TITLE

Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors

RUNNING TITLE

MET exon 14 alterations confer response to targeted therapy

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CONFLICT OF INTEREST

All Foundation Medicine authors are employees of and stock-holders in Foundation Medicine Inc.. No other conflicts of interest are declared.

ABSTRACT

Focal amplification and activating point mutation of the *MET* gene are well characterized oncogenic drivers that confer susceptibility to targeted MET inhibitors. Recurrent somatic splice site alterations at *MET* exon 14 (METex14) that result in exon skipping and MET activation have been characterized, but their full diversity and prevalence across tumor types is unknown. Here we report analysis of tumor genomic profiles from 38,028 patients to identify 221 cases with METex14 mutations (0.6%), including 126 distinct sequence variants. METex14 mutations are detected most frequently in lung adenocarcinoma (3.0%), but also frequently in other lung neoplasms (2.3%), brain glioma (0.4%), and tumors of unknown primary origin (0.4%). Further in vitro studies demonstrate sensitivity to MET inhibitors in cells harboring METex14 alterations. We also report three new patient cases with METex14 alterations in lung or histiocytic sarcoma tumors that showed durable response to two different MET targeted therapies. The diversity of METex14 mutations indicates that diagnostic testing via comprehensive genomic profiling is necessary for detection in a clinical setting.

SIGNIFICANCE

Here, we report the identification of diverse exon 14 splice site alterations in MET that result in constitutive activity of this receptor and oncogenic transformation in vitro. Patients whose tumors harbored these alterations derived meaningful clinical benefit from MET inhibitors. Collectively, these data support the role of METex14 alterations as drivers of tumorigenesis, and identify a unique subset of patients likely to derive benefit from MET inhibitors.

INTRODUCTION

Personalized medicine offers great promise in cancer treatment, by matching patients with targeted therapies that act based on the specific molecular alterations present in their tumors. Targeted therapies have the potential to be more effective than conventional cytotoxic chemotherapies, often with fewer side-effects (1). Consequently, the identification of new subsets of patients likely to benefit from targeted therapy is critically important for improving cancer patient care.

The hepatocyte growth factor receptor, encoded by the *MET* oncogene, is a receptor tyrosine kinase that plays a fundamental role in regulating development and cell growth. Upon stimulation, MET induces a cellular program known as invasive growth, which promotes mitogenesis, motility, invasion, and morphogenesis. Pathological activation of MET, through both gene copy number amplification and point mutation, are well characterized drivers of oncogenesis that occur in many different types of tumors. In cancer, activation of MET promotes tumor proliferation, invasive growth and angiogenesis (2).

Accumulating evidence suggests that patients with tumors harboring *MET* alterations can benefit from targeted therapies (3). A number of drugs have been developed that repress MET activation and/or signaling, including small molecule kinase inhibitors and monoclonal antibodies targeting MET or its ligand, HGF. For example, treatment with crizotinib has benefited patients with tumors containing high level *MET* amplifications including NSCLC, gastroesophageal cancer, glioblastoma, and carcinoma of unknown primary (4-8) and the dual MET/VEGFR2 inhibitor foretinib provided benefit to patients with MET-mutated papillary renal cell carcinoma (9). MET-targeting antibodies onartuzumab and MetMAb have elicited responses in patients with MET-amplified NSCLC and gastric cancer (10-11). In addition, high MET expression has been suggested to predict response of patients with GEJ carcinoma to therapy regimen involving rilotumumab, a monoclonal HGF-targeting antibody (12).

Somatic mutations affecting splice sites of exon 14 of the *MET* gene (METex14) were first reported in primary lung cancer specimens and in a lung cancer cell line (13-14). These METex14 alterations were shown to promote RNA-splicing based skipping of *MET* exon 14, which results in activation of MET kinase activity through a unique mechanism. The portion of the protein encoded by exon 14, most prominently Y1003 in a DpYR motif, is required for efficient recruitment of ubiquitin ligase CBL, which targets MET for ubiquitin-mediated degradation (15-17). Loss of MET exon 14 maintains the reading frame and leads to increased MET stability and prolonged signaling upon HGF stimulation, leading to increased oncogenic potential (18-19). Inclusion of MET exon 14 into an oncogenic TPR-MET fusion, in which exon 14 is conspicuously excluded, leads to reduction of TPR-MET oncogenic potential (20). Thus, in cancer, genomic alterations that promote METex14 skipping lead to oncogenic MET activation.

METex14 alterations have since been shown to occur in ~3% of lung adenocarcinoma cases (14,21-25) and have also been observed in neuroblastoma and gastric cancer cell lines (26-27). In total, fewer than twenty distinct METex14 sequence variants have been described, and their full diversity and prevalence across tumor types has not been characterized (Supplementary Table 1).

In vitro pre-clinical studies indicate that MET targeted agents can counteract oncogenesis resulting from MET exon 14 loss (13,16). This suggests that targeted therapies inhibiting MET signaling would be beneficial for patients with METex14 alterations. Recently, three case reports have demonstrated clinical response to crizotinib, a tyrosine kinase inhibitor, in lung carcinoma patients with METex14 alterations (28-30).

We present a large series of genomic profiles of advanced cancers, assayed in the course of clinical care, with METex14 alterations. We also present *in vitro* studies, further demonstrating the oncogenic potential of METex14 alterations. Finally, we report durable responses to MET targeted therapy in three patients with tumors harboring METex14 alterations.

RESULTS

Comprehensive cancer genome profiling (31) was performed on 38,028 tumor specimens, from unique patients, in the course of routine clinical care, in a CLIA certified laboratory, between April 2012 and February 2015. Base substitution, indel, copy number alteration, and rearrangement alterations were examined to identify those likely to affect splicing of exon 14 of the *MET* gene (METex14 alterations). In total, 224 distinct METex14 alterations were identified, occurring in 221 specimens. These alterations displayed remarkably diverse sequence composition, with 126 different genomic sequence variants represented. The alterations were comprised base substitutions (n=2) and indels (33) at splice acceptor sites, base substitutions (102) and indels at splice donor sites, and base substitutions (2) and indels (49) in the ~25 bp intronic noncoding region immediately adjacent to the splice acceptor site (Figure 1A). We also identified five samples with whole exon deletions of *MET* exon 14 (Figure 1B). Indels were predominantly deletions, but several insertions and complex indels were detected (Supplementary Table 2).

METex14 alterations were detected in 221 cases and were distributed among primary disease site as; lung adenocarcinoma (3.0%, 131/4402, 95% CI; 2.5-3.5%), other lung neoplasms (2.3%, 62/2669, 1.8-3.0%), brain glioma (0.4%, 6/1708, 0.1-0.8%), tumors of unknown primary origin (0.4%, 15/3376, 0.3-0.7%), and other tumor types (<0.1%, 7/25873). METex14 alterations were not found in tumors of the female reproductive system (n=7436), colon and rectum (3714), pancreas (1424), or hematopoietic system (2113). We did not observe a statistically significant difference between the rates of METex14 alterations in the various subtypes of lung carcinoma. Additionally, the distribution of the genomic position and type (base substitution, deletion, insertion or complex indel) of METex14 alterations did not vary significantly among the different sites of tumor primary origin.

We examined the other genomic alterations co-occurring with METex14, focusing on the cohort of 4,402 lung adenocarcinoma (LA) specimens (Figure 2A, Supplementary Table 3). Multiple other receptor tyrosine kinase or MAPK pathway driver mutations in LA have been described, including activating mutations in *KRAS*, *EGFR*, *ERBB2*, *BRAF* and *MET* as well as gene fusions involving *ALK*, *RET*, and *ROS1* (24, 32-33). Examining co-occurrence among mutations in each of these genes, we observed that they were mutually exclusive (Figure 2B). This exclusivity of LA driver alterations has been observed previously and is confirmed in this large cohort of LA specimens. Tumors with METex14 alterations rarely harbored the other known drivers of LA, as has been previously observed in other cohorts (22-25), supporting the role of METex14 alterations as oncogenic driver mutations. We also observed that mutations in *KRAS*, *EGFR*, *ERBB2* and *MET* each frequently co-occurred with copy number amplification of the same gene, highlighting the cumulative effect of gene activation by both mutation and amplification.

We next examined co-occurrence of other frequently occurring genomic alterations in LA (Figure 2C-D). In addition to their mutual exclusivity, each of the driver mutations had distinct pattern of co-occurring alterations, further supporting the hypothesis that they define distinct molecular sub-types of LA. Notably, METex14 splicing alterations were strongly coincident with amplification of *MDM2* and *CDK4*, on chromosome 12q. Unlike METex14 alterations, copy number amplifications of *MET* were not significant coincident with *MDM2*/*CDK4*

amplification. We also observed strong and statistically significant co-occurrence of mutations in several pairs of genes, some of which have been described previously (34-35) most notably KRAS/STK11, STK11/KEAP1, and PIK3CA/RB1. We observed significant anti-occurrence between EGFR/STK11, EGFR/KEAP1, ERBB2/NF1, and CDKN2A/RB1. Many other statistically significant gene occurrence interactions were observed (Supplementary Table 4).

In addition to the METex14 alterations, we observed two cases with MET p.Y1003N (c.3007T>A) alterations. This alteration has been observed previously (36), and likely activates MET in a fashion similar to METex14 alterations, by preventing CBL-mediated degradation of MET. Interestingly, both of these cases were NSCLC, harbored copy number amplifications of *MDM2* and *CDK4,* and lacked other characteristic NSCLC driver alterations such as *KRAS*, *EGFR*, *ERBB2*, *BRAF*, *ALK*, *RET*, and *ROS*.

We sought to further characterize the function of MET exon 14 skipping using cell line models. To model METex14 alterations occurring in human patient samples, human *MET* cDNA with exon 14 deleted ($MET^{\Delta 14}$) and mouse *Met* with the homologous exon 15 deleted ($MET^{\Delta 15}$) were generated through site-directed mutagenesis. In the human embryonic kidney cell line, HEK 293, transient expression of $MET^{\Delta 14}$ activated MEK-ERK signaling, as indicated by increased ERK activation phosphorylation under both 10% FBS and serum starvation condition (Figure 3A).

In the mouse fibroblast cell line NIH3T3, we generated clones with stable ectopic expression of wildtype MET (MET^{wt}) and $MET^{\Delta 15}$ as well as $HRAS^{G12V}$ and RFP as controls. Gain of MET activity in $MET^{\Delta 15}$ as compared with MET^{wt} was indicated by increased phosphorylation of the $MET^{\Delta 15}$ at Y1234/1235 sites. Western blotting with specific antibodies confirmed expression of MET^{wt}, $MET^{\Delta 15}$, and HRAS. We also measured the transforming ability of *Met* variants lacking exon 15 in soft agar colony forming assays. $MET^{\Delta 15}$ increased anchorage-independent colony formation 10 fold, as compared with MET^{wt} (Figure 3B). These findings are consistent with the previous reports noting the oncogenic nature of MET variants lacking exon 14-mediated CBL binding and further highlight the evolutionarily conserved role of the MET exon14-encoded portion of the juxtamembrane domain in attenuation of MET signaling.

INC280 (capmatinib) is a highly selective and potent small-molecule inhibitor of the MET receptor tyrosine kinase (half maximal inhibitory concentration [IC_{50}] value of 0.13 nM). It is highly specific, with >10,000-fold selectivity for c-MET in biochemical studies with a panel of human kinases. INC280 demonstrates potent activity (IC_{50} values of 0.2–2 nM) in cell-based biochemical and functional assays that measure c-MET signaling and c-MET-dependent cell proliferation and survival (37-38).

To determine if $MET^{\Delta 15}$ expressing cells are sensitive to INC280, proliferation was measured by CellTiterGlo assay 72 hrs after treatment. NIH3T3 cells expressing MET^{wt} or $MET^{\Delta 15}$ showed dose-dependent inhibition of cell proliferation with INC280 treatment and at 20nM concentration, cell survival rate of $MET^{\Delta 15}$ is significantly lower than RFP control. Cells expressing either $MET^{\Delta 15}$ or $HRAS^{G12V}$ were sensitive to trametinib, a MEK1/2 inhibitor. In comparison, cells expressing $HRAS^{G12V}$ were resistant to INC280, suggesting a high selectivity of INC280 to MET-driven cells. (Figure 3C-D). These *in vitro* results suggest that genomic

alterations resulting in MET exon 14 skipping or loss are oncogenic; cells expressing these forms of MET are dependent on its aberrant signaling, and potentially sensitive to inhibition with MET-selective agents.

Given preclinical evidence suggesting sensitivity to MET inhibitors, the clinical outcomes for patients harboring METex14 alterations were investigated. We were able to successfully identify a small number of patients that had been treated with appropriate targeted therapies. All cases had been subjected to comprehensive genomic profiling in the course of routine clinical care. Although only a subset of cases were available for evaluation, in this limited sampling, the outcomes for those obtaining MET inhibitors tended very strongly to favorable responses.

An 84 year old female never smoker had a palpable left upper anterior chest mass incidentally identified during the course of an examination after a minor trauma. Imaging demonstrated a mass 13 cm in the largest dimension traversing the left lung and chest wall, and was deemed to be unresectable, stage III disease. Morphologic and immunohistochemical characterization of a biopsy of the chest wall mass demonstrated histocytic sarcoma (Figure 4A-B). Comprehensive genomic profiling demonstrated that that the tumor harbored a METex14 alteration (c.2888-5_2944del62) as well as *TP53* p.R175H and *ZMYM3* c.3008-1G>A. The patient was not a candidate for surgical therapy, so systemic treatment options were investigated. On four months of treatment with crizotinib, the lesion decreased >60% in volume, deemed a partial response (PR) under RECIST criteria 1.1 (Figure 4C-E). The patient experienced minimal toxicity on crizotinib, but did have disease progression as assessed by imaging at 8 months.

In a separate clinical trial (NCT01324479), two non-small cell lung carcinoma cases with METex14 alterations were identified by comprehensive genomic profiling. This trial is a phase I open-label, dose escalation study with expansion to assess the safety and tolerability of the investigational MET inhibitor INC280 (capmatinib) in patients with MET dependent advanced solid tumors. Both patients received capmatinib, described in detail above, and were treated at Sarah Cannon Research Institute, Nashville, TN, USA.

An 82 year old female, with a 25 pack-year smoking history, was diagnosed with stage IV large cell lung carcinoma with right hilar node metastases. Initial therapy included complete surgical resection, the patient declined peri-operative chemotherapy, and was monitored until recurrence of disease three years and three months later. The patient declined treatment with standard of care chemotherapy regimens and instead elected to enter the clinical trial above. Comprehensive genomic profiling was performed on the primary resection and demonstrated that the tumor harbored a METex14 alteration (c.3028G>C) and TP53 p.N30fs*14. *MET* gene copy number was six, in a triploid cancer genome, as measured by NGS-based comprehensive genomic profiling. MET IHC performed on the same specimen was 3+ (H-score 270). MET FISH was not performed. The patient was treated with capmatinib for more than five months and had a tumor reduction of 53%, a partial response (Figure 5A-B).

A 66 year old female, with a 4 pack-year smoking history, was diagnosed with stage Ib poorly differentiated squamous cell carcinoma of lung (LSCC), which was resected, and followed immediately by adjuvant gemcitabine and carboplatin that was discontinued after a single cycle due to toxicity. The patient was monitored only until her disease recurred after nine months in the soft tissue of the axilla and chest wall; she was also later noted to have CNS, bone, and renal

metastases. The patient subsequently underwent multiple administration of radiation therapy to the body with palliative intent, whole brain radiotherapy, weekly taxol and carboplatin for 4 months, and subsequently was enrolled a phase I clinical trial for a CHK1-inhibitor, but progressed after two months on this therapy. Upon enrollment into the capmatinib study, comprehensive genomic profiling demonstrated the LSCC harbored a METex14 alteration (c.3028+1G>T). *MET* gene copy number was four. Additional molecular testing indicated MET FISH 13.8 copy number (MET:CEBP7 ratio 2.3) and IHC 3+ (H-score 300). The patient was treated with capmatinib for 13 months with tumor reduction of 61%, a partial response. On disease progression, the patient's tumor burden remained significantly decreased from baseline and disease-related pain did not recur (Figure 5C-D).

DISCUSSION

METex14 alterations are important recurrent alterations that are clinically and therapeutically relevant, occurring in approximately 3% of lung adenocarcinomas, 2% of other lung neoplasms, ½ percent of brain gliomas and ½ percent of carcinomas of unknown primary origin. Consequently, the assessment of METex14 alteration status will be appropriate for many advanced cancer patients. In the context of NSCLC, the demonstration of mutually exclusivity between METex14 alterations and other oncogenic drivers is consistent with METex14 itself being such a driver. Three cases with durable responses to MET targeted therapy presented in this study included response to crizotinib, an FDA approved inhibitor targeting MET and ALK, as well as capmatinib, a highly selective and potent small-molecule MET inhibitor that is in clinical development.

In addition, three other such reports of response to targeted therapy in cases with METex14 alterations have been recently published, further extending the evidence of potential clinical benefit (28-30). As there are no clinical trials at present focusing on the METex14 advanced cancer population, the accumulation of clinical responses presented in vignette form is the sole form of clinical evidence demonstrating the targetability of METex14. In the near future, it may come to light that cancer cases with METex14 alterations were fortuitously enrolled in trials for anti-MET targeted therapy on the basis of other eligibility criteria, and responses of such cases will further buttress the notion of possible clinical benefit presented here.

The early data presented here, suggests that METex14 alterations present a viable therapeutic target and could be added to the growing list of known oncogenic drivers in NSCLC as well as other tumor types. Moreover, the frequency of METex14 alterations in NSCLC presented here is comparable to, if not exceeding the frequency of MET amplifications in NSCLC, and effectively doubles the number of NSCLC cases that could respond to anti-MET targeted therapy. We also note that the METex14 alterations reported here are not all likely to result in the same amount of MET exon 14 skipping and pathogenicity, indicating that further study of these alterations is warranted.

It is interesting that in two of our clinical cases, as well is in one recently published case (28), a METex14 alteration was accompanied by MET overexpression by IHC, with one of those cases also containing an apparent *MET* gene copy number amplification. In the third presented case, neither IHC nor FISH analysis were performed. In preclinical studies, lack of CBL binding to both human and murine MET exon 14 regions (MET exon 15 in mouse), such as via skipping of MET exon 14, has been shown to impair MET down-regulation and degradation, leading to increased MET protein expression (13, 16, 18-19). Indeed, MET overexpression has been previously noted in lung tumors with METex14 alterations, and MET variants lacking exon 14 were noted to be preferentially overexpressed in those cases rather than the full-length MET (13). As mentioned above, MET amplification, presumably leading to MET overexpression, has been shown to be confer sensitivity to MET inhibitors in a variety of tumor types. Thus, the functional basis for MET inhibitor sensitivity may be similar in patients with METex14 alterations and MET amplification in their tumors.

The levels of MET protein lacking exon 14 compared to full-length MET in the tumors of the three patients who achieved responses to MET inhibitors is not known. Therefore, the possibility that overexpression of full-length MET being a driver alteration responsible for sensitivity to MET inhibitors can't be excluded. However, the lack of detectable *MET* amplification in two of the three sensitive tumors, the report of MET variants lacking exon 14 being preferentially expressed over full-length MET in lung cancer samples (13) and the oncogenic nature of METx14 alterations all suggest that the inhibition of MET variants lacking exon 14 contributed to the observed clinical responses.

It is also interesting to note that none of the three responders in our cohort had either *MDM2* or *CDK4* amplification in their tumors. As mentioned above, gene copy number amplification of *MDM2*, and less frequently of *CDK4* is highly coincident with METex14 alterations. Whether amplification of either *MDM2* or *CDK4* might impact sensitivity of tumors with METex14 alterations to MET inhibitors is currently unclear. Among the three recently published case studies, a patient with a METex14 alteration and amplification of *MDM2* and *CDK4* in their tumor (28) exhibited the shortest response to MET targeted agent of the six responses known to date, but a patient with a *METex14* alteration and amplification of *MDM2*, but not of *CDK4* in their tumor exhibited a major response (30). However, it is difficult to draw conclusions regarding the effect of *MDM2* or *CDK4* amplification on the responsiveness to MET inhibitors at this time. Since numerous inhibitors of MDM2 and CDK4 are currently being clinically evaluated in a variety of cancer types, including the CDK4/6 inhibitor palbociclib, which has been FDA approved for the treatment of breast cancer, the efficacy of combined MET and MDM2/CDK4 inhibition in preclinical models is worth investigating.

In summary, these results demonstrate that METex14 alterations occur in multiple tumor types, particularly lung carcinoma, and can confer clinical sensitivity to targeted therapies. Identification of this new patient population is an important step towards making appropriate targeted therapies available for all cancer patients.

METex14 alterations pose a challenge for diagnostic testing. They exhibit highly diverse sequence composition, many are novel, and more than half are indel mutations (up to 3kb in length), which are challenging to detect with high sensitivity and specificity. Consequently, assessing METex14 alteration status requires appropriate laboratory and analytic methods, capable of accurate sequencing, statistical detection, annotation and reporting of this diverse class of alterations.

As the number of targeted therapies and molecular alterations that are relevant for routine cancer patient treatment continues to grow, comprehensive genomic profiling will be increasingly required to accurately stratify patients for appropriate therapy. Finally, the diversity of METex14 alterations highlights the need for profiling of large numbers of cancer genomes to newly identify and fully elucidate cancer driver mutations that have degenerate genomic sequence signatures.

ACKNOLEDGEMENTS

XL and EAC thank support from Uniting Against Lung Cancer grant P0503003.

We thank Salil Soman for assistance with radiological figure preparation.

METHODS

COMPREHENSIVE CANCER GENOME PROFILING

Comprehensive cancer genomic profiling was performed using the FoundationOne test. The laboratory and computational methods employed in the FoundationOne DNA assay have been described in detail previously (31). Data were used from three consecutive versions of the FoundationOne test, targeting increasing numbers of genes. Hybridization capture baits for the *MET* gene were identical for all three versions of the test.

All base substitution, indel, copy number alteration, and re-arrangement variant calls were examined to identify those nearby to the splice junctions of MET exon 14. These genomic alterations were then manually inspected to identify those likely to affect splicing of exon 14, or delete the exon entirely. A table describing all genomic alterations identified as likely to affect MET exon 14 splicing is provided (Supplementary Table 2).

CELL CULTURE, TRANSFECTION, PLASMIDS AND VIRUS PACKAGING

HEK293 cell line, obtained January 2014, was gift from Davide Ruggero (UCSF). HEK293 cells were cultured in DMEM (Mediatech Inc., Cellgro) with 10% FBS (SH30910.03, Hyclone) and transfected with TransIT-LT1 reagent (MIR2300, Mirus, Madison, WI) according to manufacturer's instructions.

NIH3T3 cell line, obtained March 2014, was gift from Martin McMahon (UCSF). NIH3T3 cells infected with retrovirus were selected with 1.5ug/ml puromycin for 5 days to get stable expression of indicated protein.

pCDNA3-human-MET wt 3xFlag was a gift from Sourav Bandyopadhyay (UCSF), pBabe puro c-met WT was a gift from Joan Brugge (Addgene plasmid # 17493) (39). Exon 14 deletion in human MET and exon 15 deletion in mouse Met were created by site-directed PCR mutagenesis. pBABE-GFP and pBABE-HRASG12V were gift from Eric Collisson (UCSF). Ecotropic retrovirus were made from PLAT-E packing cells after transfection of indicated pBABE plasmid with TransIT-LT1 reagent (MIR2300, Mirus, Madison, WI) according to manufacturer's instructions.

All cell lines are tested mycoplasma negative (Mycoplasma Detection Kit, Cat. 13100-01, SouthernBiotech, AL) within 6 months of preforming the experiments. Cell line authentication was not performed.

SOFT AGAR ASSAY

Soft agar assayed were performed as described previously (20). Briefly 25,000 NIH3T3 cells were suspended in 0.4% agarose (50101, Lonza, Basel, Switzerland) with 10% calf serum in DMEM and plated in 6 well plate. The sum of colonies from 5 random fields of each well at week 3 was reported as mean of duplicates.

CELL VIABILITY ASSAY

2,500 NIH3T3 cells were plated in 96 well format and then treated with indicated concentration of INC280, trametinib, or 0.1% DMSO on the second day for 72hrs. Cell survival was measure by CellTiter-Glo assay (G7570, Promega, Madison, WI) following manufacturer's instructions. Relative cell survival rate was normalized to DMSO treated group as 100%. Each data point shows biological duplicate of triplicate well experiment.

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FIGURE LEGENDS

Figure 1

The genomic position of METex14 alterations. Genome coordinates are human genome build GRCh37/hg19. Genomic positions with alterations occurring in more than one case are indicated with * for two and the number of cases for greater than two. A) chr7:116,411,600-116,412,200. B) chr7:116,411,300-116,415,300.

Figure 2
Comprehensive genomic profiling of 4,402 lung adenocarcinomas. A) Co-mutation plot of frequently altered genes. The known clinically relevant driver genes and other most frequently altered genes are shown. The type of mutation is indicated by colors described in the legend. Data for this figure are available in Supplementary Table 4. B-D) Co- and anti- occurrence of genes containing known driver and other frequently occurring alterations in lung adenocarcinoma. Statistically significant (FDR <5%) co- and anti- occurrence was tested using the Fisher Exact test with false discovery rate correction for multiple-hypothesis testing and is indicated with *. The color scale indicates the fold change of enrichment (green) or exclusivity (green), versus random assortment. CDKN2A/CDKN2B is show in grey because they occur in close proximity on chromosome 9 and are frequently both affected within a single homozygous copy number deletion. To reduce outlying signal, the least significant boundary of a 50% binomial confidence interval around measured co-occurrence counts was used.

Figure 3
Human METΔ14 and the equivalent mouse METΔ15 transform cells and confer Met dependent growth, at least partially through activation of MEK-ERK pathway. A) Transient expression of Flag-tagged human wild type (wt) MET or exon 14 deletion (Δ14) mutant in HEK293 cells. Expression and phosphorylation of MET (pY1234/1235) and ERK1/2 (pT202/Y204) were measured by immunoblotting. B) Anchorage-independent growth was assessed by soft agar assay comparing NIH3T3 cells expressing of mouse wt Met, METΔ15, or HRAS G12V or Red Florescent Protein (RFP) control. Expression of wt MET, METΔ14 and HRAS G12V were confirmed by immunoblotting. The sum of colonies from 5 random fields at week 3 is reported as mean of duplicates (±s.d.). C) NIH3T3 cells from (B) were treated with increasing concentration of the MET inhibitor INC280 or D) MEK inhibitor trametinib for 72hrs and inhibition of proliferation was determined by cell viability assay (CellTiterGlo). * $p<0.05$, comparing METΔ15 to RFP at 20nM INC280.

Figure 4
Histiocytic sarcoma of the thorax harboring a METex14 alteration has a major response to crizotinib. A-B) Photomicrographs demonstrate a neoplasm with pleomorphic, polygonal cells with scattered mitotic activity as well as apoptotic cells. Tumor cells were focally positive for CD68 by immunohistochemical staining, consistent with a histiocytic origin. Stains for CD45 and Vimentin were positive (data not shown). CK7, CK20, CK5/6, CK AE1/3, CK CAM5.2, p63, CD43, CD30 and Smooth Muscle Actin were negative (data not shown). Contrast enhanced chest CT images at C) 0 months, D) 2 months, and E) 4 months after therapy with crizotinib.

Left anterior chest wall mass (white arrow) demonstrated decrease in bulk and heterogeneity after 2 and 4 months treatment with crizotinib. At initiation of treatment, the tumor was measured as 13.8 x 11.7 cm, and decreased to 8.9 x 6.5 cm at 4 months, a reduction of >60% volume and a partial response per RECIST 1.1 criteria.

Figure 5
Non-small lung carcinomas harboring METex14 alterations respond to capmatinib. Contrast enhanced Abdominal CT images are shown. A) Non-small cell lung carcinoma right hilar mass (white arrow) pre-treatment. B) Decrease in size after treatment with capmatinib. C) Non-small lung carcinoma with left renal midpole lesion (yellow arrow) pre-treatment. D) Decrease in size after capmatinib treatment.

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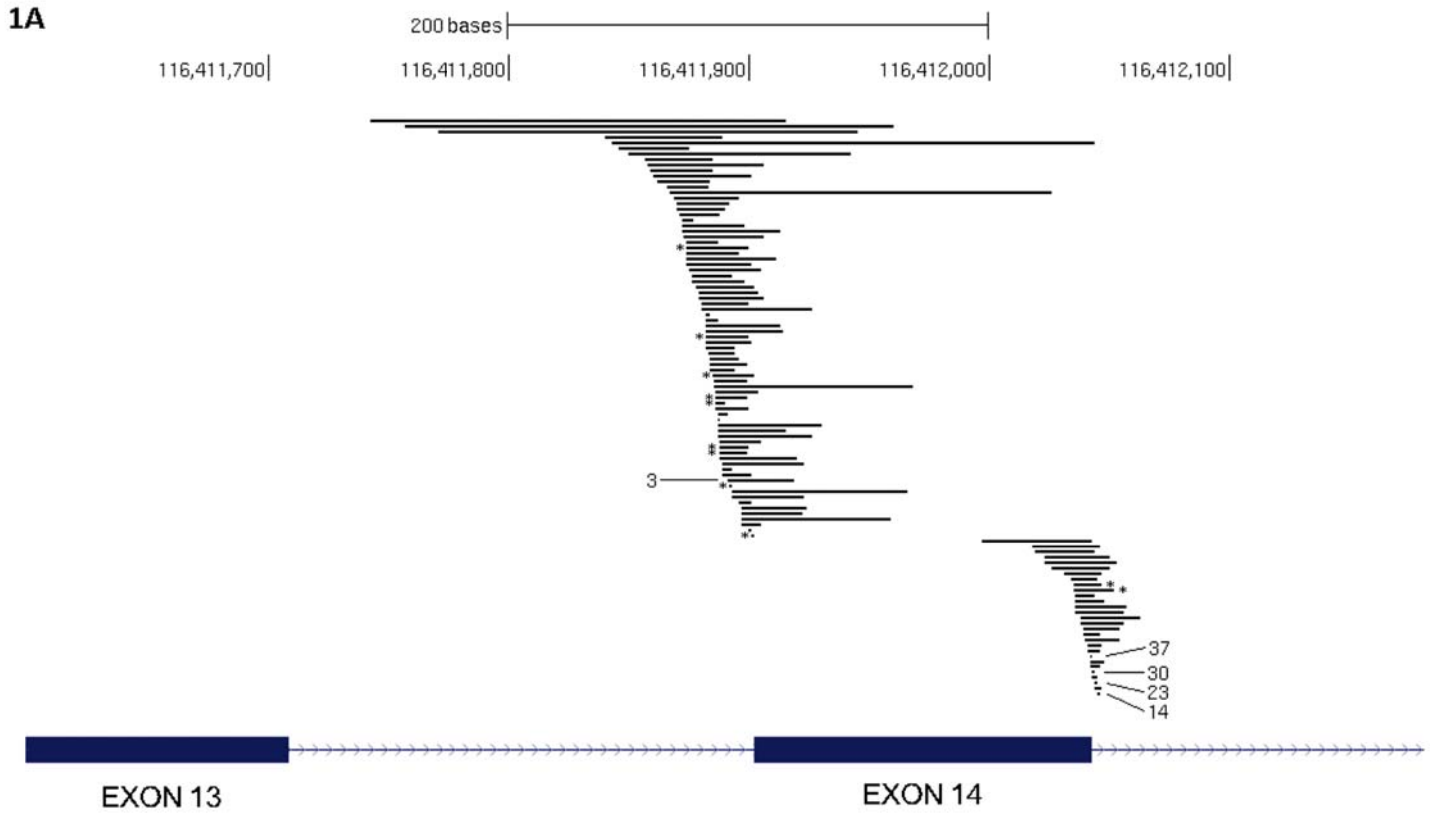

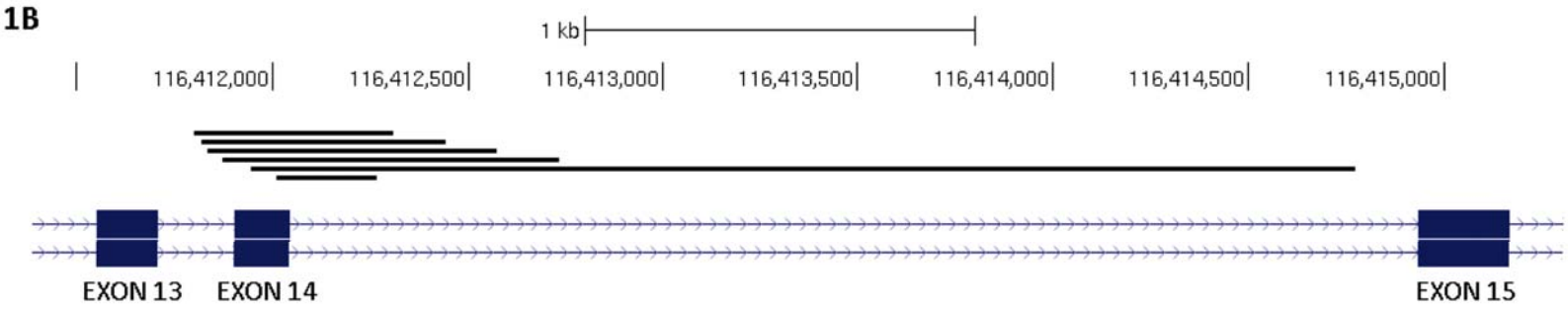

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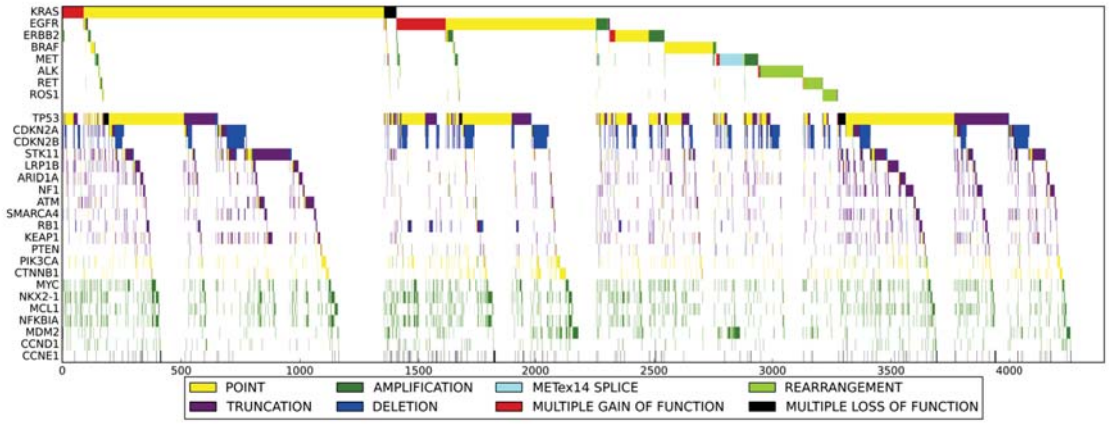

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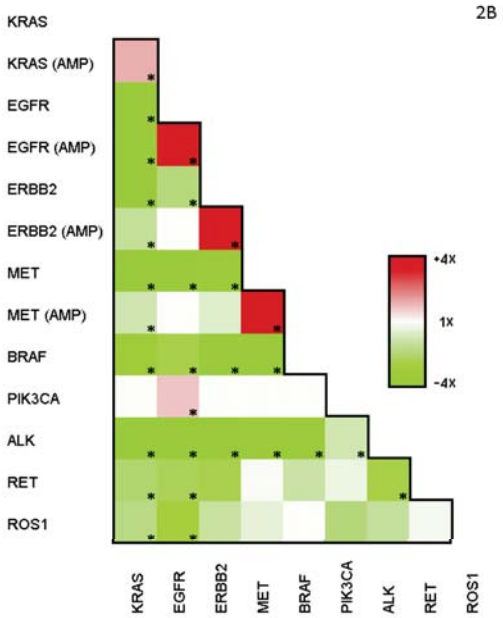

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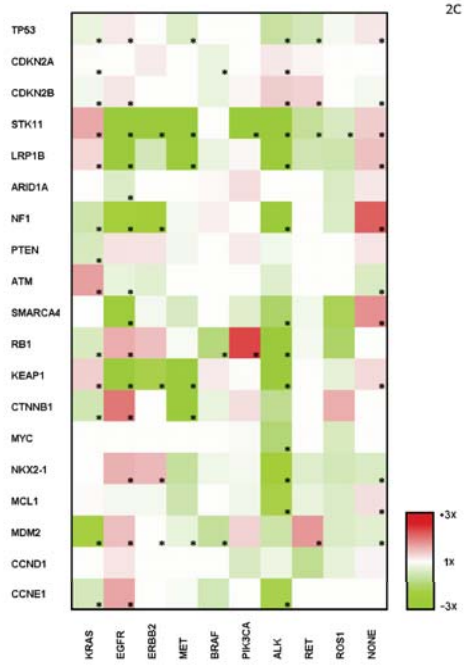

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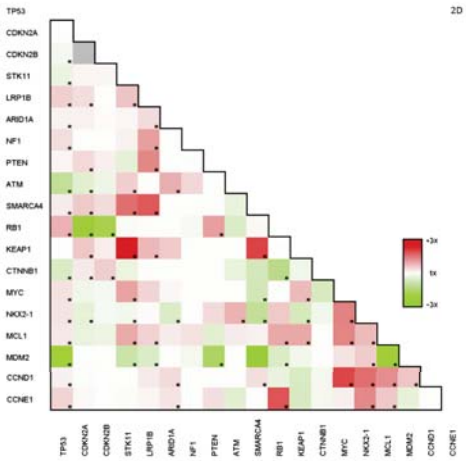

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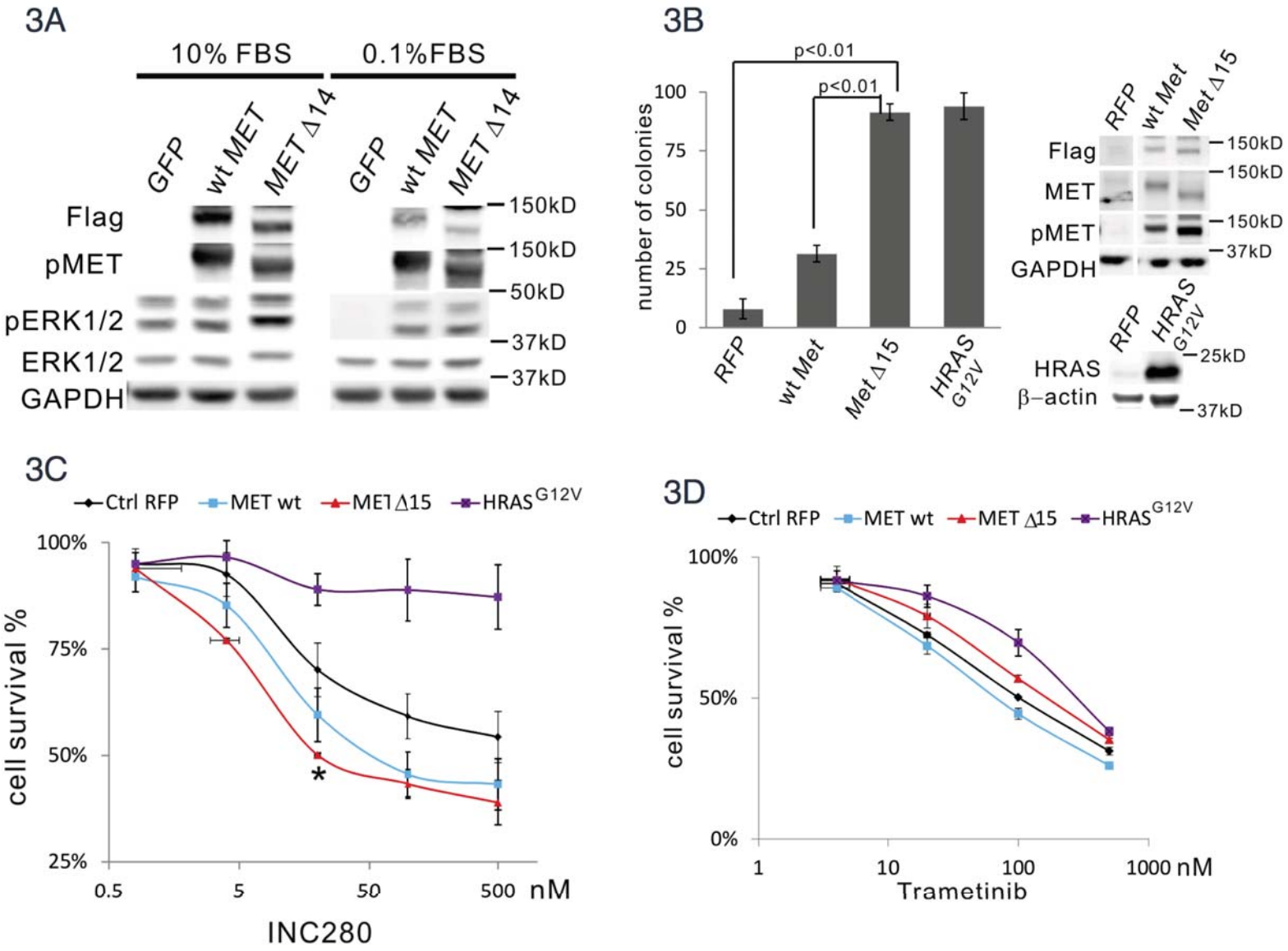

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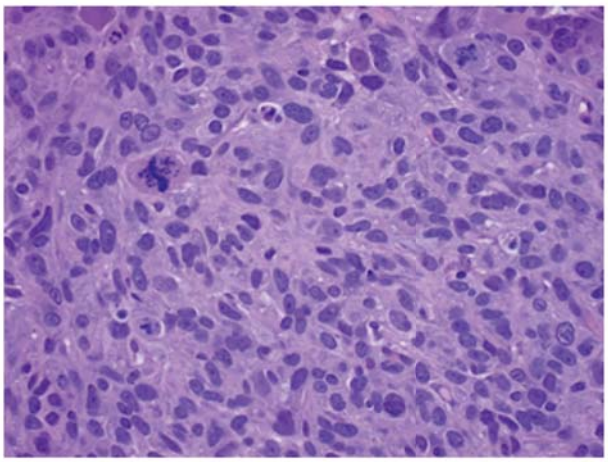

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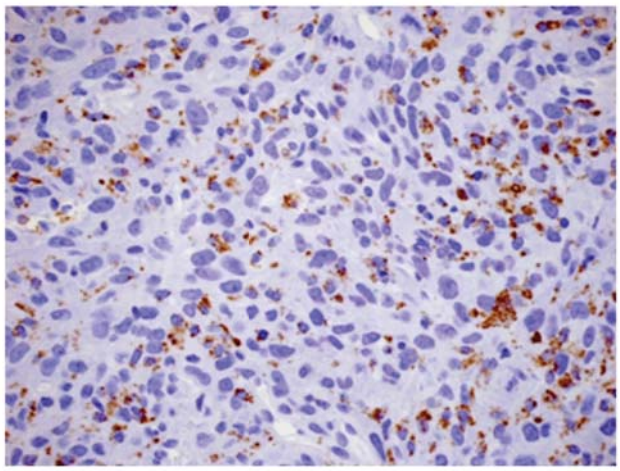

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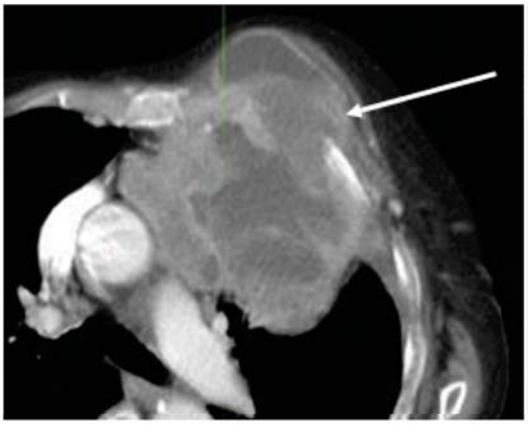

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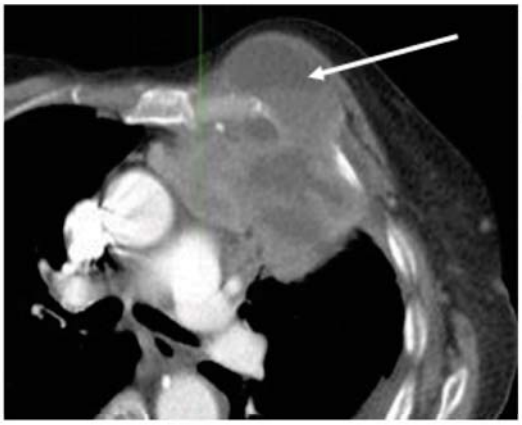

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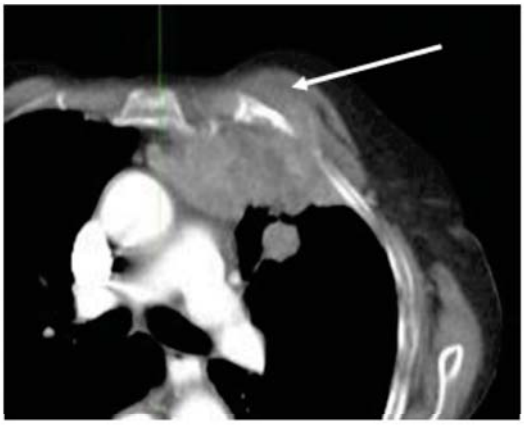

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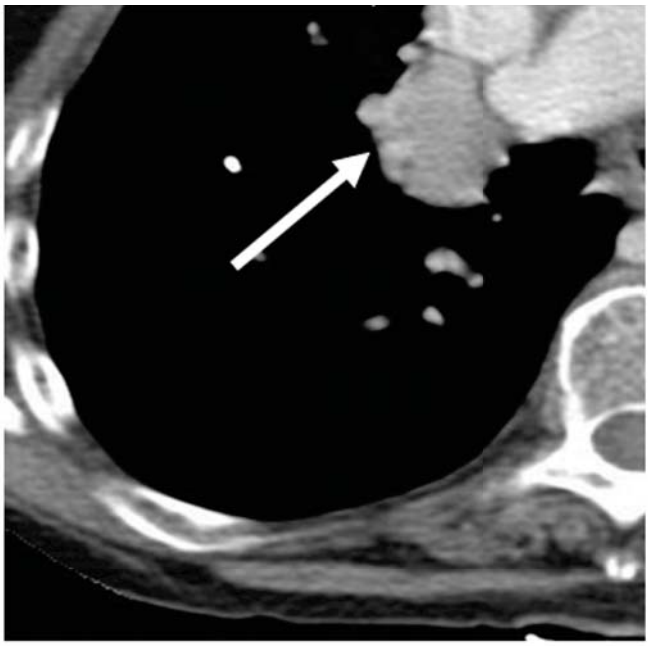

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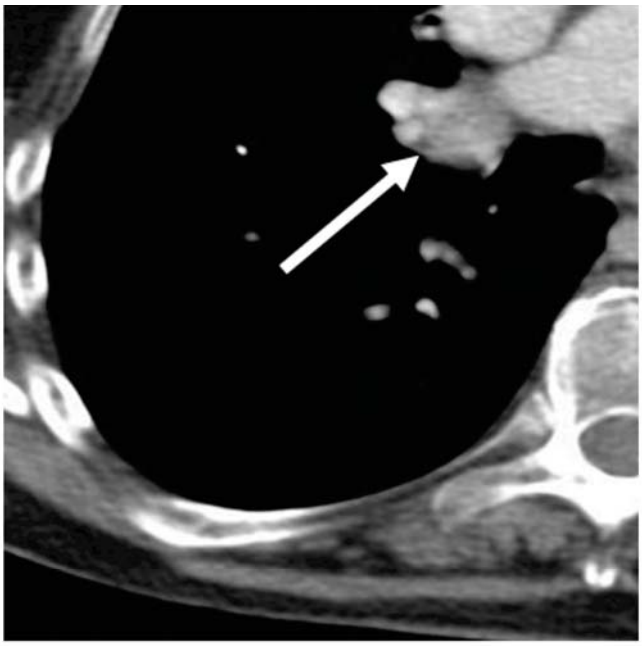

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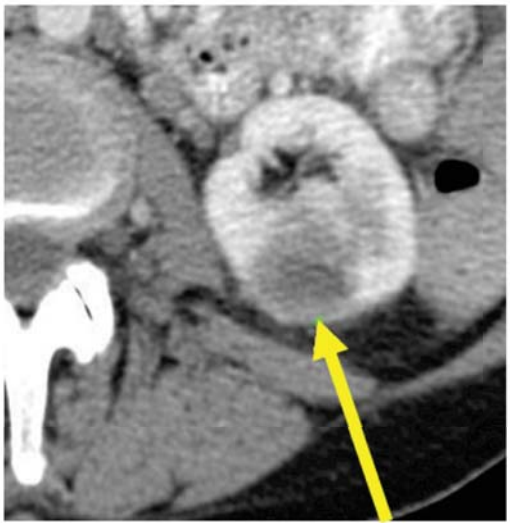

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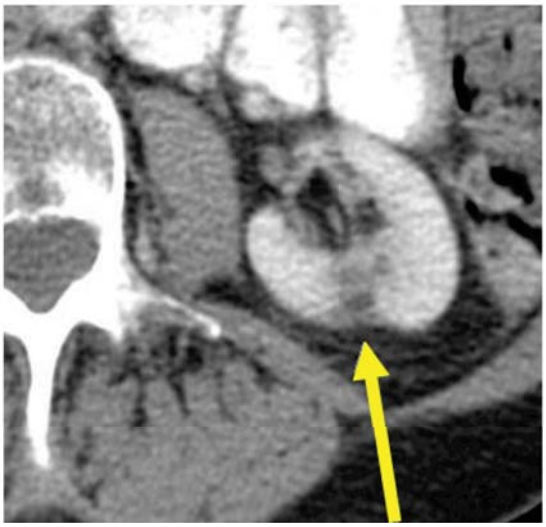

AACR American Association for Cancer Research

CANCER DISCOVERY

Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors

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Cancer Discovery Published OnlineFirst May 13, 2015.