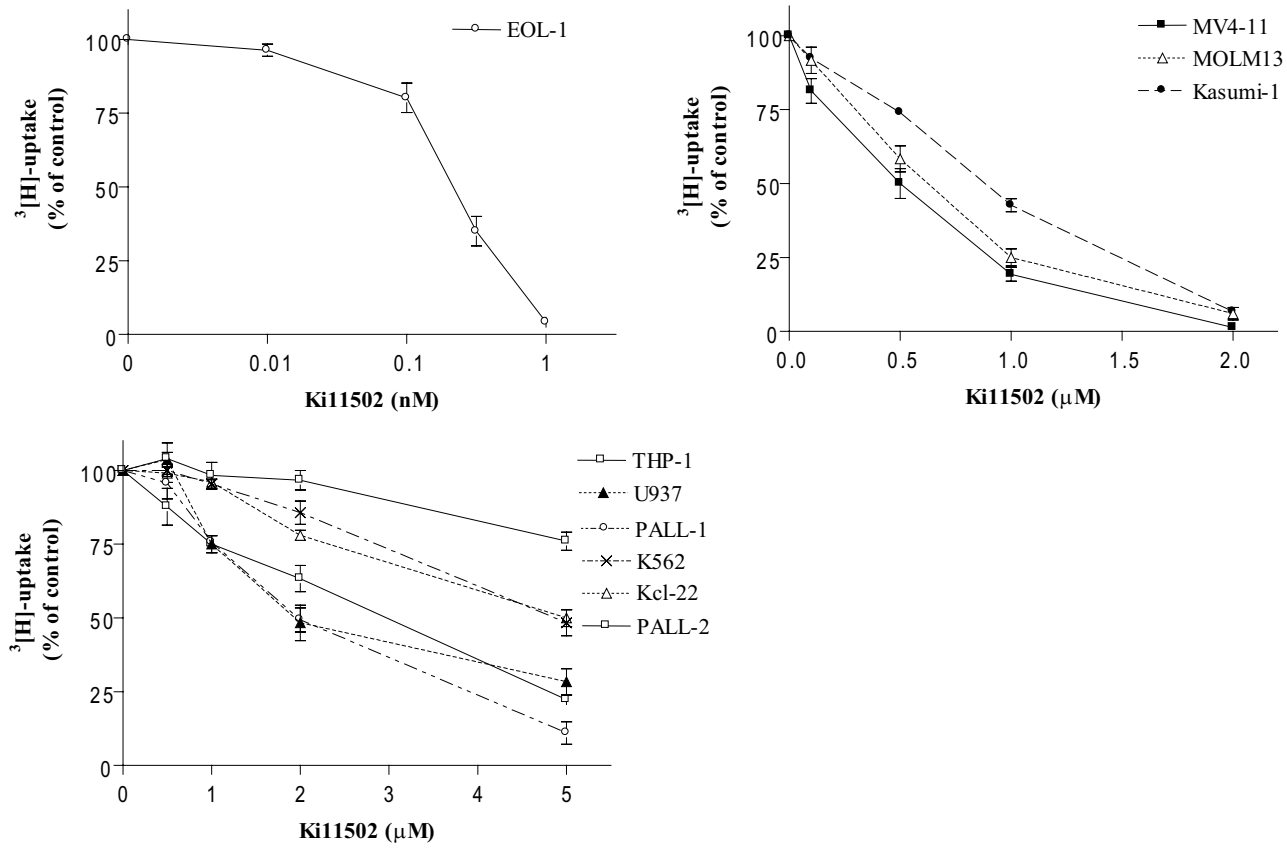

Figure 2. Ki11502 inhibits proliferation of a variety of human leukemia cells: ^{3}[H]-thymidine uptake study. Cells (5×10^5/mL) were plated in 96-well plates and cultured with various concentrations of Ki11502 (0.01 nM to 5 μM). After 2 days, proliferation was measured by ^{3}[H]-thymidine uptake study. Results represent the mean plus or minus SD of 3 experiments performed in triplicate.

addition, Ki11502 blocked kinase activity of imatinib-resistant KITT670I mutant, which was found in a GIST patient and was also resistant to nilotinib and dasatinib.[22]

Ki11502-induced growth arrest of human leukemia cells

Leukemic cells were grown in liquid culture for 2 days in the presence of various concentrations of Ki11502 (0.01 nM to 5 μM). Growth inhibition was measured by thymidine uptake; the percentage inhibition was graphed (Figure 2), and the concentration of Ki11502 that induced 50% growth inhibition (IC_{50}) of EOL-1 cells (*FIP1L1/PDGFRα* fusion) was 0.2 nM (Table 2). In addition, Ki11502 was active against FLT3/ITD-expressing MV4-11 and MOLM13, BCR/ABL-expressing Kcl-22, K562, PALL-1, and PALL-2 cells, as well as Kasumi-1 cells with gain-of-function mutation in *c-KIT* with IC_{50} values ranging from 0.5 to 5 μM (Table 2). In contrast, other types of leukemia cells, except for U937 cells, without known mutations in their RTK genes were generally more resistant to Ki11502 (Table 2).

Effect of Ki11502 on cell-cycle distribution of leukemia cells

To investigate the mechanisms by which Ki11502 inhibited the growth of EOL-1 cells, we explored the effects of Ki11502 on the cell cycle of these cells by flow cytometry (Figure 3A). Exposure of EOL-1 cells to Ki11502 (0.1-1 nM) for 24 hours induced an increase in the number of cells in the G_0/G_1 phase of the cell cycle compared with untreated controls, with a concomitant decrease in the proportion of cells in the S phase and increase in the cells in the pre-G_1 phase of cell cycle; the latter is characteristic of apoptosis (Figure 3A). Accumulation of cells in the pre-G_1 phase of the cell cycle became more prominent after exposure to Ki11502 (0.1-1.0 nM) for 48 hours (Figure 3A). The presence of apoptotic

Table 2. Inhibition of the proliferation of malignant hematopoietic cells by Ki11502

Cell line	Gene alteration	IC_{50}, μM*
Eosinophilic leukemia: EOL-1	FIP1L1/PDGFRα	0.0002
Biphenotypic leukemia: MV4-11	FLT3-ITD	0.5
Myeloid leukemia		
MOLM13	FLT3-ITD	0.6
Kasumi-1	c-KIT (Asn822Lys)	1
U937	2	
THP-1	—	Not achieved
HL-60	—	Not achieved
Kcl-22	Bcr/Abl	5
K562	Bcr/Abl	5
Lymphoblastic leukemia		
PALL-1	Bcr/Abl	2
PALL-2	Bcr/Abl	3
BALL-2	—	Not achieved
Jurkat	—	Not achieved
Multiple myeloma: U266	—	Not achieved
Adult T-cell leukemia		
MT-1	—	Not achieved
MT-2	—	Not achieved
HUT102	—	Not achieved

— indicates not applicable.

*Concentration of Ki11502 that induced 50% growth inhibition (IC_{50}) was calculated from dose-response curves.

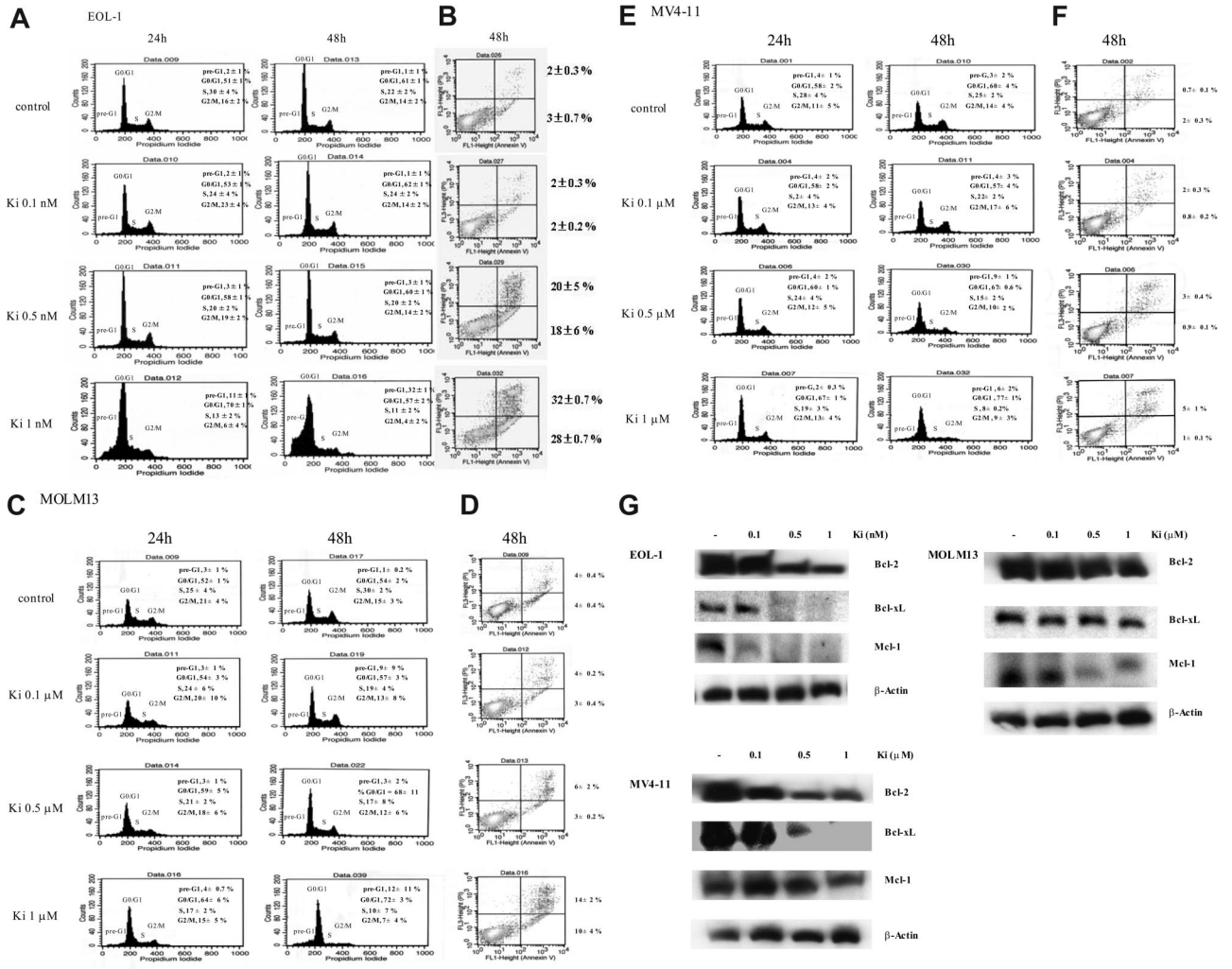

Figure 3. Ki11502 induces G_0/G_1 cell-cycle arrest and apoptosis of leukemia cells. (A,C,E) Cell-cycle analysis. EOL-1 (A), MOLM13 (C), or MV4–11 (E) cells (5×10^5/mL) were plated in 6-well plates and cultured with Ki11502. After 24 or 48 hours, cells were harvested and cell-cycle distribution was analyzed by FACScan. The numerical results represent the mean of triplicate plates, and a representative experiment is shown. (B,D,F) Annexin V binding. EOL-1 (A), MOLM13 (C), or MV4–11 (E) cells (5×10^5/mL) were plated in 24-well plates and cultured with various concentrations of Ki11502. After 48 hours, cells were harvested and stained for annexin V and propidium iodide (PI), and cells were analyzed by FACScan. Lower left quadrants: viable cells. Lower right quadrants: early apoptotic cells (annexin V^+, PI^-). Upper right quadrants: nonviable, late apoptotic/necrotic cells (annexin V and PI^+). The numerical results represent the mean of triplicate plates, and a representative experiment is shown. (G) Western blot analysis. EOL-1, MV4-11, and MOLM13 cells were cultured with various concentrations of Ki11502 (0.1-1 nM for EOL-1 and 0.1-1 μM for MV4-11 as well as MOLM13). After 48 hours, cells were harvested, and proteins were extracted and subjected to Western blot analysis. The membranes were sequentially probed with anti–Bcl-2, Bcl-xL, Mcl-1, and anti–β-actin antibodies. Results represent one of the 3 experiments performed independently, each giving similar results.

cells was assessed by measuring annexin V staining in EOL-1 cells treated with Ki11502 for 48 hours. Ki11502 (0.5 or 1.0 nM) induced a mean 38% and 60% of EOL-1 cells to become apoptotic, respectively (Figure 3B). Similarly, Ki11502 (0.1-1 μM) induced G_0/G_1 cell-cycle arrest in MOLM13 and MV4-11cells, followed by apoptosis (Figure 3C,D), although effects were less potent compared with those in EOL-1 cells.

Ki11502 down-regulated levels of Bcl-2 family members in leukemia cells

Previous studies showed that down-regulation of Bcl-2 family member was critical to inducing apoptosis of AML cells after exposure to a FLT3 kinase inhibitor.[23] We therefore explored whether Ki11502 could modulate the levels of these antiapoptotic proteins as assessed by Western blot analysis. Ki11502 (0.1-1 nM, 48 hours) profoundly down-regulated levels of Bcl-2, Bcl-xL, and Mcl-1 in EOL-1 cells, dramatically decreased levels of Bcl-xL in MV4-11, and markedly lowered expression of Mcl-1 in MOLM13 cells (Figure 3G).

Effect of Ki11502 on RTKs and its downstream signals in leukemia cells

EOL-1 cells constitutively expressed the phosphorylated forms of PDGFRα, as a result of activated FIP1L1/ PDGFRα.[12] Exposure of these cells to Ki11502 (0.1-1 nM, 3 hours) potently down-regulated levels of p-PDGFRα, as measured by coimmunoprecipitation followed by Western blot analysis (Figure 4A). Moreover, Akt, ERK, and STAT5 signals were activated in EOL-1 cells as measured by FACS using the phospho-specific antibodies (Figure 4B). Exposure of EOL-1 cells to Ki11502 (0.1 nM, 1 hour) profoundly decreased expression of p-Akt, p-ERK, as well as p-STAT5 without a decrease in levels of total amount of these proteins (Figure 4B). Consistently, Western blot analysis showed that Ki11502 (0.1 nM, 1 hour) potently down-regulated the levels

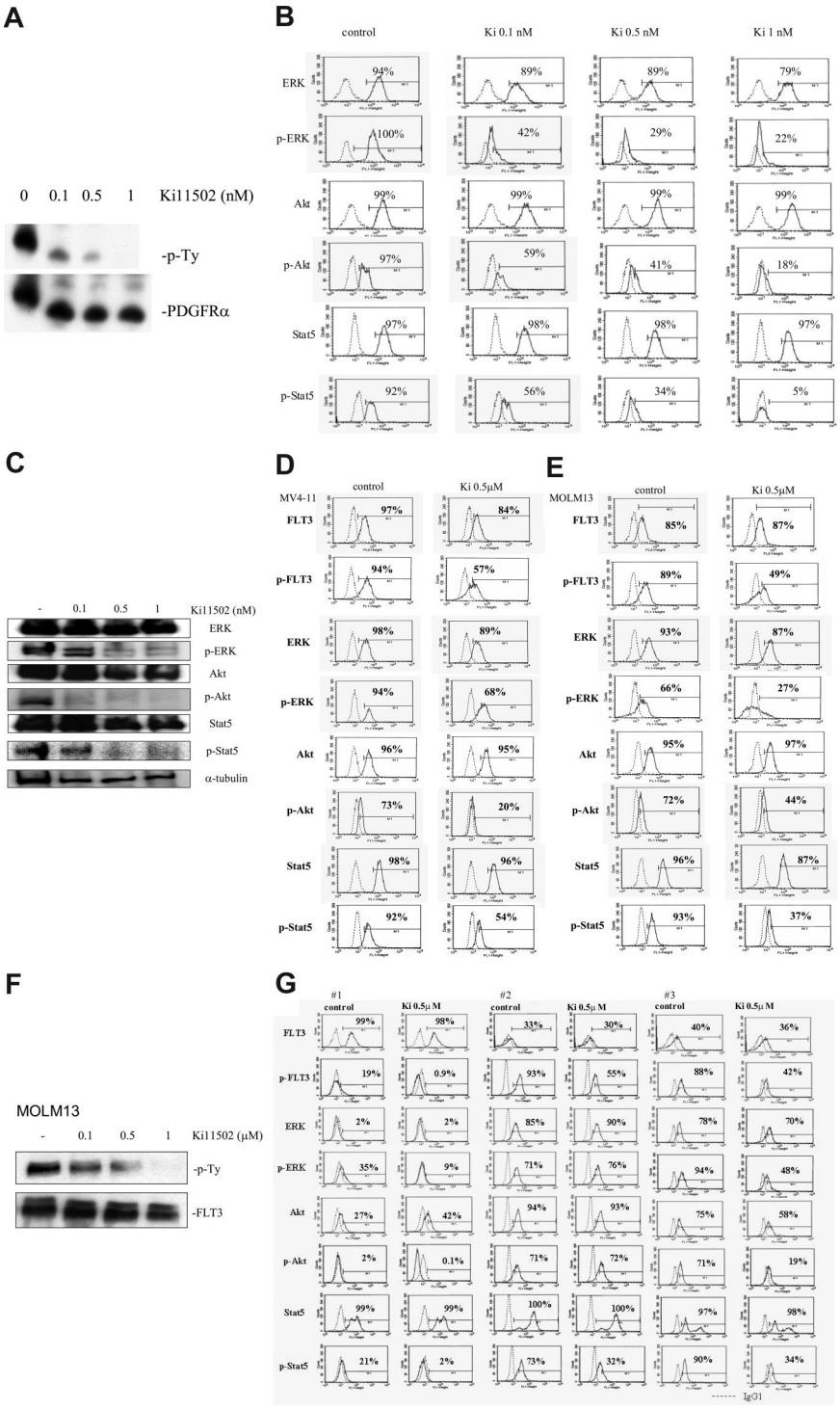

Figure 4. Ki11502 inhibits RTK and its downstream signal pathways. (A,F) Coimmunoprecipitation. EOL-1 (A) or MOLM13 (F) cells were cultured with various concentrations of Ki11502 (0.1-1 nM for EOL-1 and 0.1-1 μM for MOLM13). After 3 hours, cells were harvested and proteins were extracted. The PDGFRα or FLT3 protein was immunoprecipitated and subjected to Western blot analyses. The membrane was probed sequentially with an antiphosphotyrosine antibody (top) and an anti-PDGFRα or FLT3 (bottom). p-Ty, phosphotyrosine. (B) Analysis of ERK, Akt, and STAT5 in EOL-1 cells by FACS. EOL-1 cells were cultured with Ki11502 (0.1-1 nM) or control diluent. After 3 hours, cells were harvested, incubated with the indicated antibodies for 30 minutes at room temperature, and analyzed by flow cytometry. Results are representative of 3 experiments performed in duplicate plates. The positive population was quantified using the CellQuest software package. (C) Western blot analysis. EOL-1 cells were cultured with various concentrations of Ki11502 (0.1-1 nM). After 3 hours, cells were harvested, and proteins were extracted and subjected to Western blot analysis. The membranes were sequentially probed with the indicated antibodies. Results represent one of the 3 experiments performed independently, each giving similar results. (D,E,G) Analysis of FLT3, ERK, Akt, and STAT5 in MV4-11, MOLM13, and freshly isolated leukemia cells by FACS. MV4-11 (D), MOLM13 (E), and freshly isolated leukemia cells from patients (G, cases 1-3, Table 3) were cultured with either Ki11502 (0.5 μM) or control diluent. After 3 hours, cells were harvested, incubated with the indicated antibodies for 30 minutes at room temperature, and analyzed by flow cytometry. Results are representative of 3 experiments performed in duplicate plates.

of p-Akt, p-ERK, and p-STAT5 in EOL-1 cells (Figure 4C), suggesting that Ki11502 effectively blocked PDGFRα and its downstream signals.

We next examined the effect of Ki11502 on FLT3 and its downstream signal pathways in MV4-11 and MOLM13 cells. Both MV4-11 and MOLM13 cells expressed p-FLT3 (94% and 89%, respectively), resulting in their activation of Akt, ERK, and STAT5 (Figs 4D,E). Exposure of these cells to Ki11502 (0.5 μM, 3 hours) decreased by nearly 50% the percentage of cells expressing p-FLT3 protein associated with lower levels of activated p-ERK, p-Akt, and p-STAT5 (Figure 4D,E). Anti-FLT3 activity of Ki11502 in leukemia cells was further confirmed by immunoprecipitation followed by Western blot analysis (Figure 3F).

Moreover, we examined the effect of Ki11502 on freshly isolated leukemia cells with/without *FLT3/ITD* (Table 3; Figure 4G). FLT3 and its downstream signals were constitutively activated in leukemia cells with *FLT3/ITD* (cases 2 and 3, Table 3; Figure 4G). Ki11502 (0.1-1 μM, 48 hours) effectively inhibited the proliferation of these freshly isolated leukemia cells from cases 2 and 3 with IC_{50} values of 0.5 and 0.1 μM, respectively, in association with blockade of FLT3 (Table 3; Figure 4G). Interestingly, all of the 3 signal pathways (ERK, Akt, STAT5) were

Table 3. Patient clinical characteristics

Patient no.	Age, y/sex	FAB	WBC × 10^6/L	Blast, %	Source	FLT3	Karyotype	Previous treatment	IC_{50}, μM
1	82/M	M4	17,600	51	PB	—	Normal	No	1
2	61/M	M1	200,000	99	PB	ITD	Normal	No	0.5
3	55/F	M1	188,400	99	PB	ITD	Normal	No	0.1

FAB indicates French-American-British (leukemia classification); PB, peripheral blood; ITD, internal tandem duplication; WBC, white blood cells; and —, not applicable.
Freshly isolated leukemia cells were cultured in the presence of various concentrations of Ki11502 (0.01-1 μM). After 2 days, the proliferation of cells was measured by ^{3}[H]-thymidine uptake. Concentration of Ki11502 that induced 50% growth inhibition (IC_{50}) was calculated from the dose-response curves.

effectively blocked in leukemia cells from case 3, which were more sensitive to Ki11502-mediated growth inhibition than leukemia cells from case 2, in which only STAT5 signal was blocked (Figure 4G). On the other hand, freshly isolated leukemia cells with wild-type FLT3 (case 1, Table 3) slightly expressed p-FLT3 (19%), p-ERK (35%), p-Akt (2%), and p-STAT5 (21%), and were relatively resistant to Ki11502-mediated growth inhibition (IC_{50} of 1 μM, Table 3; Figure 4G). We examine whether Ki1152 affected viability of $CD34^+$ hematopoietic stem cells from healthy volunteers (n = 3). For this purpose, we performed the colony forming assay with methylcellulose culture system. Ki11502 (0.01-2 μM) did not inhibit colony formation of $CD34^+$ hematopoietic stem cells (Figure S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). On the other hand, Ki11502 (2 μM) completely inhibited the colony formation of FLT3/ITD-expressing AML cells from cases 1 to 3 (Table 3; Figure S1).

Ki11502 inhibited growth of EOL-1 cells growing as tumor xenografts in SCID mice

EOL-1 cells grew as tumor xenografts in SCID mice. Ki11501 (50 mg/kg, given by gavage twice daily for 5 days) completely inhibited the growth of EOL-1 cells without sign of wasting or any other toxicity (Figure 5).

Discussion

RTKs, including PDGFRα, c-KIT, and FLT3, are emerging as promising molecular targets of hematologic malignancies, including CEL and AML. Ki11502, a novel multikinase inhibitor with selectivity against PDGFR, potently caused growth arrest and apoptosis of eosinophilic leukemia EOL-1 cells in association with blockade of PDGFRα and its downstream signals (Figures 2-4). FIP1L1/PDGFRα fusion protein is involved in development of eosinophilic leukemia[12,24] and constitutively activates PDGFRα, resulting in stimulation of Akt, ERK, and STAT5. Imatinib, an inhibitor of PDGFRα, produced growth arrest and apoptosis of EOL-1 cells in vitro[12] and successfully controlled this hematologic malignancy.[15-17] However, development of imatinib resistance resulting from PDGFRαT674I mutation, which is homologous to the T315I gate keeper mutation in ABL, was observed in one patient after treatment with imatinib.[16,17] Recently, sorafenib, an orally active multitarget kinase inhibitor, effectively inhibited PDGFRαT674I mutant in vitro.[25] This study found that the IC_{50} value of Ki1152 for EOL-1 proliferation was 0.2 nM, which was almost identical to that of sorafenib (IC_{50}, 0.5 nM),[25] imatinib (IC_{50}, 0.2 nM),[26] and nilotinib (IC_{50}, 0.54 nM).[26] Interestingly, Ki11502 was also active against PDGFRαT674I mutant (Table 1), suggesting that Ki11502 could be of value for the treatment of individuals with CEL with PDGFRα/FIP1L1, as well as those possessing imatinib-resistant PDGFRαT674I mutant.

Moreover, Ki11502 was active against leukemia cells expressing FLT3/ITD (Tables 2,3). It effectively inhibited FLT3 in leukemia cells, although the effect of the drug on downstream signaling varied between cell types (Figure 4G). Ki11502 potently inhibited phosphorylation of ERK, Akt, and STAT5 in freshly isolated leukemia cells from case 3 (Figure 4G). On the other hand, Ki11502 attenuated only STAT5 signaling in leukemia cells from case 2 (Figure 4G). In parallel, the leukemia cells from case 3 were more sensitive to growth inhibition mediated by Ki11502 than those from case 2 (Table 2). Similarly, recent studies found that the cytotoxic response of leukemia cells to the FLT3 kinase inhibitors depended on the degree of deactivation of its downstream signals, such as ERK and STAT5.[27] For example, CEP701 or PKC412 failed to block phosphorylation of STAT5 in leukemia cells, and these cells were resistant to growth inhibition by these FLT3 kinase inhibitors; nevertheless, phosphorylation of FLT3 was effectively inhibited in these cells.[27] These observations suggested that concomitant blockade of FLT3 kinase and its downstream signals would provide clinical benefit for treatment of individuals with AML possessing *FLT3/ITD*. Indeed, we have recently shown that blockade of Akt/mTOR signaling by RAD001 (everolimus) potentiated the antiproliferative activity of sunitinib in *FLT3/ITD* containing leukemia cells,[19] as well as GIST cells.[28] In addition, concomitant inhibition of MEK/ERK signaling augmented the ability of ZD6474, a novel multikinase inhibitor, to induce growth arrest and apoptosis of AML cells with *FLT3/ITD*.[21] Future studies will explore whether blockade of MEK/ERK or Akt/mTOR signaling might potentiate the antiproliferative activity of Ki11502 in freshly isolated leukemia cells from patients such as case 2.

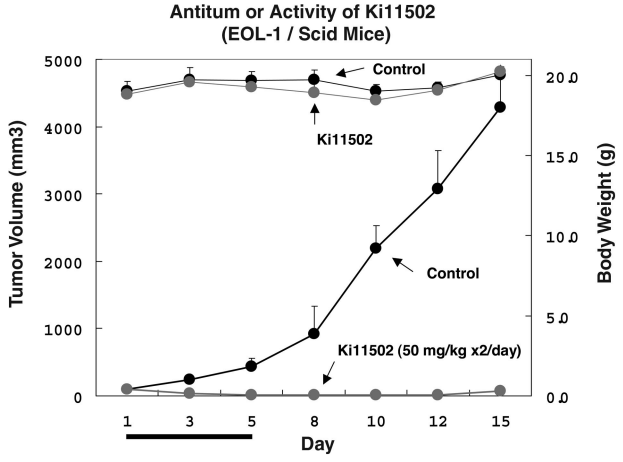

Figure 5. Ki11502 inhibits the proliferation of EOL-1 tumor xenografts in SCID mice. EOL-1 cells were injected subcutaneously into SCID mice. After EOL-1 cells formed palpable tumors, mice were randomized into 2 groups (n = 3) and treatment was initiated. Ki11502 (50 mg/kg) was orally administered twice a day for 5 days. Control mice received diluent only. Tumors were measured twice a week. Each point represents the mean plus or minus SE of 3 tumors. Body weight of mice was measured twice a week and shown at the top.

Taken together, Ki11502 may be useful for the treatment of individuals with CEL, AML, and GIST with mutations of *RTK* gene, including *PDGFRα*, *FLT3,* and *KIT*. In addition, Ki11502 could be of value for the treatment of individuals with imatinib-resistant these malignancies.

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Authorship

Contribution: T.I. contributed to the concept and design, interpreted and analyzed the data, and wrote the manuscript; C.N. performed the experiments and wrote the manuscript; J.Y. performed the experiments; A.M. synthesized and provided Ki11502; T.T., Y.K., and K.T. provided clinical samples; H.P.K. provided critical revision and intellectual content; A.Y. provided important intellectual content and gave final approval.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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