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Inhibitory Effects of Omacetaxine on Leukemic Stem Cells and BCR-ABL-Induced Chronic Myeloid Leukemia and Acute Lymphoblastic Leukemia in Mice

Yaoyu Chen[1], **Yiguo Hu**[3], **Shawnya Michaels**[2], **David Segal**[2], **Dennis Brown**[2], and **Shaoguang Li**[1]

[1]Division of Hematology/Oncology, Department of Medicine, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605, USA

[2]ChemGenex Pharmaceuticals, Inc., Menlo Park, CA 94025

[3]Current address: Dana-Farber Cancer Institute, Harvard Medical School, 44 Binney Street, Mayer 557, Boston, MA 02115, USA

Abstract

Omacetaxine mepesuccinate (formerly homoharringtonine) is a molecule with a mechanism of action that is different from tyrosine kinase inhibitors and its activity in chronic myeloid leukemia (CML) seems to be independent of BCR-ABL mutation status. Using BCR-ABL-expressing myelogenous and lymphoid cell lines and mouse models of CML and B cell acute lymphoblastic leukemia (B-ALL) induced by wild type BCR-ABL or T315I mutant-BCR-ABL, we evaluated the inhibitory effects of omacetaxine on CML and B-ALL. We demonstrated that more than 90% of the leukemic stem cells were killed after treatment with omacetaxine *in vitro*. In contrast, less than 9% or 25% of the leukemic stem cells were killed after treating with imatinib or dasatinib, respectively. After 4 days of treatment of CML mice with omacetaxine, Gr-1$^+$myeloid leukemia cells decreased in the peripheral blood of the treated CML mice. In the omacetaxine treated B-ALL mice, only 0.8% B220$^+$leukemia cells were found in peripheral blood, compared with 34% B220$^+$leukemia cells in the placebo group. Treatment with omacetaxine decreased the number of leukemia stem cells and prolonged survival of mice with BCR-ABL induced CML or B-ALL.

Keywords

Omacetaxine; leukemic stem cells; CML; B-ALL; BCR-ABL

Introduction

The Abl tyrosine kinase inhibitors (TKIs) imatinib mesylate and dasatinib, have revolutionized the treatment of Philadelphia-positive (Ph$^+$) leukemia in both chronic

Correspondence: Shaoguang Li, M.D., PhD., Department of Medicine, University of Massachusetts Medical School, Lazare Research Building #315, 364 Plantation Street, Worcester, MA 01605, USA; Tel: 508-856-1691; email: Shaoguang.Li@umassmed.edu.

myeloid leukemia (CML) and B-cell acute lymphoblastic leukemia (B-ALL) by targeting and disabling the proliferative signal coming from BCR-ABL. These TKIs elicit high rates of durable complete cytogenetic responses, particularly in CML, however, only a small proportion of treated individuals remain disease free if therapy is discontinued(1, 2). This lack of curative effect of TKIs in Ph^+ leukemias is largely due to insensitivity of primitive leukemic stem cells to these TKIs and the selection of cells expressing TKI resistant BCR-ABL mutants(3). At present, an anti-stem cell strategy has not been developed for treating these leukemia patients; however a number of agents such as farnesyl transferase inhibitors have shown activity toward leukemic stem cell that is superior to TKIs(4, 5). These findings suggest that non-TKI based therapies may prove useful in targeting leukemic stem cells in Ph^+ leukemias and raise the prospect of improved rates of durable complete molecular remission.

Omacetaxine is a cephalotaxine ester derived from the evergreen tree, *Cephalotaxus harringtonia*, native to China. The chemical structure of omacetaxine is shown in Figure 1. Omacetaxine has shown clinical activity alone and in combination with imatinib in CML patients resistant to imatinib or other TKIs(6–8). However, little is known about whether omacetaxine has an inhibitory effect on leukemic stem cells. In this study, we utilized mouse model of BCR-ABL induced leukemia to investigate the efficacy of omacetaxine toward leukemic stem cells. We show that omacetaxine inhibited the proliferation of CML and B-ALL stem cells and provided a significant survival benefit to mice with CML and B-ALL.

Materials and Methods

Cell lines

Human K562 myeloid leumkemia cell line was grown in RPMI 1640 medium containing 10% FCS. To generate the BCR-ABL–expressing pre-B cell lines, bone marrow cells were transduced with the BCR-ABL-WT- or BCR-ABL-T315I-IRES-GFP-MSCV retrovirus, followed by transplantation into recipient mice. The BCR-ABL-expressing cells were isolated from the spleen of a mouse with BCR-ABL induced B-ALL, and pre-B leukemic cells were selected through GFP sorting by fluorescence-activated cell sorter (FACS). To generate the BCR-ABL-expressing 32D line, the cells were transduced with the BCR-ABL-WT- or BCR-ABL-T315I-IRES-GFP-MSCV retrovirus, and the BCR-ABL–expressing cells were selected by GFP sorting by fluorescence-activated cell sorter (FACS).

Histology

The lungs from the placebo- or drug-treated mice were fixed in Bouin fixative (Fisher Scientific, Pittsburgh, PA) for 24 hours at room temperature, followed by an overnight rinse in water. Ten-μm sections were stained with hematoxylin and eosin (H&E) and observed by a model DMRE compound microscope (Leica, Heidelberg, Germany). All sections were imaged with a 2.5 × PH1 objective (NPLan, NA 0.25) and 10 × PH1 objective (NPLan, NA 0.40). All images were imported into MetaMorph software (Molecular Devices, Downingtown, PA) as a series of tagged image files. All images were then constructed in Adobe Photoshop 7.0 (Adobe, San Jose, CA).

Antibodies and Western blot analysis

Antibodies against c-ABL, Hsp90, Hsp70, Mcl-1 and actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Protein lysates were prepared by lysing cells in radioimmunoprecipitation (RIPA) buffer, and immunoprecipitation and Western blotting were carried out as described previously(9).

Bone marrow transduction/transplantation

The retroviral vector *MSCV-IRES-EGFP*(10) carrying the *p210 BCR-ABL* cDNA was used to make high-titer, helper-free, replication-defective ecotropic virus stock by transient transfection of 293T cells using the kat system(11) as previously described(9). 6- to 10-week-old wild-type BABL/c or C57BL/6 mice (The Jackson Laboratory, Bar Harbor, Maine, and U.S.A) were used for leukemogenesis experiments. Induction of CML(9) and B-ALL(9, 12) was as previously described. Briefly, to model CML, bone marrow from 5-FU–treated (200 mg/kg) donor mice was transduced twice with *BCR-ABL* retrovirus by cosedementation in the presence of IL-3, IL-6, and SCF. To model B-ALL, bone marrow from non–5-FU–treated donors was transduced without cytokines. Wild-type recipient mice were prepared by 900 cGy (for BABL/c) or 1100 cGy (for C57BL/6) gamma irradiation and a dose of 0.5×10^6 (CML) or 1.0×10^6 (B-ALL) cells transplanted via tail vein injection. Diseased mice were analyzed by histopathological and biochemical analyses as described previously(9).

Flow cytometry

Hematopoietic cells were collected from peripheral blood and bone marrow of diseased mice, and red blood cells were lysed with NH_4Cl red blood cell lysis buffer (pH 7.4). The cells were washed with PBS, and stained with B220-PE for B cells, Gr-1-APC for neutrophils, and Sca1-APC/c-Kit-PE for hematopoietic stem cells. After staining, the cells were washed once with PBS and subjected to FACS analysis.

Culture of leukemia stem cells

Bone marrow cells isolated from CML mice were cultured *in vitro* in the presence of stemspan SFEM, SCF, IGF-2, TPO, heparin, and FGF as reported previously for culture of hematopoietic stem cells(13, 14)

Drug treatment

Omacetaxine (ChemGenex Pharmaceuticals, Inc, Menlo Park, CA) was dissolved in 0.9% NaCl to a stock concentration of 1 mg/ml. Further dilutions were made to working concentrations using media or water. Imatinib was dissolved in water directly at a concentration of 10 mg/ml. The drugs were given by either oral gavage for the CML model or by I.P. route for the B-ALL model in a volume of <0.5 ml, once a day, at 0.5 mg or 1.0 mg per kilogram of body weight for omacetaxine and 100 mg per kilogram of body weight per dose of imatinib, beginning at 10 days after bone marrow transplantation.

Statistical analysis

Results are reported as mean ± SD. Differences were evaluated by t test or analysis of variance, and accepted as significance when P value is less than 0.05.

RESULTS

Omacetaxine suppresses myeloid leukemic cells and improves survival of mice with BCR-ABL induced CML

To investigate the therapeutic effect of omacetaxine (Supplementary Figure 1) on CML, we used a bone marrow transplantation (BMT) mouse model of CML in which bone marrow cells from BALB/c donor mice pretreated with 5-fluorouracil (5-FU), were transduced with BCR-ABL and injected into BALB/c recipient mice to induce CML(9). Mice transplanted with BCR-ABL-transduced bone marrow were treated with a placebo or omacetaxine. Omacetaxine treatment of CML mice decreased BCR-ABL–expressing (GFP^+) leukemia cells during therapy (Figure 1a and 1b, $P < 0.001$). In addition, splenomegaly in omacetaxine-treated mice (a consistent physical sign in CML) decreased by 88%, compared to placebo (Figure 1b). All placebo-treated mice developed CML and died within 3 weeks after BMT. In contrast, all omacetaxine treated CML mice survived (Figure 1c). Lung hemorrhage caused by infiltration of mature myeloid leukemia cells is a major cause of death of CML mice(9). We further evaluated the therapeutic effect of omacetaxine on CML by examining the severity of lung hemorrhages at day 14 after BMT. Compared with placebo-treated mice, much less severe hemorrhages were observed in the lungs of omacetaxine–treated CML mice (Figure 1d). We compared the effect of omacetaxine on BCR-ABL-expressing and non-BCR-ABL-expressing 32D cells, and found that omacetaxine inhibited BCR-ABL-expressing cells more strongly than non-BCR-ABL-expressing cells (Figure 1e).

Omacetaxine suppresses chronic myeloid leukemia stem cells *in vitro* and *in vivo*

Although imatinib, the BCR-ABL inhibitor, has been shown to prolong survival of mice with BCR-ABL–induced CML, it does not completely stop the progression of CML in our BMT mouse model, due to accumulation of surviving leukemia stem cells (15, 16). The Lin^- $cKit^+$ $Sca\text{-}1^+$ population has been identified as CML stem cells, as these cells confer leukemia in recipient mice in the CML model(16). To investigate whether omacetaxine has an inhibitory effect on leukemia stem cells, bone marrow cells from CML mice on day 13 after BMT were isolated and treated with omacetaxine at various doses in conditions that support survival and growth of hematopoietic stem cells(HSCs) (Figure 2a)(13, 14). After 6 days in culture, survival of $GFP^+Lin^-cKit^+$ $Sca\text{-}1^+$ cells representing leukemia stem cells, and GFP^+ cells indicating BCR-ABL-expressing cells, was determined. FACS analysis showed that compared with the placebo group, omacetaxine treatment inhibited survival of BCR-ABL expressing leukemia stem cells and total leukemia cells in a dose dependent manner (Figure 2b, $P < 0.001$).

The effect of omacetaxine on leukemia stem cells in CML mice was also examined. Mice with BCR-ABL-induced CML were treated with a placebo, omacetaxine, imatinib or both drugs in combination for 4 days from Day 10 after BMT, and the number of

$GFP^+Lin^-cKit^+Sca\text{-}1^+$ and total GFP^+ bone marrow cells was determined by flow cytometry. Consistent with our previous findings(17), imatinib treatment did not lower the percentage and number of leukemia stem cells and total leukemia cells in bone marrow, compared with the placebo group. Omacetaxine treatment greatly reduced the numbers of both leukemia stem cells and total leukemia cells, while treatment of CML mice with both drugs did not show further effects on reducing the number of leukemia stem cells or total leukemia cells, compared with the mice treated with omacetaxine alone (Figure 2c). The effect of omacetaxine on leukemia stem cells in CML mice was also compared with the effect of omacetaxine on BCR-ABL-negative stem cells in the same animals. We found that a higher percentage of leukemia stem cells were inhibited by omacetaxine as compared with the percent reduction of BCR-ABL-negative stem cells (Figure 2d).

Omacetaxine improves survival of mice with BCR-ABL-T315I induced CML and suppresses BCR-ABL-T315I leukemia stem cells

Imatinib does not suppress leukemia cells expressing BCR-ABL-T315I and does not prolong the survival of mice with BCR-ABL-T315I induced CML(17–19). To investigate whether omacetaxine also inhibits BCR-ABL-T315I induced CML and leukemia stem cells, we transduced bone marrow cells with BCR-ABL-T315I and implanted the transduced cells into BALB/c recipient mice to induce CML. Similar to wild type BCR-ABL induced CML, omacetaxine caused a decrease in peripheral blood BCR-ABL-T315I–expressing (GFP^+) leukemia cells (Figure 3a) and reduced splenomegaly (data not shown). BCR-ABL-T315I-expressing myeloid leukemic cells in peripheral blood of the treated CML mice were reduced greater than 49.5 fold compared to untreated CML mice (Figure 3a), whereas wild type BCR-ABL-expressing myeloid leukemic cells in peripheral blood of the treated CML mice were reduced only 30.2 fold (Figure 1b). These results suggest that omacetaxine was more efficient in inhibiting BCR-ABL-T315I compared to wild type BCR-ABL, which is supported by a previous report (20). Omacetaxine treatment prolonged the survival of mice transplanted with BCR-ABL-T315I expressing bone marrow cells (Figure 3b). We next examined whether omacetaxine could inhibit the BCR-ABL-T315I induced leukemia stem cells *in vivo*. Mice with BCR-ABL T315I-induced CML were treated with a placebo or omacetaxine for 4 days from Day 10 after BMT. Bone marrow and spleen cells were analyzed by FACS for GFP^+Lin^- $cKit^+CD34^-$ cells. The number of BCR-ABL-T315I expressing leukemia stem cells was significantly decreased by omacetaxine treatment (Figure 3c).

Omacetaxine caused degradation of BCR-ABL protein through inhibiting HSP90 and reduced MCL-1 protein level in myeloid leukemic cells

To understand the possible mechanisms by which omacetaxine inhibits myeloid leukemia cells, we examined the effect of omacetaxine on human myeloid leukemia cells (K562). In a dose-dependent manner, treatment with omacetaxine significantly suppressed K562 cell growth particularly at concentrations of 200 nM or more ($P<0.001$) (Figure 4a). We then compared protein levels in K562 cells after treatment with either 50 nM or 150 nM omacetaxine. After treatment, levels of BCR-ABL protein were decreased in K562 cells in a dose dependent manner (Figure 4b). As BCR-ABL protein is associated with HSP90 protein(17), we next measured the HSP90 protein levels and found that the HSP90 protein

levels were also decreased after omacetaxine treatment (Figure 4b). However, HSP70 which plays a positive role inBCR-ABL–mediated resistance to apoptosis was not changed after omacetaxine treatment (Figure 4b). Down regulation of MCL-1, a member of the BCL-2 family, is associated with a substantial decrease in viability of K562 cells, and reduced survival of imatinib-resistant K562 cells(21). The level of MCL-1 protein was also greatly reduced following treatment with 150 nM omacetaxine treatment (Figure 4b).

Omacetaxine improves survival of mice with BCR-ABL induced B-ALL

In contrast to CML(Figure 1), the development of B-ALL induced by BCR-ABL is not significantly affected by TKIs such as imatinib and dasatinib(19, 21). To induce B-ALL in mice, BCR-ABL–transduced bone marrow cells from donor mice that were not pretreated with 5-FU were transplanted into B6 mice(9, 15). To determine whether omacetaxine was effective in treating B-ALL, we used the B-ALL model, where pre-B cells express the B220 and CD19 cell surface antigens, and phenotypically resemble de novo Ph^+ B-ALL and lymphoid blast crisis of CML(9, 12). These mice were treated with a placebo, 0.5mg/kg or 1mg/kg omacetaxine daily starting at day 10. After 4 days of treatment, only 2% of cells in peripheral blood were GFP^+ B- leukemia cells in the 1mg/kg group, compared with 20% GFP^+ B-leukemia cells in 0.5mg/kg group or 50% GFP^+ B-leukemia cells in placebo group ($P < 0.001$, Figure 5a and 5b). After 10 days of treatment, less than 2% GFP^+ B-leukemia cells were detected in both drug treatment groups (Figure 5a and 5b) ($P < 0.001$). All placebo-treated recipients of BCR-ABL transduced bone marrow developed and died of B-ALL within 4 weeks after BMT (Figure 5c), and all B-ALL mice treated with omacetaxine survived.

Omacetaxine inhibits BCR-ABL expression without affecting HSP90 in B-lymphoid cells expressing BCR-ABL

We further investigated whether omacetaxine also affected the expression levels of BCR-ABL and HSP90 proteins in lymphoid cells expressing BCR-ABL. Pre-B cells expressing BCR-ABL or BCR-ABL-T315I from omacetaxine-treated B-ALL mice showed a concentration dependent inhibition of cell proliferation in response to omacetaxine (Figure 6a). We then compared BCR-ABL protein levels between placebo and omacetaxine (50 nM or 150 nM) treatment groups. After the treatment, the level of wild type BCR-ABL protein was slightly lower in omacetaxine treated B-leukemia cells, but the level of BCR-ABL T315I protein was markedly decreased. However, unlike myeloid leukemia cells (Figure 4b), the level of HSP90 protein did not change after omacetaxine treatment (Figure 6b).

Discussion

The BCR-ABL positive leukemia stem cell is a major target for curative therapy of Ph+ leukemias(3). Although current TKI based therapies have significant clinical efficacy in Ph+ leukemias, these agents do not effectively kill BCR-ABL positive leukemia stem cells and only rarely elicit long lasting complete cytogenetic remission after cessation of therapy (1, 2). This finding has led to investigation of therapeutics that selectively kill BCR-ABL positive leukemia stem cells as potential novel treatments of Ph+ leukemias (4, 5). Here we have shown in animal models of Ph^+ CML and B-ALL that omacetaxine effectively targets

BCR-ABL positive leukemia stem cells in vivo and confers a significant survival benefit for leukemic mice. In contrast, previous work has shown that the TKI imatinib did not affect the numbers of stem cells found in the circulation or the bone marrow of mice with CML (16). These findings raise the possibility that clinical efficacy of omacetaxine in CML (where it is currently in phase 2/3 trials) may be due, at least in part, to its inhibiting activity on leukemic stem cells.

The appearance of mutations in the BCR-ABL oncoprotein is a major cause of failure with imatinib therapy of BCR-ABL positive leukemias (22). Whilst the second generation TKIs dasatinib and nolotinib, inhibit many imatinib resistant mutant BCR-ABL proteins, cells expressing T315I mutant BCR-ABL are resistant to all 3 TKIs currently used to treat CML. Ongoing clinical trials have found that omacetaxine has clinical efficacy in CML patients with T315I mutant BCR-ABL (8). Here we have shown that omacectaxine significantly prolonged survival in these mice and dramatically reduced the number of T315I BCR-ABL positive leukemic cells both *in vivo* and *in vitro*. These observations support the clinical use of omacetaxine in CML patients with TKI resistant disease mediated by the T315I mutant BCR-ABL.

The mechanisms for omacetaxine inhibition of Ph^+ leukemic stem cells are unclear. As omacetaxine is an inhibitor of protein synthesis, we hypothesized that the anti-leukemic activity of omacetaxine may be due to the loss of antiapoptotic proteins. We postulated that omacetaxine may induce apoptosis in BCR-ABL positive CML cells via three potential pathways: (a) by directly reducing the expression of BCR-ABL, (b) by reducing the expression levels of BCR-ABL stabilizing proteins (such as HSP 90), which will lead to the degradation of BCR-ABL, and (c) by reducing the expression of the short lived antiapoptotic BCL-2 family protein MCL-1. We investigated the effect of omacetaxine on the expression of BCR-ABL, HSP90 and MCL-1 proteins in CML derived K562 cells and found that omacetaxine treatment induces the loss of BCR-ABL, HSP90 and MCL-1. These observations are consistent with the hypothesis that omacetaxine acts by directly or indirectly inhibiting the BCR-ABL, MCL-1 and HSP90 pathways in BCR-ABL positive CML cells and raise the possibility that omacetaxine acts on CML leukemic stem cells via reduced levels of these proteins.

Acute lymphocytic leukemia in adults is commonly caused by the expression of BCR-ABL. In general, B-ALL tends to show different symptoms, compared with CML. Omacetaxine removed most GFP^+ $B220^+$ cells from the peripheral blood with less than 1% GFP^+ $B220^+$ cells still detected by FACS and prolonged the survival of B-ALL mice. However, there was no change in HSP90 protein levels, although BCR-ABL protein level was greatly reduced by omacetaxine. In CML, treatment with omacetaxine reduced the level of both HSP90 and BCR-ABL protein demonstrating a different role for omacetaxine in treating CML and B-ALL. These results are consistent with our previous observation that BCR-ABL utilizes different signaling pathway to induce CML and B-ALL(15). Omacetaxine also has potential for treating CML and Ph^+ B-ALL resistant to TKIs (20).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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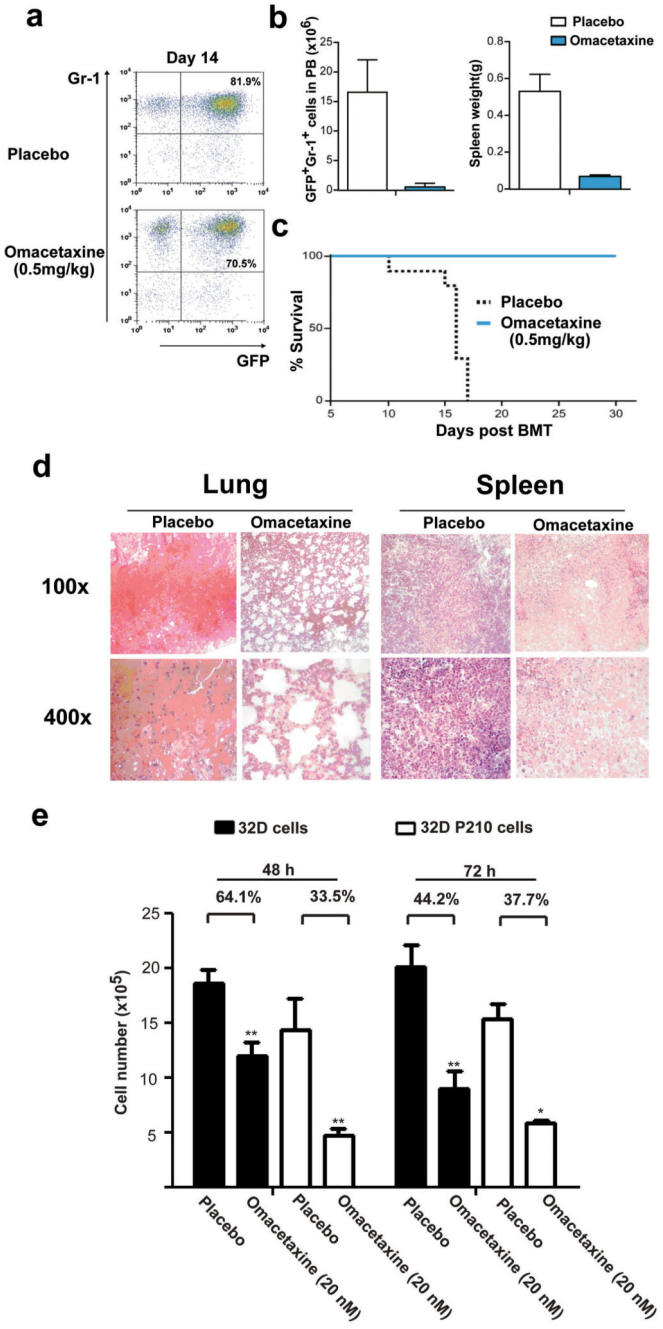

Figure 1. Omacetaxine reduces circulating GFP$^+$ cells, reduces spleen weight and improves survival in mice with BCR-ABL-WT-induced CML

a. Flow cytometric evaluation of the leukemic process in omacetaxine and placebo treated CML mice. Percentage of Gr-1$^+$GFP$^+$ cells in peripheral blood of placebo or omacetaxine-treated CML mice were analyzed at day 14 after BMT.

b. The number of circulating leukemic cells (calculated as percentage of Gr-1$^+$GFP$^+$ cells×white blood cell count) and spleen weight in mice with BCR-ABL induced CML treated with placebo or omacetaxine for 4 days was determined on day 14 after 0.5×10^6 cells transplantation.

C. Treatment with the omacetaxine prolonged survival of CML mice. Mice with BCR-ABL-induced CML were treated with placebo (n = 15) or omacetaxine (0.5 mg/kg for 4 days) (n=15).

c. Treatment with omacetaxine reduced lung hemorrhage and splenomegaly of mice with BCR-ABL induced CML after treatment with omacetaxine for 4 days.

d. The inhibitory effect of Omacetaxine on 32D and 32D P210 cells. 32D and BCR-ABL-transduced 32D cells were cultured at 5×10^5 cells per well in 24-well plates, and omacetaxine (20nM) was added to the culture for 48h or 72h. The number of viable cells at the indicated drug concentrations was determined by trypan blue.

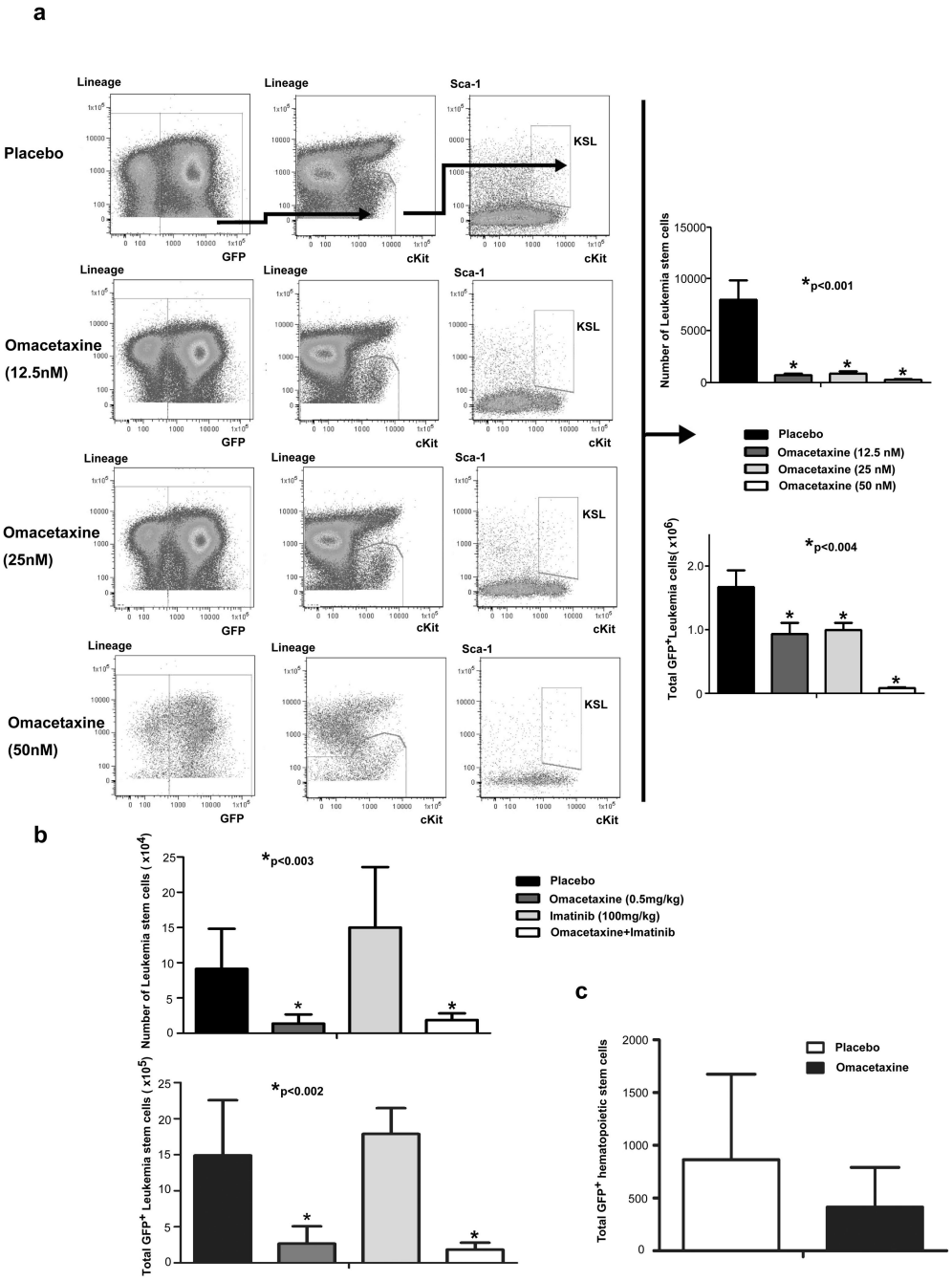

Figure 2. Omacetaxine inhibits survival of leukemic stem cells *in vitro* and *in vivo*

a. Bone marrow cells isolated from C57BL/6 (B6) mice with BCR-ABL-induced CML on day 13 afternoon transplant were cultured in vitro (5×10^6 cells/6 cm tissue culture plate) under stem cell conditions ("Materials and Methods") in the presence or absence of omacetaxine (12.5 nM, 25 nM, 50 nM)) for 6 days (changing the stem cell medium containing placebo or omacetaxine at day 3) followed by FACS analysis of leukemia stem cells ($GFP^+Lin\text{-}c^-Kit^+Sca\text{-}1^+$).

b. Mice with BCR-ABL–induced CML were treated with placebo (n=5) or omacetaxine (0.5 mg/kg, 4 days) (n=5), respectively, for 4 days beginning at day 10 after transplantation. Bone marrow cells were isolated from the treated CML mice, and leukemia stem cells were analyzed by FACS. The numbers of cells represents the average number of leukemia stem cells from the femur and tibia of each treated CML mouse.

c. Mice transplanted with *MSCV-GFP* induced bone marrow cells were treated with placebo (n=3) or omacetaxine (0.5 mg/kg) (n=3), respectively, for 4 days beginning at day 10 after transplantation. Bone marrow cells were isolated from the treated mice, and hematopoietic stem cells were analyzed by FACS. The numbers of cells represents the average number of hematopoietic stem cells from the femur and tibia of each mouse.

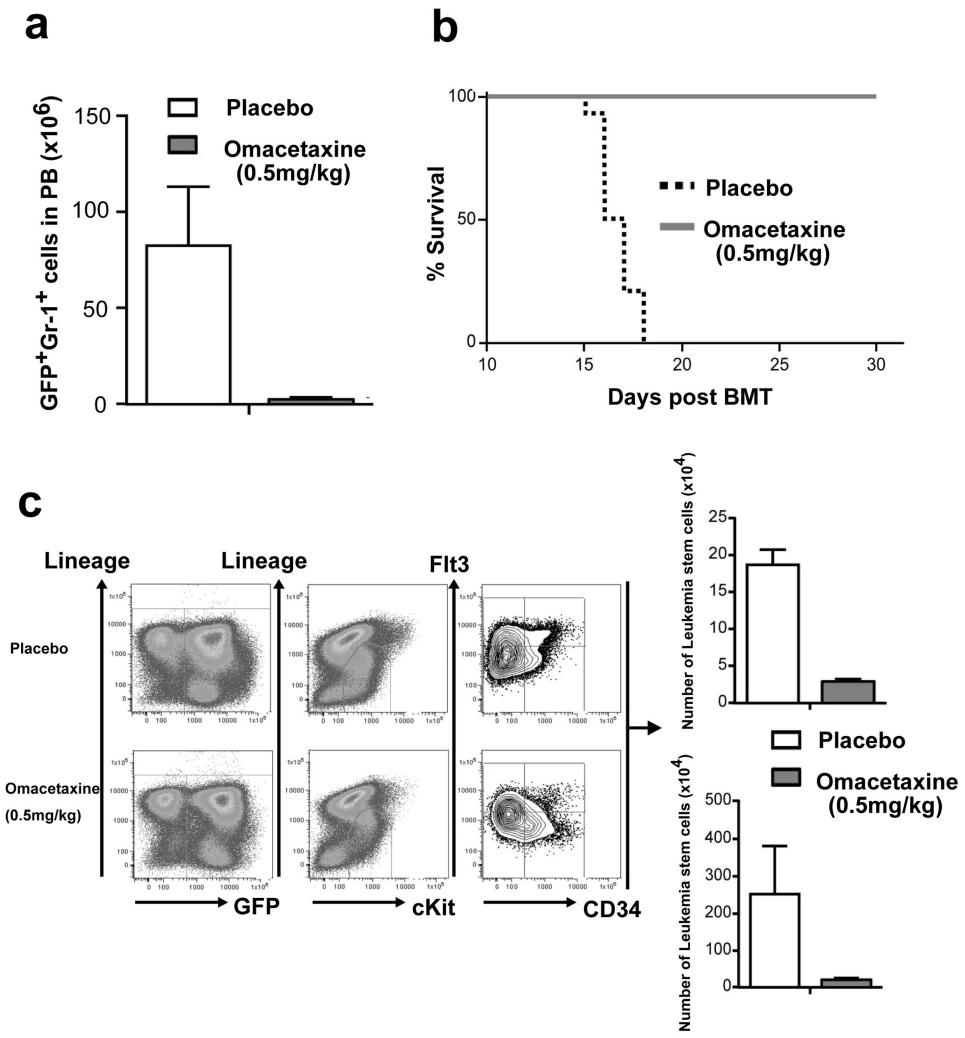

Figure 3. Omacetaxine improves survival of mice with BCR-ABL-T315I-induced CML

a. The number of circulating leukemia cells (calculated as percentage of Gr-1$^+$GFP$^+$cells×white blood cell count) in mice with BCR-ABL-T315I–induced CML treated with placebo or omacetaxine was determined on day 14 after transplantation.

b. Treatment with the omacetaxine prolonged survival of CML mice. Mice with BCR-ABL-T315I induced CML were treated with placebo (n = 15) or omacetaxine (0.5 mg/kg for 4 days) (n=15).

c. Mice with BCR-ABL-T315I induced CML were treated with a placebo (n=5) or omacetaxine (0.5 mg/kg, 4 days) (n=5), respectively, for 4 days beginning at day 10 after transplantation. Bone marrow cells were isolated from the treated CML mice, and leukemia stem cells were analyzed by FACS. The numbers of cells represents the average number of leukemia stem cells from the femur and tibia of each treated CML mouse.

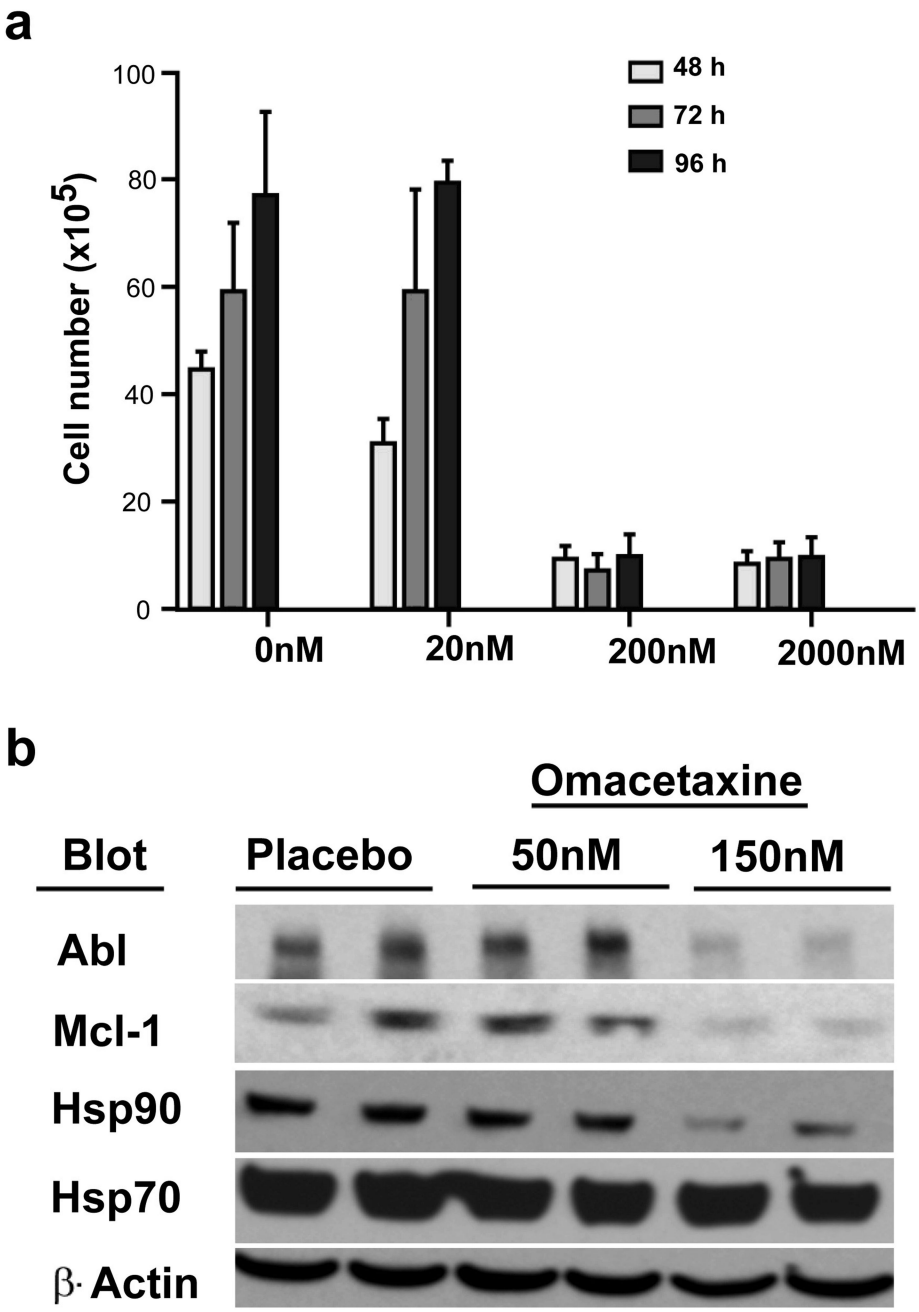

Figure 4. Omacetaxine degrades BCR-ABL by inhibiting HSP90 and suppresses MCL-1 in myeloid leukemia cells

a. Omacetaxine inhibited K562 cells in a dose dependent manner. The number of viable cells at the indicated drug concentrations was determined by trypan blue.

b. Omacetaxine inhibited the expression of HSP90, ABL and MCL-1 in K562 cells. K562 cells were treated with omacetaxine (50 nM, 150 nM) for 48 hours. Protein lysates were analyzed by Western blotting using antibodies indicated.

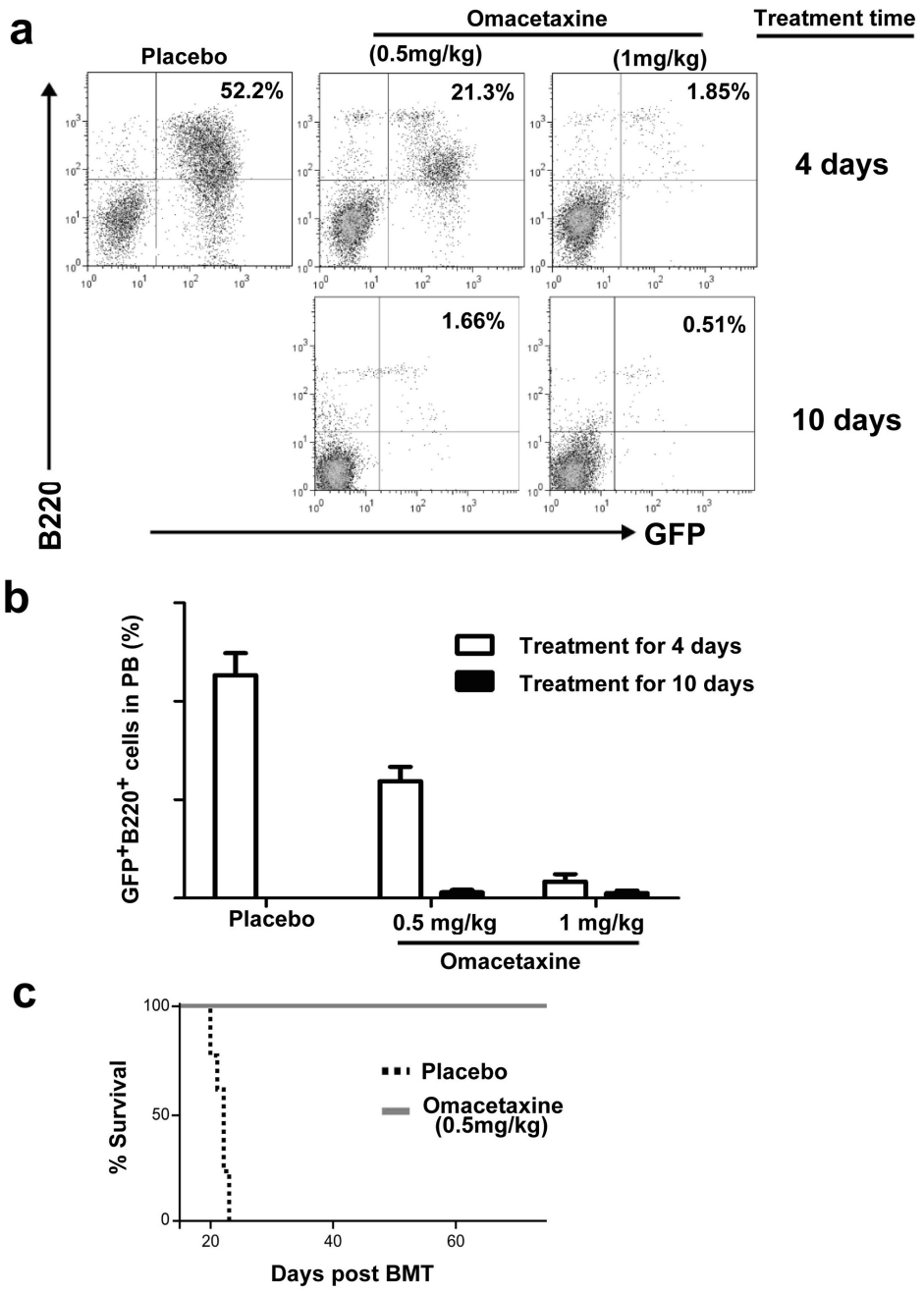

Figure 5. Omacetaxine improves survival of mice with BCR-ABL-induced B-ALL

a and b Flow cytometric evaluation of the B-lymphoid leukemic process. The B-lymphoid leukemic cells (GFP^+B220^+) were measured after placebo or omacetaxine (0.5 mg/kg or 1 mg/kg, n=4) treatment of B-ALL mice 4 days or 10 days after treatment.

c. Treatment with the omacetaxine prolonged survival of BCR-ABL induced B-ALL mice. Mice with BCR-ABL induced B-ALL were treated with placebo (n = 15) or omacetaxine (1 mg/kg/day) (n=15).

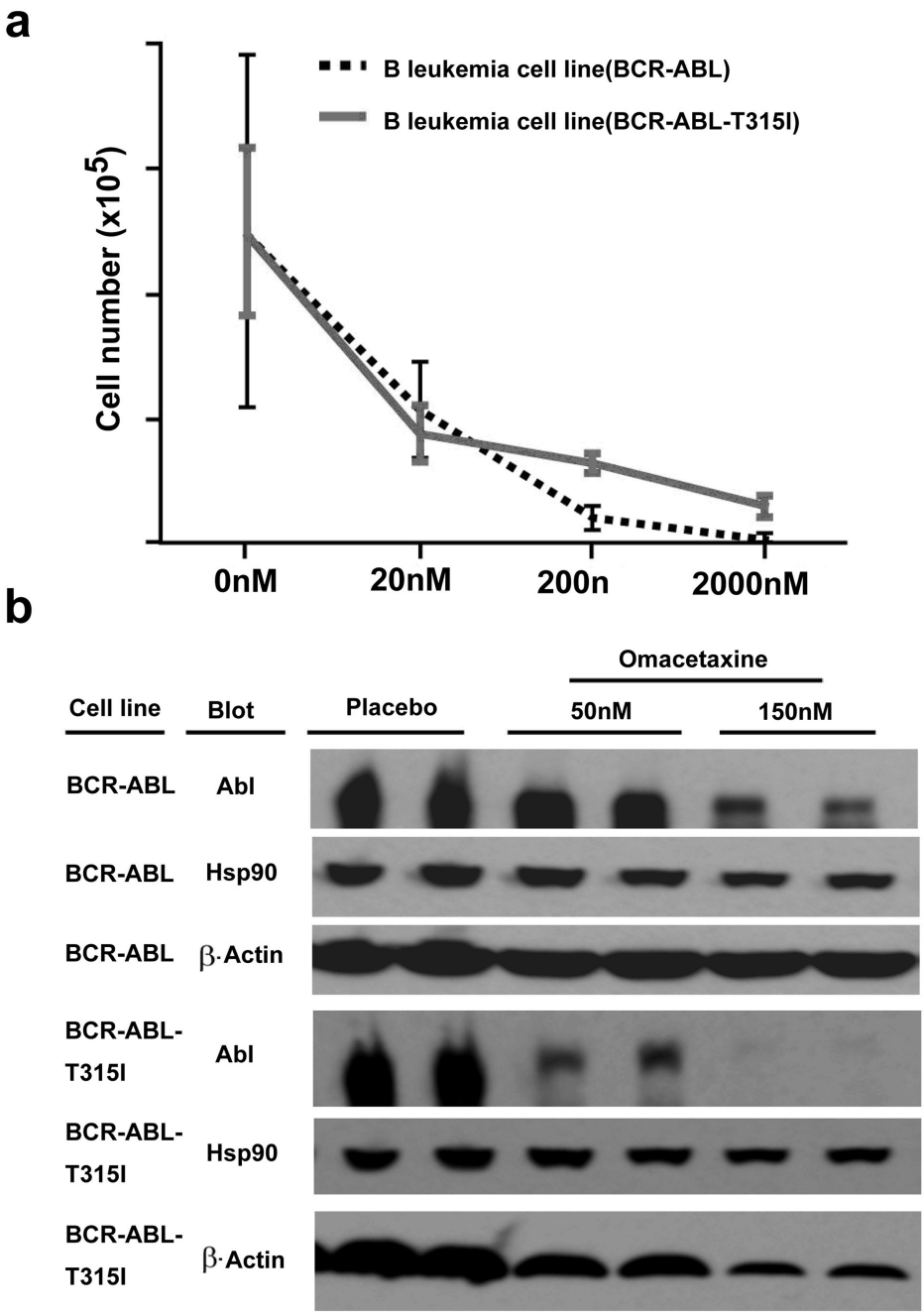

Figure 6. Omacetaxine inhibits B-ALL cells by suppressing BCR-ABL without affecting HSP90

a. Omacetaxine inhibited pre-B cells expressing BCR-ABL or BCR-ABL-T315I associated with drug concentration. The number of viable cells at the indicated drug concentrations was determined by trypan blue.

b. Omacetaxine inhibited the expression of ABL in pre-B cells expressing BCR-ABL or BCR-ABL-T315I. These pre-B cells were treated with omacetaxine (50 nM, 150 nM) for 48 hours. Protein lysates were analyzed by Western blotting using antibodies indicated.