Biochemical Characterization of AMG 102: A Neutralizing, Fully Human Monoclonal Antibody to Human and Nonhuman Primate Hepatocyte Growth Factor

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Abstract

AMG 102 is a fully human monoclonal antibody that selectively targets and neutralizes hepatocyte growth factor/scatter factor (HGF/SF). A detailed biochemical and functional characterization of AMG 102 was done to support its clinical development for the treatment of cancers dependent on signaling through the HGF/SF:c-Met pathway. In competitive equilibrium binding experiments, AMG 102 bound to human and cynomolgus monkey HGF with affinities of approximately 19 pmol/L and 41 pmol/L, respectively. However, AMG 102 did not detect mouse or rabbit HGF on immunoblots. Immunoprecipitation experiments showed that AMG 102 preferentially bound to the mature, active form of HGF, and incubation of AMG 102/HGF complexes with kallikrein protease indicated that AMG 102 had no apparent effect on proteolytic processing of the inactive HGF precursor. AMG 102 inhibited human and cynomolgus monkey HGF-induced c-Met autophosphorylation in PC3 cells with IC₅₀ values of 0.12 nmol/L and 0.24 nmol/L, respectively. AMG 102 also inhibited cynomolgus monkey HGF-induced migration of human MDA-MB-435 cells but not rat HGF-induced migration of mouse 4T1 cells. Epitope-mapping studies of recombinant HGF molecules comprising human/mouse chimeras and human-to-mouse amino acid substitutions showed that amino acid residues near the NH₂-terminus of the β-chain are critical for AMG 102 binding. Bound AMG 102 protected one trypsin protease cleavage site near the NH₂-terminus of the β-chain of human HGF, further substantiating the importance of this region for AMG 102 binding. Currently, AMG 102 is in phase II clinical trials in a variety of solid tumor indications.

Introduction

Hepatocyte growth factor [HGF; also known as scatter factor (SF)] is the only known ligand for c-Met, a receptor tyrosine kinase expressed in epithelial tissues that plays an essential role in the growth and maintenance of cells (1). Signaling through HGF/SF:c-Met mediates a number of normal cellular functions, including proliferation, survival, motility, migration, angiogenesis, and morphogenesis (1–3). Abnormal signaling via the HGF/SF:c-Met pathway has been associated with many types of cancers (1–4), with evidence for both paracrine and autocrine activation of c-Met by HGF; expression of these molecules in tumors and elevated concentrations of circulating HGF have been associated with an increased risk of tumor metastasis and poor prognosis (5–9).

Mutations in c-Met resulting in constitutive activation have been described in hereditary and sporadic human cancers (10), and expression of some of these mutant alleles in mice enhances their tumorigenicity (11, 12). Some of these c-Met mutants remain sensitive to HGF activation, and can be blocked by HGF antagonists (10, 13).

HGF is normally secreted from cells of mesenchymal origin as a monomeric, inactive precursor (pro-HGF) with molecular mass of ~90 kDa. Extracellular proteolysis at a site COOH-terminal to the fourth kringle domain generates the fully active, disulphide-linked dimeric form of HGF capable of activating signaling through binding to c-Met (1, 14–16). The NH₂-terminal portion of HGF, the α-chain, contains the NH₂-terminal hairpin domain and four kringle domains. Protein fragments derived from NH₂-terminal regions of HGF (e.g., NK1, NK2, and NK4) contain a high-affinity c-Met receptor binding domain and can act as HGF agonists or as antagonists, showing that HGF binds to c-Met...
and can function through determinants in the α-chain (17, 18). The COOH-terminal β-chain of HGF is structurally homologous to serine proteases, but lacks proteolytic activity (1). The β-chain also interacts directly with c-Met and seems to be critical for mature HGF signaling through its receptor (19, 20).

The importance of HGF/SF–c-Met signaling in cancer has led to the development of specific inhibitors of this pathway that may provide novel therapeutic strategies for intervention in human disease (21–23). In previous studies (24, 25), we reported on the biological activity of novel, fully human anti-HGF antibodies in both in vitro and in vivo models of cancer. The five antibodies analyzed were all competitive inhibitors of HGF binding to its receptor c-Met. Based on its in vitro and in vivo activities, AMG 102 was selected as the clinical candidate. Phase I clinical studies with AMG 102 have been completed (26), and phase II clinical trials in a variety of solid tumor indications are underway. Here we provide a detailed biochemical and functional characterization of this molecule in support of its clinical development.

Materials and Methods

Reagents and Cell Lines

AMG 102 was prepared as previously described (24). cDNA cloning and DNA sequencing of cynomolgus monkey HGF (submitted to GenBank; full-length HGF = GQ477910; d5 HGF = GQ477911) and rabbit HGF (submitted to GenBank; full length HGF = GQ477909) were done using standard methods and an automated sequencer. The amino acid sequences aligned with those of human and mouse full-length HGF are shown in Supplementary Fig. S1. The calculated percent similarities are shown in Supplementary Table S1. MAB294 (a mouse anti-human HGF monoclonal antibody) and AF-294-NA (a goat anti-HGF polyclonal antibody) were purchased from R&D Systems. 93R1B9 (an anti-HGF monoclonal antibody identified by phage display that detects human and rabbit HGF equally well) and the rabbit anti-mouse HGF polyclonal antibody were generated using standard immunization procedures; both were prepared at Amgen Inc. Recombinant human HGF, human d5-HGF (a naturally occurring splice-variant of HGF with a five–amino acid deletion of residues 163 to 167 in the α-chain; ref. 27), cynomolgus monkey d5-HGF, and mouse HGF were expressed by Chinese hamster ovary (CHO) cells and purified with heparin sulfate affinity chromatography and gel filtration. Rabbit HGF and chimeric and mutated HGF constructs were expressed in human embryonic kidney (HEK293) cells and collected from serum-free conditioned medium without further purification. Rat HGF was obtained from the Institute of Immunology, Japan.

Equilibrium Binding

To evaluate the physical interaction between HGF and AMG 102, a competition equilibrium binding method was developed. Analysis of AMG 102 was done on a Biacore 3000. AMG 102 was immobilized on a research-grade CM5 sensor chip via primary amine groups using the Amine Coupling Kit (Biacore Life Sciences), according to the manufacturer's suggested protocol. Two different concentrations (1 nmol/L and 3 nmol/L) of purified human d5-HGF or cynomolgus monkey d5-HGF were incubated with a dose range of AMG 102 (1 pmol/L to 25 nmol/L) in PBS with 0.005% surfactant P-20 and 0.1 mg/mL bovine serum albumin (BSA). Protein samples were incubated at room temperature for at least 6 h to allow binding to reach equilibrium. The binding signal obtained after the samples were injected over the immobilized AMG 102 surface was considered to be proportional to the free HGF in solution at equilibrium. The equilibrium dissociation constants (Kd) were obtained from nonlinear regression analysis of the competition curves using a dual-curve, one-site homogeneous binding model (KinExA software, Sapidyne Instruments Inc.).

AMG 102 Binding to Human, Mouse, and Rabbit HGF

Protein samples were resolved by reducing SDS-PAGE, transferred to Hybond ECL membranes (Amersham), and blocked with TBS, 0.25% Tween 20, and 5% BSA. The primary blotting antibody was either AMG 102 or 93R1B9 as indicated. The secondary antibody was horseradish peroxidase–conjugated goat polyclonal antibody (1:5,000 dilution). The membranes were washed and the complexes were detected using electrochemiluminescence.

Immunoprecipitation Studies

Human d5-HGF (1 μg) was mixed in an ≈1:1 molar ratio with 2 μg of either AMG 102 or MAB294 in 1 mL of 0.1% BSA-PBS buffer and incubated for 2 h at room temperature. The antibody-antigen complexes were captured on Protein G beads (Pierce), washed with PBS, and pelleted. The immunoprecipitated proteins were eluted with 2× SDS-PAGE loading buffer containing 5% β-mercaptoethanol. The mixture was boiled, and the eluted, immunoprecipitated proteins were separated and immunoblotted as above. The primary antibody was goat anti-HGF polyclonal antibody AF-294-NA (100 ng/mL), and the secondary antibody was horseradish peroxidase–conjugated goat polyclonal antibody (1:5,000). The membrane was washed and the immune-precipitated complexes were detected using electrochemiluminescence.

HGF Proteolysis/Processing

A preliminary experiment determined that AMG 102 was resistant to kallikrein proteolysis (data not shown).
Purified human d5-HGF was preincubated for 20 min at room temperature with or without AMG 102 (1:1 and 5:1 molar ratios of antibody: HGF were tested) to allow complex formation prior to the addition of kallikrein. When included together, kallikrein and HGF were added in a 1:10 (weight:weight) ratio, and reactions were further incubated at 37°C for 0, 4, or 12 h. Results were analyzed by SDS-PAGE and Coomassie Blue staining.

**Autophosphorylation Studies**

Electrochemiluminescence assays to determine the phosphorylation status of c-Met were done as described (24) using human PC3 cells (a human prostate cancer line that expresses c-Met but not HGF) treated with serial dilutions of AMG 102 that had been preincubated with 200 ng/mL human d5-HGF or cynomolgus monkey d5-HGF. Percent inhibition was calculated relative to the results for cells stimulated with HGF in the absence of AMG 102. The IC50 values were determined using the 4-parameter fit equation in XLfit (IDBS).

**Cell Invasion Assays**

Cell invasion assays were done as previously described (24). Cynomolgus monkey d5-HGF (50 ng/mL) was used as the attractant for human breast carcinoma MDA-MB-435 cells. In one experiment (Fig. 2C), rat HGF (50 ng/mL) was used as the attractant for the migration of mouse mammary tumor 4T1 cells. In this experiment, rabbit anti-mouse HGF polyclonal antibody was employed as an inhibitory control. Data represent mean ± SE, n = 3. Data are representative of two experiments.

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**Figure 1.** Binding specificity of AMG 102. AMG 102 (1 pmol/L to 25 nmol/L) was incubated with two concentrations (●, 1 nmol/L; ○, 3 nmol/L) of human (A) or cynomolgus monkey (B) d5-HGF. The fraction of free HGF in solution at equilibrium was measured by Biacore, and the values were used to calculate affinity constants (Kd). C, recombinant human, rabbit, or mouse HGF was separated by nonreducing SDS-PAGE, transferred to membranes, and probed with either 93R1B9 (anti-pan HGF, top) or AMG 102 (bottom). Top, lanes 1 to 5, 25, 50, 100, 250, and 500 ng purified human d5-HGF; lanes 6 to 8, 5, 15, and 30 μL conditioned medium from CHO cells stably expressing rabbit HGF; lane 9, 30 μL conditioned medium from human HEK293 cells transiently expressing rabbit HGF. Bottom, lanes 1 to 6, 10, 25, 50, 100, and 250 ng purified human HGF; lanes 7 to 10, 10, 100, 250, and 500 ng purified mouse HGF; lane 11, 20 μL conditioned medium from CHO cells expressing rabbit HGF; lane 12, culture medium alone.

D, the preparation of purified human HGF used in this experiment contained approximately equivalent amounts of mature HGF and pro-HGF. After immunoprecipitation of HGF, the samples were separated by reducing SDS-PAGE and analyzed with immunoblotting using goat anti-human HGF polyclonal (AF-294-NA), which detects pro-HGF as well as the larger, α-chain of mature-HGF. Lane 1, purified recombinant human HGF without immunoprecipitation; lane 2, recombinant human HGF immunoprecipitated with a 1:1 molar ratio of AMG 102; lane 3, recombinant human HGF immunoprecipitated with a 1:1 molar ratio of MAB 294. E, human HGF was incubated with or without AMG 102 at a 1:5 molar ratio, and with or without kallikrein protease. After incubation, the samples were analyzed with reducing SDS PAGE, followed by Coomassie Blue staining. Lanes 7 and 10 (arrowheads) contain Seablue molecular weight standards. Pro-HGF and mature HGF as well as the heavy and light chains of AMG 102 are as indicated.
Epitope Mapping

Human-mouse chimeric HGF-avidin fusion proteins were constructed using standard recombinant DNA and PCR methodologies as described by Burgess et al. in 2006 (24). As shown in Table 1 and Supplementary Table S2, chimeras 1, 2, and 3 comprised a portion of the NH2-terminus of mouse HGF linked to the COOH-terminus of human HGF. Chimera 7 comprised the NH2- and COOH-terminus of human HGF, with the internal amino acid residues from the mouse β-chain (because of the strong sequence identity between human and mouse HGF, there are only nine murine residues in chimera 7; Fig. 3B). Chimera 8 mirrored chimera 7, with the mouse and human sequences interchanged, thus the only human HGF residues present in chimera 8 were from the NH2-terminal portion of the β-chain (only seven unique human residues and a two–amino acid deletion relative to the mouse sequence are present in chimera 8). We also generated five human HGF-avidin constructs, each containing a single, nonconservative amino acid substitution from the mouse HGF sequence: a human HGF construct containing a two–amino acid insertion from mouse HGF, and a mouse HGF with the same two–amino acid deletion (Table 1, Supplementary Table S2, and Fig. 3).

Each avidin-HGF fusion-chimeric and point-mutated protein was transiently expressed in HEK293 cells. Conditioned media were incubated with BSA-blocked, biotin-coated beads and stained with FITC-labeled...
anti-avidin antibodies (Vector Lab) to evaluate protein expression level. The fluorescent probe allowed normalization of the amount of protein expression among different samples (24).

To evaluate AMG 102 binding to the chimeric and mutated HGF proteins, a second fluorescent probe, phycoerythrin (PE)-labeled goat anti-human F(ab)2, was used. The presence of both FITC (avidin/HGF) and PE (AMG 102) labeling, detected on a Becton Dickinson Bioscience FACScan (BD), indicated that AMG 102 bound to the HGF portion of the fusion construct. Single-color fluorescence-activated cell sorting (FACS) histograms without normalization were generated from the dual-labeled data sets. Biotin-coated beads complexed with avidin alone were used as a negative control, enabling detection of shifts in fluorescence associated with AMG 102 binding. Because the spectral properties of FITC and PE overlap, control samples labeled with FITC only or PE only were analyzed and fluorescence signals were adjusted accordingly to enhance sensitivity and to facilitate measurement of binding with constructs that were expressed at low levels.

**Protease Protection Studies**

Human HGF was preincubated with and without AMG 102 to allow complex formation (~1:1 molar ratio of antibody:HGF was tested). Digestion with trypsin was carried out at 37°C for 1 h (weight:weight trypsin:HGF = 1:30). The digested material from each sample was subjected to reverse-phase high-performance liquid chromatography (HPLC) for peptide separation. The HPLC maps were compared to identify trypsin cleavage sites protected by AMG 102 binding. NH₂-terminal protein sequencing was done to identify the protected peptides.

A complementary experiment was designed to isolate and identify the AMG 102-binding peptides of HGF. The HGF-AMG 102 complex was digested as above and then subjected to Microcon centrifugal filtration (Millipore) with a cutoff at a molecular weight of 10 kDa to capture AMG 102 with the bound peptides, while the unbound peptides flowed through the filter. AMG 102 was released from the bound peptides captured on the filter by addition of intact human HGF (HGF elution). The mixture of HGF-bound AMG 102 and free peptides was incubated overnight at 4°C and was again filtered to collect the AMG 102–binding peptides. These peptides and those eluted at the first filtration step were analyzed with reverse-phase HPLC. Bound peptides were isolated and subjected to NH₂-terminal sequencing.

**Results**

**Binding Specificity of AMG 102**

A competition binding method was used to evaluate the affinity of AMG 102 for human and cynomolgus monkey d5-HGF. The equilibrium dissociation constants (K_D) were obtained from nonlinear regression analysis of the competition curves (Fig. 1A and B) using a dual-curve, one-site binding model.

### Table 1. AMG 102 binding results for chimeric and mutant HGF molecules

<table>
<thead>
<tr>
<th>HGF chimera or mutant designation</th>
<th>(Domains or amino acid numbers)</th>
<th>AMG 102 binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human full length*</td>
<td>N, K1, K2, K3, K4, β</td>
<td>None</td>
</tr>
<tr>
<td>Mouse full length†</td>
<td>None</td>
<td>N, K1, K2, K3, K4, β</td>
</tr>
<tr>
<td>Ch 1 HGF</td>
<td>K1, K2, K3, K4, β</td>
<td>N-domain</td>
</tr>
<tr>
<td>Ch 2 HGF</td>
<td>K2, K3, K4, β</td>
<td>N, K1</td>
</tr>
<tr>
<td>Ch 3 HGF</td>
<td>K3, K4, β</td>
<td>N, K1, K2</td>
</tr>
<tr>
<td>Ch 7 HGF</td>
<td>N, K1, K2, K3, K4, β (586 to 728)</td>
<td>β 507 to 585</td>
</tr>
<tr>
<td>Ch 8 HGF</td>
<td>β (507 to 585)</td>
<td>N, K1, K2, K3, K4, β (586 to 728)</td>
</tr>
<tr>
<td>G555E HGF</td>
<td>N, K1, K2, K3, K4, β†</td>
<td>555†</td>
</tr>
<tr>
<td>C561R HGF</td>
<td>N, K1, K2, K3, K4, β†</td>
<td>561†</td>
</tr>
<tr>
<td>D592N HGF</td>
<td>N, K1, K2, K3, K4, β†</td>
<td>592†</td>
</tr>
<tr>
<td>N601S HGF</td>
<td>N, K1, K2, K3, K4, β†</td>
<td>601†</td>
</tr>
<tr>
<td>R647Q HGF</td>
<td>N, K1, K2, K3, K4, β†</td>
<td>647†</td>
</tr>
<tr>
<td>Human NK (insertion) HGF</td>
<td>N, K1, K2, K3, K4, β†</td>
<td>2 amino acid insertion of mouse 540 to 541</td>
</tr>
<tr>
<td>Mouse NK (deletion) HGF</td>
<td>None</td>
<td>N, K1, K2, K3, K4, β (540, 541 deleted)†</td>
</tr>
</tbody>
</table>

Abbreviations: N, amino terminus; K1, K2, K3, and K4, the four conserved kringle domains of HGF; NK, insertion or deletion of Asn-Lys; Ch, chimera.

*Human HGF sequence, GenBank NM_000601.
†Mouse HGF sequence, GenBank NM_010427.
‡Mutationally altered domain or amino acid substitution.
Figure 3. Epitope mapping of AMG 102 binding to HGF. A, single-color FACS histograms for binding of human/mouse chimeric and point-mutated HGF to AMG 102. PE fluorescence is a measure of AMG 102 binding to the various HGF proteins captured on avidin-coated beads (red) or beads with avidin alone as negative controls (black). Each human/mouse hybrid HGF construct is described in Table 1 and Supplementary Table S2. B, HGF was subjected to partial trypsin digestion without AMG 102 (first panel) or with bound AMG 102 (second panel), and peptides were analyzed by reverse-phase HPLC. AMG 102-bound HGF was partially digested with trypsin and then subjected to filtration to separate the unbound (third panel) from the bound peptides (fourth panel). Prior to analysis, AMG 102 was removed from the bound peptides by incubation with intact HGF. C, summary of AMG 102 epitope mapping. The amino acid sequences of the mouse and human HGF β-chain are aligned. Red boxes, both conservative and nonconservative differences between the two sequences; green box, a region of human HGF implicated in AMG 102 binding. Red stars (both closed and open), residues that were investigated using the point-mutation strategy; closed stars, residue changes that disrupted AMG 102 binding to human HGF.
homogeneous binding model. AMG 102 showed $K_D$ values of 19 pmol/L for human d5-HGF and 41 pmol/L for cynomolgus monkey d5-HGF.

To investigate the interaction of AMG 102 with HGF of other species, recombinant HGF molecules were separated, blotted, and probed with 93R189, an anti-HGF monoclonal antibody that detects human and rabbit HGF equally well (Fig. 1C, top), or with AMG 102 (Fig. 1C, bottom). Antibody 93R189 detected both human HGF (top, lanes 1 to 5) and rabbit HGF (top, lanes 6 to 8). AMG 102 detected as little as 10 ng of human HGF (bottom, lanes 1 to 6) but did not detect up to 500 ng of either mouse HGF (bottom, lanes 7 to 10) or rabbit HGF (bottom, lane 11).

To investigate the specificity of AMG 102 for pro-HGF and mature HGF, we did immunoprecipitation reactions containing ~1:1 molar ratio of AMG 102 to total HGF. The CHO-derived preparation of HGF contained a mixture of both the mature and pro forms of HGF, which separate on a reducing gel as shown (Fig. 1D, lane 1). Mature HGF was the predominant form immunoprecipitated by AMG 102 under these conditions (lane 2), whereas both forms were immunoprecipitated using a commercial monoclonal anti-HGF antibody MAB294 (lane 3). At higher molar ratios (e.g., 5:1) of AMG 102: HGF, both mature HGF and pro-HGF were immunoprecipitated (data not shown).

We next investigated whether AMG 102 alters proteolytic processing of pro-HGF to mature HGF under conditions of excess AMG 102 (at a 5:1 molar ratio; a 1:1 molar ratio was also tested; data not shown). Pro- and mature forms of HGF were stable throughout the 12-hour incubation period in the absence (Fig. 1E, lanes 1 to 3) and the presence of AMG 102 alone (Fig. 1E, lanes 4 to 6; note the prominent antibody heavy and light chains of AMG 102 in lanes 4 to 5 and 12 to 14). Incubation with kallikrein alone led to a time-dependent conversion of pro-HGF to the mature form that was complete by 12 hours (Fig. 1E, lanes 8, 9, and 11). Incubation with AMG 102 had no apparent impact on the rate or extent of pro-HGF processing catalyzed by kallikrein (Fig. 1E, lanes 12 to 14). Thus, AMG 102 binding to pro-HGF did not seem to enhance or inhibit proteolytic processing of human HGF.

**Functional Specificity of AMG 102**

The ability of AMG 102 to inhibit c-Met autophosphorylation in response to human and cynomolgus monkey HGF was tested in PC3 cells. AMG 102 treatment led to potent and complete inhibition of c-Met phosphorylation induced by either human or cynomolgus monkey HGF, with calculated IC$_{50}$ values of 0.12 nmol/L or 0.24 nmol/L (Fig. 2A and B).

The functional activity of AMG 102 was further studied using Matrigel cell-invasion assays. Treatment of human MDA-MB-435 cells with cynomolgus monkey d5-HGF (50 ng/mL for 18 hours) stimulated their migration through a Matrigel matrix-coated membrane. Incubation with 10 µg/mL AMG 102 completely inhibited monkey HGF–induced migration (Fig. 2C).

Consistent with the binding studies showing lack of interaction between AMG 102 and nonprimate HGF, a migration assay employing mouse 4T1 cells stimulated with rat HGF revealed no inhibition by AMG 102 at concentrations up to 10 µg/mL (Fig. 2D, left). In contrast, migration of these cells was significantly inhibited ($P = 0.003$) by a polyclonal rabbit anti-mouse HGF antibody (Fig. 2D, right).

**Epitope Mapping of AMG 102 Binding Sites**

Although the lack of cross-reactivity of AMG 102 for rodent HGF precluded the use of rodents in safety studies, we took advantage of the species specificity of AMG 102 to probe its binding sites on human HGF. As in our previous studies (24), we employed engineered human/mouse chimeric HGF fusion proteins (Table 1, Supplementary Table S2) and a novel two-color FACS method for these experiments. FACS results are shown in Fig. 3A and are summarized in Table 1. As was shown in our previous experiments, AMG 102 bound to fully human HGF but not to fully mouse HGF (24). AMG 102 also bound to chimeras 1, 2, and 3, which are human HGF molecules containing increasing lengths of the NH$_2$-terminal α-chain of mouse HGF including the N, K1, and K2 domains. These data suggested that AMG 102 was binding predominantly to the β-chain of human HGF. Most significantly, AMG 102 bound to chimera 8, a mouse HGF containing only seven unique human amino acids and a two–amino acid deletion relative to the mouse HGF sequence (Fig. 3A). In contrast, AMG 102 did not bind to chimera 7, a human HGF containing only nine mouse HGF-specific amino acids from an NH$_2$-terminal region of the β-chain. These data suggested that the key binding epitope for AMG 102 comprised sequences including one or more of the indicated residues.

To further refine our analysis, we identified the non-conservative amino acid differences between the human and mouse HGF sequences within the NH$_2$-terminal portion of the HGF β-chain and generated five constructs, each of which contained a point mutation that changed a human residue to a mouse residue (Table 1, Supplementary Table S2, Fig. 3C). Substitutions at position 555 (G555E HGF) or 561 (C561R HGF) completely inhibited binding (Fig. 3A). Substitutions at 592 (D592N HGF), or 647 (R647Q HGF) had little effect on binding between HGF and AMG 102. Insertion of two mouse-specific residues, 540N and 541K (Fig. 3C), which are not present in the human HGF sequence, completely disrupted binding [human NK (insertion) HGF; Table 1, Fig. 3A]. However, deletion of these same two residues from the mouse sequence did not restore AMG 102 binding [mouse NK (deletion) HGF; Table 1, Fig. 3A]. These data identified key residues within the β-chain of human HGF that seemed to be critical for AMG 102 binding.

Finally, to physically map the interaction between AMG 102 and human HGF, we identified trypsin
cleavage sites in HGF that were protected by AMG 102 binding. As the comparison of the two upper HPLC traces in Fig. 3B indicate, AMG 102 binding to human HGF protected two peptide-containing peaks, designated T33 and T38.6. Peak T38.6 contained two peptides with NH2-terminal sequences of VVNGIPTR and GIPTRT corresponding to sequences beginning at residue 495 or 498, respectively, at the NH2-terminus of the β-chain of mature HGF (Fig. 3C). Based on an observed mass of peptides 7165 and 6878 (determined by mass spec), the likely protected trypsin cleavage site is R556 (calculated masses for the two predicted peptides were 7152 and 6840, respectively). The NH2-terminal sequence of T33 (VNTADQ) corresponded to a region within the α-subunit of mature HGF (beginning at residue 65 of the NH2 domain of HGF; Supplementary Fig. S1). The interaction of AMG 102 with the α-subunit of HGF was not confirmed by other methods. However, with the use of a complementary approach to directly capture AMG 102-binding HGF peptides, the same two NH2-terminal β-chain sequences were identified (contained in peak T48; Fig. 3B, bottom). AMG 102-bound HGF was trypsin digested and unbound peptides were separated from bound peptides by filtration. Bound peptides were eluted from AMG 102 with intact HGF and separated by HPLC (Fig. 3B, bottom). NH2-terminal protein sequencing showed that the VVNGIPTR and GIPTRT peptides were contained in peak T48. Three other peaks (*, Fig. 3B, bottom) contained either no peptide or a peptide of unknown origin, unrelated to HGF or AMG 102. Taken together, the epitope mapping data strongly indicated that AMG 102 bound to the NH2-terminus of the β-chain of HGF, specifically including residues from Valine 495 or Glycine 498 to Arginine 556 of human HGF.

Discussion

In the present study, we extended the characterization of fully human anti-HGF–neutralizing monoclonal antibodies described by Burgess et al. (24) by focusing on the most promising candidate for clinical investigation, now called AMG 102 (25). AMG 102 bound to human and cynomolgus monkey d5-HGF with high affinity (Kd = 19 pmol/L and 41 pmol/L, respectively) but did not bind to rodent or rabbit HGF. AMG 102 preferentially bound to the mature, heterodimeric form of human HGF, and binding had no apparent effect on the proteolytic processing that leads to pro-HGF activation. AMG 102 completely inhibited in vitro c-Met autophosphorylation stimulated by human and by cynomolgus monkey HGF in cells with IC50 values of 0.12 nmol/L and 0.24 nmol/L, respectively. AMG 102 inhibited cynomolgus monkey d5-HGF–stimulated migration of human MDA-MB-435 cells through Matrigel but had no such effect on mouse 4T1 cells stimulated with rat HGF. Epitope mapping studies revealed that AMG 102 bound directly to amino acid residues at the NH2-terminus of the human HGF β-chain of human HGF.

The species specificity of AMG 102 binding and activity is reflected in the extent of similarity in amino acid sequence among the HGF molecules. Although the mutational epitope mapping done in this study is not exhaustive, binding of AMG 102 was disrupted by substitution of only one or two amino acids from the human sequence with those from mouse HGF. Furthermore, the changes that disrupted binding of AMG 102 were all within the region of the β-chain defined by the studies employing chimeric molecules. Substitutions outside of this region (592, 601, and 647) did not disrupt AMG 102 binding. Finally, protection from trypsin cleavage and identification of AMG 102-bound human HGF peptides point to a discrete region at the NH2-terminus of mature human HGF β-chain that is both necessary and sufficient for binding of AMG 102 to human HGF.

The functional interaction of HGF and c-Met involves multiple points of physical contact, including high-affinity binding between c-Met and the α-chain of HGF (28, 29) and the more recently described low-affinity, but functionally critical, binding between the β-chain of HGF and the Sema-domain of c-Met (19, 20). AMG 102 seemed to be unique in its ability as a single agent to completely neutralize human HGF binding to c-Met and to fully inhibit in vitro and in vivo functional assays (24); it had been suggested that at least three antibodies with overlapping epitopes are required for full activity (30). More recently, other neutralizing anti-HGF mouse monoclonal antibodies have been described (31); however, characterization of the binding epitope has not been reported. Our studies provide evidence that the fully human monoclonal antibody AMG 102 bound predominantly near the NH2-terminal portion of the β-chain of human HGF. The binding of AMG 102 to this epitope on HGF seems to confer excellent functional neutralizing properties to this clinical candidate.

The lack of binding and functional activity of AMG 102 on mouse, rat, or rabbit HGF precluded the use of these species for preclinical studies, including efficacy and safety evaluation of the antibody. As we have previously reported (24), the efficacy of AMG 102 was shown only in preclinical models that were dependent on human HGF. Kakkar et al. (32) did cross-reactivity studies using fluorescein-conjugated AMG 102 on a wide variety of human and cynomolgus monkey tissues as well as on a smaller panel of mouse, rat, and rabbit tissues. The results were consistent with the species specificity of AMG 102 binding/activity seen in our in vitro experiments. Based on the species specificity shown here and by Kakkar et al., cynomolgus monkeys were deemed to be a pharmacologically relevant species for further pharmacokinetic and safety studies of AMG 102 (32).

The therapeutic consequences of preferential binding of AMG 102 for the active, heterodimeric form of HGF are not known. However, a preexisting store of pro-HGF...
and mature HGF is expected to be present in many tissues and the tumors of cancer patients. It is possible that the apparently higher affinity of AMG 102 for processed HGF would enhance its potential anticancer properties. Stimulating the conversion of pro-HGF to mature, active HGF would be counter to the desired clinical effect, i.e., inhibition of mature, active HGF; therefore, showing that AMG 102 binding to HGF does not enhance proteolytic processing provides yet another positive characteristic of AMG 102.

AMG 102 is a fully human, neutralizing antibody to human and nonhuman primate HGF. It bound HGF with low pmol/L affinity and neutralized cell activity at low nanomolar concentrations. We recently reported data from the AMG 102 first in human/phase I study (Gordon et al in press, Clinical Cancer Research). The concentration of AMG 102 in plasma was in stoichiometric excess compared with circulating HGF levels, even in the lowest-dose group (>100-fold molar excess at 0.5 mg/kg). Epitope mapping showed that AMG 102 bound primarily to mature human HGF via the β-chain. Currently, AMG 102 is in multiple, controlled phase II clinical trials in a variety of solid tumor indications. Treatment regimens include AMG 102 in combination with chemotherapy or other targeted agents in gastric, prostate, colorectal, small cell lung, and other solid tumors.

**Disclosure of Potential Conflicts of Interest**

All authors are current or former employees of and own stock in Amgen, Inc.

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