Original Study

Alterations of *MET* Gene Copy Number and Protein Expression in Primary Non–Small-Cell Lung Cancer and Corresponding Nodal Metastases

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Abstract

Alterations of the potential therapeutic target mesenchymal epithelial transition factor (MET) were assessed in 300 non–small-cell lung cancer specimens including 93 with nodal metastases using immunohistochemistry and fluorescent in situ hybridization. We found that MET alterations were more frequently seen in metastases than primary tumors and were associated with better overall survival of patients.

Introduction: Mesenchymal epithelial transition factor (MET) is a promising therapeutic target in non–small-cell lung cancer (NSCLC) but there are limited data about MET alterations in treatment-naive NSCLC and whether or not these changes are consistent between primary tumors and metastases. We aimed to investigate concordance, clinicopathological correlations, and prognostic value of MET alterations in primary NSCLC and corresponding nodal metastases. **Materials and Methods:** *MET* gene copy number (GCN) status was evaluated using fluorescent in situ hybridization (FISH) and MET protein expression using immunohistochemistry (IHC) in tissue microarray sections from a retrospective cohort of 300 surgically resected NSCLCs including 93 cases with nodal metastases. **Results:** Primary NSCLCs were MET IHC positive in 28 (10.3%) of cases and *MET* FISH positive (high polysomy or amplification) in 22 (8.1%) but only 1 (0.4%) showed amplification. In metastases, high *MET* GCN (18.3%) and protein expression (21.3%) was more frequent compared with primary tumors. The status of MET in lymph nodes significantly correlated with MET status in the corresponding primary tumors. Squamous cell carcinomas showed lower MET overexpression compared with nonsquamous tumors but there were no other associations with clinicopathological characteristics. Patients with tumors that were either *MET* FISH positive or IHC positive had a significantly better overall survival in univariate and multivariate analyses. **Conclusion:** Alterations of MET are more commonly seen in nodal metastases than primary tumors and this might have implications for their utility as predictive biomarkers to select patients for MET inhibition. MET overexpression and MET high polysomy occur in a low proportion of primary NSCLCs and is associated with a good prognosis.

Clinical Lung Cancer, Vol. ■, No. ■, ■-■

Keywords: Biomarker, Fluorescent in situ hybridization, Hepatocyte growth factor receptor, Immunohistochemistry, Prognosis

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Submitted: Apr 10, 2015; Revised: Aug 5, 2015; Accepted: Aug 11, 2015

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1525-7304/$ - see frontmatter
http://dx.doi.org/10.1016/j.cllc.2015.08.002

Introduction

Mesenchymal epithelial transition factor (MET), also known as hepatocyte growth factor receptor—HGFR—is a 126,193-base pair gene located on chromosome 7q31. It encodes for a primary single chain precursor protein of 1390 amino acids, which is post-transcriptionally cleaved to produce the α and β subunits. The mature MET protein is a heterodimer made of an α and a β subunit, which has tyrosine kinase activity.[1]

Mesenchymal epithelial transition factor signaling is involved in regulation of cell proliferation and differentiation, organ regeneration, and embryogenesis. Activation of MET signaling through protein overexpression, gene amplification, or mutations has been implicated in many cancers including non−small-cell lung cancer (NSCLC), glioblastoma, hepatoblastoma, and hereditary papillary renal carcinomas.[2] In addition, *MET* amplification is responsible for approximately 20% of acquired resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) treatment in lung adenocarcinomas.[3]

Inhibition of MET pathway signaling such as with monoclonal antibodies or TKIs could potentially be used clinically to treat NSCLC patients with alterations in this pathway including those who have developed resistance to EGFR TKIs.[4] There is evidence from clinical trials that used anti-MET monoclonal antibodies that MET immunohistochemistry (IHC) could be a useful predictor of patient survival,[5] however, no improvement in survival was found in a confirmatory phase III trial regardless of MET status.[6] The MET/anaplastic lymphoma kinase TKI crizotinib has shown high response rates in patients with intermediate or high levels of *MET* amplification (*MET*/chromosome enumeration probe for chromosome 7 [*CEP7*] ratio > 2.2) in a phase I clinical trial.[7] Currently however, MET biomarkers in NSCLC have not been well characterized and there is virtually no information regarding the consistency of MET alterations in primary tumors compared with metastatic disease. Only 1 previous study has investigated *MET* in primary tumors and corresponding metastases and they reported a higher incidence of *MET* amplification in nodal metastases compared with primary tumors[8] but the study only included 22 patients and used reverse transcription polymerase chain reaction, a technique not commonly used in routine clinical practice. In our study we aimed to investigate the concordance of alterations of *MET* gene copy number and protein expression between primary lung NSCLCs and their corresponding nodal metastases. We also aimed to investigate the prognostic value and clinicopathological associations of *MET* gene copy number and protein expression alterations in the setting of resectable stage I-III NSCLC.

Materials and Methods

Patient Cohort and Tissue Microarray Construction

This retrospective study was conducted in a cohort of 256 treatment-naive NSCLC patients who underwent resection of a primary NSCLC at Royal Prince Alfred Hospital, Sydney, Australia. Surgery was performed by the one cardiothoracic surgeon (B.M.) from 1994 to 2002. To extend the sample size for correlative analyses between primary and lymph node metastatic tumors, an additional cohort of 44 NSCLC patients with primary and regional lymph node metastases resected from 2010 to 2012 was also selected, for a total of 300 cases. Tumor subtype was determined using the World Health Organization 2004 classification[9] by an experienced lung pathologist (W.A.C.). Staging was determined using the 7th edition American Joint Committee on Cancer tumor, node, metastases (TNM) classification.[10]

Tissue microarrays (TMAs) were constructed using a Manual Tissue Arrayer (MTA-1, Beecher Instruments Tissue Arrayer) with core size of 1 mm in diameter as previously described.[11,12] Each patient was represented by 2 to 4 tissue cores of normal bronchial epithelium obtained from bronchi or bronchioles, 1 to 2 tissue cores of normal peripheral lung parenchyma, and 3 to 6 tumor cores that were selected from areas previously marked by a specialist lung pathologist (W.A.C.). In cases with lymph node metastatic disease, 1 to 6 tumor cores of each metastatic deposit were included, with the number of cores limited by the size of the metastatic deposits. There were only 2 patients who had only 1 core of their metastatic deposit in the tissue microarrays, and both of whom were excluded from the analysis because there was no evaluable cancer cells in the sections.

Mesenchymal epithelial transition factor gene copy number was determined using fluorescent in situ hybridization (FISH). MET protein expression was determined using immunohistochemical staining. *MET* FISH and IHC were able to be evaluated in 276 (92%) and 294 cases (98%), respectively. The number of patients in whom *MET* FISH and IHC were available for analysis was 271 (90.3%) (see Supplemental Figure 1 in the online version). The study was approved by the Royal Prince Alfred Ethics Review Committee (X06-0167 and X10-0278) with all tissue specimens analyzed anonymously.

Mesenchymal Epithelial Transition Factor FISH

Fluorescent in situ hybridization was performed on 4-μm sections of tissue microarrays using Vysis *MET* SpectrumRed FISH Probe Kit (Abbott) and Vysis *CEP7* SpectrumGreen (Abbott Molecular) according to the manufacturer's instruction. In brief, after being baked at 60° C overnight, slides were submitted to deparaffinization, slide pretreatment, and protease pretreatment procedures. In the next stage, after a step of denaturation at 95° C for 5 minutes, each slide was hybridized with a 10-μL cocktail of *MET*/*CEP7* probes at 37° C for 17 hours. Finally, the slides were washed with a solution of 2× sodium chloride sodium citrate and 0.3% Nonyl Phenoxypolyethoxyethanol 40 at 75° C for 2 minutes and counterstained with 10 μL of 4′,6-diamidino-2-phenylindole.

Signals were counted in at least 50 nonoverlapping tumor nuclei per core using an epifluorescence microscope (Zeiss). The mean copy number per cell of each probe (*MET* and *CEP7*), the percentage of cells with *MET* signal clusters, and the percentage of cells with 4 copies or more of the *MET* signal were recorded for outcome interpretation.

Tumors were classified into 2 groups: *MET* FISH positive (amplification or high polysomy) and *MET* FISH negative (low copy number or normal) as previously described.[13] Cases were considered as *MET* amplification if 1 of the following conditions was satisfied: *MET*/*CEP7* ratio ≥ 2.0; presence of tight gene clusters in ≥ 10% of cells; or ≥ 10% of tumor cells that contained ≥ 15 *MET* signals. Cases were considered as high-level polysomy if they did not fulfil criteria for amplification but ≥ 40% of tumor cells contained ≥ 4 *MET* signals. Otherwise, a tumor was defined as *MET* FISH negative.[13]

Immunohistochemical Staining

A 4-μm section of each TMA was stained using a Benchmark Ultra Autostainer (Ventana) with CONFIRM anti-total c-MET (SP44) rabbit monoclonal primary antibody (Ventana), using the manufacturer's instruction. In brief, sections were incubated at 65° C for 20 minutes before being deparaffinized at 75° C for 16 minutes using EZ Prep (Ventana), then treated with Cell Conditioning 1 solution—CC1 (Ventana)—at 95° C to hydrolyze the covalent bonds formed by formalin in tissue. The slides were then incubated with anti-Total c-MET antibody at 37° C for 16 minutes. Finally, the total c-MET protein–antibody complex was visualized with UltraView DAP Universal Detection Kit (Ventana) followed by Hematoxylin II (Ventana) and Bluing Reagent (Ventana) counterstaining.

For each case, total c-MET immunohistochemical staining of primary tumor cores, bronchial mucosa cores, and metastatic lymph node cores was evaluated using light microscopy. Staining was scored by determining the percentage of cells showing weak (1+), moderate (2+), or strong (3+) membranous staining. Scoring was undertaken independently by W.A.C. and T.N.T. and any discrepant cases were re-evaluated and a consensus score reached. Tumors were considered total c-MET positive (MET IHC positive) if at least 50% of tumor cells showed cellular membrane staining at intensity 2+ or 3+ as previously described in a biomarker analysis from a clinical trial of a MET inhibitor.[5]

Statistical Analysis

R version 2.15.2 (The R Foundation for Statistical Computing) was used for all statistical analyses. The normality of variables was tested using Shapiro–Wilks test. Association with clinicopathological variables was tested using Pearson χ^2 with Fisher exact test (categorical variables) and 2-sample *t* test (continuous variables) or Cramer V and Wilcoxon–Mann–Whitney tests for parametric and nonparametric testing, respectively. Overall survival estimation and comparison were calculated using the Kaplan–Meier method and log rank tests. Statistical significance was set at $P < .05$ for 2 sides. Multivariate analysis was performed using Cox proportional hazard regression model with a forward stepwise method. The univariate and multivariate analyses were explored on the basis of the following clinicopathological factors: age, sex, TNM stage, histological type and grade, lymphatic, vessel, and perineural invasions and *MET* gene copy number and protein expression status. Only factors with a significant *P* value or at least a *P* value < .3 in univariate analysis were tested in multivariate analysis using a Cox proportional hazard regression model with a forward stepwise method.

Results

Mesenchymal Epithelial Transition Factor Gene Copy Number and Protein Expression in Primary Tumors and Nodal Metastases

In primary lung tumor 8.1% (22 of 271) of NSCLCs were *MET* FISH positive with 7.7% (21 of 271) showing *MET* high polysomy and 0.4% (1 of 271) showing *MET* amplification (Figure 1A and C). The case with amplification had a *MET:CEP7* ratio of 2.0 which just fulfilled criteria for amplification (ratio ≥ 2), and did not show any tight gene clusters. The incidence of MET protein overexpression was 10.3% (28 of 271) (Figure 1A and D). There was a statistically significant relationship between *MET* FISH positivity and MET IHC positivity in primary tumors, although the correlation was modest (Cramer V = 0.21; $P < .05$) with only 7 cases being positive for *MET* FISH and MET IHC.

There was a significantly greater incidence of *MET* FISH and MET IHC positivity in metastatic lymph nodes compared with primary tumors. Nodal metastases were *MET* FISH positive in 18.3% compared with 8.1% in primary tumors ($P < .05$). Similarly, nodal metastases were MET IHC positive in 21.3% compared with 10.3% of primary tumors (Figure 1B).

The status of *MET* FISH and MET IHC in nodal metastases were highly correlated with their corresponding primary tumors (Cramer V = 0.6 and 0.7, respectively; $P < .01$; Table 1). All patients who were positive for MET IHC in primary tumors were also positive in nodal metastases and for *MET* FISH this was true for most cases (80%). In contrast, 11.9% of nodal metastases that were MET IHC positive were negative in their matched primary tumor and 9.7% of *MET* FISH positive nodal metastases were negative in the corresponding primary tumor (Table 1).

Correlation of MET With Clinicopathological Features

In primary tumors, there was no significant association between *MET* FISH or MET IHC status and clinical features including patient age, sex, smoking status, tumor size, or TNM stage (Table 2). There was no correlation between histological subtypes of NSCLC and status of *MET* FISH, however, MET IHC protein expression level was significantly lower in squamous cell carcinoma compared with nonsquamous subtypes (adenocarcinoma and large cell carcinoma) in primary tumors ($P < .001$). In nodal metastases, MET IHC positivity was associated with adenocarcinomas ($P < .01$; Table 3) and female sex ($P < .05$) but *MET* FISH status was not significantly associated with any clinicopathological features.

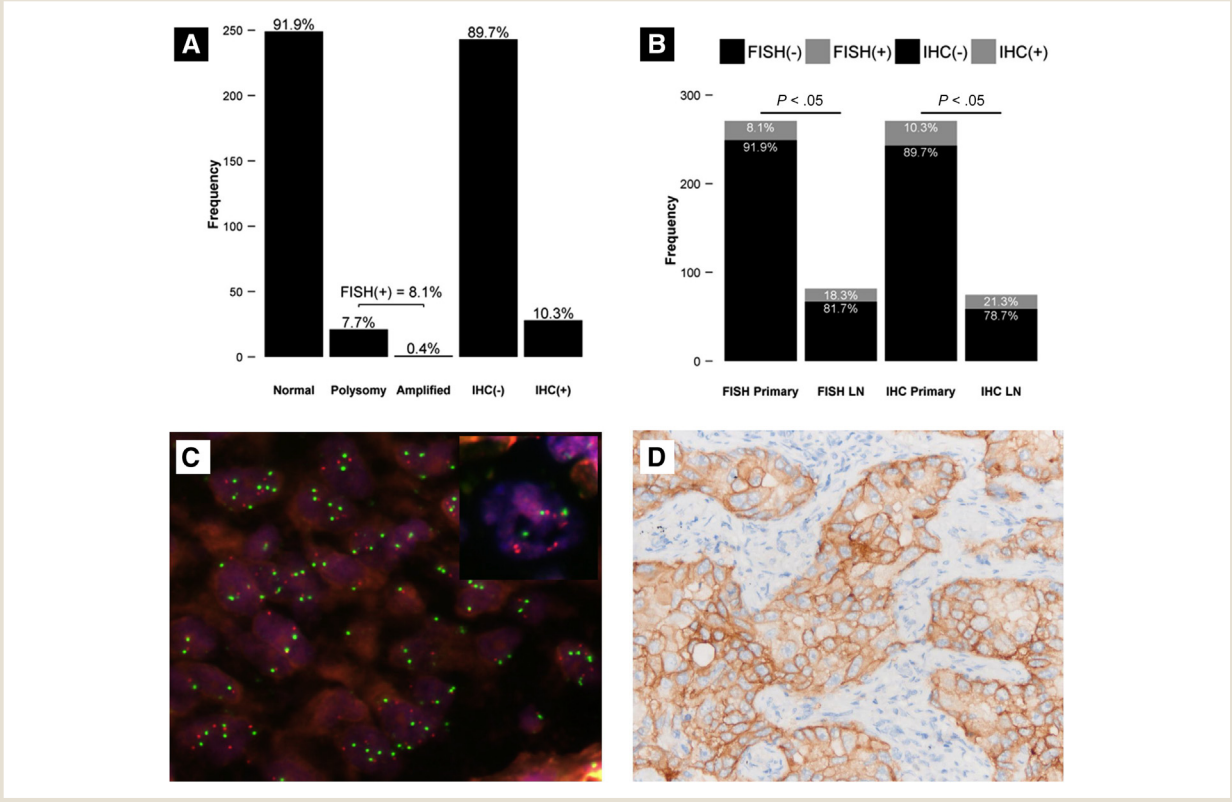
Prognostic Value of MET Gene Copy Number and Protein Expression

In survival analysis, patients with primary tumors that were either *MET* FISH positive or MET IHC positive had significantly better overall survival ($P < .05$ and .01, respectively; Figure 2). For multivariate analysis, clinicopathological features were explored individually as prognostic factors: stage (II-III vs. I), vessel invasion, lymphatic invasion, perineural invasion, histological subtype, tumor grade, *MET* FISH status, and MET IHC status. Factors that showed statistical significance ($P < .05$) were included in the Cox regression model to test their independent prognostic value. Multivariate analysis showed *MET* FISH positivity (hazard ratio [HR], 0.35; 95% confidence interval [CI], 0.15-0.8) or MET IHC positivity (HR, 0.53; 95% CI, 0.31-0.92) were independent favorable prognostic factors along with lower stage and absence of lymphatic invasion (Table 4). MET IHC or FISH status in nodal metastases was not associated with any differences in overall survival, although the number of cases in this group was relatively small.

Discussion

To our knowledge, this is the first study to investigate *MET* gene copy number and protein expression in the same cohort of primary NSCLC and corresponding nodal metastases. The frequency of *MET* high polysomy in our study was 7.7% in primary tumors,

Figure 1 (A) Frequency of *MET* FISH Positivity (High Polysomy or Amplification) and MET IHC Positivity in Primary NSCLC. (B) Correlation of *MET* FISH and MET IHC Status Between Primary Tumors and Their Corresponding Nodal Metastases. There Was a Significantly Greater Frequency of *MET* FISH Positive and MET IHC Positive Cases in Nodal Metastases Compared With Primary Tumors ($P < .05$). (C) Primary NSCLC Showing MET High Polysomy Using FISH (≥ 40% of Tumor Cells With ≥ 4 Red *MET* Signals) and Another Case Showing Amplification (Inset) (*MET/CEP* Ratio ≥ 2.0). (D) Positive MET Protein Expression Was Demonstrated by Strong Membranous IHC Staining in at Least 50% of Tumor Cells



Abbreviations: FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; LN = lymph node; MET = mesenchymal epithelial transition factor; NSCLC = non–small-cell lung cancer.

which is comparable with rates of high polysomy reported in previous studies (7.6%-12.8%) using the same scoring criteria.[14-17] Similarly, using an alternative scoring method, high *MET* copy number (≥ 5 signals) has been reported in 7.8% to 11.0% of NSCLC.[18,19] However, *MET* amplification was very rare in our study (only 1 case—0.4%) compared with a reported range of 2.1% to 4.1% in other studies of primary NSCLC.[14,15,19-21] These previous studies included patients with higher stage disease (stage I-IV)[14,19-21] than our study, however, and in at least 1 study, higher stage disease was associated with higher rates of *MET* amplification in the primary tumor.[18]

We selected our IHC scoring threshold for determining high MET protein expression on the basis of the predictive biomarker analysis from an anti-MET clinical trial[5] and found 10.3% of NSCLC had high MET expression. Using the same scoring criteria, another study found high MET protein expression in 25%

Table 1 Concordance of *MET* FISH and IHC Status in Primary Tumors and Nodal Metastases

		Metastatic Lymph Node		*P*	Cramer V
		***MET* FISH(−)**	***MET* FISH(+)**		
Primary Tumors	*MET* FISH(−)	65 (90.3%)	7 (9.7%)	<.01	0.6
	MET FISH(+)	2 (20%)	8 (80%)		
		MET IHC(−)	**MET IHC(+)**		
	MET IHC(−)	59 (88.1%)	8 (11.9%)	<.01	0.7
	MET IHC(+)	0 (0%)	8 (100%)		

Abbreviations: FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; MET = mesenchymal epithelial transition factor.

Table 2 Correlation Between MET Status and Clinicopathological Characteristics in Primary NSCLC

Category	*MET* FISH(−)	*MET* FISH(+)	*P*	MET IHC(−)	MET IHC(+)	*P*
Median Age (Range)	68 (41-87)	65.5 (50-83)	.555	67 (41-87)	69 (51-83)	.323
Median Tumor Size (Range), mm	35 (7-150)	32 (20-60)	.681	35 (7-150)	34 (15-110)	.847
Sex						
Female	88 (89.8)	10 (10.2)	.361	87 (88.8)	11 (11.2)	.836
Male	161 (93.1)	12 (6.9)		156 (90.2)	17 (9.8)	
Smoking						
Ex- or current smoker	191 (90.5)	20 (9.5)	1	187 (88.6)	24 (11.4)	.288
Never smoked	9 (100)	0 (0)		7 (77.8)	2 (22.2)	
Histology						
ADC	116 (89.9)	13 (10.1)	.798	110 (85.3)	19 (14.7)	<.01
SCC	86 (93.5)	6 (6.5)		91 (98.9)	1 (1.1)	
LCC	40 (93)	3 (7)		35 (81.4)	8 (18.6)	
Mixed	3 (100)	0 (0)		3 (100)	0 (0)	
Other	4 (100)	0 (0)		4 (100)	0 (0)	
AJCC 7 Stage						
I	94 (93.1)	7 (6.9)	.109	89 (88.1)	12 (11.9)	.712
II	120 (93.8)	8 (6.2)		115 (89.8)	13 (10.2)	
III	35 (83.3)	7 (16.7)		39 (92.9)	3 (7.1)	
T Stage						
1	70 (93.3)	5 (6.7)	.962	69 (92)	6 (8)	.207
2	132 (91)	13 (9)		126 (86.9)	19 (13.1)	
3	34 (91.9)	3 (8.1)		36 (97.3)	1 (2.7)	
4	13 (92.9)	1 (7.1)		12 (85.7)	2 (14.3)	
N Stage						
0	147 (93)	11 (7)	.192	140 (88.6)	18 (11.4)	.747
1	84 (92.3)	7 (7.7)		82 (90.1)	9 (9.9)	
2	18 (81.8)	4 (18.2)		21 (95.5)	1 (4.5)	
Grade						
1	24 (100)	0 (0)	.336	23 (95.8)	1 (4.2)	.09
2	121 (90.3)	13 (9.7)		124 (92.5)	10 (7.5)	
3	103 (92)	9 (8)		95 (84.8)	17 (15.2)	
Vascular Invasion						
Absent	207 (91.6)	19 (8.4)	1	203 (89.8)	23 (10.2)	.792
Present	42 (93.3)	3 (6.7)		40 (88.9)	5 (11.1)	
Lymphatic Invasion						
Absent	208 (92.9)	16 (7.1)	.236	202 (90.2)	22 (9.8)	.597
Present	41 (87.2)	6 (12.8)		41 (87.2)	6 (12.8)	
Perineural Invasion						
Absent	233 (92.1)	20 (7.9)	.647	225 (88.9)	28 (11.1)	.232
Present	16 (88.9)	2 (11.1)		18 (100)	0 (0)	

Data are presented as n (%), except where otherwise stated.
Abbreviations: ADC = adenocarcinoma; AJCC 7 = American Joint Committee on Cancer Seventh Edition; FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; LCC = large cell carcinoma; MET = mesenchymal epithelial transition factor; NSCLC = non–small-cell lung cancer; SCC = squamous cell carcinoma.

of tumors[19] and others have used different scoring criteria and reported high MET expression in 22.2%[16] and 13.7% of NSCLC.[15]

We have shown that alterations of *MET* gene copy number and protein expression are more frequent in metastases than primary tumors, a finding that could have implications on biomarker testing in clinical practice when biopsies might be taken from the primary tumor regardless of the disease stage. Interestingly, although the frequency of MET positivity was greater in nodal metastases, MET status in primary tumors did not significantly correlate with higher

Table 3 Correlation Between MET Status in Metastatic LNs and Clinicopathological Characteristics

Category	LN *MET* FISH(−)	LN *MET* FISH(+)	*P*	LN MET IHC(−)	LN MET IHC(+)	*P*
Median Age (Range), Years	68 (48-78)	62 (42-81)	.24	67 (42-81)	64 (48-80)	.66
Median Tumor Size (Range), mm	35 (12-100)	32 (18-75)	.76	34 (12-100)	36 (20-85)	.61
Sex						
Female	29 (76.3)	9 (23.7)	.27	22 (64.7)	12 (35.3)	<.05
Male	38 (86.4)	6 (13.6)		37 (90.2)	4 (9.8)	
Smoking						
Ex-/Current smoker	49 (79)	13 (21)	1	44 (75.9)	14 (24.1)	.254
Never smoked	1 (100)	0 (0)		0 (0)	1 (100)	
Histology						
ADC	37 (78.7)	10 (21.3)	.57	25 (64.1)	14 (35.9)	<.01
Non-ADC	30 (85.7)	5 (14.3)		34 (94.4)	2 (5.6)	
AJCC 7 Stage						
II	47 (87)	7 (13)	.13	40 (80)	10 (20)	.768
III	20 (71.4)	8 (28.6)		19 (76)	6 (24)	
T Stage						
1	18 (90)	2 (10)	.34	16 (88.9)	2 (11.1)	.328
2-4	49 (79)	13 (21)		43 (75.4)	14 (24.6)	
N Stage						
1	53 (85.5)	9 (14.5)	.18	45 (81.8)	10 (18.2)	.341
2	14 (70)	6 (30)		14 (70)	6 (30)	
Grade						
1 and 2	33 (80.5)	8 (19.5)	1	31 (83.8)	6 (16.2)	.399
3	34 (82.9)	7 (17.1)		28 (73.7)	10 (26.3)	
Vascular Invasion						
Absent	52 (80)	13 (20)	.73	46 (78)	13 (22)	1
Present	15 (88.2)	2 (11.8)		13 (81.2)	3 (18.8)	
Lymphatic Invasion						
Absent	44 (81.5)	10 (18.5)	1	42 (84)	8 (16)	.139
Present	23 (82.1)	5 (17.9)		17 (68)	8 (32)	
Perineural Invasion						
Absent	58 (80.6)	14 (19.4)	.68	49 (76.6)	15 (23.4)	.439
Present	9 (90)	1 (10)		10 (90.9)	1 (9.1)	

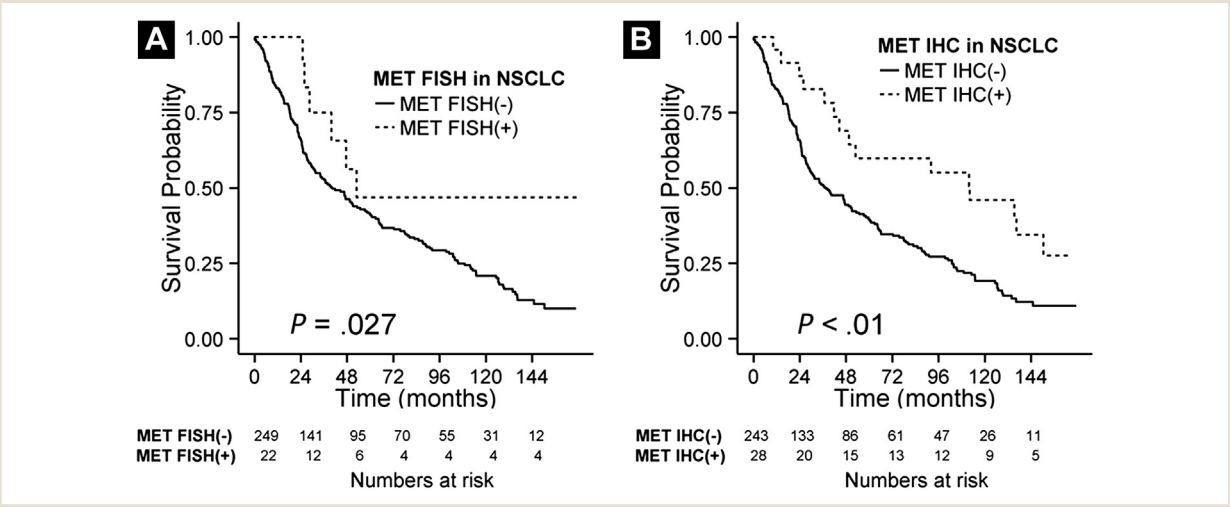
Data are presented as n (%), except where otherwise stated.
Abbreviations: ADC = adenocarcinoma; AJCC 7 = American Joint Committee on Cancer Seventh Edition; FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; LN = lymph node; MET = mesenchymal epithelial transition factor.

stage disease, suggestive that the alteration occurs later in disease progression after tumor spread has occurred. To our knowledge, only 1 previous study has been conducted to investigate *MET* in primary tumors and corresponding metastatic disease,[8] and in that small study, *MET* amplification was also significantly higher in nodal metastases than primary tumors using reverse transcription polymerase chain reaction.

In a recent study, Dziadziuszko et al showed a significant correlation between *MET* gene copy number and protein expression. In particular, all patients with > 5 *MET* copies per cell had high MET protein expression.[19] Schildhaus et al[21] also reported a significant correlation but they only analyzed IHC in a small subset of their cases. In our study, we also found a correlation between the status of *MET* FISH and MET IHC in either primary or nodal tumors, but not all cases were concordant, which suggests that there might be other factors involved in determining the expression of MET protein. Similarly, others have not found perfect concordance between *MET* FISH status and protein expression.[15,17]

As in previous studies[16,19] we did not find any association between patient sex, smoking status, age, or tumor stage and *MET* FISH status. In contrast, several studies have shown an association between *MET* FISH positivity and advanced-stage disease.[18,19] One study has shown an association between higher tumor grade and *MET* FISH positivity[16] and another reported no association.[19] In our study, all 24 cases with Grade 1 tumors (well differentiated) were *MET* FISH negative however

Figure 2 Comparison of Overall Survival in Primary NSCLC on the Basis of *MET* FISH Status (A) and MET IHC Protein Expression Status (B)



Abbreviations: FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; MET = mesenchymal epithelial transition factor; NSCLC = non–small-cell lung cancer.

there was no statistically significant association between tumor grade and *MET* FISH status. We found a significantly lower frequency of MET IHC protein expression in squamous cell carcinomas but no other significant clinicopathological correlations. Similarly, others have found that high MET IHC is more common in adenocarcinomas[16] but no association between MET IHC and patient age, sex, or tumor stage.[19]

Most studies have shown that alterations of *MET* gene copy number are not independently associated with patient outcome in NSCLC.[3,15,19,20,22-24] However, *MET* FISH positivity has been previously reported to be independently associated with shorter overall survival in 2 studies of stage I-IV NSCLC.[16,18] Similarly, MET protein expression has generally not been associated with patient survival,[16,19] or has been associated with a worse prognosis in univariate analysis only.[24] Only 1 study found that MET protein expression is an independent poor prognostic factor in NSCLC.[15]

Surprisingly, in our study, *MET* FISH positivity and MET overexpression were found to be predictors of longer overall survival in univariate and multivariate analyses. Because our findings are contrary to previous studies the results should be taken with

Table 4 Multivariate Analysis Incorporating Clinicopathological Features and MET Gene and Protein Expression Using the Cox Proportional Hazards Regression Model

		Univariate			Multivariate		
No.	**Prognostic Factor**	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
1	AJCC 7 TNM stage 2-3 versus stage 1	2.12	1.56-2.88	<.01	1.83	1.33-2.53	<.01
2	Vessel invasion (present vs. absent)	1.82	1.25-2.65	<.01	0.96	0.6-1.56	.88
3	Lymphatic invasion (present vs. absent)	2.51	1.65-3.83	<.01	2.69	1.62-4.47	<.01
4	Perineural invasion (present vs. absent)	2.09	1.2-3.62	<.05	1.74	0.91-3.35	.1
5	Histology (ADC vs. non-ADC)	1.16	0.95-1.43	.15	1.02	0.8-1.29	.89
6	Grade (3 vs. 1 or 2)	1.16	0.86-1.56	.32	1.23	0.88-1.71	.22
7	*MET* FISH (amplification vs. no amplification)	0.41	0.18-0.93	<.05	0.35	0.15-0.8	<.05
8	MET IHC (positive vs. negative)	0.47	0.27-0.8	<.05	0.53	0.31-0.92	<.05

Abbreviations: ADC = adenocarcinoma; AJCC 7 = American Joint Committee on Cancer Seventh Edition; FISH = fluorescent in situ hybridization; HR = hazard ratio; IHC = immunohistochemistry; MET = mesenchymal epithelial transition factor; TNM = tumor, node, metastases.

caution. Because all but 1 of the *MET* FISH positive cases in our study resulted from high polysomy rather than amplification, our results might suggest that high polysomy is a favorable prognostic factor. In fact, Cappuzzo et al showed a better prognosis for NSCLCs with 4 to 5 *MET* signals compared with those with < 4 signals,[18] similar to the high polysomy group in our study. Although the previous NSCLC studies that showed an association between increased *MET* gene copy number and poor prognosis used criteria similar to our study to determine FISH positivity, these studies had higher rates of *MET* amplification than in our study population (2.1%-4.1%).[14,15,18,19]

Conclusion

We comprehensively analyzed alterations of MET protein expression and gene copy number in a large cohort of treatment-naive primary NSCLC with matched nodal metastases. We showed that *MET* gene copy number correlates with protein expression although concordance is not perfect. Although there is a correlation between *MET* gene copy number status and protein expression in primary tumors and their nodal metastases, alterations of MET are more likely to be found in metastatic tumors. These findings could have implications for the utilization of these assays as potential predictive biomarkers in assessing anti-MET therapies. Furthermore, we have produced data suggestive that MET alterations (predominantly high polysomy) are prognostically favorable, challenging the previous literature and suggestive that further studies are needed to clarify the role of MET in NSCLC.

Clinical Practice Points

- Mesenchymal epithelial transition factor is a potential therapeutic target in NSCLC and increased MET protein expression or increased gene copy number are promising predictive markers. However, little is known about the concordance of these 2 alterations and no previous studies have investigated whether or not the alterations are consistent between primary tumors and nodal metastases.
- We have shown a statistically significant correlation between evaluation of MET protein expression using IHC and gene copy number alteration with FISH, which suggests that either technique might be suitable for assessment of MET status.
- Alterations of MET were significantly more frequent in nodal metastases than in primary tumors and this could have implications for assessment of the clinical utility of MET as a predictive marker because diagnostic tumor biopsies might be obtained from metastatic or primary tumors, even if the patient has advanced-stage disease. We suggest it is more appropriate to assess MET in biopsies taken from metastatic sites rather than primary tumors because it is more likely to reflect the MET status of more advanced disease.

Acknowledgments

Support from the National Foundation for Medical Research and Innovation, Cancer Institute New South Wales, Chris O'Brien Lifehouse at RPA Grant, and The Sydney Breast Cancer Foundation is gratefully acknowledged. Those providing funding support for the research were not involved in the study design, collection, analysis and interpretation of data, writing of the report or decision to submit the article for publication.

Disclosure

W.A.C. has received honoraria for participation in advisory boards and lectures from Pfizer oncology. S.A.O.T. has received honoraria for participation in advisory boards from Pfizer oncology. The remaining authors have stated that they have no conflicts of interest.

Supplemental Data

The supplemental figure accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.cllc.2015.08.002.

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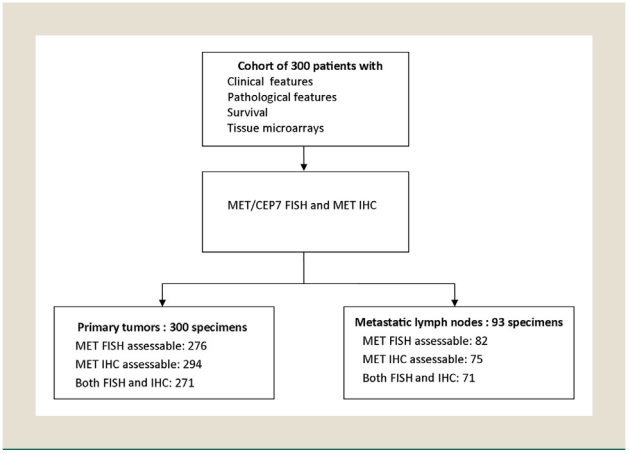
Supplemental Figure 1 Cohort Dropout Flow Diagram



Abbreviations: CEP7 = ■■■; FISH = fluorescent in situ hybridization; IHC = immunohistochemistry; MET = mesenchymal epithelial transition factor.