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

Joanna H. Tong1,2,3, Sai F. Yeung1,2,3, Anthony W.H. Chan1,2,3, Lau Y. Chung1,2,3, Shuk L. Chau1,2,3, Raymond Wai Ming Lung1,2,3, Carol Y. Tong1,2,3, Chit Chow1,2,3, Edith K.Y. Tin1,2,3, Yau H. Yu1,2,3, Hui Li1,2,3, Yi Pan1,2,3, Wing P. Chak1,2,3, Calvin S.H. Ng4, Tony S.K. Mok5,5, and Ka F. To1,2,3

**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-naive non–small cell lung carcinoma (NSCLC).

**Experimental Design:** Six hundred eighty-seven NSCLCs were tested for MET exon 14 splicing site mutation (MET14), DNA copy number alterations, and protein expression by Sanger sequencing, FISH, and IHC, respectively.

**Results:** MET14 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. MET14 mutation was not detected in squamous cell carcinoma, large cell carcinoma, and lympho-epithelioma-like carcinoma but significantly enriched in sarcomatoid carcinoma (P < 0.001). MET14 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 ≥5) was mutually exclusive to the major driver genes except MET14. Oncogenic MET14 mutation and/or high-level amplification occurred in a total of 3.3% (23/687) of NSCLC and associated with higher MET protein expression. MET14 occurred more frequently in older patients whereas amplification was more common in ever-smokers. Both MET14 and high-level amplification were independent prognostic factors that predicted poorer survival by multivariable analysis.

**Conclusions:** The high incidence of MET14 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
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 (MET Δ14) 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 MET Δ14 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 classified as MET Δ14 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 (MET Δ14) 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 IIA 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 CIUK-NTE Clinical Research Ethics 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).
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.). $\chi^2$ 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 expression was significantly higher in adenocarcinoma (P < 0.001), female (P < 0.001), and never-smokers (P < 0.001). Similarly, KRAS mutation was more common 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.

**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**
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.

**MET**14 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 MET14 mutation rates were 2.6% in adenocarcinoma, 4.8% in ADSQ, and 31.8% in PSC. No MET14 mutation was found in SCC, LCC, and LELC. A significantly higher MET14 mutation rate was found in PSC (P < 0.001). The mean age of MET14-positive patients was 73.7 years versus 64.2 years in MET14-negative patients (P = 0.001). There were no significant differences in gender distribution, smoking history, or stage between patients with or without MET14 mutation (Table 1). MET14 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 MET14-positive cases and found that all MET14 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 MET14 tumors were shown in Supplementary Table S2.

**Figure 1.**
Major driver events in NSCLC and adenocarcinoma of lung.
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 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 > 5; n = 8.
3. Low-level amplification/high gene copy number (L-Amp/H-GCN): 2 ≤ MET/CEP7 < 5, MET signal > 5; n = 7.
4. Low-level amplification/low gene copy number (L-Amp/L-GCN): 2 ≤ 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 MET14 mutation and MET CNA (P < 0.001). Among 29 FISH+ cases, 20.7% (6/29) showed MET14 mutation (Supplementary Table S4). Although in FISH+ group, only 1.8% (12/658) of the cases harbored MET14 mutation (Table 1). MET14 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 MET14, and 1 with KRAS mutation. Three of 12 L-Amp, including 1 L-Amp/L-GCN and 2 L-Amp/H-GCN, coocurred with EGFR, KRAS, or MET14. In 8 tumors with H-Amp, coexisting MET14 mutation was found in 3. Notably, H-Amp coexisted with MET14 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 SCC, and 5.6% in other histologic subtypes. MET14+/FISH+ cooccurred with EGFR, KRAS, or MET14. In 8 tumors with H-Amp, coexisting MET14 mutation was found in 3. Notably, H-Amp coexisted with MET14 only and was mutually exclusive of other driver genes (Supplementary Table S4).

Association between MET DNA alterations and MET protein expression

MET14 mutation status significantly correlated with MET IHC (P < 0.001). All MET14+ 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+.
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+.

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total n = 687</th>
<th>Positive n = 230</th>
<th>Negative n = 457</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>392</td>
<td>195</td>
<td>197</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SCC</td>
<td>180</td>
<td>10</td>
<td>170</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>LCC</td>
<td>45</td>
<td>7</td>
<td>38</td>
<td>0.008*</td>
</tr>
<tr>
<td>ADSQ</td>
<td>21</td>
<td>9</td>
<td>12</td>
<td>0.356*</td>
</tr>
<tr>
<td>LELC</td>
<td>27</td>
<td>0</td>
<td>27</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PSC</td>
<td>22</td>
<td>9</td>
<td>13</td>
<td>0.49*</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>223</td>
<td>95</td>
<td>128</td>
<td>0.001</td>
</tr>
<tr>
<td>Male</td>
<td>464</td>
<td>135</td>
<td>329</td>
<td></td>
</tr>
<tr>
<td><strong>Stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IA–IIIA</td>
<td>583</td>
<td>187</td>
<td>396</td>
<td>0.056</td>
</tr>
<tr>
<td>IIIB–IV</td>
<td>91</td>
<td>39</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking history</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>223</td>
<td>99</td>
<td>124</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ever</td>
<td>395</td>
<td>110</td>
<td>285</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>687</td>
<td>63.4 ± 12.5</td>
<td>65.0 ± 10.4</td>
<td>0.088</td>
</tr>
<tr>
<td><strong>MET DNA alterations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MET DNA alteration Positive</td>
<td>24</td>
<td>23</td>
<td>1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Negative</td>
<td>663</td>
<td>207</td>
<td>456</td>
<td></td>
</tr>
<tr>
<td><strong>MET IHC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>29</td>
<td>25</td>
<td>4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Negative</td>
<td>658</td>
<td>205</td>
<td>453</td>
<td></td>
</tr>
<tr>
<td><strong>PathVysion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>8</td>
<td>0</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Negative</td>
<td>679</td>
<td>222</td>
<td>457</td>
<td></td>
</tr>
<tr>
<td><strong>High-level amplification</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>41</td>
<td>37</td>
<td>4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Negative</td>
<td>646</td>
<td>193</td>
<td>453</td>
<td></td>
</tr>
</tbody>
</table>

*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...
the contrary, only 2 of 5 cases of L-Amp/L-GCN (40%) were IHC\(^+\) (Supplementary Fig. S1). All 4 FISH\(^+\)/IHC\(^+\)/C0 tumors, which included 3 SCC and 1 LELC, harbored L-Amp. The clinical significance of FISH\(^+\)/IHC\(^+\)/C0 SCC and LELC remained to be defined.

A total of 205 cases were IHC\(^+\)/FISH\(^-\)/C0. Among them, 12 cases had MET\(^{D14}\) 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\(^-\)/C0 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\(^{D14}\) 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 (\(P < 0.001\)), MET\(^{D14}\) 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\(^{D14}\) mutations and MET amplification are therapeutic relevant recurrent events. The clinicopathologic characteristics, ethnic distribution, and prognostic implications of MET\(^{D14}\) 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\(^{D14}\) 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 (\(2 < \text{MET/CEP7} < 5\)) and H-Amps (\(\text{MET/CEP7 ratio} \geq 5\)). 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-naive patients and thus did not fulfill the criteria of oncogenic driver (23). However, we found that high-level MET amplification (MET/CEP7 ratio ≥ 5) was mutually exclusive to the major driver events in RTK/RAS/PI3K axis except MET14. Our data showed a significant association between MET DNA CNAs and MET14 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 ≥ 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 MET14 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 MET14 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 to previous reports (1, 7–9, 20–22) and no significant ethnic difference across East Asian and Caucasian was found. MET14 mutation was not found in SCC (n = 180) in the current study. Surprisingly, we found a significantly higher frequency of MET14 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 MET14+ 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 unexplored. 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-naive NSCLC by IHC. However, only 16.1% of IHC+ tumors harbor MET DNA alterations, that is, MET14 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, Jansen, MSD, Novartis, Pfizer, Roche/Genentech, SFL, and Vertex; and is a consultant/advisory board member for ACEA Biosciences, Astrazeneca, BI, BioMarin, Clovis Oncology, Eli Lilly, GSK, Jansen, Merck Serono, MSD, Novartis, Pfizer, Roche/Genentech, SFL, and Vertex. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**

References


**Clinical Cancer Research**

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


Updated version
Access the most recent version of this article at:

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2016/02/04/1078-0432.CCR-15-2061.DC1

Cited articles
This article cites 32 articles, 6 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/22/12/3048.full#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/22/12/3048.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.