

# Inhibition of p38 $\alpha$ Mitogen-Activated Protein Kinase Prevents the Development of Osteolytic Bone Disease, Reduces Tumor Burden, and Increases Survival in Murine Models of Multiple Myeloma

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## Abstract

The bone microenvironment plays a critical role in supporting the growth and survival of multiple myeloma as well as in the development of osteolytic bone disease. Signaling through p38 $\alpha$  mitogen-activated protein kinase (MAPK) mediates synthesis of multiple myeloma cell growth factors, and its inhibition reduces proliferation *in vitro*. However, it is unclear whether targeting p38 $\alpha$  MAPK prevents multiple myeloma growth and the development of bone disease *in vivo*. In this study, we determined whether SCIO-469, a selective p38 $\alpha$  MAPK inhibitor, inhibits multiple myeloma growth and prevents bone disease in the 5T2MM and 5T33MM models. SCIO-469 decreased constitutive p38 $\alpha$  MAPK phosphorylation of both 5T2MM and 5T33MM cells *in vitro*. This was associated with decreased DNA synthesis and an induction of apoptosis when the cells were cultured with bone marrow stromal cells. Treatment of C57Bl/KaLwRij mice bearing 5T33MM cells with SCIO-469 inhibited p38 $\alpha$  MAPK phosphorylation and was associated with a significant decrease in serum paraprotein, an almost complete reduction in tumor cells in the bone marrow, a decrease in angiogenesis, and a significant increase in disease-free survival. Injection of 5T2MM murine myeloma cells into C57Bl/KaLwRij mice resulted in myeloma bone disease characterized by increased osteoclast occupation of the bone surface, reduced cancellous bone, and the development of osteolytic bone lesions. Treatment of 5T2MM-injected mice with SCIO-469 reduced this development of bone disease. Together, these data show that targeting p38 $\alpha$  MAPK with SCIO-469 decreases myeloma burden *in vivo*, in addition to preventing the development of myeloma bone disease. [Cancer Res 2007;67(10):4572–7]

## Introduction

Multiple myeloma is a plasma cell cancer characterized by the clonal expansion of malignant cells that accumulate in the bone marrow, leading to osteoclast and endothelial cell activation,

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which, in turn, results in bone destruction and angiogenesis. Despite the progress achieved in the last few years with high-dose chemotherapy and stem cell transplantation resulting in a higher success rate of remission, patients eventually develop drug-resistant disease and relapse. New therapies targeting both multiple myeloma cells and their microenvironment are urgently required.

Mitogen-activated protein kinase (MAPK) p38 $\alpha$ , a member of the MAPK family and activated by cytokines and growth factors, has been reported to be involved in the production of tumor-promoting factors by the multiple myeloma bone marrow microenvironment. Activation of p38 $\alpha$  in bone marrow stromal cells enhanced the production of interleukin (IL)-6, which is critical for multiple myeloma growth and survival (1). The p38 $\alpha$  MAPK inhibitor decreased IL-6 secretion in the bone marrow stromal cells of patients with multiple myeloma, decreasing proliferation of multiple myeloma cells adherent to bone marrow stromal cells and IL-6 secretion by the bone marrow stromal cells triggered by adherence of multiple myeloma cells to bone marrow stromal cells *in vitro* (1). The p38 $\alpha$  MAPK inhibitor SCIO-469 prevented tumor necrosis factor- $\alpha$ -induced adhesion of multiple myeloma cells to bone marrow stromal cells through an intercellular adhesion molecule 1- and vascular cell adhesion molecule-1-independent mechanism (2). Furthermore, SCIO-469 enhanced PS-341 (bortezomib)-induced cytotoxicity against multiple myeloma cell lines and patient's multiple myeloma cells (3) by inhibiting transient expression and phosphorylation of heat shock protein 27, a downstream target of p38. SCIO-469 also enhanced bortezomib-induced multiple myeloma apoptosis by up-regulation of p53 and down-regulation of Bcl-X<sub>L</sub> and Mcl-1. In a xenograft model for plasmacytosis, it was furthermore shown that inhibiting p38 augments the effects of bortezomib in decreasing multiple myeloma tumor growth *in vivo* (4). SCIO-469 also inhibits secretion and expression of the osteoclast-activating factors IL-11, receptor activator of NF- $\kappa$ B ligand, and macrophage inflammatory protein 1 $\alpha$ , and prevents human osteoclast activation *in vitro*. Recently, two articles report on the effect of inhibiting p38 on the restoration of dendritic cell function both in murine and human multiple myeloma (5, 6). In the present work, we investigated the role of p38 MAPK in the development of multiple myeloma disease *in vivo* using the specific p38 $\alpha$  MAPK inhibitor SCIO-469 in a fully immunocompetent murine multiple myeloma model.

## Materials and Methods

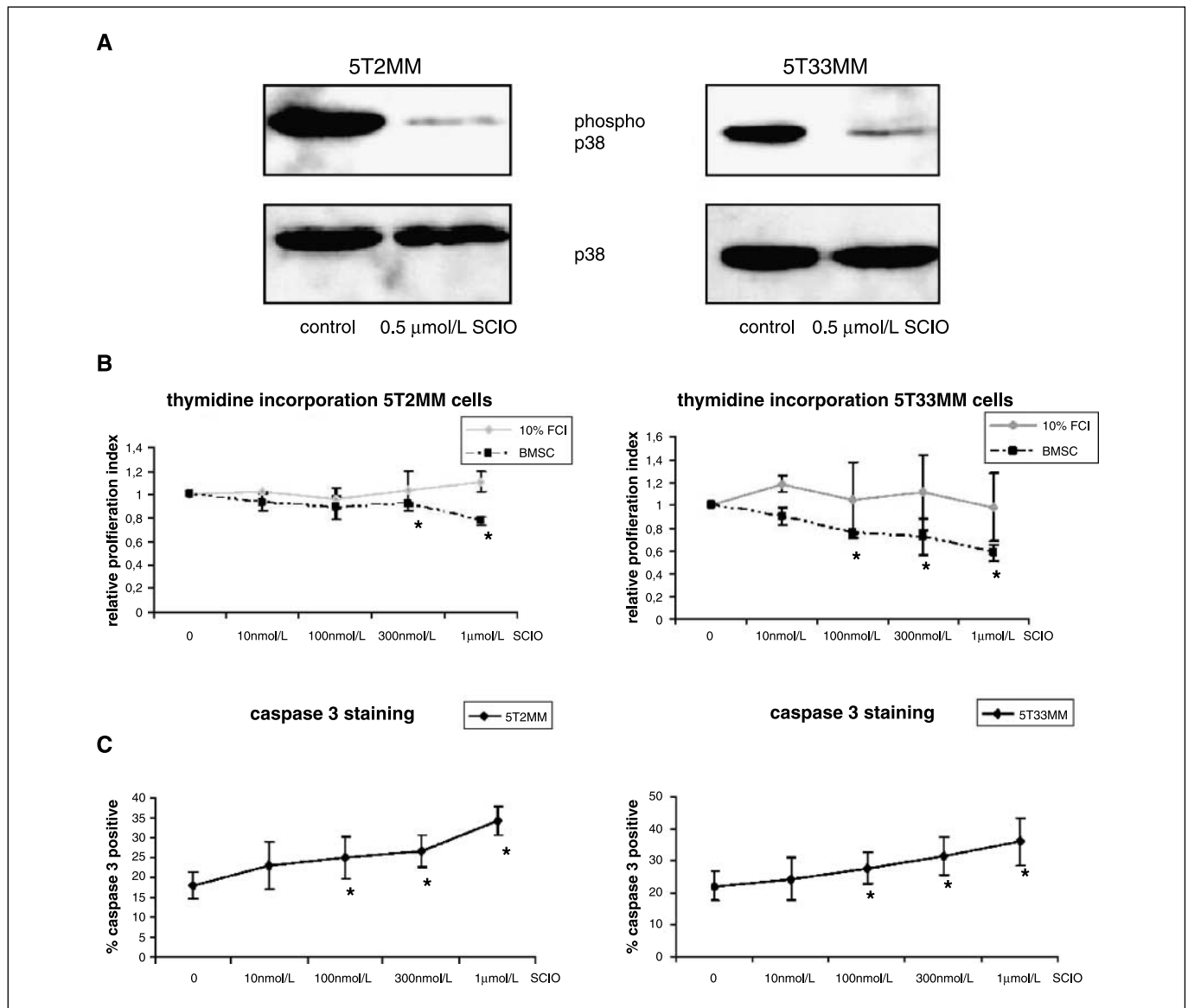
**The 5T2MM and 5T33MM murine models of myeloma.** The 5T2MM and 5T33MM murine models of myeloma originated spontane-

ously in elderly C57Bl/KaLwRij mice (7, 8). The myeloma cells are maintained *in vivo* by i.v. transfer of diseased bone marrow mononuclear cells into young syngeneic C57Bl/KaLwRij mice (Harlan) at 6 to 8 weeks of age. When the paraprotein concentration reached 10 mg/mL, the mice were sacrificed and tumor cells isolated according to previously described procedures (9). For *in vitro* experiments, myeloma cells were isolated from the bone marrow of diseased animals and purified by Lympholyte M (Cedarlane) gradient centrifugation. All procedures involving mice were approved by the local Research Ethics Committee (license no. LA1230281) and met the standards required by the UK Coordinating Committee on Cancer Research 1998 guidelines.

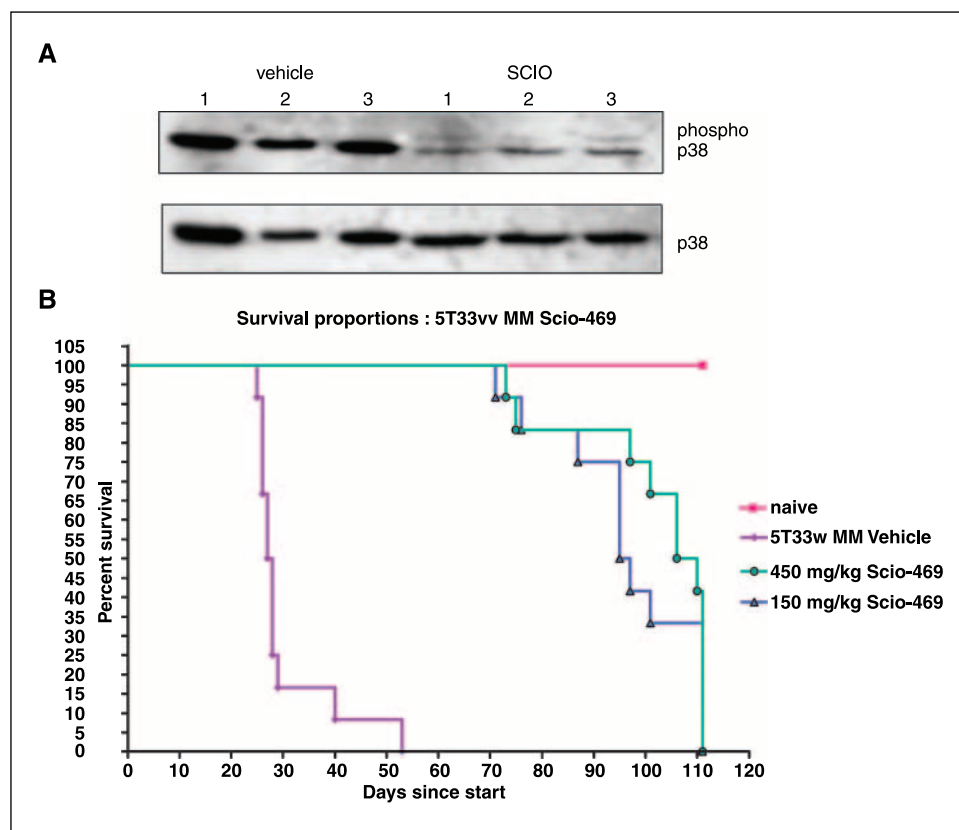
**Assessment of the effects of SCIO-469 on the development of myeloma disease *in vivo*.** For studies of the effect of SCIO-469 on myeloma development, three groups of male mice ( $n = 12$ ) were injected

i.v. with  $0.5 \times 10^6$  5T33MM cells. Mice were left untreated (naive) or, if injected with tumor cells, treated from the time of tumor cells injection with either SCIO-469 (150 or 450 mg/kg powder diet continuously available for the mice) or a vehicle (PBS) until the first mice showed signs of morbidity (at 3.7 weeks). Serum paraprotein concentration was assessed using standard electrophoretic techniques (9), bone marrow tumor burden was assessed by determining plasmacytosis on cytosmears, and bone marrow angiogenesis was assessed by determining microvessel density (see below).

To determine the effect of SCIO-469 on survival, an identical experiment to that described above was done, with the exception that treatment continued until each animal showed signs of morbidity (i.e., hind limb paralysis), at which point they were sacrificed. Kaplan-Meier analysis was done to determine the effect on time to morbidity. Tumor load was confirmed on bone marrow samples.



**Figure 1.** *In vitro* effects of SCIO-469 on 5TMM cells. **A**, 5T2MM and 5T33MM cells were preincubated with 0.5  $\mu$ mol/L SCIO-469 for 1 h before being lysed. Equivalent amounts of lysates were blotted with anti-phospho-p38 and reblotted with anti-p38 to confirm equal loading. Representative of three experiments. **B**, 5T2MM and 5T33MM cells were incubated in 10% FCI or on irradiated bone marrow stromal cells (BMSC) after a 1-h preincubation with different concentrations of SCIO-469. DNA synthesis was measured by a thymidine incorporation assay. **C**, 5T2MM and 5T33MM cells, cocultured with bone marrow stromal cells through a Transwell system, were incubated with different concentrations of SCIO-469 and stained for active caspase-3. Points, mean of three independent experiments; bars, SD. \*,  $P < 0.05$ .



**Figure 2.** *In vivo* activity of SCIO-469 in the 5T33MM model. **A**, 5T33MM mice were treated twice a day with 90 mg/kg SCIO-469 p.o. Two hours after last treatment, mice were sacrificed and bone marrow was collected for Western blotting. Equivalent amounts of lysates were blotted with anti-phospho-p38 and reblotted with anti-p38 to confirm equal loading. **B**, naïve mice or mice bearing 5T33MM cells were treated with vehicle, SCIO-469 (150 mg/kg), or SCIO-469 (450 mg/kg). Survival was assessed by a Kaplan-Meier analysis.

To determine the effect of SCIO-469 on the development of myeloma bone disease, studies were done in the 5T2MM model, which develops a characteristic myeloma bone disease (8, 10, 11). Mice were divided into the following groups: group 1 ( $n = 10$ ) remained without tumor cells (naïve group) and groups 2 to 4 ( $n = 10$  each) were injected via the tail vein with  $2 \times 10^6$  5T2MM cells. At the time of tumor cell injection, mice were treated with either zoledronic acid (120  $\mu\text{g}/\text{kg}$ , s.c., single dose at week 7) or SCIO-469 [150 or 450 mg/kg given in the diet throughout the experimental period (11 weeks)]. At 11 weeks, all mice were sacrificed and the effects of SCIO-469 and zoledronic acid on tumor burden, development of myeloma bone disease, and angiogenesis were assessed (see below).

**Radiographic and histologic analyses of bone disease.** The tibiae were X-rayed using a Hewlett Packard Faxitron. X-rays were scanned, enlarged, and the number of osteolytic lesions counted manually. Tibia were fixed in 10% neutral buffered formalin, decalcified in EDTA, embedded in paraffin, and 3- $\mu\text{m}$  sections cut using a Leica Microsystems Microtome. The sections were either stained with H&E or reacted for tartrate-resistant acid phosphatase activity to identify osteoclasts and counterstained with Gill's hematoxylin. The sections were examined by light microscopy (Leica Microsystems). The number of osteolytic lesions that penetrated the full thickness of the cortical bone in each tibia was counted manually. Cancellous bone area was measured in an area 0.56  $\text{mm}^2$ , 0.25 mm from the growth plate using the osteomeasure analysis software (Osteometrics). The number of osteoclasts per millimeter and the percentage surface covered by osteoclasts (osteoclast perimeter) were measured on 6.5 mm of the cortico-endosteal surfaces, starting 0.25 mm from the growth plate.

**Assessment of microvessel density *in vivo*.** One femur was fixed in zinc fixative, decalcified, embedded in paraffin, and 3- $\mu\text{m}$  sections cut. Vessel endothelial cells were stained for the presence of CD31 staining, as previously described (12). Microvessel density was measured in the area

with the highest blood vessel density (hotspot) and the number of blood vessels was counted per 0.22  $\text{mm}^2$ .

**Assessment of p38 $\alpha$  MAPK inhibition by Western blot.** 5T33MM and 5T2MM cells were isolated from the tibiae of diseased mice and lysed as previously described (13). For assessing the effect of SCIO-469 on *in vitro* p38 $\alpha$  phosphorylation, 5T2MM and 5T33MM cells were preincubated with 0.5  $\mu\text{mol}/\text{L}$  SCIO-469 for 1 h before being lysed. For assessing the effect of SCIO-469 on *in vivo* p38 $\alpha$  phosphorylation, 5T33MM mice were treated twice a day with 90 mg/kg SCIO-469 p.o. Two hours after last treatment, bone marrow was collected for Western blotting.

The cell debris was then removed by centrifugation (5 min,  $13,000 \times g$ ) and sample buffer added. After boiling, the samples were separated on a 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Bio-Rad). The membrane was blocked with PBS containing 5% low fat milk and 0.1% Tween 20 and probed with anti-phospho-Thr<sup>180</sup>/Tyr<sup>182</sup> p38. For measuring total protein levels, the blots were stripped and reprobed with total p38 antibody (Bioké). The bands were visualized using the enhanced chemiluminescence system (Amersham).

**Effect of SCIO-469 on thymidine incorporation.** 5TMM cells ( $1 \times 10^6/\text{mL}$ ) were pretreated with different concentrations of SCIO-469 in either serum-free medium or in 10% Fetal Clone 1 for (FC1) 1 h. The cells in serum-free medium were then incubated on irradiated (1,500 rad) syngeneic bone marrow stromal cells. Sixteen hours before harvest, cells were pulsed with 1  $\mu\text{Ci}$  [methyl-<sup>3</sup>H]thymidine (Amersham). Cells were harvested using a cell harvester (Inotech) onto fiberglass filters (Filtermat A, Wallac). Filters were dried for 1 h in a 60°C oven and sealed in sample bags (Wallac) containing 4 mL of Optiscint Scintillation Liquid (Wallac). Radioactivity was counted using a 1450 Microbeta Liquid Scintillation Counter (Wallac). Results are expressed as the relative DNA synthesis compared with untreated cells.

**Table 1.** *In vivo* activity of SCIO-469 in the 5T33MM model

5T33MM	MVD		M-compound		MM load	
	Mean (SE)	P	Mean	P	Mean	P
Naive	18 (0.6155)		0 (0)		0 (0)	
5T33 vehicle	25.36 (1.185)	0.0002	8.839 (1.36)	0.0003	67.2 (8.07)	0.0005
Low dose	19.25 (0.7191)	0.0005	0.0425 (0.0305)	0.0003	1.083 (0.528)	0.0007
High dose	19.27 (0.4684)	0.0004	0 (0)	0.0003	0 (0)	0.0004

NOTE: Naive mice or mice bearing 5T33MM cells were treated with vehicle, SCIO-469 (150 mg/kg), or SCIO-469 (450 mg/kg). Microvessel density, serum M-component, and tumor load in the bone marrow are given as mean  $\pm$  SE. Abbreviations: MVD, microvessel density; MM, multiple myeloma.

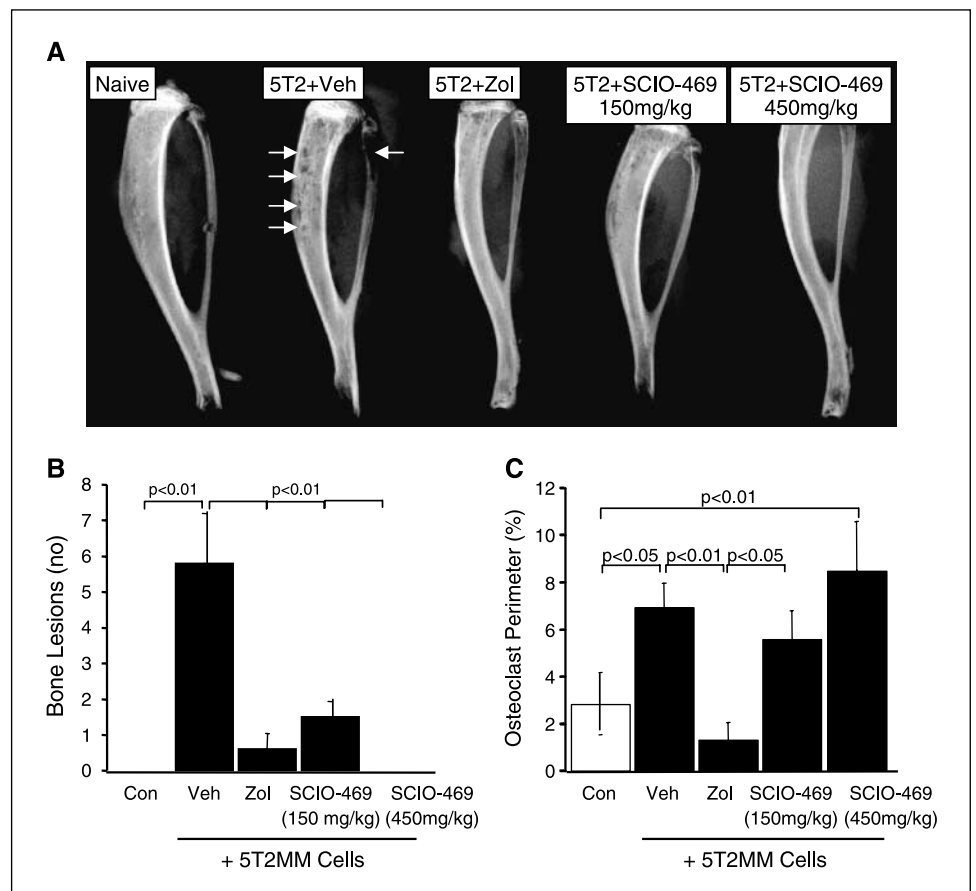
**Effect of SCIO-469 on caspase-3 activity.** 5TMM cells ( $0.5 \times 10^6$ /mL) were pretreated with different concentrations of SCIO-469 in serum-free medium and then placed in the lower compartment of a Transwell system (Elscolab). Syngeneic bone marrow stromal cells were seeded into the Transwell itself. After 18 h, the 5TMM cells were collected from the lower compartment and stained for active caspase-3 with a FITC-labeled antibody according to manufacturer's instructions (Becton Dickinson).

**Statistical analysis.** All experiments were done on a minimum of three separate occasions. The *in vitro* data were analyzed using the paired Student's *t* test whereas the data from the *in vivo* experiments were analyzed using the Mann-Whitney *U* test or ANOVA with Tukey post hoc test.

**Results and Discussion**

Multiple myeloma is a plasma cell malignancy that develops in the bone marrow and, importantly, is highly dependent on the bone marrow for critical survival signals. Furthermore, there is now increasing evidence that the bone marrow microenvironment not only supports multiple myeloma cell survival and proliferation but also regulates drug resistance. Signaling through p38 $\alpha$  MAPK has been shown to play a key role in the survival and in the proliferation of the multiple myeloma cells and may also regulate osteoclast formation *in vitro*. Furthermore, the

**Figure 3.** *In vivo* activity of SCIO-469 in the 5T2MM model. Naive mice or mice bearing 5T2MM cells were treated with either vehicle (*Veh*), zoledronic acid (*Zol*), SCIO-469 at 150 mg/kg, or SCIO-469 at 450 mg/kg. **A**, radiographs of the tibia showing the presence of lesions (*arrows*) in 5T2MM-bearing mice but not in naive mice or mice treated with either zoledronic acid or SCIO-469. **B**, effect of 5T2MM injection and treatment on the number of osteolytic bone lesions. **C**, effect of 5T2MM injection and treatment on the proportion of cortico-endosteal bone surface covered by osteoclasts. *Columns*, mean; *bars*, SE.



p38 $\alpha$  inhibitor SCIO-469 also enhances chemotherapy (bortezomib)-induced cytotoxicity of the multiple myeloma cells (3). However, in view of the bone marrow dependency of the multiple myeloma cells, it is crucial to assess the effects of such inhibition in a three-dimensional *in vivo* bone marrow microenvironment. We have chosen to do such studies in the 5TMM series of models of myeloma. The 5TMM models, initially developed by Radl (7), are syngeneic, fully immunocompetent murine models. Mice are sequentially transplanted with diseased bone marrow (without *in vitro* passage) to generate a typical multiple myeloma disease. All clinical and molecular characteristics studied until now are highly analogous to the human disease (8) and make these models suitable for preclinical analysis.

Initially, we examined the effect of SCIO-469 on the biology of 5T2MM and 5T33MM myeloma cells *in vitro*. Treatment resulted in a clear inhibition of p38 $\alpha$  phosphorylation, as assessed by Western blot analysis (Fig. 1A). This was associated with a 20% to 40% inhibition of stromal cell-mediated 5TMM proliferation and an induction of apoptosis (Fig. 1B and C). No effect was seen on the level of thymidine incorporation when the 5TMM cells were cultured in medium containing 10% FCI alone (Fig. 1B). These data are consistent with the demonstration that SCIO-469 could also reduce p38 phosphorylation of human myeloma cells and induce apoptosis *in vitro* (3).

To assess the effect of blocking the p38 $\alpha$  MAPK pathway *in vivo* on the development of multiple myeloma disease, 5T33MM injected mice were treated with SCIO-469 from the time of tumor cell injection. Pharmacokinetic analysis of the serum samples of these mice resulted in values of 1 and 3  $\mu$ mol/L, respectively, which were similar to the values obtained in patients. Treatment was associated with a reduction in p38 $\alpha$  MAPK phosphorylation, as assessed on bone marrow samples of treated animals (Fig. 2). This was also associated with a decrease in serum paraprotein ( $8.8 \pm 1.4$  g/dL to  $0.04 \pm 0.03$  g/dL with 150 mg/kg and to  $0.0 \pm 0.0$  g/dL with 450 mg/kg;  $P < 0.001$ ) and a reduction in the proportion of tumor cells in the bone marrow ( $67.2 \pm 8.1\%$  to  $1.09 \pm 0.5\%$  for the 150 mg/kg and to  $0.0 \pm 0.0\%$  for the 450 mg/kg group;  $P < 0.001$ ; Table 1). Microvessel density decreased from  $25.4 \pm 1.2$  to  $19.2 \pm 0.7$  and  $19.2 \pm 0.5$ , respectively, for the 150 mg/kg group and the 450 mg/kg group ( $P < 0.001$ ), levels similar to that of the naïve controls. This decrease could be the result of either a direct effect on angiogenesis or an indirect effect via the reduction in tumor burden. Kaplan-Meier analysis showed an increase in disease-free survival following treatment of the mice with SCIO-469 (vehicle, 27.5 days, versus SCIO-469, 96 days;  $P < 0.0001$ ; Fig. 2).

To address whether inhibiting the p38 $\alpha$  MAPK pathway also affects the development of myeloma bone disease, studies were

done in the 5T2MM model. Injection of 5T2MM murine myeloma cells into C57Bl/KaLwRij mice resulted in the growth of myeloma cells in the bone marrow and the development of bone disease characterized by increased osteoclast surface ( $P < 0.05$ ), a reduction in cancellous bone ( $P < 0.01$ ), and the presence of osteolytic bone lesions on X-ray ( $P < 0.01$ ; Fig. 3). Treatment of 5T2MM-bearing mice with SCIO-469 resulted in a 40% decrease in serum paraprotein ( $P < 0.1$ , for both the 150 mg/kg group and the 450 mg/kg group). Microvessel density was reduced from  $25.5 \pm 0.8$  for the control group to  $18.8 \pm 0.7$  for the zoledronic acid group,  $20.3 \pm 0.7$  for the SCIO-469 150 mg/kg group, and  $18.7 \pm 0.5$  for the 450 mg/kg group (all values,  $P < 0.001$ ), levels similar to that of the naïve controls. SCIO-469 treatment (both 150 and 450 mg/kg) also prevented the development of osteolytic lesions ( $P < 0.01$ ; Fig. 3). This was also seen with the bisphosphonate zoledronic acid ( $P < 0.01$ ), used as a positive control. Histologic analysis showed that zoledronic acid treatment significantly reduced the increase in bone surface covered by osteoclasts by 5T2MM cells ( $P < 0.01$ ). This is consistent with our previous report showing that repeated dosing is effective in reducing osteoclast formation and the development of lytic bone lesions in this model (11). In contrast, SCIO-469 had no effect on osteoclast surface when compared with mice treated with vehicle. Indeed, osteoclast perimeter remained significantly increased when compared with mice treated with zoledronic acid ( $P < 0.05$ ). The strong inhibition of lytic lesions yet the absence of an effect on the proportion of bone surface covered by osteoclasts is consistent with SCIO-469 inhibiting osteoclast activity and function rather than osteoclast formation.

Our results suggest that, in addition to the previous published role of SCIO-469 on suppression of soluble factors within the bone marrow microenvironment *in vitro* (2), SCIO-469 also reduces p38 $\alpha$  phosphorylation in multiple myeloma cells, both *in vitro* and *in vivo*, resulting in a decreased tumor burden, angiogenesis, and bone disease, and therefore targets the multiple myeloma disease at multiple levels. This raises the possibility that targeting p38 $\alpha$  MAPK may offer a novel therapeutic approach in the treatment of multiple myeloma.

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## References

- Hideshima T, Akiyama M, Hayashi T, et al. Targeting p38 MAPK inhibits multiple myeloma cell growth in the bone marrow milieu. *Blood* 2003;101:703-5.
- Nguyen AN, Stebbins EG, Henson M, et al. Normalizing the bone marrow microenvironment with p38 inhibitor reduces multiple myeloma cell proliferation and adhesion and suppresses osteoclast formation. *Exp Cell Res* 2006;312:1909-23.
- Hideshima T, Podar K, Chauhan D, et al. p38 MAPK inhibition enhances PS-341 (bortezomib)-induced cytotoxicity against multiple myeloma cells. *Oncogene* 2004; 23:8766-76.
- Navas TA, Nguyen AN, Hideshima T, et al. Inhibition of p38 $\alpha$  MAPK enhances proteasome inhibitor-induced apoptosis of myeloma cells by modulating Hsp27, Bcl-X(L), Mcl-1 and p53 levels *in vitro* and inhibits tumor growth *in vivo*. *Leukemia* 2006;20:1017-27.
- Wang S, Yang J, Qian J, Wezeman M, Kwak LW, Yi Q. Tumor evasion of the immune system: inhibiting p38 MAPK signaling restores the function of dendritic cells in multiple myeloma. *Blood* 2006;107:2432-9.
- Wang S, Hong S, Yang J, et al. Optimizing immunotherapy in multiple myeloma: restoring the function of patient's monocyte-derived dendritic cells by inhibiting p38 or activating MEK/ERK MAPK and neutralizing interleukin-6 in the progenitor cells. *Blood* 2006;108: 4071-7.

7. Radl J, De Glopper ED, Schuit HR, Zurcher C. Idiopathic paraproteinemia. II. Transplantation of the paraprotein-producing clone from old to young C57BL/KaLwRij mice. *J Immunol* 1979;122:609–13.
8. Vanderkerken K, Asosingh K, Croucher P, Van Camp B. Multiple myeloma biology: lessons from the 5TMM models [review]. *Immunol Rev* 2003;194:196–206.
9. Vanderkerken K, Asosingh K, Willems A, et al. The 5T2MM murine model of multiple myeloma: maintenance and analysis. *Methods Mol Med* 2005;113:191–205.
10. Vanderkerken K, Goes E, De Raeve H, Radl J, Van Camp B. Follow-up of bone lesions in an experimental multiple myeloma mouse model: description of an *in vivo* technique using radiography dedicated for mammography. *Br J Cancer* 1996;73:1463–5.
11. Croucher PI, De Hendrik R, Perry MJ, et al. Zoledronic acid treatment of 5T2MM-bearing mice inhibits the development of myeloma bone disease: evidence for decreased osteolysis, tumor burden and angiogenesis, and increased survival. *J Bone Miner Res* 2003;18:482–92.
12. Van Valckenborgh E, De Raeve H, Devy L, et al. Murine 5T multiple myeloma cells induce angiogenesis *in vitro* and *in vivo*. *Br J Cancer* 2002;86:796–802.
13. Menu E, Jernberg-Wiklund H, Stromberg T, et al. Inhibiting the IGF-1 receptor tyrosine kinase with the cyclolignan PPP: an *in vitro* and *in vivo* study in the 5T33MM mouse model. *Blood* 2006;107:655–60.