An Alert to Possible False Positives With a Commercial Assay for MET Exon 14 Skipping

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ABSTRACT

Introduction: Because molecular-targeted drugs against MET exon 14 (METex14) skipping have been approved, molecular testing of the alteration has added to clinical guidelines. There are several such assays, but methodological issues have been reported.

Methods: METex14 skipping results from three assays (Oncomine DxTT, ArcherMET, and laboratory-developed reverse-transcriptase polymerase chain reaction test [LDT RT-PCR]) were compared in a relatively small series of the specimens diagnosed as advanced NSCLC (n = 50).

Results: The ArcherMET and LDT RT-PCR results were identical for all 50 samples, but eight samples had discordant results between Oncomine DxTT and the other two assays. All eight samples had METex14 skipping with Oncomine DxTT and wild-type signals with ArcherMET and LDT RT-PCR. The discordance might be caused by the homopolymeric error of the splice donor site with Oncomine DxTT, and false positives could be distinguished by relatively low read counts.

Conclusions: Although the caution in detecting METex14 skipping focuses on false negatives in the literature, false positives were first noted at a relatively high frequency (8 of 26, 30.8%) in this study. According to the results of previous clinical trials using the other tyrosine kinase inhibitors, it could be surmised that MET inhibitor treatment in patients without METex14 skipping is detrimental. Clinicians need to be alert to the false positives that can lead to harmful treatments.

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Keywords: MET exon 14 skipping; Oncomine Dx target test; Molecular testing; NSCLC; Tepotinib; Capmatinib

Introduction

MET, a proto-oncogene located at 7q21 to q31, encodes a receptor tyrosine kinase that leads to RAS/MAPK, Rac/Rho, and PI3K/AKT signaling pathway activation. Dysregulation of these pathways is known to be involved in tumor growth, antiapoptosis, and metastasis.1 MET amplification and overexpression are often observed in several carcinomas (including colorectal cancer, gastric cancer, liver cancer, sarcoma) and are also found in up to 4% of lung adenocarcinomas and approximately 1% of lung squamous cell carcinomas.2 A humanized antibody specifically targeting the amplification and overexpression of this gene was tested in clinical trials, but sufficient efficacy was not found in the phase 3 clinical study.3 Nevertheless, it has been reported that MET amplification is acquired as one of the resistance mechanisms to EGFR tyrosine kinase inhibitor

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treatment, and EGFR tyrosine kinase inhibitors and MET inhibitors are currently being investigated as treatment methods.\(^5\) In addition, MET gene mutations have been reported in SCLC,\(^6\) and mutations in the specific intronic regions of the MET gene could result in MET exon 14 (METex14) skipping.\(^7\) Subsequently, METex14 skipping was detected widely in NSCLCs and particularly in high-grade carcinomas, such as sarcomatoid carcinoma.

Tepotinib and capmatinib were first approved in 2020 in Japan for patients with METex14 skipping based on the results of the VISION study\(^8\) and GEOMETRY mono-1 study,\(^9\) respectively. Their companion diagnostics (CDxs) for detecting METex14 skipping were assigned as ArcherMET and FoundationOne CDx, respectively, after full analytical validation with clinical trial-used assays (the Oncomine Comprehensive Assay and Guardant360 in the VISION study and a laboratory-developed reverse-transcriptase polymerase chain reaction test [LDT RT-PCR] in the GEOMETRY study). The CDx test for tepotinib is the ArcherMET in Japan, whereas no specific molecular assays have been specified in the United States and Europe. Therefore, we conducted direct comparison of the results of METex14 skipping with Oncomine DxTT, ArcherMET, and LDT RT-PCR, in this study.

Materials and Methods

Samples

We selected nearly consecutive 50 specimens diagnosed as advanced NSCLC from 568 for which METex14 skipping status was determined by Oncomine DxTT. The relevant clinicopathologic data, including patient demographics, pathologic features, and sample types for molecular testing, were listed as a Supplementary Data (Supplementary Table 1). In this series, 26 cases were positive for METex14 skipping with Oncomine DxTT and 24 were negative. Unstained sections identical to those assessed by Oncomine DxTT were submitted to and evaluated with ArcherMET and LDT RT-PCR in a commercial laboratory and the laboratory in our department, respectively. This study was approved by the institutional review board, based on the patient comprehensive consent for research (institutional review board number 2020-215).

LDT RT-PCR

LDT RT-PCR uses common fragment analysis to detect METex14 skipping. Extracted RNA was amplified using one-step RT-PCR (OneStep RT-PCR Kit, Qiagen) with a FAM-labeled primer set as follows: forward, 5’ FAM-ATTACTACTTGCTTCTCTGTG-3’, and reverse, 5’-ATACTGCACCTGTGCGCATGAA-3’. PCR products were electrophoresed on a capillary sequencer (ABI PRISM 3100) or agarose gel. When METex14 skipping was present, PCR amplified the shorter segment of DNA, creating a new peak in the electropherogram.

Statistical Analysis

Variables were compared using the \(t\) test and Fisher’s exact test for background analysis. More than two groups were compared by one-way analysis of variance (ANOVA). The threshold for significance was set at \(p\) value less than 0.05. All statistical analyses were performed using R version 3.5.3 (The R Foundation for Statistical Computing, Vienna, Austria) with the aid of EZR version 1.38 (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

Results

The results of ArcherMET and LDT RT-PCR were identical for all 50 samples, whereas discordance between the results of Oncomine DxTT and ArcherMET/LDT RT-PCR was found for eight samples, all of which had METex14 skipping with Oncomine DxTT and wild-type signals with ArcherMET/LDT RT-PCR. Only 18 of 26 samples (69.2%) concordantly revealed METex14 skipping with the three assays, and eight samples were positive only in the Oncomine DxTT results. The relationship was illustrated in Table 1. Of note, comutations of the other driver genes (\(EGFR\) Ex19Del and \(ALK\) fusion) were detected in two of eight discordant samples (Supplementary Table 1), in contrast to mutually exclusive nature of the driver mutations.\(^10\)

To explore the differences between the 18 samples with concordant results and the eight samples with discordant results, the sequencing status, including the read counts of MET(13)-MET(15) products, sample RNA concentration, specimen types, and sequencing quality control measures, was analyzed. As illustrated in Table 2, the read counts and the total unmapped reads had statistically significant differences. Markedly, most of the discordant samples had read counts less than 800, and no samples with more than 800 reads had discordance (Fig. 1). The total unmapped reads were also different, suggesting fair sequencing conditions, but the ranges between the concordant and discordant groups somewhat overlapped. Therefore, we considered that 800 reads could be a good classifier to distinguish the discordant group from the concordant one.

To validate the findings, recent six samples with METex14 skipping detected by Oncomine DxTT in another cohort were analyzed (Table 3). Three of the six had more than 800 reads of MET(13)-MET(15) product, and the other assays were positive for METex14 skipping. Nevertheless, in the other three samples, the read
counts were only 49, 58, and 56, suggesting a discordant result. Indeed, ArcherMET and LDT RT-PCR was negative for METex14 skipping in these samples.

Then, we evaluated the possible cause of discordance. When we looked at the sequencing results with the Integrative Genome Viewer, one of the thymidine repeats at the METex14 donor site (chr7:116771979–116771982) was deleted in five of the eight discordant samples (Fig. 2), and the number of deletions at the site in individual samples matched the read counts of MET(13) to MET(15) products. Therefore, we speculated that the deleted reads might be reported as the variant.

### Discussion

Currently, molecular therapy targeting driver mutations is a clinical standard, so the mutational status of driver mutations is needed to determine the therapeutic options for patients. The number of identified target genes is increasing, and single-gene tests are no longer able to cover all of the targets. Therefore, multiplex gene panel testing is recommended in the molecular testing guidelines. Oncomine DxTT is the first multiplex gene panel test, using next-generation sequencing technique, that has been approved by the Food and Drug Administration as a CDx test. This panel covers most targets that need to be assessed in current clinical practice for lung cancer treatment. Therefore, Oncomine DxTT has been used worldwide.

This panel test for detecting METex14 skipping is characterized by RNA-based amplicon sequencing using Ion PGM Dx (Thermo Fisher Scientific, Walther, MA), which is contrasted to larger panels, such as FoundationOne CDx, which is based on DNA-based hybrid capture sequencing with the Illumina system.

To date, high rates of false negatives for METex14 skipping have been highlighted, particularly in cases of either amplicon sequencing or DNA-based sequencing. Transcriptional skipping of METex14 occurs owing to genetic deletions or mutations in the intron/exon boundary sites causing...

### Table 1. METex14 Skipping Status Among the Three Assays

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArcherMET/LDT RT-PCR</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Oncomine DxTT</td>
<td>8</td>
<td>24</td>
</tr>
</tbody>
</table>

Note: ArcherMET and LDT RT-PCR were combined owing to identical results in all samples.

### Table 2. Comparison Among the Three Groups

<table>
<thead>
<tr>
<th>Sample Information and Quality Indicators</th>
<th>METex14 Concordant (n = 18)</th>
<th>METex14 Discordant (n = 8)</th>
<th>Negative METex14 Skipping (n = 24)</th>
<th>p Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>p Value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample type</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0.858</td>
<td>0.256</td>
</tr>
<tr>
<td>Mean read length (AQ20)</td>
<td>104.9 ± 1.3</td>
<td>104.8 ± 1.6</td>
<td>104 ± 2.1</td>
<td>0.858</td>
<td>0.256</td>
</tr>
<tr>
<td>Mean read length (AQ30)</td>
<td>96.5 ± 1.6</td>
<td>96.7 ± 1.9</td>
<td>95.5 ± 2.1</td>
<td>0.823</td>
<td>0.137</td>
</tr>
<tr>
<td>Total mapped fusion panel reads</td>
<td>82,281.2 ± 35,599.4</td>
<td>101,770.8 ± 34,534.6</td>
<td>86,981.6 ± 41,575.7</td>
<td>0.206</td>
<td>0.494</td>
</tr>
<tr>
<td>Total unmapped reads</td>
<td>10,842.9 ± 8911.9</td>
<td>28,006.5 ± 18,557.5</td>
<td>12,438.3 ± 9627</td>
<td>0.004</td>
<td>0.002</td>
</tr>
<tr>
<td>Average read length</td>
<td>97.8 ± 8.5</td>
<td>93.5 ± 2.5</td>
<td>90.1 ± 14</td>
<td>0.178</td>
<td>0.094</td>
</tr>
<tr>
<td>Expression controls total reads</td>
<td>37,025.7 ± 15,939.1</td>
<td>56,488.1 ± 35,457.5</td>
<td>38,098.2 ± 28,607</td>
<td>0.062</td>
<td>0.182</td>
</tr>
<tr>
<td>MET(13)–MET(15) read count</td>
<td>7478 ± 6420.2</td>
<td>73.3 ± 27.3</td>
<td>0</td>
<td>0.004</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>p value denotes statistical differences between METex14 concordant and discordant groups.

<sup>b</sup>p value denotes statistical differences between METex14 concordant, discordant and negative METex14 skipping groups.

<sup>c</sup>Denotes SDs.

EBUS-TBNA, endobronchial ultrasound transbronchial needle aspiration; Mbp, mega base pair; METex14, MET exon 14.
splicing abnormalities or whole exon deletion. Therefore, mutations and short insertions/deletions in the primer regions prevent PCR for amplicon sequencing, resulting in false negatives. In addition, half of the causative DNA alterations are either insertion or deletion; the lengths and involved sites vary significantly and some deletions involve the whole exon.\textsuperscript{10} Because of the complexity of the DNA alterations that cause METex14 skipping, assays for detecting this alteration should be designed and optimized carefully.\textsuperscript{12,14}

The Ion Torrent platform is more susceptible to errors at the homopolymeric sites, which are mostly linked to false deletions,\textsuperscript{15} and indeed, most instances of discordance in this study were found at homopolymeric sites. Nevertheless, the discordant homopolymeric site was located at the splice donor site, and several short deletions involving the homopolymeric site have been reported (Fig. 2).\textsuperscript{14} Therefore, it would be quite difficult to distinguish a true deletion from a homopolymeric error with a single assay. Although the discrepancies in this study can be resolved if the read counts are considered, this difficulty always technically accompanies any assays.

In summary, the currently used Oncomine DxTT has an issue in detecting METex14 skipping. Although caution in detecting METex14 skipping has focused on false negatives in the literature, false positives were first noted with relatively high frequency (8 of 26, 30.8%) in this study. According to the results of the IPASS study, gefitinib treatment in patients without EGFR mutations was harmful.\textsuperscript{16} Therefore, capmatinib or tepotinib treatment should be avoided in the patients without METex14 skipping. Clinicians need to be alert to false positives that can lead to harmful treatments.

### CRediT Authorship Contribution Statement

**Takashi Teishikata**: Methodology, Formal analysis, Writing—original draft.

**Kohya Shiraishi**: Data Curation, Writing—review and editing.

**Yuki Shinno, Tatsuya Yoshida**: Data collection, Writing—review and editing.

**Yoshihisa Kobayashi, Jumpei Kashima**: Methodology, Formal analysis, Writing—review and editing.

**Takako Ishiyama**: Formal analysis.

**Taisuke Mori**: Methodology, Writing—review and editing.

**Yasushi Yatabe**: Conceptualization, Project administration, Writing—review and editing.
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Supplementary Data
Note: To access the supplementary material accompanying this article, visit the online version of the Journal of Thoracic Oncology at www.jto.org and at https://doi.org/10.1016/j.jtho.2021.07.028.

References


