

# LY2801653 is an orally bioavailable multi-kinase inhibitor with potent activity against MET, MST1R, and other oncoproteins, and displays anti-tumor activities in mouse xenograft models

S. Betty Yan · Victoria L. Peek · Rose Ajamie · Sean G. Buchanan · Jeremy R. Graff · Steven A. Heidler · Yu-Hua Hui · Karen L. Huss · Bruce W. Konicek · Jason R. Manro · Chuan Shih · Julie A. Stewart · Trent R. Stewart · Stephanie L. Stout · Mark T. Uhlik · Suzane L. Um · Yong Wang · Wenjuan Wu · Lei Yan · Wei J. Yang · Boyu Zhong · Richard A. Walgren

Received: 19 October 2012 / Accepted: 3 December 2012 / Published online: 29 December 2012  
© The Author(s) 2012. This article is published with open access at Springerlink.com

**Summary** The HGF/MET signaling pathway regulates a wide variety of normal cellular functions that can be subverted to support neoplasia, including cell proliferation, survival, apoptosis, scattering and motility, invasion, and angiogenesis. MET over-expression (with or without gene amplification), aberrant autocrine or paracrine ligand production, and mis-sense MET mutations are mechanisms that lead to activation of the MET pathway in tumors and are associated with poor prognostic outcome. We report here preclinical development

**Electronic supplementary material** The online version of this article (doi:10.1007/s10637-012-9912-9) contains supplementary material, which is available to authorized users.

Study funded by Eli Lilly and Company

All gene names in this manuscript used the HUGO gene naming nomenclature (<http://www.genenames.org>)

S. B. Yan (✉) · V. L. Peek · R. Ajamie · S. G. Buchanan · J. R. Graff · S. A. Heidler · Y.-H. Hui · K. L. Huss · B. W. Konicek · J. R. Manro · J. A. Stewart · T. R. Stewart · S. L. Stout · M. T. Uhlik · S. L. Um · Y. Wang · W. Wu · L. Yan · R. A. Walgren  
Lilly Research Laboratories, Eli Lilly and Company, DC0522, 307 E. McCarty Street, Indianapolis, IN 46285, USA  
e-mail: s.betty\_yan@lilly.com

C. Shih · B. Zhong  
Formerly at Eli Lilly and Company, currently with Crown Biosciences Inc, Jiangsu, China

W. J. Yang  
Formerly at Eli Lilly and Company, currently with Johnson & Johnson, Jiangsu, China

of a potent, orally bioavailable, small-molecule inhibitor LY2801653 targeting MET kinase. LY2801653 is a type-II ATP competitive, slow-off inhibitor of MET tyrosine kinase with a dissociation constant ( $K_i$ ) of 2 nM, a pharmacodynamic residence time ( $K_{off}$ ) of 0.00132 min<sup>-1</sup> and  $t_{1/2}$  of 525 min. LY2801653 demonstrated in vitro effects on MET pathway-dependent cell scattering and cell proliferation; in vivo anti-tumor effects in MET amplified (MKN45), MET autocrine (U-87MG, and KP4) and MET over-expressed (H441) xenograft models; and in vivo vessel normalization effects. LY2801653 also maintained potency against 13 MET variants, each bearing a single-point mutation. In subsequent nonclinical characterization, LY2801653 was found to have potent activity against several other receptor tyrosine oncokinasases including MST1R, FLT3, AXL, MERTK, TEK, ROS1, DDR1/2 and against the serine/threonine kinases MKNK1/2. The potential value of MET and other inhibited targets within a number of malignancies (such as colon, bile ducts, and lung) is discussed. LY2801653 is currently in phase 1 clinical testing in patients with advanced cancer (trial I3O-MC-JSBA, NCT01285037).

**Keywords** MET inhibitor · LY2801653 · MST1R · MKNK · ROS1

## Introduction

The human *MET* proto-oncogene encodes the MET kinase, also known as HGF receptor. HGF binds with high affinity

to this trans-membrane tyrosine kinase receptor and is the only known ligand for this receptor. Binding of HGF to the extracellular domain of MET induces receptor multimerization, activates the intrinsic kinase activity of the receptor and results in the phosphorylation of multiple tyrosine residues in the intracellular region. The HGF/MET signaling pathway regulates a wide variety of normal cellular functions that can be subverted to support neoplasia including cell proliferation, survival, apoptosis, scattering and motility, invasion, and angiogenesis [1]. Dysregulated MET/HGF signaling leads to an abnormally activated cellular invasive program that plays a role in cellular transformation; epithelial-mesenchymal transition; and tumor invasion, progression and metastasis. MET over-expression (with or without gene amplification), aberrant autocrine or paracrine ligand production, and missense MET mutations are mechanisms that lead to activation of the MET pathway in tumors and are associated with poor prognostic outcome [2]. Over-expression of MET, ligand-dependent activation, or MET amplification have also been implicated as potential mechanisms of resistance to epidermal growth factor receptor (EGFR) inhibitor therapies [3–6]. Receptor cross-activation of other oncoproteins such as MST1R (also known as RON), AXL and PDGFRA by MET has also been reported [7, 8]. We report the discovery and initial in vitro and in vivo evaluation of a small-molecule inhibitor LY2801653, whose development was initiated with the intent of targeting the MET kinase. We provide data to illustrate the in vitro effects of LY2801653 on the MET pathway-dependent cell scattering and cell proliferation, as well as its in vivo anti-tumor effects in mouse xenograft models. In subsequent nonclinical characterization, LY2801653 was screened against a larger panel of kinases and was found to have potent activity against several other receptor tyrosine oncokines including MST1R (MET related tyrosine kinase), FLT3, AXL, MERTK, TEK, and ROS1, and against the serine/threonine kinases MKNK1/2. The potential value of MET and other inhibited targets within a number of malignancies is discussed. LY2801653 is currently in phase 1 clinical testing in patients with advanced cancer (trial I3O-MC-JSBA, NCT01285037).

## Materials and methods

### Materials

The cell lines U-87MG, H441, H1299, MV4-11, HT29, H460, TT, Calu1, U118MG, A375, HCT-116, DU145, T47D and H1993 were obtained from ATCC (Manassas, VA). S114 cells were licensed from National Institute of Health; HCC78 and BaF3 cells were from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany); MKN45 cells from

the Japanese Health Science Research Resources Bank (Osaka, Japan); A2780 cells from NCI DCTD repository, and the KP4 cells from RIKEN Cell Bank (Tsukuba, Japan); HUVECs were obtained from Invitrogen (Madison, WI). Cells were cultured according to manufacturers' guidelines.

### Chemical synthesis of LY2801653

The synthesis of LY2801653 is described in Example 1 of the patent [9].

### MET inhibition enzyme kinetics studies

The dissociation constant ( $K_i$ ) value, mode of inhibition (competitive, noncompetitive or uncompetitive) and the pharmacodynamic residence time ( $K_{off}$ ) value of LY2801653 for the MET kinase activity were determined using radiometric-filter binding and spin column assays. Methods for these assays are described in detail in the [Supplementary Methods](#) section.

### Co-crystallization of LY2801653 with MET and crystal structure determination

MET kinase domain (amino acid boundaries 1056–1364) was co-expressed with protein-tyrosine phosphatase 1B (PTP1B) and purified by Kinasia (Carmel, IN) similarly to previous work by Wang et al. [10]. MET protein was purified by nickel affinity and MonoQ chromatography and concentrated to 8 mg/mL in 20 mM MES (pH 6.0), 500 mM NaCl, 10 % glycerol, and 2 mM DTT. The buffer was adjusted to 10 mM HEPES (pH 7.0), 500 mM NaCl, 5 % glycerol, 5 mM DTT, and 0.2 mM n-dodecyl- $\beta$ -D-maltoside for crystallization with 10 mg/mL MET incubated with 1 mM LY2801653 (1 % DMSO). Crystals were grown by hanging drop vapor diffusion at 20 °C, with reservoir solution of 16 % PEG 10,000, 0.1 M HEPES (pH 7.0), 5 % ethylene glycol, and optimized by microseeding. Crystals were flash frozen in liquid nitrogen with 20 % glycerol. Crystals of MET/LY2801653 belong to space group P212121 with unit cell parameters  $a=40.51$  Å,  $b=63.89$  Å,  $c=111.63$  Å. The diffraction data (1.8 Å, 99.7 % complete) were collected and processed on SGX-CAT beam line at APS in Argonne National Laboratory. The crystal structure was determined by the method of molecular replacement using 1 internal MET structure as a search model. The Flynn program (OpenEye Scientific Software) was used for ligand fitting and Coot [11] was used in model building. Structure refinement ( $R_{work}=0.216$  and  $R_{free}=0.249$ ,  $RMS_{bond}=0.009$ ,  $RMS_{angle}=1.218$ ) was carried out by Refmac5 [12]. Protein Data Bank accession code for the co-crystal structure is 4EEV.

Cell-based assays to determine the inhibition ( $IC_{50}$ ) of MET auto-phosphorylation by LY2801653

Inhibition of auto-phosphorylation of MET by LY2801653 was assessed in two cell lines by ELISA. One measured the HGF stimulated MET auto-phosphorylation of Y1349 in H460 cells. Another measured the MET auto-phosphorylation at Y1230/Y1234/Y1235 in NIH3T3-derived S114 cells engineered to stably express human MET and HGF [13]. For details, see the [Supplementary Method](#) section.

In vitro inhibitory activity of LY2801653 on MET-activating mutations

Translocated promoter region-MET (TPR-MET) oncogene [14] was cloned into the pLNCX2 vector (Clontech Laboratories, Inc., Mountain View, CA) and various reported MET mutations in hereditary papillary renal cell carcinoma were introduced using the QuikChange™ Site-Directed Mutagenesis Kit (Agilent, Santa Clara, CA). BaF3 cells are an immortalized murine bone marrow-derived pro-B-cell line, the proliferation of which depends on the presence of IL3. These cells were transduced with retrovirus containing each of the TPR-MET mutation-bearing constructs as previously described [15]. BaF3 cell lines expressing these MET mutations were confirmed with bidirectional DNA sequencing, using 3730xl DNA analyzers (Applied Biosystems, Foster City, CA). Cells were maintained in RPMI 1640 media + 10 % dialyzed FBS (Invitrogen # 26400-044) and antibiotic/antimycotic (Invitrogen # 15240-067).  $4.5 \times 10^3$  cells (-IL3) or  $1.8 \times 10^3$  cells (+IL3) (10 ng/mL, R&D Systems, # 403-ml) were plated onto 96-well plates, treated with LY2801653, and incubated for 48 h at 37 °C. WST1 reagent (Roche, #11644807001) was added and incubated for 2 h; absorbance was read at OD<sub>450</sub>. The  $IC_{50}$  values were determined with the JOCKYSS program.

In vitro biochemical kinase specificity profiling of LY2801653

The specificity of the inhibitory activity of LY2801653 was determined using single-point or 3-point profiling and confirmatory 10-point or 11-point  $IC_{50}$  assays available at EMD Millipore Inc. (Dundee, UK), Cerep (Poitiers, France), or KinomeScan (DiscoverX, San Diego, CA). These assays used active full-length or receptor tyrosine kinase domain enzymes, and peptide or protein substrates unique to each enzyme. Reactions were run at or near the  $K_m$  for ATP to allow for direct comparisons of  $IC_{50}$  potency between the enzymes. For details, see the [Supplementary Method](#) section.

In vitro HGF induced cell scattering assay

$2 \times 10^3$  DU145 cells/well on poly-D-lysine 96-well black/clear plates were treated with LY2801653 (in 0.4 % DMSO), immediately followed by the addition of human HGF (20 ng/ml), and incubated for 48 h at 37 °C. 2 % formaldehyde fixed cells were stained with AlexaFluor 488 Phalloidin (#A12379, Invitrogen, Molecular Probes, Carlsbad, CA) and counterstained with Propidium Iodide (# P3566 Invitrogen). Colony counts were quantified on Acumen Explorer™ laser-scanning fluorescence microplate cytometer (TTP Labtech Ltd, Cambridge, MA). A colony was defined as  $\geq 4$  cells.

Cell-based assays to determine the inhibition ( $IC_{50}$ ) of non-MET kinases by LY2801653

Cell-based assays (anti-proliferative or inhibition of phosphorylation of target kinase) were performed to evaluate the inhibitory activity of LY2801653 against MST1R, AXL, ROS1, PDGFRA, MKNK1/2, FLT3, MERTK, TYRO3, TEK, KDR, CSF1R, RET, DDR1, DDR2. for details, see the [Supplementary Method](#) section.

In vivo mouse studies

All animal studies were performed in accordance with American Association for Laboratory Animal Care institutional guidelines. All in vivo experimental protocols were approved by the Eli Lilly and Company Animal Care and Use Committee. Athymic nude mice and CD-1 nude mice were obtained from Harlan (Indianapolis, IN) and Charles River (Wilmington, MA), respectively. In all in vivo studies, LY2801653 was formulated in 10 % acacia daily and dosed orally. The in vivo mouse studies explored the MET target inhibition and pharmacokinetics of LY2801653 in the S114 xenograft model; as well as the antitumor growth activity in xenograft (U-87MG, KP4, H441, MKN45, H1993, HT-29, and MV4-11) and in orthotopic (H1299) models. For details, see the [Supplementary Method](#) section.

Multiplexed immunofluorescence tumor tissue imaging

U-87MG xenograft tumors were excised from mice after 28 days of dosing and placed into Zinc-Tris fixative (BD Pharmingen, San Jose, CA), stained and quantified as previously described [16]. For the apoptotic and proliferative markers, slides were stained with a combination of Hoechst 33324 (Invitrogen), rat anti-mouse CD31 (Pharmingen) /anti-rat Alexa-488 (Invitrogen), rabbit anti-Ki67 (NeoMarkers) /anti-rabbit Alexa 647 (Invitrogen), and TUNEL-TMR red (Roche). For the angiogenesis markers, slides were stained with a combination of Hoechst 33324, rat anti-mouse CD31/

anti-rat Alexa-488, rabbit anti-glucose transporter 1 (GLUT1) (Chemicon) /anti-rabbit Alexa 647, and mouse anti-Smooth Muscle Actin/Cy3 (Sigma).

## Results

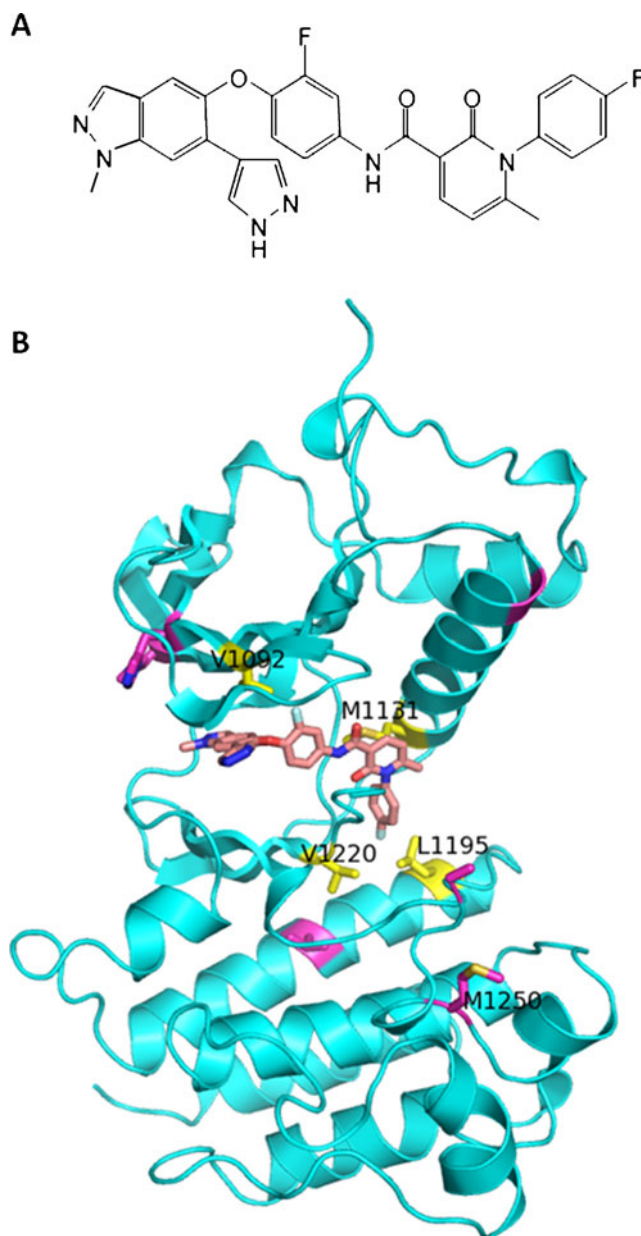
LY2801653, structure shown in Fig. 1a, was designed to target the MET kinase. During the development, the kinase specificity profile of LY2801653 was evaluated against a panel of 100 kinases and LY2801653 was determined to be both a MET and MST1R inhibitor, with very weak activity against KDR. Later, LY2801653 was more broadly screened against a panel of over 400 kinases and the additional kinase activities identified are reported at the end of the Results section.

### In vitro MET inhibitory activities

LY2801653 was evaluated for its ability to inhibit biochemical and cellular functions of MET including cell scattering/migration and proliferation. Biochemical assays using purified human MET kinase domain and a synthetic peptide substrate demonstrated LY2801653 to be a potent, competitive, slow-off inhibitor of MET tyrosine kinase (Supplementary Fig. S1) with a dissociation constant ( $K_i$ ) of 2 nM, a pharmacodynamic residence time ( $K_{off}$ ) of  $0.00132 \text{ min}^{-1}$  and a half-life ( $t_{1/2}$ ) of 525 min. The crystal structure of MET bound to LY2801653 was determined to 1.8 Å (Fig. 1b) and demonstrated that the inhibitor bound MET is in an inactive form with the activation loop DFG motif adopting a DFG-out conformation indicating that LY2801653 is a type II kinase inhibitor [17, 18].

The mean  $IC_{50}$  value ( $n=6$  determinations) of LY2801653 for inhibition of MET auto-phosphorylation in HGF-stimulated H460 cells was  $35.2 \pm 6.9 \text{ nM}$  and the  $IC_{50}$  for MET auto-phosphorylation in S114 cells was 59.2 nM. The activity of LY2801653 was also evaluated in BaF3 cells stably expressing 1 of 13 different MET-activating mutations (Table 1). Transfection with the MET variants conferred growth-factor independence and treatment with LY2801653 inhibited growth of these MET variant clones with an  $IC_{50}$  ranging from 3-fold more potent (V1092I) to approximately 6-fold less potent (L1195V) compared with the growth inhibition of cells with the MET wild-type sequence.

In vitro, LY2801653 potently blocked HGF-induced DU-145 cell scattering (Fig. 2). Among the untreated controls, approximately 300 colonies were detected. With HGF treatment scattering was induced, and the number of colonies detected was reduced to approximately 90. LY2801653, in the concentration range of 0.01–10  $\mu\text{M}$ , completely blocked the HGF-induced scattering.



**Fig. 1** **a** Structure of LY2801653; IUPAC name, N-(3-fluoro-4-([1-methyl-6-(1H-pyrazol-4-yl)-1H-indazol-5-yl]oxy)phenyl)-1-(4-(4-fluorophenyl)-6-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide); Chemical name, N-(3-fluoro-4-(1-methyl-6-(1H-pyrazol-4-yl)-1H-indazol-5-yloxy)phenyl)-1-(4-(4-fluorophenyl)-6-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide); CAS number 1206799-15-6; CAS name, 3-Pyridinecarboxamide, N-[3-fluoro-4-([1-methyl-6-(1H-pyrazol-4-yl)-1H-indazol-5-yl]oxy)phenyl]-1-(4-(4-fluorophenyl)-1,2-dihydro-6-methyl-2-oxo-. **b** Co-crystal structure of MET kinase domain with LY2801653. Residues where mutations are observed close to the inhibitor are colored in yellow while others are colored in magenta

Anti-proliferative effect of LY2801653 was evaluated against a panel of MET expressing cell lines (Supplementary Table S1). LY2801653 demonstrated more potent anti-proliferative activity in cell lines with MET gene amplification

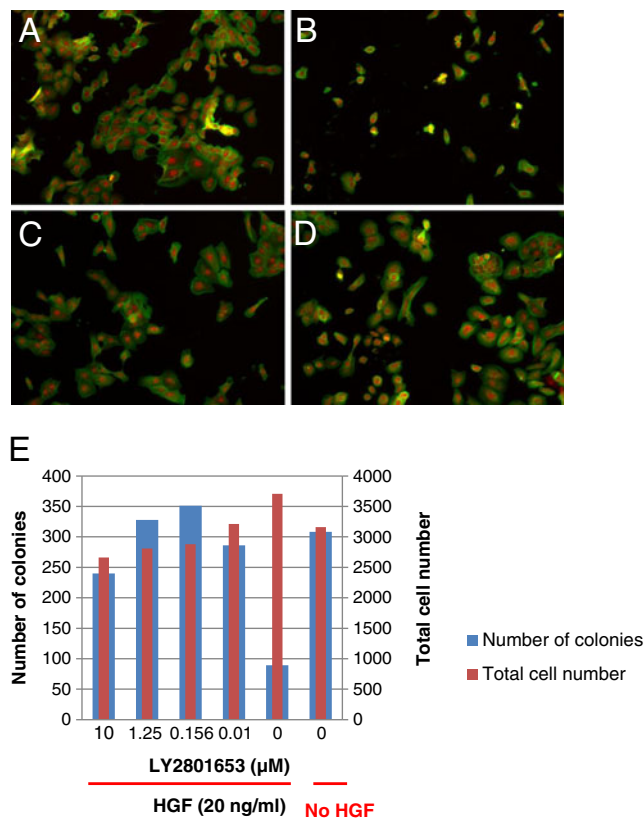


**Table 1** In vitro inhibitory activity of LY2801653 for TPR-MET bearing activating mutations in BaF3 cells cultured without IL3

MET mutation	IC <sub>50</sub> (nM)
TPR-MET wild type	42
Y1230C	54
D1228N	111
V1092I	12
M1250T	119
V1188L	23
L1195V	248
K1244R	77
M1131T	208
V1220I	18
S1040P	53
H1106D <sup>a</sup>	33
G1119V	62
H1094Y	25

<sup>a</sup>Sequencing of the clone indicated an additional unintended mutation introduced at D1117N

(MKN45, Hs746T and H1993) than the cell lines without MET gene amplification (U-87MG, KATO-III).



**Fig. 2** Field images of the scattering of DU145 cells following 48 h treatment: **a** untreated, **b** HGF (20 ng/mL), **c** LY2801653 (10 μM)+HGF, **d** LY2801653 (0.02 μM)+HGF. Images captured using Marianas Digital Imaging Workstation configured with a Zeiss Axiovert 200 M inverted fluorescence microscope (Intelligent Imaging Innovations). **e** Quantified data of the LY2801653 treatment effect. A colony was defined as  $\geq 4$  cells

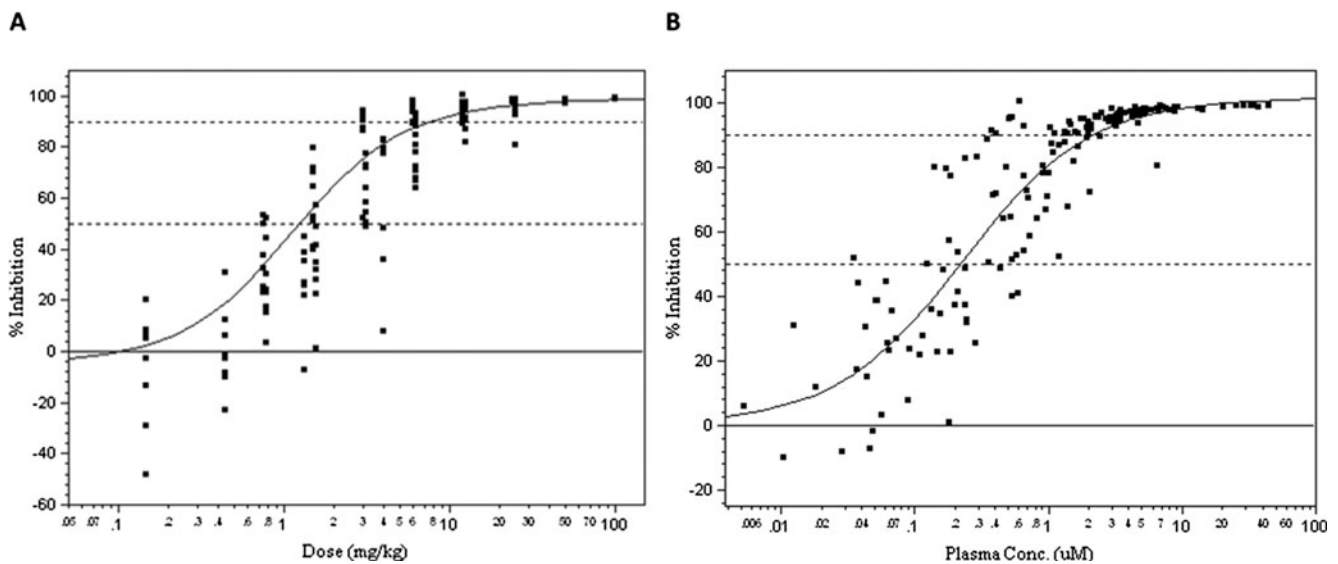
### In vivo MET target inhibition study in S114 xenograft model

Target inhibition studies to assess activity of LY2801653 were undertaken in S114 xenograft tumors created through subcutaneous implantation of S114 cells, a murine cell line engineered to express both human MET and HGF [13]. MET phosphorylation was inhibited by 98 % 2 h post-treatment with a single 50-mg/kg dose. Dose response studies were then performed at this same time point to further assess potency. LY2801653 treatment inhibited MET phosphorylation with a composite TED50 (50 % target inhibition dose) of 1.2 mg/kg and a composite TED90 (90 % target inhibition dose) of 7.4 mg/kg (Fig. 3a). The corresponding TEC50 (the concentration that inhibited 50 % MET phosphorylation) was 0.2 μM, whereas the TEC90 (the concentration that inhibited 90 % MET phosphorylation) was 2 μM (Fig. 3b). A pharmacokinetic-pharmacodynamic relationship was observed in these studies. Subsequently, a dose of 12 mg/kg (TED95) was used in a 24-h time course study to examine the duration of action. Ninety-five percent inhibition of p-MET occurred at 2 h and was maintained up to 8 h (data not shown). By 16 h, no p-MET inhibition was observed in tumors. Among the species studied, LY2801653 has the shortest elimination half life in mice of 2.9 h, compared with 14.3 h in non-human primate (data not shown).

### Anti-tumor activity in MET-dependent mouse xenograft models

MET amplification status by FISH analysis of U-87MG, H441, and MKN45 cells is shown in Fig. 4a. U-87MG and KP4 cells (Fig. 4b and c, respectively) were selected for representation of MET pathway activation via autocrine signaling. Two additional lines were selected to explore ligand-independent MET over-expression either in the presence (MKN45 cells; Fig. 4d) or low MET gene amplification (H441 cells; Fig. 4e). Whereas neither MKN45 nor H441 cells express detectable HGF, both U-87MG and KP4 cells express HGF with the highest levels being found in the latter (KP4: 4.7 ng/10<sup>6</sup> cells/24 h; U-87MG: 0.13 ng/10<sup>6</sup> cells/24 h). At doses of 4 and 12 mg/kg, similar levels of anti-tumor growth activity were observed with once or twice daily dosing of LY2801653 for the U-87MG, and H441 xenograft models.

Two non-continuous dosing regimens were also explored in the U-87MG and H441 xenograft models. The U-87MG study (Fig. 4b-2) evaluated the 9/5 schedule for 2 cycles and the 5/2 schedule for 4 cycles. The H441 study (Fig. 4e) evaluated the 9/5 schedule for 3 cycles and the 5/2 schedule for 6 cycles. Results indicated that when dosed at 12 mg/kg (around TED95), the anti-tumor growth effect in the two non-continuous dosing regimens was similar to that in the



**Fig. 3** Dose dependent inhibition of p-MET by LY2801653 in S114 tumors (a), and pharmacokinetic analysis of the dose response study (b). Animals ( $n=8$  per dose group) were treated with LY2801653.

Phospho-MET level in tumors was determined by MSD ELISA and compared with that in the vehicle treated group. Data from 4 studies were used

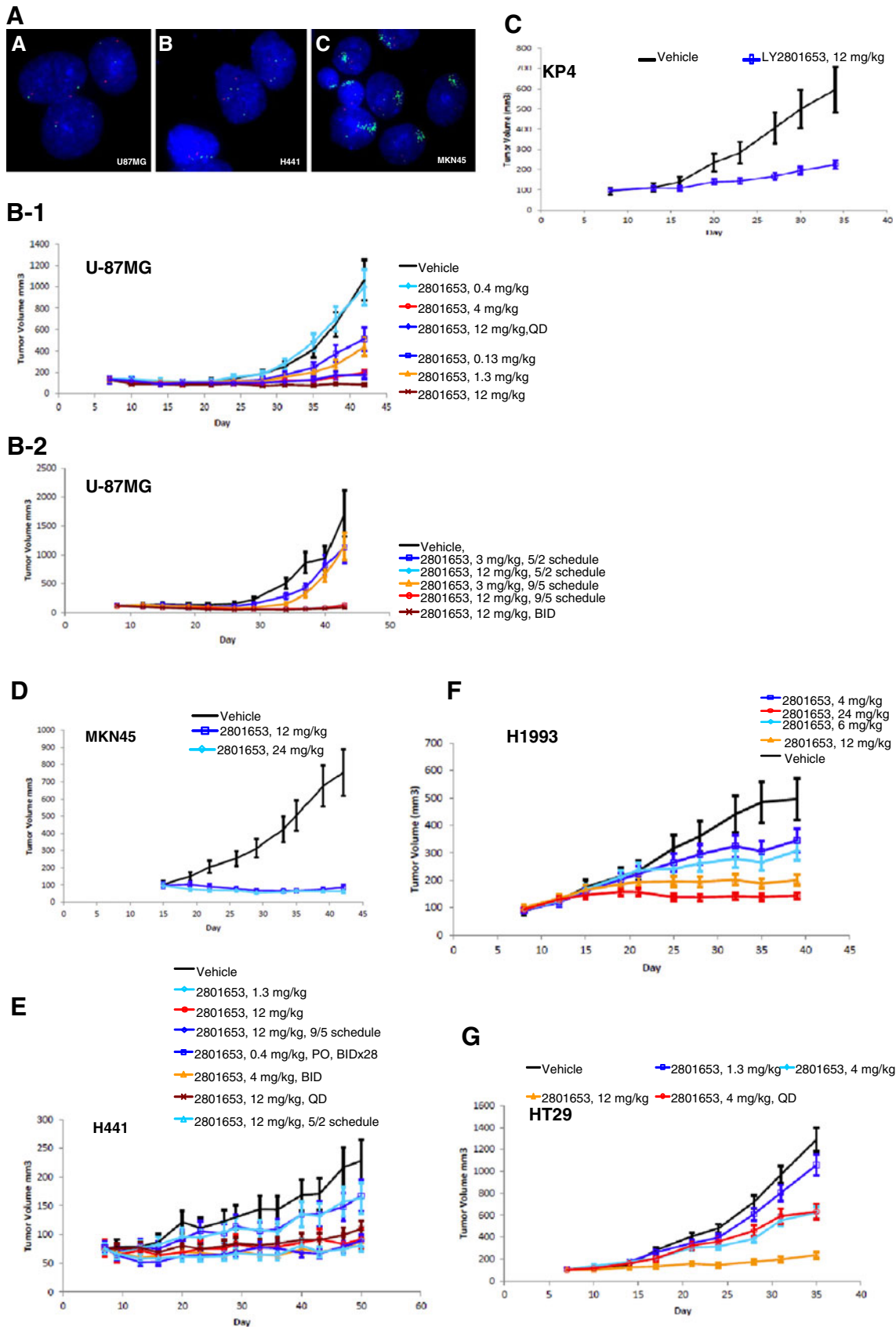
twice-daily uninterrupted dosing groups. The tumor growth delay lasted for at least a week after discontinuing dosing. When the dose was lowered to 3 mg/kg, the 9/5 schedule displayed better anti-tumor growth effect than the 5/2 schedule in U-87MG xenograft tumor models (Fig. 4b-2).

#### Vessel normalization and anti-apoptotic/anti-proliferative effect

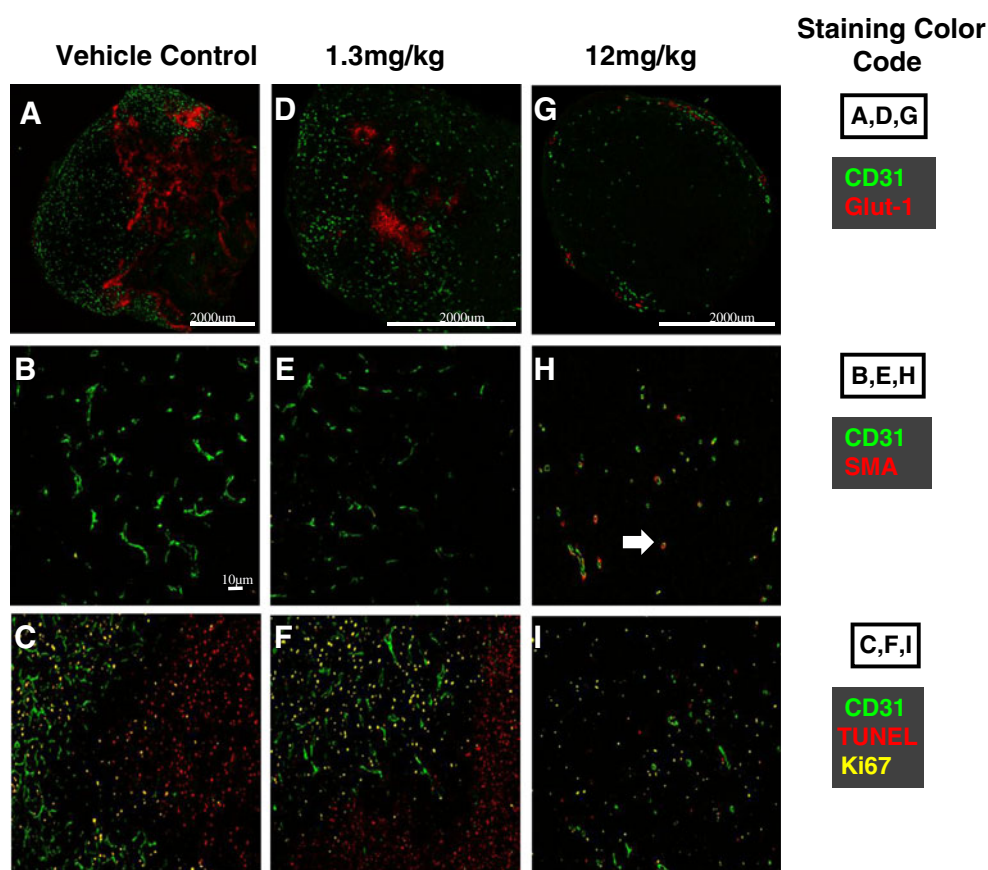
Multiplexed tissue imaging was performed on U-87MG xenograft tumors treated with vehicle (Figs. 5a–c) or LY2801653 treatment of 1.3 mg/kg (Figs. 5d–f) and 12 mg/kg (Figs. 5g–i). Qualitatively, U-87MG xenografts from vehicle-treated animals were well vascularized, with focalized areas of hypoxia and necrosis. Tumors had a tortuous network of vessels consisting of extensive neo-angiogenic vascular sprouts and mature vessels (CD31-labeled cells) that were poorly covered by pericytes and most vehicle-treated tumors had clearly demarcated hypoxic areas (marked by GLUT1 staining) located some distance from perfused vessels as well as necrotic areas spaced more distal from vessels. Low-dose (1.3 mg/kg once daily  $\times$  28 days) LY2801653 did not result in qualitative differences in any angiogenesis parameters, but did lead to a quantitative increase in vessel area (Supplementary Fig. S2). In contrast, treatment once daily with 12 mg/kg LY2801653 appeared to modulate angiogenesis as evident by a decrease in GLUT1 expression, an increase in pericyte coverage of vessels, and a qualitative difference in vessel phenotype. Specifically, vessels in tumors from the 12 mg/kg group, when compared with vehicle-treated tumors, were of smaller caliber, less tortuous, highly pericyte-covered, and lacked

staining for hypoxia (GLUT-1) in surrounding tumor cells (Fig. 5, Supplementary Fig. S2) suggesting adequate regional perfusion. Vehicle-treated tumors had proliferating tumor cells in close proximity to the vasculature, but also frequently displayed one or more large areas of necrosis that were avascular. Treatment with 1.3 mg/kg LY2801653 showed a trend of reducing the area of apoptosis (Supplementary Fig. S2), while treatment with 12 mg/kg LY2801653 significantly reduced apoptotic area. Focalized areas of necrosis/apoptosis were absent in this treatment group, and tumor cells were less densely packed and had lower intensity of Hoechst staining (mean nuclear intensity), which was consistent with a decreased proliferative potential.

**Fig. 4** In vivo anti-tumor effect of LY2801653 in mouse xenograft models. In all models, LY2801653 was given orally twice daily uninterrupted except where noted and all vehicle control groups are denoted with a black line. Two non-continuous dosing schedules were explored for the H441 and U-87 MG models: 5/2 and 9/5. In the 5/2 schedule, animals were dosed twice a day for 5 days followed by 2 treatment-free days for 4 cycles. In the 9/5 schedule, mice received twice daily dosing on the 9 days followed by 5 treatment-free days dose-free for a total of 2 cycles. **a** FISH analysis of tumor cell lines: no *MET* amplification in U-87MG cells; low level of *MET* amplification in H441 cells; high level of *MET* amplification in MKN45 cells. Red is a chromosome 7 anchor probe (RP11-161C7), green is *MET* locus specific probe (RP11-163C9). **b** A glioblastoma line U-87 MG as a *MET* autocrine model; part A-1, continuous dosing; part A-2, 5/2 or 9/5 dosing schedule compared to continuous dosing. **c** A pancreatic line KP4 as a *MET* autocrine model expressing high levels of the ligand HGF. **d** A gastric line MKN45 with *MET* gene amplification. **e** A NSCLC line H441 with over-expression of *MET*. **f** A NSCLC line H1993 with *MET* amplification and cross-activation of *MST1R*. **g** A colon line HT29 bearing *MST1R* activating mutation



**Fig. 5** Sample whole tumor (a, d, g), and field images (b, c, e, f, h, i) of the angiogenesis or apoptotic/proliferative markers of U-87MG xenograft in mice treated with LY2801653 (once daily for 28 days) compared with vehicle control group (a, b, c). Hoechst stains all cell nuclei, CD31 stains endothelial cells, SMA (smooth muscle actin) stains myofibroblasts and pericytes, and GLUT1 (glucose transporter 1) stains hypoxic cells. The scale bar in **b** represents 10  $\mu\text{m}$  and applies to (c, e, f, h and i). The white arrow in (h) shows a pericyte covered vessel



#### Kinase specificity profile for LY2801653

These *in vitro* studies demonstrated that, in addition to being a MET inhibitor, LY2801653 also has potent activity against MST1R, the TAM family of receptor tyrosine kinases (AXL, MERTK, and TYRO3), ROS1, PDGFRA, FLT3, TEK, DDR1/2 and MKNK1/2 (Table 2). A dendrogram of the *in vitro* cell-based activity of LY2801653 with  $\text{IC}_{50}$  less than 200 nM is shown in Supplementary Fig. S3.

Findings from subsequent testing of LY2801653 in xenograft or orthotopic models selected based on their dependence on MST1R (H1993, HT-29), FLT3 (MV4-11), and AXL (H1299) were consistent with the anti-growth activity of LY2801653 in the presence of these targets (Figs. 4f, and g and Supplementary Figs. S4A-C). NSCLC-derived H1993 xenograft tumors have previously been shown to have high levels of pMST1R due to transactivation by highly amplified MET [7]. The HT-29 colon xenograft tumors not only express MET, but also express a constitutively activated splice variant of MST1R, p160MST1R [19]. Growth of MV4-11 xenograft tumors, an acute myeloid leukemia cell line is driven by a FLT3-activating mutation [20], and *in vitro* anti-proliferative activity of LY2801653 for MV4-11 cells was determined to have an  $\text{IC}_{50}$  of 4 nM. Lastly, the NSCLC H1299 xenograft tumors over-express AXL and its ligand Gas6.

#### Discussion

##### MET inhibitory activity

The normal physiologic regulation of HGF and MET signaling found in development and organogenesis is prone to dysregulation in cancer and may contribute to invasion and metastasis [1, 18]. For these reasons, LY2801653 was developed through a strategy of targeting the MET receptor, and data presented here demonstrate that LY2801653 is a potent ATP-competitive inhibitor of the MET receptor tyrosine kinase, which is further capable of inhibiting MET-dependent tumor cell proliferation and migration/scattering. In addition, LY2801653 was able to induce vessel normalization in xenograft tumors. Whereas MET signaling is involved in angiogenesis, this latter effect of LY2801653 may not be exclusively due to MET inhibition. Indeed, inhibition of angiogenesis by LY2801653 may include contributions from among the limited range of other targets that it inhibits, such as TEK and AXL, that are involved in modulating angiogenesis.

*In vitro* biochemical measurements showed LY2801653 to be a potent inhibitor of MET with a 2 nM  $K_i$ , this activity was confirmed in cell-based assessments, in which LY2801653 had an  $\text{IC}_{50}$  for inhibition of MET phosphorylation of 35.2–59.2 nM. The S114 cell line is an engineered



**Table 2** In vitro inhibitory activity of LY2801653 against MET and other kinases

Kinase	Biochemical activity IC <sub>50</sub> or Kd <sup>k</sup> (nM)	Cell-base activity <sup>c</sup> IC <sub>50</sub> (nM)
MET	4.7 <sup>m</sup>	35–52 <sup>a</sup>
MST1R	12 <sup>m</sup>	11
AXL	11 <sup>m</sup>	2
ROS1	43 <sup>k</sup>	23–170 <sup>b</sup>
MKNK1/2	130 <sup>k</sup> /109 <sup>m</sup>	7
PDGFRA	620 <sup>k</sup>	41
FLT3	31 <sup>m</sup>	7
MERTK	0.8 <sup>k</sup>	10
TYRO3	1210 <sup>c</sup>	28
TEK	4 <sup>m</sup>	63
DDR1	0.95 <sup>k</sup>	0.1
DDR2	41 <sup>m</sup>	7
CSF1R	32 <sup>m</sup>	300
FLT1	76 <sup>m</sup>	>1000
FLT4	23 <sup>m</sup>	>316
TIE1	0.9 <sup>k</sup>	>1000
KDR	53 <sup>m</sup>	347
RET	590 <sup>c</sup>	2000

<sup>m</sup> EMD Millipore assay<sup>c</sup> Cerep assay<sup>k</sup> KINOMEScan assay<sup>a</sup> 35nM from H460 cells and 52 nM from S114 cells<sup>b</sup> 23nM from U118MG cells and 170 nM from HCC78 cells<sup>c</sup> cell-based activity was cell proliferation or target inhibition, see Supplementary Method section for each

mouse cell line expressing both human MET and HGF. In mice implanted subcutaneously with S114 tumorigenic cells, LY2801653 given orally at 12 mg/kg resulted in 95 % inhibition of MET phosphorylation in the tumors for up to 8 h. With elimination  $t_{1/2}$  being the shortest in mice of 2.9 h (e.g. compared to 14.3 h in non-primate), but with the kinase active site occupancy  $t_{1/2}$  of approximately 8.5 h, LY2801653 can be dosed orally twice a day in mouse tumor model studies.

The crystal structure of MET bound with LY2801653 indicates that LY2801653 is a type II inhibitor and illustrates that the mechanism of inhibition is through the stabilization of the inactive ‘DFG-out’ conformation [17, 18]. This binding mode often leads to a long dissociation time constant, a feature that was experimentally confirmed by the observation that LY2801653 has a slow-off rate with an active site occupancy  $t_{1/2}$  of approximately 8.5 h.

Type-II kinase inhibitors are typically more tolerant of mutations in the kinase domain than type-I inhibitors [17]. For LY2801653, this feature was experimentally confirmed, and the molecule does retain inhibitory activity with a

variety of MET-activating point mutations. Of the 13 point mutations examined, 12 are within the kinase domain. The exception, S1040P, is in the juxtamembrane domain. The MET crystal structure with bound LY2801653 provides a basis to interpret the impact of these mutations on the IC<sub>50</sub> of LY2801653. The 2 mutants causing the greatest increases in IC<sub>50</sub> (L1195V and M1131T) are located at the hydrophobic binding site of the inhibitor where changing from a hydrophobic side chain to a polar one, or reducing the size of hydrophobic side chain, is expected to weaken the inhibitor binding. The 2 mutants (D1228N, M1250T) with the next largest increases in IC<sub>50</sub> bear mutations in the activation loop. This region is distal from the inhibitor. One of the mutations (D1228N) was not observed in the structure due to loop disorder. Thus the impact of the mutation is not through direct interaction with the inhibitor. One hypothesis for the increased IC<sub>50</sub> is that these activating mutations stabilize the active conformation of the activation loop (DFG-in conformation), therefore reducing the potency of the inhibitor. The 2 mutations with noticeable decreases in IC<sub>50</sub> (V1092I, V1220I) are located near the inhibitor. Both mutations slightly increase the bulk of the hydrophobic side chain, likely leading to stronger Van der Waals and/or hydrophobic interactions with the inhibitor.

Activating mutations of MET have been reported in sporadic and inherited papillary renal carcinomas, hepatocellular carcinomas, head and neck squamous cell carcinomas (HNSCC) and other neoplasms [21]. The majority of these mutations are kinase domain mutations with homology to cancer-inducing mutations in other receptor tyrosine kinases such as EGFR. In HNSCC, cells harboring multiple kinase domain mutations have been observed and are postulated to be selected during metastatic spread [22]. The 13 MET mutations evaluated here represent approximately half of the kinase domain mutations currently reported in the literature [23]. The LY2801653-binding configuration to MET appears to contribute 2 clinically intriguing features; specifically, the potential ability to retain potency versus a spectrum of mutations and the ability to sustain target inhibition due to its slow off-rate.

Because the HGF/MET pathway has also been shown to be involved in cancer cell survival and growth, LY2801653 was evaluated for its ability to inhibit cell growth against a panel of MET expressing tumor cell lines. LY2801653 showed several-fold more potent anti-proliferative activity against tumor cell lines with high MET gene amplification (MKN45, Hs746T, H1993) than the cell lines without MET amplification (U-87MG, KATO III). These potency differences were less evident in the in vivo evaluation in xenograft models.

The in vivo effects in xenograft mouse models observed for LY2801653 were otherwise consistent with the in vitro data. LY2801653 treatment demonstrated significant anti-

tumor growth effect in two MET autocrine models (U-87MG, KP4), a model with very high MET gene amplification (MKN45), and a model with MET low MET amplification, but over-expression, (H441). Similar tumor growth inhibition effect was observed following LY2801653 dosing of 4 to 12 mg/kg either once or twice a day. In the U-87MG and H441 models, similar anti-tumor effects were seen with interrupted dosing on a 9/5 or 5/2 schedule. Whereas the mechanism for maintaining tumor growth suppression with the non-continuous dosing schedules is unclear, it may be related to the observed slow off-rate of LY2801653 released from the ATP-binding site of the kinase target.

#### Kinase selectivity profile

Whereas type-II inhibitors are often characterized by a broad spectrum of kinase activity, LY2801653 retains a limited repertoire of engagement. The selectivity profile was examined through biochemical screening against 442 kinases, followed by confirmation with cell-based activity assays. LY2801653 was found to have potent activity against 11 additional kinases besides MET. A dendrogram shows that the 11 kinases are clustered in 3 regions, 2 of them in the receptor tyrosine kinase (RTKs) families and 1 (MKNK1/2) in the CAMK family (calcium/calmodulin-dependent protein kinases). Because LY2801653 does not have significant activity against ALK and LTK, it is somewhat surprising to find potent activity against ROS1, which is in the member of the ALK/LTK family. The x-ray crystal structure data may offer some insight. The inactive form of the ALK structure has been shown to be distinct from the typical features, such as the DFG-out conformation, observed in other RTKs [24]. Instead, it appears that the DFG-in conformation is more stable in the inactive form of ALK, explaining the lack of inhibition by LY2801653. As MKNK1/2 are inhibited by LY2801653 despite these being serine/threonine kinases rather than tyrosine kinases, this inhibition is likely due to the inactive form of MKNK1/2 adopting a DFG-out (DFD in MKNK1/2) conformation, stabilized in part by the replacement of Asp (D) for Gly (G) in the canonical DFG motif [25, 26].

LY2801653 is an inhibitor of MET and MST1R (Table 2) *in vitro* and demonstrates tumor activity in 2 xenograft models (H1993, HT29) that co-express MET and MST1R. Tumor cells such as H1993 that have high levels of MET gene amplification have been shown to cross-activate MST1R [7]. The ability to potently block both MET and MST1R pathway signaling may be advantageous in treating tumors with this phenotype.

Some of the other kinase targets of LY2801653 presented in Table 2 were also examined further in *in vivo* models. The potent *in vitro* anti-proliferative activity ( $IC_{50}=4$  nM) and *in vivo* anti-tumor growth activity of LY2801653 with

the MV4-11 tumor cells may be explained by its inhibitory activity against FLT3 as this tumor cell line bears a FLT3-activating mutation [20]. The *in vivo* anti-tumor growth activity of LY2801653 against AXL was demonstrated in a murine orthotopic model of NSCLC (H1299) with AXL over-expression. MET has been reported to be capable of transactivating AXL and PDGFRA *in vitro* in tumor cells [8]. In human bladder cancer, co-expression of these RTKs was associated with poor patient survival, and over-expression of MET/AXL/PDGFR was shown to correlate with poor survival, and contributed to the progression of human bladder cancer [8]. Up-regulation of AXL was reported recently to be a resistance mechanism to EGFR targeted therapy [27].

MKNK1/2 are activated by ERK or p38 [28], leading to the phosphorylation of several substrates including eIF4E and Sprouty2, thereby enhancing tumorigenesis [29]. An increase in Sprouty-2 protein in tumor cells was associated with an increase in MET expression and in tumor malignancy, especially in colon cancer cells [28]. LY2801653 treatment produces anti-tumor effect in multiple murine xenograft models bearing KRAS-activating mutation as illustrated in H441 NSCLC cells and KP4 pancreatic cells, and treatment outcomes for such tumors may benefit from combined MET and MKNK1/2 inhibition.

ROS1 is an orphan RTK that has been reported to be constitutively activated via gene translocation leading to formation of fusion proteins in glioblastoma, NSCLC, ovarian cancer and cholangiocarcinoma [30–33]. Over-expression of DDR1/2 has been reported in multiple tumor types such as sarcoma [34]. Activating mutations for DDR2 in squamous cell lung cancer were also reported [34, 35]. In view of the reported transforming activity of constitutively activated ROS1 kinase with various fusion partners, and the potent inhibitory activity of LY2801653 for ROS1 and DDR1/2, there may be clinical merit in evaluating LY2801653 in tumor types bearing ROS1 or DDR1/2 aberrations.

Tumor vessel normalization describes a process whereby a tortuous, poorly perfused vascular network is converted to one that consists of directionally-aligned, highly perfused, pericyte-covered vessels [36]. Treatment of U-87MG tumor-bearing mice with daily doses of 12 mg/kg LY2801653 resulted in phenotypic events that are hallmarks of vessel normalization. Whereas the tumors treated with 12 mg/kg LY2801653 displayed a more functional vasculature, the tumors themselves had less apoptotic and proliferative markers compared with vehicle-treated tumors, consistent with a cytostatic phenotype. As LY2801653 has little or no inhibitory activity against KDR, the displayed anti-angiogenic effects *in vivo* were most probably via a non-VEGFR-dependent mechanism. The HGF/MET [37], the AXL and MERTK [38], and the TEK [39] pathways were all reported

to play a role in tumor angiogenesis and were inhibited by LY2801653.

In summary, we report here a potent type II ATP-competitive inhibitor of MET, LY2801653, that has in vivo anti-tumor activities in multiple mouse xenograft models, as well as anti-angiogenic, and anti-proliferative/cytostatic activities. In addition, this molecule has potent inhibitory activity against several other oncogenic proteins, including MST1R, FLT3, AXL, PDGFRA, ROS1, TEK, DDR1/2 and MKNK-1/2. Nonclinical testing suggests that LY2801653 may have merits for clinical evaluation in tumors with a number of MET pathway phenotypic profiles such as over-expression, activating mutation, and gene amplification. Furthermore, as MKNK-1/2 is down-stream of the RAS/MEK/ERK signaling pathway, LY2801653 may have anti-tumor activity in tumors bearing KRAS-activating mutations in addition to the over-expression of the above mentioned RTK targets. These observations have supported the progression of LY2801653 into clinical testing in an ongoing phase I study (JSBA, NCT01285037).

**Acknowledgments** The authors wish to thank Robert Foreman, Adam Phillips, Jennifer Stephens, Spring Weir for performing the mouse xenograft studies; Patti Moore for editing and managing the manuscript writing; Colleen Burns/Nick Loizos, Jason Cunningham and Jacqueline Doody/Yanxia Li for performing cell-based assays for PDGFRA, MST1R and CSF1R respectively; Laura Bloem for in vitro biochemical kinase assay management; Steve Demarest/Arlene Sereno for providing recombinant human Gas6; Beverly Falcon for constructing the figure of the tumor images; Shaoyou Chu for development of the cell scattering assay and Megan Thobe for review of the manuscript.

**Disclosure of potential conflicts of interest** All authors: current or former employees of Eli Lilly and Company

**Open Access** This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

## References

- Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF (2003) Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 4:915–25
- Comoglio PM, Giordano A, Trusolino L (2008) Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nat Rev Drug Disc* 7:504–16
- Turke AB, Zejnullahu K, Wu YL, Song Y, Dias-Santagata D, Lifshits E et al (2010) Preexistence and clonal selection of Met amplification in EGFR mutant NSCLC. *Cancer Cell* 17:77–88
- Yamada T, Takeuchi S, Kita K, Bando H, Nakamura T, Matsumoto K et al (2011) Hepatocyte growth factor induces resistance to anti-epidermal growth factor receptor antibody in lung cancer. *J Thoracic Oncol* 7:272–80
- Bean J, Brennan C, Shih JY, Riely GJ, Viale A, Wang L et al (2007) Met amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci USA* 104:20932–7
- Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO et al (2007) Met amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 316:1039–43
- Benvenuti S, Lazzari L, Arnesano A, Chiavi GL, Gentile A, Comoglio PM (2011) RON kinase trans-phosphorylation sustains MET oncogene addition. *Cancer Res* 71:1945–55
- Yeh CY, Shih SM, Yeh HH, Wu TJ, Shin JW, Chang TY et al (2011) Transcriptional activation of the Axl and PDGFR- $\alpha$  by cMet through a ras- and Src-independent mechanism in human bladder cancer. *BMC Cancer* 11:139
- Li T, Pobanz MA, Shih C, Wu Z, Yang WJ, Zhong B (2011) Amidophenoxyindazoles useful as inhibitors of c-Met. *US 8030302B2*
- Wang W, Marimuthu A, Tsai J, Kumar A, Krupka HI, Zhang C et al (2006) Structural characterization of autoinhibited c-MET kinase produced by coexpression in bacteria with phosphatase. *Proc Natl Acad Sci USA* 103:3563–8
- Emsley P, Cowtan K (2004) Coot: model-building tools for molecular graphics. *Acta Cryst Section D* D60:2126–32
- Murshudov GN, Vagin AA, Dodson EJ (1997) Refinement of macromolecular structures by the maximum-likelihood method. *Acta Cryst Section D* D53:240–55
- Rong S, Oskarsson M, Faletto D, Tsarfaty I, Resau JH, Nakamura T et al (1993) Tumorigenesis induced by coexpression of human hepatocyte growth factor and the human met protooncogene leads to high levels of expression of the ligand and receptor. *Cell Growth Differ* 4:563–9
- Park M, Dean M, Kaul K, Braun MJ, Gonda MA, Vande Woude GF (1987) Sequence of MET protooncogene cDNA has features characteristic of the tyrosine kinase family of growth-factor receptors. *Proc Natl Acad Sci USA* 84:6379–83
- Aarts LH, Roovers O, Ward AC, Touw IP (2004) Receptor activation and 2 distinct COOH-terminal motifs control G-CSF receptor distribution and internalization kinetics. *Blood* 103:571–9
- Meier T, Uhlik MT, Chintharlapalli S, Dowless M, Van Horn R, Stewart J et al (2011) Tasisulam sodium, an antitumor agent that inhibits mitotic progression and induces vascular normalization. *Mol Cancer Ther* 10:2168–78
- Underiner TL, Herbertz T, Miknyoczki SJ (2010) Discovery of small molecule c-Met inhibitors: evolution and profiles of clinical candidates. *Anti-cancer agents Med Chem* 10:7–27
- Gherardi E, Birchmeier W, Birchmeier C, Vande Woude GF (2012) Targeting MET in cancer: rationale and progress. *Nat Rev Cancer* 12:89–103
- Wang MH, Kurtz AL, Chen YQ (2000) Identification of a novel splicing product of the RON receptor tyrosine kinase in human colorectal carcinoma cells. *Carcinogenesis* 21:1507–12
- Quentmeier H, Reinhardt J, Zaborski M, Drexler HG (2003) Flt3 mutations in acute myeloid leukemia cell lines. *Leukemia* 17:120–4
- Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P et al (1997) Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. *Nat Genet* 16:68–73
- Di Renzo MF, Olivero M, Martone T, Maffe A, Maggiora P, De Stefani A et al (2000) Somatic mutations of the MET oncogene are selected during metastatic spread of human HNSC carcinomas. *Oncogene* 19:1547–55
- Sattler M, Hasina R, Reddy MM, Gangadhar T, Salgia R (2011) The role of the c-Met pathway in lung cancer and the potential for targeted therapy. *Ther Adv Med Oncol* 3:171–84
- Lee CC, Jia Y, Li N, Sun X, Ng K, Ambing E et al (2010) Crystal structure of the ALK (anaplastic lymphoma kinase) catalytic domain. *Biochem J* 430:425–37

25. Jauch R, Cho MK, Jakel S, Netter C, Schreiter K, Aicher B et al (2006) Mitogen-activated protein kinases interacting kinases are autoinhibited by a reprogrammed activation segment. *EMBO J* 25:4020–32
26. Jauch R, Jäkel S, Netter C, Schreiter K, Aicher B, Jäckle H et al (2005) Crystal structures of the Mnk2 kinase domain reveal an inhibitory conformation and a zinc binding site. *Structure* 13:1559–68
27. Zhang Z, Lee JC, Lin L, Olivas V, Au V, LaFramboise T et al (2012) Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. *Nat Genet* 44:852–60
28. DaSilva J, Xu L, Kim HJ, Miller WT, Bar-Sagi D (2006) Regulation of sprouty stability by Mnk-1-dependent phosphorylation. *Mol Cell Biol* 26:1898–907
29. Ueda T, Sasaki M, Elia AJ, Chio LIC, Hamada K, Fukunaga R et al (2010) Combined deficiency for MAP kinase-interacting kinase 1 and 2 (Mnk1 and Mnk2) delays tumor development. *Proc Natl Acad Sci USA* 107:13984–90
30. El-Deeb IM, Yoo KH, Lee SH (2010) ROS receptor tyrosine kinase: a new potential target for anticancer drugs. *Med Res Rev*. doi:10.1002/med.20206:1-25
31. Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H et al (2007) Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell* 131:1190–203
32. Gu TL, Deng X, Huang F, Tucker M, Crosby K, Rimkunas V et al (2011) Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. *PLoS One* 6:e15640
33. Bergethon K, Shaw AT, Ou SHI, Katayama R, Lovly CM, McDonald NT et al (2012) ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol* 30:863–70
34. Bai Y, Li J, Fang B, Edwards A, Zhang G, Bui M et al (2012) Phosphoproteomics identifies driver tyrosine kinases in sarcoma cell lines and tumors. *Cancer Res* 72:2501–11
35. Hammerman PS, Sos ML, Ramos AH, Xu C, Dutt A, Zhou W et al (2011) Mutations in the DDR2 kinase gene identify a novel therapeutic target in squamous cell lung cancer. *Cancer Dis* 1:78–89
36. Jain RK (2005) Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 307:58–62
37. Sengupta S, Gherardi E, Sellers LA, Wood JM, Sasisekharan R, Fan TPD (2003) Hepatocyte growth factor/scatter factor can induce angiogenesis independently of vascular endothelial growth factor. *Arterioscler Thromb Vasc Biol* 23:69–75
38. Verma A, Warner SL, Vankayalapati H, Bearss DJ, Sharma S (2011) Targeting Axl and Mer kinases in cancer. *Mol Cancer Ther* 10:1763–73
39. Huang H, Bhat A, Woodnutt G, Lappe R (2010) Targeting the ANGPT-TIE2 pathway in malignancy. *Nat Rev Cancer* 10:575–85