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Response to crizotinib in a patient with lung adenocarcinoma harboring a MET splice site mutation

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Introduction

MET (c-MET; mesenchymal-epithelial transition factor) is a receptor tyrosine kinase that was first characterized as a proto-oncogene in 1984, in a chemically transformed osteosarcoma cell line [1]. Located on chromosome 7q21-31, the *MET* gene encodes a cell surface receptor tyrosine kinase comprised of a 50-kDa α -chain and a 140-kDa transmembrane β -chain linked by a disulfide bond. The natural ligand for cMet is hepatocyte growth factor (HGF), also known as scatter factor [2]. Following binding of HGF the c-MET receptor undergoes dimerization and phosphorylation, which in turn promotes recruitment of downstream effector proteins, leading to activation of multiple signaling cascades, including the MAPK, PI3K/Akt, STAT and NF- κ B pathways [3]. Physiologic roles for HGF/MET signaling include embryogenesis, development, and wound healing [3–5].

Aberrant activation of the c-Met receptor tyrosine kinase promotes oncogenicity in a subset of lung adenocarcinomas. A variety of mechanisms can result in constitutive c-Met signaling, including *MET* gene amplification, protein overexpression, activating point mutations, and induction of its ligand HGF [3, 6, 7]. Crizotinib, a multitargeted tyrosine kinase inhibitor (TKI) that is FDA approved for the therapy of lung adenocarcinomas harboring *ALK* or *ROS1* fusions [8–10], was also recently found to be clinically active in tumors with high level *MET* amplification [11]. These findings have prompted the clinical development of more selective c-MET inhibitors for evaluation in this particular patient population.

MET splice site mutation has also been demonstrated to induce constitutive activity and confer sensitivity to c-Met inhibition *in vitro* [12, 13]. Indeed, frequent activating *MET*

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splice site mutations were recently described in whole exome sequencing (WES) discovery efforts in lung adenocarcinoma [14]. However, the clinical activity of c-Met inhibition in this context remains unknown. We identified one such mutation using targeted next generation sequencing (NGS) in a patient with lung adenocarcinoma and treated him with crizotinib.

Case Report

An 86 year-old never smoking male presented with left lower extremity weakness and unsteadiness. A brain magnetic resonance imaging (MRI) scan revealed three enhancing lesions measuring up to 1.4 cm in the right frontal lobe and left cerebellar hemisphere, suggestive of metastatic disease. Chest and abdominopelvic computed tomography (CT) scans revealed a 5.6 cm right lower lobe mass and mediastinal lymphadenopathy, as well as a right adrenal nodule. Positron emission tomography confirmed mild to moderate fluorodeoxyglucose (FDG) avidity of the lung mass, subcarinal lymph node, and adrenal nodule. Core biopsy of the lung mass identified TTF-1 positive lung adenocarcinoma. Standard genotyping for alterations in EGFR, KRAS, ALK, ROS1, and RET was negative.

The patient underwent stereotactic radiotherapy (2000 cGy) to each brain lesion, followed by palliative radiation (3900 cGy) to the obstructing lung mass. A single cycle of pemetrexed was complicated by cellulitis and not pursued further. Biopsy of the right adrenal lesion was then performed for more comprehensive profiling. Targeted NGS identified a *MET* splice site mutation predicted to be activating based on comparison to similar mutations reported in the literature [14] (Figure 1A, B). Immunohistochemistry (IHC) revealed 2+ phosphorylated c-Met in 50% of cells, consistent with c-Met activation (Figure 1B, C).

Given these findings and the clinical activity of crizotinib in *MET*-amplified lung adenocarcinoma [11], the patient was started on crizotinib. After 5 weeks, chest/abdominopelvic CT scans revealed significant improvement of the lung mass and decrease in size of the unirradiated adrenal lesion from 2.0×1.9 cm down to 1.4×1.2 cm (Figure 2A, B). Unfortunately, associated pneumonitis necessitated crizotinib discontinuation. Disease progression occurred 8 weeks later, upon which palliative care was pursued.

Discussion

This is the first report to our knowledge that associates clinical response to crizotinib with *MET* splice site mutation in lung adenocarcinoma. Since this elderly patient was unable to tolerate even single agent pemetrexed, we empirically treated him with crizotinib because of the reported activity in *MET* amplified NSCLC and acceptable toxicity profile [8]. Although difficult to separate response of his lung mass from continued effects of palliative radiation, we observed a clear decrease in size of the unirradiated adrenal lesion from which the *MET* splice site mutation was identified. Since more selective c-Met inhibitors remain in clinical development, we were unable to transition him to alternative agents following the development of crizotinib toxicity, and thus could not assess durability of activity.

Recent WES data in lung adenocarcinoma revealed *MET* exon 14 splice site mutations in 4.3% of patients, twice the frequency of *MET* amplification [14]. This intronic deletion, c. 2887-18_2887-7del12, resides in the polypyrimidine tract just upstream of the exon 14 5' splice acceptor site (Figure 1A). Mutations affecting this tract or the 3' splice donor site cause in-frame skipping of exon 14 [13]. The resulting protein lacks a portion of the juxtamembrane domain, which eliminates binding of Cbl, an E3 ubiquitin ligase involved in c-Met downregulation. Consequently, studies show that c-Met protein lacking exon 14 has enhanced activity, consistent with the IHC results in this case.

Importantly, clinical targeted NGS assays that include *MET* exon primers typically read sequence into splice sites (Figure 1A), highlighting the importance of examining these key intron/exon junctions from patient sequencing data. Without bioinformatics platforms that analyze and capture these additional reads, it is likely that such mutations are frequently overlooked. Their potential clinical relevance in lung adenocarcinoma suggests that heightened efforts should be made to identify and report these mutations in such patients.

Conclusion

In conclusion, although clinical trials are necessary to confirm our findings, these results suggest targeted therapy benefit in a patient population greater than *ROS1*- and approaching the frequency of *ALK*-rearranged lung cancer. Indeed, additional efforts to identify *MET* splice site mutations in lung adenocarcinoma patients and enroll them in genotype directed studies may expand the repertoire of genotype-directed therapy and further realize the goal of personalized cancer medicine.

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Clinical Practice Points

Lung adenocarcinoma has been increasingly subdivided based on the presence of certain driver oncogenes that confer susceptibility to targeted therapy. Indeed, similar to *ALK* and *ROS1* rearranged lung adenocarcinomas, those that harbor *MET* amplification were recently found to respond to the multitargeted tyrosine kinase inhibitor crizotinib. Here we describe clinical activity of crizotinib in a patient with lung adenocarcinoma with a *MET* splice site mutation. Although these mutations occur at nearly twice the frequency of *MET* amplification, special attention is required to detect them on clinical next generation sequencing platforms. Our findings suggest the need to expand efforts to identify these mutations and evaluate responsiveness to c-Met inhibition in genotype directed clinical trials.

Fig. 1. Activating MET splice site mutation

A) Schematic demonstrating how MET intronic deletions adjacent to exon 14 interfere with splicing and result in deletion of the juxtamembrane cytoplasmic domain, which leads to impaired c-MET downregulation. Expanded view of exon 14 reveals that targeted next generation sequencing primers are able to capture these mutations at the exon/intron junctions in general. B) Adrenal biopsy specimen subjected to targeted NGS identified a *MET* intronic deletion, c. 2887-18_2887-7del12. C) IHC against a variety of oncogenic pathways converged to identify phosphorylated c-Met positivity.

Fig. 2. Response to crizotinib therapy

A) Chest CT scans 2 months following irradiation and then pre- and post-crizotinib showing improvement in the right lower lobe mass and obstructed lung (arrows), but associated diffuse pneumonitis that extended outside of the radiation field. B) Abdominopelvic CT scan showing decrease in size of the unirradiated right adrenal mass (arrows) after 5 weeks of crizotinib therapy.