**Abstract**

**Purpose:** MET is a potential therapeutic target in lung cancer and both MET tyrosine kinase inhibitors and monoclonal antibodies have entered clinical trials. MET signaling can be activated by various mechanisms, including gene amplification. In this study, we aimed to investigate MET amplification status in adenocarcinomas and squamous cell carcinomas of the lung. We propose clearly defined amplification scores and provide epidemiologic data on MET amplification in lung cancer.

**Experimental Design:** We evaluated the prevalence of increased MET gene copy numbers in 693 treatment-naïve cancers by FISH, defined clear cutoff criteria, and correlated FISH results to MET IHC.

**Results:** Two thirds (67%) of lung cancers do not have gains in MET gene copy numbers, whereas 3% show a clear-cut high-level amplification (MET/centromer7 ratio ≥2.0 or average gene copy number per nucleus ≥6.0 or ≥10% of tumor cells containing ≥15 MET copies). The remaining cases can be subdivided into intermediate (6%) and low-level gains (24%). Importantly, MET amplifications occur at equal frequencies in squamous and adenocarcinomas without or with EGFR or KRAS mutations.

**Conclusion:** MET amplification is not a mutually exclusive genetic event in therapy-naïve non–small cell lung cancer. Our data suggest that it might be useful to determine MET amplification (i) before EGFR inhibitor treatment to identify possible primary resistance to anti-EGFR treatment, and (ii) to select cases that harbor KRAS mutations additionally to MET amplification and, thus, may not benefit from MET inhibition. Furthermore, our study provides comprehensive epidemiologic data for upcoming trials with various MET inhibitors.

**Introduction**

Lung cancer is the leading cause of cancer-related deaths in the Western world, and an estimated number of 230,000 newly diagnosed cases occurred in the year 2013 in the United States with approximately 160,000 deaths (1). Better understanding of the molecular pathophysiology of non–small cell lung cancer (NSCLC) has led to the development of more selective and targeted treatment options with improved outcome rates (2, 3). With the discovery of EGFR mutations, _EMLA-ALK_ (Echinoderm microtubule-associated protein like 4-anaplastic lymphoma kinase) translocations and the ROSI translocation, lung cancer treatment has been fundamentally changed and shown to be much more effective than ancient chemoradiation therapy regimens (4–7).

More recently, the MET receptor tyrosine kinase and its ligand, the hepatocyte growth factor (HGF), also known as scatter factor, were identified as further therapeutically relevant targets in lung cancer (3). MET is a heterodimeric transmembrane receptor tyrosine kinase, composed of an extracellular α-chain and a transmembrane spanning β-chain linked via disulfide bonds (2) and its gene is localized to chromosome 7q (8). Binding of HGF to MET triggers receptor dimerization and transphosphorylation, which lead to conformational changes of the receptor...
Met is one of the most promising new therapeutic targets in lung cancer. An important activating mechanism is met amplification. There are, however, conflicting data on the prevalence of met amplification and the criteria for measurement of met changes in pulmonary carcinomas. Therefore, in situ hybridization has not yet been fully evaluated as a potential biomarker. In this work, we describe our met FISH findings on a cohort of nearly 700 well-characterized therapy-naïve lung cancers. Based on this to-date largest series, we describe prevalence and patterns of met amplification and propose clear-cut evaluation criteria. Furthermore, we provide evidence that met amplification occurs in adeno- and squamous cell carcinomas at the same frequency and can be found even in EGFR- and KRAS-mutated tumors. These findings are relevant for the treatment of lung cancer with both EGFR and met inhibitors.

Translational Relevance

with subsequent activation of the tyrosine kinase (TK). Activation of the TK domain in turn mediates downstream signaling via the PI3K/AKT, RAS-RAC/RHO, MAPK, and phospholipase C pathways (8, 9). During embryogenesis, MET receptors are expressed on epithelial cells as well as on muscle precursor cells to mediate epithelial-to-mesenchymal transition which is essential for limb bud development (10). MET signaling is maintained throughout adult life where it plays an important physiologic role for cell migration, cell growth, cell differentiation, angiogenesis, and cell survival (11). Dysregulation of the MET-HGF signaling pathway has been reported in many different cancer types (3) and results in cancer cell proliferation, survival, invasion, motility, and the formation of metastasis (12–14). MET overexpression was shown in various cancer tissues and correlated with disease progression (15–17). However, MET expression in NSCLC is reported controversially and it ranges from 3% to 10%, depending on the evaluation criteria. Furthermore, we provide evidence that MET amplification has not yet been fully evaluated as a potential biomarker.

Materials and Methods

Patients

The study was carried out with a total of 693 NSCLC tumor samples with sufficient material for molecular diagnostics. Two hundred and seventy-seven (40%) female patients and 416 male patients (60%) were included into the study with a mean age of 65.8 years (range 30–92 years). About two third of the samples consisted of biopsy specimens, the remaining cases were surgical resection samples. Unselected consecutive lung cancer patients who did not undergo prior nonsurgical treatment were screened for MET amplification as part of the routine molecular diagnostics program (27) of the Network Genomic Medicine (Cologne, Germany) between January 2011 and July 2013. The study was conducted in accordance with the local ethical guidelines and was reviewed by the institutional ethics committee.

Samples and subgroups

The entire cohort of 693 NSCLC tumor samples consisted of 651 primary tumors, 16 lymph node metastases, and 26 samples of distant metastases from NSCLC.

A total of 519 adenocarcinomas and 174 SCCs were included into the study. The group of adenocarcinomas consisted of three genetically characterized subgroups: (i) KRAS-mutated carcinomas, (ii) EGFR-mutated adenocarcinomas, and (iii) adenocarcinomas lacking KRAS and EGFR mutations (in the following referred to as wild-type adenocarcinomas). It is noteworthy that among the EGFR-mutated tumors, there were two lesions that showed clear histologic and immunohistological criteria for SCC. However, these tumors were not included into the group of SCCs but were kept in the group of EGFR-mutated carcinomas. Beside these two incidentally found tumors, SCCs were not systematically examined for EGFR mutations. A subset of SCCs was checked for KRAS mutations, but all were negative.

According to these data, a total of four subgroups were defined: (i) wild-type adenocarcinomas (n = 212, 30%), (ii) KRAS-mutated adenocarcinomas (n = 171, 25%), (iii) EGFR-mutated carcinomas (n = 138, 20%, including the two SCCs), and (iv) SCCs (n = 172, 25%).

As already mentioned, we included unselected consecutive patients from our screening program. The given frequencies of the four groups, however, do not reflect the prevalence of these genetic findings in our entire screening cohort, because the selection of the patients was done on the basis of their molecular characteristics to adjust the frequencies of patients in the four groups. The same range (from 20% to 30%) was important for further statistical analysis. Despite the fact that the analyzed cohort was numerically adjusted to the same size of the included molecular subgroups, the entire cohort consisted of unselected representative cases that were consecutively included.
(solely based on the four molecular subtypes that were collected prospectively in parallel). The subgroups are, therefore, representative and suitable for the evaluation of prevalence and the comparison between subgroups.

No cytology specimens were included. All tumor tissues were fixed in buffered formalin and embedded in paraffin blocks. Mutational analyses for EGFR and KRAS were carried out as previously described (28).

All primary diagnoses were reviewed by two experienced pathologists, according to current recommendations and tumor diagnoses were made in accordance to the current World Health Organization classification system (29).

**FISH assay**

FISH was carried out as previously described (30, 31). Briefly, 3- to 4-μm tissue sections were hybridized overnight with the Zyto-Light SPEC MET/CEN7 Dual Color Probe (ZytoVision). Normal tissue, including vessels, fibroblasts, lymphocytes, or nonneoplastic lung tissue, served as internal controls. Tumor tissue was entirely scanned for amplification hot spots by using a ×63 objective and appropriate filter sets (DM5500 fluorescent microscope; Leica). If MET signals showed a homogenous distribution, random areas were used for reading the slides. Twenty contiguous tumor cell nuclei from three areas, either hot spots or from randomly selected regions, resulting in a total of 60 nuclei, were individually evaluated with the ×100 or ×63 objectives by counting green MET and orange centromere 7 (CEN7) signals.

MET/CEN7 ratio, the percentage of tumor cells with ≥4, ≥5, and ≥15 MET signals, and the average MET copy number per cell were calculated. All FISH assays were evaluated by one reader (H.-U. Schildhaus).

Patients were classified into the following four groups of MET amplification status:

1. **High-level amplification** was defined in tumors with
   a. a MET/CEN7 ratio ≥2.0 or
   b. an average MET gene copy number per cell of ≥6.0 or
   c. ≥10% of tumor cells containing ≥15 MET signals.

2. **Intermediate level** of gene copy number gain being defined as
   a. ≥50% of cells containing ≥5 MET signals and
   b. criteria for high-level amplification are not fulfilled

3. **Low level** of gene copy number gain was defined as
   a. ≥40% of tumor cells showing ≥4 MET signals and
   b. criteria for high-level amplification or intermediate-level of gene copy number gain are not fulfilled

4. All other tumors were classified as **negative**.

The evaluation and interpretation criteria were derived from our observation on several hundred tumors (see below) and influenced by earlier published data in which a modified Colorado score was recommended for the evaluation of MET amplification (15, 32, 33).

**MET IHC**

We selected 65 cases from all subgroups (EGFR-mutated carcinomas, KRAS-mutated adenocarcinomas, wild-type adenocarcinomas, and SCCs) with sufficient tumor material for further IHC staining. Cases were selected on the basis of previous FISH results, that is, five tumors with high-level MET amplification, five low/intermediate cases, and 55 negative tumors. Stainings were carried out by using the CONFIRM SP44 anti-MET monoclonal antibody (Ventana Medical Systems; cat No. 790-4430). Staining was done on an automated stainer system (Bench Mark ULTRA) where slides had been dewaxed, pretreated by cell conditioner No 1 at 95°C for 8 minutes, treated with CC1 (four times at 20, 36, 52, and 64 minutes), and thereafter incubated with SP44 for 16 minutes. Finally, slides were counterstained with hematoxylin II. Immunohscoring was done according to the criteria recently proposed for NSCLC (34, 35) by evaluating both staining intensity (negative, weak, moderate, or strong) and the percentage of these intensities. Four diagnostic subgroups (‘immunoscores’) have been delineated: 3+ (≥50% of tumor cells stained exhibiting strong staining intensity); 2+ (≥50% of tumor cells with moderate or higher staining intensity but <50% strong intensity); 1+ (≥50% of tumor cells with weak or higher staining intensity but <50% with moderate or higher intensity); or 0 (no staining or <50% of tumor cells with any intensity). MET expression status was determined in a blinded fashion (by J. Rüschoff) in particular without any knowledge of FISH data.

**Statistical analysis**

For statistical analysis, the SPSS software, version 21.0, (IBM) was used. χ², Fisher exact, and t tests were used, if appropriate. All tests were two sided, with a 95% confidence interval.

**Results**

**Detection of MET amplification by FISH**

We obtained valid and easily evaluable FISH data for all 693 samples. On the basis of our findings in this representative cohort of pulmonary adenocarcinomas and SCCs, we have noticed, that nearly two thirds of these tumors do not harbor any copy number gains of the MET gene. Thus, the majority of NSCLCs is disomic for chromosome 7 with a regular number of MET gene copies, indicated by one to two MET and CEN7 signals in the FISH analysis (Fig. 1). However, one third of the tumors showed an increase in either MET or CEN7 signals or in both of them. It is noteworthy, that most of those cases are characterized by an uneven, that is, heterogeneous or focal distribution of signals. We have observed focally amplified tumor clones in a background of tumor tissue with only slight increase in copy numbers as well as cancers in which isolated amplification occurred patchily distributed throughout the entire tumor tissue. On the basis of these experiences, we were able to establish evaluation criteria for MET FISH (see Materials and Methods section for details) in which we also included previously published data. By applying these criteria, we categorized lung carcinomas into four amplification patterns (Fig. 2). The average MET gene copy number per tumor cell showed a great variability throughout the entire cohort (range, 1.3–31.0; mean, 3.2). Mean copy numbers per nucleus were 2.5, 3.7, 4.9, and 9.3 for negative, low-level, intermediate-level, and high-level tumors, respectively. Another unique finding in MET FISH on NSCLC is the occurrence of amplicons, which include parts of or the entire centromeric region (Fig. 1H) without a numerically balanced MET copy number gain. This phenomenon may result in a MET/CEN7 ratio below 1.0. Thus, we noticed ratios from only 0.2 up to 14.1 (mean, 1.2, 1.3, 1.5, 2.9, and 1.3 for negative, low-level, intermediate-level, high-level cases, and the entire cohort, respectively). Tumor cells containing large clusters with 15 or more MET gene copies were nearly restricted to the group of high-level cases and occurred only rarely in all other subgroups.
Distribution of amplification patterns

A total of 227 tumors (33%) showed criteria for any type of MET copy number gains (high-level amplification, intermediate-level, or low-level copy number gain). Twenty-two carcinomas fulfilled the criteria for high-level amplification (3%; Table 1 and Fig. 2), 43 (6%) tumors reached the criteria for intermediate-level, and 162 tumors (24%) for low-level gene copy number gain (Fig. 2).

The different subgroups, that is, EGFR-mutated carcinomas, KRAS-mutated adenocarcinomas, wild-type adenocarcinomas, and SCCs did not show any statistically significant difference in the frequency of low-, intermediate-, or high-level MET amplification (Fig. 2). Furthermore, MET/CEN7 ratio, gene copy number per cell, percentage of tumor cells containing ≥4, ≥5, or ≥15 signals were not significantly different between the different tumor groups (Fig. 3). We found three high-level amplified cases...
in the group of EGFR-mutated carcinomas, four high-level amplified tumors among KRAS-mutated adenocarcinomas, and six highly amplified cases in the SCC cohort. The highest number of nine high-level amplified cases was found among wild-type adenocarcinomas. This distribution, however, was not statistically significant. To note, one of the EGFR-mutated tumors with histologic and immunohistologic characteristics for SCC showed low-level MET copy number gain.

According to sex, the group of high-level amplified tumors was distributed as follows: 14 (64%) male patients and 8 (36%) female patients.

Table 1. Characteristics of 22 pulmonary carcinomas with high-level MET amplification

<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Subgroup</th>
<th>MET/CEN7 ratio (threshold: ≥2.0)</th>
<th>Average MET gene copy number per nucleus (threshold: ≥6.0)</th>
<th>Percentage of tumor cells containing ≥15 MET signals (threshold: ≥10%)</th>
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<tbody>
<tr>
<td>45</td>
<td>Female</td>
<td>EGFR</td>
<td>1.03</td>
<td>11.22</td>
<td>35</td>
</tr>
<tr>
<td>79</td>
<td>Male</td>
<td>EGFR</td>
<td>1.52</td>
<td>6.22</td>
<td>5</td>
</tr>
<tr>
<td>68</td>
<td>Female</td>
<td>EGFR</td>
<td>2.94</td>
<td>15.03</td>
<td>50</td>
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<tr>
<td>71</td>
<td>Male</td>
<td>KRAS</td>
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<td>6.2</td>
<td>2</td>
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<tr>
<td>69</td>
<td>Male</td>
<td>KRAS</td>
<td>2.79</td>
<td>14.33</td>
<td>48</td>
</tr>
<tr>
<td>45</td>
<td>Female</td>
<td>KRAS</td>
<td>1.46</td>
<td>7.68</td>
<td>8</td>
</tr>
<tr>
<td>60</td>
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<td>KRAS</td>
<td>1.88</td>
<td>6.25</td>
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<tr>
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<td>6.15</td>
<td>0</td>
</tr>
<tr>
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<td>Male</td>
<td>Wt</td>
<td>8.96</td>
<td>15.98</td>
<td>45</td>
</tr>
<tr>
<td>57</td>
<td>Male</td>
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<td>3.82</td>
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<tr>
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<td>3.88</td>
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<tr>
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<td>6.39</td>
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<td>49</td>
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<td>14.1</td>
<td>31.0</td>
<td>95</td>
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<tr>
<td>68</td>
<td>Female</td>
<td>Wt</td>
<td>2.44</td>
<td>4.95</td>
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<tr>
<td>75</td>
<td>Female</td>
<td>Wt</td>
<td>1.55</td>
<td>6.97</td>
<td>15</td>
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<tr>
<td>48</td>
<td>Male</td>
<td>Wt</td>
<td>1.42</td>
<td>8.62</td>
<td>10</td>
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<tr>
<td>70</td>
<td>Male</td>
<td>SCC</td>
<td>1.61</td>
<td>6.97</td>
<td>10</td>
</tr>
<tr>
<td>74</td>
<td>Male</td>
<td>SCC</td>
<td>3.58</td>
<td>8.6</td>
<td>8</td>
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<tr>
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<td>5.78</td>
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<tr>
<td>52</td>
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<td>SCC</td>
<td>1.5</td>
<td>6.08</td>
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</tr>
<tr>
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<td>Male</td>
<td>SCC</td>
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<td>5.37</td>
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</tr>
<tr>
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<td>Male</td>
<td>SCC</td>
<td>1.46</td>
<td>6.82</td>
<td>10</td>
</tr>
</tbody>
</table>

Mean (range) Mean (range) Mean (range) Mean (range)

2.9 (1.0–14.1) 9.2 (3.8–31) 18.9 (9–95)

NOTE: Gray boxes indicate fulfilled criteria. Note that all criteria for high-level amplification are fulfilled in only five cases. High-level amplified tumors represent 3.2% of all pulmonary carcinomas and can occur in the background of EGFR and KRAS mutations of adenocarcinomas as well as in SCC.

Abbreviations: EGFR, EGFR-mutated carcinoma; KRAS, KRAS-mutated adenocarcinomas; Wt, wild-type adenocarcinoma; SCC, squamous cell carcinoma.
female patients without significant enrichment for any sex when comparing the amplification negative and positive patients \( (P = 0.87) \). Furthermore, there was no difference in the mean age at diagnosis. The mean age of the entire cohort was 65.8 years (range 30–92 years) with a mean age of all high-level amplified cases of 63.7 years (range 45–91, \( P = 0.35 \)). However, the group of \( EGFR \)-mutated patients (irrespective of the \( MET \) status) showed a higher age at diagnosis (mean, 68.0 years) when compared with \( KRAS \)-mutated tumors (mean age, 63.7 years) or wild-type adenocarcinomas (mean age, 64.5 years; \( t \) test: \( P = 0.001 \) and \( P = 0.008 \), respectively).

Of all high-level amplified cases \( (n = 22) \), 18 tumors showed an average number of \( MET \) signals \( \geq 6 \) (mean, 4.5), 11 cases showed a \( MET/CEN7 \) ratio \( \geq 2 \) (mean 1.5), and 11 of these cases exhibited \( \geq 10\% \) of tumor cells containing \( \geq 15 \) \( MET \) signals or large clusters. Only five cases fulfilled all three criteria (Table 1).

Correlation between \( MET \) amplification and protein expression

To evaluate whether our FISH evaluation score is related to and can be confirmed by c-MET receptor protein expression, 55 tumor cases were selected comprising the different amplification levels. Overall, IHC and FISH results were statistically significantly correlated (Table 2; \( \chi^2: P < 0.0001 \)). Even if one subdivides IHC stainings into "positive" (scores 2+ and 3+) and "negative" (scores 0 and 1+), those categories were significantly correlated with FISH results (high level vs. negative/low level/intermediate level; Fisher exact test: \( P = 0.001 \)). Most interestingly, immunoscore 3+ was strongly correlated with high-level amplification. Four out of five cases (80%) with high-level amplification were intensely stained resulting in immunoscore 3+ (Table 2 and Fig. 4). One highly amplified tumor reaching immunoscore 2+ showed markedly lower \( MET \) gene copy numbers compared with the 3+ cases, but yet fulfilled the criteria of high-level amplified cases. Moreover, immunoscore 3+ was exclusively noticed in tumors with high-level amplification. On the other hand, negative or weak immunostainings ("IHC negative" cases; immunoscores 0 or 1+) were associated with FISH negativity. Tumors with low-level/intermediate-level \( MET \) gain showed mostly a 2+ immunoscore (three out of five low/intermediate level cases). However, 2+ staining was also seen in FISH-negative tumors as well as in one highly amplified carcinoma. Therefore, we conclude (i) that our FISH findings could be confirmed by IHC.

### Table 2. Correlation of \( MET \) amplification status with \( MET \) protein expression

<table>
<thead>
<tr>
<th>IHC</th>
<th>FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
</tr>
</tbody>
</table>

NOTE: For the definition of IHC scores and FISH categories, see the Materials and Methods section.
as an independent method, (ii) that IHC and FISH are basically correlated to each other, and (iii) that this FISH–IHC correlation is best in clearly positive and negative categories (FISH: negative and high-level amplification, IHC: scores 0/1+ and score 3+), whereas IHC and FISH are only weakly correlated in borderline categories (FISH: low/intermediate level, IHC score 2+), which, however, account for a significant proportion of lung cancers [FISH, 205/693 (30%); IHC, 14/65 (22%) in our cohort].

**Discussion**

Aktivated MET is a potential therapeutic target in lung cancer, and several drugs with anti-MET activity have entered early or even late clinical trials (3). Most of them are small-molecule TKIs, but also monoclonal antibodies and anti-HGF compounds have been developed and introduced to clinical application. It is important to keep in mind that MET signaling can be the result of various activated mechanisms in tumor cells. Gene amplification is a major pro-oncogenic event leading to MET receptor activation. Other known mechanisms such as gain-of-function-mutations, protein overexpression, paracrine and autocrine activation via HGF, as well as epigenetic changes have been reported and need to be taken into account (15, 16, 18, 36). Furthermore, some of these mechanisms may be related to each other. To date, two major issues are still unsatisfactorily addressed: (i) which type of MET activation is relevant for which tumor entity and its initiation and propagation, and (ii) in terms of biomarkers, which type of activation is predictive for which anti-MET compound.

In this study, we aimed to investigate MET amplification as one relevant mechanism of MET activation in NSCLC. By analyzing a large cohort of SCCs and adenocarcinomas, to the best of our knowledge, we provide the currently most comprehensive dataset on the prevalence of MET amplifications in therapy-naive NSCLC. Second, we are able to define amplification patterns of the MET gene and third, we correlate our findings with common genetically defined subgroups of pulmonary carcinomas. Our findings may be useful in the context of screening programs for patients in upcoming clinical trials with c-MET inhibitors. Furthermore, they may also have impact on the future application of c-MET TKI therapy administration because our data provide evidence that MET amplification occurs also in EGFR-mutated carcinomas before TKI treatment and hence may represent a mechanism of primary resistance to TKIs in these tumors.

One major finding of our study is the fact that MET amplification can occur in both adenocarcinomas and SCCs of the lung and that MET amplifications appear even in the background of EGFR and KRAS mutations. Thus, MET amplification does not fulfill the criteria of a genetically epistatic oncogenic driver lesion at least in these cases (37). However, here we show unambiguously that MET amplification is not a mutually exclusive event to KRAS and/or EGFR mutation in adenocarcinomas. Our data reveal that both, high-level amplification as well as low/intermediate-level copy number gains occur in treatment-naive carcinomas of different genetic background and that there is no statistically significant difference in the frequency amongst the subgroups.

It is well known that MET gene amplification shows higher frequencies in patients who received and relapsed after therapies with EGFR inhibitors (23). However, it remained unclear whether MET activation occurs before treatment (primary resistance) or after the exposure to EGFR inhibitors (secondary resistance). To date, only very few data about the MET amplification status in treatment-naive NSCLC patients are available. Data from our study indicate that 2% (3/138) of EGFR-mutated lung cancers and 2% (4/171) of KRAS-mutated lung adenocarcinomas harbor simultaneous high-level MET amplification, a percentage that even increases if one includes intermediate- and low-level cases. These findings might be an explanation for at least a part of the nonresponders to EGFR TKI therapies. Therefore, it might be necessary to determine the MET status in all EGFR-mutated NSCLC cases before treatment, particularly when administrating EGFR inhibitors. As a possible result, the addition of MET inhibitors to EGFR TKIs might be necessary in amplified cases.

Furthermore, one has to keep possible KRAS mutations in mind when administrating unselected MET inhibition therapy and being confronted with treatment resistance. On the basis of our data, it might be wise to determine the KRAS status of patients who are candidates for an anti-MET therapy.

In a recent report by The Cancer Genome Atlas (TCGA) network (38), the frequency of MET amplifications was 2.2%, which is basically in the range of our findings. Data from this particular publication, however, suggest that high-level MET amplification is mutually exclusive with EGFR and KRAS mutations because MET amplification could not be detected in these molecular subgroups. This finding is in contrast with our aforementioned observation. One explanation for this discrepancy might be the number of analyzed samples. The TCGA cohort consisted of 230 pulmonary adenocarcinomas, among them 32% KRAS (n = 75) and 11% EGFR (n = 26) mutants and a total of six MET amplified cases. On the other hand, 171 KRAS- and 138 EGFR-mutated cases were included in our study, and we could detect a total of 22 samples with high-level MET amplification. We found that EGFR/MET and KRAS/MET coalterations are rare with 0.6% (3 out of 519 cases) and 0.4% (2/519) of all adenocarcinomas, respectively. Therefore, this phenomenon might not be perceptible in smaller cohorts. As mentioned before we are, however, convinced that coalterations of MET and canonical oncogenic drivers might have
Our criteria for low- and/or high-level amplification of "MET" attempts of facilitate the definition of categories can be considered as certain, to some extent independently from classification and scoring systems (see below).

Interestingly, nearly 30% of pulmonary non–small cell carcinomas in our study showed a moderate increase in "MET" gene copy numbers. Because this cohort was more heterogeneous and showed larger variations in gene copy numbers and "MET/CEN7" ratio than negative or high-level amplified cases, we defined the groups of intermediate-level copy number gain (tumors showing ≥50% of cells with ≥5 "MET" gene copies per cell) and low-level copy number gain (≥40% of cells with ≥4 "MET" copies). Intermediate level cases account for 6% and low-level tumors for 24% of all investigated NSCLCs. To date, it is unclear whether patients with these tumors will also benefit from anti-MET treatment.

Our aim was to propose an easily applicable "MET" amplification classification system, which might serve as a reliable basis and as a potential biomarker for consecutive studies of "MET" inhibitors. The defined thresholds are suggested on the basis of the experiences in our large cohort of nearly 700 consecutive lung cancer cases and previous attempts (15, 18, 32, 33). Our proposal allows to define a clear-cut high-level amplification category, which is defined by certain and easily reproducible criteria (i.e., "MET/CEN7" ratio ≥2.0 or average gene copy number ≥6.0 per tumor cell or ≥10% of tumor cells containing ≥15 "MET" signals). These parameters are widely accepted as criteria for true amplification of many genes, and, based on our findings, are suitable to select cases with the highest level of "MET" amplification for ongoing clinical trials (the top 3%). On the other hand, our scoring system clearly defines a large cohort (67% of all squamous cell and adenocarcinomas), which does not show any significant "MET" gene copy alteration. Application of our proposed low- and intermediate-level categories is to date somewhat arbitrary because, as already mentioned, the clinical relevance is still unclear. We decided to introduce those categories only (i) to facilitate the definitions of negative and high-level amplified categories and (ii) to allow a better comparability with previous attempts of "MET" scoring. Some of those publications have used our criteria for low- and/or high-level amplification as parameters for "MET" positivity. These attempts resulted in a high proportion of "MET-positive" cases whereas most of these authors did not discriminate between polysomic tumors and true amplifications.

In a subgroup of 65 cases, we could finally verify our FISH findings by IHC, in which "MET" protein overexpression was highly associated with high-level amplification, indicating that our FISH findings basically have a biologic relevance. Larger studies, however, are needed to clarify whether "MET" amplification status and "MET" protein expression (measured by IHC) are correlated and which biomarker assay has the best predictive value. Our findings indicate that the IHC 2+ cases represent a genetically diverse group of tumors. Therefore, IHC might have limited diagnostic value especially in these tumors and "MET" in situ hybridization could be superior to IHC, especially in characterizing genetically diverse borderline cases. Therefore, we propose to include the genomics-based information, that is, the amplification status, obtained by FISH, in the panel of potential biomarkers.

**Disclosure of Potential Conflicts of Interest**

H.-U. Schildhaus reports receiving speakers bureau honoraria from Abbott Molecular, Novartis, Pfizer, Roche, and Zytomed Systems and is a consultant/advisory board member for Abbott Molecular, Aigen, Pfizer, and Roche. A.M. Schultheis reports receiving speakers bureau honoraria from Roche. J. Ruschoff is a consultant/advisory board member for DAKO and Roche. S. Merklebach-Bruse reports receiving speakers bureau honoraria from Roche. M. Serke is a consultant/advisory board member for Pfizer. S. Krüger reports receiving speakers bureau honoraria from Roche and is a consultant/advisory board member for Boehringer Ingelheim and Roche. T. Zander is consultant/advisory board member for Aigen, Merck, Novartis, and Roche. No potential conflicts of interest were disclosed by the other authors.

**Authors' Contributions**

Conception and design: H.-U. Schildhaus, M. Serke, J. Wolf, R. Buettner

Development of methodology: H.-U. Schildhaus, R. Buettner


Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H.-U. Schildhaus, A.M. Schultheis, J. Ruschoff, E. Binot, S. Merklebach-Bruse, T. Zander, J. Wolf, R. Buettner

Writing, review, and/or revision of the manuscript: H.-U. Schildhaus, A.M. Schultheis, J. Ruschoff, M. Bos, M. Serke, M. Reiser, S. Krüger, J. Wolf, R. Buettner

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H.-U. Schildhaus, E. Binot, A. Schlesinger, M. Brockmann, U. Gerigk, K. Helm, E. Stoelben

Study supervision: H.-U. Schildhaus

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**References**

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Hans-Ulrich Schildhaus, Anne M. Schultheis, Josef Rüschoff, et al.


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