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Reclassification of Noncanonical MET Splice-Site Variants Using RNA-Based Validation: A Practical Diagnostic Approach in Non-Small Cell Lung Cancer

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ABSTRACT

MET exon 14 (*MET*ex14) skipping alterations represent actionable oncogenic drivers in non-small cell lung cancer (NSCLC). However, noncanonical intronic splice-site variants are frequently classified as variants of uncertain significance (VUS) on DNA-based next-generation sequencing (NGS), limiting timely therapeutic decision making.

We describe two patients with advanced NSCLC harboring novel intronic deletions affecting the *MET* exon 14 splice acceptor region, initially classified as VUS. Integrated transcript level analysis using RNA sequencing and orthogonal validation by reverse transcription PCR (RT-PCR) was performed to assess functional impact.

RNA-based analysis in both cases demonstrated *MET*ex14 skipping through identification of exon 13–15 junction reads, which was further confirmed by RT-PCR. Based on functional evidence, both variants were reclassified as clinically actionable alterations (AMP/ASCO/CAP Tier II), directly influencing therapeutic management with *MET*-targeted tyrosine kinase inhibitors. Early clinical stabilization was observed in both patients.

These findings support the routine incorporation of RNA-based validation strategies to improve accurate interpretation of noncanonical *MET* splice-site variants. We propose a practical diagnostic workflow for evaluation and reclassification of *MET* splice-site VUS, emphasizing the role of transcript-level evidence in enabling accurate molecular reporting and guiding targeted therapy.

Keywords: *MET* exon 14 skipping, Non-canonical splice-site variants, Transcript-level analysis, RNA sequencing (RNA-Seq), Lung adenocarcinoma, Precision oncology

INTRODUCTION

MET exon 14 (*MET*ex14) skipping is a recurrent, independent, targetable oncogenic driver in approximately 3-4% of cases of non-small cell lung cancer (NSCLC), enriched in lung adenocarcinoma and pulmonary sarcomatoid carcinoma (1-3). It tends to occur in older individuals, particularly those with a history of tobacco exposure.

At the cellular level, loss of exon 14 disrupts the juxtamembrane domain of the *MET* receptor, impairing CBL-mediated ubiquitination and leading to sustained *MET* signaling through downstream pathways including MAPK and PI3K-AKT that contributes to aggressive tumor behaviour (4).

Clinically, this molecular phenotype shows limited responsiveness to conventional chemotherapy and immunotherapy, while showing marked sensitivity to *MET*-selective tyrosine kinase inhibitors (TKIs) such as tepotinib and capmatinib (5,6). Hence, it emphasizes the crucial need for their accurate molecular identification.

Genomically, *MET*ex14 can arise from a heterogeneous spectrum of alterations affecting splice regulatory regions flanking exon 14, such as changes within intronic splice acceptor site or donor site, polypyrimidine tract, branch points, or adjacent regulatory motifs (7,8). Despite advances in combined DNA-RNA based next-generation sequencing approaches, non-canonical intronic variants are frequently classified as variants of uncertain significance (VUS) due to limited functional evidence (9,10).

Here, we report two cases of advanced NSCLC harboring novel *MET* splice-site deletions within the exon 14 acceptor region, initially annotated as VUS. Transcript-level analysis using reverse transcription PCR confirmed *MET*ex14 skipping in both cases, enabling variant reclassification and directly informing targeted therapy. These find-

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ings support the routine incorporation of transcript-level assays for the evaluation of *MET* splice-site variants and highlight the need for systematic integration of functionally validated variants into clinical databases to improve molecular interpretation and therapeutic decision making.

CASE REPORT

Two patients with advanced NSCLC underwent comprehensive molecular evaluation following initial standard of care workup.

Case 1

A 71-year-old woman presented with metastatic lung adenocarcinoma involving the left upper lobe with mediastinal and osseous metastasis. Tumor cells were positive for thyroid transcription factor-1 (TTF-1), with wild-type EGFR status (PCR), ALK (IHC) negativity, and PD-L1 tumor proportion score (TPS) of 90%. The patient received one cycle of gemcitabine-carboplatin chemotherapy and remained symptomatic but clinically stable.

Comprehensive molecular profiling using a customized NGS lung cancer panel performed on formalin-fixed paraffin-embedded (FFPE) tumor tissue identified a novel intronic MET splice-site deletion (NM_001127500.3: c.2942-18_2942-4delCTTTCTCTCTGTTTT;VAF 93.78%) within the exon 14 acceptor region, initially classified as a VUS. Matched RNA sequencing demonstrated exon 13–15 junction reads (total mapped reads: 37,066; 132372 reads per million), consistent with *MET*ex14 skipping (Figure 1A). No additional actionable genomic alterations were detected. Functional validation by reverse transcription PCR (RT-PCR) performed on cDNA synthesized from freshly extracted RNA using primers flanking exons 13-15, yielded a shorter 140-bp amplicon (Figure 1B), confirming *MET*ex14 skipping alteration leading to reclassification of the variant as a Tier II variant of potential clinical significance according to AMP/ASCO/CAP guidelines (Table I).

In accordance with current guidelines and evidence from the VISION trial, the patient was initiated on oral tepotinib (225 mg twice daily). At early follow-up, the patient was

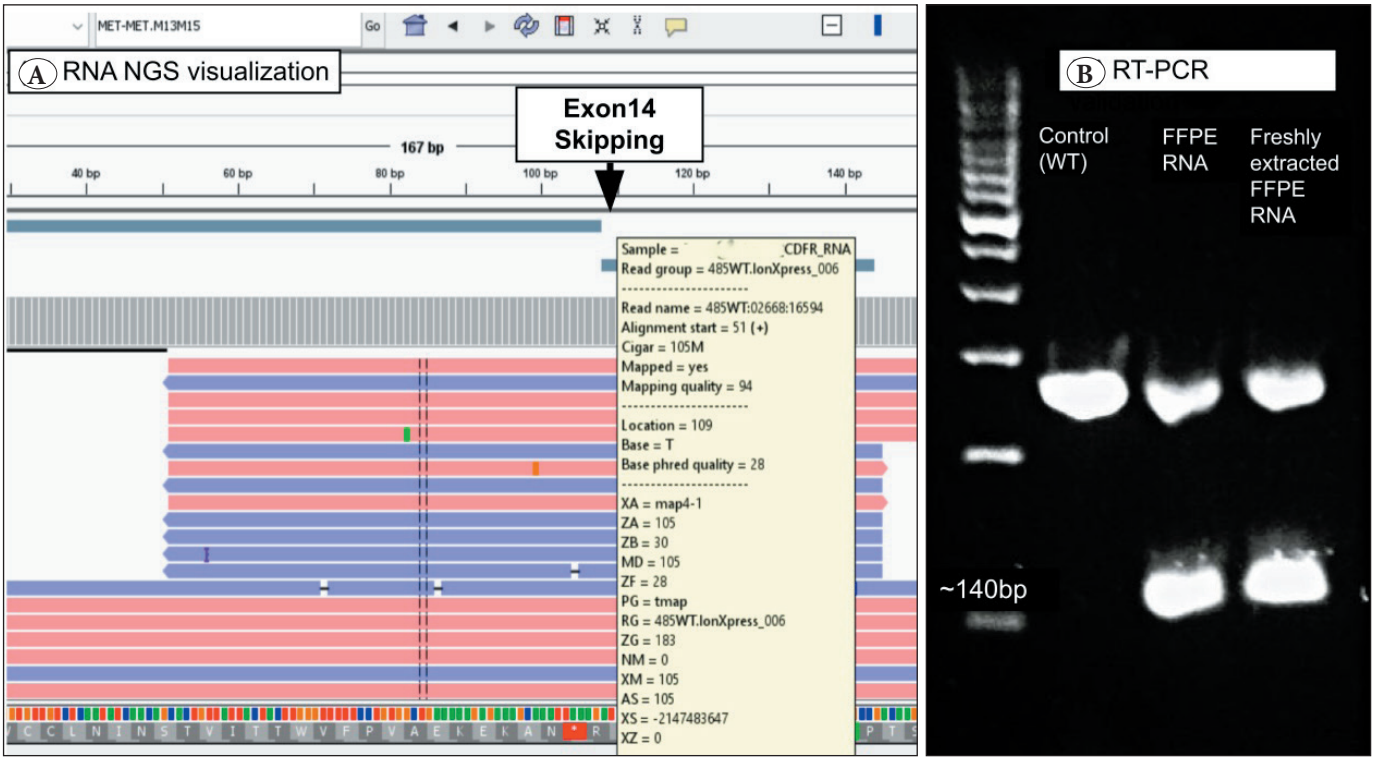

Figure 1: Integrated RNA-based validation of MET exon 14 skipping in non-canonical splice-site variants.
A) RNA sequencing visualization demonstrating split reads spanning exon 13 to exon 15, consistent with METEx14 skipping alteration. **B)** Reverse Transcription PCR (RT-PCR) using primer flanking exons 13-15 demonstrates a shorter amplicon (~140bp) corresponding to METex14 skipping in the tumor sample compared with the wild-type control. Lane M: Molecular weight marker; WT: wild-type control; FFPE RNA: Tumor sample; Freshly extracted FFPE RNA: Repeat RNA extraction from the same FFPE tissue block to exclude contamination.
Representative data from one case is shown; similar findings were observed in both cases.

Table I: Final Interpretation: Likely pathogenic, clinically actionable MET exon 14 skipping alteration (AMP/ASCO/CAP Tier II)

Evidence Type	Observation in Present Cases	Interpretation
Functional evidence	RNA NGS + RT-PCR confirmed MET exon 14 skipping	Strong (PS3)
Variant location	Intronic splice acceptor region (intron 13)	Supporting (PVS1_supporting)
Population data	Not reported in population databases	Supporting (PM2)
Clinical context	NSCLC with known METex14 phenotype	Supporting (PP4)

clinically stable with mild gastrointestinal toxicity and continued tepotinib with supportive care.

Case 2

A 70-year-old man with metastatic pulmonary sarcomatoid carcinoma presented with a left upper lobe mass and skeletal metastases. Tumor profiling showed PDL1 TPS of 100%, with negative EGFR (PCR) and ALK (IHC) testing. The patient was started on weekly paclitaxel-carboplatin with symptomatic improvement.

Subsequent DNA–RNA NGS using the same customized panel identified a novel *MET* splice-site deletion (NM_001127500.3: c.2942-19_2942-10delTCTTTCTCTC; VAF 20.87%) in the exon 14 acceptor region, along with a truncating *TP53* frameshift mutation (p. Cys229Tyrfs*10; VAF 24.72%). Similar to case 1, matched RNA sequencing demonstrated exon 13–15 junction reads (total mapped reads: 50,179; 92,125.5 reads per million), consistent with *MET*ex14 skipping, which was orthogonally confirmed by RT-PCR. The variant was reclassified as a clinically actionable *MET*ex14 alteration (Table I), prompting transition to oral tepotinib (225 mg twice daily) with symptomatic improvement at early follow-up.

To summarise, both cases with noncanonical intronic *MET* splice-site variants initially classified as VUS on DNA-based analysis were functionally validated by RNA sequencing and RT-PCR, enabling reclassification and directly guiding targeted therapy decisions.

DISCUSSION

These findings demonstrate that noncanonical *MET* splice-site variants involving the exon 14 acceptor region, initially classified as VUS on DNA-based analysis, can represent functionally relevant *MET*ex14 skipping alteration when supported by transcript level evidence, directly enabling targeted therapy (10,11). Both patients exhibited clinicopathological features typical of *MET*ex14 NSCLC, including older age, advanced metastatic disease, and high PD-L1 expression, and achieved early clinical stability following initiation of tepotinib after limited exposure to cytotoxic chemotherapy (5,6).

Biological and Molecular Context

*MET*ex14 skipping results in loss of juxtamembrane domain of the *MET* receptor, impairing CBL-mediated ubiquitination, prolonging receptor stability, and leading to sustained downstream signaling through pathways such as RAS-RAF-MEK-ERK and PI3K-AKT (4). Genomically, these alterations arise from a heterogeneous spectrum of variants, including substitutions, small insertions/deletions, and complex rearrangements affecting splice regulatory regions flanking exon 14 (7,8).

Large sequencing datasets have shown enrichment of such variants within the intron 13 splice acceptor region (7), consistent with deletions identified in this study, which were not previously catalogued in curated databases and therefore initially classified as VUS.

Role of RNA-Based Confirmation: NGS and RT-PCR

A key diagnostic challenge highlighted by these cases is the limitations of DNA-only NGS approaches in interpreting noncanonical splice-site variants. Such alterations are frequently intronic, variably covered, and not reliably interpreted by standard bioinformatic pipelines, leading to under-recognition of clinically actionable events (9,11). In contrast, RNA-based assays overcome this limitation by directly detecting exon 14-skipping transcripts, providing definitive functional evidence (10,12). In both cases, RNA sequencing demonstrated high-confidence exon 13-15 junction reads, confirming the functional consequence of the identified variants.

Importantly, orthogonal validation using RT-PCR provided an additional rapid and cost-effective method to confirm exon 14 exclusion. This is particularly relevant in settings where RNA NGS is unavailable or inconclusive and supports the incorporation of RT-PCR as a practical adjunct in molecular diagnostic workflow (13). Together, these findings support the use of transcript-level evidence

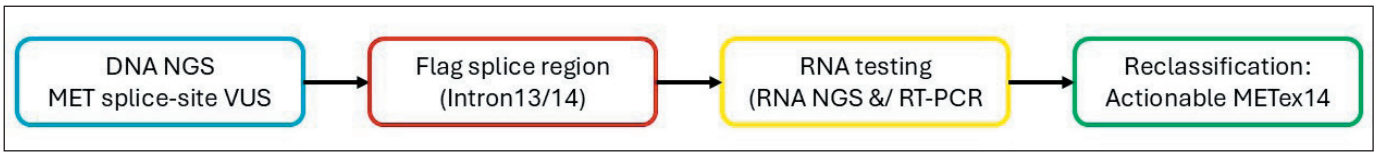

Figure 2: Proposed diagnostic workflow for evaluation of MET splice-site variants.

as strong functional data (PS3) for variant interpretation and reclassification.

Clinical Implications and Therapeutic Relevance

From a clinical perspective, accurate identification of *MET*ex14 alterations has direct therapeutic implications. *MET*ex14-positive NSCLC is associated with inferior outcomes when treated with chemotherapy or immunotherapy, even in the context of high PD-L1 expression (6,14). In contrast, *MET*-targeted tyrosine kinase inhibitors have demonstrated robust and durable activity. Prospective trials have reported meaningful response rates, prolonged progression-free survival, and manageable toxicity with agents such as tepotinib and capmatinib, supporting prioritization of *MET*-targeted therapy once *MET*ex14 is confirmed (5,6).

In the present cases, integrated RNA-based enabled reclassification of ambiguous splice-site VUS as likely pathogenic Tier II *MET*ex14 alterations, prompting a timely transition from chemotherapy to tepotinib. Early clinical stability in both patients is consistent with outcomes reported in prospective trials and reinforces the clinical utility of transcript-level confirmation (5,11,12).

Proposed Diagnostic Workflow for *MET* Splice-Site Variants (Figure 2)

Based on our findings and existing literature, we propose a practical diagnostic framework for the evaluation of *MET* splice-site variants identified on DNA-based NGS:

1. **Variant Localization:** Intronic variants located within the exon 14 flanking regions (particularly intron 13 splice acceptor and intron 14 splice donor sites) should be flagged for potential splicing impact, even when noncanonical.

2. **Initial Classification**: Variants should not be dismissed as VUS solely based on lack of database annotation if they occur within known splice regulatory regions.

3. **Splice Impact Assessment**: In silico splice prediction tools may be used as supportive evidence but should not be solely relied upon for classification.

4. **RNA-Based Validation**: RNA sequencing should be performed to directly assess exon 14 skipping by identifying exon 13–15 junction transcripts.

5. **Orthogonal Confirmation**: In resource-limited settings or where RNA NGS is inconclusive, RT-PCR using primers flanking exons 13–15 provides a cost-effective and reliable alternative for detecting exon 14 exclusion.

6. **Variant Reclassification**: Detection of *MET*ex14 skipping at the transcript level should be considered strong functional evidence (PS3) supporting reclassification under AMP/ASCO/CAP or ACMG frameworks.

7. **Clinical Reporting**: Reports should integrate DNA and RNA findings, explicitly stating when transcript-level evidence supports classification of a previously reported VUS as a clinically actionable *MET*ex14 alteration.

8. **Therapeutic Implication**: Confirmed *MET*ex14 alterations should prompt consideration of *MET*-targeted therapies in accordance with current clinical guidelines.

Limitations of this study include short follow-up duration, precluding assessment of long-term outcomes. However, the primary objective was to demonstrate a reproducible diagnostic and interpretative framework for evaluation of noncanonical *MET* splice-site variants. The concordance between RNA sequencing and RT-PCR strengthens the validity of the proposed approach with RT-PCR serving as an additional low-cost orthogonal validation, strengthening confidence in accurate variant classification and reporting.

CONCLUSION

Noncanonical *MET* splice-site variants involving the exon 14 acceptor region, often classified as variants of uncertain significance on DNA-based testing, can present clinically actionable *MET*ex14 skipping alterations when supported by transcript level evidence. Integration of RNA-based assays, including RNA sequencing and cost-effective RT-PCR, enables functional validation, accurate variant reclassification, and directly informs targeted therapy selection.

These findings highlight a broader diagnostic gap in molecular oncology and support routine incorporation of transcript-level evaluation into molecular diagnostic workflows for *MET* splice site variants to improve detection of actionable *MET* alterations.

Systematic curation of such functionally validated alterations in genomic databases such as ClinVar and COSMIC

is essential to improve future variant interpretation, reduce VUS burden, and facilitate timely precision oncology care in NSCLC.

Conflict of Interest
The authors declare no conflict of interest.

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Authorship Contributions
Concept: **AM, RS,** Design: **AM, RS,** Data collection or processing: **SP, MP, SG,** Analysis or Interpretation: **AM, RS, SP,** Literature search: **RS,** Writing: **RS,** Approval: **AM.**

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