The Novel ATP-Competitive Inhibitor of the MET Hepatocyte Growth Factor Receptor EMD1214063 Displays Inhibitory Activity against Selected MET-Mutated Variants

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Abstract

The receptor tyrosine kinase MET is a prime target in clinical oncology due to its aberrant activation and involvement in the pathogenesis of a broad spectrum of malignancies. Similar to other targeted kinases, primary and secondary mutations seem to represent an important resistance mechanism to MET inhibitors. Here, we report the biologic activity of a novel MET inhibitor, EMD1214063, on cells that ectopically express the mutated MET variants M1268T, Y1248H, H1112Y, L1213V, H1112L, V1110I, V1206L, and V1238I. Our results show a dose-dependent decrease in MET autophosphorylation in response to EMD1214063 in five of the eight cell lines (IC50 2–43 nmol/L). Blockade of MET by EMD1214063 was accompanied by a reduced activation of downstream effectors in cells expressing EMD1214063-sensitive mutants. In all sensitive mutant-expressing lines, EMD1214063 altered cell-cycle distribution, primarily with an increase in G1 phase. EMD1214063 strongly influenced MET-driven biologic functions, such as cellular morphology, MET-dependent cell motility, and anchorage-independent growth. To assess the in vivo efficacy of EMD1214063, we used a xenograft tumor model in immunocompromised mice bearing NIH3T3 cells expressing sensitive and resistant MET-mutated variants. Animals were randomized for the treatment with EMD1214063 (50 mg/kg/d) or vehicle only. Remarkably, five days of EMD1214063 treatment resulted in a complete regression of the sensitive H1112L-derived tumors, whereas tumor growth remained unaffected in mice with L1213V tumors and in vehicle-treated animals. Collectively, the current data identifies EMD1214063 as a potent MET small-molecule inhibitor with selective activity towards mutated MET variants. Mol Cancer Ther; 12(11); 2415–24. ©2013 AACR.

Introduction

During the last three decades, deregulated activity of the hepatocyte growth factor (HGF)/HGF receptor MET system has been strongly correlated with the pathogenesis of numerous human malignancies (reviewed in refs. 1–3). Consequently, MET is considered a significant molecular target in cancer therapy. Current understanding of the role of the MET receptor tyrosine kinase (RTK) signaling supports three main classes of therapeutic approaches to inhibit its activity in clinical setups: (i) interventions on the receptor–ligand interaction level by HGF or MET monoclonal antibodies or use of HGF analogs, (ii) inhibition of the tyrosine kinase activity by small-molecule inhibitors, and (iii) blockade of the receptor downstream association with its signaling adaptors and effectors (4).

In addition to dysregulated paracrine/autocrine loops and MET gene overexpression, which are among the most prevalent mechanisms that contribute to deregulated function of the MET/HGF system, MET oncogenic activity could be unleashed also by activating mutations in the tyrosine kinase domain of the receptor. Tyrosine kinase domain mutations in the MET gene were first identified in hereditary renal papillary cell carcinoma (5) and subsequently were described also in the sporadic form of this disease as well as in head and neck squamous cell carcinoma, hepatoblastoma, and glioma (reviewed in ref. 6). Generally, the presence of these mutations was shown to be sufficient to increase MET tyrosine kinase activity in a ligand-independent manner with subsequent increase in cellular proliferation, motility, and in vivo metastatic potential (5, 7). MET tyrosine kinase domain structural...
studies indicate that activating mutations interfere with the receptor autoinhibition (8) and that various mutations have different biologic effects, presumably due to coupling to distinct downstream effectors, consequently activating discrete signaling cascades (9–11).

In the last 10 years, the development of specific small-molecule tyrosine kinase inhibitors (TKI), which target the catalytic activity of the receptor primarily, but not only, via competing with ATP on its binding to the ATP pocket of the kinase domain, is critical for successful treatment of MET-driven tumors. First attempts to identify ATP-competitive MET inhibitors led to the characterization of the staurosporine analog K252a, a broad-spectrum kinase inhibitor that blocks MET kinase activity at submicromolar concentrations (12). Later, the highly selective indole-2-one core anti-MET small-molecule compounds SU11274 and PHA665752 were discovered and developed by SUGEN, but their limited pharmacokinetic properties restricted the use of these inhibitors to in vitro and limited in vivo studies (13–16). However, the encouraging observations that were made with these two prototypic tool molecules stimulated rapid development of new generation available anti-MET compounds whose integration in clinical practice is highly awaited.

Over the last decade, accumulating clinical experience with TKIs that target primarily BCR-ABL, KIT, and EGFR receptor (EGFR) have proven that the efficacy of these inhibitors in imposing tumor growth control could be significantly compromised by primary or secondary mutations, leading respectively either to resistance to initial treatment response or to tumor relapse (17–19). These observations have important implications in the case that similar resistance would be also observed with MET inhibitors, which are currently evaluated in clinical trials.

Approximately 20 different selective as well as nonspecific MET TKIs have been reported so far (6, 20), of which about 10 are currently in different phases of clinical trials (21). In that category, EMD1214063 and EMD1204831 are selective, reversible, and highly potent indole-2-one core oral ATP-competitive inhibitors of the MET kinase domain, which have recently completed phase I clinical trial (22–24). As a recent study by Bladt and colleagues reported the impact of this novel and highly potent MET inhibition by EMD1214063.

Our results identify five of the eight MET variants as responsive to EMD1214063 based on its ability to block MET autophosphorylation with IC_{50} values ranging between 2.2 and 43 nmol/L. MET kinase inhibition was associated with reduction of downstream signaling and MET-driven biologic activities. Three of the MET mutated variants, Y1248H, L1213V, and V1206L did not exhibit kinase inhibition even at a concentration of 75 nmol/L. An in vivo mouse subcutaneous model confirmed the in vitro observations with cells expressing the L1213V variant that maintained growth when compared with H1112L-derived tumors, which displayed a dramatic shrinkage following only five sessions of daily 50 mg/kg EMD1214063 administration.

Materials and Methods

Cell lines

The construction of the vectors expressing the MET mutations M1268T, Y1248H, H1112Y, L1213V, H1112L, V1110I, V1206L, and V1238I was previously described and their identities were verified by sequencing of both strands of DNA in the region of interest (25). NIH3T3 cell lines that stably express these MET-mutated variants were provided by Dr. Laura Schmidt (National Cancer Institute, Frederick, MD). Cells were maintained in Dulbecco’s Modified Eagle Medium (GIBCO, Invitrogen Corp.) supplemented with 10% fetal calf serum (Sigma), antibiotic–antimycotic, and 0.5 mg/mL Geneticin/G-418 sulfate (GIBCO, Invitrogen Corp.) and used for experiments within 2 months being in culture.

EMD1214063

EMD1214063 (3-(1-(3-(5-(1-Methylpiperidin-4-ylmethoxy)-pyrimidin-2-yl)-benzyl)-1,6-dihydro-6-oxo-pyridazin-3-yl)-benzonitrile) was obtained from Merck, diluted in DMSO and kept at –20 °C. Working solutions were prepared freshly in the corresponding media.

Western blot analysis and antibodies

Cells were treated with EMD1214063 as indicated in the legends to the figures and lysed in a lysis buffer containing 1% Triton X-100, 0.5% NP-40, 1 mmol/L EGTA, 1 mmol/L EDTA, 150 mmol/L NaCl, 10 mmol/L Tris-HCl, 1 mmol/L Na2VO4, 10 mmol/L NaF, 1 mmol/L ZnCl2, 50 μmol/L Na2MoO4, and a cocktail of proteases inhibitors (Complete Mini, Roche). Cell extracts were cleared by centrifugation and total protein concentration was determined by using the Bio-Rad protein quantification reagent (Bio-Rad Laboratories, Inc.) and data were processed by Excel (Microsoft) to obtain IC_{50} values for MET kinase inhibition by EMD1214063.

Rabbit polyclonal anti-phospho-MET (Tyr1234/1235) and mouse monoclonal anti-phospho-AKT (Ser473) antibodies were from Cell Signaling (Cell Signaling Technology, Inc.). The rabbit polyclonal anti-MET and anti-phospho-PLCγ1 (Tyr783) antibodies were purchased from Santa Cruz (Santa Cruz Biotechnology, Inc.). The mouse monoclonal anti-β-Actin and the rabbit polyclonal anti-

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phospho-ERK1/2 antibodies were purchased from Millipore (Chemicon and Upstate, respectively).

**Cell-cycle analysis by flow cytometry**

Cells were plated in 6-well plates and the next day treated overnight with 30 nmol/L EMD1214063. After treatment, cells were trypsORIZED, washed, fixed with 70% ethanol in PBS, pH 7.4, and stored at +4°C. On the day of measurements, fixed cells were treated with 100 μL of RNase A + Propidium iodide (PI) cocktail (0.1% Triton-X100, 20 μg/mL PI, 400 μg/mL RNase A in PBS) at 4°C for 60 minutes and subsequently analyzed with a FACScan (Becton Dickinson). The results shown represent the mean of three independent experiments, ± SEM, and were determined using the FlowJo software.

**Wound healing assay**

Cells were seeded in a 6-well plate (2 × 10⁵ cells/well) and treated with 50 nmol/L EMD1214063 as indicated in the legend to the figure. After 4 hours of treatment, a portion of the monolayer was scratched with a pipette tip. Cells were examined for resealing of the "wounded" monolayer 24 hours later by staining with 0.005% crystal violet in PBS, pH 7.4, for 3 minutes before photodocumentation with a Leica DC 300F camera (4× objective). Analysis was done using a Leica IM-50 Software. In addition, EMD1214063-mediated changes in cellular morphology were observed by microscopy, documented with a Leica DC 300F camera (10× objective), and processed with the Leica IM-50 software at the same time-points.

**Growth in soft agarose**

Cells were plated in 6-well plates and 24 hours later, 15 nmol/L of EMD1214063 or DMSO as a negative control was added. One day after addition of EMD1214063 or DMSO, the cells were harvested by trypsinization, counted, and plated on seaplaque agarose (1% for bottom layers and 0.55% for top layers, ± 15 nmol/L EMD1214063). After solidification, plates were incubated until macroscopic colonies were visible (average period of 18 days) and gels were further supplemented with 15 nmol/L of EMD1214063 or DMSO every 7 days. Colonies were stained by adding 0.5 mL of 0.005% crystal violet per well for overnight, excess solution was aspirated and caliper measurements, excess solution was aspirated and pictures taken with a digital camera. Colonies were counted using the Bio-Rad Quantity One software.

**In vivo studies**

NIH3T3 expressing MET-mutated variants were transduced by the lentivirus pCR-XL-FLUL that expresses the firefly luciferase (Luc), which was constructed by Dr. Mario Tschan, Department of Pathology, University of Bern, Bern, Switzerland.

A total of 20,000 Luc-expressing NIH3T3 MET H1112L or L1213V cells were injected subcutaneously into the right flank of 16-week-old female Rag2 common gamma-null mice and the growth of the tumors was regularly monitored by luminescence using the NightOwl and by caliper measurements. As soon as all the tumors became visible and palpable, mice were randomly divided into the treatment by TKI (50 mg/kg of EMD1214063 per day) or the control (vehicle only, Solutol HS 15, BASF ChemTrade GmbH) group, treated per os for 5 consecutive days and following 2 days without treatment. After 5 days of treatment, tumors were visualized and measured as aforementioned.

**IC₅₀ values**

The inhibitory effects of EMD1214063 (0–1 μmol/L) on MET autophosphorylation (Tyr1234/1235; pMET) were inferred from Western blot analysis. The IC₅₀ values were obtained following densitometry of pMET bands normalized to β-Actin expression. A least-squares fit of the observed data with the response function

\[ r(c) = r_0 + (1 - r_0) / (1 + (c / IC_{50})^k) \]

where \( r_0 \) is the baseline relative autophosphorylation, IC₅₀ is the half maximal effective concentration, and \( k \) is the Hill coefficient, was conducted.

**Statistical analysis**

Statistical significance was calculated using the Student t test, \( P < 0.05 \) were considered significant. Where not indicated, the data represent the mean of at least three independent experiments ± SEM.

**Results**

**Effect of EMD1214063 on MET phosphorylation and its downstream signaling**

Initially, we tested the capacity of the specific MET small-molecule TKI EMD1214063 (Fig. 1A) to reduce the receptor Tyr1234/1235 autophosphorylation in NIH3T3 cell lines, which stably express the M1268T, Y1248H, H1112Y, L1213V, H1112L, V1110I, V1206L, and V1238I MET-mutated variants (Fig. 1B). Calculation of the corresponding IC₅₀ values yielded values of 42.6 nmol/L for H1112L, H1112Y, M1268T, V1206L, and V1238I

values were in good agreement with data from a recent study by Bladt and colleagues who reported an average IC₅₀ of 23 nmol/L for inhibition of tyrosine autophosphorylation of the wild-type form of MET (22). On the other hand, the V1206L, L1213V, and Y1248H variants displayed considerably higher IC₅₀ values of 224.0, 270.1, and 42.6 nmol/L for H1112L, H1112Y, M1268T, V1238I, and V1110I, respectively (Fig. 1C and Table 1). These values are in good agreement with data from a recent study by Bladt and colleagues who reported an average IC₅₀ of 23 nmol/L for inhibition of tyrosine autophosphorylation of the wild-type form of MET (22). On the other hand, the V1206L, L1213V, and Y1248H variants displayed considerably higher IC₅₀ values of 224.0, 270.1, and >1,000 nmol/L, respectively (Fig. 1C and Table 1).

The decrease in MET activation by EMD1214063 within a range of up to 75 nmol/L was further associated with an inhibition of the activation state of the MET downstream signaling molecules AKT, ERK, and PLCγ (Fig. 2), albeit with particular differences between the highly sensitive variants. Thus, while reduction in ERK1/2 phosphorylation was most evident in the case of variants M1268T and H1112L, a rather more moderate decrease was detected in cells expressing the H1112Y variant. Similar observations...
were made in respect with the phosphorylation status of PLCγ. Although the molecular basis for this phenomenon has yet to be determined, similar findings have been previously reported with respect to the effect of the MET tool TKI SU11274 on the phosphorylation status of PLCγ Y783 in different MET variants (26).

Table 1. IC₅₀ values for MET autophosphorylation (Tyr1234/1235) in eight studied MET-mutated variants

<table>
<thead>
<tr>
<th>MET-mutated variant</th>
<th>M1268T</th>
<th>Y1248H</th>
<th>H1112Y</th>
<th>L1213V</th>
<th>H1112L</th>
<th>V1110I</th>
<th>V1206L</th>
<th>V1238I</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ (nmol/L)</td>
<td>17.3</td>
<td>&gt;1000</td>
<td>6.9</td>
<td>270.1</td>
<td>2.2</td>
<td>42.6</td>
<td>224.0</td>
<td>26.1</td>
</tr>
</tbody>
</table>

NOTE: The values are based on least-squares fits shown in Fig. 1C.

Figure 1. A, structures of EMD1214063 (MSC2156119) and EMD1204831 (MSC2156996). B, impact of increasing concentrations of EMD1214063 (16 hours) on MET autophosphorylation (Tyr1234/1235) levels in NIH3T3 mouse fibroblast cells stably expressing MET-activating point mutations M1268T, Y1248H, H1112Y, L1213V, H1112L, V1110I, V1206L, and V1238I. C, impact of EMD1214063 treatment (0–1 mmol/L; for 16 hours) on MET autophosphorylation inferred from Western blotting analysis (Fig. 1B) and the least-squares fit of the values at different concentrations with the dose response function. Resulting values for IC₅₀ are reported in Table 1.
Impact of EMD1214063 on MET-Mutated Variants

Contrary to the observations with M1268T, H1112Y, H1112L, V1238I, and V1110I, EMD1214063 doses up to 75 nmol/L did not display any inhibition of the MET enzymatic activity in cells expressing the Y1248H, L1213V, and V1206L mutants as judged from the levels of phosphorylated MET. Consequently, EMD1214063 also did not alter the activation of the aforementioned MET downstream signaling effectors in these three cell lines, suggesting their resistance to this drug. Determination of the IC50s of EMD1214063 for autophosphorylation blocking of these variants resulted in values of 224.0, 270.1, and >1,000 nmol/L for V1206L, L1213V, and Y1248H, respectively (Fig. 1C and Table 1).

EMD1214063 selectively attenuates MET-dependent cell-cycle progression of cell lines expressing drug-sensitive forms of the MET receptor

To assess the biologic consequences of the inhibition of MET-driven signaling pathways by EMD1214063, we first investigated its impact on cell-cycle distribution in the cell lines expressing the various MET variants. As the results of Fig. 3 suggest, variants V1238I, M1268T, and H1112L exhibited the highest sensitivity to 30 nmol/L of EMD1214063 in terms of growth arrest as manifested by 57%, 47%, and 43% respective reduction in S-phase cells. All these three cell lines displayed a concomitant accumulation of cells in G1, indicating a G1 arrest. Cells expressing the H1112L and V1110I variants showed a 30% reduction of cells found in S-phase, whereas V1206L cells showed a very moderate reduction in S-phase and a similar increase in G1 content. On the other hand, no changes in cell-cycle progression in terms of G1 and S-phase populations were observed in Y1248H and L1213V cells treated with the EMD1214063, indicating a resistance of these MET variants.

In addition, data from a viability assay (Supplementary Materials and Methods) show that cell-cycle arrest is also accompanied by increases in cell death upon EMD1214063 treatment for 48 hours, M1268T, and V1238I being the most sensitive variants with 9.6- and 7.4-fold increase, respectively (Supplementary Fig. 1; Supplementary Table S1).

EMD1214063 impacts cellular morphology, motility, and anchorage-independent growth in drug-sensitive MET variants

Signaling pathways that are activated by MET not only promote proliferation but also cellular transformation, motility, migration and invasion, which are crucially implicated in tumor progression. Therefore, we studied the effects of MET inhibition by EMD1214063 on biologic endpoints, in particular on cellular morphology, motility, and anchorage-independent growth.

The morphology of the cells was examined following exposure to 50 nmol/L of EMD1214063 for 24 hours. As can be seen in Fig. 4A, of the eight lines that express the constitutively active MET mutations, M1268T, H1112Y, and V1238I cells exhibited considerable morphologic changes such as cell flattening and resumption of cell-cell contact upon exposure to EMD1214063. Similar changes, although at lesser extent, were seen in cell lines expressing the variants H1112L and V1110I. A minor effect of EMD1214063 was seen with V1206L cells. On the other hand, the transformed morphology of cells expressing the MET mutants Y1248H and L1213V did not show any alterations following EMD1214063 treatment. In that respect, the lack of MET downstream signaling inhibition by EMD1214063 seems to be very well correlated with cell scattering and motility, as evaluated by in vitro wound healing/scratch assay. Predictably, the motility of the five sensitive MET mutants was either completely hindered as in the case of M1268T, H1112Y, H1112L, V1238I, or considerably diminished in the case of V1110I (Fig. 4B). As expected, in the case of the three resistant mutations (Y1248H, L1213V, and V1206L), EMD1214063 had almost no effect on the wound closure and, similar to the controls, the scratches were hardly visible due to effective cell invasion.

As MET RTK signaling is required for invasiveness of MET-expressing tumor cells, we next studied the ability of EMD1214063 to reduce anchorage-independent growth of MET mutated variants. Taking into consideration the IC50 values of EMD1214063 to inhibit MET tyrosine phosphorylation (the IC50 values were ranging between 2 and 43 nmol/L), we decided to use 15 nmol/L of EMD1214063 to assess its effects on cellular growth in soft agar. Surprisingly, at this concentration, there was a consistent and remarkable reduction in colony formation in all
EMD1214063-sensitive MET-mutated variants (Fig. 5). However and again, no hindrance of this feature was observed in Y1248H and L1213V variants.

**EMD1214063 represses tumor growth in a xenograft animal model**

To test the efficiency of the MET inhibitor EMD1214063 in vivo and based on our in vitro data, we established a mouse xenograft tumor model using NIH3T3 cells expressing MET-mutated variants H1112L and L1213V. We have chosen to use these mutants as we have detected more than 120-fold difference for in vitro IC$_{50}$ values between H1112L and L1213V (2.2 vs. 270.1 nmol/L, respectively). We injected 20,000 of tumor cells expressing Luc gene into the right flank of a Rag2 common gamma-null mice and let them to form palpable and visible tumors. Animals bearing H1112L or L1213V tumors were randomized for the treatment with 50 mg/kg/d of EMD1214063 or vehicle.
only. Surprisingly, after only 5 days of treatment with EMD1214063, the H1112L tumors completely regressed (Fig. 6). On the other hand, tumor growth continued in the vehicle-treated mice with H1112L tumors as well as in mice bearing the L1213V tumors (both vehicle- and EMD1214063-treated). These results strongly support the data obtained in in vitro studies and show the enormous capacity of EMD1214063 to inhibit certain forms of deregulated MET.

Discussion

Primary and secondary acquired resistances to MET small-molecule inhibitors result from preexisting and newly appearing point mutations, respectively. This observation follows the accumulating experience with other TKIs that target molecular pathways such as those related to EGFR and ABL signaling (27, 28). As already ascertained for Gleevec in chronic myelogenous leukemia and gastrointestinal stromal tumors, information related to presence of mutations and response of particular variants to given inhibitors is critical for treatment outcome. Since first described in 1997 (5), approximately 30 MET mutated variants have been so far reported (29). Nonetheless, it is rather surprising to note that little data are available that systematically documents their responsiveness towards selective and nonselective MET TKIs, which are presently evaluated in clinical trials (21).

In the current study, we used cells expressing mutated MET variants to characterize the inhibitory capacity of EMD1214063, a novel selective and reversible ATP competitor that targets the MET receptor. We report that EMD1214063 showed effective inhibition of MET tyrosine autophosphorylation of five of the eight MET variants analyzed in this study. Sensitive MET mutations targeted with EMD1214063 resulted in a loss of cellular proliferation, change in cell-cycle redistribution, motility impairment, and reduced the ability of tumor cells to grow independently of an anchorage as well as to sustain tumor growth in an in vivo xenograft model.

IC50 values for the studied mutants ranged between 2.2 and 43 nmol/L for the H1112L and V1110I variants, respectively. In this regard, EMD1214063 displays anti-MET IC50 values, which are in the range of crizotinib, a MET small molecule that is currently in phase III clinical trials (30). These figures are also in agreement with IC50 values of EMD1214063 towards the wild-type form of MET as recently reported (22). Intriguingly, and with respect to inhibition of downstream MET-dependent signaling, we observed variations between the different variants. For example, while reduction in tyrosine phosphorylation of PLCγ is evident in cells expressing the

Figure 4. A, EMD1214063 treatment induces selective and differential reversion of transformed morphologies of NIH3T3 cells stably expressing mutated MET receptor. B, MET inhibition by EMD1214063 causes selective inhibition of spontaneous cell motility in a scratch assay. Experiments were carried out independently at least three times; representative pictures are shown.

Figure 5. EMD1214063 selectively affects anchorage-independent growth of eight distinct MET-mutated variants. The columns represent the average number of colonies (±SEM) of six repetitions as a percentage of the corresponding controls.
M1268T and V1238I variants, H1112Y and H1112L cells display only a moderate reduction in phosphorylation of this MET signaling effector. Similar observations for this phenomenon, which remains still to be clarified, have been previously reported with the MET tool small-molecule inhibitor SU11274 (26).

As EMD1214063 at a daily dose of 50 mg/kg has been recently shown to very effectively reduce in vivo tumor growth of human KP-4 pancreatic tumor cells that express wild-type MET (22), we have used this dosage in our current mouse model. Interestingly, our data underscore a compelling activity of EMD1214063 on the responsive MET-mutated variant H1112L in vivo, which indicated dramatic tumor shrinkage within only 5 days of overall treatment, which are by far more pronounced comparing the effects detected with this drug in the in vitro assays. We tend to attribute this observation to potential effects that the inhibitor may exert on MET-dependent tumor–stroma interactions, which largely mediate tumor growth and progression (31–33).

Of major importance is our observation that the autophosphorylation status of the MET-mutated variants Y1248H, L1213V, and V1206L displays a rather resistant-like response towards EMD1214063. In that respect, Y1248H and L1213V exhibit a similar resistant pattern towards EMD1214063 as to the MET tool compound.

![Image of Figure 6](https://example.com/figure6.png)

**Figure 6.** The specific MET inhibitor EMD1214063 leads to regression of the MET-driven xenograft drug-sensitive tumors bearing the H1112L MET mutation but not the drug-resistant MET mutants L1213V. Tumor growth was evaluated by both luminescence and caliper measurements.
which apart of MET targets also VEGFR2, RET, KIT, FLT3, that the nonselective class II inhibitor, cabozantinib, recent study, Yakes and colleagues provided evidence unlike BCR-ABL, KIT and EGFR, for which mutations for MET-driven and MET-positive tumors. respect to their emerging significance in targeted therapy stream signaling seem to be unique to this RTK system. variants developed tumors of different histotypes (41). who showed that knock-in mice expressing MET-mutated diverse biologic consequences (10, 37–40). These observa-
couple with different signaling pathways, leading to not activated by the wild-type receptor (9). This is further supported by the observation that MET mutants can couple with different signaling pathways, leading to diverse biologic consequences (10, 37–40). These observa-
tions also support the findings by Graveel and colleagues who showed that knock-in mice expressing MET-mutated variants developed tumors of different histotypes (41). The specific complexity of MET mutations and down-stream signaling seem to be unique to this RTK system. Moreover, their importance is further manifested with respect to their emerging significance in targeted therapy for MET-driven and MET-positive tumors.

Finally, it is probably worthwhile mentioning that unlike BCR-ABL, KIT and EGFR, for which mutations that confer resistance to targeted therapies were first identified in the clinic due to lack of tumor response or relapse, MET inhibitors still have not enter routine clinical practice. Yet, the high number of already identified MET variants along with experience made with the aforementioned clinical targets strongly suggest for an early screening and characterization of responses of clinically-relevant MET TKIs for particular mutations, similar to the current study with EMD1214063.

Finally, to better elucidate the molecular basis for the currently reported observations, structural studies that use crystallographic approaches will have to be carried out using these MET mutants in combination with EMD1214063.

Disclosure of Potential Conflicts of Interest
A. Blaukat has ownership interest (including patents) in Merck KGaA. F. Bladt is employed as a Director of Biomarker Discovery (other than primary affiliation; e.g., consulting) in Merck Serono and has ownership interest (including patents) in EMD1214063 patents. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Medová, B. Pochon, B. Streit, P. Franzica, D. Stroka, A. Keogh
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Medová, B. Pochon, B. Streit, D. Stroka, Y. Zimmer
Writing, review, and/or revision of the manuscript: M. Medová, D. Stroka, A. Blaukat, F. Bladt, Y. Zimmer
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Study supervision: Y. Zimmer

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