Preclinical Evaluation of AMG 337, a Highly Selective Small Molecule MET Inhibitor, in Hepatocellular Carcinoma

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Abstract

Aberrant hepatocyte growth factor (HGF)/MET signaling has been implicated in hepatocarcinogenesis, suggesting that MET may serve as an attractive therapeutic target in hepatocellular carcinoma. We sought to investigate the in vitro and in vivo antitumor activity of AMG 337, a potent and highly selective small molecule MET kinase inhibitor, in preclinical models of hepatocellular carcinoma. The antiproliferative activity of AMG 337 was evaluated across a panel of hepatocellular carcinoma cell lines in a viability assay. Daily oral administration was used to evaluate the in vivo antitumor activity of AMG 337 in two patient-derived xenograft (PDX) models of hepatocellular carcinoma (LI0612 and LI1078). AMG 337 exerted potent antiproliferative activity against 2 of 4 hepatocellular carcinoma cell lines, namely, MHCC97H (IC50 0.015 μmol/L) and HCCLM3 (IC50 0.025 μmol/L). Both sensitive cell lines showed MET amplification (MET/CEN-7 >2.0) assessed by FISH, and high MET expression (3+ IHC) assessed by IHC. AMG 337 potently inhibited p-MET in all cell lines with detectable levels of total MET. However, the dose-dependent inhibition of downstream effectors of HGF/MET signaling, including p-GAB1, p-AKT, and p-ERK, was limited to those cell lines sensitive to AMG 337 in a viability assay (MHCC97H and HCCLM3). AMG 337 significantly inhibited tumor growth at all doses tested in the MET-amplified and MET-high–expressing hepatocellular carcinoma PDX model LI0612 and had no effect on tumor growth in the non-MET–amplified and MET-low–expressing hepatocellular carcinoma PDX model LI1078. AMG 337 represents a promising and novel therapeutic strategy for targeting hepatocellular carcinomas with a dependence on HGF/MET signaling. Mol Cancer Ther; 15(6); 1227–37. ©2016 AACR.

Introduction

MET, a receptor for hepatocyte growth factor (HGF) is widely expressed in epithelial tissues, such as liver, lung, gastrointestinal tract, and kidney, both during embryogenesis and throughout adulthood (1). Binding of HGF to the extracellular domain of MET leads to its homodimerization and the autophosphorylation of multiple tyrosine residues within the kinase domain, resulting in subsequent phosphorylation of Y1349 and Y1356 in the carboxy-terminal tail (2, 3). The tyrosine phosphorylation of MET results in its activation and the recruitment of signaling effectors, such as the adaptor proteins Grb2 and Gab-1, which in turn promote the activation of diverse downstream signaling pathways, including the PI3K/AKT, Ras/RAF/MEK/ERK, PLC-γ, FAK, and STAT3 pathways (4). Thereby, HGF-induced MET activation regulates a diverse set of biologic responses that include cell proliferation, survival, migration, invasion, angiogenesis, and branching morphogenesis (5–7).

Abnormal MET signaling has been observed in many types of human cancer, including hepatic, gastric, lung, colorectal, breast, and pancreatic cancers (8–13). Activation of the HGF/MET signaling in cancer can occur via many different mechanisms, such as MET gene amplification, overexpression, mutation, or paracrine and autocrine activation of MET by HGF. Each of these mechanisms has been implicated in the development and progression of numerous cancers, including hepatocellular carcinoma (14–18). Although surgical resection and transplantation have significantly improved the survival of patients with an early diagnosis, hepatocellular carcinoma is still the second leading cause of cancer-related deaths worldwide (19), and hepatocellular carcinoma continues to have a relatively poor prognosis for patients with late-stage disease. Therefore, targeting the HGF/MET signaling axis represents a rational therapeutic strategy for the treatment of hepatocellular carcinoma.

Many strategies have been employed to target abnormal HGF/MET signaling in cancer, including therapeutic antibodies targeting MET and HGF and ATP-competitive small molecule inhibitors of the MET tyrosine kinase. Recently, considerable effort has been focused on the development of selective small molecule MET kinase inhibitors (1, 16, 20). AMG 337, a novel, potent, and highly selective small molecule MET inhibitor, has
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been evaluated in a phase I clinical study in patients with a variety of solid tumors where it showed favorable pharmacokinetics and acceptable tolerability and toxicity profiles (21).

In this study, we have characterized the antiproliferative activity of AMG 337 across a panel of hepatocellular carcinoma cell lines and we report a strong correlation between sensitivity to AMG 337 and MET amplification and protein overexpression. We also demonstrate that the inhibition of downstream effector signaling within the HGF/MET pathway is limited to those cell lines exhibiting sensitivity to AMG 337 in a viability assay. Furthermore, we show that AMG 337 has robust antitumor efficacy in a hepatocellular carcinoma patient–derived tumor xenograft (PDX) model that harbors MET amplification and high MET protein expression. Our results provide insight into the relationship between the status of MET gene and protein expression and the preclinical response to AMG 337, which will help to inform therapeutic strategies in patients with MET-driven hepatocellular carcinoma.

Materials and Methods

AMG 337

For in vitro studies, AMG 337 was prepared in 100% DMSO at room temperature and diluted in relevant growth media. For in vivo studies, AMG 337 was formulated in a solution of 30% hydroxypropyl-β-cyclodextrin (HPβCD; w/v) plus 10% Pluronic F68 (w/v). Animals were given AMG 337 or vehicle control once per day by oral gavage.

Cell lines and cell proliferation assay

Human hepatocellular carcinoma cell lines were purchased from one of five organizations (ATCC in 2008, 2009, and 2012, Japanese Collection of Research Bioresources (JCRB) in 2008, Korean Cell Line Bank (KCLB) in 2010, Shanghai Institutes of Biological Sciences, CAS (SIBS) in 2008, or Zhongshan Hospital Fudan University (ZHFU) in 2009; see Table 1), and all the cell lines have been authenticated using viability, morphology, and genetics analysis on a regular basis, and tested negative for Mycoplasma. The cell lines were cultured in growth media at 37°C, 5% CO₂, and 95% humidity. Cells were seeded at optimized densities in 96-well plates and were allowed to adhere to the bottom of the plates during an overnight incubation. The cells were then treated with AMG 337 [10-step (1:3) serial dilution, starting at 10 μmol/L], and staurosporine was used as the positive control. Cell viability was measured after 72 hours using a CellTiter-Glo Luminescence Cell Viability Assay Kit (Promega). Luminescence was measured with a PerkinElmer EnVision plate reader. The effects on viability were reported as POC (percent of control), which was calculated as follows: POC = (compound-treated signal/DMSO-treated signal) × 100. GraphPad Prism software (version 5) was used to calculate IC₅₀ values from a 4-parameter logistic curve fit of the POC values. Viability measurements for vehicle-treated controls were collected at the time of compound addition (time 0 hour, T0), and these measurements were reported as a broken line on dose–response curves.

SNP 6.0 assay

Among the 40 cell lines, SNP 6.0 data for 27 cell lines were downloaded from the CCLE project (http://www.broadinstitute.org/ccle/home). Data for the remaining 13 cell lines (BEL7402, HCC-9810, HCCLCM3, MHCC97H, NOZ, OCUG-1, OZ, QGY7701, QGY7703, SMMC7721, SNU354, SNU368, and SNU370) were produced using the Affymetrix Genome-Wide Human SNP Array 6.0. All raw data were processed with the PICNIC software program (https://www.adherewebdesign.com/picnic-software-website/) and are presented as the number of MET copies.

ELISA

Hepatocellular carcinoma cell lines were lysed in cold lysis buffer (Sigma) containing a phosphatase inhibitor cocktail (Sigma), and the lysates were centrifuged at 12,000 rpm for 10 minutes at 4°C to remove cell debris. The levels of total-MET and phospho-MET in the cell protein extracts were determined using corresponding ELISA kits according to the manufacturer’s instructions (total-MET ELISA, R&D Systems DYC358-5; and phospho-MET ELISA, R&D Systems DYC2480-5). All the data presented were normalized to the total amounts of protein present in the extracts.

Western blot analysis

Six hepatocellular carcinoma cell lines, including MHCC97H, HCCLCM3, JHH-5, JHH-4, Hep3B, and SNU398, were selected for Western blot signaling analysis. Cell lines were treated with different concentrations of AMG 337 or DMSO (control) for 2 hours at 37°C. The cells were washed with PBS and lysed using cell lysis buffer (Sigma, C2978). Cell lysates were collected and then centrifuged at 13,000 rpm for 20 minutes at 4°C to remove cell debris. The supernatant proteins were quantified using a BCA protein assay, normalized for protein content, and subsequently mixed with 4X LDS (Lithium dodecyl sulfate) sample buffer plus reducing agent. Lysates were then boiled at 70°C for 10 minutes. The lysates were centrifuged briefly and then loaded on 4% to 15% SDS–PAGE gels. Proteins were transferred onto polyvinylidene difluoride membranes using the Bio-Rad Trans-Blot System.
The membranes were blocked with 5% milk plus 0.1% Tween in TBS at room temperature for 1 hour. The membranes were then incubated with primary antibodies diluted in blocking buffer at 4°C overnight. After washing, the membranes were incubated with RDIY 800/680 anti-rabbit/anti-mouse antibody (LI-COR Biosciences) diluted in blocking buffer (1:5,000) or an horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (1:2,000) for 1 hour at room temperature. The bands were detected with the Odyssey System (LI-COR Biosciences). The following antibodies were purchased from Cell Signaling Technology: anti-p-MET Y1234/1235 (3077S), anti-MET (3148S), anti-p-Gab1 Y627 (3231S), anti-Gab1 (3232S), anti-p-ERK Thr202/Tyr204 (4370S), anti-ERK (9107S), anti-p-AKT Ser473 (4060S), and anti-AKT (2920S).

**AlphaScreen assay**

Cell lysates from six hepatocellular carcinoma cell lines were prepared following the protocol described above for Western blot analysis. The cell lysates were then adjusted to contain the same total protein concentration based on the results of a pilot experiment. Four microliters of each sample were then transferred to a 384-well Proxiplate for the SureFire AlphaScreen assay. Positive and negative controls were supplied in the AlphaScreen kits, and lysis buffer alone was used as a blank control. A total of 5 μL of acceptor mix was then added to each well, and the samples were incubated for 2 hours at 22°C. Subsequently, 2 μL of donor mix was added to each well, and the samples were incubated overnight at 22°C. The plate was then read on an Envision plate reader using standard AlphaScreen settings. Triplicate samples were tested for each treatment condition. The relative MET expression was calculated by subtracting the background control signal and was referred to as the Net AlphaScreen Signal.

**MET FISH**

MET gene amplification was analyzed by FISH using the Dako MET/CEN-7 I QISH Probe Mix (RUO). The CEN-7 centromere probe was used as a reference control according to the manufacturer's instructions. Formalin-fixed, paraffin-embedded specimens (FFPE, cell pellets or PDX models) were prepared for sections, and the sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin. Heat pretreatment was then performed using a pretreatment solution in a microwave oven for 3 minutes 50 seconds, and the samples were then performed using a pretreatment solution in a microwave oven for 3 minutes 50 seconds, and the samples were then incubated with primary antibodies diluted in blocking buffer at 4°C overnight. After washing, the membranes were incubated with RDIY 800/680 anti-rabbit/anti-mouse antibody (LI-COR Biosciences) diluted in blocking buffer (1:5,000) or an horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (1:2,000) for 1 hour at room temperature. The bands were detected with the Odyssey System (LI-COR Biosciences). The following antibodies were purchased from Cell Signaling Technology: anti-p-MET Y1234/1235 (3077S), anti-MET (3148S), anti-p-Gab1 Y627 (3231S), anti-Gab1 (3232S), anti-p-ERK Thr202/Tyr204 (4370S), anti-ERK (9107S), anti-p-AKT Ser473 (4060S), and anti-AKT (2920S).

**MET IHC**

MET protein levels were characterized by IHC in a subset of hepatocellular carcinoma cell lines and both PDX models. First, the FFPE slides were dewaxed, hydrated, and washed with water. For MET detection by the Dako MET IHC (RUO) assay on an automatic immunostainer (Dako), antigen retrieval of FFPE slides was performed at 97°C for 20 minutes in EDTA buffer (pH 8.5–9.5), and the slides were then cooled at 65°C and rinsed for 5 minutes. The 4-μm sections were then rinsed, incubated with endogenous peroxidase–blocking reagent for 5 minutes, and rinsed in TBST (Tris-buffered saline with Tween 20). The slides were then labeled with a primary antibody against human-MET (Dako). After washing, the HRP-labeled polymer and diaminobenzidine (DAB) substrate were added, and the samples were counterstained using an EnVision Hematoxylin Kit (Dako), dehydrated, and sealed. The MET IHC staining intensity was evaluated by experienced pathologists using the following scoring criteria: 0, any tumor cells with membrane staining at intensity of no staining; 1+, weak staining; 2+, moderate staining; or 3+, strong staining; High MET expression was defined as the presence of any tumor cells with membrane staining at IHC 3+ intensity.

**In vivo xenograft study**

Two hepatocellular carcinoma PDX mouse models were established by subcutaneously injecting female BALB/c nude mice with human primary hepatocellular carcinoma tumor tissues (2–4 mm in diameter/mouse). These mice (8–10 weeks old) were purchased from the Shanghai Laboratory Animal Center. All animal experiments were performed according to the guidelines approved by the Institutional Animal Care and Use Committee. Treatment was initiated when the average tumor size reached approximately 180 mm³. Mice were randomly allocated to four experimental groups (12 animals per group) according to their tumor sizes.

Mice were dosed with vehicle (30% HBPCD and 10% Pluronic F68) or AMG 337 at doses of 3, 10, or 30 mg/kg once per day by oral gavage for 14 days. Tumor size and body weight were measured twice weekly. The length (L), width (W), and height (H) of tumors were measured with a caliper. Tumor volume was calculated ($0.5 \times L \times W \times H$) and was expressed in mm³. The percentage of tumor growth inhibition was calculated to evaluate the antitumor efficacy as follows: %TGI = 100% – (VTi – VTO)/(VCI – VCO) × 100%; where VTi is the mean tumor size in the treatment group on day i; VTO is the mean tumor volume in the treatment group on day 0; VCI is the mean tumor size in the vehicle group on day i; and VCO is the mean tumor size in the vehicle group on day 0.

The data are expressed as the means ± SEs and are plotted as a function of time. The statistical significance of the observed differences between the growth curves of treatment groups and the control group was evaluated by repeated measures analysis of covariance of the log-transformed tumor volume data with Dunnett-adjusted multiple comparisons. An adjusted value of $P < 0.05$ was considered to be statistically significant. The analysis was done using SAS procedures mixed with model effects of the baseline log tumor volume, day, treatment, and day-by-treatment interaction with a Toeplitz covariance structure.
PDX tumors were harvested for protein extraction after 14 days of AMG 337 treatment. Five tumors per treatment group, including vehicle control, were lysed and 50 mg of each protein extract was subjected to Western blot analysis.

**Results**

*In vitro* efficacy of AMG 337 is correlated with dysregulated MET signaling

AMG 337 is a potent, highly selective, ATP-competitive small-molecule inhibitor of MET (21). To characterize the activity of AMG 337 in the hepatocellular carcinoma setting, we performed an *in vitro* antiproliferation screen across a panel of 40 hepatocellular carcinoma cell lines. The neoplastic activity of cancer cells was assessed after 72 hours of treatment with AMG 337, and the IC50 values were calculated (Table 1). Of the 40 hepatocellular carcinoma cell lines examined, only two cell lines (MHCC97H and HCCLM3) showed sensitivity to AMG 337 (IC50 values of 0.015 µmol/L and 0.025 µmol/L, respectively; Fig. 1).

*MET* gene copy number and protein expression across the 40 hepatocellular carcinoma cell line panel were analyzed by a SNP 6.0 array and ELISA assay, respectively. Both of the AMG 337-sensitive cell lines, MHCC97H and HCCLM3, had elevated *MET* gene copy number (Table 2) and higher protein expression compared with the other tumor cell lines displaying lack of response to AMG 337 (IC50 > 10 µmol/L; Fig. 2). These data suggest that the inhibitory activity of AMG 337 correlates with dysregulation of the MET pathway, and that this pathway plays a dominant role in the proliferation of these two tumor cell lines.

*MET* gene amplification and high protein expression in hepatocellular carcinoma cell lines predict for sensitivity to AMG 337

We next investigated biomarkers that could help identify tumors sensitive to MET inhibition. FISH analysis and IHC staining were used to confirm the presence of *MET* amplification and high MET protein expression. Two sensitive cell lines (MHCC97H and HCCLM3) and four insensitive cell lines (JHH-4, JHH-5, SNU398, and Hep3B) were selected for the analyses (Fig. 3). Both sensitive cell lines had high levels of total MET expression with strong membrane staining (IHC 3+) and *MET* gene amplification (*MET/CEN-7* > 2.0). These cell lines were deemed *MET* positive by IHC. The other cell lines had low MET protein expression (IHC 2+) and a normal *MET* copy number (*MET/CEN-7* ≤ 2.0). These cell lines were considered to be *MET* negative by IHC. Notably, hepatocellular carcinoma cell lines with either moderate expression of total MET (IHC 3+) or low to no expression of total MET (IHC 1+ or 0) failed to exhibit sensitivity to AMG 337. These findings demonstrated a significant and favorable response to AMG 337 in the *MET*-amplified and *MET*-positive hepatocellular carcinoma lines, thus highlighting
the relevance of MET gene copy number and protein expression to AMG 337 response.

**AMG 337 inhibits MET phosphorylation and downstream pathway signaling**

To examine the effects of AMG 337 on MET signaling in vitro, the six hepatocellular carcinoma cell lines previously described for MET copy number and protein expression analysis were treated with a dose titration of AMG 337. Cell lysates were prepared and subjected to Western blot analysis to detect changes in the levels of total and phosphorylated MET as well as downstream signaling nodes, including GAB1, ERK1/2, and AKT. AMG 337 potently inhibited p-MET in all tumor cell lines with detectable levels of total MET (Fig. 4A). However, dose-dependent inhibition of p-

![Graph](image)

**Table 2.** MET gene copy number in the 40 hepatocellular carcinoma cell lines as determined by SNP 6.0 array

<table>
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<th>Cell line</th>
<th>Average genome ploidy</th>
<th>MET copy number</th>
<th>MET amplification</th>
<th>AMG 337 IC50 efficacy</th>
<th>Cell line</th>
<th>Average genome ploidy</th>
<th>MET copy number</th>
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*NOTE: MET gene copy number and average genome ploidy were analyzed by the Affymetrix SNP 6.0 array in 40 hepatocellular carcinoma cell lines, and the data were processed using PICNIC software. Gray boxes indicate the AMG 337-responsive cell lines.*

![Graph](image)

**Figure 2.** The phospho/total MET protein levels in the 39 hepatocellular carcinoma cell lines as determined by ELISA. A, the total and phospho-MET protein levels were measured in the cell lysates by an ELISA assay. The protein expression (ng) per total protein (mg) is shown for the indicated cell lines. The boxes indicate the AMG 337-sensitive cell lines. Total MET protein expression level of the SNU398 cell line is not shown because the MET concentration was below the lowest level of the standard working range.
GAB1, p-AKT, and p-ERK was limited to those cell lines with MET amplification and high MET protein expression (MHCC97H and HCCLM3), highlighting a key association between AMG 337 sensitivity and inhibition of downstream signaling in MET-amplified and high-MET expressing cell lines.

Consistent with the Western blot findings, inhibition of MET signaling was also observed when using the AlphaScreen assay to profile lysates generated from cells treated with a dose titration of AMG 337 (2 hours). AMG 337 treatment resulted in a dose-dependent reduction in p-ERK in the AMG 337–sensitive cell lines.
These results suggest that the antiproliferative activity of AMG 337 in the MET-amplified (MET/CEN-7 ratio > 2.0 as determined by FISH) hepatocellular carcinoma cell lines was mediated via the inhibition of MET kinase activity and subsequent inhibition of downstream signaling.

To explore the incidence of MET gene amplification and protein overexpression in hepatocellular carcinoma tumors, 26 Chinese hepatocellular carcinoma PDX samples were profiled by FISH and IHC analysis, two widely used clinical platforms for detecting MET copy number and protein expression. This analysis revealed a MET gene amplification rate of 8% (2/26; MET/CEN-7 > 2.0; Table 3). Furthermore, 23% of the PDX samples exhibited strong membranous MET staining (IHC 3+; Table 4), whereas the remaining 77% of samples were negative, low, or moderate for MET staining (IHC 0, 1+, or 2+). The association of MET amplification with elevated MET protein expression was also analyzed (Table 5). Notably, 100% (2/2) of the MET-amplified PDX samples were positive for elevated MET expression (IHC 3+). Intriguingly, four samples stained high for MET expression (IHC 3+) but had normal MET copy number. The data indicate that although the majority of tumors with MET amplification show high MET expression, there are exceptions where high MET expression is not associated with MET amplification.

Table 3. The MET gene copy numbers in the 26 Chinese hepatocellular carcinoma PDX samples were determined by FISH

<table>
<thead>
<tr>
<th>Patient classification for FISH</th>
<th>Patient samples, n (%)</th>
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<td>Normal</td>
<td>24 (92)</td>
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<tr>
<td>Gene amplification</td>
<td>2 (8)</td>
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<tr>
<td>Total</td>
<td>26 (100)</td>
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NOTE: MET/CEN-7 > 2.0 was defined as a normal MET copy number. MET/CEN-7 > 2.0 was defined as MET gene amplification.
expression, the cases with high MET expression can also be found to be MET nonamplification.

AMG 337 inhibits tumor growth in a MET-amplified and MET-high–expressing hepatocellular carcinoma PDX model

To determine the effects of AMG 337 on tumor growth in vivo, we performed two in vivo efficacy studies in primary patient-derived hepatocellular carcinoma xenograft models (LI0612 and LI1078). The MET copy number and protein expression were characterized for these models using FISH and IHC assays, respectively. LI0612 exhibited MET amplification [MET/CEN-7 > 2 (6.39)] and high MET protein expression (IHC 3+), whereas LI1078 showed no evidence of MET amplification (MET/CEN-7 < 2.0) or MET overexpression (IHC 1+; Fig. 5A).

Mice with subcutaneously established tumor tissues were randomly grouped and treated with vehicle or AMG 337 at doses of 3, 10, or 30 mg/kg once daily by oral administration for 14 days. In the LI0612 model, AMG 337 significantly inhibited tumor growth compared with vehicle, exhibiting tumor growth inhibition (%TGI) values of 101.13% (P = 0.0003), 114.59% (P < 0.0001), and 120.35% (P < 0.0001) at doses of 3, 10, and 30 mg/kg, respectively (Fig. 5B). In addition, 10 and 30 mg/kg of AMG 337 led to significant tumor regression (Fig. 5B). These results indicated that AMG 337 acted in a dose-dependent manner in the LI0612 model. For the LI1078 model, AMG 337 had no significant inhibitory effect on tumor growth at any of the doses tested (%TGI values of 0.96%, 11.68%, and 16.87% at doses of 3, 10, and 30 mg/kg, respectively; Fig. 5B). No significant changes in body weight were observed following the administration of AMG 337 at any of the dose levels in either model, and AMG 337 was well tolerated in the antitumor study, which was indicative of acceptable tolerability and toxicity. Thus, AMG 337 demonstrated dramatic efficacy in the MET-amplified and MET-high–expressing PDX model (LI0612), but failed to exhibit efficacy in the non–MET-amplified and MET-low–expressing PDX model (LI1078).

We next examined the effects of AMG 337 on MET signaling in the sensitive LI0612 mouse model (Supplementary Fig. S1). Tumors were harvested after 14 days of treatment with vehicle or AMG 337 at doses of 3, 10, or 30 mg/kg. The phosphorylation of MET and a downstream signaling effector, GAB1, were dramatically inhibited in the AMG 337-treated groups compared with vehicle control.

Discussion

Hepatocellular carcinoma, in adult men, is the fifth most frequently diagnosed cancer worldwide and is the second leading cause of cancer-related death in the world (19). The expression of MET has been demonstrated during liver regeneration, hepatocyte survival, and tissue repair (22, 23). In addition, the HGF/MET signaling pathway has been shown to be activated during the genesis and progression of hepatocellular carcinoma. Many reports have highlighted the prevalence of MET gene and protein expression in hepatocellular carcinoma, and have investigated the role of MET signaling in hepatocellular carcinoma progression and maintenance (24–28). The importance of MET signaling in these processes suggests that the status of MET gene amplification and/or protein expression in patients with hepatocellular carcinoma may serve as potential biomarkers for prognosis or response to anti-HGF/MET therapy. Much work is underway to investigate these two endpoints and their predictive capacity in treating hepatocellular carcinoma.

Multiple therapies targeting the MET/HGF axis have been investigated in several different settings. Preclinical studies have shown that both antibodies and small molecules targeting MET or HGF, such as onartuzumab, rilotumumab, INC 280, and veltinib, are effective agents for treating MET-dependent tumors. Here we describe the activity of AMG 337 in preclinical models of hepatocellular carcinoma. AMG 337 is a novel and highly selective small molecule kinase inhibitor of MET. A phase I study of AMG 337 in patients with advanced solid tumors evaluated the safety, tolerability, pharmacokinetics, and efficacy of AMG 337 monotherapy (NCT01253707). The study showed that AMG 337 had robust pharmacokinetics and a favorable toxicity profile, with clinical activity in MET-amplified gastroesophageal junction, gastric, or esophageal cancer (21).

In the current study, we characterized MET gene copy number and protein expression levels in a panel of 40 hepatocellular carcinoma cell lines and found that the in vitro antiproliferative activity of AMG 337 was limited to two cell lines with aberrant HGF/MET signaling. Furthermore, the antitumor efficacy of AMG 337 can be attributed to the inhibition of MET signaling, and correlates with MET amplification and overexpression. An in vivo study indicated that AMG 337 significantly inhibited the growth of a MET-amplified and high-MET expressing primary PDX model of hepatocellular carcinoma. Thus, our data emphasized the robust efficacy of AMG 337, a novel MET kinase inhibitor focused on the hepatocellular carcinoma treatment. This study provides the first evidence of the translational significance of alterations in MET for determining the sensitivity to AMG 337 in hepatocellular carcinoma.

Many studies have reported on the association between alterations in MET and poor prognosis and/or disease severity in human cancers (25–28). These findings support the hypothesis that specific alterations in MET may provide predictive capacity for identifying patients most likely to benefit from MET inhibitor therapy. In this study, we employed FISH and IHC to characterize the MET gene copy numbers and quantify MET protein levels in a panel of hepatocellular carcinoma cell lines and PDX models. Both approaches utilized routine technologies and commercially
Overexpression (IHC 2+) of MET was used to assess MET in inhibitory effects on MET-dependent tumor cell lines and studies. One such inhibitor, INC280, has demonstrated growth in vivo in mouse models of glioblastoma tumors. Our data indicate that AMG 337 is a promising candidate for the treatment of hepatocellular carcinomas exhibiting MET gene amplification (FISH ratio > 2.0) and high MET expression.

Additional highly selective MET kinase inhibitors are currently being evaluated in preclinical and early-stage clinical studies. One such inhibitor, INC280, has demonstrated growth inhibitory effects on MET-dependent tumor cell lines and suppressed tumor growth in vivo in mouse models of glioblastoma and gastric tumors (29, 30). A phase 1 clinical trial of INC280 showed preliminary antitumor efficacy in 50% of patients with EGFR wild-type non–small cell lung carcinoma with MET FISH ratio ≥ 2.0 or MET CN ≥ 5, or 50% of tumor cells with a staining intensity of IHC 2+ or 3+ for MET (31). Volitinib is another highly selective small molecule inhibitor of MET. Preclinical studies showed that MET-amplified and MET-overexpressing tumor xenograft models were highly responsive to volitinib as a single agent or in combination with other therapies (32–35). Here, FISH analysis was applied to assess MET amplification (MET/CEN-7 ratio ≥ 2) and IHC (SP44 Ventana Medical System, Roche) was used to assess MET overexpression [IHC 2+ or 3+ (≥10% of tumor cells showing membrane or cytoplasmic staining with moderate or strong intensity)]. MET-FISH or MET-IHC positivity was considered as a predictive marker for response to therapy and determined patient selection criteria (32–35). In our study, the tumor cells with extremely high MET expression (IHC 3+), such as MHCC97H and HCCLM3, could respond to AMG 337, whereas those with the mid-level of MET expression (IHC 2+), such as JHH-4 did not. So based on our preclinical results, we suggested that the patients with MET-driven hepatocellular carcinoma with MET amplification plus high protein expression (IHC 3+) might be the right enrollment subpopulation who could respond to MET-targeted therapy.

In vitro experiment, although AMG 337 can inhibit the phosphorylation of MET in JHH-4 cell line which showed the moderate level of MET expression (IHC 2+), the activation of downstream signaling effectors, such as the phosphorylation of ERK and AKT were not suppressed (Fig. 4). The results indicated a key association between AMG 337 sensitivity and inhibition of downstream signaling in MET-amplified and high-MET expressing cell lines.

Taken together, the current data demonstrate that AMG 337, a highly selective MET kinase inhibitor, exhibits potent antitumor effects against a subset of MET-driven hepatocellular carcinoma cell lines and primary human hepatocellular carcinoma tumors. Our data indicate that AMG 337 is a promising candidate for the treatment of hepatocellular carcinomas exhibiting MET gene amplification (FISH ratio > 2.0) and high MET expression.
protein expression (IHC 3+). These patient stratification biomarkers may be further investigated to help screen MET-driven hepatocellular carcinoma and predict clinical efficacy to MET-targeted therapy.

Disclosure of Potential Conflicts of Interest

S. Caenepeel has ownership interest in Amgen Inc. stock. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

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