Molecular mechanisms of acquired resistance to MET tyrosine kinase inhibitors in patients with MET exon 14 mutant NSCLC

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Running Title: Resistance mechanisms to MET tyrosine kinase inhibitors

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Statement of significance:

Genomic mechanisms of resistance to MET tyrosine kinase inhibitors where identified in 20 patients experiencing disease progression to type I or type II MET inhibitors using tumor and/or plasma next-generation sequencing. On-target (secondary MET kinase domain mutations, MET amplification) and off-target genomic alterations (ERBB family of receptor tyrosine kinase gene amplification, MAPK pathway gene regulation) are discussed.
amplification and KRAS mutations) were detected. Switching between type I and type II MET inhibitors resulted in partial responses in two patients. The understanding of the biological mechanisms of resistance to MET inhibitors may guide the development of novel treatment strategies.

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Abstract

Purpose: Molecular mechanisms of acquired resistance to MET TKIs are poorly understood. We aimed to characterize the genomic mechanisms of resistance to type I and type II MET TKIs and their impact on sequential MET TKI therapy outcomes in patients with metastatic MET exon 14 mutant NSCLC.

Experimental Design: Genomic alterations occurring at the time of progression on MET TKIs were studied using plasma and tissue next-generation sequencing (NGS).

Results: A total of 20 patients had tissue or plasma available for analysis at the time of acquired resistance to a MET TKI. Genomic alterations known or suspected to be mechanisms of resistance were detected in 15 patients (75%). On-target acquired mechanisms of resistance, including single and polyclonal MET kinase domain mutations in codons H1094, G1163, L1195, D1228, Y1230, and high levels of amplification of the MET exon 14 mutant allele, were observed in 7 patients (35%). A number of off-target mechanisms of resistance were detected in 9 patients (45%), including KRAS mutations and amplifications in KRAS, EGFR, HER3, and BRAF; one case displayed both on- and off-target mechanisms of resistance. In two patients with on-target resistant mutations, switching between type I and type II MET TKIs resulted in second partial responses.

Conclusions: On-target secondary mutations and activation of bypass signaling drive resistance to MET TKIs. A deeper understanding of these molecular mechanisms can support the development of sequential or combinatorial therapeutic strategies to overcome resistance.
Introduction

MET exon 14 alterations occur in ~3% of non-small cell lung cancers (NSCLCs) and predict for response to treatment with MET tyrosine kinase inhibitors (TKIs)\(^{(1,2)}\).

Several MET TKIs are under clinical development for the treatment of patients with advanced MET exon 14 mutant NSCLC. Type I MET TKIs, such as crizotinib, capmatinib, tepotinib, and savolitinib bind to MET in its catalytically active conformation where the aspartic acid-phenylalanine-glycine (DFG) motif projects into the ATP-binding site (DFG-in)\(^{(3–7)}\). In contrast, type II MET TKIs such as cabozantinib, merestinib, and glesatinib, bind to MET in its inactive DFG-out conformation\(^{(8–10)}\). Type I MET inhibitors are further subclassified as type Ia (crizotinib) when the drug interacts with the solvent front G1163 residue, and type Ib (capmatinib, tepotinib and savolitinib) when drug binding to the kinase domain is independent from this interaction. Early reports indicate that response rates with type I MET TKIs range from 32% to 55%, and the median progression-free survival (PFS) with these drugs varies between 5 and 12 months, and is limited by the invariable emergence of acquired resistance to these therapies \(^{(4,5,11–13)}\).

The landscape of resistance mechanisms to MET TKIs in patients is not well characterized. Acquired MET kinase domain mutations in residues D1228 and Y1230 confer resistance to type I MET TKIs in vitro by weakening chemical bonds between the drug and the MET kinase domain \(^{(8,14)}\). In addition, the solvent front G1163R mutation confers resistance to crizotinib but not to type Ib MET inhibitors like tepotinib, savolitinib or capmatinib in vitro \(^{(15)}\). By contrast, resistance to type II MET inhibitors can occur by mutations affecting residues L1195 and F1200 \(^{(9,15)}\).
Preclinical studies suggest that mutations in specific residues can cause resistance to drugs that bind in a similar fashion and could be potentially overcome by switching to a type of MET TKI with different mode of binding (8,9,16). Off-target mechanisms of resistance have also been described in patients treated with MET TKIs, including but not limited to wild-type KRAS amplification and activating KRAS mutations (17,18). Nevertheless, the relative frequency of on-target and off-target resistance mechanisms in patients is not well known.

In this study, using tissue and blood-based NGS we assessed the genomic mechanisms of acquired resistance in tumors from patients with advanced MET exon 14 mutant NSCLC treated with MET TKI.

Materials and methods

Patients and clinical outcomes

Patients with MET exon 14 altered NSCLC treated at the Dana-Farber Cancer Institute (DFCI) with available paired tumor biopsies and/or plasma samples at baseline and at the time of resistance to treatment with a MET TKI were identified. Patients were included in this study if they had achieved a confirmed or unconfirmed partial response or any degree of initial target lesion shrinkage followed by disease progression, or if they had experienced disease progression after 6 months of stable disease while on treatment with a MET TKI. Patients experiencing primary progression to their first MET TKI were not included. Progression-free survival and time to treatment discontinuation of MET TKIs were estimated using the Kaplan-Meier method and groups were
compared using the log-rank test. All patients provided written consent to institutional review board-approved protocols at the Dana-Farber/Harvard Cancer Center (DF/HCC) allowing for chart review and genomic sequencing on tissue and plasma samples [DF/HCC protocols #02-180, #16-374 (NCT02920996) and #14-147 (NCT022790049)]. The study was conducted in accordance with the Declaration of Helsinki.

Tissue and plasma next-generation sequencing

Baseline and MET TKI-resistant samples were analyzed using targeted NGS with the DFCI OncoPanel platform, as previously described (19). For patients who received prior care outside of DFCI, Clinical Laboratory Improvement Amendments (CLIA)-approved NGS assays were retrieved from electronic medical records. When plasma samples were available, cell-free DNA (cfDNA) was extracted and analyzed using the Guardant360 panel from Guardant Health, as previously reported (20).

Bioinformatic Analysis

Targeted next generation sequencing (NGS) was performed using the validated OncoPanel assay (19,21) at the Dana-Farber Cancer Institute Center for Cancer Genome Discovery for 277 (OncoPanel version 1), 302 (version 2), or 447 (version 3) cancer-associated genes. Briefly, tumor DNA was prepared as previously published, hybridized to custom RNA bait sets (Agilent SureSelectTM, San Diego, CA) and sequenced using Illumina HiSeq 2500 with 2x100 paired-end reads. Sequence reads were aligned to reference sequence b37 edition from the Human Genome Reference Consortium, using bwa, and further processed Picard (version 1.90,
http://broadinstitute.github.io/picard/) to remove duplicates and Genome Analysis Toolkit (GATK, version 1.6-5-g557da77) to perform localized realignment around indel sites (22). Single nucleotide variants were called using MuTect v1.1.46 and insertions and deletions were called using GATK Indelocator. Variants were filtered to remove potential germline variants as previously published and annotated using Oncotator (23). In order to remove additional germline noise, variants were excluded which were annotated as benign/likely benign in ClinVar or were present at a population maximum allele frequency of 0.1%, retaining variants in either case if they were annotated as confirmed somatic in at least two samples in COSMIC (24,25). Copy number variants and structural variants were called using the internally-developed algorithms RobustCNV and BreaKmer (26). For each gene, the absolute copy number was estimated based on the tumor purity (p) and the weighted average of segmented log2 ratios across the gene (l) using the formula: \[ ACN = \frac{2^{(l+1)} - 2(1-p)}{p} \].

Due to regulatory constraints, the files required to estimate ploidy were unavailable for analysis. Our analyses assume a diploid status for all tumors which we recognize could impact the absolute copy number in aneuploid tumors. Arm-level copy number changes were generated using an in-house algorithm specific for panel copy number segment files. Chromosome arms were classified as amplified or deleted if more than 70% of the covered portion of arm was altered.

MET molecular modelling methods

Molecular dynamics (MD) simulations were performed for savolitinib and glesatinib in wild type and H1094Y MET kinase domain. The co-crystal structure of savolitinib with
MET (pdbcode: 6SDE) was used as initial structure for MD simulation. Missing side chains and loops were built using Prime in schrodinger suite (release 2019-2)(27,28), and MD simulation of 500ns was carried out using Desmond on GPU. Trajectories were saved every 50ps for production runs. Default equilibration protocol prior to production was applied, and the long range interaction cut-off was set to 10 Angstrom. 1800 frames were uniformly sampled from MD trajectories of last 450ns for binding free energy calculation using MM-GBSA algorithm (Schrodinger Prime MM-GBSA module)(29). MD simulation for the H1094Y mutation was carried out with the same procedure and settings. The mutation was introduced directly to the initial structure and energy optimized prior to MD setup and simulations. The protocol applied to MD simulations for glesatinib as well. Since the cocrystal structure for glesatinib with MET was not available at the time of calculation, glesatinib was modeled based on one of the cocrystal structures of a close analog (pdbcode: 3C1X). Similarly, missing side chains and loops were modeled with Prime prior to MD simulations. All binding free energies were calculated by Prime MM-GBSA with default settings.

Antibodies and Compounds

Antibodies 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, crizotinib, cabozantinib, and glesatinib were purchased from Selleckchem; 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. The amplicon was subcloned into pDNR-dual (BD Biosciences) via the HindIII and XHOI restriction sites as described previously (30). Exon 14 was deleted from the full length MET cDNA construct using the USB® Change-IT™ Multiple Mutation Site Directed Mutagenesis Kit and (5’phospho) forward mutagenic primer:

gctgaaaaagagaagcaataaaagatcagttctcaattcatacagaacg, along with the reverse primer provided with the kit. The MET H1094Y mutation was introduced using the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies) and the following mutagenic primers: Forward: 5’- agagggcattttgttttgtatatattatggactttttgg -3’;
Reverse: 5’- ccaacaagtccataatacaacacccaaatggtccctct -3’. All constructs were confirmed by DNA sequencing. Constructs were shuttled into the retroviral expression vector JP1540 using the BD Creator™ System (BD Biosciences).

Cell lines

HEK-293T cells were purchased from ATCC (in 2009), cultured in DMEM, supplemented with 10% FBS, streptomycin and penicillin and authenticated using the Promega GenePrint 10 System at the RTSF Genomics Core at Michigan State University in August 2016. All cell lines used in the study tested negative for mycoplasma as determined by the Mycoplasma Plus PCR Primer Set (Agilent).
Drug treatments and Western blotting

For transient MET (WT or mutant) overexpression, 5 x 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 6 hours and subsequently lysed for Western blotting. Cell lysis, Western blotting, and immunoblotting were done as described previously (31). Blots were developed on Amersham Imager 600 (GE Healthcare Life Sciences).

Results

Patients

Between April 2014 and June 2019, 114 patients with NSCLC harboring MET exon 14 splicing alterations were identified, of which 71 (62%) had metastatic disease (supplementary Figure 1). Of these, thirty-nine patients (55%) received at least one MET TKI and were evaluable for treatment response. In total, 29 patients had documented disease progression on a MET TKI, and in 20 cases, tissue and/or plasma NGS was performed at the time of progression. For their best objective response, thirteen patients experienced a partial response, five had stable disease lasting ≥ 6 months and two patients experienced stable disease ≤ 6 months with some degree of tumor reduction.

Baseline clinical and molecular characteristics of patients included in genomic profiling at progression are summarized in supplementary Table 1. Among the 20
patients who underwent NGS analysis of pre- and post-treatment samples, 14 received one MET TKI, and 6 received sequentially both type I and II MET TKIs. NGS was performed after progression on both types of MET TKIs in five cases, and in one case, treatment with a second-line MET TKI was still ongoing at the time of study cut-off.

Genomic mechanisms of resistance to MET TKIs

Mechanisms of resistance conferred by on- and off-target genomic alterations were identified in 15 cases (75%). On-target mechanisms of resistance, including MET kinase domain mutations or acquired focal amplification of the MET exon 14 mutant allele, were found in 7 cases (35%). Acquired KRAS mutations or acquired amplification of wild-type EGFR, KRAS, HER3, and BRAF, as single or compound mechanisms of resistance, were detected in 9 cases (45%). One case displayed both on- and off-target resistance with a D1228N MET kinase domain mutation as well as amplification of EGFR and HER3. In 5 cases (25%), the genomic mechanism of resistance to MET TKIs was not identified (Figure 1).

On-target resistance mechanisms: MET mutations and amplification

Focusing on MET-dependent resistance mechanisms, secondary MET kinase domain mutations in residues H1094, G1163, L1195, D1228, and Y1230 were acquired in 6 cases, and high focal amplification of the MET exon 14 mutant allele was identified in one case (summarized in Table 1 and supplementary Table 2). Single MET resistance mutations were identified in four cases: D1228H in one patient (case #1), D1228N in two patients (cases #2 and #6), and Y1230C in one patient (case #3)
Multiple MET kinase domain mutations were detected in two cases. In an updated analysis from case #4, which we previously described (9), after acquired resistance to crizotinib, tissue showed only a Y1230H mutation, but plasma NGS detected multiple MET kinase domain resistance mutations including G1163R, L1195V, D1228N, D1228H, Y1230H, and Y1230S (Figure 2B, supplementary Figure 2A). In this case, by plasma NGS analysis, D1228 and Y1230 mutations were confirmed to be in trans on different alleles, but the allelic distribution of the G1163R and L1195V mutations relative to the other mutations could not be determined due to their distance from the other genomic alterations (Supplementary Figure 3). In case #5, after treatment with the type II MET TKI glesatinib, tissue NGS detected a MET H1094Y mutation, however plasma NGS detected both the H1094Y and L1195V mutations (Figure 2B, supplementary Figure 2B). The H1094Y substitution (also referred to as H1112Y when referencing the MET variant 1 transcript, which is 18 amino acids longer (32)) is an activating MET kinase domain mutation commonly found in papillary renal cell carcinoma (33), but its impact on TKI resistance is unknown. By immunoblot analysis, we found that MET H1094Y modestly reduces the ability of glesatinib to dephosphorylate MET compared to wild type H1094 (supplementary Figure 4). Interestingly, the H1094Y mutation appeared to be particularly sensitive to the type I MET TKI savolitinib compared to wild type H1094 MET (supplementary Figure 4). By structural modelling, this is likely due to the increased hydrophobic interaction between Y1094 and the imidazo-pyridine head group of savolitinib. Molecular dynamics simulation and binding free energy calculations indicate that H1094Y mutation increases the binding energy to savolitinib by 3.7 kcal/mol. In contrast, H1094Y reduces
the binding affinity of glesatinib by 2.7 kcal/mol. Due to the distance between
nucleotides encoding for H1094Y and L1195V, it was not possible to determine whether
these mutations were present in cis or in trans by phasing analysis on plasma NGS.

In addition to secondary MET kinase domain mutations, on-target mechanisms of
resistance also involved high levels of MET amplification. Prior to treatment with MET
TKIs, case #7 had an estimated 4 copies of MET at baseline, but upon acquired
resistance to the type II MET TKI glesatinib, 17 copies of the MET exon 14 mutant allele
were detected. After subsequent treatment with crizotinib after glesatinib, this case
developed further amplification of the MET exon 14 mutant allele (up to 47 copies)
(Figure 2B, Figure 4B).

Off-target resistance mechanisms

Acquired amplification of the ERBB family of receptor tyrosine kinase genes
EGFR and HER3, and amplification of the mitogen-activated protein kinase (MAPK)
pathway effector genes KRAS and BRAF, as well as acquired activating KRAS
mutations were recurrently found at resistance to MET TKIs (Figure 3, supplementary
Table 3). As a single event, EGFR amplification was detected in one case (#9), and
KRAS amplification in a second case (#13) (supplementary Figure 5). Amplification of
more than one gene was also commonly detected, including: KRAS/EGFR amplification
in two patients (cases #10 and #12, Figure 3A), EGFR/HER3 amplification in two
patients (case # 6, Figure 2B and case #8 Figure 3A), and KRAS/BRAF/EGFR/MET
amplification in one case (#11 Figure 3A, supplementary Figure 5D). We previously
reported three of these cases (cases #10, #11, #13) in an earlier description of bypass mechanisms of resistance to MET TKIs in MET exon 14 mutant NSCLC (17).

KRAS mutations were also implicated in resistance to MET TKIs. A KRAS G12D mutation was acquired in one case driving resistance to crizotinib (#14, Figure 3B). In a second patient previously treated with crizotinib, chemotherapy, and nivolumab, for which no other resistance mechanisms were found in tissue NGS, a KRAS G60D mutation was found in plasma NGS prior to treatment with glesatinib. This patient received treatment with a type II MET TKI but did not experience a clinical benefit (#15, Figure 3B and supplementary Figure 2C). The KRAS G60D mutation was not detected by tissue NGS at the time of progression to glesatinib.

In 5 cases (25%), NGS of post-treatment samples did not detect genomic alterations with a clear role in resistance to MET TKIs (supplementary Table 4, supplementary Figure 6).

Overcoming acquired resistance with sequential MET TKI treatment

We next examined the efficacy of sequential treatment with MET TKIs in six patients, according to the resistance mechanisms acquired with the previous line of MET inhibition. Four patients received a type I followed by a type II MET TKI: crizotinib followed by glesatinib in two patients, crizotinib followed by merestinib in one patient, and capmatinib followed by merestinib in one case. In addition, two patients received sequential treatment with glesatinib (type II) followed by crizotinib (type I) (supplementary Table 1).
Overall, four of the six patients had detectable on-target resistance mechanisms prior to switching treatment (Cases #3-5 and #7, Figure 2B). In a fifth case, with resistance to capmatinib, *EGFR* and *HER3* amplification were detected on tissue whereas the *MET* D1228N mutation was found in plasma only (Case #6). In a sixth patient, a *KRAS* mutation was present at the time of treatment initiation with the second MET TKI (Case #15, Figure 3B).

Sequential treatment with a structurally different MET TKI was effective in overcoming single on-target resistance mechanisms in two patients (2/6, 33%). In one patient with an acquired crizotinib-resistant *MET* Y1230C mutation detected in both tissue and plasma, switching treatment to merestinib resulted in a confirmed partial response (case #3, Figure 4A). In case #7, the patient’s cancer developed focal amplification of the *MET* exon 14 mutant allele (from 4 to 17 copies) at the time of glesatinib resistance, and after switching treatment to crizotinib, the patient experienced a confirmed partial response lasting for 10 months (Figure 4B). However, at the time of crizotinib resistance, higher levels of amplification of the *MET* exon 14 mutant allele (47 copies) were detected, suggesting that incremental levels of focal *MET* amplification can confer resistance to both type I and II MET TKIs.

In four patients, sequential treatment with an alternative MET TKI did not result in clinical benefit. Among these, in two patients, multiple *MET* mutations were found at the time of progression on the first MET TKI treatment. One patient (case #4) with acquired resistance to crizotinib not only had multiple secondary *MET* kinase domain mutations that confer resistance to crizotinib (D1228N/H, Y1230S/H and G1163R), but also had a *MET* L1195V mutation detected at a low allele fraction of 0.04%, and this mutation has
been reported to confer resistance to both crizotinib and type II MET TKIs in vitro (Figure 2B, supplementary Figure 2A) (15). Switching treatment to glesatinib was ineffective, and higher levels of the MET L1195V mutation were detected by plasma monitoring during treatment with glesatinib and at the time of progression (allelic frequency increased from 0.04% to 0.8%) (case #4, supplementary Figure 2A). In the second patient, sequential treatment with crizotinib after progression on glesatinib in the context of both MET L1195V and H1094Y mutations was also ineffective (case #5, supplementary Figure 2B), highlighting the challenges of targeting the L1195V mutation due to its broad impact on sensitivity to both crizotinib and type II MET TKIs.

In the remaining two cases harboring off-target resistance mechanisms (cases #6 and #14), sequential MET treatment strategies were ineffective. In one patient (case #6, Figure 2B) in which EGFR and HER3 amplification (detected by tissue NGS) and a MET D1228N mutation (detected only by plasma NGS) were found at capmatinib resistance, primary progression occurred in the lungs after switching treatment to merestinib. At merestinib progression, the MET D1228N mutation was no longer detected by plasma NGS, suggesting that merestinib was active against MET D1228N mutant clones, but resistance was most likely driven by the co-occurring EGFR and/or HER3 bypass signaling (case #6, Figure 2B). In another patient (case #14, Figure 3B), who had a partial response to crizotinib as the first MET TKI lasting for 13 months, no resistance mechanisms were detected on tissue or blood NGS. However, after treatment with chemotherapy and immunotherapy, a KRAS G60D mutation was detected in plasma NGS (AF: 1.08%) prior to starting treatment with glesatinib. Switching to the type II MET TKI resulted in a short disease stability lasting for only 3
months with persistence of the KRAS mutation by plasma monitoring at progression (case #15, supplementary Figure 2C).

Concordance between plasma and tissue NGS to assess resistance to MET TKIs

We compared the detection rate of genomic resistance mechanisms in both plasma and tissue NGS in eleven paired samples. Concordant results, defined as the detection of genomic alterations in plasma and tissue NGS, were observed in three cases (supplementary Figure 2D). Discordant results were observed in six cases (55%): in two patients, plasma NGS detected multiple MET kinase domain mutations whereas tissue NGS detected only single MET mutations; in one case a KRAS G60D mutation was detected in plasma but not in tissue, and in the remaining three cases, tissue NGS detected MET amplification or EGFR gene amplification which were not found in plasma NGS. Plasma NGS was not informative in 2 cases in which there was no evidence of tumor DNA shedding.

Clinical outcomes of patients treated with MET TKI and resistance mechanisms.

In EGFR-mutant lung cancer with acquired resistance to TKIs, clinical outcomes may differ depending on whether the cancers developed on-target versus off-target resistance (34,35); therefore, we also sought to explore clinical outcomes in our cohort based on MET TKI resistance mechanism. Among patients in which resistance to MET TKIs was assessed (N = 20), the median PFS to the first line of MET TKI was 6.7 months (95% CI: 4.7-29.4) and median time to treatment discontinuation (TTD) was 8.3 months (95% CI: 5.7-28.7). There were no significant differences in median PFS
according to the type of resistance mechanisms: 7.4 months (95% CI: 5.2-15.9) in patients with on-target versus 5.7 months (95% CI: 3.2-14.0) with off-target resistance (p = 0.9) (supplementary Figure 7A). TTD was also similar between groups, 8.3 months (95% CI: 5.9-21.7) versus 8.8 months (95% CI: 3.2-14.0), respectively (p = 0.4) (supplementary Figure 7B, supplementary Figure 8).

Discussion

MET TKIs are expected to become a standard treatment option for patients with MET exon 14 mutant lung cancer. The present study provides clinical and molecular evidence suggesting that resistance driven by genomic alterations recurrently fall in two broad categories: on-target resistance mediated by secondary MET kinase domain mutations and/or amplification of the MET exon 14 mutant allele; and off-target resistance resulting from activation of bypass signaling due to amplification of ERBB family of receptor tyrosine kinase genes, BRAF amplification, KRAS amplification, and KRAS mutations.

MET kinase domain mutations were frequently involved in resistance to type I and type II MET TKIs as single or polyclonal events. Hotspot mutations in codons D1228 and Y1230 were detected in about a third of patients experiencing disease progression on a type I MET TKI. In concordance with preclinical studies showing activity of type II inhibitors in this setting, we showed that resistance to crizotinib, driven by the MET Y1230C mutation, was clinically targetable with merestinib (15,36).

However, in two cases, switching treatment to a type II MET TKI was not effective, potentially hampered by the co-occurrence of alternative mechanisms of resistance like
EGFR amplification or the selection of cancer subclones harboring the MET-L1195V mutation, which confers resistance to crizotinib as well as to type II MET TKIs (15). The complex nature of on-target alterations reflects the diversity of subclonal selection under treatment pressure (37). Most of the on-target resistance mutations detected have been reported previously in studies that employed genomic data or in vitro modelling, in which clinical correlation was missing (15,38). Our study provides further information on the clinical context in which these mutations emerge and the efficacy of subsequent treatments with MET TKIs.

In one case, resistance to the type II MET TKI glesatinib appeared to be driven by high levels of focal amplification of the MET exon 14 mutant allele, and this patient had a subsequent response to the type I MET TKI crizotinib. One possible explanation for this response to crizotinib may be a difference in potency between TKIs. While a MET exon 14 mutant-expressing NIH/3T3 spheroid growth model showed greater potency with crizotinib compared to glesatinib (with a 50% inhibitory concentration [IC\textsubscript{50}] of 28.9 nanomolar [nM] compared to IC\textsubscript{50} 80.6 nM, respectively (9), by contrast, a Ba/F3 cell model suggests similar in vitro activity against MET exon 14 between crizotinib and glesatinib with IC\textsubscript{50} values of 22 nM versus 21 nM, respectively (15). Alternatively, differences in pharmacokinetics or tolerability may have limited the maximal drug concentration of glesatinib compared to crizotinib; plasma levels of these drugs were not available for study in this case. Similar on-target amplification has been reported to drive resistance in ALK rearranged lung cancers, where ALK amplification can cause resistance to crizotinib but is not observed with more potent ALK TKIs (39). Hence, in light of the complex landscape of on-target molecular alterations, the optimal
sequencing of MET-targeting strategy needs further prospective exploration through clinical trials which also aim to identify the mechanism of resistance to the prior MET inhibitor. In the setting of on-target resistance, additional MET-targeted strategies employing MET antibodies or MET antibody-drug conjugates, should also be explored prospectively through clinical studies (40–42).

Off-target genomic bypass mechanisms were detected in about half of the patients in the present study. MET amplification is a common mechanism of resistance to EGFR inhibitors in EGFR-mutant lung cancer, and combining MET and EGFR inhibitors can be an effective strategy in overcoming this mechanism of resistance (43–45). Given that focal EGFR amplification was frequently involved in resistance to type I MET TKIs, exploring the role of dual MET and EGFR inhibition in this setting is warranted (46). In concordance with previous reports, KRAS oncogenic mutations and wild type KRAS and BRAF amplification constituted a common cause of resistance to MET TKIs in our study (17,18). In the setting of oncogenic KRAS activation, preclinical evidence suggests an additive effect of combining MET with MEK or EGFR/HER2 inhibition (18,47). Our results further support the need to explore optimal combination strategies to delay or overcome resistance secondary to bypass activation of the MAPK pathway (18).

As with other targeted therapy populations, we also observed discordance between tissue and plasma genotyping (48,49). While plasma genotyping can potentially reflect greater inter-tumoral heterogeneity of resistance than an analysis of a single tumor biopsy site, some cancers do not shed sufficient quantities of tumor DNA into the circulation to allow for accurate detection of resistance mechanisms using a
blood sample alone (50). Furthermore, gene copy number gains and losses can be
more difficult to ascertain in plasma than in tissue (51), which could limit detection of
bypass mechanisms of resistance using plasma only. Additionally, plasma samples can
contain other sources of somatic mutations other than from the primary tumor of
interest, such as from clonal hematopoietic cell populations which can complicate the
elucidation of TKI-resistance mechanisms (52). With the various advantages and
disadvantages of plasma versus tissue analysis, we will need to continue exploring the
optimal sequencing platform that enables the most sensitive and specific identification
of resistance mechanisms safely and rapidly.

This study has several limitations. Firstly, genomic testing on tissue samples was
not performed with a single NGS assay in all patients. However, OncoPanel testing was
performed in most cases (87.5%). In addition, as only patients experiencing disease
progression were included in the study, the mechanisms of resistance we describe here
may encompass those occurring in a population of patients experiencing earlier disease
progression with MET TKI. Patients with long-term response to TKIs might not be
represented here and alternative mechanisms of resistance could develop in this
setting. Lastly, the sample size is relatively small and thus, the frequency of the reported
genomic alterations could vary in larger cohorts. Nevertheless, this is the largest study
to date exploring genomic mechanisms of resistance to MET TKI in the clinical setting.

Conclusions

Our study indicates that the mechanisms of resistance to MET TKIs are heterogeneous.
Systematic genomic profiling of tumor biopsies and plasma from patients progressing
on treatment with MET TKIs can be informative in the treatment decision process and can identify patients who might benefit from sequential MET inhibitor strategies and contribute to the design of future clinical trials.

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Mizuki Nishino’s work is supported by NIH grants R01CA203636 and U01CA209414. Mark Awad’s work is supported by the Conquer Cancer Foundation of the American Society of Clinical Oncology, grant number 6298701.

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Available from: https://doi.org/10.1016/j.jtho.2018.08.299

of the GEOMETRY mono-1 phase II study for evaluation of the MET inhibitor
capmatinib (INC280) in patients (pts) with METΔex14 mutated advanced non-
small cell lung cancer (NSCLC). Ann Oncol [Internet]. 2018;29:mdy424.090-
mdy424.090. Available from: http://dx.doi.org/10.1093/annonc/mdy424.090

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Amplified NSCLC after Progression on Prior EGFR-TKI. J Thorac Oncol [Internet].
Elsevier; 2017;12:S1768. Available from:
https://doi.org/10.1016/j.jtho.2017.09.377

METD1228V Mutation and Resistance to MET Inhibition in Lung Cancer. Cancer

Glesatinib Exhibits Antitumor Activity in Lung Cancer Models and Patients


27. Jacobson MP, Friesner RA, Xiang Z, Honig B. On the role of the crystal


Table 1. Summary of on-target MET mutations found upon resistance to each of the list MET tyrosine kinase inhibitors (TKIs) used in this cohort.

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Figure Legends

**Figure 1.** Distribution of on-target, off-target and unknown mechanisms of resistance to MET tyrosine kinase inhibitors. # D1228N detected only in plasma and EGFR/HER3 amplification in tissue. * detected in plasma after previous treatment with crizotinib, chemotherapy, and nivolumab.

**Figure 2.** Oncoprint of genomic alterations detected in tissue (T) and plasma (B) next-generation sequencing in tumors harboring on-target resistance mechanisms to single (A) or multiple lines (B) of MET tyrosine kinase inhibitors. Pre: pretreatment, R1: resistance to first line of MET TKI, R2: resistance to second line of MET TKI, PR: partial response, SD: stable disease, NE: not evaluable, TTD: time to treatment discontinuation, NR: not reportable at the request of the sponsor. Low copy number gain is defined as 3-5 copies and gene amplification is defined as \( \geq 6 \) copies.

**Figure 3.** Oncoprint of genomic alterations detected in tissue (T) and plasma (B) next-generation sequencing in tumors harboring off-target resistance mechanisms to single (A) or multiple lines (B) of MET tyrosine kinase inhibitors. Pre: pretreatment, R1: resistance to first line of MET TKI, R2: resistance to second line of MET TKI, PR: partial response, SD: stable disease, NE: not evaluable, TTD: time to treatment discontinuation, NR: not reportable at the request of the sponsor. Low copy number gain is defined as 3-5 copies and gene amplification is defined as \( \geq 6 \) copies.
Figure 4. Clinical cases of effective sequential treatment with type I and type II MET TKI. (A) A patient with extensive pleural disease (case #3) received treatment with crizotinib as first line of MET TKI treatment. At the time of progression, both plasma and tissue NGS detected a MET Y1230C mutations and switching treatment to merestinib resulted in a rapid partial response. (B) Baseline levels of MET copy number are shown (case #7), reflecting MET polysomy by NGS and fluorescence in situ hybridization (FISH). After an initial partial response with glesatinib, MET amplification was detected on tissue NGS (17 copies). Tumor responses were observed after switching treatment to crizotinib lasting for 10 months. At the time of progression, a tissue biopsy showed higher levels of MET amplification by NGS (47 copies) and FISH.
### Figure 2

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Figure 4

A. Baseline | Crizotinib Response | Progression \(MET\ Y1230C\) | Merestinib Response
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Crizotinib PFS 15 months

B. Baseline | Glesatinib Response | Glesatinib Resistance | Crizotinib Response | Crizotinib Progression
---|---|---|---|---

Glesatinib PFS 5.5 months | Crizotinib PFS 10.3 months

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Clinical Cancer Research

Molecular mechanisms of acquired resistance to MET tyrosine kinase inhibitors in patients with MET exon 14 mutant NSCLC

Gonzalo Recondo, Magda Bahcall, Liam F Spurr, et al.

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