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Supplemental Material to:

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Biochemical and pharmacological characterization of human c-Met neutralizing monoclonal antibody CE-355621

mAbs 2012; 4(6)
http://dx.doi.org/10.4161/mabs.22160

http://www.landesbioscience.com/journals/mabs/article/22160

Supplementary Methods

Handling of Cynomolgus monkeys

Two male and two female Cynomolgus monkeys who were IgG2 naïve and ranged in weight from 3.0-3.3 kg, were administered an intravenous bolus dose of CE-355621 at 5 mg/kg. The monkeys had free access to food at their regular scheduled times and unrestricted access to water prior to and during the study. CE-355621 was dosed as a solution in sterile pH 5.5 acetate buffer (20 mM acetate, 140 mM NaCl) on the day of the study, and administered through the cephalic vein. Blood samples (2 mL) were collected from the femoral vein at various times following single dose drug administration. Whole blood samples were collected into heparinized vacutainers. Plasma was prepared by centrifugation and stored at -20 °C prior to analysis.

The monkeys were housed individually in stainless steel cages and transferred to clean cages approximately every 2 weeks. The temperature and humidity in the animals' quarters (70°F-78°F; 30%-70% RH) were monitored and the airflow in the room was sufficient to provide several exchanges per hour with 100% fresh filtered air. An automatic timing device provided an alternating 12-hour light (6 AM-6 PM)/dark cycle. Lab Diet® 5LR9 Certified HI-Fiber Primate Diet was provided to animals twice daily and diet was supplemented with fruit and/or vegetables. Individual food consumption of all animals was assessed daily by visual inspection. Water was supplied *ad libitum* via an automatic system and analyzed periodically for the presence of contaminating substances that could interfere with interpretation of the study. All animals were acclimated to the study room for at least 3 days prior to the study. Manual restraint and/ or a pole and collar system were used to restrain the animals for dosing and sample collection. The animals were acclimated to these restraints prior to the start of the study. CE-355621 was diluted in sterile pH 5.5 acetate buffer (20 mM

acetate, 140 mM NaCl) and filter-sterilized through a 0.2 μm filter and given intravenously. Intravenous dosing was accomplished by clipping the hair over the cephalic vein, prepping the site aseptically, and inserting a butterfly catheter for the bolus infusion. Animals were observed predose, immediately postdose, and daily throughout the course of the study. Whole blood samples were taken at multiple time points throughout the study from the femoral vein in the inguinal region. The site was clipped of hair and swabbed with alcohol prior to whole blood collection with a vacutainer system.

Supplementary Figures

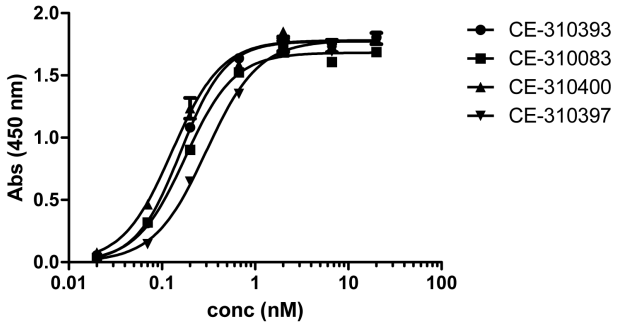

Fig S1. Binding of anti-c-Met antibodies to the isolated c-Met extracellular domain. Lead antibodies were serially diluted and binding to immobilized recombinant human c-Met ECD-Fc protein was detected by ELISA after 4 hr incubation with each antibody.

A

Light chain germline gene usage V=L5, J=JK4

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CE-310393 LC  DIQMTQSPSSVSASVGDRVTITCRASQGINTWLAWYQQKPGKAPKLLIYAASSLKS
CE-355621 LC  DIQMTQSPSSVSASVGDRVTITCRASQGINTWLAWYQQKPGKAPKLLIYAASSLKS
Germline      DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQS
                                       CDR1                    CDR2
CE-310393 LC  GVPSRFSGSGSGADFTLTISSLQPEDFATYYCQQANSFPLTFGGGTKVEIK
CE-355621 LC  GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFPLTFGGGTKVEIK
Germline      GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFPLTFGGGTKVEIK
                                             CDR3
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Heavy chain germline gene usage V=1-18, D=D2-15, J=JH4b

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CE-310393 HC  QVQLVQSGAEVKKPGASVKVSCEASGYTFTSYGFSWVRQAPGQGLEWMGWISASNGNTYYAQKLQG
CE-355621 HC  QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGFSWVRQAPGQGLEWMGWISASNGNTYYAQKLQG
Germline      QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQG
                                       CDR1                    CDR2
CE-310393 HC  RVTMTTDTSTSSAYMELRSLRSDDTAVYYCARVYADYADYWGQGTLVTVSS
CE-355621 HC  RVTMTTDTSTSTAYMELRSLRSDDTAVYYCARVYADYADYWGQGTLVTVSS
Germline      RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR-YS-YFDYWGQGTLVTVSS
                                             CDR3
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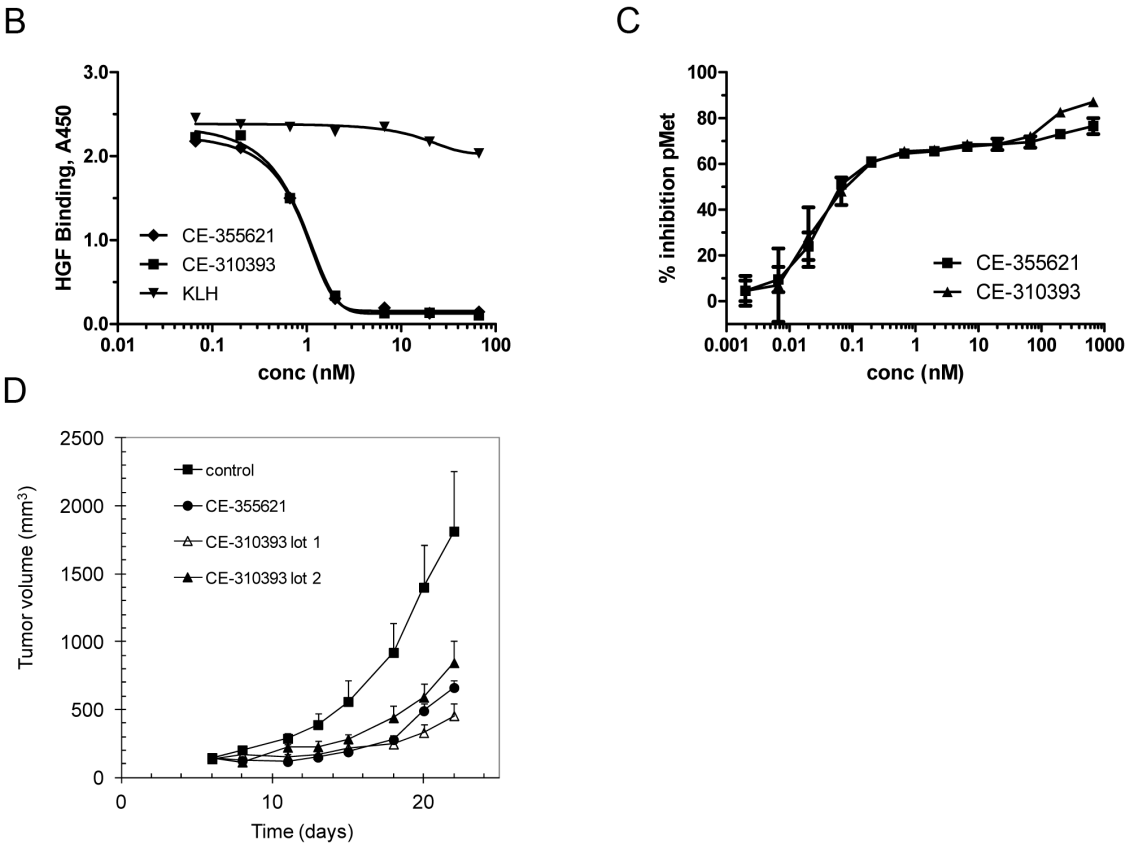

Figure S2. The activity of germlined CE-355621 is identical to that of parental CE-310393 *in vitro* and *in vivo*. (A) Sequence alignment of CE-310393 and CE-355621 compared to germline sequence. Sequence differences from germline are represented in bold red text. CDRs are highlighted by underlined text. (B) Serially diluted CE-310393, CE-355621 and anti-KLH were preincubated with Met ECD-Fc coated in microtiter plates prior

to challenge with recombinant HGF (100 ng/ml) for 15 min. (C) Antibodies were titrated and were applied to A549 cells 4 hrs before addition of recombinant HGF (200 ng/ml). c-Met activation was assessed following 15 min stimulation with HGF in a capture ELISA using PY20 to detect pY-Met. (D) U87 xenograft tumors were established to a size of ~150 mm3 in athymic mice, and 7 mice per group were dosed with 100 μg of CE-355621 or CE-310393 from two different lots. Caliper measurements were performed every 2-3 days to determine tumor size. The size of tumors on days 8-22 for each of the antibody treatments is statistically different ($P<0.05$) from the vehicle control. There were no statistically significant differences in tumor size for either lot of CE-310393 compared with CE-355621 for days 8-22 ($P>0.05$).

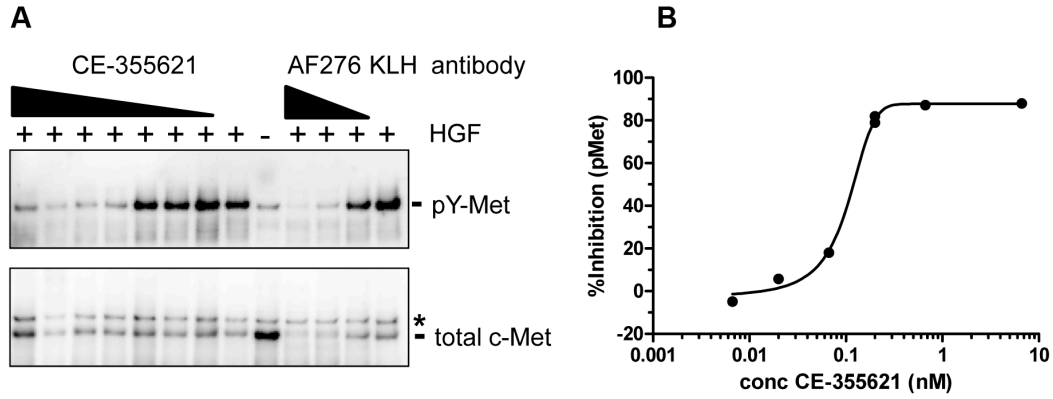

Figure S3. CE-355621 inhibits activation of c-Met in U87 by exogenous HGF. U87 cells were pretreated with vehicle or varying concentrations of CE-355621, AF276, or anti-KLH for 24 hrs then stimulated with HGF (200 ng/ml) for 15 min, and cell lysates were made. (A) Western blots were performed to evaluate c-Met activation with PY20 and total Met protein with Santa Cruz sc-10. (B) Quantitation of bands in (A) by LumiImager demonstrates the dose-dependent inhibition of c-Met activation (pMet) by CE-355621. Preincubation of U87 cells with CE-355621 for 1-48 hrs has similar effects on HGF-induced c-Met activation (data not shown).

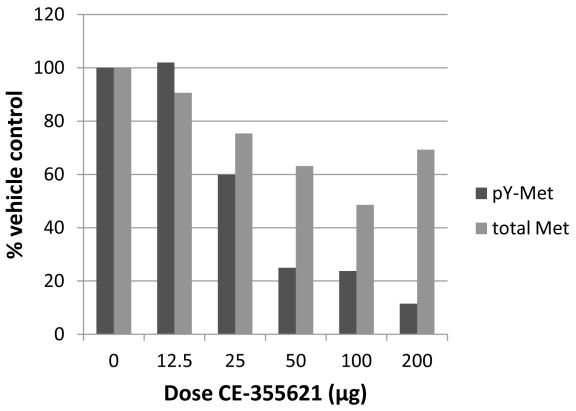

Figure S4. Dose-dependent effects of CE-355621 on phospho- and total Met in U87 xenografts at 24 hrs. The levels of phospho- and total Met determined from the PK/PD experiment presented in Figure 5A are plotted as % vehicle control according to dose. Mice bearing U87 tumors were dosed i.p. with various amounts of CE-355621 and excised 24 hrs later. c-Met was immunoprecipitated from lysates; phospho- and total Met levels were determined by Western blot and quantitated by LumiImager.

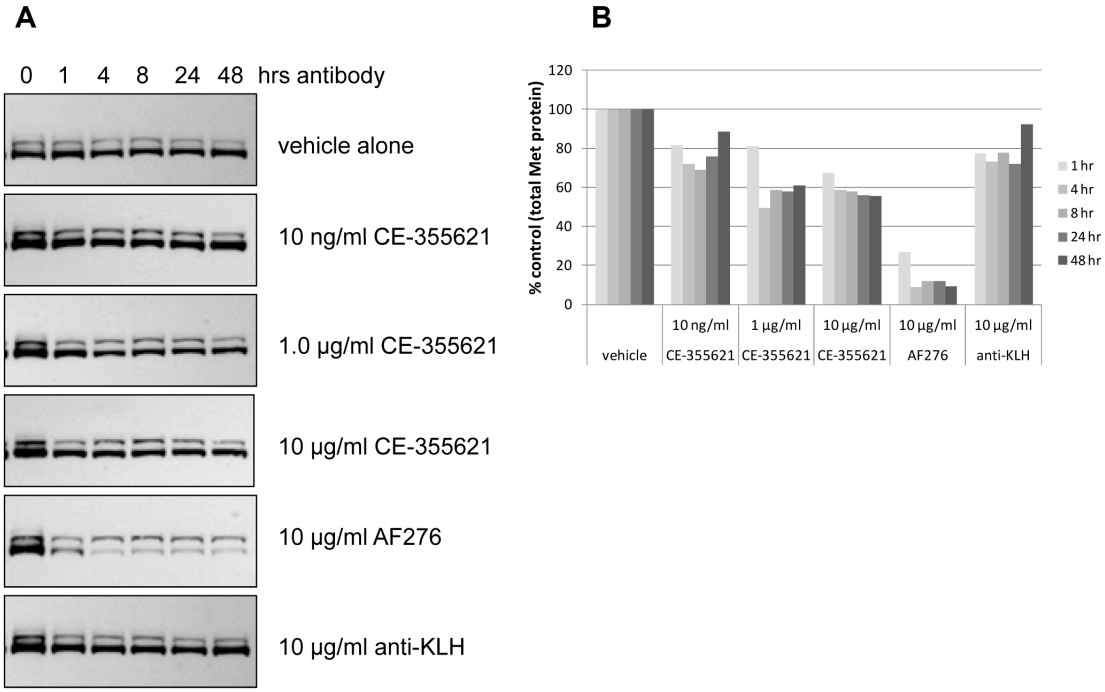

Figure S5. CE-355621 induces c-Met turnover in U87 cells. U87 cells were treated with vehicle, varying concentrations of CE-355621, A276 or anti-KLH over a 48 hr period. (A) Total c-Met levels were assessed by Western blot with Santa Cruz sc-10. (B) Bands from the Westerns displayed in (A) were quantitated by LumiImager and plotted.

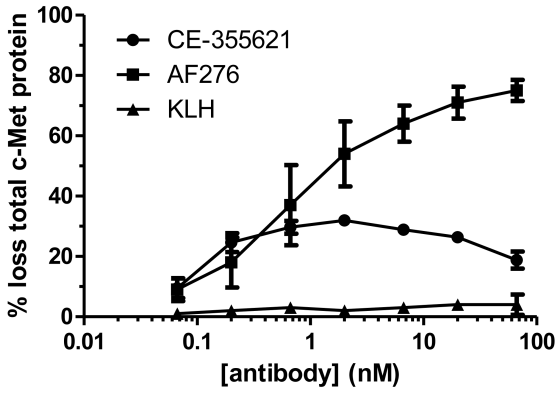

Figure S6. CE-355621 induces loss of total c-Met in A549 cells. A549 cells were treated with varying concentrations of CE-355621, AF276, or anti-KLH for 4 hours and the level of total c-Met was determined by ELISA.

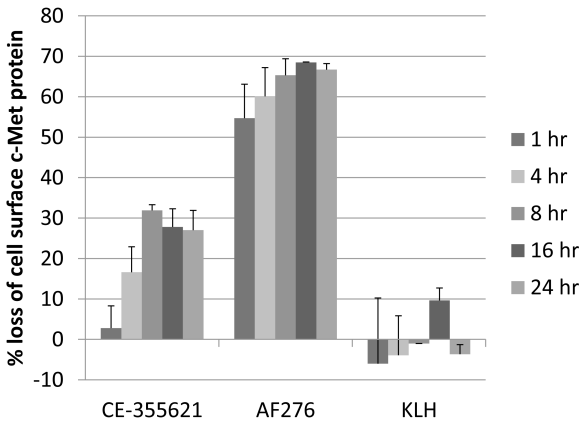

Figure S7. c-Met antibodies induce loss of c-Met from the cell surface. Each antibody (10 µg/ml) was incubated with A549 cells for various times (1-24 hrs). Cells were detached and fixed with paraformaldehyde. The level of c-Met remaining on the cell surface was determined using flow cytometry to detect the binding of biotinylated antibody BAF358 to the c-Met ECD. Values are means of triplicate samples ± SD.

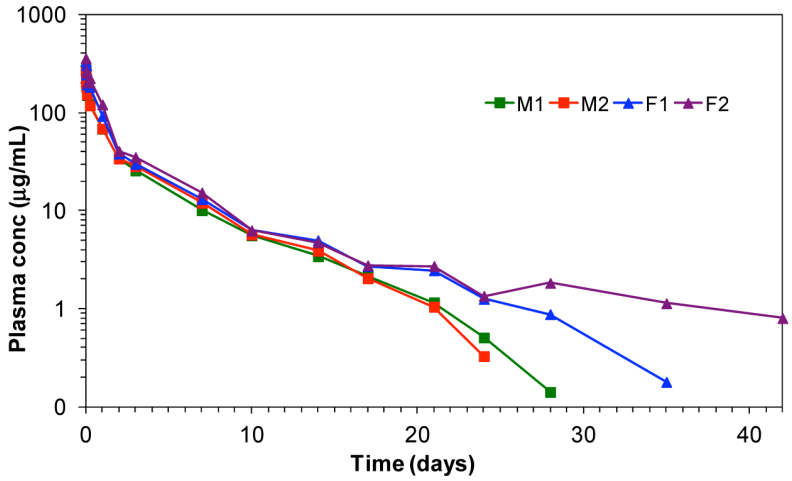

Figure S8. Pharmacokinetics of CE-355621 in cynomolgus monkeys. Two male and two female cynomolgus nonhuman primates were injected i.v. with a 5 mg/kg bolus dose of CE-355621. At various times, blood was drawn and the level of circulating intact CE-355621 in plasma capable of binding to Met ECD-Fc was quantitated by ELISA.

		Biacore		flow cytometry	Ligand Binding	Cell pMet
antibody	target species	K_D (pM)	k_{off} (1/s)	EC_{50} (pM)	(IC_{50}, pM)	(IC_{50}, pM)
CE-310393	human c-Met	220	1.5×10^{-4}	39 ± 7	461 ± 110	152 ± 22
CE-355621	human c-Met	200	1.8×10^{-4}	56 ± 7	466 ± 111	185 ± 88
CE-310393	cyno c-Met	600	2.9×10^{-4}	48 ± 7		
CE-355621	cyno c-Met	610	4.0×10^{-4}	73 ± 26		

Table S1. Comparison of binding and *in vitro* neutralizing activity for CE-310393 and CE-355621 for human and cynomolgus c-Met. Binding affinity (KD) and off-rate (1/s) of parental antibody CE-310393 and its germlined progeny CE-355621 to human and cynomolgus c-Met ECD-Fc was measured with Biacore. Antibody affinity for human and cynomolgus c-Met was estimated by binding of varying concentrations of antibody to native c-Met on human A549 and cynomolgus kidney cells for 6 hrs and measured by flow cytometry. Neutralizing activity of the two antibodies was assessed in the ligand binding assay and in the A549 cellular c-Met autophosphorylation (Cell pMet) assays. In the ligand binding assay, antibodies were preincubated with immobilized human c-Met ECD-Fc prior to addition of recombinant HGF (100 ng/ml) for 15 min. In the Cell pMet assay, A549 cells were preincubated with antibodies for 4 hrs prior to treatment with HGF (200 ng/ml) for 15 min. c-Met activity was measured by capture ELISA using PY20-HRP as the detection antibody. Values for the flow cytometry, ligand binding and Cell pMet assays represent means ± SEM of multiple dose titration experiments. IC_{50} values were calculated using GraphPad Prism software.

	Vehicle	CE-355621
p-Met	**2+**	**1+**
Ki-67	**2+**	**1+**
Cleaved caspase 3	**1+**	**2+**

Table S2. CE-355621 affects phospho-Met (p-Met) levels, tumor cell proliferation and apoptosis in U87MG xenograft tumors. U87MG tumors were treated with vehicle or a single i.p. 200 μg dose of CE-355621, excised 72 hrs later, formalin-fixed then paraffin-embedded. Immunohistochemical staining intensities from excised tumors stained for phospho-Met (pY1234/1235), Ki-67 (proliferation marker), and cleaved caspase 3 (apoptosis marker) were scored by microscopic evaluation on a scale of 0-3+.