BRIEF REPORT

Emergence of Preexisting MET Y1230C Mutation as a Resistance Mechanism to Crizotinib in NSCLC with *MET* Exon 14 Skipping

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ABSTRACT

Introduction: *MET* proto-oncogene, receptor tyrosine kinase gene exon 14 skipping (*MET*ex14) alterations represent a unique subset of oncogenic drivers in NSCLC. Preliminary clinical activity of crizotinib against *MET*ex14-positive NSCLC has been reported. The full spectrum of resistance mechanisms to crizotinib in *MET*ex14-positive NSCLC remains to be identified.

Methods: Hybrid capture–based comprehensive genomic profiling performed on a tumor specimen obtained at diagnosis, and a hybrid capture–based assay of circulating tumor DNA (ctDNA) at the time of progression during crizotinib treatment was assessed in a pairwise fashion.

Results: A *MET*ex14 alteration (D1010H) was detected in the pretreatment tumor biopsy specimen, as was MET proto-oncogene, receptor tyrosine kinase (MET) Y1230C, retrospectively, at very low frequency (0.3%). After a confirmed response during crizotinib treatment for 13 months followed by progression, both *MET* proto-oncogene, receptor tyrosine kinase gene Y1230C and D1010H were detected prospectively in the ctDNA.

Conclusion: Emergence of the preexisting MET Y1230C likely confers resistance to crizotinib in this case of *MET*ex14-positive NSCLC. Existence of pretreatment MET Y1230C may eventually modulate the response of *MET*ex14-positive NSCLC to type I MET tyrosine kinase inhibitors. Noninvasive plasma-based ctDNA assays can provide a convenient method to detect resistance mutations in patients with previously known driver mutations.

Keywords: MET Y1230C; Resistance mutation; Crizotinib; *MET* exon 14 skipping; Circulating tumor DNA

Introduction

MET proto-oncogene, receptor tyrosine kinase gene (*MET*) exon 14 skipping (*MET*ex14) alterations are now recognized as important driver mutations in NSCLC.[1] Crizotinib, a MET proto-oncogene, receptor tyrosine kinase (MET) tyrosine kinase inhibitor (TKI), has demonstrated activity against NSCLC with *MET*ex14 alterations.[2] As with the use of targeted therapy in other oncogene-driven tumors, specific mechanisms of

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acquired resistance to MET TKI are anticipated and have been recently described.[3] Structural insights from the binding interaction of crizotinib in the MET kinase domain suggest that certain amino acid changes may confer broader resistance to other type I MET TKIs.[4] It is important to identify the mechanisms of resistance to crizotinib in *MET*ex14-positive NSCLC to help guide subsequent therapy with structurally divergent inhibitors. However, repeat tumor biopsy at progression can be difficult. The availability of a noninvasive plasma-based assay provides an alternate method to assess resistance. Here, by using a plasma-based circulating tumor DNA (ctDNA) assay, we have identified a clinically novel MET mutation after progression during crizotinib treatment in a patient with *MET*ex14-positive NSCLC after a confirmed durable response.

Methods

Patients with *MET*ex14-positive NSCLC as identified by comprehensive genomic profiling (CGP) of tissue specimens[5] were enrolled into the *MET*ex14-positive expansion cohort of the phase 1 crizotinib trial (ClinicalTrials.gov identifier NCT00585195). For this case, at time of progression, a ctDNA assay (FoundationACT [Foundation Medicine, Inc., Cambridge, MA]) was performed to assess potential resistance mechanisms and inform therapy for the patient after cessation of treatment on the clinical trial. Specifically, the Clinical Laboratory Improvement Amendments–validated FoundationACT assay was conducted as follows. Two 10-mL aliquots of peripheral whole blood were collected in cell-free DNA blood collection tubes. A double-spin protocol was used to isolate plasma, and 50 to 100 ng of ctDNA was extracted to create adapted sequencing libraries before hybrid capture and sample-multiplexed sequencing on an Illumina HiSeq 2500 sequencer (Illumina, San Diego, CA). The FoundationACT ctDNA test covers 62 genes to $\times$5000 unique coverage and uses proprietary algorithms to call alterations at low allele frequencies (0.1% for substitutions, 1% for indels and rearrangements, and 20% for copy number amplifications). Mutant allele frequency (MAF) for the tissue-based assay represents the percentage of DNA obtained from the mutation-containing tumor on which a biopsy was performed. In the blood, MAF represents the percentage of ctDNA in the bloodstream that is harboring the mutation on a given day and time. For this reason, MAF for the tissue assay and ctDNA assays cannot be directly compared.

Results

Stage IV metastatic adenocarcinoma of the lung was diagnosed in a 67-year-old Asian female never-smoker when she presented with superior vena caval syndrome and a 7-pound weight loss. Imaging studies revealed large right hilar and subcarinal lymphadenopathy with compression of the right mainstem bronchus, moderate right pleural effusion, and a right lower lung mass (Fig. 1) and brain metastasis. Endobronchial ultrasound–guided biopsy of the mediastinal lymph nodes revealed poorly differentiated adenocarcinoma (CK7 positive, TTF-1–positive, CK20 negative). The patient received stereotactic radiosurgery to the brain and one cycle of carboplatin/paclitaxel. CGP of the initial tissue specimen from the biopsy performed as part of standard of care revealed a *MET*ex14 alteration (MET D1010H) (44% MAF) but no concurrent *MET* amplification. Of note, retrospective analysis of the original CGP testing revealed that two of 762 sequencing reads (0.26%) showed the Y1230C mutation present at well below reportable levels of the assay (Fig. 2 [*upper panel*]). She was enrolled onto a phase 2 trial of crizotinib in patients with *MET*ex14-positive NSCLC (ClinicalTrials.gov Identifier NCT00585195). She achieved a confirmed

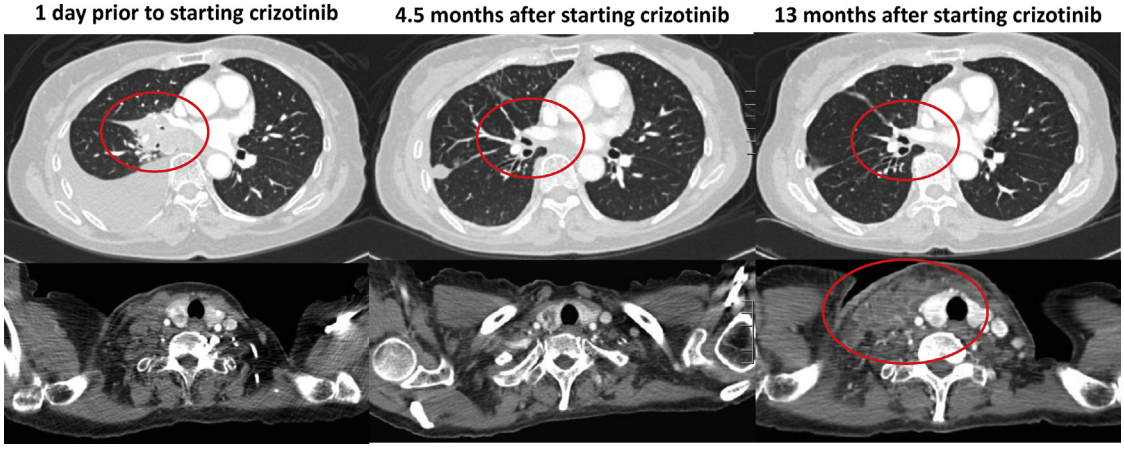

Figure 1. Computed tomography scans of the patient before crizotinib treatment, confirmed response to crizotinib, and at progression during crizotinib treatment.

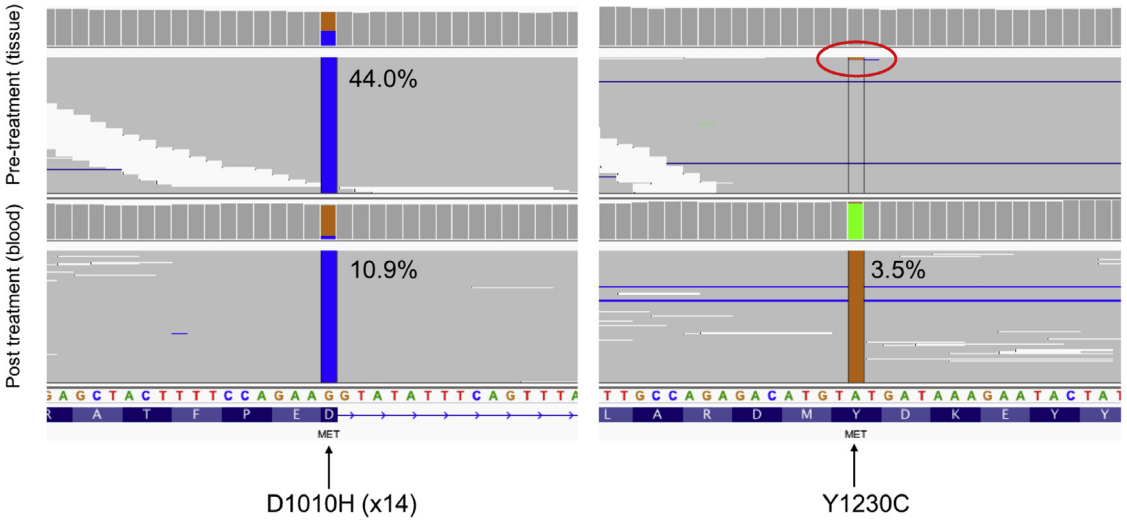

Figure 2. Comprehensive genomic profiling profiling of a pre-crizotinib treatment tumor tissue sample showing MNNG HOS Transforming gene (*MET*) exon 14 skipping alteration (D1010H) and the presence of Y1230C mutation (*red circle*) at a very low frequency (*upper panel*). Circulating tumor DNA assay detecting the presence of the original *MET* exon 14 skipping alteration and the Y1230C resistance mutation, both at reportable levels (*lower panel*). Percentages indicate the mutant allele frequency for the given assay.

partial response (PR) after 2 months of treatment (see Fig. 1) and maintained a PR for nearly 13 months, at which point metastasis to her right cervical lymph nodes developed. Restaging of the patient revealed progression of her brain metastases (see Fig. 2). Because of ongoing response in the right hilar mass and the vascular cervical metastasis, a tissue sample could not be safely obtained. A ctDNA assay was performed; it confirmed the presence of the previously detected primary *MET*ex14 (D1010H) alteration at 10.9% MAF and also detected the MET Y1230C resistance mutation at a MAF of 3.5% (see Fig. 2 [*lower panel*]). The patient received whole brain radiation and radiation to the right side of the neck but declined further chemotherapy or an alternate MET TKI and returned to her native country.

Discussion

This is the first clinical report that Y1230C mutation occurs in *MET*ex14-positive NSCLC after progression during crizotinib treatment. The MET receptor tyrosine kinase adopts a unique inactive conformation, with the activation loop spanning the adenosine triphosphate binding site through several critical interactions, including the salt pair involving an aspartate residue at 1228 (D1228) with a lysine residue at 1110 (K1110) that stabilizes the position of the tyrosine residue at 1230 (Y1230).[4] Y1230 lies in the activation loop and is locked inside the adenosine triphosphate binding area through interaction with A1226. Type I MET TKIs such as crizotinib bind to the MET unique autoinhibitory conformation through interaction of the aromatic ring of the inhibitor (π stacking) with Y1230 in the MET activation loop.[4] Substitution of tyrosine with a cysteine abolishes this interaction and weakens the binding affinity of crizotinib and other type I inhibitors with the MET kinase domain.[4] In vitro mutagenesis experiments have indicated that mutations at Y1230 and D1228 are the two most common types of acquired mutations identified in the setting of type I MET TKI resistance, including resistance to crizotinib.[5,6] A D1228N acquired resistance mutation to crizotinib in *MET*ex14-positive NSCLC has recently been reported clinically.[3] Thus, mutations at Y1230 and D1228 are likely to be the Achilles' Heel for all type I MET TKIs. Indeed, another type I MET TKI, AMG337, is ineffective against Y1230 and D1228 mutations.[7] On the other hand, merestinib, a type II MET TKI, is less dependent on the π stacking interaction with Y1230 given its additional interaction with the hydrophobic back pocket of the MET kinase domain, and it has inhibitory activity against wild-type MET similar to that against MET Y1230C.[8] Currently, several clinical trials involving type I MET TKIs such as crizotinib (NCT00585195, NCT02465060 [NCI-MATCH], and NCT02499614 [METROS]) capmatinib (NCT02750215), and tepotinib (NCT02864992) and type II inhibitors such as cabozatinib (NCT01639508) and glesatinib (NCT02544633) in *MET*ex14-positive NSCLC are ongoing. CGP of tissue or assay of ctDNA at time of disease progression will likely help guide optimal treatment with the two major classes of MET TKIs on the basis of the resistance mechanisms detected.

Importantly, in this report Y1230C was already detectable at a very low frequency at the time of diagnosis before any treatment, when it was looked for

retrospectively. The preexistence of low-level *MET* Y1230C before treatment is novel and similar to the low level of *EGFR* T790M mutation or *MET* amplification observed in patients with treatment-naive *EGFR*-mutated NSCLC.[9,11] The existence of the T790M mutation negatively modulated the response and progression-free survival of *EGFR*-positive patients to first-generation EGFR TKIs.[9] Our patient did achieve a confirmed PR to crizotinib for 13 months before progressing, indicating that the low level of Y1230C did not confer upfront resistance but likely did play a role in the disease progression. The prospective detection of *MET* Y1230C as an acquired resistance mechanism to a type I MET TKI in a patient with *MET*ex14-positive NSCLC using a clinically validated assay points to the importance of developing type II MET TKIs and/or utilizing total blockade of the MET axis with neutralizing antibodies to the MET receptor or its ligand.[10] Although the MAF of Y1230C in pretreatment tissue cannot be directly compared with the MAF as detected in the posttreatment blood sample, given the enrichment of Y1230C relative to the D1010H primary mutation in the posttreatment sample as well as the structural and preclinical data indicating that Y1230C is a resistance mechanism, given the clinical context, the emergence of Y1230C likely contributed to disease progression in our patient.

Patients with *MET*ex14-positive NSCLC are generally elderly, and the incidence of brain metastasis at the time of diagnosis, and more importantly at the time of progression during treatment with a MET TKI, remains to be determined.[12] Crizotinib has limited central nervous system (CNS) penetration.[13] Thus, whether CNS progression in our patient was due to limited penetration of crizotinib and/or the Y1230C mutation is unknown. For patients with *MET*ex14-positive NSCLC with CNS progression during crizotinib treatment, the practical utility of a convenient ctDNA assay to detect the presence or absence of known or predicted resistance mutations may direct future therapy toward type II MET TKIs with CNS activity such as cabozantinib[14] or to type I MET TKIs with better CNS activity, respectively. Limitations to the plasma-based detection method include the inability to detect histologic changes such as epithelial-mesenchymal transition[15] small cell transformation[16] or potential upregulation of the MET ligand.[10] Nonetheless, given the specific binding interactions of type I MET TKIs in the MET kinase domain, enrichment of preexisting resistance mutations or development of acquired ones such as Y1230 or D1228 will likely be the prevailing mode of resistance, and plasma-based ctDNA assays will provide a noninvasive method to detect these likely dominant resistance mutations to MET TKIs.

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