

Incidence and PD-L1 Expression of *MET* 14 Skipping in Chinese Population: A Non-Selective NSCLC Cohort Study Using RNA-Based Sequencing

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Background: Mesenchymal–epithelial transition (*MET*) exon14 skipping mutations represent a clinically unique molecular subtype of NSCLC. The prevalence rates of *MET* exon 14 skipping in lung adenocarcinoma (ADC) range from 0.9% to 4.0% in Asian populations. Since some somatic variants that do not encompass the *MET* exon 14 splice sites might also induce *MET* exon 14 skipping, the RNA-based sequencing is speculated as the most accurate method for detecting exon 14 skipping.

Patients and Methods: A total of 951 NSCLC patients from two hospitals were enrolled in this study. *MET* exon14 skipping was detected using RNA-based next-generation sequencing (NGS). Also, immunohistochemistry (IHC) was performed in 405 samples simultaneously.

Results: The overall estimated prevalence of *MET* exon 14 skipping was approximately 1.8% in ADCs and 1.7% in NSCLCs. The detection rate of *MET* exon 14 skipping from surgical resection specimen was 2.3% in NSCLCs and 2.0% in ADCs. The *MET* exon 14 skipping was identified in 6.6% of *EGFR/KRAS/ALK/ROS1/RET*-negative ADCs. Additionally, PD-L1 was found to be highly expressed in NSCLC patients harboring *MET* exon 14 skipping ($P < 0.01$).

Conclusion: The prevalence of *MET* exon14 skipping in lung ADCs in the East Asian population was similar to that of the Western population as assessed by RNA-based NGS. The NSCLC patients with *MET* exon 14 skipping were older than those with other oncogenic driver mutations, such as *EGFR*, *ALK*, and *ROS1*. In addition, PD-L1 was highly expressed in NSCLC patients with *MET* exon 14 skipping.

Keywords: non-small cell lung cancer, *MET* exon14 skipping, next-generation sequencing, PD-L1

Introduction

Currently, lung cancer is the leading cause of cancer-related deaths worldwide.¹ Non-small cell lung cancer (NSCLC) accounts for about 85% of all lung cancers; of these, ADC and squamous cell carcinoma (SCC) are the most frequent histological subtypes, accounting for 50% and 30%, respectively. In the past decade, remarkable progress has been made in the treatment of NSCLC in patients whose tumors harbor targetable somatic mutations.² Recently, the activating mutations in the *MET* gene have been recognized as potential therapeutic targets in NSCLC.^{3,4}

MET is a receptor tyrosine kinase that is activated upon binding to the hepatocyte growth factor (HGF) ligand, forming homodimers and subsequently activating the kinase domain. This process triggers the downstream signaling cascade that promotes proliferation, cell cycle progression, cell migration, and invasion.⁵ The

aberrant activation of *MET* triggers a constitutive activation of downstream signaling pathways, *PI3K/AKT/mTOR* and *RAS/ERK/MAPK*, involved in tumorigenesis and tumor development.^{6–8}

MET exon14 skipping as a therapeutically targetable mutation has been reported in lung cancer and comprises of 4.3% of lung ADCs in The Cancer Genome Atlas dataset.⁹ *MET* exon 14 skipping results in the deletion of the juxtamembrane domain that is essential for the efficient binding of E3 ubiquitin protein ligase (CBL). These alterations lead to increased *MET* stability and oncogenic potential and confer sensitivity to *MET* tyrosine kinase inhibitors (TKIs), such as crizotinib and cabozantinib.^{3,4,10}

The prevalence rates of *MET* exon 14 skipping in Asian populations with lung ADC are reported to be approximately 4.0% in Taiwan, 2.6% in Hong Kong, 0.9% in China, and 2.13% in Korea^{11–14} as compared to the rates reported in the USA, 3% (131/4403), 2.9% (205/7140), and 2.1% (18/873).^{10,15,16} The diverse detection rate of *MET* exon 14 skipping might be attributed to the methodology, specimen source, and ethnic differences of the patients. In this study, we detected *MET* exon 14 alterations in 951 Chinese NSCLC patients using targeted RNA-based next-generation sequencing (NGS). In addition, the molecular alterations of the main oncogenic drivers and PD-L1 expression was elucidated in these patients.

Patients and Methods

Study Cohort

Formalin-fixed paraffin-embedded (FFPE) tumor tissues were obtained from patients with NSCLC, who had undergone surgical resection, percutaneous transthoracic needle biopsy (PTNB), and transbronchial lung biopsy (TBLB) from January to December 2018 at the Beijing Chest Hospital, Capital Medical University and Henan Provincial People's Hospital, Zhengzhou University. The patients were reviewed according to the 2015 World Health Organization classification.¹⁷ The demographic data and clinicopathological parameters were collected from the electronic medical records. The staging of patients was assessed according to the 8th edition of the tumor, node, and metastasis (TNM) classification for lung cancer.¹⁸ A total of 951 patients with NSCLC, who fulfilled the selection criteria were included in the present study that was approved by the Ethics Committee of the Beijing Chest Hospital. This study was conducted in accordance with the Declaration of Helsinki and all

patients signed the informed consent to allow the use of the tumor samples in future studies. All clinical data and samples were received anonymously.

DNA and RNA Preparations

According to the manufacturer's instructions, DNA was isolated using TIANamp Genomic DNA Kit (Tiangen, Beijing, China) and the qualitative check was performed on 1% agarose gel electrophoresis. RNA was isolated using the RecoverAll™ Total Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Waltham, MA, USA), and RNA integrity was evaluated by qPCR and agarose gel electrophoresis.

DNA/RNA-Based NGS

An equivalent of 10 ng DNA served as a template to construct the amplicon libraries using an Ion AmpliSeq™ Library Kit 2.0 (Thermo Fisher Scientific), followed by targeted NGS as reported previously.¹⁹ The custom-designed panel encompassed 21 cancer-related genes, including *AKT1*, *ALK*, *BRAF*, *CTNNA1*, *DDR2*, *EGFR*, *ERBB2*, *ERBB4*, *FGFR1*, *FGFR2*, *FGFR3*, *KRAS*, *MAP2K1*, *MET*, *NOTCH1*, *NRAS*, *PIK3CA*, *PTEN*, *SMAD4*, *STK11*, and *TP53* ([Supplementary Table 1](#)).

RNA-based NGS was conducted using a custom-designed panel which included probes spanning the *MET* exon 13–15 junction. Also, fusions of *ALK*, *RET*, *ROS1*, and *NTRK1* with other genes were detected. The libraries were prepared using the one-step PCR amplification method. Then, the amplicon libraries were sequenced on an Ion Torrent Systems Proton system and a PI chip with barcoding performed using an Ion Xpress Barcode Adapter 1–96 Kit (Thermo Fisher Scientific).

NGS Data Analysis

Torrent Suite Software (version 5.0) was used for signal processing, base calling, quality score assignment, and adapter trimming after sequencing reaction. The mutant allele frequency $\geq 1\%$, fusion mutants with $\geq 1000\times$ coverage, and Normalized Detection Fractions (NDF) ≥ -2.8 were accepted.

RT-PCR and Sanger Sequencing

In order to confirm *MET* exon 14 skipping mutation in the specimens of patients, FFPE tumor samples were assessed by quantitative RT-PCR using the following primers: forward, 5'-AGATCAGAATTCACAGGATTGATTGC-3'; reverse, 5'-CTGTCAG AGGATACTGCACTTGTC-3'. The forward primer probed *MET* exon 13, and the reverse primer targeted

MET exon 15. Finally, the PCR products were analyzed by agarose gel electrophoresis and sequenced by standard procedures.

MET and PD-L1 Immunohistochemistry (IHC) Assay

The specimens were subjected to IHC staining to evaluate the expression of PD-L1 and *MET* using a mouse monoclonal PD-L1 antibody (22-c3, Dako, California, USA) and a rabbit monoclonal *c-Met* antibody (SP44, 1:50 dilution, Ventana Medical Systems, AZ, London, UK). The PD-L1 protein expression was scored using the tumor proportion score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining.²⁰ The staining intensity was classified into TPS \geq 50% (high expression), 1–49% (low expression), and 0 (negative). The IHC staining intensity of *MET* was scored semi-quantitatively on a 4-titer scale as follows: 0 (none), 1+ (weak membrane staining in $>$ 10% of the tumor cells), 2+ (moderate staining of the entire membrane in $>$ 10% of the tumor cells), or 3+ (strong staining of the entire membrane in $>$ 10% of the tumor cells).²¹ The *MET*-positive specimens were defined by a score of 2+ or 3+. Representative IHC images of *c-MET* and PD-L1 are shown in [Supplementary Figure 1](#).

Statistical Analysis

The correlation between the clinicopathological variables and *MET* status was analyzed by χ^2 test, Fisher's exact test, and Student's *t*-test using the Statistical Package for the Social Sciences 19 (SPSS, Chicago, IL, USA) software. All tests were two-sided. *P*-value $<$ 0.05 was considered as statistically significant.

Results

Patient Characteristics

A total of 951 NSCLC patients were enrolled in this study, and the clinical characteristics are summarized in [Table 1](#). The median age of the patient cohort was 63 (range, 25–91) years. The cohort comprised of 38 patients (4.0%) who were $<$ 45-years-old. Moreover, 55.3% of the patients (526/951) were male, and 44.7% (425/951) were female. Regarding smoking history, 51.1% (454/888) were never-smokers and 48.9% (434/888) were ever-smokers (63 cases had missing data). The histological subtypes of the cancer were primarily ADC (81.2%, 772/951) and SCC (15.9%, 151/951). Other histological subtypes included adenosquamous carcinoma (ASC, 1.2%, 11/951), large cell carcinoma

(LCC, 0.7%, 7/951), sarcomatoid carcinomas (SC, 0.4%, 4/951), and mucoepidermoid carcinoma (MEC, 0.1%, 1/951). In addition, the histological subtype of 5 (0.5% 5/951) patients was not specified (NOS). Among 608 patients with complete pathological stage information, 17.3% were stage I (105/608), 4.4% were stage II (27/608), 16.4% were stage III (100/608), and 61.9% were stage IV (376/608).

Prevalence of MET Exon 14 Skipping in Patients with NSCLC

MET exon 14 skipping was identified in 16 cases, which accounted for 1.7% (16/951) of all the NSCLC patients ([Figure 1A](#)). Of these, 14 cases were ADC (14/16; 87.5%), 1 case was ASC (1/16, 6.3%), and 1 case was SC (1/16; 6.3%). *MET* exon 14 skipping was not detected in squamous cell carcinoma or other histological types in this study. The incidence was 1.8% (14/772, [Figure 1B](#)) in ADCs and 2.0% (16/800) in non-squamous NSCLCs. In the present study, tumor samples were acquired in three different ways: surgical resection, PTNB, and TBLB. The detection rate of *MET* exon 14 skipping from surgical resection specimen was 2.3% (8/344) in NSCLCs and 2.0% (6/307) in ADCs ([Figure 1A](#)). The prevalence in the surgical resected sample was a representative of the overall population.

Other known tumor genetic alterations have also been evaluated using a DNA- and RNA-based NGS panel. The oncogenic driver mutation status of 951 NSCLC patients is shown in [Figure 1A](#): *EGFR* mutation ($n = 444$, 46.7%), *KRAS* mutation ($n = 99$, 10.4%), *ALK* rearrangement ($n = 52$, 5.5%), *ERBB2* mutation ($n = 31$, 3.3%), *BRAF* mutation ($n = 18$, 1.9%), *ROS1* rearrangement ($n = 13$, 1.4%), *ERBB4* mutation ($n = 12$, 1.3%), and *RET* rearrangement ($n = 10$, 1.1%). The proportion of genetic alterations was also evaluated in the ADC population ([Figure 1](#), left panel). Consequently, the proportion of *EGFR* mutation and *ALK* rearrangement in ADCs was slightly higher as compared to that in the NSCLC population.

Clinical Characteristics of Patients with MET Exon 14 Skipping

The clinicopathological characteristics of 16 patients harboring *MET* exon 14 skipping are demonstrated in [Table 1](#). Except 1 patient (59-year-old), all were $>$ 60-years-old. Significant differences were observed in the median age between NSCLC patients with *MET* exon 14 skipping and

Table 1 Patient Characteristics and Correlation Between Clinical Features and *MET* Exon 14 Skipping in NSCLC Patients

Clinical Features		No. (n=951)	Met ex14 Skipping		P-value
			Negative N=935 (98.3%)	Positive N=16 (1.7%)	
Age (years)	≥ 60	626	611 (97.6%)	15 (2.4%)	0.018
	< 60	325	324 (99.7%)	1 (0.3%)	
Gender	Male	526	515 (97.9%)	11 (2.1%)	0.275
	Female	425	420 (98.8%)	5 (1.2%)	
Smoking history	Never-smoker	454	447 (98.5%)	7 (1.5%)	0.551
	Ever-smoker	434	425 (97.9%)	9 (2.1%)	
	Unknown	63	63 (100%)	0 (0%)	
Location	Left lung	394	384 (97.5%)	10 (2.5%)	0.053
	Right lung	539	534 (99.1%)	5 (0.9%)	
	Unknown	18	17 (94.4%)	1 (5.6%)	
Stage	I-III	232	226 (97.4%)	6 (2.6%)	0.756
	IV	376	369 (98.1%)	7 (1.9%)	
	Unknown	343	340 (99.1%)	3 (0.9%)	
Histological subtypes	Adenocarcinoma	772	758 (98.2%)	14 (1.8%)	0.015
	Squamous	151	151 (100%)	0 (0.0%)	
	Adenosquamous	11	10 (90.9%)	1 (9.1%)	
	Sarcomatoid carcinoma	4	3 (75.0%)	1 (25.0%)	
	Others	13	13 (100%)	0 (0%)	
PD-L1 (TPS)	< 50%	328	324 (98.8%)	4 (1.2%)	<0.001
	≥ 50%	77	68 (88.3%)	9 (11.7%)	
c-MET IHC	0	33	33 (100%)	0 (0.0%)	0.152
	1+	122	121 (99.2%)	1 (0.8%)	
	2+	160	157 (98.1%)	3 (1.9%)	
	3+	49	46 (93.9%)	3 (6.1%)	
	unknown	587	578 (98.5%)	9 (1.5%)	

other oncogenic driver mutations, such as *ALK* (66 vs 54, $P = 0.001$), and *ROS1* (66 vs 59, $P = 0.010$; [Figure 2A](#)), indicating that patients with *MET* exon 14 skipping were older than those with other oncogenic driver mutations.

Further statistical analyses assessed the gender and smoking history. Between *MET* exon 14 skipping and wild-type population, no significant difference was observed in the proportion of gender and never-smokers. Furthermore, no significant association was detected between *MET* exon 14 skipping and tumor location and stage ([Figure 2B](#) and [C](#)).

c-MET Expression in NSCLC Patients with *MET* Exon 14 Skipping

A total of 364 patients underwent c-*MET* IHC assay and were enrolled in the c-*MET* cohort. According to the staining intensity (negative, weak, moderate, or strong) and

prevalence in tumor cells, patients in the c-*MET* cohort were divided into four subgroups: 3+ (n = 49, 13.5%), 2+ (n = 160, 44.0%), 1+ (n = 122, 33.5%), and 0 (n = 33, 9.0%). Furthermore, *MET* exon 14 skipping was identified in 7 cases ([Figure 3](#)), 3 cases (42.9%) scored 3+, 3 cases (42.9%) scored 2+, and 1 case (14.2%) scored 1+. According to the definition of c-*MET*-positive described before, *MET* overexpression occurred in 85.7% (6/7) *MET* exon 14 skipping NSCLCs and in 56.9% (203/357) *MET* exon 14 wild-type NSCLCs. As shown in [Supplementary Table 2](#), the χ^2 test did not show any significant difference ($P = 0.253$) between the two groups.

PD-L1 Expression in NSCLC Patients with *MET* Exon 14 Skipping

Simultaneously, the PD-L1 expression status was estimated by IHC when sufficient tissue is available. The tumor

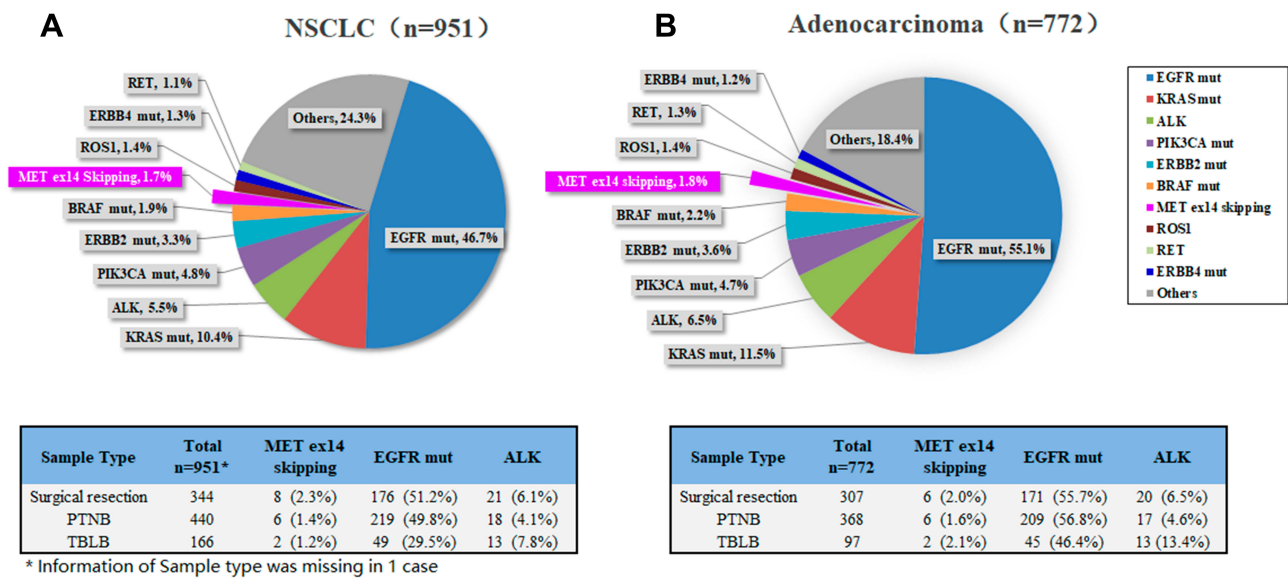


Figure 1 Pie chart with the frequency of driver alterations detected in 951 NSCLCs and 772 adenocarcinomas. (A) Prevalence of *MET* exon 14 skipping and other oncogenic mutations in NSCLC patients. (B) Prevalence of *MET* exon 14 skipping and other oncogenic mutations in adenocarcinoma patients. Detection rate of *MET* exon 14 skipping, *EGFR*, and *ALK* mutation in three sample types are listed.

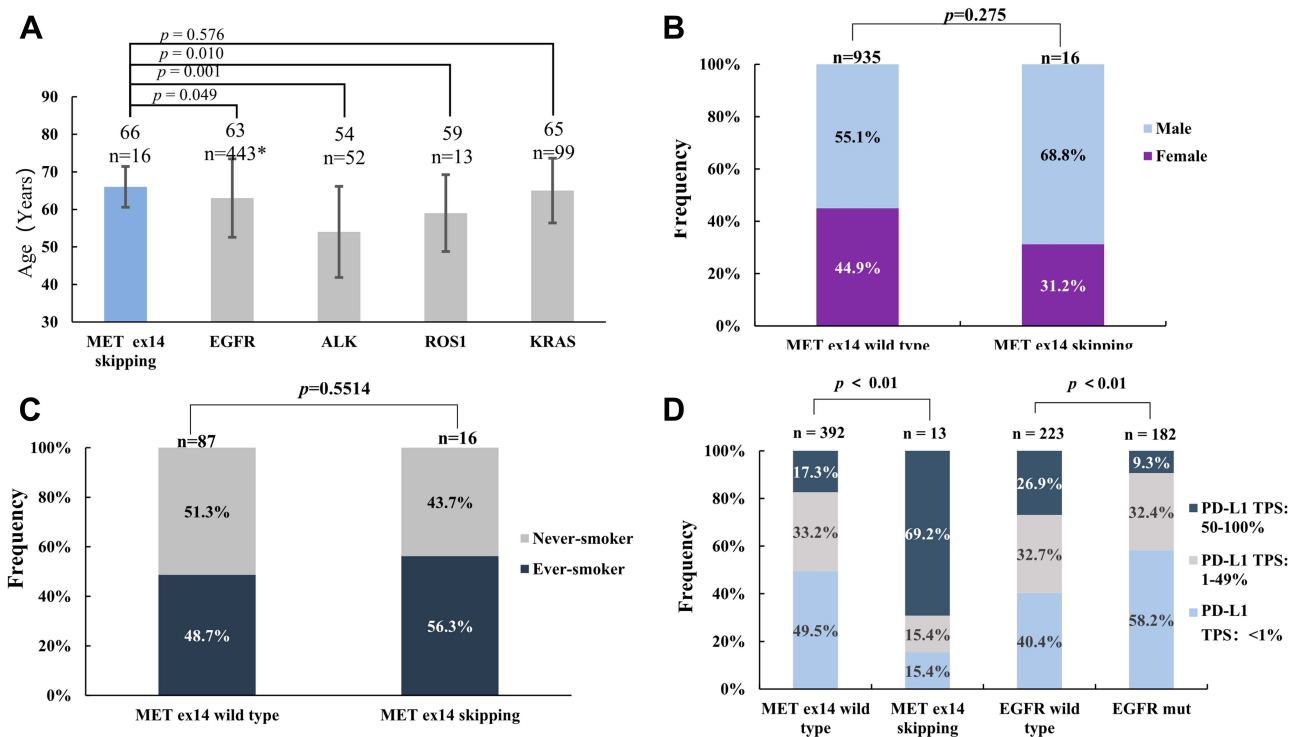


Figure 2 Clinical characteristics of patients with *MET* exon 14 skipping. (A) Median age of patients harboring different oncogenic driver mutations. (B) Gender proportion in *MET* exon 14 skipping and *MET* exon 14 wild-type population. (C) Proportion of never-smokers and ever-smokers in *MET* exon 14 skipping and *MET* exon 14 wild-type population. (D) PD-L1 expression status in *MET* exon 14 skipping, *MET* exon 14 wild-type, *EGFR* mutant, and *EGFR* wild-type population. *Although there are a total of 444 *EGFR* mutations, only 443 cases were shown because the age of one patient was not found.

proportion score (TPS) was applied to evaluate the PD-L1 protein expression as introduced before. Among 401 patients who were subjected to PD-L1 IHC assay, 196 cases (48.9%) were negative (TPS < 1%), 132 cases

(32.9%) showed low expression (1% ≤ TPS ≤ 49%), and 73 cases (18.2%) showed high expression (TPS ≥ 50%; Table 1). The PD-L1 expression status of 13 patients harboring the *MET* exon 14 skipping is shown in Figure 3. Of

ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16
c-Met IHC	+++	+++	+++	++	++	++	+	NA	NA	NA	NA	NA	NA	NA	NA	NA
PDL-1 (TPS)	90%	70%	Negative	10%	60%	Negative	5%	95%	70%	95%	60%	90%	85%	NA	NA	NA
Age (Years)	59	73	70	63	61	74	61	67	66	63	79	66	68	61	71	63
Histological subtypes	ADC	ADC	ADC	ADC	ADC	ADC	ADC	ADC	SC	ASC	ADC	ADC	ADC	ADC	ADC	ADC
Stage	IV	IV	IV	I	IV	I	I	NA	II	II	IV	NA	I	NA	IV	IV
Gender	M	M	M	M	M	F	M	M	F	F	M	M	F	F	M	M
Co-mutations	TP53	c.524G>A	c.578A>T	c.830G>A				c.736A>C	c.844C>T			c.644G>A				
	PTEN				c.G44C c.C892T											
	PIK3CA				c.1624G>A c.1633G>A											
	EGFR															c.2240_2257 del
	MET								c.2950_2951 insT							

Figure 3 C-MET IHC, PD-L1 expression status, and co-mutations profile in patients harboring the *MET* exon 14 mutations. Patient ID are shown in the first line. The stage of presentation is indicated, and patients with stage IV are highlighted in bold. c-MET staining is shown in brown and the intensity represents the expression status. PD-L1 expression is indicated similarly. Patients with high expression of PD-L1 is shown in dark purple, while low expression is shown in light purple.

these, 9 (69.2%) patients showed high PD-L1 expression, and 4 (30.8%) patients showed low or negative PD-L1 expression (Table 1). The prevalence of PD-L1 expression status in patients with *MET* exon 14 skipping was significantly different from those with *MET* exon 14 wild-type. As shown in Figure 2D, PD-L1 tended to be highly expressed in NSCLC patients harboring *MET* exon 14 skipping (69.2% vs 17.3%, $P < 0.01$). Conversely, compared to the *EGFR* wild-type cohort, the proportion of PD-L1 high expression was significantly lower in the *EGFR* mutant cohort (9.3% vs 26.9%, $P < 0.01$; Figure 2D).

Co-Occurrence of Genetic Alterations in NSCLC Patients with *MET* Exon 14 Skipping

DNA- and RNA-based NGS was performed to identify the lung cancer-associated genetic alterations including fusions, truncations, and in-frame and missense mutations. Among the 16 patients harboring *MET* exon 14 skipping, *EGFR* exon 19 deletion was detected with low mutation abundance in 1 case (Figure 3, *EGFR* c.2240_2257del, 1.41%). The other common oncogenic driver genes, such as *KRAS*, *ALK*, *ROS1*, and *RET* were not found to be co-mutated with *MET* exon 14 skipping. As shown in Table 2, the incidence of *MET* exon 14 skipping was 4.7% (16/340) in *EGFR/KRAS/ALK/ROS1/RET*-negative NSCLCs.

As a major tumor suppressor, *TP53* co-mutations were widespread in the driver gene mutation-positive population. In this study, *TP53* mutations were detected in 48% (444/935)

NSCLC patients, 46.2% (205/444) showed *EGFR* mutants, 49.5% (49/99) showed *KRAS* mutants, 21.2% (11/52) showed *ALK*-rearrangements, 38.5% (5/13) patients showed *ROS1* rearrangement, and 20% (2/10) presented *RET* rearrangements (Figure 4). The proportion of *TP53* co-mutation was 37.5% (6/16) and 47.5% (444/935) in *MET* exon 14 skipping and wild-type patients, respectively (Supplementary Figure 2). However, no significant difference was observed in both groups.

Discussion

Lung ADC patients harboring *MET* exon14 skipping mutations have been reported to represent a clinically unique molecular subtype of NSCLC. In the present study, RNA-based NGS was used for the detection of *MET* exon 14 skipping in 951 NSCLC patients. Since some somatic variants not encompassing the *MET* exon14 splice sites might induce *MET* exon

Table 2 Co-Mutation Between *MET* Ex14 Skipping and *EGFR/KRAS/ALK/ROS1/RET* in NSCLC and ADC Populations

	NSCLC			ADC		
	N	MET ex14-	MET ex14+	N	MET ex14-	MET ex14+
<i>EGFR</i> mut	444	443	1 (0.2%)	425	424	1 (0.2%)
<i>KRAS</i> mut	99	99	0	89	89	0
<i>ALK</i>	52	52	0	50	50	0
<i>ROS1</i>	13	13	0	11	11	0
<i>RET</i>	10	10	0	10	10	0
Quintuple-negative	340	324	16 (4.7%)	196	183	13 (6.6%)

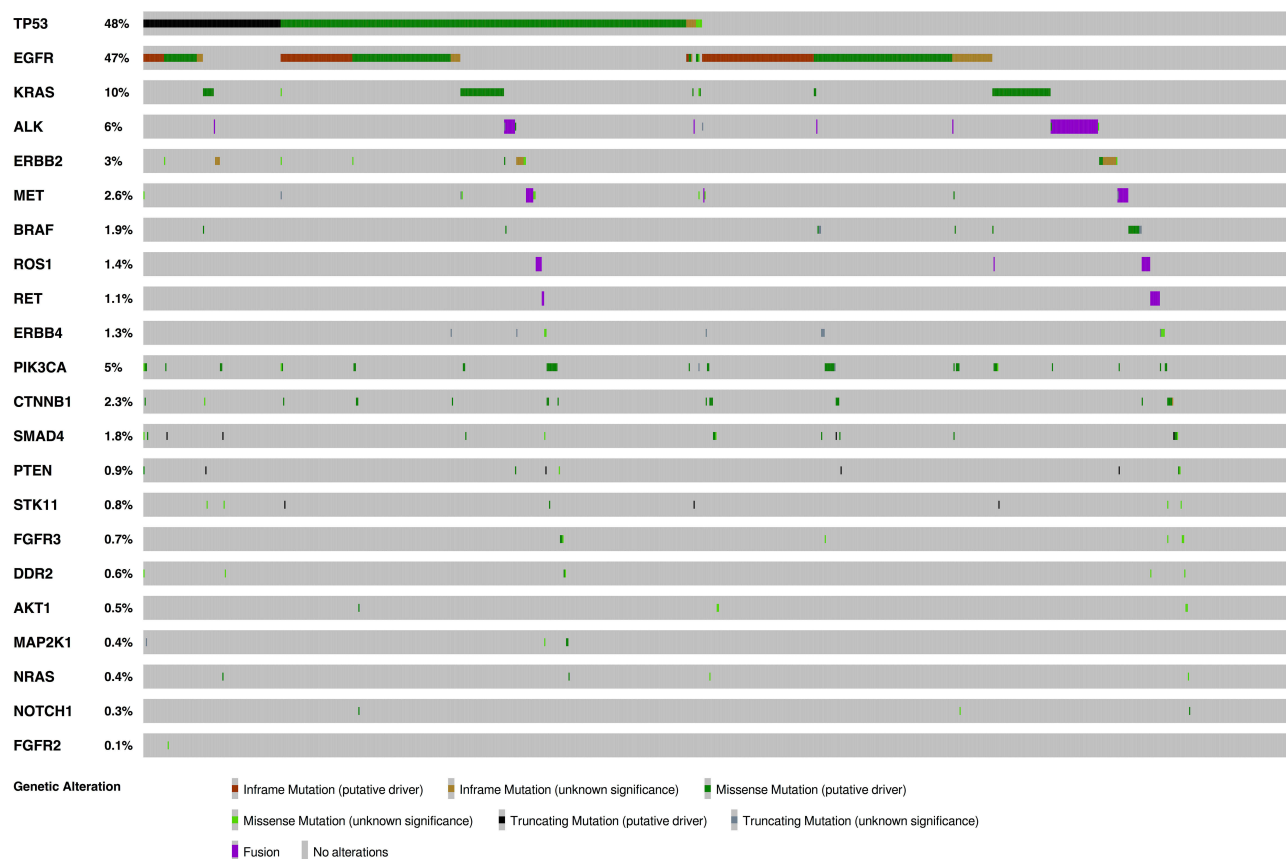


Figure 4 Landscape of oncogenic mutations. Each sample occupies a vertical column. The alterations of genes are classified as fusions, truncations, as well as in-frame and missense mutations. *MET* exon 14 skipping was defined as fusion since the molecular mechanisms were similar.

14 skipping, we speculated that RNA could serve as a favorable source of material for analyzing the phenomenon.

The prevalence rates of *MET* exon 14 skipping in lung ADC were 0.9–4.0% in Asian populations and 2.1–3% in the Western population. In the current study, the overall estimated prevalence of *MET* exon 14 skipping was 1.9% in ADCs and 1.7% in NSCLCs (Figure 1). Furthermore, we compared the detection rate of *MET* exon 14 skipping from different specimen sources, including surgical resection, PTNB, and TBLB. As shown in Figure 1A, the detection rate of *MET* exon 14 skipping from surgical resection specimen was 2.3% in NSCLC and 2.0% in ADC, respectively. Liu et al reported that the prevalence rate of *MET* exon 14 skipping in Chinese population with lung ADC was 0.9% as assessed by biopsy specimens in 2016. The low detection rate of *MET* exon 14 skipping in the study by Liu et al might be attributed to diverse specimen sources rather than ethnic differences.

Notably, *MET* exon 14 skipping is mutually exclusive of other known driver gene alterations.^{10,12} In this study, *MET* exon 14 skipping was identified in 6.6% of *EGFR/KRAS/ALK/ROS1/RET*-negative ADCs (13/196). This incidence

was lower than that reported previously. Furthermore, the prevalence rate of *MET* exon 14 skipping was 19% (10/54 NSCLC cases) in Western patients without the mutation of other driver genes, such as *EGFR*, *KRAS*, *BRAF*, *ERBB2*, *ALK* and *ROS1*, while it is 37.8% (17/45) in East Asian patients.^{14,22} Thus, it is beneficial to screen *MET* exon14 skipping mutations in patients without other oncogenic driver mutations, as described previously²³.

TP53 mutations were detected in 37.5% of the samples with *MET* exon 14 skipping, 46.2% of the samples with *EGFR* mutations, and 21.2% of the samples with *ALK* fusions. A previous study demonstrated that *TP53* mutations reduced the responsiveness to crizotinib and worsened the prognosis in NSCLC patients with *ALK* rearrangements.²³ Thus, additional studies are essential to determine the impact of *TP53* mutations in response to crizotinib in NSCLC patients with *MET* exon 14 skipping. One patient with ADC harbored the *MET* exon 14 skipping and *EGFR* exon19 deletions (Figure 3), which could be attributed to tumor heterogeneity.²⁴ Presently, the treatment of patients with multiple mutations lacks consensus

on the appropriate TKI combination for optimal efficacy, thereby necessitating further investigation.

Several recent studies^{25,26} reported that PD-L1 expression in NSCLCs is inversely correlated with *EGFR* mutation and positively correlated with *TP53* mutation and *MET* amplification. The current results also showed that PD-L1 is highly expressed in NSCLC patients with *MET* exon 14 skipping (69.2% vs 17.3%, $P < 0.01$), and significantly lower in patients with *EGFR* mutant cohort (9.3% vs 26.9%, $P < 0.01$). These data suggested that in the *EGFR* wild-type patients, *MET* mutation status and PD-L1 expression might be critical factors for personalized targeted therapy that downregulates the *RAS/MAPK* pathway.

The present study has several inherent limitations due to the retrospective design and insufficient follow-up period. The prevalence of *MET* exon 14 skipping in lung ADCs in the East Asian population was similar to that in the Western population as assessed using RNA-based NGS. The NSCLC patients with *MET* exon 14 skipping were older than those with other oncogenic driver mutations, such as *EGFR*, *ALK*, and *ROS1*. However, no significant differences were observed in the proportion of gender, smoking history, tumor location, and tumor stage between *MET* exon 14 skipping and wild-type NSCLCs. Moreover, PD-L1 was highly expressed in NSCLC patients with *MET* exon 14 skipping.

Conclusion

The prevalence of *MET* exon14 skipping in lung ADCs in the East Asian population was similar to that in the Western population as assessed by RNA-based NGS. The NSCLC patients with *MET* exon 14 skipping were older than those with other oncogenic driver mutations, such as *EGFR*, *ALK*, and *ROS1*. In addition, PD-L1 was highly expressed in NSCLC patients with *MET* exon 14 skipping.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Beijing Chest Hospital (2017CHB06), and written informed consent was obtained from all patients.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The abstract of this paper was presented at the 2019 ASCO Annual Meeting as a poster presentation with interim findings. The poster's abstract was published in "Identification of *MET* exon 14 skipping by targeted RNA-based next-generation sequencing in Chinese Patients with NSCLC" in *Journal of Clinical Oncology*, DOI: 10.1200/JCO.2019.37.15_suppl.e20018. Drs Ziguang Xu and Lingfei Kong report grants from Major Science and Technology Projects in Henan Province (No.161100311400), during the conduct of the study. Peng Cheng and Fang Luo are employees of Novogene Co., Ltd., Beijing, China. Shijun Fu and Min Gao are employees of Pfizer Inc. The authors declare no other potential conflicts of interest.

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