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# Responses to the multitargeted MET/ALK/ROS1 inhibitor crizotinib and co-occurring mutations in lung adenocarcinomas with *MET* amplification or *MET* exon 14 skipping mutation

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## Abstract

**Introduction—**Genomic aberrations involving *ALK*, *ROS1* and *MET* can be driver oncogenes in lung adenocarcinomas. Identification of tyrosine kinase inhibitors (TKIs) with activity against these tumors and of preclinical systems to model response are warranted.

**Methods—**We analyzed cases with lung adenocarcinomas for representative genomic aberrations, evaluated the response to the multitargeted MET/ALK/ROS1 crizotinib TKI in cases with *MET* aberrations and profiled lung cancer cell lines with the aforementioned genomic changes.

**Results—**Lung cancer cell lines with *ALK* rearrangement, *ROS1* rearrangement or *MET* amplification had expected *in vitro* responses to crizotinib and the ALK/ROS1 TKI ceritinib. However, a commercially-available cell line with *MET* exon 14 skipping mutation and co-occurring *PIK3CA*-p.Glu545Lys mutation did not respond to crizotinib; suggesting the latter abrogated response. 10% of *MET* exon 14 skipping mutation co-occurred with *PIK3CA* mutation in the TCGA cohort. Putative crizotinib-responsive somatic mutations (*ALK* rearrangements, *ROS1* rearrangements, high level *MET* amplification or *MET* exon 14 skipping mutations) were present in 10% of lung adenocarcinomas analyzed at our service and in 9.5% of the TCGA lung adenocarcinoma database. One patient each whose advanced tumors harbored high level *MET* amplification with wild-type *PIK3CA* or *MET* exon 14 skipping mutation with *PIK3CA*-p.

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**CONFLICT OF INTEREST STATEMENT**

Susan E. Jorge; Sol Schulman; Jason A. Freed; Paul A. VanderLaan; Deepa Rangachari; Susumu S. Kobayashi; and Mark S. Huberman have no conflicts to disclose.

No other conflict of interest is stated.

Glu542Lys had significant responses to crizotinib; suggesting that *PIK3CA* co-mutation did not affect clinical response.

**Conclusions—**Approximately 10% of lung adenocarcinomas harbor aberrations that are targetable using the approved multitargeted TKI crizotinib. *MET* exon 14 skipping mutation predicts for response to MET TKIs in human lung adenocarcinomas but co-occurrence of *PIK3CA* mutation needs to be better evaluated as a modifier of response to TKI therapy. MET TKIs should not be omitted from *MET* exon 14 skipping mutated tumors until further preclinical and clinical data can confirm or refute mechanisms of primary or acquired resistance to crizotinib and other MET TKIs in these recalcitrant cancers.

### Keywords

mutation; lung cancer; adenocarcinoma; MET; exon 14; amplification; PIK3CA; ALK; ROS1; crizotinib; ceritinib

## INTRODUCTION

Precision medicine is an important palliative modality in the management of advanced lung adenocarcinomas, with oral tyrosine kinase inhibitors (TKIs) approved for advanced tumors with epidermal growth factor receptor (*EGFR*) mutations (gefitinib, erlotinib and afatinib [1]) or anaplastic lymphoma kinase (*ALK*) rearrangements (crizotinib and ceritinib [2–5]). Cell line models of *EGFR* mutated or *ALK* rearranged non-small-cell lung cancers (NSCLCs) have consistently matched clinical responses and highlighted that these tumors are oncogene addicted to their mutated kinase; underscoring the susceptibility exploited with TKIs.

The clinical availability of approved well-tolerated oral TKIs for lung adenocarcinoma has sparked interest in identifying additional driver genomic aberrations (be it rearrangements, mutations or amplifications) that may be targetable by the aforementioned drugs. Interestingly, preclinical models have established that crizotinib is a multitargeted TKI with *in vitro* activity against the kinase domains of ALK, hepatocyte growth factor receptor (MET) and c-ros oncogene 1 (ROS1) and *in vivo* effects against tumors driven by somatic aberrations in these genes [6–12]. A significant proportion of lung adenocarcinomas - as recently confirmed by the massive sequencing efforts of the The Cancer Genome Atlas (TCGA) and the Lung Cancer Mutation Consortium - harbor genomic aberrations that encompass putative targets of ALK, ROS1 and MET TKIs [13, 14]: *ALK* rearrangements (2–7% of tumors), *ROS1* rearrangements (1–2% of tumors), high level amplification of *MET* (1–2% of tumors) or heterogeneous *MET* mutations that lead to exon 14 skipping (1–4% of tumors). The clinical experience of how the latter changes predict for response to crizotinib are mounting. In the case of lung adenocarcinomas with *ROS1* rearrangements it is now well established in a multitude of cases, from ongoing clinical trials and retrospective cohorts, that crizotinib leads to tumor reduction in the majority of patients [10, 11] and an expanded approval label for this genomic subgroup is eagerly awaited. Preclinical models and clinical data to support the use of crizotinib in lung adenocarcinomas with de novo high level *MET* amplification or *MET* exon 14 skipping mutation are sparse but clinical responses have been reported [9, 12, 15, 16].

Here; we confirm the significant frequency of *ALK*, *ROS1* and *MET* somatic genomic aberrations in lung adenocarcinomas, add to the reported cases of response to crizotinib in tumors with *MET* amplification or *MET* exon 14 skipping mutation, and evaluate preclinical models that may or may not adequately exemplify response to TKIs against MET abnormalities in lung adenocarcinomas with a focus on how phosphoinositide-3-kinase, catalytic, alpha polypeptide (*PIK3CA*) mutations may affect the response to TKI therapy.

## MATERIALS AND METHODS

### Tumor and data collection

Sixty nine patient-tumor pairs followed at Beth Israel Deaconess Medical Center (BIDMC) with a diagnosis of lung adenocarcinoma over a 6-month period and whose cancers were submitted for genomic profiling (52 cases only by single gene assays for *EGFR*, *KRAS*, *ALK* and *ROS1*; and an additional 17 using next generation sequencing assays [17, 18]) were identified through an ongoing Institutional Review Board-approved study [19]. Pathologic, tumor genotype and radiographic parameters were gathered from chart extraction. Data was collected and managed using REDCap electronic data capture hosted at BIDMC. In addition, the TCGA lung adenocarcinoma mutation database was reviewed and collated for putative crizotinib-responsive genotypes and co-existing mutations [13].

### Cell culture, cell proliferation assays and reagents

NCI-H3122 (H3122) and HCC78 cells were obtained as described previously [7]. NCI-H1993 (H1993) and NCI-H596 (H596) were purchased from ATCC (Manassas, VA). All cells were maintained in RPMI 1640 medium (Mediatech, Manassas, VA) supplemented with 10% fetal bovine serum (FBS). All cells were grown at 37°C in a humidified atmosphere with 5% $CO_2$. Cells were plated in 96-well plates, allowed to attach overnight and then treated with or without kinase inhibitors for 72 hours. Cell viability was determined by CellTiter 96 Aqueous One solution proliferation kit (Promega, Madison, WI) according to the manufacture's protocol. Inhibitory proliferation curves and the 50% inhibitory concentration ($IC_{50}$) were generated using the GraphPad Prism 6 software (GraphPad Software, La Jolla, CA). Crizotinib and ceritinib were purchased from LC Laboratories (Woburn, MA) and Active Biochem (Maplewood, NJ), respectively. All reagents were dissolved in dimethyl sulfoxide (DMSO) and stored at −80°C.

### Western blot and antibodies

Cells were lysed in cell lysis buffer after exposure to 12 hours of inhibitors or DMSO, lysates were separated by 8% SDS-polyacrylamide gel, transferred to PVDF membranes and analyzed with the use of Pierce ECL western blotting substrate (Thermo Scientific, Waltham, MA); as previously described [7, 20]. Phospho-MET (pTry1234/1235) and MET antibodies were purchased from Cell Signaling Technology (Beverly, MA), and β-actin antibody from Santa Cruz Biotechnology (Dallas, TX). All primary antibodies were diluted 1:1000 and their recommended secondary antibodies were diluted 1:10000.

# RESULTS

## Preclinical cell lines models

To identify lung adenocarcinoma cell lines that could be used for modeling response to TKIs we selected four cell lines each with a putative crizotinib-sensitive genotype: H3122 (*EML4-ALK* E13:A20), HCC78 (*SLC34A2-ROS1*), H1993 (high level *MET* amplification with 15 copies of MET [21]) and H596 (*MET* homozygous point mutation in the 3p splice donor site of exon 14 [c.3251spl+1 G>T], leading to exon 14 skipping [22]). We profiled these lines against increasing concentrations of crizotinib and of the dual ALK/ROS1 TKI ceritinib. The use of crizotinib led to expected dose-dependent abrogation of proliferation in the *ALK*, *ROS1* and *MET* amplification driven cells (Figure 1A). In the same systems, ceritinib – as expected – only led to dose-dependent abrogation of proliferation in the *ALK* and *ROS1* rearranged cells and not in H1993 with MET amplification (Figure 1B). When we analyzed MET protein expression on H1993 and the ability of crizotinib and not ceritinib to inhibit MET phosphorylation, we observed the expected high expression level of MET and dephosphorylation of MET upon crizotinib treatment, respectively, in this preclinical system (Figure 1C).

H596 cells with *MET* exon 14 skipping were not inhibited by nanomolar concentrations of crizotinib to the same extent as the other cell lines used (Figure 1A). Despite a slightly lower size of total MET protein corresponding to the expression of a shortened MET (from exon 14 skipping and removal of 47 amino-acids [L964-D1010]), we were unable to detect constitutive activation of MET phosphorylation in H596 cells *in vitro* (Figure 1C) without the addition of the MET ligand HGF that can readily activate p-MET in H596 [22–23]. H596 are known to also harbor a *PIK3CA*-p.Glu545Lys mutation and other groups have reported the prerequisite for dual MET and PI3K inhibitors for full anti-proliferative effects and downstream (AKT and ERK) inhibition [23]. Therefore, this preclinical system does not accurately represent isolated *MET* exon 14 skipping and infers that co-existence of activating *PIK3CA* mutations may be a mechanism of primary resistance to MET TKIs [23].

## Tumor genotype at BIDMC and in the TCGA cohort

We compiled the mutational profile of 69 adenocarcinomas genotyped consecutively at our service to identify crizotinib-sensitive genomic events. 7 cases had putative crizotinib-responsive aberrations: 3 with *ALK* rearrangement, 1 with *ROS1* rearrangement, 1 with high level *MET* amplification and 2 with *MET* exon 14 skipping mutation (Figure 2A). 1 out of 2 lung adenocarcinomas with *MET* exon 14 skipping mutation also harbored a *PIK3CA*-p.Glu542Lys mutation. The other 62 tumors had other genomic changes (including 11 *EGFR* mutations, 22 *KRAS* mutations, 2 *ERBB2* mutations, 1 *RET* rearrangement and 1 *MAP2K1* mutation) or had limited genomic testing following analysis of *EGFR*, *KRAS*, *ALK* and *ROS1*.

To confirm our findings in a cohort of lung adenocarcinomas analyzed with identical comprehensive genomic studies, we turned to the 2014 TCGA lung adenocarcinoma database [13]. The frequency of putative crizotinib-responsive aberrations equaled 9.5% in the 230 resected lung adenocarcinoma cases available from TCGA (Figure 2A).

Interestingly, TCGA disclosed that 4.3% of lung adenocarcinomas had *MET* exon 14 skipping mutations that were mutually exclusive with other known driver mutations [13]. Interestingly, *MET* exon 14 skipping co-occurred with other genomic events including 1 out of 10 (10%) of tumors with a *PIK3CA*-p. Glu542Lys mutation (Figure 2B).

### Clinical and radiographic responses to crizotinib in lung adenocarcinomas harboring *MET* aberrations

A focused review of the available literature on the ability of the multitargeted MET/ALK/ROS1 TKI crizotinib to induce clinical and radiographic responses disclosed >60% change of initial tumor regression in most series of lung adenocarcinomas with *ALK* rearrangements, *ROS1* rearrangements, high level (≥ 5 copies) *MET* amplification or *MET* exon 14 skipping mutation (Table 1). None of the reported TKI-responsive *MET* exon 14 skipping mutated adenocarcinomas harbored *PIK3CA* mutations.

Turning back to our BIDMC cohort, two patients with *MET* genomic aberrations in their lung adenocarcinomas received off-label crizotinib at a dose of 250 mg twice daily after progression on first line chemotherapy with carboplatin, pemetrexed and maintenance pemetrexed. The first case was that of a 72-year old former smoker (12 pack-years) woman with advanced lung adenocarcinoma whose clinical course has been partially described previously [24]. Genomic profiling with targeted next generation sequencing [17] showed *MET* amplification (10 copies) as the main oncogenic driver alteration and the tumor was wild-type for *PIK3CA* [24]. Off-label crizotinib was commenced with marked clinical improvement. Radiographic re-assessments after 1, 2 and 5 months of therapy (Figure 2C) disclosed improvement of pulmonary and nodal tumor lesions. The calculated decrease of selected target lesions was calculated at 38.7% using RECIST 1.1. The clinical response is ongoing at time of this report (11 months of therapy).

The second case was that of a 68 year-old former smoker (24 pack-years) woman with advanced lung adenocarcinoma. Genomic profiling with targeted next generation sequencing [18] showed a *MET* p.D1010_splice (c.e14 1 G>A) mutation leading to *MET* exon 14 skipping. Other major somatic genomic findings in the tumor included *PIK3CA*-p.Glu542Lys (c.1624 G>A) and TP53 p.Arg337Leu (c.1010 G>T) mutations. Off-label crizotinib was commenced with clinical improvement of cough and dyspnea. Radiographic re-assessment after 1 month of therapy (Figure 2D) disclosed improvement of pulmonary and nodal lesions. The calculated decrease of target lesions was calculated at 45.1% using RECIST 1.1. Interestingly, a measurable brain metastatic lesion also decreased in size upon crizotinib use. The clinical and radiographic response lasted for 7 months of therapy.

## DISCUSSION

The ability to use comprehensive molecular profiling (i.e., target next generation sequencing assays) in clinical practice using DNA purified from either small biopsies or surgical procedures [17, 18] is exposing Thoracic Oncologists to a multitude of potentially actionable driver oncogenes that had previously been relegated to the research realm [13]. Although the landscape of precision oncology TKI drug approvals for lung adenocarcinoma is restricted to tumors with *EGFR* mutations or *ALK* rearrangements, it is clear that a long tail of other

potentially actionable non-overlapping driver genomic kinase events may be targetable with off-label use of approved TKIs. The use of robust cell line preclinical models of genotype-TKI response has been an invaluable resource to identify TKIs in novel driver oncogene genotypes [7].

In this report, we explored the ability to identify and target aberrations in the MET receptor tyrosine kinase. *MET* amplification or *MET* exon 14 skipping mutations were present in a significant percentage of our and in the TCGA series [13] of lung adenocarcinomas. If one takes all putative genomic aberrations that can be targeted by the MET/ALK/ROS1 TKI crizotinib, we observed that approximately 10% of all lung adenocarcinomas will have either ALK, ROS1 or MET actionable aberrations. The clinical course of our patients treated with off-label crizotinib emphasized that significant clinical and radiographic responses can be attained when the tumor harbors high level *MET* amplification or *MET* exon 14 skipping mutation. Our results hold well with single case reports and ongoing clinical trials of crizotinib in patients selected based on their tumors' expression of somatic *MET* genomic aberrations (Table 1).

We also were able to confirm that available cell line models with an *ALK* rearrangement, *ROS1* rearrangement or *MET* amplification are adequate preclinical systems to model response to the effects of multitargeted TKIs. These same preclinical tools have been used by our group and others to both understand intracellular responses and mechanisms of resistance to TKIs in lung adenocarcinomas with *ALK* and *ROS1* rearrangements [7, 25], and we believe the H1993 *MET* amplified cell line will be similarly used to understand resistance to crizotinib in the preclinical setting. However, our results also highlight that not all cell line systems with a genomic event can engage the kinase pathway and serve as a reliable model for preclinical studies in the absence of experimental manipulations and may not reflect clinical responses. The *MET* exon 14 skipping mutated H596 cell line was unable to recapitulate the human clinical pattern of response seen with crizotinib. The need to use high levels of endogenous MET ligands to engage MET activity in H596 *in vitro* and to induce xenograft *in vivo* systems [22] and the co-occurrence of *PIK3CA*-p.Glu545Lys mutation in this cell [23] make it alone an inadequate system to study TKI response *in vitro* without experimental manipulations or co-treatment with PI3K inhibitors [23]. Novel cell lines or animal models of *MET* exon 14 skipping mutations are warranted to model preclinically response and resistance to MET TKIs in these lung adenocarcinomas. The preclinical experience with H596 would suggest that concurrent *MET* and *PIK3CA* mutations lead to insensitivity to MET TKI monotherapy. However, our clinical case with concurrent *MET* p.D1010_splice mutation and *PIK3CA*-p.Glu542Lys had a significant and prolonged response to crizotinib (Figure 2D). The latter highlights that crizotinib should not be omitted from lung adenocarcinomas with *MET* exon 14 skipping mutations irrespective of co-existing mutations until further preclinical and clinical experiments can confirm or refute mechanisms of primary and acquired resistance to TKIs in these unique tumors.

In summary, our results confirm that the multitargeted MET/ALK/ROS1 TKI crizotinib induces anti-tumor activity in lung adenocarcinomas driven by clinically-relevant *MET* amplification or *MET* exon 14 skipping mutation (even in the presence of co-occurring

*PIK3CA* mutation) and highlight the need for robust preclinical models to better understand oncogene addiction in NSCLC.

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## HIGHLIGHTS

- *MET* amplification and *MET* exon 14 skipping mutation happen in lung adenocarcinomas
- These genomic changes predict for response to the MET/ALK/ROS1 inhibitor crizotinib
- Co-occurrence of PIK3CA mutations should be evaluated as modifiers of response

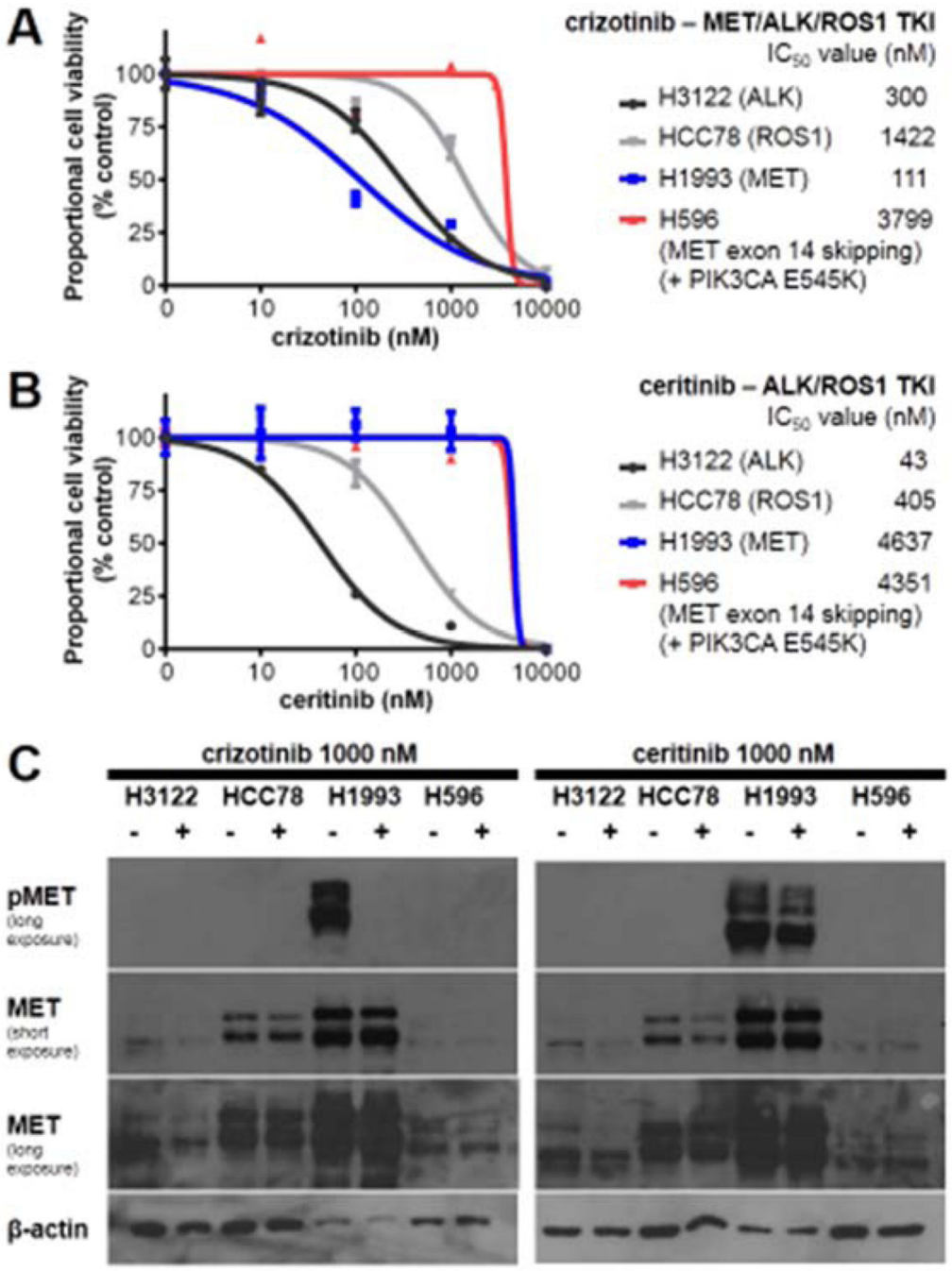


**Figure 1.**

Preclinical models of genomic aberrations of *ALK*, *ROS1* and *MET* in lung adenocarcinomas. A. 72-hour proliferation assay of increasing concentrations of crizotinib. The 50% inhibitory ($IC_{50}$) concentration is depicted for each cell line. B. 72-hour proliferation assay of increasing concentrations of ceritinib. $IC_{50}$ concentration is depicted for each cell line. Proliferation assays were performed in triplicate and error bars indicate standard deviation. C. Western blot analysis of cells treated with or without 1000 nM crizotinib (left panel) or 1000 nM ceritinib (right panel) for 12 hours. Levels of

phosphorylated MET at positions pTry1234/1235. total MET (long and short exposures) and β-actin are shown.

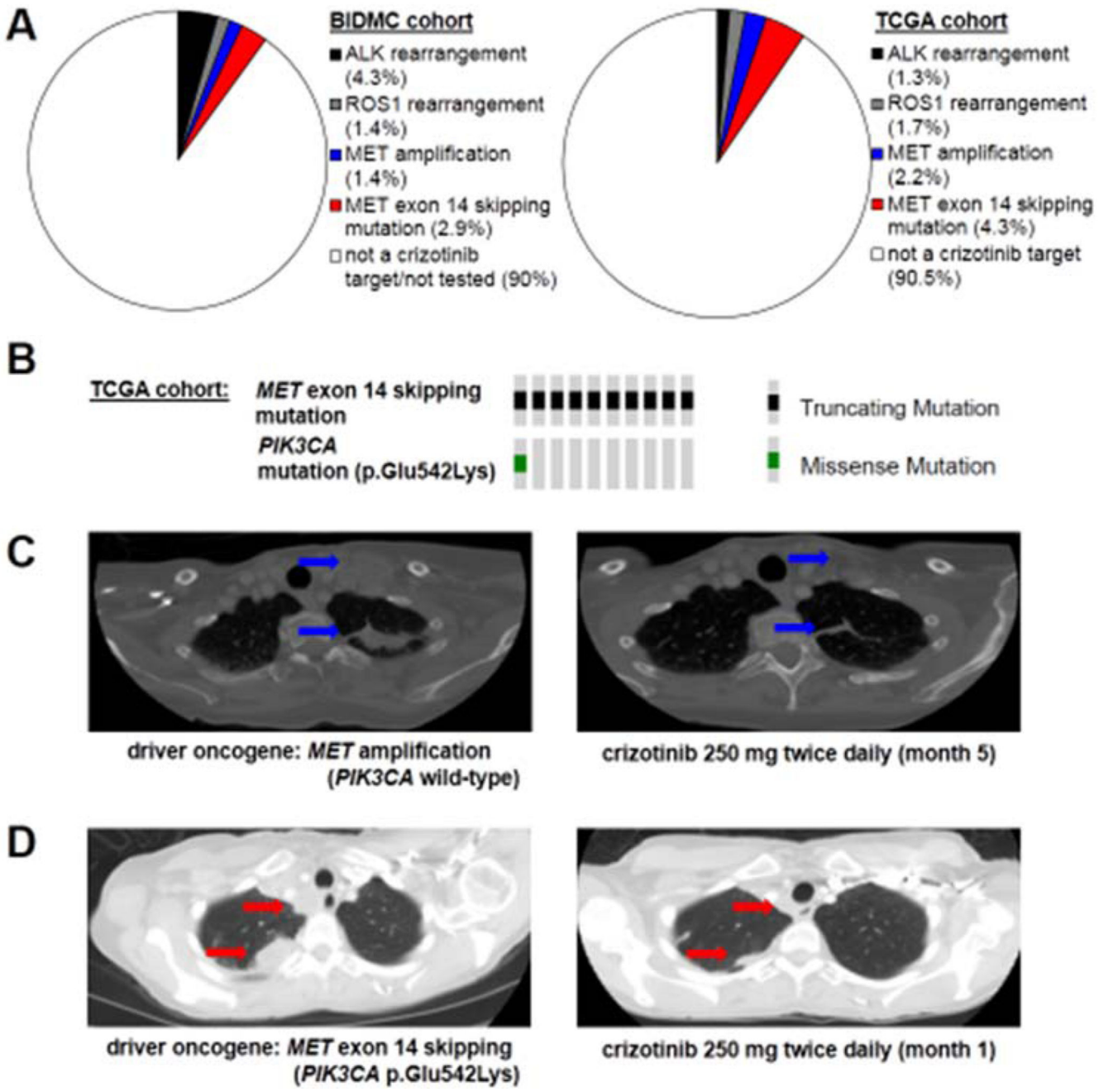


**Figure 2.**

Genomic aberrations of *ALK*, *ROS1* and *MET* in lung adenocarcinomas. A. Pie charts of the frequency of *ALK* rearrangement, *ROS1* rearrangement, *MET* amplification and *MET* exon 14 skipping mutation in lung adenocarcinomas from BIDMC (left panel) and the TCGA cohort (right panel). B. Co-occurrence of *MET* exon 14 skipping mutation and *PIK3CA* mutations in lung adenocarcinomas from the TCGA cohort. C. Computed tomography before and after crizotinib from a patient whose lung adenocarcinoma harbored *MET* amplification. The blue arrows highlight nodal and lung lesions. D. Computed tomography

before and after crizotinib from a patient whose lung adenocarcinoma harbored *MET* exon 14 skipping. The red arrows highlight nodal and lung lesions.

## Table 1

Prior and current publications demonstrating the clinical efficacy of the multitargeted MET/ALK/ROS1 tyrosine kinase inhibitor crizotinib in advanced lung adenocarcinomas with selected genomic aberrations.

| genomic abnormality | clinical trial (name) | number of cases | response rate to crizotinib | Reference no. |
|---|---|---|---|---|
| *ALK* rearrangement | Yes (PROFILE1001) | 143 | 60.8% | 3 |
| | Yes (PROFILE1007) | 173 | 65% | 4 |
| | Yes (PROFILE1014) | 172 | 74% | 5 |
| *ROS1* rearrangement | Yes (PROFILE1001) | 50 | 72% | 10 |
| | No (EUROS1 cohort) | 32 | 80% | 11 |
| *MET* amplification (high level) | Yes (PROFILE1001) | 6 | 66.6% | 9 |
| | No (case report) | 1 | 100% | 12 |
| | No (case report) | 1 | 100% | 24, current report |
| *MET* exon 14 skipping mutation | No (case report) | 1 | 100% | 15 |
| | No (case series) | 4 | 75% | 16 |
| | No (case report) | 1 | 100% | current report |