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Title: Co-occurring alterations in the RAS-MAPK pathway limit response to MET inhibitor treatment in *MET* exon 14 skipping mutation positive lung cancer

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Running Title: RAS-MAPK pathway at MET TKI resistance in *MET*ex14 NSCLC

Statement of Translational Relevance: This report describes targeted sequencing of the largest reported cohort of advanced-stage non-small cell lung cancers (NSCLCs) with a *MET* exon 14 skipping (*MET*ex14) mutation. While MET tyrosine kinase inhibitors (TKIs) are active in *MET*ex14-mutated NSCLC, response rates are lower than those seen to TKI therapy in other forms of oncogene-driven NSCLC. The data presented here uncover enrichment for both primary and acquired RAS-MAPK pathway alterations in *MET*ex14-mutated NSCLC, events that may limit initial response magnitude or duration to MET TKI treatment. Combined MET plus MEK inhibitor treatment can overcome RAS-MAPK pathway-mediated resistance, suggesting a novel polytherapy strategy for evaluation in prospective clinical trials.

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Disclosures: B.C.B is a consultant to Lilly. E.A.C. is a consultant/advisor to Celgene, Qiagen, Guardant Health and Takeda and has received research funding from Merck KgA, Ignyta, Gilead, and Daiichi Sankyo and is a shareholder in Clara Health, Illumina, Guardant Health, Tatara Therapeutics and Pacific Biosciences. C.E.M has received honoraria from Honoraria from Guardant, Takeda, and Novartis. C.M.B. is a consultant to Revolution Medicines and Jazz pharmaceuticals, and has received research funding from Novartis, AstraZeneca, MedImmune, Ignyta, Mirati, Spectrum, and Clovis Oncology. J.S.F. is a consultant, has received research funding, and has a financial stake in Relay Therapeutics. R.B.C. is a consultant/advisory board member for Amgen, Astex Pharmaceuticals, Avidity Biosciences, BMS, Fog Pharma, Genentech, LOXO, Merrimack, N-of-one, Roche, Spectrum Pharmaceuticals, Symphogen, Taiho, and Warp Drive Bio and has received research funding from AstraZeneca and Sanofi. JP is a consult to AstraZeneca and Novartis. Has received honoraria from Genentech and Takeda. Has received research funding from Pfizer. T.L. has received honoraria from Foundation Medicine and research funding from Pfizer, Hengrui, and Merck. T.G.B is a consultant/advisor to Novartis, AstraZeneca, Revolution Medicines, Takeda, and Array Biopharma and has received research funding from Novartis, Revolution Medicines, and Ignyta. V.M.R. and R.B.L are employees and shareholders of Guardant Health, Inc.

Word Count: 2570

Figures: 4

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Abstract:

PURPOSE: While patients with advanced-stage non-small cell lung cancers (NSCLCs) harboring *MET* exon 14 skipping mutations (*MET*ex14) often benefit from MET tyrosine kinase inhibitor (TKI) treatment, clinical benefit is limited by primary and acquired drug resistance. The molecular basis for this resistance remains incompletely understood. METHODS: Targeted sequencing analysis was performed on cell-free circulating tumor DNA obtained from 289 patients with advanced-stage *MET*ex14-mutated NSCLC. RESULTS: Prominent co-occurring RAS-MAPK pathway gene alterations (e.g. in *KRAS, NF1*) were detected in NSCLCs with *MET*ex14 skipping alterations as compared to *EGFR*-mutated NSCLCs. There was an association between decreased MET TKI treatment response and RAS-MAPK pathway co-occurring alterations. In a preclinical model expressing a canonical *MET*ex14 mutation, KRAS overexpression or NF1 downregulation hyperactivated MAPK signaling to promote MET TKI resistance. This resistance was overcome by co-treatment with crizotinib and the MEK inhibitor trametinib. CONCLUSION: Our study provides a genomic landscape of co-occurring alterations in advanced-stage *MET*ex14-mutated NSCLC and suggests a potential combination therapy strategy targeting MAPK pathway signaling to enhance clinical outcomes.

Introduction:

Somatic *MET* mutations leading to splicing-mediated loss of exon 14 and subsequent MET overexpression are an emerging therapeutic target present in 2-4% of lung adenocarcinomas (1,2). MET tyrosine kinase inhibitor (TKI) treatment was associated with improved overall survival in a retrospective study of patients with *MET*ex14-mutated NSCLC (3). In ongoing prospective studies, response rates of 32%, to the multi-kinase inhibitor crizotinib, 41%, to the MET TKI tepotinib, and up to 72% for treatment-naïve patients to the MET TKI capmatinib have been reported (4-6). Crizotinib has recently received FDA breakthrough designation for use in the treatment of *MET*ex14-mutated NSCLC.

While MET remains an attractive therapeutic target, both primary and acquired resistance limit the long-term survival of patients with *MET*ex14-mutated NSCLC. Second-site *MET* mutations and downstream signaling reactivation via acquired *KRAS* amplification have been reported at acquired resistance to MET TKI therapy, and may inform treatment decisions (7-10). By contrast, the mechanisms mediating both primary MET TKI resistance and tumor cell persistence during initial MET TKI treatment remain largely undefined, as does the full landscape of alterations promoting acquired resistance.

Recent studies show that advanced-stage NSCLCs often harbor multiple oncogenic alterations, which may impact response to targeted therapies (11-13). Analysis of cell-free circulating tumor DNA (cfDNA) utilizing next-generation sequencing (NGS) provides one avenue to describe the genomic landscape within a cancer patient and offers the potential to capture genomic changes reflecting heterogeneity across distinct metastatic

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tumor sites (11,14). A more detailed understanding of the mutational landscape that co-exists with oncogenic *MET* alterations in NSCLC may facilitate an improved understanding of the determinants of MET TKI response and identify rational polytherapy strategies to improve clinical outcomes.

Here, we describe the spectrum of co-occurring genomic alterations observed within the cfDNA of patients with *MET*ex14-mutated, advanced-stage NSCLC and identify prominent co-alteration of RAS pathway genes as a contributing factor to disease progression.

Results:

Co-occurring genomic alterations are common in advanced-stage METex14-mutated lung cancer

We analyzed a cohort of 289 patients with advanced-stage NSCLC who had a *MET*ex14 mutation identified upon cfDNA analysis of 68 cancer-relevant genes using a clinically validated assay (Guardant360). This is the largest reported cohort to-date describing the genomic landscape of advanced-stage *METex14*-mutated NSCLC. We evaluated the frequency with which *MET*ex14 mutations co-occur with other cancer-associated mutations (Supplemental Table 1) (11,15). Synonymous mutations and those with predicted neutral or unknown functional impact were excluded, as previously described (11), as were mutations previously associated with clonal hematopoiesis (16). 86.5% of samples contained co-occurring genomic alterations, with a mean of 2.74 alterations per sample (range 0-22), in addition to the *MET*ex14 mutation. The most commonly altered genes, co-occurring with the *MET*ex14 mutation in at least 10% of patients, were *TP53* (49.5% of patients), *EGFR* (16.3%), *NF1 (neurofibromatosis-1)* (15.6%), *BRAF* (10.7%), and *CDK4* (10.4%). Additional *MET* gene alterations were also common in this patient population, in which 9.3% of patients had co-occurring *MET* copy number gain and 12.5% had a co-occurring second-site *MET* mutation (Figure 1A).

Seventeen of 34 second-site *MET* mutations were located in the tyrosine kinase domain. All identified *MET* second-site mutations, regardless of predicted functional impact, were included in this analysis. Of these, fourteen (G1163R, L1195V/F, F1200I, D1228H/N/Y, and Y1230H/S) were located at residues previously associated with MET TKI resistance (7-9,17-19). Nine of these occurred in patients with known prior MET TKI exposure and five in patients with unknown prior treatment history. The remaining three mutations (H1094Y, R1336W, and I1084L) occurred in patients without available prior treatment history. While the *MET* H1094Y mutation is known to lead to MET activation (20), the effects of the other two *MET* mutations remain uncharacterized.

The co-occurrence of other established oncogenic driver alterations (*KRAS, EGFR, ALK, ROS-1, BRAF*) was uncommon except for the presence of activating *KRAS* mutations in 5.2% of patients (G12C/D/S/V 3.5%, G13C 1%, Q22K 0.3%, and Q61H 0.3%), canonical *EGFR*-activating mutations in 3.5% (del19 3.1%, L858R 0.7%, T790M 2%), and an *ALK* gene rearrangement in 0.7%. In addition, a *HER2* exon 20 insertion was detected in one

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patient. In a patient with known clinical outcomes data, with both *EGFR* del19/EGFR T790M mutations and a *METex*14 mutation, there was partial response (RECIST 1.1) to treatment with the EGFR TKI osimertinib, which lasted 13.8 months. While in the overall dataset the *MET*ex14 mutation detected was predominantly clonal (80.6% of samples), in samples with a detectable co-occurring oncogenic driver alteration the *MET*ex14 alteration was more likely to be subclonal (21.4% of samples clonal, p-value <0.0001). The converse was also true; co-occurring established oncogenic driver mutations that were detected were more likely to be clonal than the other detected co-occurring genomic alterations (68.6% versus 46.3%, p-value = 0.0144) (Supplemental Figure S1).

RAS pathway alterations are more common in METex14-mutated NSCLC than in EGFR-mutated NSCLC

To understand how the genomic landscape in advanced-stage *MET*ex14-mutated NSCLC compares to NSCLCs with a different targetable oncogenic driver mutation associated with high upfront response rates to TKI therapy, we used identical cfDNA profiling to compare a cohort of patients with known *MET*ex14-mutated NSCLC to a previously unpublished, independent cohort of 1653 samples from 1489 patients with advanced-stage *EGFR*-mutant (del19, L858R) NSCLC (Supplemental Table 1). This comparison demonstrated differential frequency of co-occurring genomic alterations in 17 genes (Figure 2A). In the *MET*ex14-mutated cohort, co-occurring alterations in *NF1*, *CDK4, STK11*, *ALK, KRAS*, *ATM*, *CDKN2A*, *NRAS, TSC1*, and *ESR1* were more commonly identified. In the *EGFR*-mutated cohort, co-occurring alterations in *AR, ERBB2*, *CCNE1*, *PIK3CA, BRAF*, *CTNNB1,* and *MYC* were more commonly identified, independently validating our prior findings identifying these as common co-occurring alterations in *EGFR*-mutated NSCLC (11). Among the 10 genes with a greater frequency of co-occurring genomic alteration in patients with a *MET*ex14 mutation, three (*NF1*, *KRAS*, and *NRAS*) are key components of the RAS-MAPK signaling pathway.

A propensity towards genomic alterations promoting downstream hyperactivation of the RAS pathway may favor primary resistance to TKI therapy and help explain the comparatively lower TKI response rates in *MET*ex14-mutated NSCLC. Examination of the subset of cfDNA samples obtained from patients with *MET*ex14-mutated NSCLC prior to known MET TKI treatment (n=61) demonstrated high rates of genomic alterations capable of promoting RAS-MAPK pathway activation (Figures 2B, 2C). When compared to a previously published cohort (11) of cfDNA samples from TKI-naïve patients with *EGFR*-mutated NSCLC (n=20), RAS-MAPK pathway alterations that were detected before treatment remained more common in *MET*ex14-mutated NSCLC (35.2% vs 10%, p-value = 0.042).

Both MET second-site mutations and RAS pathway alterations are newly detectable following MET TKI treatment.

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We identified twelve patients with cfDNA obtained following treatment with crizotinib and an available matched sample obtained prior to known crizotinib exposure (Figure 3A, B, Supplemental Table 3). Details regarding one patient (patient #5) in this dataset have previously been published by other groups (7,9).

Newly detectable *MET* second-site mutations (Y1230H/S, D1228H/N, F1200I, L1195V) were detected in four of twelve patients following MET TKI treatment (Figure 3C, Supplemental Table 3). While some of these mutations have been reported at acquired MET TKI resistance (7-9,17-19), the *MET* F1200I has not yet been described in a patient sample. Identification of specific acquired second-site mutations at resistance to MET TKI therapy may inform treatment decisions. MET TKIs can be classified as type I TKIs (e.g. crizotinib, capmatinib) or type II TKIs (e.g. cabozantinib) based on the kinase domain conformation to which they bind and differ in their activity against second-site MET mutations. For example, the *MET* Y1230X and D1228X mutations develop at resistance to type I MET TKIs and may predict response to subsequent type II MET TKI treatment (7-9,17-19). Conversely, the *MET* L1195V mutation has been reported at resistance to type II MET TKI treatment (7).

The development of acquired *MET* F1200 mutations as a resistance mechanism to MET TKIs has previously been predicted in a preclinical drug resistance screen, in which *MET* F1200 mutations were the dominant resistance mechanism to a type II MET TKI and were observed, though less common, at resistance to a type I MET TKI (21). Molecular modeling studies suggest that *MET* F1200I alters the conformation of the kinase domain such that it interferes with both the binding of type II MET TKIs within the DFG-out binding pocket, and to a lesser extent, may promote type I MET TKI resistance through disruption of an autoinhibitory MET conformation (Supplemental Figure S2) (7). Moreover, the F1200 residue is conserved across multiple tyrosine kinases, including ALK, ROS1, NTRK, and ABL, in which mutations at the corresponding residue have been linked to TKI resistance (Supplemental Table 4) (21-27).

In contrast, parallel and downstream pathway alterations with the potential to provide alternative input for RAS-MAPK pathway signaling were newly detectable in eight of twelve patients following MET TKI treatment as compared to cfDNA samples obtained prior to MET TKI treatment (Figure 3C, Supplemental Table 3). In addition to two patients with *KRAS* amplification (Figure 4), which was recently implicated in MET TKI resistance (10), an *NF1* frameshift mutation, and copy number gain in *EGFR* and *KIT* were identified. In one patient with acquired KRAS amplification, addition of the MEK inhibitor trametinib to crizotinib treatment rapidly decreased detectable circulating tumor cfDNA. In the second patient with acquired *KRAS* amplification, an activating *KRAS* G12D mutation was also present within a progressing lesion on crizotinib. Further details regarding the two patients with acquired *KRAS* amplification are presented in Supplemental Figure 3.

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MEK inhibition overcomes crizotinib resistance induced by RAS-MAPK pathway alterations

The clinical data suggested that preexisting or acquired genomic changes leading to RAS-MAPK pathway activation (e.g. in *KRAS, NF1*) may limit response and induce resistance to MET TKI treatment in *MET*ex14-mutated NSCLC. In one patient with *KRAS* amplification detectable at acquired resistance to crizotinib treatment, combination treatment off-label with both crizotinib (250 mg po BID) and trametinib (2 mg po daily) resulted in rapid loss of both detectable *MET*ex14 and *KRAS* amplification by cfDNA suggestive of treatment response. However, despite molecular evidence of tumor response this combination therapy was poorly tolerated in the context of overall clinical decline, with fatigue, fluid retention, and diarrhea. Despite dose reduction to trametinib 2 mg every other day, the patient expired before radiographic response assessment (Figure S3).

To assess the functional impact of RAS-MAPK pathway hyperactivation on sensitivity to MET TKI treatment, we engineered a new Ba/F3 cell-based system. The IL-3 dependent Ba/F3 cell line, while not of epithelial origin, is an established system to assess oncogenic capacity and putative drug resistance mechanisms (28,29). Stable expression of human *MET*ex14 in Ba/F3 cells in the presence of the MET ligand human HGF (Hepatocyte Growth Factor) induced IL-3-independent growth and increased downstream Erk phosphorylation compared to expression of wild type *MET* (Figure 4D, 4F). *MET*ex14-mutant expressing cells were sensitive to treatment with crizotinib, as measured by reduced cell growth in standard cell viability assays (Figure S4).

Overexpression of wild type *KRAS* or knockdown of wild type *NF1* in *MET*ex14-expressing Ba/F3 cells induced resistance to crizotinib (Figure 4A, 4D). Overexpression of wild type *KRAS* increased the IC50 (inhibitory concentration of drug that decreases cell viability to 50%) to crizotinib from 0.16 μM to 1.68 μM (p-value < 0.001) (Figure 4A, 4G) and *NF1* downregulation increased the IC50 to crizotinib from 0.16 μM to 0.75 μM (p-value < 0.001) (Figure 4D, 4H). Treatment of cells harboring both *MET*ex14 and *KRAS* overexpression with the combination of crizotinib and the MEK inhibitor trametinib in order to block both MET signaling and downstream MAPK pathway signaling restored sensitivity to treatment (crizotinib IC50 of 1.68 μM in the absence of trametinib versus 0.25 μM with trametinib co-treatment, *p*-value < 0.001) (Figure 4B, 4G). Similarly, co-treatment of cells harboring both *METex14* and *NF1* downregulation with both trametinib and crizotinib restored sensitivity to treatment (crizotinib monotherapy IC50 0.75 μM versus 0.18 μM upon addition of trametinib, *p*-value < 0.001 (Figure 4E, 4H). The selected trametinib dose modestly reduced but did not eliminate cell growth in the absence of crizotinib (Figure S4). Immunoblotting demonstrated sustained Erk phosphorylation despite crizotinib treatment in samples with *KRAS* overexpression or *NF1* downregulation, which was abrogated by the addition of trametinib (Figure 4C, 4F). In both genomic contexts, combination treatment was associated with increased levels of

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cleaved PARP, indicative of apoptosis, which was absent with monotherapy (Figure 4C, 4F).

Discussion:

The challenge of therapeutic resistance in patients harboring *MET*ex14 mutations is of increasing clinical relevance given the emergence of MET-targeted therapies into the clinic. While off-label use of MET TKI therapy has demonstrated clinical activity, objective reported response rates of approximately 30-70% as reported in early studies (4-6) are generally lower than those seen in response to TKI treatment in NSCLC driven by other canonical oncogenes (EGFR, ALK), where response rates greater than 80% have been reported (30,31).

RAS-MAPK pathway hyperactivation has an established role in promoting resistance to EGFR, ALK, BRAF, and ROS1 targeted therapies via diverse mechanisms including *KRAS* amplification and *KRAS, BRAF*, and *NF1* mutations (32-40). While *KRAS* amplification has also recently been reported at acquired MET TKI resistance in *MET*ex14-mutated NSCLC (10), the broader role of compensatory genomic events providing signaling pathway re-activation remains less well-understood in *MET*ex14-mutated NSCLC. Here, we describe frequent co-occurring RAS-MAPK pathway alterations in *MET*ex14-mutated NSCLC as compared to *EGFR*-mutated NSCLC. RAS-MAPK pathway alterations were detected even in TKI-naïve patients; our preclinical and clinical data suggest these co-occurring alterations may promote resistance to MET TKI therapy. More specifically, RAS-MAPK pathway alterations when present as co-occurring genomic events in *MET*ex14-mutated NSCLC prior to MET TKI treatment may induce not only primary resistance but also contribute to tumor cell persistence, thus limiting response magnitude and potentially duration during initial treatment. This notion is supported by our findings in the Ba/F3 preclinical system we engineered. We also describe the spectrum and relative frequencies of newly detectable genomic alterations following MET TKI treatment as measured by cfDNA, which included alterations within the RAS-MAPK pathway or within RTKs upstream of the RAS-MAPK pathway (41) in two thirds of patients. While our reported dataset is limited by lack of complete clinical outcomes data, it highlights the importance of developing future patient cohorts incorporating outcomes data to link the understanding of the genomic landscape to treatment response and prognosis, particularly for those patients with less common or emerging driver mutations.

Clinically, while many *MET* second-site mutations acquired during type I MET TKI (e.g. crizotinib) treatment may be overcome by use of type II MET TKIs (e.g. cabozantinib) (7-9), the genomic alterations favoring RAS-MAPK pathway activation described here will likely require a combination therapy strategy. In our *MET*ex14-mutated preclinical model system, RAS-MAPK pathway hyperactivation via *KRAS* overexpression or *NF1* downregulation induced MET TKI resistance that was overcome by the addition of the

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MEK inhibitor trametinib. Changes in the cfDNA profile suggested early evidence of molecular response to treatment with a crizotinib and trametinib combination therapy in a patient with acquired *KRAS* amplification, but treatment was poorly tolerated. Future efforts to develop combination therapies against these targets will require attention to agent selection, dosing, and scheduling to achieve both tolerability and efficacy.

This study enhances the understanding of the role of co-occurring genomic alterations in *MET*ex14-mutated NSCLC, with implications for the development of personalized therapeutic strategies to enhance the initial response magnitude and duration to MET TKI and delay or overcome acquired resistance to improve clinical outcomes. Given the prominence of genomic alterations favoring RAS-MAPK pathway hyperactivation, the addition of a MEK or potentially an ERK inhibitor to MET TKI therapy is a promising combination therapy strategy which warrants further prospective study.

Methods:

Patients: Patient samples were obtained and analyzed in accordance with IRB-approved protocols. The cfDNA analysis included 332 consecutive samples from 289 patients with advanced (stage IIIB/IV) non-small cell lung cancer with a *MET*ex14 mutation obtained between October 2015 and March 2018, and 1653 consecutively tested samples from 1489 patients with *EGFR*-mutated NSCLC (L858R and del19) obtained between April 2016 and May 2017, as well as a previously published (11) cohort of TKI-naïve EGFR-mutated NSCLC.

Cell lines and reagents: Ba/F3 cells were purchased from ATCC (ATCC® HB-283™) and maintained in culture for a total of approximately 2-3 months in DMEM supplemented with 1 ng/mL IL-3 (Peprotech). Mycoplasma testing was not performed. Retrovirus was generated using TransIT-LT1 transfection reagent (Mirus). Cells were infected with filtered retrovirus, expressing either mCherry, human MET^{WT} or human MET^{ex14} in a pBABE-puro vector backbone as previously described (9) and selected in puromycin (2 µg/mL). Expression was confirmed by immunoblotting. KRAS-overexpressing cells were obtained by retroviral infection with a pBABE-hygro *KRAS4B* construct and selected in hygromycin (800 µg/mL). Knockdown of NF1 was achieved by lentiviral transduction of the following sequence: 5'-CCGGGCCAACCTTAACCTCTCTAATCTCGAGATTAGAGAGGTTAAGGTTGGCTTTTTG-3', expressed from a pLKO.1-hygro plasmid backbone (Addgene #24150). Three days after lentiviral transduction, cells were selected via treatment with hygromycin B and knockdown was confirmed by immunoblotting. All drugs were purchased from Selleck Chemicals.

Transformation and cell proliferation assays: Transformation assay was performed by removing IL-3 through centrifugation and adding 50 ng/mL human HGF (Peprotech 100-39H). For proliferation assays cells were seeded in 96-well plates at 5,000 cells/well

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and the following day were exposed to crizotinib (Selleck Chemicals, #S1068) at 0 to 10 μM and/or trametinib (Selleck Chemicals, #S2673) at 0.01 μM. After 72 hours of drug exposure, CellTiter-Glo (Promega) reagent was added and luminescence was measured on a Spectramax spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) according the manufacturer's instructions. Data are presented as percentage of viable cells compared with control cells (vehicle treatment).

Immunoblotting: Cells were washed in PBS and lysed with 25 mM Tris-HCL (pH 7.6), 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS supplemented with Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific) and Halt Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific). Lysates were separated in a 4%–15% SDS-PAGE gel and transferred onto a nitrocellulose membrane (Bio-Rad). Membranes were blocked with 5% fetal bovine serum (FBS) in Tris-buffered saline (TBS) containing 0.1% Tween and incubated with the appropriate antibodies. Detection was performed via ECL Prime (Amersham Biosciences). Antibodies against the following were obtained from Cell Signaling Technology (Danvers, MA, USA) and were used at a dilution of 1:1000: MET (#3148), p-MET Y1349 (#3121), pMEK S217/221 (#9121), ERK1/2 (#3493), p-ERK1/2 T202/Y204 (#9106), HSP90 (#4874), PARP (#9546), NF1 (#14623), horseradish peroxidase (HRP)-conjugated anti-mouse (#7076) and HRP-conjugated anti-rabbit (#7074). The following antibody was obtained from EMD Millipore (Burlington, MA, USA): RAS (05-516, 1:2000 dilution). Detection was performed via ECL Prime (Amersham Biosciences).

Cell-free DNA analysis: Samples were shipped to a Clinical Laboratory Improvement Act (CLIA)-certified, College of American Pathologists–accredited laboratory (Guardant Health, Redwood City, CA). cfDNA was extracted from whole blood collected in 10-mL Streck tubes. After double centrifugation, 5–30 ng of cfDNA was isolated for digital sequencing of either a 70 or 73-gene panel (Supplementary Table 6) as previously described (15). Only those genes in common to these two panels were included in subsequent analysis. Nonsynonymous mutations were further processed with the R statistical computing program (version 3.3). Variants with unknown or neutral predicted functional significance were filtered prior to analysis as previously described (11). Mutations previously reported as associated with clonal hematopoiesis were also excluded (16). Assignment as clonal or subclonal was performed by normalized mutational allele frequency to percentage detected using a cut off of 0.2 as previously described(11). Residue numbering was standardized to MET UniProtKB-P08581.

Next Generation Sequencing: Tumor sample next generation sequencing (NGS) was performed in CLIA-approved laboratories. The Foundation One and Foundation ACT assays are commercially available assays which were used in the clinical standard-of-care setting. The UCSF500 assay sequences 479 cancer-associated genes to target 200X coverage, utilizing sequencing of a PBMC sample, target 100X coverage, as a control. The University of Florida GatorSeq NGS assay, utilized for both tumor tissue and PBMC analysis, performs sequencing of 76 cancer-associated genes with target 500X

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coverage (Supplemental Table 2). Germline mutations were subtracted utilizing sequencing of buccal swab samples to a target depth of 100X.

Droplet Digital PCR: Isolated genomic DNA extracted from FFPE was amplified using ddPCR Supermix for Probes (Bio-Rad) using KRAS and MET assays (PrimePCR ddPCR Mutation Assay, Bio-Rad, and custom-designed). DNA template (8 µL) was added to 10 µL of ddPCR Supermix for Probes (Bio-Rad) and 2 µL of the primer/ probe mixture. This 20-µL reaction mix was added to a DG8 cartridge together with 70 µL of Droplet Generation Oil for Probes (Bio-Rad) and used for droplet generation. Droplets were then transferred to a 96-well plate (Eppendorf) and then thermal cycled with the following conditions: 5 minutes at 95°C, 40 cycles of 94°C for 30 seconds, 55°C for 1 minute followed by 98°C for 10 minutes (ramp rate 2°C/second). Droplets were analyzed with the QX200 Droplet Reader (Bio-Rad) for fluorescent measurement of FAM and HEX probes. Gating was performed based on positive and negative controls, and mutant populations were identified. The ddPCR data were analyzed with QuantaSoft analysis software (Bio-Rad) to obtain fractional abundance of the mutant DNA alleles in the wild-type (WT)/normal background. The quantification of the target molecule was presented as number of total copies (mutant plus WT) per sample in each reaction. Fractional abundance is calculated as follows: F.A. % = (Nmut/(Nmut + Nwt)) × 100), where Nmut is number of mutant events and Nwt is number of WT events per reaction. Multiple replicates were performed for each sample. ddPCR analysis of normal control genomic DNA (gene fragment obtained from IDT) and no DNA template (water) controls was performed in parallel with all samples, including multiple replicates as contamination-free controls.

Statistical Analysis: Pairwise sample group comparisons for cfDNA analysis were carried out using a two-tailed Fisher's exact t-test, with Benjamini-Hochberg correction for multiple comparisons, using a false discovery rate of less than 0.2. For cell viability curves, comparisons were performed using the two-sided student t-test, with significance threshold of p-value < 0.05.

Acknowledgements:

The authors acknowledge funding support from NIH / NCI U54CA224068 (to R.B.C.); R01CA227807, R01CA239604, R01CA230263 (to E.A.C.); NIH / NCI U01CA217882, NIH / NCI U54CA224081, NIH / NCI R01CA204302, NIH / NCI R01CA211052, NIH / NCI R01CA169338, and the Pew-Stewart Foundations (to T.G.B.); and the Damon Runyon Cancer Research Foundation, Doris Duke Charitable Foundation, V Foundation, and American Cancer Society to C.M.B.

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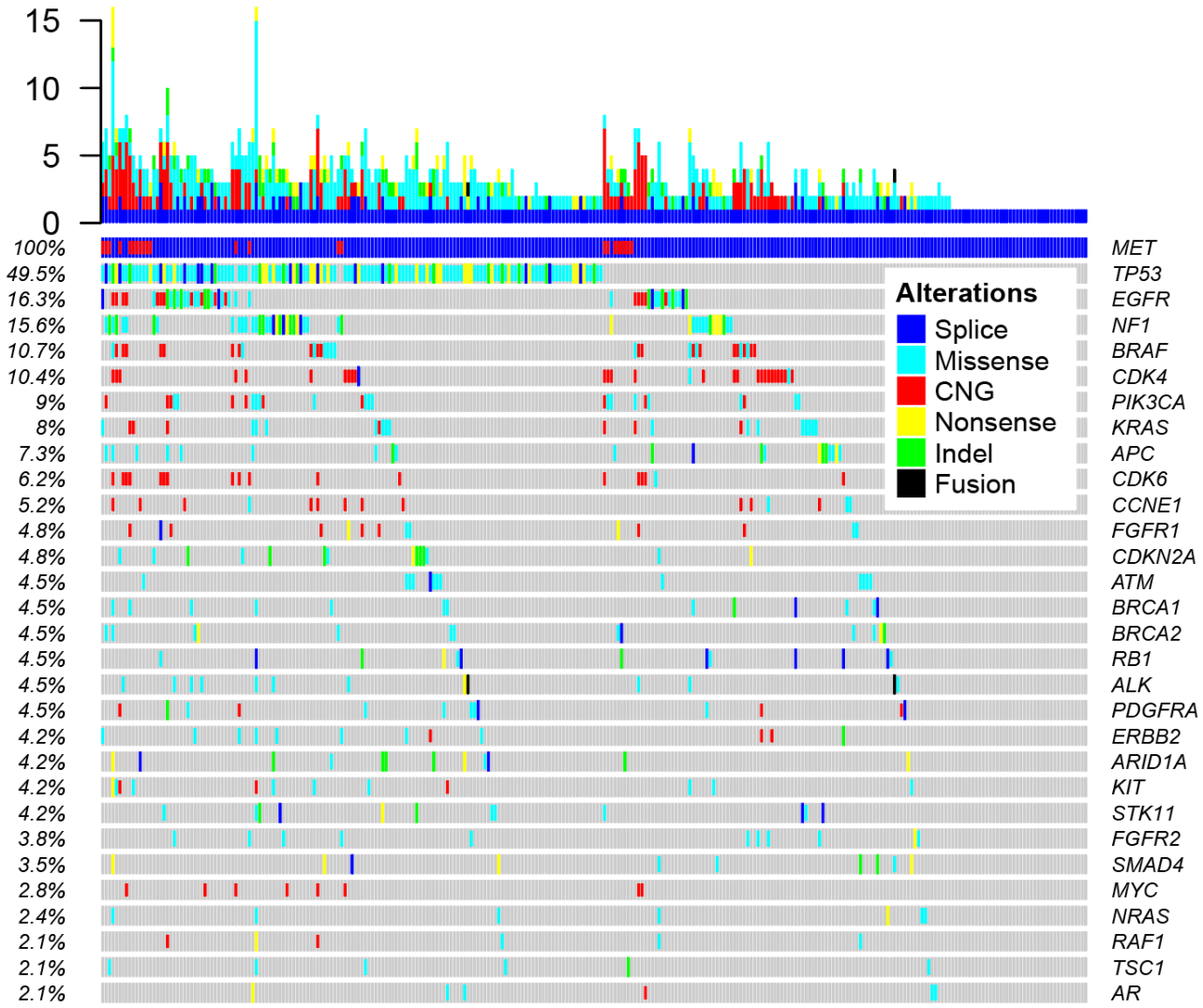

Figure 1. Co-occurring genomic alterations are common in non-small cell lung cancer (NSCLC) with a *MET* exon 14 mutation. A. Distribution of co-occurring genomic alterations in a targeted list of cancer-associated genes (Supplemental Table 2) as detected by cfDNA in 332 samples from 289 patients with advanced-stage *MET*ex14-mutated NSCLC. Results are filtered to exclude synonymous variants, variants predicted to result in an unknown or neutral function impact (via COSMIC v83, GENIE, ClinVar, and mutation assessor prediction algorithms) and mutations previously reported as associated with clonal hematopoiesis. Only genomic alterations occurring at a 2% or greater frequency are displayed. CNG, copy number gain.

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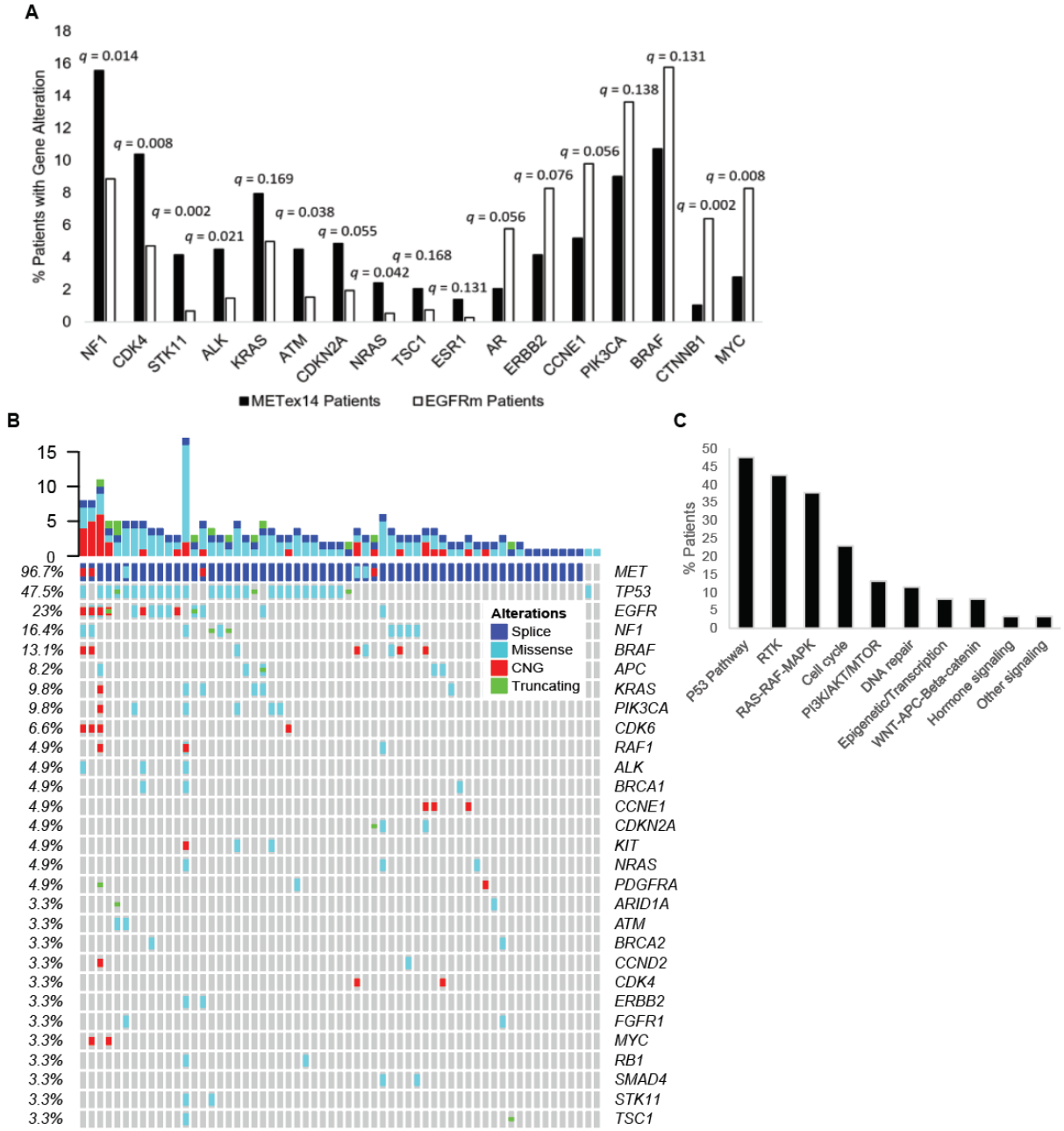

Figure 2. RAS-MAPK pathway alterations are common in *MET*ex14-mutated NSCLC
A. Comparative frequency of genomic alterations as measured by cfDNA in a cohort of advanced-stage *MET*ex14 NSCLC patients (n=289) compared to an independent cohort of patients (n=1489) with a known canonical *EGFR* activating mutation (*EGFR* exon 19 deletions, *EGFR* L858R). Gene alterations with a statistically significant difference are displayed. q-values calculated by two-tailed Fisher's exact test with Benjamini-Hochberg multiple hypothesis testing for false discovery rate < 0.2. **B**. Spectrum of gene alterations detectable by cfDNA in the subset of METex14-mutated NSCLC patients (n=61) without

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prior MET TKI treatment. Genomic alterations with a frequency of greater than 2% displayed. **C.** Genomic alterations detectable by cfDNA in the same patient cohort classified by functional category. CNG, copy number gain.

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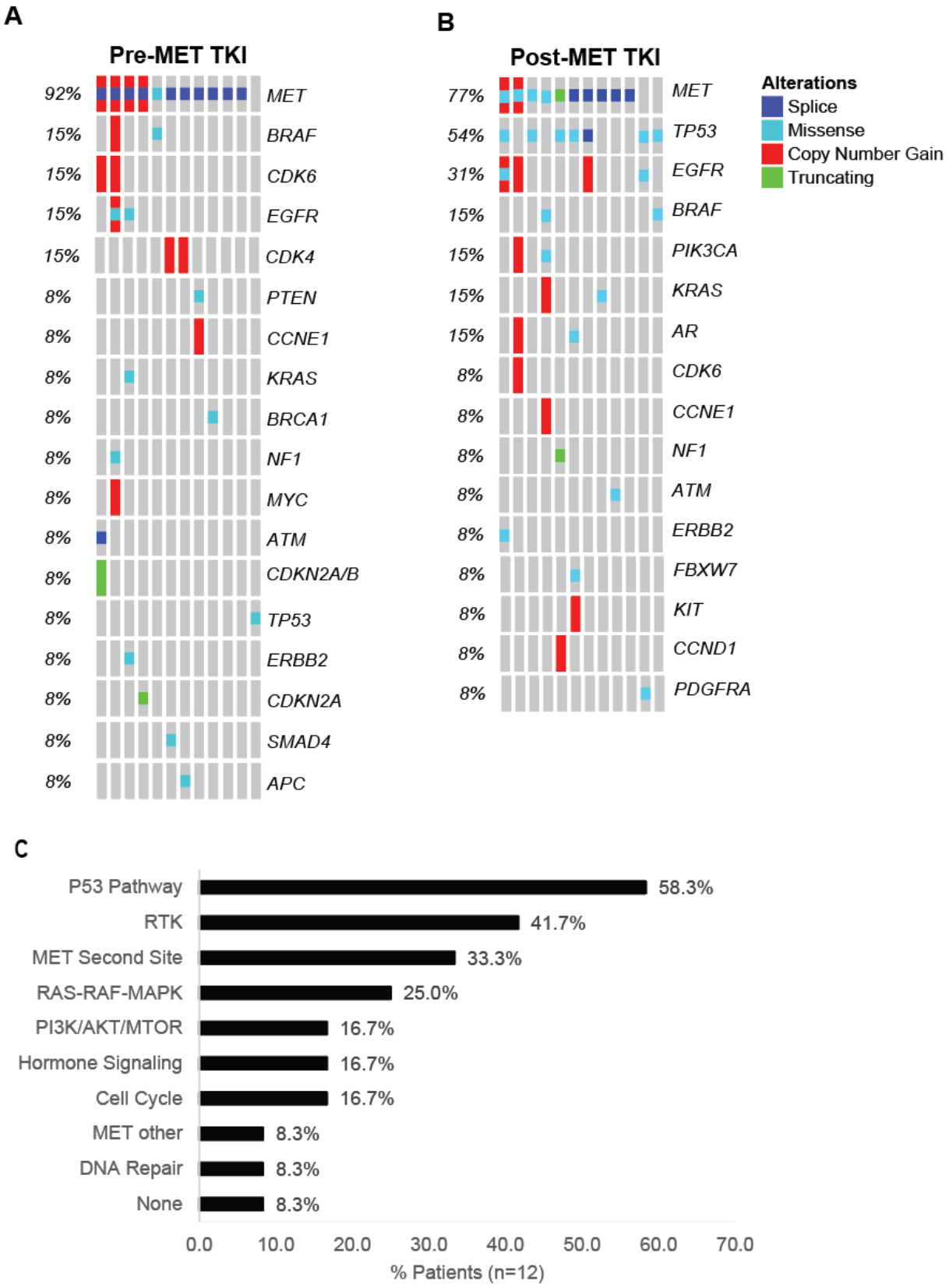

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Figure 3. *MET* second-site and bypass pathway alterations are newly detectable after MET TKI treatment. Oncoprints show detectable genomic alterations by in paired samples from 12 patients, obtained either **(A)** before known MET TKI exposure or **(B)** after known MET TKI exposure. All samples reflect the results of cfDNA analysis (Guardant360), with the exception of three patients for whom only tissue NGS was available pre-MET TKI. Genes not common to the cfDNA panel were excluded from analysis. Full details available in Supplemental Table 3. **C.** Spectrum of newly detectable genomic alterations following MET TKI exposure by functional category.

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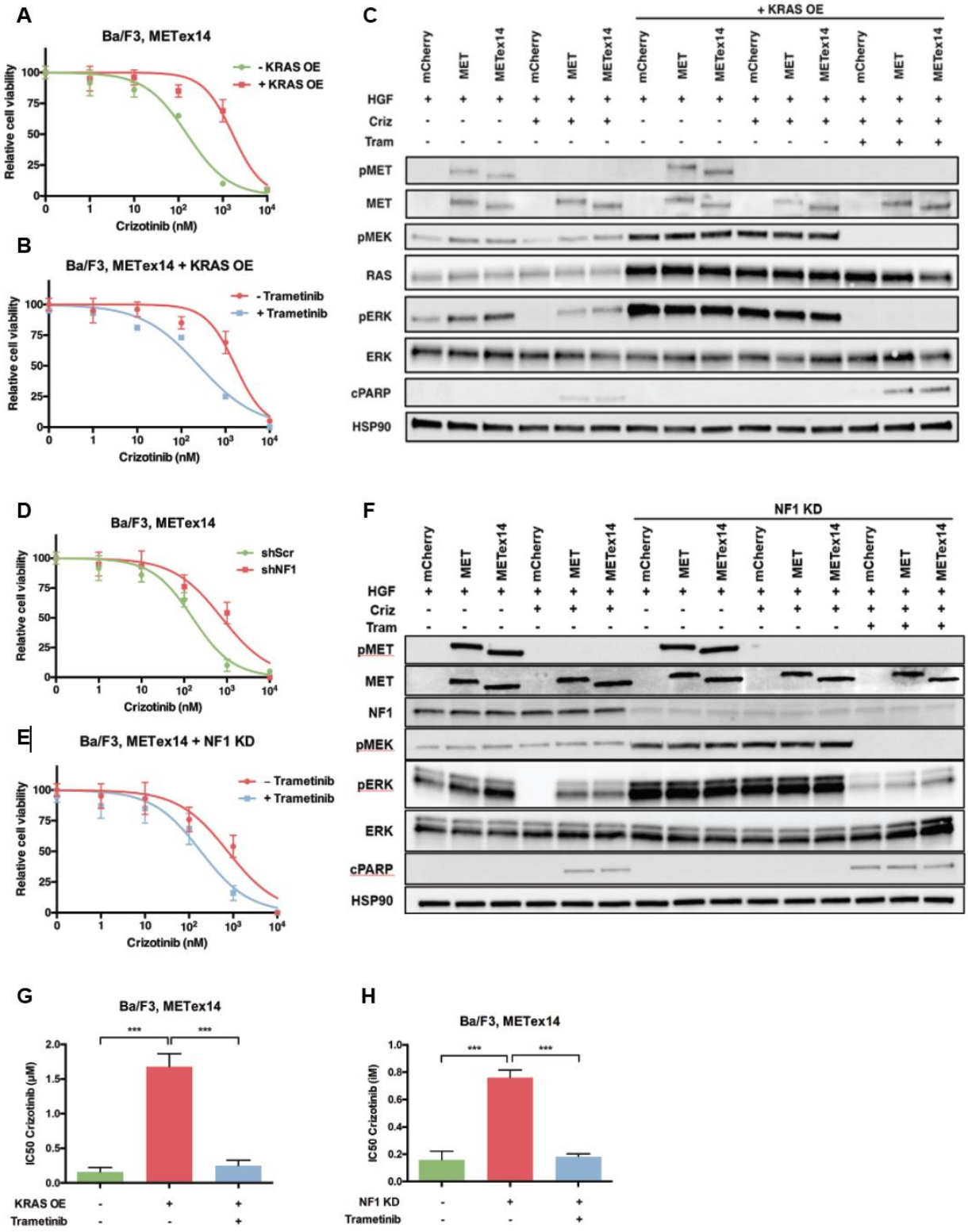

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Figure 4. *KRAS* overexpression or *NF1* downregulation promotes MET TKI resistance that is overcome by combined crizotinib and trametinib polytherapy in *MET*ex14-mutated preclinical models. A. Cell viability curves demonstrating a shift in the half maximal inhibitory concentration (IC50) to crizotinib with overexpression of wild type *KRAS* in *MET* exon 14-mutant expressing Ba/F3 cells. The cells were grown in culture with HGF supplementation (50 ng/mL). **B.** Cell viability curve for crizotinib treatment in Ba/F3 cells with both *MET*ex14 expression and wild-type *KRAS* overexpression (KRAS OE), in the setting of treatment with trametinib at 0.01 μM and supplementation with HGF 50 ng/mL. The KRAS OE, trametinib negative curves in panels A and B reflect the same experimental data, displayed on two graphs for clarity. **C.** Cell viability curves for crizotinib-treated MET exon 14-mutant expressing Ba/F3 cells with either NF1 knockdown (shNF1) or a negative control scrambled shRNA (shScr). **D.** Cell viability curves for crizotinib-treated MET exon-14 mutant expressing Ba/F3 cells with NF1 knockdown, with and without addition of 0.01 μM trametinib. The shNF1, trametinib negative curves in panels C and D reflect the same data displayed on two graphs for clarity. **E.** Ba/F3 cells with stable expression of wild type MET, MET with an exon 14 skipping mutation (METex14), or mCherry control were treated with 50 ng/mL HGF with or without 24 hours treatment with crizotinib at 0.1 μM and/or trametinib (0.01μM). Treatment with crizotinib inhibits MET phosphorylation and inhibits downstream Erk phosphorylation, with associated increase in apoptosis as measured by cleaved PARP (cPARP). KRAS overexpression (KRAS OE) restored downstream Erk phosphorylation and reduced cleaved PARP, despite crizotinib treatment. Addition of trametinib inhibited Erk phosphorylation and increased cleaved PARP, consistent with induction of apoptosis, despite the presence of KRAS overexpression. **F.** NF1 knockdown (NF1 KD) restores downstream Erk phosphorylation and decreases cleaved PARP in crizotinib-treated tumor cells. The addition of trametinib reduced Erk phosphorylation and restored PARP cleavage consistent with induction of apoptosis despite the presence of NF1. **G.** Summary graph of IC50 to crizotinib with trametinib with and without KRAS overexpression. **H.** IC50s of crizotinib with trametinib with or without NF1 downregulation. *** p-value < 0.001 by student's t-test.

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References:

1. Frampton GM, Ali SM, Rosenzweig M, Chmielecki J, Lu X, Bauer TM*, et al.* Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. Cancer Discov 2015;5(8):850-9.
2. Comprehensive molecular profiling of lung adenocarcinoma. Nature 2014;511(7511):543-50.
3. Paik PK, Drilon A, Fan PD, Yu H, Rekhtman N, Ginsberg MS*, et al.* Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. Cancer Discov 2015;5(8):842-9.
4. Felip E, Sakai H, Patel J, Horn L, Veillon R, Griesinger F*, et al.* Phase II Data for the MET Inhibitor Tepotinib in Patients with Advanced NSCLC and MET Exon 14-Skipping Mutations. IASLC 19th World Conference on Lung Cancer. Toronto, Canada2018.
5. Drilon AC, J. W., Weiss J, Ou I, Camidge R, Solomon B, Otterson GA*, et al.* Updated Antitumor Activity of Crizotinib in Patients with MET Exon 14-Altered Advanced Non-Small Cell Lung Cancer. 2018 9/25/18; Toronta, Canada.
6. Wolf J, Seto T, Han J-Y, Reguart N, Garon EB, Groen H*, et al.* Results of the GEOMETRY mono-1 phase II study for evaluation of the MET inhibitor capmatinib (INC280) in patients (pts) with METex14 mutated advanced non-small cell lung cancer (NSCLC). Annals of Oncology 2018;29(Supplement 8).
7. Engstrom LD, Aranda R, Lee M, Tovar EA, Essenburg CJ, Madaj Z*, et al.* Glesatinib Exhibits Antitumor Activity in Lung Cancer Models and Patients Harboring MET Exon 14 Mutations and Overcomes Mutation-mediated Resistance to Type I MET Inhibitors in Nonclinical Models. Clin Cancer Res 2017;23(21):6661-72.
8. Bahcall M, Sim T, Paweletz CP, Patel JD, Alden RS, Kuang Y*, et al.* Acquired METD1228V Mutation and Resistance to MET Inhibition in Lung Cancer. Cancer Discov 2016;6(12):1334-41.
9. Lu X, Peled N, Greer J, Wu W, Choi P, Berger AH*, et al.* MET Exon 14 Mutation Encodes an Actionable Therapeutic Target in Lung Adenocarcinoma. Cancer Res 2017;77(16):4498-505.
10. Bahcall M, Awad MM, Sholl LM, Wilson FH, Xu M, Wang S*, et al.* Amplification of Wild-type KRAS Imparts Resistance to Crizotinib in MET Exon 14 Mutant Non-Small Cell Lung Cancer. Clin Cancer Res 2018.
11. Blakely CM, Watkins TBK, Wu W, Gini B, Chabon JJ, McCoach CE*, et al.* Evolution and clinical impact of co-occurring genetic alterations in advanced-stage EGFR-mutant lung cancers. Nat Genet 2017;49(12):1693-704.
12. Jordan EJ, Kim HR, Arcila ME, Barron D, Chakravarty D, Gao J*, et al.* Prospective Comprehensive Molecular Characterization of Lung Adenocarcinomas for Efficient Patient Matching to Approved and Emerging Therapies. Cancer Discov 2017;7(6):596-609.
13. McCoach CE, Blakely CM, Banks KC, Levy B, Chue BM, Raymond VM*, et al.* Clinical Utility of Cell-Free DNA for the Detection of ALK Fusions and Genomic Mechanisms of ALK Inhibitor Resistance in Non-Small Cell Lung Cancer. Clin Cancer Res 2018;24(12):2758-70.
14. Russo M, Siravegna G, Blaszkowsky LS, Corti G, Crisafulli G, Ahronian LG*, et al.* Tumor Heterogeneity and Lesion-Specific Response to Targeted Therapy in Colorectal Cancer. Cancer Discov 2016;6(2):147-53.
15. Odegaard JI, Vincent JJ, Mortimer S, Vowles JV, Ulrich BC, Banks KC*, et al.* Validation of a Plasma-Based Comprehensive Cancer Genotyping Assay Utilizing Orthogonal Tissue- and Plasma-Based Methodologies. Clin Cancer Res 2018.

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16. Ptashkin RN, Mandelker DL, Coombs CC, Bolton K, Yelskaya Z, Hyman DM*, et al.* Prevalence of Clonal Hematopoiesis Mutations in Tumor-Only Clinical Genomic Profiling of Solid Tumors. JAMA Oncol 2018.
17. Heist RS, Sequist LV, Borger D, Gainor JF, Arellano RS, Le LP*, et al.* Acquired Resistance to Crizotinib in NSCLC with MET Exon 14 Skipping. J Thorac Oncol 2016;11(8):1242-5.
18. Ou SI, Young L, Schrock AB, Johnson A, Klempner SJ, Zhu VW*, et al.* Emergence of Preexisting MET Y1230C Mutation as a Resistance Mechanism to Crizotinib in NSCLC with MET Exon 14 Skipping. J Thorac Oncol 2017;12(1):137-40.
19. Schrock AB, Lai A, Ali SM, Miller VA, Raez LE. Mutation of MET Y1230 as an Acquired Mechanism of Crizotinib Resistance in NSCLC with MET Exon 14 Skipping. J Thorac Oncol 2017;12(7):e89-e90.
20. Schmidt L, Junker K, Nakaigawa N, Kinjerski T, Weirich G, Miller M*, et al.* Novel mutations of the MET proto-oncogene in papillary renal carcinomas. Oncogene 1999;18(14):2343-50.
21. Tiedt R, Degenkolbe E, Furet P, Appleton BA, Wagner S, Schoepfer J*, et al.* A drug resistance screen using a selective MET inhibitor reveals a spectrum of mutations that partially overlap with activating mutations found in cancer patients. Cancer Res 2011;71(15):5255-64.
22. Kodityal S, Elvin JA, Squillace R, Agarwal N, Miller VA, Ali SM*, et al.* A novel acquired ALK F1245C mutation confers resistance to crizotinib in ALK-positive NSCLC but is sensitive to ceritinib. Lung Cancer 2016;92:19-21.
23. Hallberg B, Palmer RH. Mechanistic insight into ALK receptor tyrosine kinase in human cancer biology. Nat Rev Cancer 2013;13(10):685-700.
24. Davare MA, Vellore NA, Wagner JP, Eide CA, Goodman JR, Drilon A*, et al.* Structural insight into selectivity and resistance profiles of ROS1 tyrosine kinase inhibitors. Proc Natl Acad Sci U S A 2015;112(39):E5381-90.
25. Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J*, et al.* Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell 2002;2(2):117-25.
26. Hughes T, Saglio G, Branford S, Soverini S, Kim DW, Muller MC*, et al.* Impact of baseline BCR-ABL mutations on response to nilotinib in patients with chronic myeloid leukemia in chronic phase. J Clin Oncol 2009;27(25):4204-10.
27. Fuse MJ, Okada K, Oh-Hara T, Ogura H, Fujita N, Katayama R. Mechanisms of Resistance to NTRK Inhibitors and Therapeutic Strategies in NTRK1-Rearranged Cancers. Mol Cancer Ther 2017;16(10):2130-43.
28. Mandanas RA, Boswell HS, Lu L, Leibowitz D. BCR/ABL confers growth factor independence upon a murine myeloid cell line. Leukemia 1992;6(8):796-800.
29. Greulich H, Chen TH, Feng W, Janne PA, Alvarez JV, Zappaterra M*, et al.* Oncogenic transformation by inhibitor-sensitive and -resistant EGFR mutants. PLoS Med 2005;2(11):e313.
30. Soria JC, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH*, et al.* Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. N Engl J Med 2018;378(2):113-25.
31. Peters S, Camidge DR, Shaw AT, Gadgeel S, Ahn JS, Kim DW*, et al.* Alectinib versus Crizotinib in Untreated ALK-Positive Non-Small-Cell Lung Cancer. N Engl J Med 2017.
32. Hrustanovic G, Olivas V, Pazarentzos E, Tulpule A, Asthana S, Blakely CM*, et al.* RAS-MAPK dependence underlies a rational polytherapy strategy in EML4-ALK-positive lung cancer. Nat Med 2015;21(9):1038-47.
33. Crystal AS, Shaw AT, Sequist LV, Friboulet L, Niederst MJ, Lockerman EL*, et al.* Patient-derived models of acquired resistance can identify effective drug combinations for cancer. Science 2014;346(6216):1480-6.

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34. Zhu YC, Lin XP, Li XF, Wu LX, Chen HF, Wang WX*, et al.* Concurrent ROS1 gene rearrangement and KRAS mutation in lung adenocarcinoma: A case report and literature review. Thorac Cancer 2018;9(1):159-63.
35. Cargnelutti M, Corso S, Pergolizzi M, Mevellec L, Aisner DL, Dziadziuszko R*, et al.* Activation of RAS family members confers resistance to ROS1 targeting drugs. Oncotarget 2015;6(7):5182-94.
36. Ohashi K, Sequist LV, Arcila ME, Moran T, Chmielecki J, Lin YL*, et al.* Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF gene mutations but lack mutations in KRAS, NRAS, or MEK1. Proc Natl Acad Sci U S A 2012;109(31):E2127-33.
37. de Bruin EC, Cowell C, Warne PH, Jiang M, Saunders RE, Melnick MA*, et al.* Reduced NF1 expression confers resistance to EGFR inhibition in lung cancer. Cancer Discov 2014;4(5):606-19.
38. Tricker EM, Xu C, Uddin S, Capelletti M, Ercan D, Ogino A*, et al.* Combined EGFR/MEK Inhibition Prevents the Emergence of Resistance in EGFR mutant Lung Cancer. Cancer Discov 2015.
39. Ercan D, Xu C, Yanagita M, Monast CS, Pratilas CA, Montero J*, et al.* Reactivation of ERK signaling causes resistance to EGFR kinase inhibitors. Cancer Discov 2012;2(10):934-47.
40. Ho CC, Liao WY, Lin CA, Shih JY, Yu CJ, Chih-Hsin Yang J. Acquired BRAF V600E Mutation as Resistant Mechanism after Treatment with Osimertinib. J Thorac Oncol 2017;12(3):567-72.
41. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. Nat Rev Cancer 2011;11(11):761-74.
42. Talevich E, Shain AH, Botton T, Bastian BC. CNVkit: Genome-Wide Copy Number Detection and Visualization from Targeted DNA Sequencing. PLoS Comput Biol 2016;12(4):e1004873.
43. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215(3):403-10.