MET Exon 14 Skipping in Non-Small Cell Lung Cancer

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ABSTRACT

Background. Non-small cell lung cancers (NSCLCs) harboring specific genetic alterations can be highly sensitive to targeted therapies.

Materials and Methods. We performed a targeted rearrangement assay on 54 NSCLCs across all stages that were from patients who were never smokers and did not have driver mutations. Because MET exon 14 skipping was the most frequent alteration found, we surveyed the results for MET exon 14 skipping at Massachusetts General Hospital (MGH) since the inclusion of this alteration into our current molecular profiling panel.

Results. In a cohort of 54 never-smokers with lung cancers that were wild-type for known driver mutations, MET exon 14 skipping was the most frequently recurring alteration, occurring in 10 cancers (19%). Clinical testing at MGH via our next-generation sequencing (NGS) and NGS-rearrangement panels showed an additional 16 cases of MET exon 14 skipping, for an overall estimated frequency of 5.6%. A clinical case of a patient with MET exon 14 skipping treated with the MET inhibitor crizotinib is also described.

Conclusion. MET exon 14 skipping is a targetable gene alteration found in NSCLC. Patients with these alterations may respond well to MET inhibition. The Oncologist 2016;21:481–486

Implications for Practice: MET exon 14 skipping occurs with an approximately 5% frequency in NSCLC and is seen in both squamous and adenocarcinoma histology. Patients whose cancers have MET exon 14 skipping can respond well to MET inhibitors. Molecular testing for MET exon 14 skipping should be performed on all lung cancers because this is a targetable alteration.

INTRODUCTION

The discovery of oncogenic driver mutations and translocations has transformed the treatment of lung cancer, and patients with sensitizing EGFR mutations or ALK or ROS1 translocations can have remarkable responses to targeted inhibition, leading to significant clinical benefit [1–8]. The advent of new technologies has spurred more comprehensive molecular profiling of lung cancers, and the mutational spectrum of lung cancers, both adenocarcinoma and squamous cell carcinoma, is becoming better defined [9–12].

Molecular testing of tumors for genomic changes has been integrated into the oncology clinic as a part of standard care at Massachusetts General Hospital since 2009 with the SNaPshot platform (LifeTechnologies/Applied Biosystem, ThermoFisher Scientific, Foster, CA, https://www.thermofisher.com), a validated, Clinical Laboratory Improvement Amendments-approved, multiplexed tumor genotyping assay that is used for real-time testing of tumors. More than 50 commonly mutated loci in 14 key oncogenes were tested in the original SNaPShot panel, and fluorescent in situ hybridization (FISH) assays for other genetic changes of interest, such as ALK and ROS1 translocation, were performed separately [13, 14]. However, a substantial number of cancers (~40%–50%) have no mutations identified by this platform.

Gene rearrangements and fusions can lead to constitutive activation of a kinase and are a key mechanism by which some cancers become “oncogene addicted.” The ability to target these oncogenic fusions is exemplified by the examples of ALK and ROS1 rearranged lung cancer. We performed targeted rearrangement sequencing using the recently described anchored multiplex polymerase chain reaction PCR (AMP) method [15] on a cohort of 54 never-smokers with lung cancer whose tumors were known to be wild-type on SNaPshot testing. We hypothesized that such a cohort would be enriched for driver genetic alterations. The overall goal was to identify fusion
drivers in lung cancer that might lead to new targets for therapy. A unique feature of our investigation was the enrichment for patients whose cancers were known to be wild-type on a large panel of driver mutations.

**Materials and Methods**

We performed a targeted rearrangement assay on 54 NSCLC patients across all stages who were never-smokers and were not known to have driver mutations on SNaPshot testing. Specifically, we excluded patients known to have EGFR, KRAS, BRAF, or ERBB2 mutations or ALK or ROS1 rearrangements. Supplemental online Table 1 shows the patient characteristics. We used a gene enrichment method, anchored multiplex PCR, to perform next-generation sequencing (NGS) using HiSeq (Illumina, San Diego, CA, https://www.illumina.com/) as previously described in detail [15]. Total nucleic acid (TNA) containing total RNA and genomic DNA were extracted from formalin-fixed, paraffin-embedded tissue by using the Agencourt FormaPure Kit (Beckman Coulter, Indianapolis, IN, https://www.beckmancoulter.com). We used at least 50 ng of TNA for RNA rearrangement analysis using the AMP method with exonic anchored primers. The genes covered in each primer panel are shown in supplemental online Table 2.
Table 3. Summary of clinical characteristics of all patients identified with MET exon 14

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (minimum, maximum), yr</td>
<td>43, 84</td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>17</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>20</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>4</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Adeno/pleomorphic carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Stage I</td>
<td>13</td>
</tr>
<tr>
<td>Stage II</td>
<td>3</td>
</tr>
<tr>
<td>Stage III</td>
<td>3</td>
</tr>
<tr>
<td>Stage IV</td>
<td>7</td>
</tr>
<tr>
<td>Never-smoker</td>
<td>12</td>
</tr>
<tr>
<td>Former smoker</td>
<td>14</td>
</tr>
<tr>
<td>Current smoker</td>
<td>0</td>
</tr>
<tr>
<td>0 pack-years</td>
<td>12</td>
</tr>
<tr>
<td>1–20 pack-years</td>
<td>9</td>
</tr>
<tr>
<td>&gt;20 pack-years</td>
<td>5</td>
</tr>
</tbody>
</table>

Coexisting alterations

- Classic EGFR sensitizing mutations: 0
- ALK rearrangement: 0
- ROS1 rearrangement: 0
- PI3K: 2
- PS3: 3
- CTNNB1: 1
- PTEN: 1
- CDKN2A: 1
- SMAD4: 1

Unless otherwise noted, values are the numbers of patients.

*Although no sensitizing EGFR mutations were detected, one patient had an Arg277Gln mutation in EGFR of unknown significance, and two patients had EGFR amplification.

*Although no ROS1 rearrangements were detected, one patient had a Leu2035Phe mutation of unknown significance.

Targeted DNA sequencing using intronic primers was then performed to confirm genomic DNA alterations when RNA MET exon 14 skipping events were found. In our validation studies, we found that AMP requires 15% tumor content for a successful analysis and that 10 unique sequencing reads spanning the exon 13–15 boundary are the minimum needed for confident calls. All patients provided written informed consent under an institutional review board-approved protocol at Dana-Farber/ Harvard Cancer Center.

RESULTS

MET Exon 14 Skipping Is Frequent Among Never-Smokers With Tumors Wild-Type for Other Drivers

Figure 1 shows the rearrangement events that were found in this cohort of 54 never-smokers with lung cancers that were wild-type on SNaPshot testing for known drivers. Specifically, we excluded patients known to have EGFR, KRAS, BRAF, or ERBB2 mutations or ALK or ROS1 rearrangements. MET exon 14 skipping was the most frequently recurring alteration, occurring in 10 patients (19%). MET exon 14 skipping occurred with both adenocarcinoma and squamous histology. One patient had coexisting PI3KCA E545K and CTNNB1 mutations. We confirmed genomic DNA alterations in MET in 9 cases; 1 case had no further available tumor nucleic acid. Six of these had deletions in the 3’ splice site of intron 13; the length of the intronic deletions ranged from 10 to 32 base pairs. Three had point mutations in the 5’ splice site of intron 14 (Table 1).

Rearrangements in RET and NRG1 were also recurring events in this cohort of patients (Fig. 1). Interestingly, two patients who were thought to be ALK-negative tested positive for an ALK translocation on the AMP panel; one of these patients had tested ALK FISH-negative at our institution, and the other had ALK testing outside, results of which were negative. One patient who had tested ROS1 negative by FISH at our institution tested positive for ROS1 rearrangement on the AMP panel.

Clinical Testing of MET

Clinical testing for MET exon 14 skipping has since been incorporated into the current standard tumor molecular profiling at Massachusetts General Hospital (MGH). The current tumor molecular profiling at MGH uses anchored multiplex PCR technology [15] to provide targeted NGS for single-nucleotide variant (SNV) and insertions/deletions of interest (SNapShot-NGS), as well as rearrangements (NGS-rearrangement panel). Both tests are ordered on all lung cancer patients seen at MGH to provide comprehensive profiling of tumor tissue.

Detection of MET exon 14 skipping has been incorporated in a phased manner: Since May 2014, the SNaPshot-NGS panel has provided targeted next-generation DNA sequencing of specific exons in MET; this allowed us to identify MET exon 14 skipping events that result from specific SNVs (e.g., a common point mutation affecting position 1010 that leads to exon 14 skipping). However, this method does not identify all of the genomic events that can lead to exon 14 skipping, many of which may occur in intronic segments. Therefore, since March 2015, the NGS-rearrangement panel has been tailored to identify MET exon 14 skipping events, thus allowing broad capture of this event regardless of the specific genomic change that produced it. Specifically, the NGS-rearrangement panel uses targeted RNA sequencing to detect gene rearrangements from clinical formalin-fixed, paraffin-embedded material, by using exonic anchor primers in MET exon 15. This allows detection of MET exon 14 skipping (which always appears as the same sequence at the RNA level) regardless of the specific DNA change (which varies from case to case) that produced it.

Because we perform tumor genomic profiling using both SNaPshot-NGS and NGS-rearrangement panel on all cases of non-small cell lung cancer seen in our thoracic oncology clinic, regardless of smoking status or histology, we are able to determine the frequency of the MET exon 14 skipping mutations in a cohort that is not selected for any specific histology or smoking status. Among 89 NGS-rearrangement panel cases run since March 2015, 5 have been identified to have MET exon 14 skipping (~5.6%). In addition, another 11 cases of MET exon 14...
Case Vignette

A 73-year-old man presented with dyspnea was found to have extensive bilateral pulmonary emboli with right ventricular strain. He underwent bilateral pulmonary thromboendarterectomy; pathology of the right and left pulmonary artery clots revealed metastatic squamous cell carcinoma. SNaPshot testing revealed the D1010N mutation in MET, which leads to MET exon 14 skipping. FISH testing was borderline for MET amplification, with a MET-to-centromere 7 ratio of 2.2. No other alterations were detected on SNaPshot. Chest, abdomen, and pelvic computed tomography (CT) showed a lytic lesion on the left third rib with associated soft tissue mass, a right adrenal nodule, and small liver lesions consistent with metastatic disease. The patient was treated with crizotinib off-label at 250 mg twice daily. CT scans obtained after 4 weeks of crizotinib treatment revealed near-complete resolution of the soft tissue mass around the lytic rib lesion and resolution of the right adrenal and liver lesions, as well as decreased thrombus in the right main pulmonary artery (Fig. 2). The patient has continued to receive crizotinib and at the time of this writing was starting his seventh month of therapy, with scans showing that the initial response has been maintained.

DISCUSSION

We report here our experience with MET exon 14 skipping in NSCLC. We found 10 cases of MET exon 14 skipping among a group of 54 never-smokers whose cancers were wild-type for other drivers. Since incorporating MET exon 14 skipping into our standard clinical testing, we have found multiple additional cases and estimate a frequency of approximately 5%. Most of these occurred in the absence of other key driver alterations, supporting the notion that this is a driver event. MET exon 14 skipping events occurred in both adenocarcinoma and squamous cell carcinoma histology. Although enriched among wild-type never-smokers, MET exon 14 skipping is found across all smoking histories and histologic types. As noted in other published reports further described in this section, we saw a dramatic clinical response in a patient with widespread metastatic lung squamous cell cancer with MET exon 14 skipping who was treated with crizotinib.

MET exon 14 skipping results from somatic mutations in the introns of MET; these mutations lead to an alternatively spliced transcript of MET in which deletion of the juxtamembrane domain results in the loss of Cbl E3-ligase binding [16, 17]. The alternatively spliced MET receptor exhibits decreased ubiquitination and delayed downregulation, leading to prolonged activation of MET and MAP kinase, which is thought to be transforming [16]. Overall, reports of MET exon 14 skipping have ranged from 1.5% to 6% of NSCLC [10, 11, 16, 18–20], and a recent report noted 22% of pulmonary sarcomatoid carcinomas had MET exon 14 skipping [21]. Among 38,028 patients with advanced cancers who underwent comprehensive genomic profiling using the Foundation platform (Foundation Medicine, Cambridge, MA, http://www.foundationmedicine.com), the highest rates of MET exon 14 skipping occurred in lung cancers, with rates of 3% (131 of 4,402) in lung adenocarcinoma and 2.3% (62 of 2,669) in other lung neoplasms [18]. Eight patients with lung adenocarcinoma and MET exon 14 skipping were identified with comprehensive profiling at Memorial Sloan Kettering Cancer Center; although a denominator is not reported, the authors estimate a frequency of 4% [22]. Three cases of MET exon skipping were identified among 87 lung adenocarcinomas from Korea [10]. In The Cancer Genome Atlas, 10 of 230 lung adenocarcinomas had MET exon 14 skipping. In 9 of these, a 5’ or 3’ splice site mutation or deletion was found; 1 case of MET exon 14 skipping occurred in the setting of METY1003* stop codon [11]. Across all the studies, MET exon 14 skipping occurred in the absence of other known drivers.

Preclinical data have shown that cell lines with MET exon 14 skipping may respond well to MET inhibition [16, 18], although data are mixed [23, 24]. Kong-Beltran showed prolonged phosphorylation of MET and MAPK with stimulation by HGF in a cell line with MET exon 14 skipping, and increased cell proliferation, which was inhibited by treatment with a MET inhibitor [16]. Frampton et al. showed that human MET exon 14 skipping and the homologous mouse MET exon 15 skipping are transforming in cell lines, at least partly through activation of the MEK/ERK pathway. A mouse model of the

Figure 2. Computed tomographic scans obtained after 4 weeks of crizotinib treatment in a 73-year-old man with metastatic squamous cell lung cancer and MET exon 14 skipping.
### Table 4. Summary of case reports of patients with MET exon 14 and responses to MET treatment

<table>
<thead>
<tr>
<th>MET exon 14</th>
<th>MET amplification</th>
<th>Selected other alterations</th>
<th>Drug</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Smoking</th>
<th>Histology</th>
<th>Reported estimated PFS (mo)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.3028G&gt;C</td>
<td>MET amp</td>
<td>MDM2 amp and multiple others</td>
<td>Cabozantinib</td>
<td>80</td>
<td>Female</td>
<td>Never</td>
<td>Adenocarcinoma</td>
<td>5.1+</td>
<td>22</td>
</tr>
<tr>
<td>c.3028+1G&gt;T</td>
<td>MET amp IHC A</td>
<td>Multiple</td>
<td>Crizotinib</td>
<td>78</td>
<td>Male</td>
<td>Former</td>
<td>Adenocarcinoma</td>
<td>3.6+</td>
<td>22</td>
</tr>
<tr>
<td>c.3028G&gt;C</td>
<td>No amp IHC C</td>
<td>Multiple</td>
<td>Crizotinib</td>
<td>65</td>
<td>Male</td>
<td>Former</td>
<td>Adenocarcinoma</td>
<td>4.6+</td>
<td>22</td>
</tr>
<tr>
<td>c.3028G&gt;C</td>
<td>No amp IHC C</td>
<td>MDM2 amp</td>
<td>Crizotinib</td>
<td>90</td>
<td>Female</td>
<td>Never</td>
<td>Adenocarcinoma</td>
<td>3.1+</td>
<td>22</td>
</tr>
<tr>
<td>c.2887-5_2944del2</td>
<td>NA</td>
<td>TPS3 ZMYM3</td>
<td>Crizotinib</td>
<td>84</td>
<td>Female</td>
<td>Never</td>
<td>Histiocytic sarcoma</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>c.3028G&gt;C</td>
<td>FISH not done 3 + IHC</td>
<td>TPS3</td>
<td>INC280</td>
<td>82</td>
<td>Female</td>
<td>Former</td>
<td>Large cell</td>
<td>5+</td>
<td>22</td>
</tr>
<tr>
<td>c.3028+1G&gt;T</td>
<td>Copy number 4; MET:CEP7 2.3</td>
<td>None reported</td>
<td>INC280</td>
<td>66</td>
<td>Female</td>
<td>Former</td>
<td>Squamous</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>c.3028G&gt;A</td>
<td>Borderline MET:CEP7 2.2</td>
<td>SNApshot wt</td>
<td>Crizotinib</td>
<td>73</td>
<td>Male</td>
<td>Former</td>
<td>Squamous</td>
<td>6+</td>
<td>This study</td>
</tr>
<tr>
<td>c.3028G&gt;C</td>
<td>BA (presume negative, Foundation)</td>
<td>MDM2 amp</td>
<td>Crizotinib</td>
<td>76</td>
<td>Female</td>
<td>Former</td>
<td>Squamous</td>
<td>NA</td>
<td>25</td>
</tr>
<tr>
<td>Chr7:g.116412043G&gt;C</td>
<td>No amp MET:CEP7 0.96</td>
<td>NA</td>
<td>Crizotinib</td>
<td>71</td>
<td>Male</td>
<td>Former</td>
<td>Adenocarcinoma</td>
<td>6+</td>
<td>26</td>
</tr>
<tr>
<td>c.2887-18&gt;2887-7del2</td>
<td>NA</td>
<td>CDKN2A/B loss, CDM4 amp, MDM2 amp</td>
<td>Crizotinib</td>
<td>86</td>
<td>Male</td>
<td>Former</td>
<td>Adenocarcinoma</td>
<td>Crizotinib discontinued because of pneumonitis</td>
<td>27</td>
</tr>
<tr>
<td>Intron 14 + 3A&gt;G</td>
<td>9 copies</td>
<td>None reported</td>
<td>Crizotinib</td>
<td>74</td>
<td>Female</td>
<td>Former</td>
<td>Sarcomatoid</td>
<td>NA</td>
<td>21</td>
</tr>
</tbody>
</table>

Abbreviations: amp, amplification; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry; NA, not available; PFS, progression-free survival.
Collection and/or assembly of data: Rebecca S. Heist, Hyo Sup Shim, Shalini Gingipally, Mari Mino-Kenudson, Long Le, Justin F. Gainor, Zongli Zheng, Junfeng Xia, Peilin Jia, Hailing Jin, Zhongming Zhao, William Pao, Jeffrey A. Engelman, A. John Iafrate

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Disclosures

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References


