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Acquired *MET* D1228V mutation and resistance to MET inhibition in lung cancer

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Abstract

Amplified and/or mutated MET can act as both a primary oncogenic driver and as a promoter of tyrosine kinase inhibitor (TKI) resistance in non-small cell lung cancer (NSCLC). However, the landscape of MET-specific targeting agents remains underdeveloped and understanding of mechanisms of resistance to MET TKIs is limited. Here we present a case of a patient with lung adenocarcinoma harboring both a mutation in *EGFR* and an amplification of *MET*, who after progression on erlotinib, responded dramatically to combined MET and EGFR inhibition with

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Conflicts of Interest:

GRO has received consulting fees from AstraZeneca, Ariad, Boehringer-Ingelheim, Genentech, Inivata; and honoraria from AstraZeneca and Boehringer-Ingelheim. PAJ has received consulting fees from Astra Zeneca, Boehringer-Ingelheim, Pfizer, Roche/Genentech, Merrimack Pharmaceuticals, Chugai, Ariad Pharmaceuticals, Ignyta and LOXO Oncology; receives post-marketing royalties from DFCI owned intellectual property on EGFR mutations licensed to Lab Corp; is under sponsored research agreement with AstraZeneca; and has stock ownership in Gatekeeper Pharmaceuticals. CPP has received honoraria from Clovis Oncology and BioRad. MMA has received consulting fees from Abbvie, Ariad, Clovis, Pfizer, Nektar, Genentech, AstraZeneca, Bristol Myers Squibb, and Merck. AGS has received travel funding from AstraZeneca and Genentech-Roche. LMS has received consulting fees from Genentech. All remaining authors have no conflicts of interest.

savolitinib and osimertinib. When resistance developed to this combination, a new *MET* kinase domain mutation, D1228V, was detected. Our *in vitro* findings demonstrate that *MET* D1228V induces resistance to type I MET TKIs through impaired drug binding while sensitivity to type II MET TKIs is maintained. Based on these findings, the patient was treated with erlotinib combined with cabozantinib, a type II MET inhibitor, and exhibited a response.

Keywords

Lung cancer; drug resistance; mutation; MET proto-oncogene; receptor tyrosine kinase

Introduction

The majority of patients with advanced non-small cell lung cancer (NSCLC) harboring mutations in the epidermal growth factor receptor (EGFR) gene can initially be successfully treated with EGFR tyrosine kinase inhibitors (TKIs); however, the emergence of resistance to these agents is inevitable in most patients (1). Several mechanisms of resistance have now been clinically recognized, the most common being the *EGFR* T790M mutation, which can be successfully treated with third-generation EGFR TKIs like osimertinib (2). *MET* amplification, which can occur as an acquired or *de novo* resistance mechanism, is a second well-established resistance mechanism known to bypass EGFR inhibition and impart resistance to EGFR TKIs, and as such requires a therapeutic strategy directed at both EGFR and MET (3). Indeed, patients with such signature of TKI resistance have benefited from various off-label combinations of EGFR TKIs and the MET inhibitor crizotinib (4).

The *MET* oncogene has long been a candidate for drug development in NSCLC but with modest success. Notably, multiple phase III trials of agents aiming to target MET signaling have recently failed (5-7). However, the identification of oncogenic *MET* alterations in subsets of lung cancers has again led to increased interest in targeting this oncoprotein. Three scenarios now exist in which oncogenic *MET* alterations have been described as potentially targetable – *de novo* amplification of *MET* (8), exon 14 skipping mutations in *MET* (9), and acquired amplification of *MET* after resistance develops to EGFR TKI (3).

Multiple MET TKIs are in development for advanced NSCLC, but much of the clinical data on drug activity is preliminary. Like many other TKIs, MET inhibitors fall into two functionally distinct classes: type I inhibitors which preferentially bind the active conformation of MET, such as crizotinib, and type II inhibitors which bind the inactive conformation, such as cabozantinib (10). Type II inhibitors often inhibit a broader array of kinase targets, potentially resulting in more off-target side effects. While the availability of multiple MET inhibitors lends itself to sequential targeted therapies, specific molecular factors impacting drug sensitivity and resistance have not been well characterized.

Here, we describe a patient with metastatic NSCLC with MET-mediated resistance to EGFR TKI who responded to treatment with a type I MET inhibitor, savolitinib (11), given in combination with a third-generation EGFR inhibitor, osimertinib (2). The patient then developed acquired resistance mediated by a novel MET kinase domain mutation.

Results

Case report

The patient was a 30 year-old female never-smoker who presented with dyspnea and was found to have a large left upper lobe mass and mediastinal adenopathy. Bronchoscopic biopsy demonstrated lung adenocarcinoma positive for an *EGFR* exon 19 deletion mutation. Due to the extent of locally advanced disease and the presence of a potentially targetable genotype, she was initiated on induction afatinib prior to definitive therapy. Surprisingly, scans after 4 weeks demonstrated mild growth of the lung mass. She was switched to induction chemotherapy with cisplatin and pemetrexed and had a radiographic response, which was then followed by concurrent chemoradiation. Unfortunately, she developed progression as she was completing radiation with a growing left cervical lymph node. This was biopsied and demonstrated recurrent lung adenocarcinoma. Imaging further demonstrated new bone metastases. She received a brief course of erlotinib but her disease continued to progress (Fig. 1A).

Targeted next-generation sequencing (NGS) of the patient's biopsy at recurrence confirmed the presence of the known *EGFR* exon 19 deletion and additionally identified high level *MET* amplification, potentially explaining her drug resistance (Supplementary Fig. S1A); no other resistance mechanisms, including *EGFR* T790M, were detected. The patient then enrolled in a phase I clinical trial (NCT02143466) combining the MET TKI savolitinib (800mg once daily) with the mutant-selective EGFR TKI osimertinib (80mg once daily). She experienced a dramatic clinical response, with near complete resolution of progressive cervical lymphadenopathy after 4 weeks of treatment (Fig. 1B). However, after 36 weeks on therapy, the patient developed a new growing pulmonary nodule consistent with objective disease progression (Fig. 1C). The lung nodule was removed surgically in an attempt at achieving local control of her progressive disease, but the patient had further disease progression in the lung and was removed from study therapy.

Tumor sequencing identifies a secondary MET kinase domain mutation

In search of genomic changes mediating resistance to combined EGFR and MET inhibition, we performed targeted next-generation sequencing (NGS) of the biopsy obtained following the development of resistance to savolitinib and osimertinib. Tumor NGS confirmed the high level *MET* amplification and the *EGFR* exon 19 deletion which both had been present on prior tumor NGS (Supplementary Fig. S1A-B). In addition, tumor NGS found 8 variants present at >5% allelic fraction (AF), 6 of which were also detected in a biopsy specimen from before treatment with savolitinib and osimertinib (Supplementary Table S1). Of the 2 acquired variants detected, one affected the *MET* kinase domain, D1228V (3683A>T), and was selected as a candidate variant for explaining drug resistance (Supplementary Fig. S1C).

Serial plasma genotyping detects an acquired MET mutation as resistance developed

We performed serial genotyping of plasma cfDNA collected during the treatment period using droplet digital PCR (ddPCR). Plasma ddPCR detected high levels of the *EGFR* exon 19 deletion prior to therapy and rapid reduction of these levels after therapy was started, with levels becoming undetectable after 12 weeks of treatment (Fig. 1D). At the time of disease

progression (36 weeks), we could again detect the *EGFR* exon 19 deletion. We developed a new ddPCR assay for *MET* D1228V and found that this mutation emerged concurrently with the progression of the *EGFR* exon 19 deletion in plasma. Levels of mutant *EGFR* and *MET* in plasma continued to increase as the patient progressed further on post-progression therapy (Fig. 1C).

Protein modeling predicts differences in drug binding between type I and type II MET inhibitors

We developed models of the wildtype and mutant MET protein to study drug binding. Savolitinib, just as with other type I MET TKIs, binds to the MET kinase in its active conformation. Our modeling demonstrates that the binding with wildtype MET relies upon a key π-π interaction with Tyr1230, present in the MET kinase activation loop (Fig. 2A). MET Asp1228 and Lys1110 residues are critical for maintaining the activation loop in a position conducive to savolitinib binding through their strong electrostatic interactions. The presence of the D1228V mutation interrupts the salt bridge connecting Asp1228 and Lys1110, which leads to the repositioning of the activation loop and loss of the π-π interaction between MET and savolitinib (Fig. 2B), predicting impaired drug binding. We subsequently modeled the binding of the MET kinase to cabozantinib, a type II inhibitor, which binds MET in its inactive conformation. The binding to wildtype MET does not rely on the availability of Tyr1230 (Fig. 2C), and drug binding is unaffected by the D1228V mutation (Fig. 2D).

In vitro studies demonstrate that MET D1228V induces resistance to type I but not type II MET inhibitors

To validate our protein modeling studies, we performed a signaling analysis in 293T cells engineered to express either full-length wildtype MET or full-length MET D1228V. We found that both type I and II MET inhibitors are able to inhibit autophosphorylation of wildtype MET. However, the MET D1228V mutation rendered all type I MET inhibitors ineffective, as evidence by the failure of savolitinib, crizotinib, and INC280 to inhibit MET phosphorylation (Fig. 3A). In contrast, type II MET inhibitors cabozantinib, MGCD265, and merestinib (LY2801653) were able to effectively suppress MET phosphorylation in the presence of the D1228V mutation. To further validate our biochemical findings, we introduced the oncogenic *TPR-MET* fusion (TPR-MET^{WT}) or *TPR-MET* harboring the D1228V mutation (TPR-MET^{D1228V}) into Ba/F3 cells or into the *EGFR* mutant EGFR TKI-sensitive PC9 cell line, and analyzed the ability of savolitinib alone, cabozantinib alone, or either agent in combination with gefitinib to inhibit viability and/or downstream effectors of the MET and EGFR signaling axes (Fig. 3B-D). The TPR-MET^{WT} Ba/F3 cells were highly sensitive to both savolitinib or cabozantinib in the dose-escalated MTS viability assay (Fig. 3B). In contrast, the TPR-MET^{D1228V} Ba/F3 cells failed to respond to savolitinib but maintained sensitivity to cabozantinib (Fig. 3B). In addition, the introduction of the WT or the D1228V mutant *TPR-MET* fusion into PC9 cells (Supplementary Fig. S2) imparted resistance to gefitinib in both cases as expected (Fig. 3C), but the D1228V mutant showed a differential response to the combination of gefitinib and savolitinib, as compared to the wild type. While the TPR-MET^{WT} PC9 cells responded to gefitinib in combination with either savolitinib or cabozantinib, the D1228V mutant was only sensitive to the gefitinib and

cabozantinib combination (Fig. 3C). A Western blot analysis of the EGFR and MET signaling in the TPR-MET bearing PC9 cell derivatives further supported our cell viability studies. In the TPR-MET^{WT} PC9 cells, phosphorylation of both EGFR and MET along with the MAPK/ERK pathway were completely inhibited following exposure to either the gefitinib/cabozantinib or gefitinib/savolitinib treatments (Fig. 3D). In contrast, the gefitinib/savolitinib combination failed to inhibit MET phosphorylation in the TPR-MET^{D1228V} PC9 cells, consequently resulting in sustained MAPK/ERK signaling. However, the Met D1228V mutant retained sensitivity to the gefitinib/cabozantinib combination, as evidenced by the suppression of MET and EGFR phosphorylation as well as inhibition of downstream signaling pathways (Fig. 3D). These cellular and biochemical studies further support our conclusions that Asp1228 is a critical residue for the binding of type I MET inhibitors, and that type II MET inhibitors may successfully address resistance caused by a mutation at this residue.

Patient response to treatment with a type II MET inhibitor

Based upon the preclinical data, we switched the treatment of the patient to an EGFR TKI combined with a type II MET inhibitor following development of acquired resistance to osimertinib/savolitinib. Given the reported tolerability of the combination of cabozantinib and erlotinib (12), the patient was started on off-label therapy with erlotinib (100mg once daily), which the patient's cancer had previously been refractory to, in combination with the type II MET inhibitor cabozantinib (60mg once daily). The patient soon felt symptomatic improvement in cough, though treatment was complicated by diarrhea requiring dose reduction of both agents. Scans after 4 weeks on combination therapy demonstrated a dramatic response in her progressive lung disease (Fig. 3E), and the patient continues to benefit from the combination 5 months following start of therapy.

Discussion

The *MET* oncogene represents an exciting therapeutic target in advanced NSCLC, with at least six MET TKIs currently in clinical development across the three different settings where genomic alterations suggest dependence upon oncogenic MET (Supplementary Table S2): de novo *MET* amplification, *MET* exon 14 skipping mutations, and *MET* amplification in resistance to EGFR TKIs. At least 8 different combinations of MET and EGFR TKIs are being investigated in the context of EGFR TKI resistance. Our finding of a *MET* kinase domain mutation that leads to resistance to type I but not type II MET TKIs highlights the potentially value of both classes of drugs in the management of NSCLC, as patients resistant to type I MET inhibitors may subsequently still respond to a type II MET inhibitor. Our findings are consistent with prior preclinical work using a mutagenesis-based resistance screen which found that MET mutations at D1228 cause resistance to NVP-BVU972 but not to AMG 458 (13). This is not dissimilar to the experience in chronic myelogeous leukemia, where type II 3rd generation Bcr-Abl kinase inhibitors (ponatinib, rebastinib) can overcome resistance to type I inhibitors (dasatinib, bosutinib) mediated by secondary mutations, including the gatekeeper mutation T315I (14, 15). Conversely, in gastrointestinal stromal tumors resistant to type II KIT inhibitors (imatinib), secondary *KIT* mutations can interfere with type II inhibitor binding while maintaining sensitivity to type I inhibitors (dasatinib,

crenolanib) (16, 17). While type II TKIs can have more off-target activity and treatment related side-effects, the alternate spectrum of activity may prove to be valuable for the management of drug resistance.

The emergence of the *MET* D1228V mutation following savolitinib treatment suggests effective on-target pressure against the MET kinase. Another mutation at this residue, D1228N, has also been recently reported in a patient with *MET*-driven NSCLC and acquired resistance to crizotinib, suggesting that this mechanism of resistance is likely to become a recurring clinical problem (18). The development of secondary kinase domain mutations, as has been seen for targeted therapies against EGFR, ALK, and ROS1 (19-21), often marks effective and specific inhibition of an oncogenic target. Indeed, the absence of such an on-target resistance mechanism can raise concern over effective target inhibition. For example, one study performed plasma cfDNA genotyping on 19 patients with acquired resistance to osimertinib; of 15 with detectable tumor DNA, 6 (40%) were positive for an *EGFR* C797S mutation, which impairs covalent binding of osimertinib to the EGFR protein (22). A more recent study performed plasma cfDNA genotyping of 43 patients with acquired resistance to rociletinib, another third-generation EGFR TKI; of 40 with detectable tumor DNA, only 1 (2.5%) was positive for *EGFR* C797S, perhaps indicating less effective on-target inhibition by this drug (23). Given that the commercially available multikinase MET TKIs, crizotinib and cabozantinib, received regulatory approval as agents targeting other oncoproteins, our finding of a secondary *MET* resistance mutation supports MET inhibition as the etiology of the dramatic response seen in cases like the one we present. Moreover, Asp1228 is highly conserved among receptor tyrosine kinases and may be a mutation hotspot in MET and other tyrosine kinases, as evidenced by prior reports of mutations at this residue (Table 1), warranting closer scrutiny when evaluating TKI resistance.

The development of MET inhibitors in advanced NSCLC has been challenging, with recent negative phase III trials for onartuzumab, a monoclonal antibody targeting MET (5), and tivantinib, a putative oral MET inhibitor (6, 24). Yet there is a surging interest in MET inhibition in NSCLC due to both the identification of oncogenic splice-site mutations near exon 14 of *MET* in ~3% of NSCLC (9), and due to the regulatory approval of osimertinib for T790M-positive resistance to EGFR TKI (2), which elevates interest in treatments for other resistance mechanisms like *MET* amplification (1). With such a range of MET inhibitors in active development, we are hopeful that an improved understanding of their activity and mechanisms of resistance will lead to more treatment options for the emerging populations of MET-driven lung cancers.

Methods

Patient

The patient provided written informed consent for treatment on trial NCT02143466. She additionally consented to a correlative protocol allowing genomic analysis of tumor and plasma specimens. Both studies were approved by the Dana-Farber Cancer Institute Institutional Review Board (IRB) and conducted in accordance with the Declaration of Helsinki.

Tumor and plasma analysis

Targeted NGS was performed at the Center for Advanced Molecular Diagnostics at Brigham and Women's Hospital using a 282-gene panel, as described previously (9). Genotyping of plasma cell-free DNA (cfDNA) was performed at the Belfer Center for Applied Cancer Science at the Dana-Farber Cancer Institute using droplet digital PCR (ddPCR). The development of the ddPCR assay for *EGFR* mutations has been described previously (25); a new ddPCR assay was developed and optimized for detection of *MET* D1228V (see Supplementary Methods).

Protein modeling

Based on the co-crystal structures of the MET kinase domain in complex with PF-04254644 (type I kinase inhibitor) or foretinib (type II kinase inhibitor), molecular docking studies of savolitinib and cabozantinib on the MET kinase domain (wildtype and D1228V mutant) were carried out using GLIDE module. Molecular dynamics simulations (500 ns isothermal and isobaric simulation) of the complexes of the MET kinase domain (wildtype and D1228V mutant) with these inhibitors (savolitinib and cabozantinib) were performed using Desmond software package (see Supplementary Methods).

Antibodies and Compounds

Antibody against phospho-MET (Tyr1234) was purchased from Santa Cruz Biotechnology; total-MET (D1C2) and anti-rabbit IgG-HRP from Cell Signaling Technology; α–tubulin from Sigma Aldrich; and anti-mouse IgG-HRP from GE Life Sciences. Savolitinib was kindly provided by AstraZeneca; crizotinib, cabozantinib, INCB28060 and MGCD-265 were purchased from Selleckchem; and merestinib from MedChem Express.

Expression vectors

Full length human *MET*, transcript variant 2, cDNA (NM_000245.2) was amplified from a banked tumor specimen with an unrelated genetic alteration, and the *TPR-MET* fusion was amplified from the pBABE-puro *TPR-MET* plasmid created by the lab of Dr. Robert Weinberg (Addgene plasmid # 10902). Both amplicons were subcloned into pDNR-dual (BD Biosciences) via the *HindIII* and *XHOI* restriction sites as described previously (26). The *MET* D1228V mutation was introduced using the QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies) and the following mutagenic primers: Forward: 5'-gctgattttggtcttgccagagtcatgtatgataaagaatactata-3'; Reverse: 5'-tatagtattctttatcatacatgactctggcaagaccaaaatcagc-3'. All constructs were confirmed by DNA sequencing. Constructs were shuttled into the lentiviral expression vector JP1698 or JP1722 using the BD Creator^{TM} System (BD Biosciences).

Cell lines

293T and PC9 cells were purchased from ATCC (in 2009) and Sigma Aldrich (in 2014), respectively. Ba/F3 cells were a generous gift from the lab of Dr. David Weinstock (in 2014). 293T cells were cultured in DMEM, supplemented with 10% FBS, streptomycin and penicillin. PC9 and transformed Ba/F3 cells were maintained in RPMI1640 supplemented with 10% FBS, streptomycin and penicillin. The human 293T and PC9 cell lines were

authenticated using the Promega GenePrint 10 System at the RTSF Genomics Core at Michigan State University in August 2016. Ba/F3 cells were not authenticated, since their STR profile has not been made publicly available. All cell lines used in the study tested negative for mycoplasma as determined by the Mycoplasma Plus PCR Primer Set (Agilent) in August 2016.

Transient transfections

For transient MET overexpression, 5×10^5 293T cells were transfected with 1 μg DNA and 6 μL FuGENE® HD (Promega) in Opti-MEM® media (Gibco). Media were replaced 16 hours post-transfection with complete DMEM. 72 hours after transfection, cells were treated with inhibitors for 5 hours and subsequently lysed for Western blotting.

Viral transductions

For stable TPR-MET expression, PC9 and Ba/F3 cells were transduced with lentivirus according to standard procedures (27). Briefly, 293T cells were transfected with the lentiviral construct in combination with the packaging plasmids VSVg and Δ8.2 (Addgene) using FuGENE® HD Transfection Reagent (Promega) per manufacturer's protocol. Viral supernatants were harvested 48 hours post-transfection, filtered, added to preplated cells along with 10 μg/mL polybrene and centrifuged for 1 hour at 2100 rpm. Cells with successful lentiviral integration were selected with 15 μg/mL blasticidin.

Viability assays

Stable TPR-MET driven and IL-3 independent Ba/F3 cells were treated with dose-escalated savolitinib or cabozantinib over the course of 72 hours. TPR-MET transduced PC9 cells were treated with dose-escalated gefitinib alone or in combination with 1 μM savolitinib or 1 μM cabozantinib over the course of 72 hours. Growth and inhibition of growth was assessed by MTS assay according to previously established methods (24). All experimental points were set up in 6 to 12 wells and all presented data are representative of several replicates.

Drug treatments and western blotting

TPR-MET PC9 derivatives were treated with each inhibitor alone or in combination with gefitinib, each at 1 μM, for 6 hours. Cell lysis, Western blotting, and immunoblotting were done as described previously (28). Blots were developed on Amersham Imager 600 (GE Healthcare Life Sciences).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Significance

With several structurally distinct MET inhibitors undergoing development for treatment of NSCLC, it is critical to identify mechanism-based therapies for drug resistance. We demonstrate that an acquired *MET* D1228V mutation mediates resistance to type I, but not type II, MET inhibitors, having therapeutic implications for the clinical use of sequential MET inhibitors.

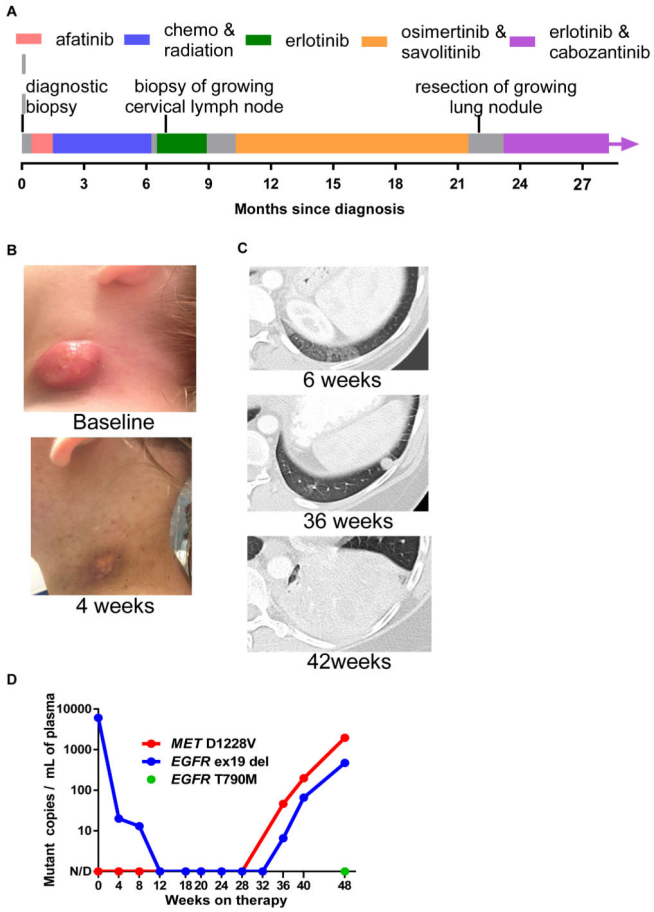

Figure 1.

Response and resistance to savolitinib and osimertinib a patient with *EGFR*-mutant NSCLC harboring *MET* amplification. **A**, treatment timeline. **B**, despite being refractory to prior EGFR inhibitors, a dramatic response to the combination was seen after 4 weeks. **C**, after 36 weeks on therapy, an isolated lung nodule appeared and was resected, however further progression was seen. **D**, serial plasma genotyping demonstrates response of the known *EGFR* exon 19 deletion followed by progression with development of an acquired D1228V mutation, in the absence of *EGFR* T790M.

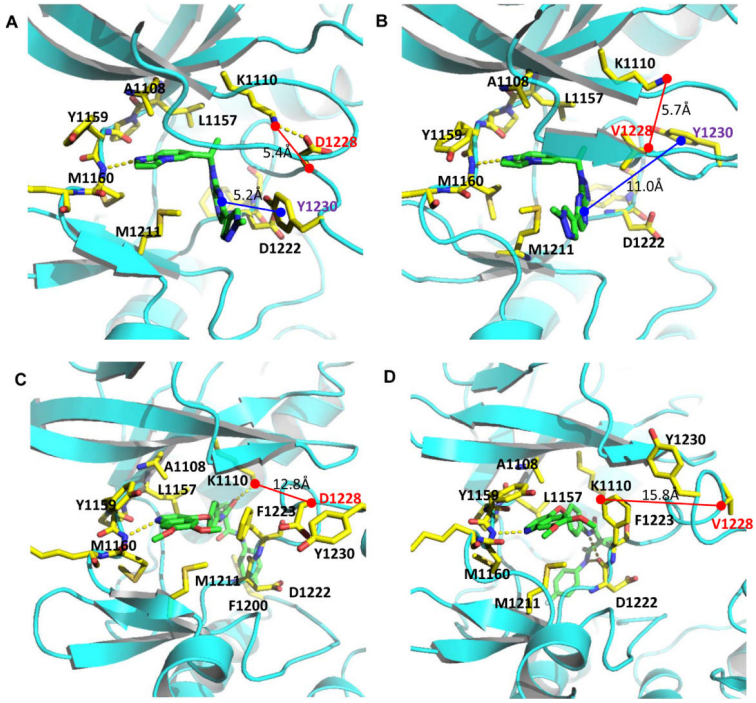

Figure 2.

Simulations of savolitinib (A, B) and cabozantinib (C, D) binding to the wildtype (A, C) and mutant (B, D) MET kinase. Kinase inhibitors are shown in green and amino acids are shown in yellow. **A**, savolitinib binding to wildtype MET relies upon a π-π interaction with Y1230 in the activation loop (blue line), stabilized by a salt bridge between D1228 and K1110. **B**, *MET* D1228V results in loss of this salt bridge, resulting in repositioning of Y1230 and loss of π-π stacking (blue line). **C**, cabozantinib binds to the inactive conformation of wildtype MET and does not rely upon this π-π interaction with Y1230. **D**, cabozantinib binding is not impacted by V1228 which is remote from the drug binding sites.

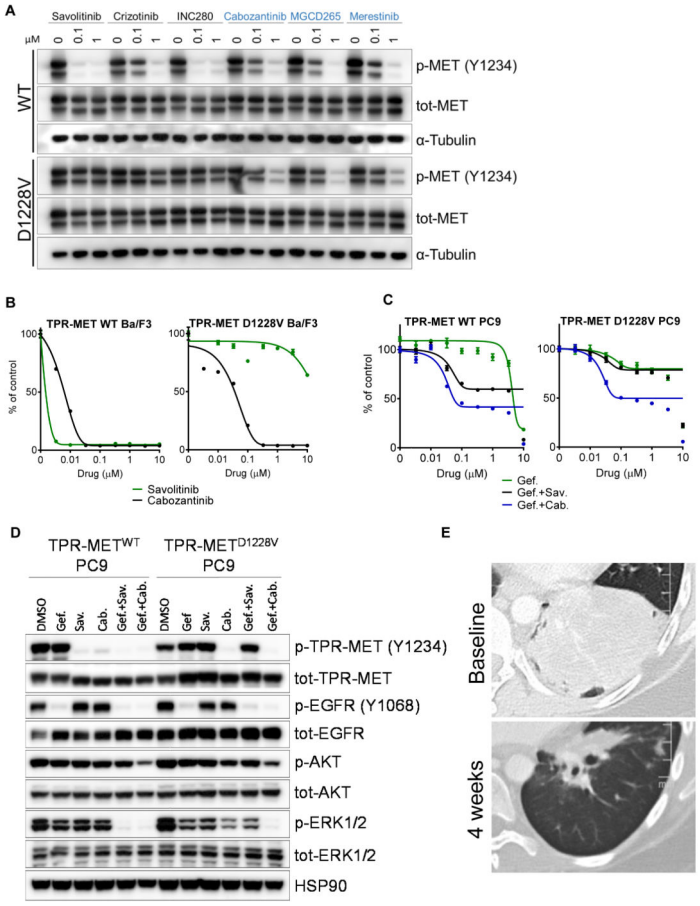

Figure 3.

MET D1228V causes resistance to type I MET inhibitors, while sensitivity to type II MET inhibitors is maintained. **A**, MET D1228V reduces the ability of type I MET kinase inhibitors (black) to dephosphorylate MET, but does not impact the activity of type II MET kinase inhibitors (blue). **B**, MTS growth inhibition assay of TPR-MET^{WT} or TPR-MET^{D1228V} transformed Ba/F3 cells exposed to dose-escalated savolitinib or cabozantinib shows differential sensitivity of TPR-MET^{D1228V}. **C**, MTS growth inhibition assay of TPR-MET^{WT} or TPR-MET^{D1228V} transduced PC9 cells exposed to dose-escalated gefitinib alone or in combination with savolitinib or cabozantinib shows differential sensitivity of TPR-MET^{D1228V} PC9 to the MET inhibitors. **D,** TPR-MET D1228V reduces the ability of savolitinib to dephosphorylate TPR-MET and inhibit downstream signaling in combination with gefitinib, but does not impact the activity of cabozantinib in combination with gefitinib. **E**, as predicted, the cancer exhibited a response after 4 weeks of cabozantinib combined with erlotinib. Abbreviations: Gef., gefitinib; Sav., savolitinib; Cab., cabozantinib

Table 1

Asp1228 in MET is conserved among related receptor tyrosine kinases, some of which have been reported to be mutated in cancer.

Protein	Aspartate Position	Sequence Context*	Mutation Examples	References
MET	D1228	DFGLARDMYDKEYYSV	D1228X†	(29, 30)
ALK	D1276	DFGMARDIYRASYYRK		
IGF-1R	D1159	DFGMTRDIYETDYYRK		
NTRK1	D674	DFGMSRDIYSTDYYRV		
FGFR1	D647	DFGLARDIHHIDYYKK		
FGFR2	D650	DFGLARDINNIDYYKK	D650_D654del	Cancer.sanger.ac.uk (31)
FGFR3	D641	DFGLARDVHNLDYYKK	D641N	(32)
ROS1	D2108	DFGLARDIYKNDYYRK		
VEGFR1	D1046	DFGLARDIYKNPDYVRK	D1046Y	(33)
VEGFR2	D1052	DFGLARDIYKDPDYVRK	D1052X	(34, 35)
VEGFR3	D1061	DFGLARDIYKDPDYVRK	D1061N	Cancer.sanger.ac.uk (31)
VEGFR4	D1027	DFGLARDVYKDPDYVRK		
FLT3	D835	DFGLARDIMSDSNYVVR	D835X	(36) (37)
RET	D898	DFGLSRDVYEEDSYVKR	D898_E901del	(38)
KIT	D816	DFGLARDIKNDSNYVVK	D816Y	(39)

*Red: Aspartate corresponding to residue D1228 in MET. Green: Tyrosine corresponding to Y1230 in MET. Blue: Recurring DFG motif. Purple: Additional tyrosines in the activation loop.

†Published as D1246X mutations based on an alternate numbering system.