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MET Exon 14 Variants in Non-Small Cell Lung Carcinoma: Prevalence, Clinicopathologic and Molecular Features

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Abstract: Somatic MET exon 14 skipping mutations (MET ex14) are targetable driver mutations for non-small cell lung cancer (NSCLC), responsive to MET inhibitors. Objective: This study seeks to further characterize the clinicopathologic features and mutational profile of MET ex14 variant NSCLC. Design: Retrospective review of all MET ex14 tested NSCLC. Testing for selected BRAF, EGFR, HER2, KRAS, and MET mutations was performed using a clinically validated NGS assay, followed by MiSeq sequencing. Variants were classified as significant (Tier1/2) or variants of uncertain significance (VUS) per 2017 AMP/ASCO/CAP Joint Consensus Guidelines. PD-L1 expression was assessed by immunohistochemistry. Results: Of 2296 NSCLCs tested between 2017-7/2019, MET ex14 variants were present in 44 (1.9%). A total of 32 of 44 variants were MET exon 14 skipping, while the other 12 mutations were significant missense (3) or VUS (9). Of nine VUS, five were adjacent to the canonical splice site and likely to impact splicing. Four cases had concomitant mutations. Of 35 cases with known clinical staging, stage 1–2 = 20 (57%), stage 3 = 3 (9%), and stage 4 = 12 (34%). Of 19 resected NSCLSs, histological types and growth pattern included 7 lepidic pattern-predominant. A high percentage of tumors with MET ex14 mutations are positive for PD-L1, and the percentage of cases with PD-L1 expression >50% trends higher in more advanced disease. Conclusions: Most MET variants identified in our cohort (73%) are MET ex14 skipping. The prevalence of MET ex14 variants is 1.9%, and a large percentage of tumors has lower clinical stage and less aggressive pathologic features.

Keywords: MET; exon 14; variants; non-small cell lung carcinoma; NSCLC; prevalence; clinicopathologic features; molecular features

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1. Introduction

The mesenchymal-to-epithelial transition (*MET*) proto-oncogene encodes a tyrosine kinase receptor that controls cell growth and migration and plays an important role in tumor invasion and metastasis [1]. *MET* exon 14 encodes the juxta membrane region of the receptor which is known to modulate receptor downregulation via Cbl binding and ubiquitin-mediated degradation; mutations to *MET* exon 14 exert their effect through altered splicing that results in exon 14 skipping [2]. *MET* exon 14 skipping leads to loss of a phosphorylation site required for Cbl binding, reducing ubiquitin-mediated degradation and promoting oncogenesis [3]. Splice site mutations in exon 14 of *MET* are reported to occur in 3% of lung adenocarcinomas and 7.7–32% of sarcomatoid carcinomas [4–6]. *MET* amplification can also lead to *MET* activation in tumors and has been reported to occur up to 5.6% of non-small cell lung cancer (NSCLC) [6,7]. Similar to other driver oncogenes such as epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*), that can be targeted with small molecule tyrosine kinase inhibitors (TKIs); driver alterations in the *MET* gene have also been identified as a target in NSCLC. Currently, FDA has approved Capmatinib and Tepotinib for use in patients with NSCLC harboring *MET* exon 14 skipping mutations [8].

With the advent of improved sequencing technologies, routine detection of *MET* exon 14 mutations has become more feasible. This study seeks to further characterize

the clinicopathologic features and mutational profile of cases with *MET* ex14 variants in patients with NSCLC. As the development of acquired resistance to targeted therapy is very common in NSCLC patients with actionable driver alterations, immunotherapy also plays an important role in the management of these patients. We thus further analyzed the percentage of tumors with *MET* ex14 mutations that are positive for program death ligand 1 (PD-L1), and the percentage of cases with PD-L1 expression >50% in more advanced disease.

2. Subjects and Methods

A retrospective review of all NSCLC cases at the Cleveland Clinic tested for *MET* ex14 skipping mutations was conducted. For *MET* exon 14 testing, genomic DNA was extracted from FFPE or FNA tumor specimens and tested for selected *BRAF, EGFR, HER2, KRAS,* and *MET* mutations using a clinically validated targeted next generation sequencing (NGS) assay. Testing utilized the customized Cancer Hotspot Panel v1 (cCHPv1, Thermo Fisher, Waltham, MA, USA) amplification-based library preparation customized to include MET exon 14 splice site variants, followed by sequencing on the MiSeq NGS platform (Illumina, San Diego, CA, USA). A primary bioinformatics analysis was performed using MiSeq Real Time Analysis (RTA), and sequence alignment and variant calls were performed using NextGENe® software (Softgenetics®, State College, PA, USA). Coverage of *MET* included the following hot spots and immediately flanking sequences: codons 168, 375, 830, 991, 992, 1001–1010, 1094, 1099, 1100, 1106, 1112, 1230, 1235, 1253 and intronic loci at the intron 14 consensus donor splice site c.3028+1 and c.3028+2 (NM_000245.3). Variants were classified as significant (Tier1/2) or variants of uncertain significance (VUS) per 2017 AMP/ASCO/CAP Joint Consensus Guidelines[10]. *ALK, RET,* and *ROS1* fusions were detected in parallel by FISH.

Definitions of variant categories are as follows: (1) "splice": involve consensus splice site (−1, −2, +1, +2) or has been previously reported in the literature as exon 14 skipping; (2) "VUS, possible splice": occurs near the exon/intron junction (in the region of other known exon 14 skipping variants) but does not involve consensus splice site (−1, −2, +1, +2) and has not been previously reported in the literature as *MET* exon 14 skipping; (3) "missense, significant": has been previously reported as a significant somatic mutation in lung carcinoma but does not involve MET exon 14 splicing; (4) "VUS, missense": not expected to involve *MET* exon 14 splicing and has not been previously reported as a significant somatic mutation in NSCLC. PD-L1 expression was assessed by immunohistochemistry (IHC) using a mouse monoclonal PD-L1 antibody (22-c3, Dako, CA, USA). Clinicopathologic features for MET ex14 positive cases were evaluated by chart review.

The study was performed in accordance with the Institutional review board (IRB) of the Cleveland Clinic (IRB# 19-1245).

3. Results

Of the 2296 cases of NSCLC analyzed by DNA-based NGS between 2017-7/2019, *MET* ex14 variants were present in 44 cases (1.9%), of which 26 (59%) were males and 18 (41%) were females. The median age of positive cases was 76 years (±9.6, 59% men; 41% women). Specimen type was FNA in 46.7%. A total of 32 of 44 variants were *MET* exon 14 skipping mutations (previously reported and/or involve the canonical recognition site), while the other 12 mutations were significant missense (3) or VUS (9). Of nine VUS, five were adjacent to the canonical splice site and likely to impact splicing, while four were missense variants (Figure 1A).

The average variant allele fraction (VAF) was 33.6%. Four cases (9%) had concomitant mutations (3 = KRAS, 1 = EGFR) (Figure 1B). In the three tumors with *KRAS* concomitant mutations, *MET* mutations were present at a much lower VAF compared with *KRAS*. However, the tumor with *EGFR* concomitant mutation had *EGFR* mutation at only 3% allele frequency but *MET* mutation at 34% allele frequency (supplementary information). All cases tested for ROS, RET, and ALK by FISH were negative (20 of 44 were tested for

ROS, RET, and ALK, 19 were tested for ALK only, 1 was tested for RET only, and 1 was tested for RET and ROS).

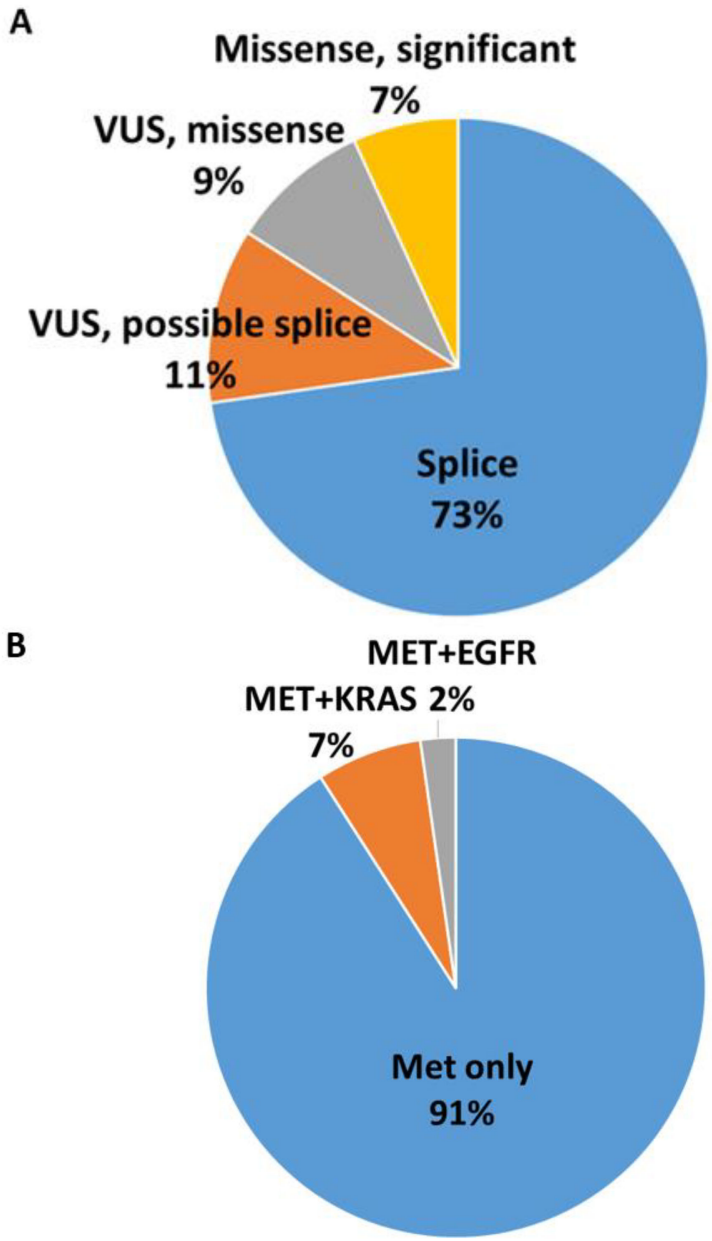

Figure 1. (**A**) MET ex14 variants present in 44 cases: 32 MET exon 14 skipping, 3 significant missense, 9 VUS. Of 9 VUS, 5 were adjacent to the canonical splice site and likely to impact splicing, and 4 were missense variants. (**B**) Four cases had concomitant mutations including 3 with KRAS concomitant mutations and 1 with EGFR concomitant mutations; none were classic exon 14 skipping.

Out of the 35 cases with known clinical staging, 20 cases (57%) were stage 1 or 2, 3 cases (9%) were stage 3, while 12 cases (34%) were stage 4. Of the 19 tumors that were resected, the histological types and growth pattern of NSCLC included 7 lepidic pattern-predominant, 6 acinar pattern-predominant, 2 micropapillary-predominant, 1 solid-predominant, 1 sarcomatoid, and 2 adenosquamous (Figure 2, representative cases with MET variants. Specifically, lepidic pattern, case #1; acinar pattern, case #3; micropapillary pattern, case #15; solid, case #29; sarcomatoid, case #10; and adenosquamous, case #13). PD-L1 expression was grouped into three categories, with 19% showing no expression, 41% each showing 1–49% expression and >50% expression. High PD-L1 expressing tumors

showed disproportionately high tumor stage, with 4 of 6 (67%) evaluable stage 4 tumors showing >50% expression compared with 6 of 18 (33%) stage 1–3 tumors. In contrast, although four of five lepidic-predominant tumors were positive for PD-L1, they were all at the level of 1–49%. The clinicopathologic correlation is summarized in Table 1. Additional details on MET variants and clinicopathologic features are shown in Table 2.

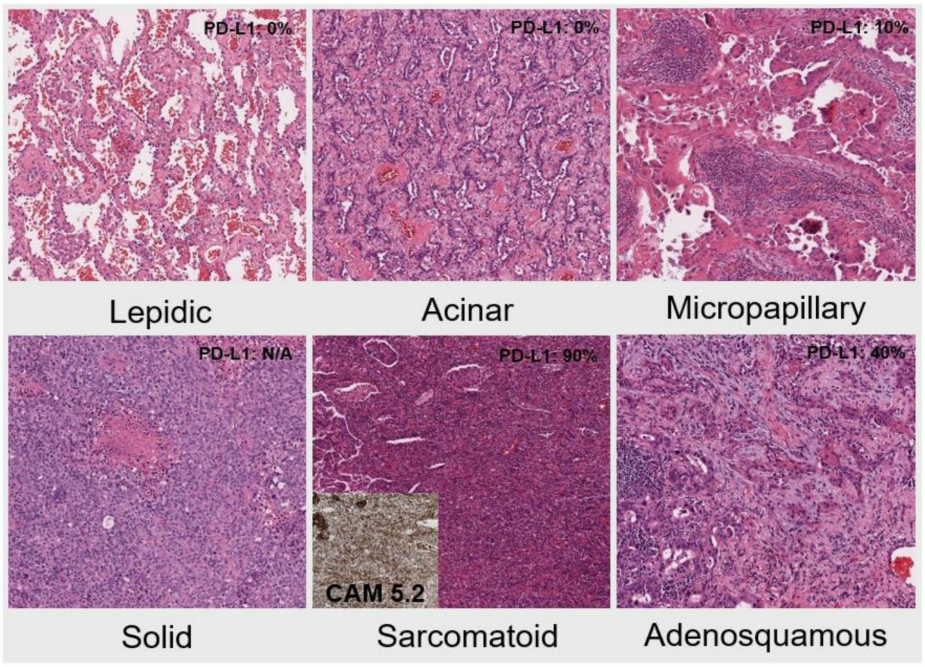

Figure 2. The histological types and growth pattern of NSCLC include lepidic pattern-predominant, acinar pattern-predominant, micropapillary-predominant, solid-predominant, sarcomatoid (inlet: CAM5.2 immunostain), and adenosquamous (IHC, ×100).

Table 1. Clinicopathologic correlation of tumors with METex14 mutations.

Prevalence of MET Exon 14 Mutations			1.9%			
Total patients	44 patients: 26 men, 18 women Mean age: 76 years					
Clinical stage available	Total 35 cases Stage 1 and 2: 20 cases (57%)		Stage 3: 3 cases (9%)		Stage 4: 12 cases (34%)	
Tumor resected	Total 19 cases Histologic type and growth pattern Lepidic pattern-predominant 7	Acinar pattern-predominant 6	Micropapillary-predominant 2	Solid-predominant 1	Adeno-squamous 2	Sarcomatoid 1
PD-L1 expression	Total 27 cases, positive in 22 cases (82%) 0% 5 cases (18%) Stage 1–3 PD-L1 < 50% 12 cases (67%)		1–49%: 11 cases (41%) PD-L1 > 50% 6 cases (33%)	 Stage 4 PD-L1 < 50% 2 cases (33%)	>50% 11 cases (41%) PD-L1 > 50% 4 cases (67%)	

Table 2. Additional details on MET variants and clinicopathologic features.

Case #	Result	AF	Significance	Type of MET Mutation	Exon14 Skipping	Other Mutations	Surgical Pathology Diagnosis on Resection
1	c.3028+2T>A	41	significant	splice	yes		Adenocarcinoma, lepidic predominant, with additional acinar component
2	c.3280C>T (p.His1094Tyr)	11	significant	missense	no	KRAS	
3	c.3028+2del	11	significant	splice	yes		Adenocarcinoma, acinar predominant (70%) with lepidic pattern (30%).
4	c.3028G>C (p.Asp1010His)	3	significant	splice	yes		
5	c.3023_3028+7delinsTC	91	significant	splice	yes		
6	c.3028+3A>G (p.?)	25	significant	splice	yes		
7	c.3028+1delG	71	significant	splice	yes		
8	c.3028G>C(p.Asp1010His)	76	significant	missense/splice	yes		
9	c.3028G>T (p.Asp1010Tyr)	71	significant	missense/splice	yes		
10	c.3028+1_3028+2delinsTC	30	significant	splice	yes		Sarcomatoid carcinoma, pleomorphic type with spindle cell and adenocarcinoma components
11	c.3025_3028+3delGAAGGTA (p.?)	66	significant	splice	yes		Adenocarcinoma, lepidic predominant
12	c.3320G>C(p.Cys1107Ser)	52	VUS	missense	no		
13	c.3028+3_3028+9delinsTTTTTTT (p.?)	34	VUS	splice?	no	EGFR	Adenosquamous carcinoma
14	c.3082+1delG (p.?)	44	significant	splice	yes		Adenocarcinoma, acinar predominant
15	c.3028+1G>C (p.?)	30	significant	splice	yes		Adenocarcinoma, micropapillary predominant (60%), with additional acinar (20%), solid (10%) and lepidic (10%) components
16	c.3747G>T, (p.Trp1249Cys)	21	VUS	missense	no		
17	c.3017_3028delCTTTTCCAGAAG (p.Thr1006_Asp1010delinsAsn)	29	significant	splice	yes		
18	c.3028+1G>C (splice)	20	significant	splice	yes		
19	c.3301G>A(p.Asp1101Asn)	4	VUS	missense	no		

Table 2. *Cont.*

Case #	Result	AF	Significance	Type of MET Mutation	Exon14 Skipping	Other Mutations	Surgical Pathology Diagnosis on Resection
20	c.3028+3A>T	24	VUS	splice?	no		Adenocarcinoma, lepidic predominant
21	c.3002_3027delTAGACTACCGAGCTACTTTTCCAGAA (p.Val1001Glyfs*5)	7	VUS	splice?	no		Adenocarcinoma (micropapillary 60%, acinar 40%, papillary 10%)
22	c.3028G>C(p.Asp1010His)	92	significant	missense/splice	yes		
23	c.3017_3028del (p.Thr1006_Asp1010delinsAsn)	14	VUS	splice?	no		Primary lung adenosquamous carcinoma
24	c.3752C>T (p.Ala1251Val)	5	VUS	missense	no	KRAS	
25	c.3028 + 3A>G	23	VUS	splice?	no		Adenocarcinoma, acinar predominant (80%) with lepidic (20%) pattern
26	c.3028G>T(p.Asp1010Tyr)	23	significant	missense/splice	yes		Adenocarcinoma, acinar-predominant
27	c.3028+1G>A (p.?)	3	significant	splice	yes		
28	c.3017_3028+2del	40	significant	splice	yes		
29	c.3028+2T>C (p.?)	11	significant	splice	yes		Adenocarcinoma, solid predominant (80%) with additional acinar pattern (20%) pattern.
30	c.3028G>A (p.Asp1010Asn)	15	significant	missense/splice	yes		
31	c.3028G>C (p.Asp1010His)	77	significant	missense/splice	yes		
32	c.3028+1G>C	73	significant	splice	yes		Adenocarcinoma, acinar predominant (60%), with additional solid (30%) and micropapillary (10%) patterns.
33	c.3028+2T>C	54	significant	splice	yes		
34	c.3028+1G>T	25	significant	splice	yes		Adenocarcinoma with predominant acinar pattern
35	c.3028+2T>C (p.?)	31	significant	splice	yes		Adenocarcinoma, lepidic predominant (55%) with acinar pattern (45%).
36	c.3028G>C (p.Asp1010His)	52	significant	missense/splice	yes		Adenocarcinoma, lepidic-predominant (70%) with acinar (20%) and solid (10%) patterns.

Table 2. *Cont.*

Case #	Result	AF	Significance	Type of MET Mutation	Exon14 Skipping	Other Mutations	Surgical Pathology Diagnosis on Resection
37	c.3028+1G>A	35	significant	splice	yes		
38	c.3028G>C (p.Asp1010His)	3	significant	missense/splice	yes		Adenocarcinoma, lepidic predominant (80%), with additional acinar pattern (20%).
39	c.3027_3028+6delAAGGTATAT	3	significant	splice	yes		
40	c.3281A>G (p.His1094Arg) and c.3340+1G>A (intron 16)	4	significant and VUS	missense	no and no	KRAS	
41	c.3007T>C(p.Tyr1003His)	6	significant	missense	no		
42	c.3028G>T(p.Asp1010Tyr)	73	significant	missense/splice	yes		
43	c.3028G>C	40	significant	splice	yes		Adenocarcinoma, lepidic predominant (60%), with additional acinar pattern (40%).
44	c.3028+2T>C	20	significant	splice	yes		

Light blue: cases with missense mutations. Light green: cases with missense/splice mutations. Orange: cases with possible splice mutations. Green: cases resected.

4. Discussion

Lung cancer continues to be the leading cause of cancer-related mortality among men and women worldwide [9], driving the need to discover new targetable genomic mutations. *MET* exon 14 skipping is a primary oncogenic driver sensitive to *MET* inhibition. In addition to existing FDA approved therapeutic options for *MET* exon 14 skipping positive tumors such as capmatinib, crizotinib, and tepotinib [1], several selective *MET* inhibitors are being investigated, including emibetuzumab and savolitinib, and are promising therapies with maximized efficacy and reduced off-target toxicity [10].

Previous studies have reported the prevalence of *MET* ex14 to be approximately 3.0% in NSCLC with highest prevalence in the pulmonary sarcomatoid subtype [3]. While Awad et al. [4] and Schrock et al. [5] reported a prevalence of 3% and 2.7% of *MET* ex14 variants, in studies conducted by Zheng et al. [11] and Liu et al. [12], the prevalence was observed to be 1.3% and 1%, respectively, possibly reflecting a lower incidence in Asians. In our cohort, *MET* ex14 variants were observed in 1.9% cases (44/2296). Our lower observed prevalence may be due in part to universal testing of NSCLC at our institution rather than testing of only advanced disease. Additionally, although our *MET* test is expected to detect most relevant exon 14 skipping variants, our lower reported prevalence may be partially attributable to variants lying outside the test's covered regions and/or alterations not efficiently detected by next generation sequencing methodology such as large deletions.

Recent studies by Poirot et al. and Davies et al. have demonstrated the superiority of RNA-based assays in detecting MET exon 14 skipping mutations compared with DNA-based assays [13,14]. In both these studies, DNA-based assays were able to identify only approximately 60% of the mutations as any variant outside the amplified area or preventing binding due to mutation of the primer would not be detected. In contrast, in RNA-based assays, any variant causing skipping of exon 14 in vivo is identified as fusion on exon 13 to exon 15. In general, RNA-based targeted approach analyses and quantifies directly fusion transcripts and is more accurate than DNA panels on tumor tissue, but it can be limited by RNA quality and quantity. However, amplicon-based RNA NGS panels can detect gene fusions in poor quality RNA samples, such as those obtained from FFPE tissue samples (PMID: 32726941). This is a major limitation of our study, as adding RNA-based NGS assays or using combined DNA-RNA testing might have allowed us to better classify the samples with non-canonical DNA mutations, including missense and VUS variants in this cohort.

In contrast with other targetable mutations seen in NSCLC such as *ALK* and *ROS1* rearrangements and *KRAS, EGFR,* and *BRAF* mutations, *MET* ex14 mutations have been identified in older patients. In a review of 933 patients with NSCLC, Awad et al. identified *MET* exon 14 skipping mutations in 28 patients with a median age of 72.5 years [4]. Whereas 68% and 60.4% patients with *MET* ex14 variants were females in the study conducted by Awad et al. [4] and Shrock et al. [5], respectively, in a study conducted by Wang et al., 66% patients were males [15]. In our cohort, the mean age of the patients was 76 years (53 to 91 years) which is consistent with previous studies. However, there is male preference is our study, with 59% (26) males and 41% (18) females.

MET ex14 mutations have been observed across various histological subtypes of non-small cell lung carcinoma, with a decreasing order of prevalence seen in sarcomatoid carcinoma, adenosquamous carcinoma, adenocarcinoma, and squamous cell carcinoma [4,16]. In our study, of the 19 patients who underwent resection, 16 patients had adenocarcinoma (including five tumors with lepidic-predominant pattern), 2 adenosquamous carcinoma, and 1 sarcomatoid carcinoma. As primary lung tumors, adenosquamous carcinoma and sarcomatoid carcinoma are relatively rare, comprising 0.3–1.3% and 0.4–4% of all lung carcinomas, respectively [17,18]. Our data are consistent with the previous studies showing that MET ex14 appears enriched in these two aggressive histologic subtypes [17,18]. However, a large percentage of tumors also had lower clinical stage and less aggressive pathologic features, both possibly reflecting sampling differences attributed to universal

testing of NSCLC at our institution rather than testing of only advanced diseases was common in early studies due to the emphasis on targeted therapies for advanced disease. This seemingly paradoxical observation is consistent with studies from other centers where *MET* ex14 variants testing is performed routinely for all cases of NSCLC [4].

The spectrum of known *MET* exon 14 skipping mutations must be considered when designing diagnostic tests for their detection. In literature, hundreds of distinct genetic alterations leading to *MET* exon 14 skipping have been reported, including base substitutions and insertions or deletions at the splice acceptor site, at the splice donor site, and in intronic non-coding regions immediately adjacent to the splice acceptor site, as well as whole exon deletions [19]. In our cohort, 32 of the 44 (73%) *MET* ex14 mutations were previously reported and/or canonical recognition sites skipping variants. Nine patients had VUS, five of which were adjacent to a canonical splice site and were likely to impact splicing and four were missense variants. Three patients had significant missense mutations. To detect these potential alterations, it is important to sequence both exon 14 and its surrounding regions, although a plurality of *MET* exon 14 skipping events occur at the donor site [20].

MET exon 14 skipping mutations are usually mutually exclusive of other characteristic driver mutations in NSCLC. Whereas the cancer genome atlas research network [21] and Awad et al. [4] identified no concomitant mutation with *MET* ex14 skipping mutation, in the study conducted by Shrock et al., concurrent *KRAS* mutation and *EGFR* amplification was observed in 3% and 6.4% cases, respectively [5]. In our cohort, four cases (9%) had concomitant mutations with three harboring KRAS mutations and one with *EGFR* mutation. However, none of the co-mutations occurred with a known functional pathogenic *MET* ex14 skipping mutation. The *EGFR* mutation was co-mutated with a VUS possible splice of *MET* ex14. The *KRAS* mutations were co-mutated with a significant missense mutation of *MET*e x14, a VUS missense of *MET* ex14, and a VUS missense mutation together with a possible splice site mutation of *MET* exon 16, respectively. Further investigations of these cases would be required to confidently establish true functionally co-mutated status. Moreover, the testing methodology used does not distinguish whether the mutations are part of the same clone (co-mutational status as a subclone) or reflect tumoral heterogeneity.

The development of monoclonal antibodies against PD-1 receptor and its ligand, PD-L1 has provided a major breakthrough in the management of patients with NSCLC in the last decade. In a review of 147 patients with *MET* exon 14 skipping mutations, PD-L1 expression of > 1% was seen in 57 0f 111 patients, although the overall survival did not improve in patients who were administered PD-L1 immunotherapy [22]. In a similar study conducted by Xu et al., 205 of 401 patients with NSCLC and *MET* exon14 skipping had PD-L1 expression of > 1% [23]. In our cohort, 27 patients had tissue available for evaluation of PD-L1 expression. PD-L1 expression of 0%, 1–49%, and >50% was observed in 19% (5/27), 41% (11/27), and 41% (11/27) of patients, respectively, which is higher compared with previous studies (approximately 80% versus 50%). Patients with stage 4 tumors showed a significantly higher percentage of PD-L1 expression of >50% (67%, 4/6) compared with stage 1–3 tumors (33%, 6/18). In contrast, although four of five lepidic-predominant tumors were positive for PD-L1, they were all at the level of 1–49%.

Engagement of programmed cell death-1 (PD-1) receptor by its ligands PD-L1 is an important adaptor immune mechanism by tumor cells. This leads to downregulation of T-cells in the tumor microenvironment which can be reversed by PD1 and PD-L1 blocking antibodies such as pembrolizumab, nivolumab, atezolizumab, and durvalumab [24]. These have shown promise in the treatment of patients with non-small cell lung carcinoma with response rates of over 20% in treatment-naïve patients [25]. Testing for PD-L1 expression remains current standard in patients with NSCLC who are likely to respond to immunotherapy. It has been seen that in patients with higher PD-L1 expression have better response to immunotherapy than patients with lower PD-L1 expression [26,27]. Presently, IHC remains the gold standard for quantifying PD-L1 expression in tumor samples [28]. Although these findings have potential therapeutic implications, NCCN recognizes that "Patients with MET ex14 skipping mutations have a modest response (16%, single-agent

ICIs) to immunotherapy, even those with high PD-L1 levels." (NCCN NSCLC version 1.2023, MS-21). Additionally, the guidelines support that any patient with a targetable oncogenic driver should receive targeted therapy before immunotherapy. Larger studies are required to clarify the PD-L1 status in patients with *MET* exon 14 skipping mutations and the potential contribution of immunotherapies in these patients, especially in patients with high-stage diseases.

In conclusion, roughly three quarters of *MET* variants identified in our cohort are *MET* ex14 skipping, another one tenth likely result in exon 14 skipping, while the other 16% are missense variants presumably unrelated to splicing. Our prevalence of *MET* ex14 variants is 1.9%, and a large percentage of tumors has lower clinical stage and less aggressive pathologic features. Notably, a high percentage of tumors with *MET* ex14 mutations are positive for PD-L1, and the percentage of cases with PD-L1 expression >50% trends higher in more advanced stage disease. It is unclear whether certain *MET* exon 14 mutations are more responsive to c-Met inhibition than others and more prospective clinical trials will be necessary to determine if immunotherapy or combination strategies (i.e., immunotherapy and chemotherapy) in addition to targeted therapy in this population will provide survival advantage. The presence of existing and potential targeted therapies for *MET* inhibition highlight the importance of including testing for *MET* in the molecular evaluation of NSCLC.

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Informed Consent Statement: No patient involved.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Fujino, T.; Suda, K.; Mitsudomi, T. Lung Cancer with MET exon 14 Skipping Mutation: Genetic Feature, Current Treatments, and Future Challenges. *Lung Cancer: Targets Ther.* **2021**, *12*, 35–50. [CrossRef] [PubMed]
2. Huang, C.; Zou, Q.; Liu, H.; Qiu, B.; Li, Q.; Lin, Y.; Liang, Y. Management of Non-small Cell Lung Cancer Patients with MET Exon 14 Skipping Mutations. *Curr. Treat. Options Oncol.* **2020**, *21*, 33. [CrossRef] [PubMed]
3. Vuong, H.G.; Ho, A.T.N.; Altibi, A.M.; Nakazawa, T.; Katoh, R.; Kondo, T. Clinicopathological implications of MET exon 14 mutations in non-small cell lung cancer—A systematic review and meta-analysis. *Lung Cancer* **2018**, *123*, 76–82. [CrossRef] [PubMed]
4. Awad, M.M.; Oxnard, G.R.; Jackman, D.M.; Savukoski, D.O.; Hall, D.; Shivdasani, P.; Heng, J.C.; Dahlberg, S.E.; Jänne, P.A.; Verma, S.; 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. [CrossRef]
5. Schrock, A.B.; Frampton, G.M.; Suh, J.; Chalmers, Z.R.; Rosenzweig, M.; Erlich, R.L.; Halmos, B.; Goldman, J.; Forde, P.; Leuenberger, K.; et al. Characterization of 298 Patients with Lung Cancer Harboring MET Exon 14 Skipping Alterations. *J. Thorac. Oncol.* **2016**, *11*, 1493–1502. [CrossRef]
6. Tong, J.H.; Yeung, S.F.; Chan, A.W.H.; Chung, L.Y.; Chau, S.L.; Lung, R.W.M.; Tong, C.Y.; Chow, C.; Tin, E.K.Y.; Yu, Y.H.; 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. [CrossRef]
7. Ou, S.-H.I.; Kwak, E.L.; Siwak-Tapp, C.; Dy, J.; Bergethon, K.; Clark, J.W.; Camidge, D.R.; Solomon, B.J.; Maki, R.G.; Bang, Y.-J.; et al. Activity of Crizotinib (PF02341066), a Dual Mesenchymal-Epithelial Transition (MET) and Anaplastic Lymphoma Kinase (ALK) Inhibitor, in a Non-small Cell Lung Cancer Patient with De Novo MET Amplification. *J. Thorac. Oncol.* **2011**, *6*, 942–946. [CrossRef]
8. Jørgensen, J.T.; Mollerup, J. Companion Diagnostics and Predictive Biomarkers for MET-Targeted Therapy in NSCLC. *Cancers* **2022**, *14*, 2150. [CrossRef]

9. Lung Cancer Statistics. Available online: https://www.cancer.org/cancer/lung-cancer/about/key-statistics.html (accessed on 14 February 2021).
10. Salgia, R.; Sattler, M.; Scheele, J.; Stroh, C.; Felip, E. The promise of selective MET inhibitors in non-small cell lung cancer with MET exon 14 skipping. *Cancer Treat. Rev.* **2020**, *87*, 102022. [CrossRef]
11. Zheng, D.; Wang, R.; Ye, T.; Yu, S.; Hu, H.; Shen, X.; Li, Y.; Ji, H.; Sun, Y.; Chen, H. MET exon 14 skipping defines a unique molecular class of non-small cell lung cancer. *Oncotarget* **2016**, *7*, 41691–41702. [CrossRef]
12. Liu, S.-Y.; Gou, L.-Y.; Li, A.-N.; Lou, N.-N.; Gao, H.-F.; Su, J.; Yang, J.-J.; Zhang, X.-C.; Shao, Y.; Dong, Z.-Y.; et al. The Unique Characteristics of MET Exon 14 Mutation in Chinese Patients with NSCLC. *J. Thorac. Oncol.* **2016**, *11*, 1503–1510. [CrossRef]
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. [CrossRef] [PubMed]
14. Davies, K.D.; Lomboy, A.; Lawrence, C.A.; Yourshaw, M.; Bocsi, G.T.; Camidge, D.R.; Aisner, D.L. DNA-Based versus RNA-Based Detection of MET Exon 14 Skipping Events in Lung Cancer. *J. Thorac. Oncol.* **2019**, *14*, 737–741. [CrossRef]
15. Wang, S.X.; Zhang, B.M.; Wakelee, H.A.; Koontz, M.Z.; Pan, M.; Diehn, M.; Kunder, C.A.; Neal, J.W. Case series of MET exon 14 skipping mutation-positive non-small-cell lung cancers with response to crizotinib and cabozantinib. *Anti-Cancer Drugs* **2019**, *30*, 537–541. [CrossRef]
16. Saffroy, R.; Fallet, V.; Girard, N.; Mazieres, J.; Sibilot, D.M.; Lantuejoul, S.; Rouquette, I.; Thivolet-Bejui, F.; Vieira, T.; Antoine, M.; et al. *MET* exon 14 mutations as targets in routine molecular analysis of primary sarcomatoid carcinoma of the lung. *Oncotarget* **2017**, *8*, 42428–42437. [CrossRef] [PubMed]
17. Filosso, P.L.; Ruffini, E.; Asioli, S.; Giobbe, R.; Macri, L.; Bruna, M.C.; Sandri, A.; Oliaro, A. Adenosquamous lung carcinomas: A histologic subtype with poor prognosis. *Lung Cancer* **2011**, *74*, 25–29. [CrossRef] [PubMed]
18. Maneenil, K.; Xue, Z.; Liu, M.; Boland, J.; Wu, F.; Stoddard, S.M.; Molina, J.; Yang, P. Sarcomatoid Carcinoma of the Lung: The Mayo Clinic Experience in 127 Patients. *Clin. Lung Cancer* **2018**, *19*, e323–e333. [CrossRef]
19. Socinski, M.A.; Pennell, N.A.; Davies, K.D. *MET*Exon 14 Skipping Mutations in Non–Small-Cell Lung Cancer: An Overview of Biology, Clinical Outcomes, and Testing Considerations. *JCO Precis. Oncol.* **2021**, *5*, 653–663. [CrossRef]
20. Awad, M.M.; Lee, J.K.; Madison, R.; Classon, A.; Kmak, J.; Frampton, G.M.; Alexander, B.M.; Venstrom, J.; Schrock, A.B. Characterization of 1,387 NSCLCs with MET exon 14 (METex14) skipping alterations (SA) and potential acquired resistance (AR) mechanisms. *J. Clin. Oncol.* **2020**, *38*, 9511. [CrossRef]
21. Cohen, D.; Hondelink, L.M.; Solleveld-Westerink, N.; Uljee, S.M.; Ruano, D.; Cleton-Jansen, A.-M.; von der Thüsen, J.H.; Ramai, S.R.S.; Postmus, P.E.; van Roggen, J.F.G.; et al. Optimizing Mutation and Fusion Detection in NSCLC by Sequential DNA and RNA Sequencing. *J. Thorac. Oncol.* **2020**, *15*, 1000–1014. [CrossRef]
22. Sabari, J.; Leonardi, G.; Shu, C.; Umeton, R.; Montecalvo, J.; Ni, A.; Chen, R.; Dienstag, J.; Mrad, C.; Bergagnini, I.; et al. PD-L1 expression, tumor mutational burden, and response to immunotherapy in patients with MET exon 14 altered lung cancers. *Ann. Oncol.* **2018**, *29*, 2085–2091. [CrossRef]
23. Xu, Z.; Li, H.; Dong, Y.; Cheng, P.; Luo, F.; Fu, S.; Gao, M.; Kong, L.; Che, N. Incidence and PD-L1 Expression of MET 14 Skipping in Chinese Population: A Non-Selective NSCLC Cohort Study Using RNA-Based Sequencing. *OncoTargets Ther.* **2020**, *13*, 6245–6253. [CrossRef] [PubMed]
24. Patel, S.A.; Weiss, J. Advances in the Treatment of Non–Small Cell Lung Cancer. *Clin. Chest Med.* **2020**, *41*, 237–247. [CrossRef] [PubMed]
25. Garon, E.B.; Hellmann, M.D.; Rizvi, N.A.; Carcereny, E.; Leighl, N.B.; Ahn, M.-J.; Eder, J.P.; Balmanoukian, A.S.; Aggarwal, C.; Horn, L.; et al. Five-Year Overall Survival for Patients With Advanced Non-Small-Cell Lung Cancer Treated With Pembrolizumab: Results From the Phase I KEYNOTE-001 Study. *J. Clin. Oncol.* **2019**, *37*, 2518–2527. [CrossRef] [PubMed]
26. Rittmeyer, A.; Barlesi, F.; Waterkamp, D.; Park, K.; Ciardiello, F.; von Pawel, J.; Gadgeel, S.M.; Hida, T.; Kowalski, D.M.; Dols, M.C.; et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): A phase 3, open-label, multicentre randomised controlled trial. *Lancet* **2017**, *389*, 255–265. [CrossRef]
27. Antonia, S.J.; Villegas, A.; Daniel, D.; Vicente, D.; Murakami, S.; Hui, R.; Kurata, T.; Chiappori, A.; Lee, K.H.; De Wit, M.; et al. Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC. *N. Engl. J. Med.* **2018**, *379*, 2342–2350. [CrossRef] [PubMed]
28. Imyanitov, E.N.; Iyevleva, A.G.; Levchenko, E.V. Molecular testing and targeted therapy for non-small cell lung cancer: Current status and perspectives. *Crit. Rev. Oncol.* **2021**, *157*, 103194. [CrossRef] [PubMed]