LY2875358, a Neutralizing and Internalizing Anti-MET Bivalent Antibody, Inhibits HGF-Dependent and HGF-Independent MET Activation and Tumor Growth

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Abstract

**Purpose:** MET, the receptor for hepatocyte growth factor (HGF), has been implicated in driving tumor proliferation and metastasis. High MET expression is correlated with poor prognosis in multiple cancers. Activation of MET can be induced either by HGF-independent mechanisms such as gene amplification, specific genetic mutations, and transcriptional upregulation or by HGF-dependent autocrine or paracrine mechanisms.

**Experimental Design/Results:** Here, we report on LY2875358, a novel humanized bivalent anti-MET antibody that has high neutralization and internalization activities, resulting in inhibition of both HGF-dependent and HGF-independent MET pathway activation and tumor growth. In contrast to other bivalent MET antibodies, LY2875358 exhibits no functional agonist activity and does not stimulate biologic activities such as cell proliferation, scattering, invasion, tubulogenesis, or apoptosis protection in various HGF-responsive cells and no evidence of inducing proliferation in vivo in a monkey toxicity study. LY2875358 blocks HGF binding to MET and HGF-induced MET phosphorylation and cell proliferation. In contrast to the humanized one-armed 5D5 anti-MET antibody, LY2875358 induces internalization and degradation of MET that inhibits cell proliferation and tumor growth in models where MET is constitutively activated. Moreover, LY2875358 has potent antitumor activity in both HGF-dependent and HGF-independent (MET-amplified) xenograft tumor models. Together, these findings indicate that the mechanism of action of LY2875358 is different from that of the one-armed MET antibody.

**Conclusions:** LY2875358 may provide a promising therapeutic strategy for patients whose tumors are driven by both HGF-dependent and HGF-independent MET activation. LY2875358 is currently being investigated in multiple clinical studies. Clin Cancer Res; 20(23); 6059–70. ©2014 AACR.
Translational Relevance

Dysregulation of the MET/hepatocyte growth factor (HGF) pathway has been implicated in a spectrum of tumor biology including proliferation, metastasis, and resistance to therapy, resulting in worse outcomes for patients. MET can be activated either by HGF-dependent autocrine or paracrine mechanisms or by HGF-independent mechanisms such as gene amplification, mutations, or transcriptional upregulation. Efforts to inhibit HGF-independent MET activation with bivalent MET antibodies have been limited by their tendency to induce agonist activity. Here, we report that the bivalent MET antibody LY2875358 is able to inhibit both HGF-independent and HGF-dependent MET activation without induction of functional agonist activity, as characterized by multiple functional assays that represent a wide range of biologic activities that can be induced by HGF. These preclinical data support the clinical evaluation of LY2875358 in patients with tumors driven by both HGF-dependent and HGF-independent MET activation.

Materials and Methods

Antibody generation and purification

Mice were immunized with recombinant human MET extracellular domain fused to human Fc (R&D Systems 358-MT), and antigen-specific spleen cells from serum-positive mice were harvested. Phage-expressed Fab libraries were prepared from spleen cell lysates and screened by the filter lift method (24). A subset of antigen-specific Fabs was converted to full-length mouse IgG1 and further characterized. Select mAbs were humanized and affinity matured. The high-affinity variant LY2875358 was converted to human IgG4 and further characterized.

LY2875358 was expressed in Chinese hamster ovary (CHO) cells. Hybridoma HB-11895 expressing mlgG1 5D5 anti-human MET bivalent antibody was obtained from ATCC and antibodies were purified by protein A affinity chromatography and size exclusion chromatography. Humanized one-armed 5D5 (huOA-5D5) MET antibody was constructed on the basis of publicly available information (see ref. 25 for example) and was expressed in CHO cells.

HepG2 cell invasion assay

HepG2 cells were starved overnight in MEM and then added in serum-free MEM to the top chamber of a Matrigel invasion chamber with the bottom chamber containing antibody treatments in serum-free medium followed by 48-hour incubation. Noninvading cells were removed from the top chamber with a swab. Chamber membrane was fixed with 95% ethanol, stained with 0.2% crystal violet, and invading cells were counted from a 2.5× microscopy field.

Primary human hepatocytes agonist proliferation assay

Primary human hepatocytes (PHH) were added at 3.5 × 10^4 cells per well in 96-well collagen I–coated plates and cultured for 24 hours in InVitroGRO CP medium (Celsis). MET antibody treatments were added in InVitroGRO HI medium (Celsis) + 0.1% BSA and incubated for 48 hours during which cells were pulsed with 1 μCi [3H]-thymidine for the last 6 hours.

FACS analysis of cell surface MET

Cells were treated overnight with a final concentration of 33 nmol/L LY2875358 or 67 nmol/L huOA-5D5 and dissociated with enzyme-free dissociation solution. For LoVo cells, 50 nmol/L antibody treatments were used. Dissociated cells were labeled for 1 hour with 2 μg/mL of a Alexa Fluor 488–labeled Lilly proprietary MET detection antibody (which binds a separate epitope than LY2875358 or huOA-5D5). Nearly 10,000 events were acquired by FACS for each sample.

In vivo mouse studies

All in vivo mouse experimental protocols were approved by the Eli Lilly and Company Institutional Animal Care and Use Committee or Oncotest GmbH. For the xenograft models (U-87 MG, MKN45, EBC-1, and SNU-5), 5 × 10^6 cells in PBS, mixed 1:1 with Matrigel (BD Biosciences), were implanted subcutaneously into the flank of athymic nude female mice obtained from Harlan. When average tumor volume reached 100 to 150 mm^3, animals were randomized.
to treatment groups according to tumor volume and body weight. LY2875358, huOA-5D5, and hIgG4 were diluted in sterile PBS before dosing via intravenous injection on a once weekly schedule. 5-Fluorouracil (5-FU; Sigma) was formulated in PBS before administration via intraperitoneal injection on a once weekly schedule. Cisplatin (Teva Pharmaceutical Industries) was diluted in saline before administration via intraperitoneal injection on a once weekly schedule. Tumor volume and body weight were measured biweekly as described previously (26). The statistical analysis of tumor volume data has also been previously described (27). Methods of implantation for the patient-derived xenograft NSCLC model LXFA-1647 have been described elsewhere (28). For the LXFA-1647 study conducted at Oncotest GmbH, animals were randomized into groups and treated with LY2875358 or hulgG4 once weekly via intravenous injection when average tumor volume reached a designated size range. Data from Affymetrix Genome Wide SNP6.0 provided by Oncotest showed that this PDX model carried 6 copies of MET to chromosome 7 copy was 3.

Measurement of levels of MET and pMET in the MKN45 xenograft tumors in response to treatment with LY2875358

MKN45 cells (5 × 10⁶ cells in PBS, mixed 1:1 with Matrigel) were implanted subcutaneously into the flank of athymic nude mice. When tumors reached an average size of 100 to 125 mm³, the animals were randomized into treatment groups. LY2875358 was administered intravenously at 2.5, 10, or 20 mg/kg and human IgG4 was administered intravenously at 20 mg/kg. Whole blood was collected from the treated animals via mandibular bleeding 30 minutes post dose and serum (~50 μL) was prepared. Animals were sacrificed using CO₂ and cervical dislocation at 24 (for 10 mg/kg dose group only), 72, 168, or 336 hours post dose. Xenograft tumors were collected; half of each tumor was frozen for preparation of tumor lysates for ELISA analyses and the other half was fixed in 10% neutral-buffered formalin for immunohistochemical (IHC) analyses. Whole blood was collected via cardiac puncture and serum was prepared for determination of LY2875358 concentrations. Total MET and pMET were measured by IHC staining. The method and reagents were described previously (29). Total MET and pMET were also analyzed by Mesoscale Discovery (MSD) ELISA from tumor lysates as previously described (27). For the determination of total MET and pMET levels, the Total MET Whole Cell Lysate Kit (K151DMD) and Phospho-MET Whole Cell Lysate Kit from MSD were used, respectively.

Monkey toxicology repeat-dose study

LY2875358 was administered to cynomolgus monkeys (n = 3/sex/group + 3/group/sex in control and high dose for recovery) weekly by intravenous bolus injection for 5 weeks at dose levels of 0, 0.3, 7, and 180 mg/kg. Animals were evaluated 1 week after the last dose or were monitored for an additional 16 weeks for delayed effects. Endpoints monitored included toxicokinetics and antidrug antibody levels; standard toxicology and safety pharmacology endpoints; total MET levels in kidney, skin, and liver; urine biomarkers of kidney injury [neutrophil gelatinase-associated lipocalin (NGAL), microalbumin, and clusterin]; and markers of cellular proliferation (Ki67) in liver, kidney, and spleen. This in vivo toxicity study was approved by the Charles River Institutional Animal Care and Use Committee.

Results

In vitro and in vivo MET/HGF neutralization properties of LY2875358

LY2875358 is a humanized IgG4 mAb that binds to human and cynomolgus monkey MET ECD-Fc with Kᵩ < 0.2 nmol/L on the basis of measurements using Biacore analysis (Supplementary Fig. S1). Because of the slow off-rate of the interaction, the binding affinity could not be accurately measured with Biacore so an upper limit was estimated. An orthogonal approach, KinEXA analysis, was used to accurately determine the affinity. This analysis demonstrated that LY2875358 binds to human MET ECD-Fc with a Kᵩ of 0.8 pmol/L [95% confidence interval (CI), 0.4–1.4 pmol/L]. LY2875358 does not bind to mouse or rat MET ECD-Fc. Hydrogen–deuterium exchange mass spectrometry (HDXMS) and diethyl pyrocarbonate (DEPC) labeling analyses were used to identify the epitope for LY2875358. Several regions of MET located in the Sema domain were shown to be protected upon LY2875358 binding. These regions are residues 123D–128D, 144H–156S, 192F–195F, and 220K–227M and are shown graphically in Supplementary Fig. S2. These regions are conserved functionally in Supplementary Fig. S3, and this was comparable with the huOA-5D5, which has previously been shown to functionally neutralize HGF (32). Second, LY2875358 was shown to inhibit HGF-induced proliferation of H596, an NSCLC cell line (Fig. 1A). LY2875358 was characterized in 2 assays. First, LY2875358 was shown to inhibit HGF-stimulated proliferation of H596, an NSCLC cell line (Fig. 1A). LY2875358 neutralization of HGF-induced proliferation was comparable with that of the huOA-5D5, which has previously been shown to functionally neutralize HGF (32). Second, LY2875358 was shown to inhibit HGF-induced motility of H441 cells (Supplementary Fig. S3), and this was comparable with that of the huOA-5D5. To determine whether LY2875358 inhibition of HGF-dependent MET activity observed in vitro can also be observed in vivo, LY2875358 was tested in the U87MG tumor xenograft model. U87MG glioblastoma cells express both MET receptor and HGF, constituting an autocrine loop, and this tumor line is a well-established HGF-dependent model (33). LY2875358 (10 mg/kg) demonstrated a significant inhibition (P < 0.001) of tumor growth (Fig. 1B), similar to huOA-5D5 (6.7 mg/kg) when dosed at the same molar equivalent.
LY2875358 has no functional agonist activities

One major challenge in developing therapeutic anti-MET antibodies is that most anti-MET antibodies have medium-to-strong agonist activity, triggering a variety of activities that are known to be induced by HGF (21–23, 32). Thus, whether LY2875358 would trigger MET receptor signaling and related biologic activities was systematically examined. As HGF is a pleiotropic factor and elicits bioactivity in different cell types, the agonist activity of LY2875358 was evaluated in 7 different assays: phosphorylation of MET and pan-AKT (pMET and pAKT), cell motility, invasion, proliferation, survival, and tubulogenesis. The cells used for these assays are further detailed in Supplementary Table S1. In all these assays, LY2875358 was compared with HGF and bivalent 5D5 as positive controls of agonist activity. Bivalent 5D5 antibody is a previously reported mouse IgG1 antibody against MET with agonist activity (23). The results of these assays are shown in Fig. 2 and in Supplementary Figs. S4–S7.

DU145 prostate tumor cells were treated with LY2875358, HGF, or bivalent 5D5 and levels of pMET and pAKT were quantified by ELISA. LY2875358 elicited a weaker and more transient phosphorylation of MET than HGF and bivalent 5D5 (Fig. 2A). More importantly, the pMET signal induced by LY2875358 did not reach the threshold necessary to translate to an increase of pAKT in DU145 cells (Fig. 2B), although it did stimulate a weak and transient pAKT signal in H441, HepG2, and Caki-1 cells (Supplementary Fig. S4). However, LY2875358 does not trigger any biologic activities in these HGF-responsive cells. LY2875358 treatment did not elicit scattering of DU145 cells, whereas cell scattering was clearly detected for bivalent 5D5 and HGF treatment (Fig. 2C). The effect of LY2875358 on cell motility was evaluated in a scratch assay using H441 NSCLC cells. LY2875358 did not induce the motility of H441 cells as compared with medium or IgG isotype control. Under the same experimental conditions, HGF and bivalent 5D5 stimulated cell migration into the gap by 339% and 265%, respectively (Fig. 2D).

An in vitro assay measuring the ability of HepG2 cells to migrate through a Matrigel-coated Transwell membrane was used to evaluate the ability of LY2875358 to stimulate invasiveness. Under the same conditions, HGF and the bivalent 5D5 antibody, but not LY2875358, stimulated HepG2 cell invasion (Fig. 2E). The mitogenic activity of LY2875358 was also evaluated in human primary hepatocytes. Hepatocytes were shown to proliferate in response to HGF treatment (34, 35). Both 200 ng/mL of HGF and 20 nmol/L (or higher) bivalent 5D5 stimulated a 5-fold increase of 3H-thymidine uptake in primary human hepatocytes (Fig. 2F). In contrast, LY2875358 was unable to induce proliferation even at a high concentration of 67 nmol/L. LY2875358 also did not show mitogenic activity in renal carcinoma Caki-1 cells (Supplementary Fig. S5) or morphogenic activity in HepG2 cells in Matrigel (Supplementary Fig. S6). HGF and bivalent 5D5 were able to reduce apoptosis induced by staurosporin in renal carcinoma Caki-1 cells, but not LY2875358 (Supplementary Fig. S7).

Evaluation of LY2875358 in monkey

The cynomolgus monkey (Macaca fascicularis) is a relevant species to assess the toxicity of LY2875358 because the epitope for LY2875358 in MET is conserved in humans and monkeys, and it binds with similar affinity to both human and cynomolgus MET. The toxicity of LY2875358 was assessed after weekly intravenous bolus doses for 5 weeks...
in cynomolgus monkeys. In this study, the no-observed adverse effect level (NOAEL) was the highest dose tested, 180 mg/kg. At this dose level, measured levels of LY2875358 (C\text{max} exceeding 7 mg/mL) were far in excess of any potential interference by endogenous MET ECD or anti-drug antibodies (36). Treatment-related findings consisted of effects on thyroid morphology and heart rate. Nonadverse dose-related thyroid follicular dilation, accompanied by increased thyroid weight and gross observations of thyroid enlargement, were observed in male and female monkeys. In a separate tissue cross-reactivity study, LY2875358 bound to thyroid epithelium in monkeys but not in humans (data not shown). Cardiovascular effects consisted of mild decreases in heart rate (11%–17%) for up to 4 hours after dose administration, accompanied by a physiologically normal and expected increase in the QT interval. No increase in cellular proliferation (Ki67) was detected in liver, kidney, or spleen of animals treated with LY2875358 compared with vehicle control–treated animals. Except as described in this section or Supplementary Table S2, there were no LY2875358-related effects on any tissue or endpoint examined.

**Internalization and degradation of MET receptor by LY2875358**

Amplification of the MET gene that leads to protein overexpression and constitutive kinase activation has been reported in several human cancers, including gastric, esophageal, and NSCLC. High MET gene amplification correlates to strong staining (3+) of MET protein by IHC as well as poor prognosis in gastric cancer (8, 10, 14, 37). LY2875358 was investigated for its ability to induce internalization and degradation of MET using multiple methods. First, LY2875358-induced MET internalization and degradation was measured using colocalization of MET and lysosomes with confocal microscopy. Using the MET-amplified gastric line MKN45, we observed that overnight LY2875358 treatment at 37°C resulted in decreased cell surface MET staining.

![Figure 2. Effect of LY2875358 on induction of MET and pan-AKT phosphorylation (A and B), cell scattering (C), motility (D), invasion (E), and proliferation (F) in HGF-responsive cells. All data are shown as mean ± SE unless otherwise noted. A and B, starved DU145 cells were treated with antibodies at 5 μg/mL or HGF at 50 ng/mL for the indicated durations and lysed. pMET and pAKT were measured by ELISA. Results are shown as the mean ± SE and are pooled from 2 independent experiments, each with duplicate treatments. C, DU145 cells were treated with LY2875358 (20 μg/mL), hIgG4 (20 μg/mL), bivalent 5D5 (5 μg/mL), or HGF (20 ng/mL) for 48 hours and then measured for the percentage of objects classified as colonies. Results are pooled from 2 independent experiments, each with 6 or 12 replicate treatments. D, the motility of starved H441 cells in a scratched cell monolayer was monitored after 16-hour treatment with 10 μg/mL of LY2875358 or bivalent SDS or 200 ng/mL HGF. Results are pooled from 2 independent experiments, each with duplicate treatments. Data shown are representative of 3 independent experiments for the untreated, LY2875358, hIgG4, and HGF treatment groups. E, the invasion of HepG2 cells through a Matrigel-covered membrane was measured after a 48-hour incubation with 10 μg/mL LY2875358 or bivalent SDS or 50 ng/mL HGF in the bottom chamber. Results are pooled from 2 independent experiments, each with duplicate or quadruplicate treatments. F, the proliferation of PHH was measured by 3H-thymidine uptake after 48-hour treatment with antibody dose titrations or 200 ng/mL HGF. Results are shown as the mean of triplicate treatments ± SD and are representative of 3 independent experiments. ^\text{*}, \text{P < 0.001}, \text{**}, \text{P < 0.0001}, antibody versus isotype control or HGF versus untreated. C, DU145 cells were treated with LY2875358 (20 μg/mL), hIgG4 (20 μg/mL), bivalent 5D5 (5 μg/mL), or HGF (20 ng/mL) for 48 hours and then measured for the percentage of objects classified as colonies. Results are pooled from 2 independent experiments, each with 6 or 12 replicate treatments. D, the motility of starved H441 cells in a scratched cell monolayer was monitored after 16-hour treatment with 10 μg/mL of LY2875358 or bivalent SDS or 200 ng/mL HGF. Results are pooled from 2 independent experiments, each with duplicate treatments. Data shown are representative of 3 independent experiments for the untreated, LY2875358, hIgG4, and HGF treatment groups. E, the invasion of HepG2 cells through a Matrigel-covered membrane was measured after a 48-hour incubation with 10 μg/mL LY2875358 or bivalent SDS or 50 ng/mL HGF in the bottom chamber. Results are pooled from 2 independent experiments, each with duplicate or quadruplicate treatments. F, the proliferation of PHH was measured by 3H-thymidine uptake after 48-hour treatment with antibody dose titrations or 200 ng/mL HGF. Results are shown as the mean of triplicate treatments ± SD and are representative of 3 independent experiments. ^\text{*}, \text{P < 0.001}, \text{**}, \text{P < 0.0001}, antibody versus isotype control or HGF versus untreated.
with the concomitant formation of intracellular granules that colocalized with lysosome staining (Fig. 3A). In addition, treatment with the lysosome inhibitor leupeptin (38) resulted in enhanced MET accumulation in the lysosome upon LY2875358 treatment, suggesting that lysosomal degradation may be part of the mechanism for LY2875358-induced MET reduction (Supplementary Fig. S8). Second, FACS analysis confirmed that LY2875358 induced cell surface MET depletion as evidenced by a 50% decrease of cell surface MET after overnight LY2875358 treatment in MKN45 cells (Fig. 3B). Under the same experimental conditions, huOA-SD5 did not reduce cell surface MET. This degradation is unlikely due to HGF, as we observed no HGF expression in MKN45 as measured by qPCR and by ELISA of the conditioned media (data not shown). The lack of HGF expression in MKN45 is consistent with one report (39) but conflicts with another report (40). This discord among reports may be due to different sources of the MKN45 cells. Depletion of cell surface MET by LY2875358 treatment was observed in a variety of cells bearing MET mutations, including MET kinase domain mutations (NIH-3T3 cells engineered to carry M1149T MET mutation; ref. 6), MET juxtamembrane mutations (H1437 with MET mutation R988C; ref. 41), and a posttranslationally unprocessed single-chain form of 190 kD MET (LoVo cells; ref. 42; Fig. 3B). Third, Western blot analysis of MET-amplified (MKN45, SNU-5, and EBC-1) and nonamplified (H596) cell line lysates showed that LY2875358, but not

Figure 3. Effect of LY2875358 on MET receptor internalization, degradation, and downstream signaling. A, MKN45 cells were treated with LY2875358 or hlgG4 (5 μg/mL) overnight at 37°C; LysoTracker Deep Red (75 nmol/L) was added for the last 2 hours. Cells were fixed, permeabilized, and stained with a Lilly proprietary MET detection antibody followed by Alexa 488-labeled anti-mouse IgG and viewed using confocal microscopy. B, MKN45, LoVo, H1437, or NIH3T3 cells bearing MET kinase mutation M1149T were treated overnight at 37°C with 33 nmol/L LY2875358, 67 nmol/L humanized one-armed 5D5 (huOA-SD5), or 33 nmol/L hlgG4 isotype control (LoVo cells were treated with 50 nmol/L antibodies), stained with Alexa 488-labeled Lilly proprietary MET detection antibody that recognizes a separate MET epitope from LY2875358 and huOA-SD5, and measured by FACS for remaining cell surface MET. Results are shown as the mean ± SE and are pooled from 3 independent experiments. ∗, P < 0.01, LY2875358 versus hlgG4. C, Western blot analysis of MKN45 total and pMET, pAKT, and pMAPK1/3 (pErk1/2) after overnight treatment with LY2875358 (100 nmol/L), huOA-SD5 (200 nmol/L), or an IgG4 isotype control antibody (100 nmol/L). D, Western blot analysis of total MET and pMET after overnight treatment with LY2875358 (100 nmol/L), huOA-SD5 (100 nmol/L), PF-04217903 (SMI; 250 nmol/L), or HGF (50 ng/mL).
LY2875358, Anti-MET Bivalent Antibody with Antitumor Effect

Figure 4. Effect of LY2875358 on proliferation (A) and apoptosis (B) of cancer cell lines with MET gene amplification. A, MKN45, SNU-5, or EBC-1 cells were treated with LY2875358, humanized one-armed 5D5 (huOA-5D5), or hlgG4 isotype control for 5 days (MKN45, EBC-1) or 6 days (SNU-5), and cell proliferation/survival was measured using CellTiter Glo. Results are shown as the mean ± SE and are pooled from 3 independent experiments, each with duplicate or triplicate treatments. B, MKN45 and SNU-5 cells were treated with 100 nmol/L LY2875358, 200 nmol/L huOA-5D5, or 100 nmol/L hlgG4 isotype control for 96 hours. EBC-1 cells were treated with 100 or 0.5 nmol/L LY2875358, 200 or 1 nmol/L huOA-5D5, or 100 or 0.5 nmol/L hlgG4 isotype control for 96 hours. Apoptosis was measured with caspase-3/7 activity induced by treatment normalized to cell confluence or total objects. Results are shown as the mean ± SE and are pooled from 3 independent experiments. * P < 0.0001, compared with hlgG4.

Inhibition of proliferation of tumor cell lines with MET amplification

LY2875358 not only induced MET degradation and a decrease of pAKT and pMAPK1/3 (pErk1/2), but also inhibited proliferation of MKN45 tumor cells (Fig. 4A). Further exploration of the mechanism of action demonstrated that LY2875358 induced MKN45 cells to undergo apoptosis (Fig. 4B). To corroborate the finding that LY2875358 could inhibit proliferation of tumor cells with MET gene amplification, data were generated for 2 additional MET-amplified gastric and NSCLC cell lines (SNU-5 and EBC-1; refs. 39, 43) treated with LY2875358. Similar to MKN45, MET was constitutively phosphorylated in these cell lines in the absence of HGF. LY2875358 treatment also significantly inhibited the proliferation and induced apoptosis of these MET-amplified cell lines (Fig. 4A and B). However, under the same experimental conditions, huOA-5D5 and the Fab fragment of LY2875358 did not inhibit proliferation of these MET-amplified gastric and NSCLC tumor lines (Fig. 4 and Supplementary Fig. S9). These findings suggest that LY2875358-induced MET internalization and degradation translate to antiproliferation and induction of apoptosis in tumor cells in vitro. Unlike DN-30 MET antibody (44), LY2875358 does not...
alter shedding of MET extracellular domain in culture medium (data not shown).

**Extent and duration of downregulation of MET receptor in vivo with LY2875358 treatment**

To characterize the pharmacodynamics of LY2875358, the magnitude and durability of MET and pMET reduction with LY2875358 treatment were evaluated in the MKN45 xenograft tumor model. Qualitative evaluation of total MET and pMET by IHC labeling revealed that LY2875358 treatment at 10 or 20 mg/kg decreased both proteins by 72 hours posttreatment and that this decrease persisted to 14 days after LY2875358 single-dose administration. A representative IHC image at 10 mg/kg dose of LY2875358 is shown in Fig. 5A. The levels of tumor-associated MET and pMET were quantified more precisely by ELISA assays. Compared with the IgG4-treated group, LY2875358 reduced MET and pMET in the tumors by approximately 50% at both the 10 and 20 mg/kg doses by 72 hours post dose, and the reductions persisted to 14 days (Fig. 5B). LY2875358 treatment at 2.5 mg/kg did not significantly reduce total MET or pMET in the tumor as compared with the IgG4-treated mice at any time point. There was a dose-dependent increase in blood levels of LY2875358 at 2.5, 10, and 20 mg/kg doses (Fig. 5C). The data suggest that in this MKN45 xenograft model, the maximum pharmacodynamic effect of LY2875358 (reduction of MET and pMET) was achieved at around the 10 mg/kg dose. Despite of higher blood levels of LY2875358 using a 20 mg/kg dose, no significant further reduction of MET and pMET was observed as compared with the 10 mg/kg dose of LY2875358.
In vivo antitumor activity of LY2875358 in gastric and NSCLC xenograft models

To determine whether the HGF-independent antiproliferative activity of LY2875358 observed in vitro is translatable to in vivo, LY2875358 was evaluated in several different xenograft models. Treatment with LY2875358 at 10 mg/kg once weekly resulted in a marked reduction in tumor growth in the MKN45 gastric xenograft tumors (Fig. 6A), consistent with in vitro cell proliferation results. Furthermore, combination of LY2875358 with gastric cancer standard-of-care chemotherapeutic agents cisplatin or 5-FU further enhanced antitumor effects compared with LY2875358 alone (Fig. 6B). The in vitro antiproliferative effect of LY2875358 on SNU-5 and EBC-1 was also translated to marked antitumor effect in xenograft tumors derived from SNU-5 and EBC-1 (Fig. 6A) with tumor regression or stasis observed, respectively. In addition, LY2875358 treatment also resulted in tumor regression in a MET-amplified patient tumor–derived xenograft lung cancer model, LXFA-1647 (refs. 28, 29; Fig. 6A).

Discussion

Numerous studies have implicated the MET signaling pathway as important for tumor progression and metastasis; targeting MET/HGF may offer a promising therapeutic strategy for treatment of MET-expressing cancers. We report here the generation and characterization of a novel, humanized, bivalent anti-MET IgG4 antibody, LY2875358. This antibody binds to human MET with high affinity (0.8 pmol/L), with similar affinity to monkey MET, but does not bind to murine MET. LY2875358 blocks HGF from activating MET both in vitro and in vivo and also induces MET receptor internalization and degradation. LY2875358 treatment resulted in decreased cell surface MET, total MET, pMET, and inhibition of cell proliferation in MET-amplified
MKN45, SNU-5, and EBC-1 tumor cells; however, HGF and huOA-5D5 did not induce MET degradation under the same conditions. In non–MET-amplified H596 cells, LY2875358 treatment only slightly decreased total MET, similar to HGF. These data suggest that the internalization and degradation dynamics may differ from one cell type to the other. We observed that bivalent binding was required for LY2875358-induced depletion of cell surface MET. Fab generated from LY2875358 retained the ability to block HGF binding to MET ECD-Fc but was unable to induce depletion of cell surface MET or inhibit HGF-independent cell proliferation. Interestingly, LY2875358-induced MET degradation did not require MET tyrosine kinase activity as cotreatment with MET kinase inhibitor PF-04217903 did not abrogate this activity. Furthermore, we demonstrated that LY2875358 treatment resulted in MET colocalization to the lysosome, suggesting a mechanism for LY2875358-induced MET degradation.

Other previously reported efforts of bivalent MET antibody development were largely unsuccessful because the antibodies tended to have agonistic rather than antagonistic properties (21–23). A battery of in vitro bioassays was used to characterize the agonist properties of LY2875358 using HGF and agonist bivalent MET antibody 5D5 as positive controls. Although LY2875358 induction of weak and transient phosphorylation of MET and pan-AKT was observed, it did not stimulate biologic activity in 7 functional MET agonist assays. The observed weak and transient phosphorylation of MET and pan-AKT was likely due to LY2875358-mediated MET dimerization. LY2875358 binding to MET overlaps the HGFβ-binding site in blades 2 and 3 of the Sema domain 7-propeller blade motif. In contrast, 5D5 binds to propeller blades 4, 5, and 6 of the Sema domain and overlaps with the HGFα-binding site (45); the bivalent form of 5D5 induces a wide range of functional MET agonist activity. This epitope difference may account for the different functional agonist properties of LY2875358 and bivalent 5D5. It has been demonstrated that bivalent MET antibodies with different epitopes can elicit different levels of agonist activity (21, 46). Our data are consistent with these reported findings and suggest that the unique MET epitope of LY2875358 is responsible for its ability to inhibit HGF binding to MET and to induce MET internalization and degradation but results in only a low and transient level of MET activation without stimulating functional biologic activities. During preparation of this article, Basilico and colleagues (46) published an article detailing a panel of MET antibodies with antagonism and agonism of MET phosphorylation; however, these results did not necessarily result in corresponding antagonism and agonism in a functional biologic assay of cell scattering. This phenomenon reflects the possibility that other factors in addition to receptor tyrosine phosphorylation contribute to functional biologic activity, including receptor internalization dynamics, signal duration, and differential activation of signaling pathways.

Another bivalent MET antibody, CE-355621, was recently described as blocking HGF-dependent activation of MET; however, functional agonist assay results were not shown for CE-355621 (47). Here, the agonist properties of LY2875358 were investigated in 7 functional assays and 5 signaling assays. To fully characterize the agonist properties of MET antibodies, previously published data indicate that it is important to use assays that reflect the wide range of pleiotropic biologic activities on multiple cell types induced by HGF (21, 48). A MET antibody with partial functional agonist activity may have a different safety profile than an antibody lacking any functional agonist activity. In addition to the in vitro agonist analysis, we demonstrated that in a 5-week toxicity study in cynomolgus monkey that the NOAEL for LY2875358 was 180 mg/kg, the highest dose tested. Non-human primate toxicity of CE-355621 was not reported by Michaud and colleagues, and CE-355621 does not appear to be in clinical development, as it is not registered at ClinicalTrials.gov.

In this study, we also demonstrated that the ability of LY2875358 to internalize and degrade MET receptors translates to in vivo tumor cell line–derived xenograft tumors in mice. A single dose of LY2875358 via intravenous injection at 10 mg/kg resulted in downregulation of MET receptor in the MKN45 model out to 14 days. At a dose of 10 mg/kg once weekly, LY2875358 showed significant antitumor effect in in vivo models of HGF-dependent U87MG xenograft tumors and HGF-independent gastric (MKN45 and SNU-5) and NSCLC xenograft tumors (EBC-1). As a single agent, LY2875358 showed tumor regression in the SNU-5 xenograft model. The addition of standard-of-care chemotherapeutic agent 5-FU or cisplatin with LY2875358 resulted in further antitumor effects in the MKN45 gastric xenograft model than LY2875358 alone. LY2875358 also showed potent antitumor effects in a MET-amplified, NSCLC patient tumor–derived xenograft model (LXFA-1647).

These preclinical data suggest that LY2875358 may be a promising therapeutic to inhibit tumor growth driven by elevated HGF as well as by constitutive activation of MET through overexpression, gene amplification, or genetic mutation. LY2875358 has completed a phase I dose-escalation study in patients with advanced solid tumors (49) and is currently being evaluated in phase II studies in combination with erlotinib in patients with NSCLC (ClinicalTrials.gov NCT01909652, NCT01897480). These studies have an accompanying biomarker program to evaluate several tumor biomarkers, including MET expression, to inform the future development and tailoring strategy for this compound. 

Disclosure of Potential Conflicts of Interest

Authors’ Contributions
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Liu, W. Zeng, M.A. Wortinger, J.R. Stephens, J. Xiu, K.M. Credille, P. Brown-Augburger, C.-K. Chow, L. Huang, I. Denning, P. Vaillancourt.


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S.B. Yan, J.W. Tetreault, J.R. Manro, I. Denning.

Study supervision: S.B. Yan, V. Wachcek, Y. Tang, P. Vaillancourt.

Other (mapping the epitope by HDXMS): L. Huang

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LY2875358, a Neutralizing and Internalizing Anti-MET Bivalent Antibody, Inhibits HGF-Dependent and HGF-Independent MET Activation and Tumor Growth

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