Therapeutic potential of hepatocyte growth factor/scatter factor neutralizing antibodies: Inhibition of tumor growth in both autocrine and paracrine hepatocyte growth factor/scatter factor:c-Met-driven models of leiomyosarcoma

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Abstract

Hepatocyte growth factor/scatter factor (HGF/SF) and its receptor, c-Met, have been implicated in the growth and progression of a variety of solid human tumors. Thus, inhibiting HGF/SF:c-Met signaling may provide a novel therapeutic approach for treating human tumors. We have generated and characterized fully human monoclonal antibodies that bind to and neutralize human HGF/SF. In this study, we tested the effects of the investigational, human anti-human HGF/SF monoclonal antibody, AMG 102, and a mixture of mouse anti-human HGF/SF monoclonal antibodies (Amix) on HGF/SF-mediated cell migration, proliferation, and invasion *in vitro*. Both agents had high HGF/SF-neutralizing activity in these cell-based assays. The HGF/SF:c-Met pathway has been implicated in the growth of sarcomas; thus, we also investigated the effect of AMG 102 on the growth of human leiomyosarcoma (SK-LMS-1) in HGF/SF transgenic C3H severe combined immunodeficient mice engineered to express high levels of human HGF/SF, as well as tumor growth of an autocrine variant of the SK-LMS-1 cell line (SK-LMS-1TO) in nude mice. The results indicate that interrupting autocrine and/or paracrine HGF/SF:c-Met signaling with AMG 102 has profound antitumor effects. These findings suggest that blocking HGF/SF:c-Met signaling may provide a potent intervention strategy to treat patients with HGF/SF:c-Met–dependent tumors. [Mol Cancer Ther 2009;8(10):2803–10]

Introduction

Activation of the c-Met receptor tyrosine kinase by hepatocyte growth factor/scatter factor (HGF/SF) promotes cell proliferation, survival, and invasion, and also plays a role in angiogenesis (1, 2). Aberrant activation of the HGF/SF:c-Met signaling pathway via autocrine, paracrine, or mutational mechanisms has been implicated in the tumorigenesis and metastasis of numerous human solid tumors (2, 3), making it an important candidate for antitumor targeted therapies (4–6). Human cell lines established from various sarcomas and primary tumors from human osteosarcomas, chondrosarcomas, and leiomyosarcomas express high levels of activated c-Met receptor and display extensive tumor cell heterogeneity with regard to both paracrine and autocrine stimulation, suggesting that HGF/SF:c-Met signaling may contribute to the tumorigenic process in human sarcomas (7).

Various strategies are being exploited to block HGF/SF:c-Met signaling in the clinic, including the use of fully human antibodies to HGF/SF (8, 9), modified antibodies to c-Met (10), moderately selective small-molecule c-Met kinase inhibitors (11), and multikinase inhibitors that have some activity against c-Met (4, 6).

We have previously shown that a mixture of at least three mouse-anti-human HGF/SF monoclonal antibodies was required to neutralize the activity of human HGF/SF in cell-based assays or significantly inhibit tumor growth in autocrine xenograft tumor models (12). More recently, we and others have shown that individual, anti-human HGF/SF monoclonal antibodies are completely neutralizing *in vitro* (13) and significantly inhibit the growth of both s.c. and orthotopic models of glioblastoma tumors (8, 14).

In this study, the inhibitory effect of the fully human anti-human HGF/SF antibody, AMG 102 (8), was compared with that of the mouse monoclonal antibody mixture, Amix (11), on HGF/SF-mediated cell migration, proliferation, and invasion. We also investigated the effect of AMG 102 on the growth of human leiomyosarcoma tumor xenografts

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(SK-LMS-1) in transgenic C3H severe combined immunodeficient (SCID) mice engineered to express high levels of human HGF/SF. These mice provide a largely paracrine environment for human HGF/SF-dependent tumor growth (14)—mouse HGF/SF is not a potent agonist of the human c-Met receptor; thus, SKL-MS-1 tumors grow poorly in nontransgenic mice. In addition, we selected an autocrine variant of the SK-LMS-1 cell line (SK-KMS-1TO) by *in vivo* passage in nude mice, and evaluated the activity of AMG 102 in this novel model. The results of these studies indicate that interrupting paracrine and/or autocrine HGF/SF:c-Met signaling with AMG 102 may provide a potent intervention strategy to treat patients with HGF/SF:c-Met–dependent tumors.

Materials and Methods

Antibodies and Reagents

AMG 102 and human IgG2 were provided by Amgen, Inc. (8). Amix is a mixture of three mouse anti-human HGF/SF monoclonal antibodies (A-1, A-7, and A-10), as described by Cao et al. (12). Human HGF/SF was purified as described previously (15).

Animals

Female human HGF/SF transgenic SCID mice (huHGF/SF-SCID) and their nontransgenic littermates (C3H-SCID) were obtained as described previously (16). Female CD1 Nu/Nu mice were purchased from Charles River Laboratories, Inc. All animal studies were done in accordance with federal animal care guidelines and were approved by the Institutional Animal Care and Use Committees of both Amgen and the Van Andel Research Institute.

Cells and Cell Culture

Madin-Darby canine kidney (MDCK) cells and SK-LMS-1 cells (a cell line derived from a leiomyosarcoma tumor expressing high levels of Met but very low levels of HGF/SF; ref. 17) were purchased from American Type Culture Collection. To develop a more robust *in vivo* model of SK-LMS-1 (17), we grew SK-LMS-1 tumors in nude mice, reestablished the resulting SK-LMS-1TO cells in culture, and then established SK-LMS-1TO tumors in mice.

MDCK cells were cultured in DMEM supplemented with 5% fetal bovine serum (HyClone). SK-LMS-1 and SK-LMS-1TO cells were cultured in DMEM plus 10% fetal bovine serum.

Cell Assays

Cell Scatter Assay. To evaluate the inhibitory effect of AMG102 and Amix on cell migration, cell scatter assay was done. MDCK cells were cultured in DMEM (Invitrogen) containing 10% fetal bovine serum, seeded into 96-well plates (1.5×10^3 cells per well) as previously described (18) and, 24 h later, treated with 20 ng/mL purified human HGF/SF (15). Immediately following the HGF/SF treatment, antibodies were added (AMG 102 at concentrations ranging from 4–500 nmol/L, Amix at 133 and 400 nmol/L, and a negative control, nonspecific, isotype-matched human IgG2 monoclonal antibody at 500 nmol/L). After an additional 24-h incubation, the cells were washed with phenol-free DMEM, stained with Diff-Quick-Stain kit (Dade Behring, Inc.) then viewed and photographed at ×4 magnification using a light microscope (Olympus CK2 equipped with a Nikon Coolpix 4500 digital camera).

Urokinase-Type Plasminogen Activator/Plasmin Activation Assay. The effect of AMG102 and Amix on urokinase-type plasminogen activator (uPA) activity was evaluated by measuring the conversion of plasminogen to plasmin (18, 19). MDCK cells were seeded in 96-well plates (1,500 cells per well) and, 24 h later, treated with human HGF/SF (50 ng/mL). Immediately following the HGF/SF treatment, antibodies were added at a range of concentrations (0–20 nmol/L for AMG 102 and 0–100 nmol/L for Amix). After an additional 24-h incubation, the cells were washed with phenol-free DMEM and incubated with 200 μL of reaction solution [50% volume per volume (v/v) 0.05 U/mL plasminogen in phenol-free DMEM, 40% v/v 50 nmol/L Tris buffer (pH 8.2), and 10% v/v 3 nmol/L chromozyme PL (Roche Molecular Biochemicals) in 100 nmol/L glycine solution; refs. 18, 19] and further incubated for 4 h. The uPA/plasmin activity levels were determined by measuring absorbance at 405 nm using a spectrophotometric plate reader (Benchmark Plus; Bio-Rad).

Cell Proliferation Assay. The effect of AMG 102 and Amix on HGF-induced cell proliferation was determined using [^{3}H] thymidine incorporation assay (20). SK-LMS-1 cells were seeded in 96-well plates (2×10^3 cells per well) and incubated in 5% CO_2 at 37°C for 24 h. After serum starvation for an additional 36 h, antibodies were added at a range of concentrations (0.16–20 nmol/L), with or without HGF/SF (100 ng/mL). IgG2 (20 nmol/L) was used as a negative control. After incubation at 37°C for 12 h, 0.5 μCi [^{3}H] thymidine (GE-Amersham Biosciences) was added to each well, and cells were incubated for an additional 4 h. Cells were treated with trichloroacetic acid to precipitate DNA, and the precipitates were solubilized and suspended in a scintillation cocktail. Incorporation of [^{3}H] thymidine was measured by liquid scintillation counting (Microβ TriLux; PerkinElmer).

Cell Invasion Assay. AMG 102 and Amix were evaluated for their ability to inhibit HGF/SF-induced invasion of SK-LMS-1 cells in three-dimensional Matrigel (BD Biosciences; ref. 20). Serum-starved cells (5×10^3) were seeded in a 24-well invasion chamber (BD BioCoat Growth Factor Reduced Matrigel Invasion Chamber; BD Biosciences). Antibodies (0.27–33 nmol/L AMG 102, 33 nmol/L Amix, or 33 nmol/L IgG2) were preincubated with 100 ng/mL of HGF/SF for 30 min and then added to the lower wells of the invasion chamber. After 24 h, cells remaining in the upper chamber were removed, and the invading cells attached to the lower surface of the insert were stained with Diff-Quick and counted by microscopic (Nikon TS-100) visualization under ×200 magnification.

In vivo Assays

SK-LMS-1. Female human HGF/SF transgenic SCID (huHGF/SF-SCID) mice (16) and their nontransgenic littermates (C3H-SCID) were injected s.c. in the right subscapular region with 5×10^5 SK-LMS-1 cells in 0.1 mL serum-free

Dulbecco's Modified Eagle Medium (DMEM). Tumor volumes were measured with calipers twice weekly and calculated as length × width × height in mm^3. When the tumor volume was ~100 mm^3, the animals were randomized 1:1 into AMG 102 versus IgG2 isotype control groups and injected i.p. with 300 μg AMG 102 or IgG2, in a volume of 0.1 mL twice weekly for 3 wk. Tumor volumes (measured twice weekly) are expressed as mean ± SEM. For both xenograft models, the studies were terminated when tumor volumes in the respective control groups approached 2,000 mm^3. Terminal blood samples were collected for measurement of circulating human HGF/SF concentrations.

SK-LMS-1TO. Female CD1 nude mice (*CD1 Nu/Nu*) were injected s.c. with 5×10^6 SK-LMS-1TO cells in 0.2 mL MEM with one-third volume Matrigel. Two days after inoculation, mice were assigned to individual treatment groups (10 per group) and injected i.p. with 30, 100, or 300 μg AMG 102 or 300 μg IgG2 twice per week. Measurements of tumor volume were done as described for the SK-LMS-1 tumor model. Tumor volumes are expressed as mean ± SEM.

Measurement of Circulating Human HGF/SF. At the end of the *in vivo* studies, blood samples were collected from the mice and serum was prepared. Levels of human HGF/SF were measured using an ELISA kit (Human HGF Quantikine ELISA kit; R&D Systems).

Statistical Analysis. Tumor volume data were analyzed using repeated-measures ANOVA (RMANOVA) with Scheffé's *post hoc* test (StatView software, version 5.0.1). Statistic analysis for uPA/plasmin activity, proliferation, and invasion assays was analyzed with Welch's two-sample *t* test.

Results

Inhibition of HGF/SF-Met Signaling

To evaluate the therapeutic potential of anti-HGF antibodies on human sarcomas, we first tested their effect on HGF/SF-induced Met signaling on various sarcoma cell lines. As shown in Supplementary Fig. S1, high level of Met protein were detected in leiomyosarcoma cell line SK-LMS-1, fibrosarcoma cell line HT1080, and rhabdomyosarcoma cell line RD. HGF/SF induced dramatic activation of Met receptor, as well as its downstream molecules extracellular signal-regulated kinase and AKT, as assayed by Western blotting with phosphorylation-specific antibodies. However, phosphorylation of Met, extracellular signal-regulated kinase, and AKT were significantly inhibited by bothAmix and AMG102. Nevertheless, the osteosarcoma cell line, MG63, displayed low level of Met receptor and HGF/SF-induced activation of Met receptor, extracellular signal-regulated kinase, and AKT were not detectable.

Inhibition of Cell Scattering

One of the classic activities of HGF/SF is its ability to induce epithelial cell migration, also known as scattering. MDCK cells were induced to migrate by adding 20 ng/mL HGF/SF and qualitatively evaluated by visual inspection and photography. As a positive control, Amix (133 and 400 nmol/L) was shown to inhibit HGF/SF-induced cell migration (Fig. 1). Treatment of HGF/SF-stimulated MDCK cells with AMG 102 inhibited migration in a concentration-dependent manner, whereas the control IgG2 antibody was inactive (Fig. 1). The individual monoclonal antibodies comprising Amix were not active in this assay as previously reported (data not shown; ref. 12).

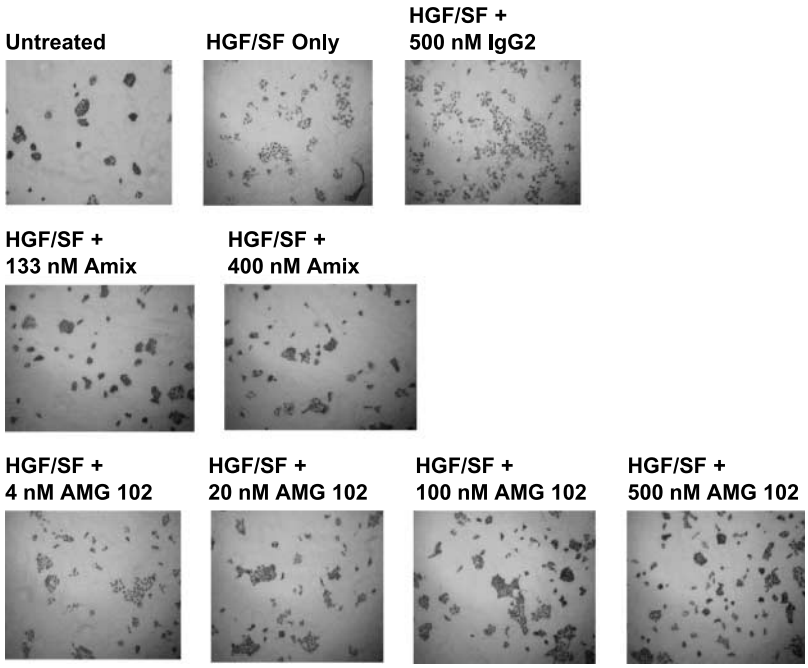

Figure 1. Inhibition of HGF/SF-mediated migration of MDCK cells by anti-HGF/SF antibodies. MDCK cells (1.5×10^3) were incubated in 20 ng/mL huHGF/SF plus a range of concentrations of AMG 102 or Amix for 24 h. Inhibition of HGF/SF-mediated migration was observed with AMG 102 (≥20 nmol/L) and Amix (≥133 nmol/L). The assay was done in triplicate and repeated twice. Representative images are magnified ×100.

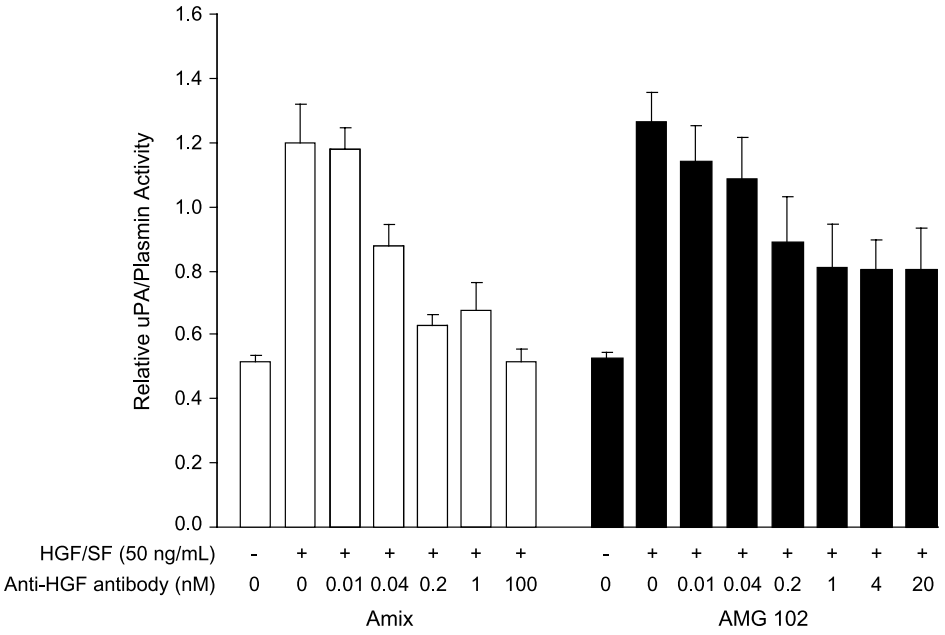

Figure 2. Inhibition of HGF/SF-induced uPA/plasmin activity in MDCK cells by anti-HGF/SF antibodies. MDCK cells (1.5×10^3) were incubated with 50 ng/mL HGF/SF plus a dose-range of AMG 102 or Amix. Concentration-dependent inhibition of uPA/plasmin activity was observed with each agent. *Columns,* mean; *bars,* SD.

Inhibition of Ligand-Induced uPA/Plasmin Activation

The HGF/SF-mediated invasion response is often accompanied by the induction of uPA and/or its receptor (2), resulting in the conversion of plasminogen to plasmin (18, 19). Amix and AMG 102 each inhibited HGF/SF-induced uPA-plasmin activity in MDCK cells in a concentration-dependent manner ($P < 0.05$ for both Amix and AMG102 at or higher than 0.04 nmol/L; Fig. 2 and Supplementary Table S1 for *t* test). Amix was somewhat more potent at inhibiting HGF/SF-mediated uPA/plasmin activation than was AMG 102 (IC_{50} = 0.035 and 0.13 nmol/L, respectively). As expected, uPA/plasmin activity was not inhibited by the control IgG2 antibody (data not shown). Neither antibody showed inhibitory effect on uPA/plasmin activity in MG63 cells with low Met receptor, suggesting both antibodies are specifically target HGF/SF-Met signaling (Supplementary Fig. S2).

Inhibition of Cell Proliferation

The HGF/SF:c-Met pathway has been implicated in sarcoma growth (2); therefore, we sought to determine if AMG 102 or Amix could inhibit the proliferation of SK-LMS-1 cells. HGF/SF induced the proliferation of SK-LMS-1 cells,

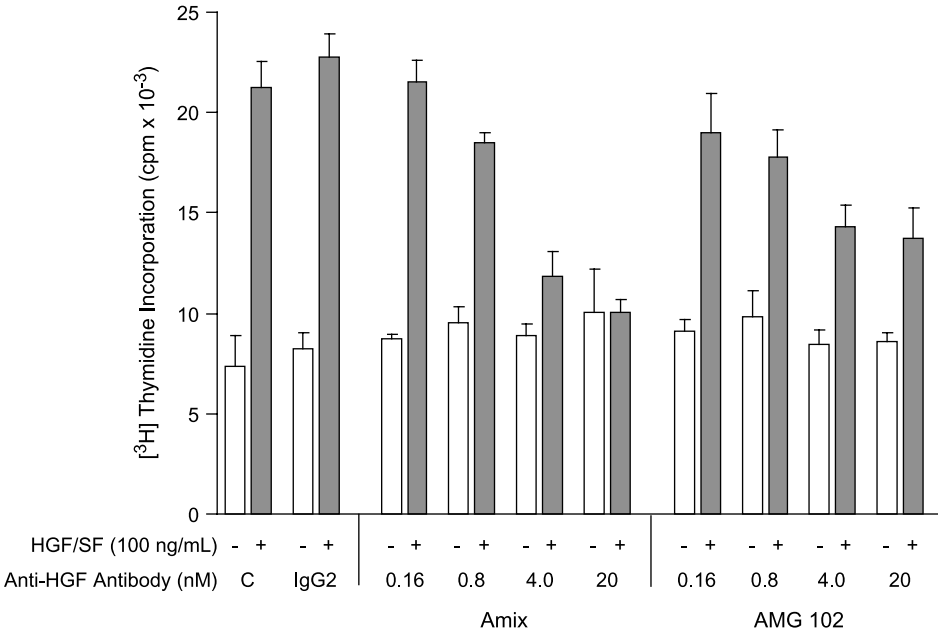

Figure 3. Inhibition of HGF/SF-mediated proliferation of SK-LMS-1 cells by anti-HGF/SF antibodies. SK-LMS-1 cells (2×10^3 cells per well) were untreated (*C*) or incubated with IgG2 control antibody or with a range of concentrations of AMG 102 or Amix in the absence or presence of HGF/SF (100 ng/mL) for 12 h at 37°C. Both AMG 102 and Amix effectively blocked HGF/SF-induced cell proliferation as measured by inhibition of [^{3}H] thymidine incorporation. *Columns,* mean; *bars,* SD.

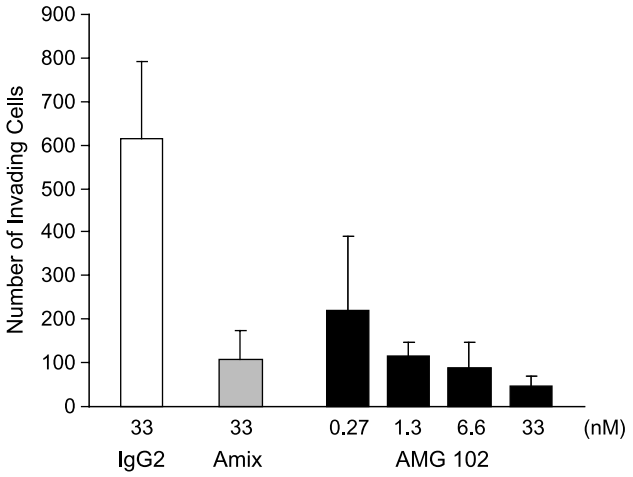

Figure 4. Inhibition of HGF/SF-mediated invasion of SK-LMS-1 cells by anti-HGF/SF antibodies. Serum-starved SK-LMS-1 cells (5×10^3) were incubated with AMG 102, Amix, or IgG2 plus 100 ng/mL of HGF/SF for 24 h. Invading cells were counted by microscopic visualization. Both AMG 102 and Amix inhibited HGF/SF-induced cell invasion compared with the control IgG2 antibody. *Columns,* mean; *bars,* SD.

as revealed by an increase in [^{3}H] thymidine incorporation in these cells (Fig. 3). Both AMG 102 and Amix inhibited HGF/SF-mediated cell proliferation in a concentration-dependent manner ($P < 0.05$ for concentration of or higher than 0.8 nmol/L; Fig. 3 and Supplementary Table S2 for *t* test). The IC_{50} for cell proliferation was calculated from two independent experiments, and was found to be similar for each agent (IC_{50} = 2.99 and 2.632 nmol/L, respectively; Supplementary Fig. S3). There was no inhibition of cell proliferation in the presence of the IgG2 control antibody (Fig. 3). Both antibodies showed little inhibitory effect on proliferation in MG63 cells (Supplementary Fig. S4).

Inhibition of Cell Invasion

The HGF/SF:c-Met pathway has been implicated in tumor cell migration and invasion, and is thought to play an important role in invasion and metastasis in human cancer. HGF/SF induced dramatic increase in cell invasion through Matrigel insert within 24 h (Supplementary Fig. S5). The increase in number of invading cells did not result from increased cell proliferation because HGF/SF did not significantly increase cell number within 24 hours (Supplementary Fig. S6). We therefore, quantitatively evaluated AMG 102 for the ability to inhibit invasion using this model with Amix serving as positive control. AMG 102 showed dose-dependent inhibition of HGF/SF-induced invasion of SK-LMS-1 cells through a Matrigel coating ($P < 0.05$ for all tested concentration; Fig. 4 and Supplementary Table S3 for *t* test).

HGF/SF Expression in SK-LMS-1 and SK-LMS-1TO Models of Leiomyosarcoma

Human HGF/SF levels were measured in the serum of C3H-SCID and huHGF/SF-SCID mice bearing SK-LMS-1 tumors to compare the amount of HGF/SF produced by the tumor cells (autocrine) with the amount of HGF/SF produced by the transgenic huHGF/SF-SCID mice (paracrine; Table 1). Approximately 1 and 10 ng/mL HGF/SF were detected in the serum of CH3-SCID and huHGF/SF-SCID mice, respectively. The higher concentration of circulating HGF/SF in huHGF/SF-SCID mice provides a "paracrine" source of HGF/SF, which drives the SK-LMS-1 tumors to establish and grow faster than in CH3-SCID mice. Although SK-LMS-1TO grew rapidly in CD1 Nu/Nu mice (see later section), the level of serum HGF/SF in this mouse model was 0.14 ng/mL (Table 1).

Inhibition of Tumor Growth in Mouse Xenograft Models of Sarcoma by AMG 102

Twenty-two huHGF/SF-SCID and 20 C3H-SCID mice were randomized into AMG 102 or IgG2 groups at 25 and 43 days, respectively, after injection of SK-LMS-1 cells. Tumor volumes increased more rapidly in huHGF/SF-SCID mice compared with their C3H-SCID littermates (Fig. 5A) due to transgenic (paracrine) HGF/SF stimulation of c-Met (16). Treatment with AMG 102 (300 μg i.p. twice weekly) significantly inhibited tumor growth in both huHGF/SF-SCID (78% inhibition on day 46; $P < 0.0001$) and C3H-SCID mice (69% inhibition on day 64; $P = 0.0012$) compared with the isotype control antibody (Fig. 5A). In a second experiment (data not shown), inhibition of tumor growth with AMG 102 was again statistically significant: for huHGF/SF/SCID mice, $P = 0.00055$ on day 46; for C3H-SCID mice, $P = 0.0108$ on Day 64. These results show that SK-LMS-1 is an HGF/SF-dependent model, and that treatment with AMG 102 can inhibit the growth of these tumors when used as a single agent in both paracrine (huHGF/SF/SCID mice) and autocrine (C3H-SCID mice) settings. It should be noted that there is a small but significant autocrine component to SKLMS-1 tumor growth in the huHGF/SF/SCID mice, in addition to paracrine HGF–mediated growth.

Table 1. Levels of HGF/SF in mice bearing SK-LMS-1 or SK-LMS-1TO tumors

Mouse host	Tumor	Source of human HGF	Treatment	Human HGF (mean ± SD, ng/mL)
C3H-SCID	SK-LMS-1	SK-LMS-1 tumors (autocrine)	IgG2 (n = 11)	1.05 ± 1.04
huHGF/SF-SCID	SK-LMS-1	huHGF/SF-SCID and SK-LMS-1 (paracrine and autocrine)	IgG2 (n = 10)	10.3 ± 9.10
CD1 Nu/Nu	SK-LMS-1TO	SK-LMS-1TO (autocrine)	None (n = 5)	0.14 ± 0.45

Abbreviation: Hu, human.

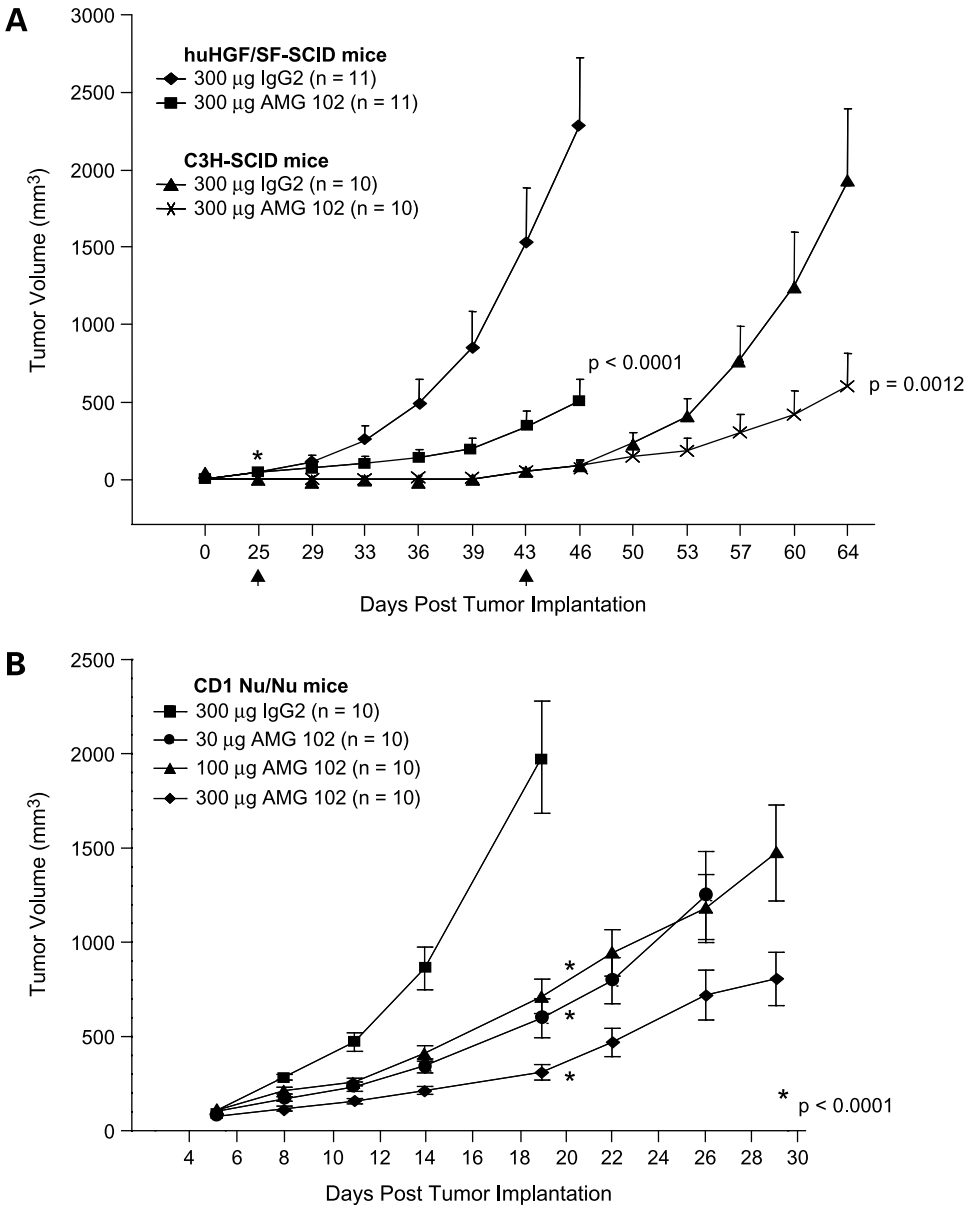

Figure 5. Inhibition of autocrine and paracrine growth of SK-LMS tumors in mice by AMG 102. **A,** huHGF/SF-SCID mice were injected with SK-LMS-1 cells and treated with AMG 102 or IgG2 after day 25 (↑). SK-LMS-1 tumor cells were similarly implanted in C3H-SCID mice, but treatment with AMG 102 or IgG2 began after day 43 (↑). AMG 102 statistically significantly inhibited tumor growth compared with IgG2. **B,** female nude mice were injected s.c. with 5×10^6 SK-LMS-1TO cells. Treatment began 2 d later with IgG2 (300 μg) or AMG 102 (30, 100, or 300 μg) twice per week. AMG 102 significantly inhibited tumor growth at all doses tested compared with the IgG2 group ($P < 0.0001$ on day 19). *Points,* mean; *bars,* SEM. Statistical analysis was done by repeated-measures ANOVA with Scheffé's *post hoc* test.

We also selected a novel variant of the SK-LMS-1 cell line (SK-LMS-1TO) by *in vivo* passage in nude mice. Although the level of HGF/SF in nude mice bearing SK-LMS-1TO tumors was lower than the level in CH3-SCID mice bearing SK-LMS-1 tumors (Table 1), SK-LMS-1TO tumors grew more aggressively, reaching ~2,000 mm^3 in 20 days (Fig. 5B). Because of the more robust tumor growth in this model, we began treatment 2 days after tumor cell implantation. AMG 102 also significantly inhibited tumor growth in this variant of SK-LMS-1 at all concentrations tested ($P < 0.0001$; Fig. 5B).

Discussion

c-Met activation by its ligand HGF/SF can lead to multiple cellular responses, including proliferation, motility, and invasion, all of which are critical for tumor initiation and malignant progression. In this study, we tested the effects of the anti-HGF/SF antibodies, AMG 102 and Amix, on cellular responses using *in vitro* cell-based assays and on HGF/SF-dependent tumor growth using a unique huHGF/SF transgenic SCID mouse model (16).

Previous studies showed that cell migration and invasion correlate with uPA activity and play an important role in tumor invasion and metastasis (18). In the present study, both AMG 102 and Amix inhibited HGF/SF-induced cell migration, proliferation, and invasion as well as uPA/plasmin activity. In general, we found that both Amix and AMG 102 are potent inhibitors of these activities; however, there were differences between the two agents in their ability to inhibit HGF/SF-induced cell activities in specific assays.

For example, although AMG 102 and Amix were essentially equipotent at inhibiting cell proliferation, Amix was more efficient at inhibiting induction of uPA, whereas AMG 102 seemed to be more efficient at blocking cell invasion. Because these differences were observed in replicate experiments, it does not seem to be due to assay variability. Similarly in a previous study (8), we observed that individual anti-HGF/SF monoclonal antibodies showed different potency profiles across different *in vitro* cell-based assays. Interestingly, all of the fully human monoclonal antibodies characterized by Burgess et al. (8), including AMG 102, were shown to bind primarily to the β chain/light chain of HGF and were all able to fully neutralize HGF. This result fits nicely with the structural data demonstrating that the interaction of the β chain of HGF with c-Met is critical for receptor activation (21). In contrast, individual components of Amix all bind to the α/heavy chain of HGF (data not shown); perhaps binding to these different epitopes confers different activities on the antibodies.

We also tested the *in vivo* effects of AMG 102 in three different models of SK-LMS-1 sarcoma. SKL-MS-1 tumors grow poorly in SCID or nude mice due to a lack of a species-compatible ligand provided by the host environment for the human c-Met receptor (16, 17): mouse HGF/SF binds human c-Met, but it is not a potent agonist of the human receptor (7, 15), primarily because of differences in the sequence of the HGF/SF β domain.[5] To overcome this, we previously developed a transgenic human HGF-SCID mouse model (huHGF/SF-SCID) in which SKL-MS-1 tumors grow robustly due to transgenic, "paracrine" huHGF/SF expression (16). In the present study, we found that AMG 102 significantly inhibited the growth of SK-LMS-1 tumors in huHGF/SF-SCID mice, suggesting that it can efficiently block paracrine HGF/SF:c-Met signaling. This result is consistent with the report that single anti-HGF antibody decrease carcinogen-induced lung carcinogenesis in human HGF transgenic mice (22), suggesting that targeting paracrine HGF/SF is potential therapeutic strategy for various human cancer.

Furthermore, AMG 102 was able to significantly inhibit the growth of the SK-LMS-1 tumors in the slower-growing, autocrine setting in SCID mice. We also selected a more robust variant of SK-LMS-1 that grew more rapidly in nude mice. This tumor was also significantly inhibited by AMG 102 in a dose-dependent manner. We originally speculated that this enhanced growth may have been due to increased production of human HGF/SF by SK-LMS-1TO. However, this did not seem to be the case, and although the reasons for the enhanced growth remain undetermined, this tumor was still highly dependent on HGF/SF.

In summary, both AMG 102 and Amix are highly effective in blocking HGF/SF-induced cellular activities of human c-Met. *In vivo*, AMG 102 shows activity in paracrine and autocrine models of HGF/SF:c-Met-driven sarcoma. These data, along with emerging evidence from other HGF/SF:c-Met inhibitors (4), provide a compelling case for moving these agents into clinical settings, especially diseases such as sarcoma, where there is good evidence that HGF/SF:c-Met plays an important role in tumor growth. AMG 102 is currently in phase II trials, with a variety of tumor types to test whether inhibition of HGF/SF can provide clinical benefit to patients.

[5] Unpublished data.

Disclosure of Potential Conflicts of Interest

J. Sun, K. Rex, T. Osgood, A. Coxon, and T.L. Burgess: employees with stock ownership, Amgen Inc. No other potential conflicts of interest were disclosed.

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