BRIEF REPORT

DNA-Based versus RNA-Based Detection of *MET* Exon 14 Skipping Events in Lung Cancer

Kurtis D. Davies, PhD,[a,*] Aprille Lomboy, BS,[a] Carolyn A. Lawrence, PhD,[a] Michael Yourshaw, JD, PhD,[a] Gregary T. Bocsi, DO,[a] D. Ross Camidge, MD, PhD,[b] Dara L. Aisner, MD, PhD[a]

[a]*Department of Pathology, Division of Pathology, University of Colorado-Anschutz Medical Campus, Aurora, Colorado*
[b]*Department of Medicine, Division of Medical Oncology, University of Colorado-Anschutz Medical Campus, Aurora, Colorado*

Received 25 September 2018; revised 7 December 2018; accepted 29 December 2018
Available online - 9 January 2019

ABSTRACT

Introduction: Genomic variants that lead to MET proto-oncogenem receptor tyrosine kinase (*MET*) exon 14 skipping represent a potential targetable molecular abnormality in NSCLC. Consequently, reliable molecular diagnostic approaches that detect these variants are vital for patient care.

Methods: We screened tumor samples from patients with NSCLC for *MET* exon 14 skipping by using two distinct approaches: a DNA-based next-generation sequencing assay that uses an amplicon-mediated target enrichment and an RNA-based next-generation sequencing assay that uses anchored multiplex polymerase chain reaction for target enrichment.

Results: The DNA-based approach detected *MET* exon 14 skipping variants in 11 of 856 NSCLC samples (1.3%). The RNA-based approach detected *MET* exon 14 skipping in 17 of 404 samples (4.2%), which was a statistically significant increase compared with the DNA-based assay. Among 286 samples tested by both assays, RNA-based testing detected 10 positives, six of which were not detected by the DNA-based assay. Examination of primer binding sites in the DNA-based assay in comparison with published *MET* exon 14 skipping variants revealed genomic deletion involving primer binding sequences as the likely cause of false negatives. Two samples positive via the DNA-based approach were uninformative via the RNA-based approach due to poor-quality RNA.

Conclusions: By circumventing an inherent limitation of DNA-based amplicon-mediated testing, RNA-based analysis detected a higher proportion of *MET* exon 14 skipping cases. However, RNA-based analysis was highly reliant on RNA quality, which can be suboptimal in some clinical samples.

Keywords: MET exon 14 skipping; Next-generation sequencing; Non–small cell lung cancer; Molecular testing

Introduction

Genomic point mutations, insertions, and deletions that alter or eliminate the splice donor, acceptor, or branch sites adjacent to MET proto-oncogenem receptor tyrosine kinase (*MET*) exon 14, or deletions that completely eliminate exon 14, are found in approximately 3% of patients with NSCLC.[1–4] These alterations result in exclusion of exon 14 from the *MET* transcript, thus eliminating the Tyr1003 residue that mediates ubiquitination of the *MET* gene product hepatocyte growth factor receptor (HGFR).[5,6] As a result, proper degradation of HGFR protein is impaired, leading to enhanced oncogenic signaling.[4,7] Several published case reports have demonstrated clinical responses in patients with lung cancer positive for these variants to HGFR

*Corresponding author.

Disclosure: Dr. Davies reports sponsored travel from ArcherDx. Dr. Camidge reports research funding from Pfizer and Symphogen outside the submitted work. Dr. Ainser reports consulting fees from Bristol-Meyers Squibb, AbbVie, and Bayer outside the submitted work. The remaining authors declare no conflict of interest.

Address for correspondence: Kurtis D. Davies, PhD, University of Colorado Anschutz Medical Campus, Department of Pathology, BioScience 2, Room 4105, 12705 E. Montview Blvd., Aurora, CO 80045. E-mail: kurtis.davies@ucdenver.edu

ISSN: 1556-0864
https://doi.org/10.1016/j.jtho.2018.12.020

inhibitors (also referred to as MET inhibitors).[2,4,6,8,9] Thus, robust approaches for detection of *MET* exon 14 skipping events are critical for clinical management.

Molecular detection of *MET* exon 14 skipping events can be achieved by sequencing DNA or RNA (or by other less common approaches). If DNA is being sequenced, a genomic variant that alters or ablates a splicing site (or deletes the whole exon), must be detected. If RNA is being sequenced, the direct result of altered splicing (observed as "fusion" of exon 13 to 15) must be detected, and this result is constant regardless of the underlying genomic event. An additional consideration is that RNA is inherently more vulnerable to degradation than DNA, and consequently RNA of sufficient quality is harder to acquire from clinical cases. In this study, we used both a DNA-based approach and an RNA-based approach to assess samples from patients with NSCLC for *MET* exon 14 skipping. The prevalence of positive samples was determined for both assays, and instances of discordance between the assays were investigated further.

Materials and Methods

Molecular Testing

All testing in this study was performed in the Colorado Molecular Correlates Laboratory (Department of Pathology, University of Colorado–Anschutz Medical Campus). Samples included formalin-fixed, paraffin-embedded processed biopsy and resection material and formalin-fixed, paraffin-embedded or Diff-Quik processed cytopathology material (Table 1). All samples contained at least 15% tumor cells. Only samples tested as part of routine clinical analysis were considered. A flow diagram demonstrating sample quality assessment and attrition can be found in Supplementary Figure 1.

DNA-Based NGS

The Illumina TruSight Tumor 26 assay (Illumina, San Diego, CA) is a DNA-based next-generation sequencing (NGS) assay that uses amplicon-mediated target enrichment to assess mutational status in select regions of 26 genes, including *MET*. This assay was performed as previously described.[10] DNA or total nucleic acid was used as the starting input material. Libraries were sequenced with the Illumina MiSeq sequencer and raw sequence data was processed using a custom-designed bioinformatic analysis algorithm. A minimum read depth of 500 reads was required to accept a negative result for each amplicon.

RNA-Based NGS

The ArcherDx FusionPlex Solid Tumor assay (ArcherDx, Boulder, CO) is an RNA-based NGS assay that uses anchored multiplex polymerase chain reaction–mediated target enrichment to interrogate for gene fusions and oncogenic gene isoforms involving select regions in 53 genes, including *MET*. The assay was performed as previously described.[11] Total nucleic acid was used as the input material. Libraries were sequenced via the Illumina MiSeq or NextSeq instruments and raw sequence data was processed with the ArcherDx Analysis software package (version 4.1.1.7, ArcherDx). Detailed information regarding RNA quality assessment has been published previously.[12] Briefly, for a sample to pass quality metrics, the average number of unique read start sites originating from primers to four housekeeping genes must exceed a defined threshold.

Results

During the period from December 2013 to April 2018, a total of 856 samples classified as NSCLC or lung cancer not otherwise specified were assessed by routine clinical application of the Illumina TruSight Tumor 26 assay. Of these, 11 samples were positive for alterations expected to result in *MET* exon 14 skipping (Table 2), for a detected prevalence of 1.3%. During the period from November 2016 to August 2018, 404 samples classified

Table 1. Sample Characteristics

Characteristic	By DNA Assay	By RNA Assay	Tested in Both
Histologic type[a]			
Adenocarcinoma	731 (85.4%)	331 (81.9%)	239 (83.6%)
Adenosquamous carcinoma	1 (0.1%)	1 (0.2%)	0 (0.0%)
Squamous cell carcinoma	77 (9.0%)	40 (9.9%)	27 (9.4%)
Large cell/large cell neuroendocrine	4 (0.5%)	4 (1.0%)	3 (1.0%)
Other or NOS	43 (5.0%)	28 (6.9%)	17 (5.9%)
Sample type			
Biopsy (FFPE)	419 (48.9%)	198 (49.0%)	141 (49.3%)
Resection (FFPE)	250 (29.2%)	117 (29.0%)	82 (28.7%)
Cytopathology preparation[b]	187 (21.8%)	89 (22.0%)	53 (22.0%)

[a]Histologic classification based on diagnosis from submitting institution.
[b]Cytopathology preparations include FFPE-processed cell blocks and Diff-Quik smears.
NOS, not otherwise specified; FFPE, formalin-fixed, paraffin-embedded.

Table 2. Genomic *MET* Exon 14 Skipping Variants Detected by DNA-Based Assay

Patient Sample	Genomic Change	Variant Frequency %
1	c.3028+1G>C	22.6%
2	c.3028+2T>C	21.3%
3	c.3028+1G>T	78.5%
4	c.3028+2T>G	45.8%
5	c.3018-3028+1 del	41.8%
6	c.3028G>C	77.9%
7	c.3028+2T>A	38.2%
8	c.3028+1G>T	47.0%
9	c.3023-3028+8 del	47.7%
10	c.3028+1G>A	8.9%
11	c.3028G>C	43.8%

MET, MET proto-oncogenem receptor tyrosine kinase.

as NSCLC or lung cancer not otherwise specified were assessed by routine clinical application of the ArcherDx FusionPlex Solid Tumor assay. Of these, 17 samples were positive for reads in which exon 13 was "fused" with exon 15 (i.e., exon 14 was omitted from the transcript) (see Fig. 1 for an example). The prevalence of *MET* exon 14 skipping detected by using the RNA-based assay was 4.2%. The proportion positive according to the RNA-based assay was greater than that according to the DNA-based assay, as determined by using Fisher's exact test ($p < 0.01$). Tumor histologic types and sample types for all tested samples are summarized in Table 1. Positive samples included 22 adenocarcinomas and two squamous cell carcinomas.

In all, 286 samples were tested by both assays. Within this subset of samples, the RNA-based assay detected 10 positives. Of these, only four were detected by the DNA-based assay. In the remaining six cases, sequencing coverage of *MET* exon 14 was well above the lower limit of 500, meaning that the false-negative results were not due to poor sequencing. In this DNA-based assay, *MET* exon 14 is covered by two amplicons (named *tile 1* and *tile 2*). Figure 2 schematically represents the positions of the primer binding sites for both amplicons in relation to exon 14. Figure 2 also demonstrates the approximate positions of selected point mutations and deletions leading to *MET* exon 14 skipping that have been reported in the literature.[1] Importantly, any genomic deletion that ablates sequence within a primer binding site would be expected to impair primer binding. Consequently, no amplification of the deleted allele would occur and no evidence of a deletion would be called by the analysis algorithm.

Among the samples tested by both assays, six positives were detected by the DNA-based assay, which included the four samples that were also positive in the RNA-based assay. In the remaining two cases, a *MET* transcript lacking exon 14 was not detected in the RNA-based assay; however, the RNA metrics in the assay revealed poor-quality RNA and the samples were reported as uninformative rather than negative. In total, 61 samples (15.1%) tested by the RNA-based assay failed postsequencing RNA quality assessment.

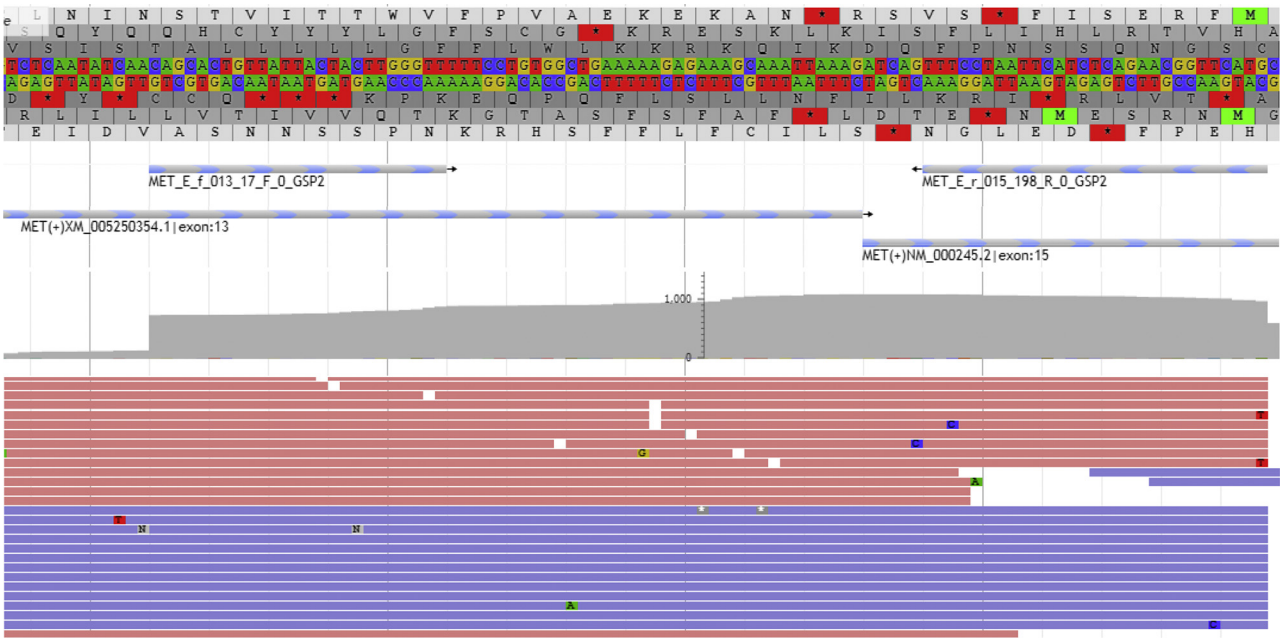

Figure 1. Example of positive MET proto-oncogenem receptor tyrosine kinase (*MET*) exon 14 skipping sample detected by the RNA-based assay. J-Browse view of next-generation sequencing reads supporting a *MET* transcript in which exon 14 is excluded (reads demonstrate direct "fusion"' of exon 13 to exon 15). Primer binding positions are noted (labeled as GSP2s). Gray histogram represents sequence depth of reads supporting this transcript.

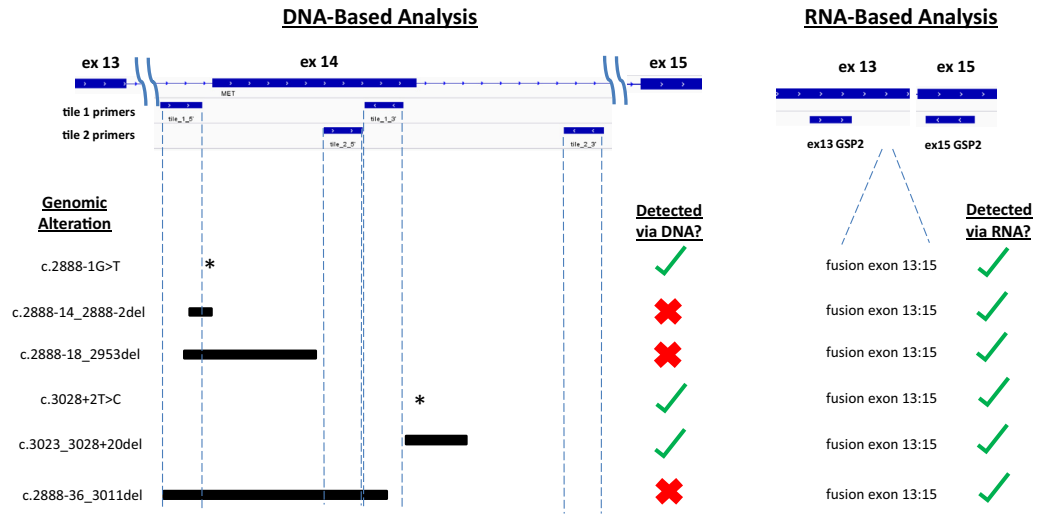

Figure 2. Schematic description of shortcomings in the DNA-based assay. (*Top left*) Representation of primers in the DNA-based assay used in this study in relation to MET proto-oncogenem receptor tyrosine kinase (*MET*) exon 14. (*Bottom left*) Approximate positions (in relation to exon 14) of previously reported point mutations and deletions (from Frampton et al.[1]). Deletions that remove significant portions of primer binding sites are predicted to not be detected by the DNA-based assay. (*Top right*) Representation of exon 14 exclusion from the *MET* transcript and positions of primers used in the RNA-based assay. All variants depicted at bottom left are expected to be detected by the RNA-based assay since exons 13 and 15 remain intact. del, deletion; ex, exon.

Discussion

In this study, we compared a DNA-based assay and an RNA-based assay with regard to their ability to detect *MET* exon 14 skipping events. Both assays use amplicon-mediated target enrichment followed by NGS. We found that the incidence of identified *MET* exon 14 skipping events in NSCLC clinical samples was higher when assessed by the RNA-based assay (4.2% versus 1.3%). Although we cannot confirm that the two subsets of samples tested by only one of the assays were equivalent, the large number of cases involved lessens the risk of a spurious difference due to cohort bias. In addition, among the samples tested by both assays, 60% that were positive according to the RNA-based assay were negative according to the DNA-based assay. Two samples that were positive via the DNA-based assay were uninformative in the RNA-based assay due to poor-quality RNA.

The shortcomings of amplicon-mediated target enrichment for DNA-based detection of *MET* exon 14 skipping have been described previously by Poirot et al.[13] In that study, an *in silico* analysis of eight different DNA-based assays (including the assay assessed in this study) that used amplicon-mediated target enrichment demonstrated that none of them was able to detect more than 63% of known *MET* exon 14 skipping variants. As was observed in our study, the limitation of the DNA-based assays was primer design that was not intended to capture all known *MET* exon 14 skipping events.[13] Specifically, any variant found outside of the amplified area(s) or any variant that would prevent proper primer binding (i.e., deletions that involve one or more primer binding sites) will not be detected by these assays. Consistent with the *in silico*–predicted deficiency, in our large real-world clinical utilization study, 60% of the samples positive by the RNA-based assay were negative in the DNA-based assay.

It should be noted that DNA-based assays using hybrid capture–mediated target enrichment are less likely to produce false-negative results in cases of genomic deletions, particularly if probe/bait design sufficiently covers the region of interest and algorithms to detect large deletions are used. This is exemplified by the studies from Frampton et al. and Awad et al., in which hybrid capture assays detected a wide variety of *MET* exon 14 skipping variants, including large deletions.[1,2]

In conclusion, in this study we found that RNA-based testing identifies a higher rate of *MET* exon 14 skipping than does amplicon-mediated DNA-based testing. This is likely the result of overcoming an inherent limitation to the DNA-based approach. In RNA-based analysis, detection is simplified such that, regardless of the underlying genomic event (even in cases of large deletions), exon 14 skipping simply manifests as a "fusion" of exon 13 to exon 15 (see Fig. 2). In a broader sense, this study also highlights the need for careful consideration of molecular assay limitations. Not all assays that include *MET* in covered gene content will detect all known *MET* exon 14 skipping variants. Therefore, it is critical that clinicians be aware of assay limitations to properly interpret results.

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.2018.12.020.

References

1. Frampton GM, Ali SM, Rosenzweig M, et al. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. *Cancer Disc*. 2015;5:850–859.
2. Awad MM, Oxnard GR, Jackman DM, et al. MET exon 14 mutations in non-small-cell lung cancer are associated with advanced age and stage-dependent MET genomic amplification and c-Met overexpression. *J Clin Oncol*. 2016;34:721–730.
3. Tong JH, Yeung SF, Chan AW, et al. MET amplification and exon 14 splice site mutation define unique molecular subgroups of non-small cell lung carcinoma with poor prognosis. *Clin Cancer Res*. 2016;22:3048–3056.
4. Lu X, Peled N, Greer J, et al. MET exon 14 mutation encodes an actionable therapeutic target in lung adenocarcinoma. *Cancer Res*. 2017;77:4498–4505.
5. Awad MM. Impaired c-Met receptor degradation mediated by MET exon 14 mutations in non-small-cell lung cancer. *J Clin Oncol*. 2016;34:879–881.
6. Drilon A, Cappuzzo F, Ou SI, Camidge DR. Targeting MET in lung cancer: will expectations finally be MET? *J Thorac Oncol*. 2017;12:15–26.
7. Kong-Beltran M, Seshagiri S, Zha J, et al. Somatic mutations lead to an oncogenic deletion of met in lung cancer. *Cancer Res*. 2006;66:283–289.
8. Paik PK, Drilon A, Fan PD, et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. *Cancer Disc*. 2015;5:842–849.
9. Heist RS, Shim HS, Gingipally S, et al. MET exon 14 skipping in non-small cell lung cancer. *Oncologist*. 2016;21:481–486.
10. Mulcahy Levy JM, Zahedi S, Griesinger AM, et al. Autophagy inhibition overcomes multiple mechanisms of resistance to BRAF inhibition in brain tumors. *Elife*. 2017;6, e19671.
11. Davies KD, Ng TL, Estrada-Bernal A, et al. Dramatic response to crizotinib in a patient with lung cancer positive for an HLA-DRB1-MET gene fusion. *JCO Precis Oncol*. 2017;2017(1).
12. Davies KD, Le AT, Sheren J, et al. Comparison of molecular testing modalities for detection of ROS1 rearrangements in a cohort of positive patient samples. *J Thorac Oncol*. 2018;13:1474–1482.
13. Poirot B, Doucet L, Benhenda S, Champ J, Meignin V, Lehmann-Che J. MET exon 14 alterations and new resistance mutations to tyrosine kinase inhibitors: risk of inadequate detection with current amplicon-based NGS panels. *J Thorac Oncol*. 2017;12:1582–1587.