Clinical Cancer Research

MET Amplification and Exon 14 Splice Site Mutation Define Unique Molecular Subgroups of Non–Small Cell Lung Carcinoma with Poor Prognosis

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Abstract

Purpose: Activation of *MET* oncogene as the result of amplification or activation mutation represents an emerging molecular target for cancer treatment. We comprehensively studied MET alterations and the clinicopathologic correlations in a large cohort of treatment-naïve non–small cell lung carcinoma (NSCLC).

Experimental Design: Six hundred eighty-seven NSCLCs were tested for *MET* exon 14 splicing site mutation (*METΔ*14), DNA copy number alterations, and protein expression by Sanger sequencing, FISH, and IHC, respectively.

Results: *METΔ*14 mutation was detected in 2.62% (18/687) of NSCLC. The mutation rates were 2.6% in adenocarcinoma, 4.8% in adenosquamous carcinoma, and 31.8% in sarcomatoid carcinoma. *METΔ*14 mutation was not detected in squamous cell carcinoma, large cell carcinoma, and lymphoepithelioma-like carcinoma but significantly enriched in sarcomatoid carcinoma ($P < 0.001$). *METΔ*14 occurred mutually exclusively with known driver mutations but tended to coexist with *MET* amplification or copy number gain ($P < 0.001$). Low-level *MET* amplification and polysomy might occur in the background of EGFR or KRAS mutation whereas high-level amplification (*MET*/CEP7 ratio $\geq$5) was mutually exclusive to the major driver genes except *METΔ*14. Oncogenic *METΔ*14 mutation and/or high-level amplification occurred in a total of 3.3% (23/687) of NSCLC and associated with higher MET protein expression. *METΔ*14 occurred more frequently in older patients whereas amplification was more common in ever-smokers. Both *METΔ*14 and high-level amplification were independent prognostic factors that predicted poorer survival by multivariable analysis.

Conclusions: The high incidence of *METΔ*14 mutation in sarcomatoid carcinoma suggested that MET inhibition might benefit this specific subgroup of patients. *Clin Cancer Res; 22(12); 3048–56. ©2016 AACR.*

See related commentary by Drilon, p. 2832

Introduction

Non–small cell lung cancer (NSCLC) represents a paradigm for the development of targeted cancer therapy. EGFR and ALK are well-known examples demonstrating that matched actionable oncogenic mutations with appropriate tyrosine kinase inhibitor (TKI) therapies improve patients' life quality and survival. Recent genomic studies in lung adenocarcinoma have found actionable oncogenic mutations involving RTK/RAS/RAF/PI3K axis such as EGFR, KRAS, HER2, BRAF, ARAF, CRAF, PIK3CA, MET, RIT1, MAP2K1, NRAS, HRAS mutations and ALK, NRG1, NTRK, ERBB4, RET, ROS1, and BRAF translocations, suggesting more than 70% of lung adenocarcinoma could be defined by gene mutations (1, 2).

MET is a high-affinity receptor tyrosine kinase (RTK) that could initiate an array of pathways promoting cell proliferation, survival, and metastasis upon stimulation. Gain-of-function alterations of MET by DNA amplification, mutation, and protein overexpression are driver events of oncogenesis in many cancer types. Dysregulation of MET enhances the malignant properties and predict poor prognosis that represents a possible target for personalized therapy (3). MET-directed anticancer strategies by blocking different MET pathway components are under preclinical and clinical trials. These include antibodies targeting MET or HGF, selective, and unselective small molecules targeting MET RTK activity.

NSCLCs harboring *MET* DNA amplification are dependent on MET for growth and survival (4). NSCLC patients with *de novo* *MET* DNA amplification are responsive to crizotinib, suggesting

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-15-2061

Translational Relevance

MET oncogene is an emerging molecular target for non–small cell lung carcinoma (NSCLC). Multiple mechanisms contribute to MET activation. The incidence and clinicopathologic characteristics of tumors with MET alterations are yet to be established. This study represents the first comprehensive parallel screening of MET alterations including *METΔ14* mutation, DNA copy number alteration, and protein expression in a large cohort of NSCLC. Oncogenic *METΔ14* mutation and high-level amplification (*MET*/CEP7 ratio ≥5), which are mutually exclusive to other major driver mutations, occur in 3.3% of NSCLC that define a distinct subset of NSCLC with sarcomatoid histology and aggressive clinical course. The finding suggests a pivotal role of MET signaling in tumorigenesis of sarcomatoid carcinoma and raises the possibility that MET inhibition may aid in treating this highly aggressive and chemotherapy-resistant subtype of NSCLC.

that *MET* amplification is a primary oncogenic driver and a valid clinical target (5, 6). *MET* mutations affecting exon 14 splicing elements occur in up to 5% of lung adenocarcinoma. These mutations result in a juxtamembrane domain lacking MET protein (METΔ14) with extended half-life after HGF stimulation that has been considered an oncogenic driver event. *In vitro* and *in vivo* studies have demonstrated that tumors harboring *MET* exon 14 mutation responded to MET inhibitors (1, 7, 8). Clinical response to MET inhibitors in patients with METΔ14$^+$ lung adenocarcinoma has been reported (9), further supporting MET as a novel therapeutic target. However, a recent phase III randomized clinical trial failed to demonstrate additional benefit of onartuzumab, an anti-MET mAb, on advanced stage NSCLC patients treated with erlotinib whose tumors were identified as MET$^+$ by IHC (10). This underscores the importance of appropriate predictive biomarker for patient stratification in the new era of personalized medicine.

We have reported the driver mutation profile of 154 lung adenocarcinoma and adenosquamous cell carcinoma (ADSQ) and demonstrated that MET DNA alterations defined a subgroup of patients with aggressive diseases that might potentially benefit from anti-MET targeted therapy (11). The clinical implication of MET alterations in different histologic subsets of NSCLC remains undefined. In the current study, we aimed to determine the prevalence of *MET* DNA alterations, including exon 14 skipping mutations (*METΔ14*) and amplifications, in a large cohort of Chinese patients, and define the clinicopathologic characteristics of MET-positive tumors.

Materials and Methods

Patients and samples

Patients with primary NSCLC who underwent surgical resection at Prince of Wales Hospital, Hong Kong, between 1995 and 2011 were selected for the retrospective study. All available formalin-fixed paraffin-embedded (FFPE) surgical resection specimens were reviewed by two pathologists (K.F. To and A.W. Chan) to confirm the histologic diagnosis and select the representative tumor blocks with appropriate tumor content. Medical records were reviewed to extract data on clinicopathologic parameters. The pathologic stages were determined according to the 7th edition of American Joint Committee on Cancer tumor–node–metastasis classification system. Early stage referred to stage I to IIIA whereas advanced stage referred to stage IIIb to IV. Patients were categorized into either never-smoker (smoke less than 100 cigarettes in their lifetime) or ever-smoker (smoke more than 100 cigarettes in their lifetime; ref. 1). Patients who received neoadjuvant chemotherapy or radiotherapy were excluded from the study. A total of 687 treatment-naïve NSCLC met the selection criteria that were included in the current study. The study protocol was approved by the Joint CUHK-NTE Clinical Research Ethnics Committee. The driver mutation profile of 154 adenocarcinoma and ADSQ has been reported in a previous study (11).

Construction of tissue microarray

Tissue microarrays (TMA) were constructed using a tissue arrayer (Beecher Instruments). The location of tumor area on the donor FFPE tissue block was first marked on the hematoxylin and eosin–stained histologic section. Three representative 1-mm cores were obtained from each tumor and were inserted to a recipient paraffin block. For FISH and IHC, 4-μm tissue sections were prepared and mounted onto Superfrost Plus microscope slides.

IHC

IHC was carried out using Benchmark XT autostainer (Ventana) using Ultraview detection system. MET IHC was performed using Confirm anti-Total c-MET (SP44) rabbit mAb (Ventana) according to the manufacturer's instruction. Expression level of MET protein was determined by a scoring system considering both staining intensity and prevalence of intensities in tumor cells. The four staining scores were defined as following: 3+ (≥50% of tumor cells staining with strong intensity); 2+ (≥50% of tumor cells with moderate or higher staining but < 50% with strong intensity); 1+ (≥50% of tumor cells with weak or higher staining but < 50% with moderate or higher intensity); or 0 (no staining or < 50% of tumor cells with any intensity; ref. 12). Tumors with moderate to strong MET protein expression (score 2+ and 3+) were considered IHC$^+$, whereas score 0 and 1+ were regarded as IHC$^-$ for MET expression.

Mutational analysis

DNA was extracted from FFPE tissue using QIAamp DNA mini kit (Qiagen) according to the manufacturer's protocol. Manual microdissection was performed to ensure more than 70% tumor content in each DNA sample for subsequent analysis. Sanger sequencing was performed to screen for *MET* exon 14 splice site mutations.

FISH

MET gene copy number/amplification status were investigated by MET/CEP7 FISH probe (Abbott Molecular) as reported previously (11). Copy number per cell and *MET*/CEP7 ratio were counted in at least 50 nonoverlapping tumor cell nuclei. As there is no consensus approach in *MET* FISH scoring, we used three scoring systems for *MET* FISH assay:

1. Tumors with ≥5 *MET* signals per cell were classified as FISH$^+$ according to Capuzzo scoring system (13).

2. Tumor with *MET*/CEP7 ratio ≥2 were defined as FISH$^+$ by PathVysion (14, 15).
3. High-level amplification (H-Amp) was defined as clustered *MET* signals or *MET*/CEP7 ratio ≥5 (6).

Screening for major driver events

EGFR exons 18–21, KRAS exons 2 and 3 were screened by PCR-direct sequencing. ALK and ROS1 translocations were examined by dual color break-apart FISH analysis as described previously (11).

Statistical analysis

Statistical analysis was performed using SPSS 19.0 (IBM Corp.). χ^2 and Fisher exact test were used to analyze associations of mutational, protein expression, and gene copy number status with clinical characteristics. We compared MET status in each histologic subtype versus all other subtypes. For example, MET status in adenocarcinomas was compared with all the non-adenocarcinomas and so on. Overall survival (OS) was defined as the time from disease diagnosis to patient's death due to disease progression. The Kaplan–Meier method was used to estimate the survival rates for different groups. The equivalences of the survival curves were tested by log-rank statistics. The Cox proportional hazards model was employed for univariable and multivariable survival analyses. The variables found to be statistical significant in the univariable survival analysis were further evaluated in the multivariable survival analysis. A two-tailed *P* value of <0.05 was considered to be statistically significant.

Results

Patient characteristics

A total of 687 treatment-naive NSCLC, comprising 392 (57.1%) adenocarcinoma, 180 (26.2%) squamous cell carcinoma (SCC), 45 (6.6%) large cell carcinoma (LCC), 21 (3.1%) ADSQ, 27 (3.9%) lymphoepithelioma-like carcinoma (LELC), and 22 (3.2%) pulmonary sarcomatoid carcinoma (PSC) were recruited. The median age of the patients was 66 years (range, 27–94 years) and male to female ratio was 2.1:1. Ever-smokers represented 63.9% of all patients and were more common in SCC than other histologies ($P < 0.001$). Adenocarcinoma was more common in female ($P < 0.001$) and never-smokers ($P < 0.001$). LELC occurred more frequently in female ($P = 0.043$), never-smokers ($P = 0.004$), and younger patients ($P = 0.005$). The demographic information was shown in Supplementary Table S1. Major driver events including EGFR, KRAS, ALK, and ROS1 were found in 26.2%, 8.9%, 3.9%, and 1.5% of NSCLC, respectively. The mutation rates of the above-mentioned genes were 41.6%, 12%, 6.1%, and 2.3%, respectively in adenocarcinoma (Fig. 1). EGFR mutation rate was significantly higher in adenocarcinoma ($P < 0.001$), female ($P < 0.001$), and never-smokers ($P < 0.001$). Significantly higher KRAS mutation rate was found in adenocarcinoma ($P = 0.001$), male ($P = 0.012$), and ever-smokers ($P = 0.012$). ALK translocation was more frequently detected in adenocarcinoma ($P = 0.001$), advanced stage ($P = 0.012$), never-smokers ($P = 0.021$), younger age ($P < 0.001$), and smaller tumor size ($P = 0.001$). ROS1 translocation was detected in 10 cases which associated with adenocarcinoma ($P = 0.049$), advanced stage ($P = 0.001$), and younger age ($P = 0.002$). Table 1 summarized the clinicopathologic associations of the driver events.

METΔ14 mutations in NSCLC

By PCR-direct sequencing, 18 (2.6%) NSCLCs harboring mutations that disrupt the consensus sequences for MET exon 14 splicing sites were identified. The METΔ14 mutation rates were 2.6% in adenocarcinoma, 4.8% in ADSQ, and 31.8% in PSC. No *METΔ14* mutation was found in SCC, LCC, and LELC. A significantly higher METΔ14 mutation rate was found in PSC ($P < 0.001$). The mean age of METΔ14-positive patients was 73.7 years versus 64.2 years in METΔ14-negative patients ($P = 0.001$). There were no significant differences in gender distribution, smoking history, or stage between patients with or without *METΔ14* mutation (Table 1).

METΔ14 mutations were comprised point mutations ($n = 8$) and small deletions ($n = 2$) affecting the consensus sequence of the splice donor site elements, point mutation ($n = 1$) and small deletions ($n = 4$) affecting the splice acceptor sites, point mutation at branching point ($n = 1$) and small deletions ($n = 2$) disrupting the polypyrimidine tract at intron 13 (Fig. 2A). We retrospectively examined other driver mutations on RTK/RAS/PI3K pathways in METΔ14-positive cases and found that all *METΔ14* mutations occurred mutually exclusively with oncogenic driver mutations, i.e., EGFR, KRAS, HER2, BRAF, NRAS, PIK3CA, MAP2K1 as well as ALK and ROS1 translocations. The clinicopathologic characteristics of patients with METΔ14 tumors were shown in Supplementary Table S2.

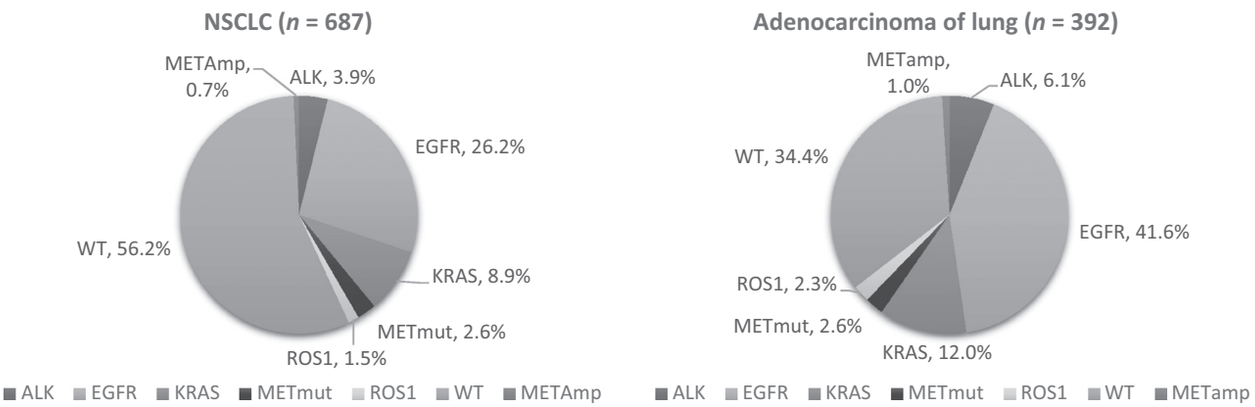

Figure 1.
Major driver events in NSCLC and adenocarcinoma of lung.

Table 1. Clinicopathologic features of NSCLC patients with MET DNA alterations and other major driver events

	Total	METΔ14		MET H-Amp		EGFR+ve		KRAS+ve		ALK+ve		ROS1+ve	
Characteristics	***n* = 687**	***n* = 18**	***P***	***n* = 8**	***P***	***n* = 180**	***P***	***n* = 61**	***P***	***n* = 27**	***P***	***n* = 10**	***P***
Histology													
AD	392	10	1[a]	4	0.73[a]	163	<0.001[a]	47	0.001[a]	24	0.001[a]	9	0.049[a]
SCC	180	0	0.006[a]	0	0.119[a]	10	<0.001[a]	2	<0.001[a]	0	0.002[a]	0	0.07[a]
LCC	45	0	0.623[a]	1	0.42[a]	0	<0.001[a]	4	0.998[a]	1	1[a]	1	0.498[a]
ADSQ	21	1	0.432[a]	0	1[a]	5	0.789[a]	4	0.096[a]	2	0.198[a]	0	1[a]
LELC	27	0	1[a]	0	1[a]	1	0.003[a]	1	0.501[a]	0	0.619[a]	0	1[a]
PSC	22	7	<0.001[a]	3	0.001[a]	1	0.014[a]	3	0.434[a]	0	1[a]	0	1[a]
Gender													
Female	223	7	0.612	1	0.448	98	<0.001	11	0.012	13	0.077	6	0.083
Male	464	11		7		82		50		14		4	
Stage													
IA–IIIA	583	15	0.723	4	0.056	149	0.306	51	0.989	19	0.012	4	0.001
IIIB–IV	91	3		3		28		8		8		6	
Smoking history													
NS	223	9	0.222	0	0.053	112	<0.001	11	0.012	14	0.021	4	0.253
ES	395	9		7		53		43		10		3	
Age													
Mean ± SD	687	73.7 ± 11.6	<0.001	65.5 ± 11.7	0.788	63.5 ± 10.9	0.201	64.7 ± 9.1	0.878	55.0 ± 13.7	<0.001	53.9 ± 16.2	0.002
Tumor size													
Mean ± SD	671	3.7 ± 1.3	0.479	4.5 ± 2.1	0.675	3.7 ± 1.7	0.003	4.1 ± 2.5	0.986	2.6 ± 1.2	0.001	3.5 ± 2.7	0.4
MET IHC													
Positive	230	18	<0.001	8	<0.001	79	0.001	35	<0.001	18	<0.001	4	0.738
Negative	457	0		0		101		26		9		6	
MET FISH													
Positive	29	6	<0.001	\		6	0.49	2	1	0	0.622	0	1
Negative	658	12		\		174		59		27		10	

Abbreviations: ES, ever smoker; NS, never smoker.
[a]Versus all other histologic types.

MET gene copy number alteration

FISH analysis was performed to investigate the copy number alteration (CNA) of *MET* gene in NSCLC. As there is no consensus scoring system for *MET* CNA, we employed three different scoring systems for *MET* FISH analysis as described in methodology. The results from the original scoring systems were summarized in Supplementary Table S3. The final FISH status was the integration of three scoring systems according to the patterns of DNA CNAs.

Twenty-nine cases were classified as FISH$^+$ by at least one scoring system (Supplementary Table S4). Four distinct patterns of *MET* CNA were identified (Fig. 2B and Supplementary Fig. S1):

1. H-Amp: ratio of *MET*/CEP7 $\geq$ 5; $n = 8$.
2. Polysomy: *MET* signal $\geq$ 5, without gene amplification; $n = 9$.
3. Low-level amplification/high gene copy number (L-Amp/H-GCN): 2 $\leq$ *MET*/CEP7 < 5, *MET* signal $\geq$ 5; $n = 7$.
4. Low-level amplification/low gene copy number (L-Amp/L-GCN): 2 $\leq$ *MET*/CEP7 < 5, *MET* signal < 5; $n = 5$.

High-level amplification-Amp was more common in PSC (3/22, 13.6%, $P < 0.001$) than other histologic subtypes whereas polysomy was exclusively found in adenocarcinoma (9/392, 2.3%, $P < 0.001$). A significant enrichment of SCC was observed in L-AMP/L-GCN group (4/5, 80%). *MET* gene amplification but not polysomy associated with positive smoking history (Supplementary Table S3).

There was a significant association between *METΔ14* mutation and *MET* CNA ($P < 0.001$). Among 29 FISH$^+$ cases, 20.7% (6/29) showed METΔ14 mutation (Supplementary Table S4). Although in FISH$^-$ group, only 1.8% (12/658) of the cases harbored *METΔ14* mutation (Table 1). *METΔ14* mutations occurred more frequently in old-age patients whereas DNA amplifications were more commonly seen in ever-smokers.

MET CNA may occur in the background of other driver events. Almost all polysome (8/9) coexisted with other driver mutations: 5 with EGFR mutation, 2 with *METΔ14*, and 1 with KRAS mutation. Three of 12 L-Amp, including 1 L-Amp/L-GCN and 2 L-Amp/H-GCN, cooccurred with EGFR, KRAS, or *METΔ14*. In 8 tumors with H-Amp, coexisting *METΔ14* mutation was found in 3. Notably, H-Amp coexisted with *METΔ14* only and was mutually exclusive of other driver genes (Supplementary Table S4).

MET protein expression in NSCLC

Moderate to strong MET protein expression was detected in 33.5% (230/687) of NSCLC (Fig. 2C). MET IHC-positive rates were 49.7% in adenocarcinoma, 42.9% in ADSQ, 40.9% in PSC, 15.6% in LCC, and 5.6% in SCC. All LELCs were negative for MET protein expression. Compared with other histologic subtypes, NSCLC with adenocarcinoma component (including adenocarcinoma and ADSQ) had a significantly higher positive rate for MET IHC ($P < 0.001$). This is in keeping with previous report that MET expression was more prevalent in adenocarcinoma than SCC (16; Table 2).

Association between *MET* DNA alterations and MET protein expression

METΔ14 mutation status significantly correlated with MET IHC ($P < 0.001$). All *METΔ14*$^+$ tumors, including 10 adenocarcinoma, 1 ADSQ, and 7 PSC demonstrate strong MET immunoreactivity. Overall, there was a good correlation between *MET* FISH and IHC ($P < 0.001$, Table 2). Concordant results were seen in 478 (69.7%) cases, with IHC−/FISH− in 453 (65.9%) and IHC$^+$/FISH$^+$ in 25 (3.6%) cases. IHC$^+$/FISH$^-$ and IHC$^-$/FISH$^+$ were observed in 205 (29.8%) and 4 (0.6%) samples, respectively.

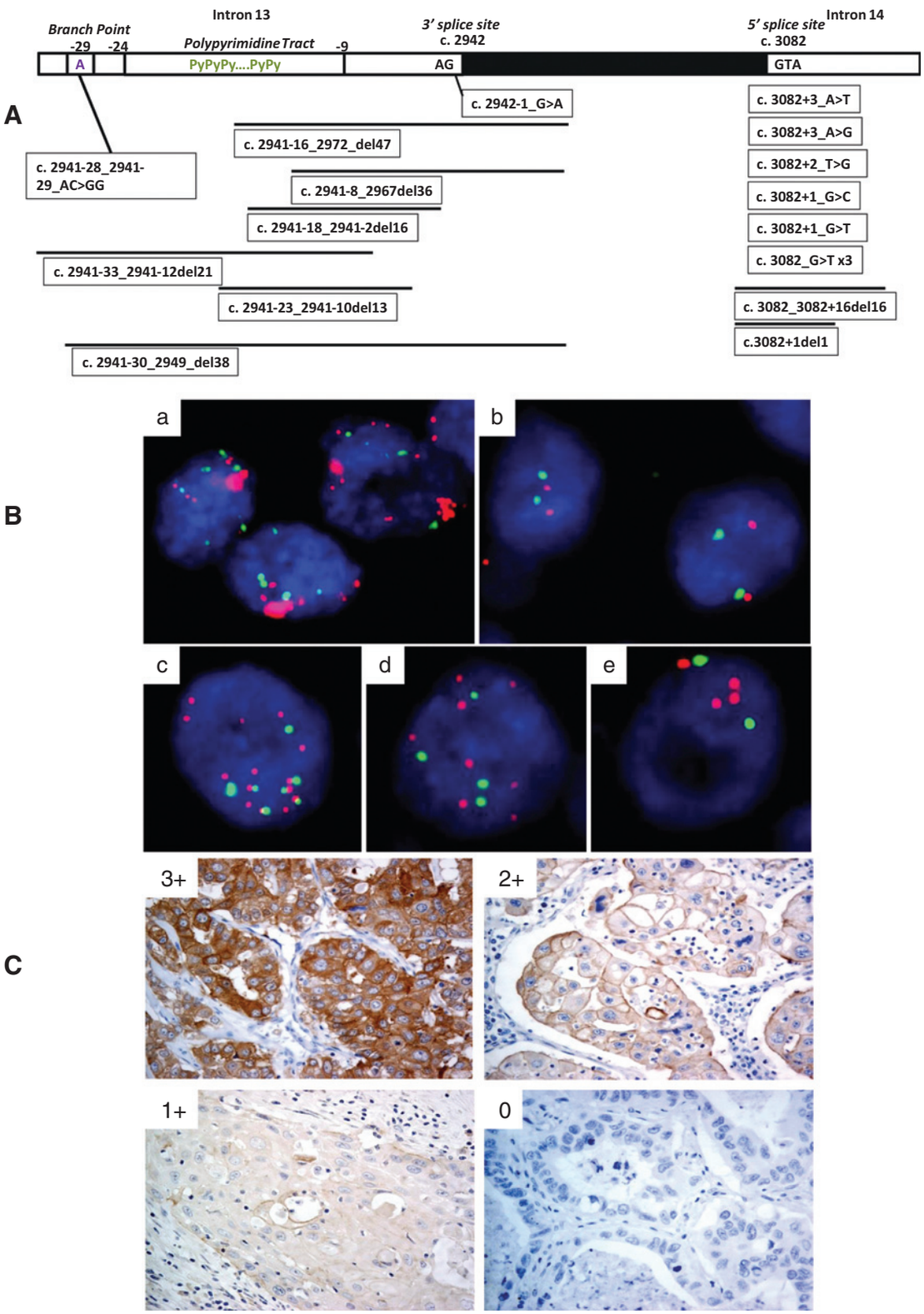

Figure 2.
A, schematic illustration of the spectrum of *MET* exon 14 skipping mutations identified in this study ($N = 18$). B, representative images showing *MET* DNA CNAs determined by FISH analysis. H-Amp (a); disomy (b); polysomy (c); L-Amp/high gene copy number (d); L-Amp/low gene copy number (e). C, representative images of MET IHC showing tumors with MET IHC scores 0–3+.

Table 2. Clinicopathologic features of NSCLC patients according to MET protein expression by immunohistochemical analysis

		MET Protein expression by IHC		
Characteristics	**Total** ***n*** **= 687**	**Positive** ***n*** **= 230**	**Negative** ***n*** **= 457**	***P***
Histology				
AD	392	195	197	<0.001[a]
SCC	180	10	170	<0.001[a]
LCC	45	7	38	0.008[a]
ADSQ	21	9	12	0.356[a]
LELC	27	0	27	<0.001[a]
PSC	22	9	13	0.49[a]
Gender				
Female	223	95	128	0.001
Male	464	135	329	
Stage				
IA–IIIA	583	187	396	0.056
IIIB–IV	91	39	52	
Smoking history				
Never	223	99	124	<0.001
Ever	395	110	285	
Age				
Mean ± SD	687	63.4 ± 12.5	65.0 ± 10.4	0.088
Tumor size				
Mean ± SD	671	3.5 ± 1.7	4.4 ± 2.3	<0.001
METΔ14 mutation				
Positive	18	18	0	<0.001
Negative	669	212	457	
Cappuzzo				
Positive	20	16	4	<0.001
Negative	667	214	453	
PathVysion				
Positive	8	8	0	<0.001
Negative	679	222	457	
High-level amplification				
Positive	24	23	1	<0.001
Negative	663	207	456	
MET FISH$^+$				
Positive	29	25	4	<0.001
Negative	658	205	453	
H-Amp	8	8	0	
Polysomy	9	9	0	
L-Amp/H-GCN	7	6	1	
L-Amp/L-GCA	5	2	3	
MET DNA alterations				
Positive	41	37	4	<0.001
Negative	646	193	453	

[a]Versus all other histologic types.

All tumors with MET H-Amp ($n = 8$) and polysomy ($n = 9$) displayed strong protein expression. MET IHC thus had 100% sensitivity and negative predictive value for the detection of *MET* H-Amp and polysomy (Supplementary Table S5). Good correlation between IHC and FISH was also observed in L-AMP/H-GCN group. Six of 7 L-AMP/H-GCN (85.7%) tumors were IHC$^+$. On

Table 3. Univariable and multivariable OS analysis in patients with NSCLC

	Univariable analysis		Multivariable analysis	
Parameter	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Older age	1.288 (0.979–1.695)	0.07	\	\
Male gender	1.251 (0.952–1.644)	0.109	\	\
Ever-smoking	1.338 (1.013–1.768)	0.04	1.265 (0.927–1.725)	0.138
Advanced pathologic stage	4.444 (3.285–6.031)	<0.001	4.707 (3.387–6.541)	<0.001
Nodal metastasis[a]	2.843 (2.184–3.702)	<0.001	\	\
Tumor size (cm)[a]	1.105 (1.038–1.176)	0.002	\	\
METΔ14$^+$	1.993 (1.023–3.882)	0.043	2.156 (1.096–4.242)	0.026
MET high-level Amp	4.904 (2.017–11.927)	<0.001	3.444 (1.398–8.482)	0.007
MET IHC$^+$	0.886 (0.673–1.166)	0.387	\	\
EGFR$^+$	0.693 (0.516–0.931)	0.015	0.748 (0.531–1.054)	0.097

[a]Nodal metastasis and tumor size were not included in multivariable analysis to avoid multicolinearity.

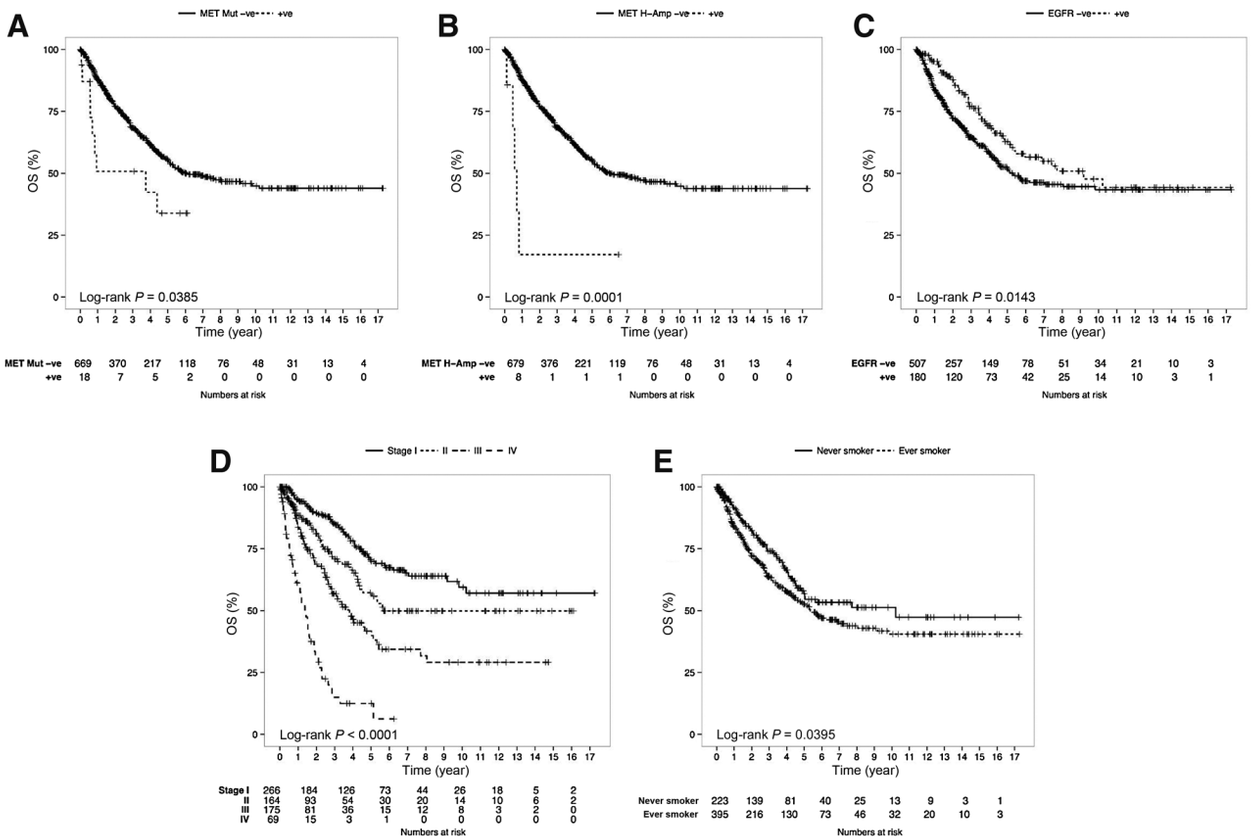

Figure 3.
Kaplan–Meier survival curve for OS in NSCLC according to *MET*Δ14 mutation (A); *MET* H-Amp (B); EGFR mutation (C); pathologic stage (D); and smoking status (E).

the contrary, only 2 of 5 cases of L-Amp/L-GCN (40%) were IHC^+ (Supplementary Fig. S1). All 4 $FISH^+/IHC^-$ tumors, which included 3 SCC and 1 LELC, harbored L-Amp. The clinical significance of $FISH^+/IHC^-$ SCC and LELC remained to be defined.

A total of 205 cases were IHC(+)/FISH(−). Among them, 12 cases had *MET*Δ14 mutation. The remaining 193 cases were heterogeneous in mutation status comprising EGFR mutation ($n = 73$), KRAS mutation ($n = 33$), ALK translocation ($n = 18$), and ROS1 translocation ($n = 4$). Mutation on these genes was not detected in 65 IHC(+)/FISH(−) cases.

Survival analysis

The median follow-up time was 31.6 months (range, 0.5–207.8 months). Univariable analysis revealed that advanced pathologic stage ($P < 0.001$), ever-smoking history ($P = 0.04$), presence of nodal metastasis ($P < 0.001$), larger tumor size ($P = 0.002$), *MET*Δ14 mutation ($P = 0.043$), high-level *MET* amplification ($P < 0.001$), and EGFR mutation ($P = 0.015$) associated with shorter overall disease-specific survival (Table 3). Representative Kaplan–Meier curves using the log-rank test showing the OS of all NSCLC patients were showed in Fig. 3.

Multivariable analysis of patients with NSCLC demonstrated that in addition to stage ($P < 0.001$), METΔ14 mutations (HR, 2.156; 95% CI, 1.096–4.242; $P = 0.026$) and high-level *MET* amplification (HR, 3.444; 95% CI, 1.398–8.482; $P = 0.007$) were independent poor prognostic factors (Table 3).

Discussion

MET DNA alterations including *MET*Δ14 mutations and *MET* amplification are therapeutic relevant recurrent events. The clinicopathologic characteristics, ethnic distribution, and prognostic implications of *MET*Δ14 mutations and *MET* amplification in treatment-naive NSCLC are yet to be defined. Furthermore, most studies were conducted in Caucasian populations (1, 7, 17–20) and data concerning MET DNA alterations in Asian is scanty (8, 21, 22). To our knowledge, current study represents the largest cohort for the parallel assessment of *MET*Δ14 mutations, *MET* copy number, and protein expression in NSCLC.

As there is no consensus in MET FISH scoring, we adopted three commonly used scoring systems for the interpretation of MET FISH results. Cappuzzo system considered both polysomy and true amplification as evidence of $FISH^+$. PathVysion revealed true amplification only which included both L-Amps ($2 \leq$ MET/CEP7 < 5) and H-Amps (*MET*/CEP7 ratio ≥ 5). We also included a stringent cut-off system that only considered amplification-Amp as $FISH^+$. This category is more clinically relevant as data from clinical studies have suggested the responsiveness to anti-MET therapy in patients with H-Amp (5, 6).

Our result showed that almost all polysomy tumors (8/9) harbored other driver mutations, that is, EGFR or KRAS, suggesting polysomy is unlikely a driver event in NSCLC. Concurrent low-level *MET* amplification was detected in 0.56% (1/180) of EGFR mutant and 1.64% (1/61) KRAS mutant NSCLC. This is in

keeping with previous report demonstrating *MET* amplification was not mutually exclusive to EGFR/KRAS mutations in treatment-naïve patients and thus did not fulfill the criteria of oncogenic driver (23). However, we found that high-level *MET* amplification (*MET*/CEP7 ratio $\geq$5) was mutually exclusive to the major driver events in RTK/RAS/PI3K axis except METΔ14. Our data showed a significant association between *MET* DNA CNAs and *METΔ*14 mutation. Such observations have been reported in EGFR, KRAS, and other oncogenes that activation mutations positively correlated with gene copy number though the underlying mechanisms remain to be elucidated. Mutant allele specific imbalance of oncogenes has been noted in human cancers (24). Although activating in one single allele of an oncogene is believed to be sufficient to drive tumorigenesis, concurrent mutation, and copy number gain are frequently found in tumors harboring mutations. These genetic alterations may have synergistic effect playing a greater role in development and maintenance of malignant phenotype. Although occurred in a low frequency, H-Amp associated with strong MET protein expression and poorer prognosis. We further demonstrated that H-Amp was an independent prognostic factor by multivariable analysis. Our result suggested that H-Amps (*MET*/CEP7 ratio $\geq$5) might be a good criterion that defines a molecular subset with poorer prognosis and potentially benefit from MET inhibitors.

MET exon 14 skipping mutations are not common but have been reported in diverse cancer types including lung cancer, glioblastoma multiforme, head and neck SCC as well as in cancer cell lines H596 (lung ADSQ; ref. 7), Hs746T (gastric cancer; ref. 25) and HCC2218 (breast cancer; ref. 26). Although *METΔ*14 mutations are less common than EGFR and KRAS, it has been detected in up to 5% of lung adenocarcinoma, a figure that is comparable with ALK translocations. This represents an additional target with proven sensitivity toward MET mAb METMab (7) and MET TKIs (crizotinib and carbozantinib; refs. 9, 17, 19). Given the high prevalence of NSCLC worldwide, anti-MET targeted therapy could potentially benefit thousands of patients each year.

The incidences of *METΔ*14 mutation and *MET* H-Amp were 2.6% and 1.0%, respectively, in lung adenocarcinoma, and 2.6% and 1.2% in NSCLC. The mutation rates are comparable with previous reports (1, 7–9, 20–22) and no significant ethnic difference across East Asian and Caucasian was found. *METΔ*14 mutation was not found in SCC ($n = 180$) in the current study. Surprisingly, we found a significantly higher frequency of *METΔ*14 mutation in PSC (31.8%) compared with other histologic subtypes. This is the first study parallel comparing MET status across different histologic subtypes of NSCLC and demonstrating high METΔ14$^+$ in PSC. We also found frequent high-level *MET* amplification in PSC (13.6%). The result is in keeping with recent studies showing frequent MET alterations in PSC (16, 27). PSC is a group of poorly differentiated NSCLC containing components of sarcoma or sarcoma-like elements according to 2014 WHO classification. Five subtypes are recognized: pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma, and pulmonary blastoma. It is considered a rare but distinct entity comprising approximately 1% of all malignant neoplasms of lung. The biology of sarcomatoid carcinoma is poorly understood. They generally run an aggressive clinical course and are resistant to chemotherapy due to heterogeneity (28–30). The genetics of sarcomatoid carcinoma is largely unexplorered. Identification of MET activating mutation in NSCLC with sarcomatoid differentiation is encouraging. As MET is implicated in the epithelial mesenchymal transition process (3, 31), activation of MET might affect the differentiation state of the tumor cells. This raises a possibility that MET inhibition may aid in treating this specific subtype of lung cancer.

MET protein overexpression was detected in 33.5% of treatment-naïve NSCLC by IHC. However, only 16.1% of IHC$^+$ tumors harbor *MET* DNA alterations, that is, *METΔ*14 and/or CNA. Majority of the IHC$^+$ tumors do not have MET genetic alteration in DNA level. This might be one of the reasons that MET IHC failed to predict response to anti-MET mAb therapy in a recent phase III trial (32). Although MET protein overexpression can be found in up to 65% of lung adenocarcinoma (11), most of them are driven by secondary events that promote tumor growth and progression but not oncogenic drivers for the individual tumor. As a matter of fact, the most common mechanism for MET activation in cancer is protein overexpression as a consequence of transcriptional upregulation. Many factors include other oncogenes, hypoxia-induced factors, cytokines, or proangiogenic factors secreted by the reactive stroma or ligand-dependent autocrine or paracrine loop contribute to the transcriptional upregulation of MET (33). According to oncogene addict model, such cases may have additional genetic lesions attenuating the dependence of tumor cells on MET signaling and therefore fail to respond to anti-MET targeted therapy. This underscores the importance of identifying key driver events that define specific subset of patients likely to benefit from targeted therapy for patient management in personalized medicine.

Nevertheless, our results demonstrated good correlation between MET IHC and DNA alterations. Especially for the detection of high-level *MET* amplification and polysomy, IHC was highly sensitive and had a 100% negative predictive value. IHC is a routine technique in most pathologic laboratory for sensitive and reliable detection of protein expression. The high negative predictive value of MET IHC for the presence of MET DNA alteration allows for a fast screening for patients with NSCLC to join proper molecular test.

In conclusion, oncogenic *MET* DNA alterations defined 3.3% of NSCLC patients with aggressive diseases and older age. We found significant enrichment of *MET* DNA alterations in PSC. MET inhibition may aid in treating this specific subtype of lung cancer.

Disclosure of Potential Conflicts of Interest

T.S.K. Mok has received speaker's bureau honoraria from ACEA Biosciences, Amgen, Astrazeneca, BI, BioMarin, Clovis Oncology, Eli Lilly, GSK, Janssen, MSD, Novartis, Pfizer, Roche/Genentech, SFJ, and Vertex; and is a consultant/advisory board member for ACEA Biosciences, Astrazeneca, BI, BioMarin, Clovis Oncology, Eli Lilly, GSK, Janssen, Merck Serono, MSD, Novartis, Pfizer, Roche/Genentech, SFJ, and Vertex. No potential conflicts of interest were disclosed by the other authors.

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Received August 25, 2015; revised January 11, 2016; accepted January 16, 2016; published OnlineFirst February 4, 2016.

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