Increased MET Gene Copy Number but Not mRNA Level Predicts Postoperative Recurrence in Patients with Non–Small Cell Lung Cancer

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Abstract
The aim of the present study was to investigate the relationship of MET copy number (CN) and MET mRNA expression to other molecular alterations, clinicopathologic characteristics, and survival of patients with resected non–small cell lung cancer. One hundred fifty-one paired surgical samples of tumor and tumor-distant normal lung tissues were analyzed by comparative quantitative polymerase chain reaction (PCR) methods with commercially available assays and the CopyCaller software v. 1.0 for post-PCR data processing (downloadable from www.appliedbiosystems.com). MET copy gain (set as more than 3.0 copies per cell) was found in 18.5% of the samples and occurred more frequently in the adenocarcinomas (ADCs) with an increased epidermal growth factor receptor (EGFR) or human epidermal growth factor receptor 2 (HER2) CN (P = .001 and .030 for EGFR and HER2, respectively) and in the ADCs with EGFR activating mutations (P = .051) but did not correlate with KRAS dosage or mutational status. MET mRNA level was 1.76-fold higher [95% confidence interval (CI), 1.29-2.40] in the tumor compared to unaffected lung tissue and associated significantly with MET CN (beta coefficient, 1.51; 95% CI, 1.22-1.87; P<.001). In the multivariable analysis, patients diagnosed with ADC with increased MET CN had a significantly higher risk of disease recurrence (hazard ratio, 1.76; 95% CI, 1.20-2.57; P = .004). An increased MET CN in combination with histologic type appears to be a prognostic factor in patients with ADC after a curative surgery.

Introduction
High mortality rate of non–small cell lung cancer (NSCLC) patients after a curative surgery [1] suggests that the tumor-node-metastasis (TNM) staging system is insufficient for patient’s prognosis and therapeutic decisions and that new prognostic factors are needed [2]. Aberrations of MET proto-oncogene, frequently observed in cancer [3,4], are one of the molecular factors with a possible prognostic potential [5]. An association between MET copy gains and a worse prognosis in patients with NSCLC has been found previously [6–9], but the data are limited and inconsistent. Recently, an increase in MET copy number (CN) has been demonstrated to be responsible for about 20% cases of the acquired resistance to EGFR tyrosine kinase inhibitors (TKIs) in patients with NSCLC [10,11], suggesting that, as a pre-existing condition occurring before treatment, it may provide a primary lack of response [12], although a number of researchers deny that possibility [10,13]. The rate of MET copy gain in NSCLC reported thus far ranges significantly from 3% to 21% depending on the detection technique used [6,7,14–17] and patient cohort differences [15]. Moreover, although a few studies examined the association between MET CN...
alterations and protein level in cancers [16–18], no data regarding MET mRNA expression in lung cancer are available.

The aim of the present study was to evaluate MET CN and mRNA expression level in stage I to IIIA NSCLC tumor samples and to assess their associations with clinicopathologic characteristics of the patients including the postoperative outcome. In addition, the relations between the mutational status of epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), and KRAS genes and MET CN alterations were analyzed.

Materials and Methods

Study Subjects and Tissue Samples

The study was performed on pairs of freshly frozen cancerous and unaffected lung tissue specimens obtained from patients with NSCLC stage I to IIIA (pTNM, 7th edition, 2009) who underwent a curative surgery at the Bialystok Medical University Hospital between 2003 and May 2010 and were followed-up for at least 3 years. None of the patients received chemotherapy or radiotherapy before the surgery.

Tissue samples were collected intraoperatively and processed immediately after surgical resection: After the macroscopic visual assessment, the tumors were divided into two sections. One of them was fixed in formalin followed by paraffin embedding and the other, as well as the unaffected lung tissue specimen from the same lobe or lung of the patient, was frozen in liquid nitrogen followed by storage at −80°C. Routine hematoxylin-eosin and immunohistochemical examination of formalin-fixed paraffin-embedded tumor samples, including p63, cytokeratin 5/6 (CK5/6), and thyroid transcription factor 1 (TTF1), and chromogranin detection, was performed to determine tumor histologic type. Before nucleic acid extraction, the cryosections of frozen tissue specimens were stained with hematoxylin-eosin and evaluated for tumor cell content. Only the tumor samples that contained at least 50% of tumor cells on a microscopic section were used for further processing. Consequently, 151 pairs of cancerous and matched unaffected lung tissues were selected for the study.

Clinicopathologic data and previously detected EGFR, KRAS, and HER2 gene mutational status were available for all the patients. For survival analysis, the overall survival (OS) was estimated as the time from the date of the surgery to the date of death due to lung cancer recurrence or metastases (event) or to the date of the last control visit (censoring). The disease-free survival (DFS) was defined as the time from the date of the surgery to the date of disease relapse or death, whichever occurred first (events), or to the date of the last visit (censoring).

The study was approved by the Ethics Committee of the University, and written informed consent for specimen collection was obtained from each patient before the surgery.

Nucleic Acid Extraction

DNA and RNA were isolated simultaneously using a magnetic extraction method. Briefly, about 40 to 50 mg of tissue was disrupted in lysis buffer (Biomerieux, Marcy l’Etoile, France) with TissueRupter (Qiagen, Hilden, Germany) and incubated with Proteinase K for 2 hours at 56°C. Nucleic acids from deproteinated cell lysates were extracted automatically on the EasyMag machine (bioMérieux) according to the producer’s protocol. Both DNA and RNA were present in the 100-μl resulting extracts. Nucleic acid quality was assessed electrophoretically.

For gene expression analysis, RNA was transcribed into cDNA in a reaction with High Capacity RNA-to-cDNA Master Mix (Applied Biosystems, Foster City, CA) according to the producer’s recommendations.

MET CN

MET CN was analyzed by a quantitative real-time duplex polymerase chain reaction (qPCR) on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) with a commercially available predesigned MET TaqMan Copy Number Assay (Hs0143282_cn) and a Reference RNase P Assay (PN4412907), both from Applied Biosystems. The qPCR was done in a 20-μl reaction mixture containing 10 μl of Applied Biosystems TaqMan Universal PCR Master Mix with UNG, 1 μl of the CN assay solution, 1 μl of the reference assay solution, and 5 μl of DNA solution according to the following cyclic conditions: 50°C for 2 minutes followed by holding for 10 minutes at 95°C and 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Each sample was analyzed in quadruplicate. The raw post-PCR data were used for MET CN calculation by the relative quantification method using the CopyCaller v.1.0 software (PN4412907) downloadable from www.appliedbiosystems.com. MET copy gain was defined as more than three copies per cell.

MET mRNA Expression

MET mRNA expression level in the tumor and unaffected lungs was evaluated with the comparative real-time reverse transcription–PCR method. Ribosomal 18S RNA (18srRNA) gene with a relatively low level of the expression variability in lung tissue [19,20] was used to normalize for the differences in the input cDNA concentration. The amplification was performed in a 20-μl mixture containing 10 μl of TaqMan Universal PCR Master Mix with UNG, 1 μl of the MET (Hs01565584_m1) or 18S rRNA (Hs99999901_s1) TaqMan Gene Expression Assay (all reagents from Applied Biosystems), and 5 μl of cDNA solution. Each sample was analyzed in triplicate on an ABI PRISM 7900HT Sequence Detection System equipped with the SDS v.2.4 software for baseline and Ct calculations. MET expression was inversely proportional to the difference between Ct for MET and Ct for 18S rRNA gene (ΔCt = CtcMET − Ct18S rRNA). Fold changes (FCs) in MET expression between the tumor and paired normal lung tissues from the same patient were calculated as FC = 2 − ΔΔCt, where ΔΔCt equaled MET expression in tumor (ΔCtT) calibrated by its expression in the corresponding nonmalignant tissue (ΔCtN) as follows: ΔΔCt = ΔCtT − ΔCtN.

EGFR, HER2, and KRAS Alterations

EGFR and KRAS activating mutations were detected with direct sequencing of the PCR-amplified EGFR exons 19 and 21 and KRAS 2 exons. EGFR, HER2, and KRAS CNs were analyzed like MET CN with the corresponding TaqMan Copy Number Assays from Applied Biosystems (Hs014326560_cn, Hs00159103_cn, and Hs02802859_cn for EGFR, HER2, and KRAS, respectively). Gene copy gain was defined as more than three copies per cell.

Statistical Analysis

The nonparametric Mann-Whitney test, Kruskal-Wallis test, or Pearson chi-squared test was used to analyze the associations between clinicopathologic characteristics and MET CN. The differences in MET expression between the tumor and unaffected lung tissues were analyzed with paired t test. The linear regression model was used to estimate the relation between MET CN and the expression level. The associations between MET gene copy number (CNG) and EGFR, HER2, and KRAS gene status were analyzed with Pearson chi-squared test. OS and DFS were calculated and plotted with
Kaplan-Meier method with the log-rank test for the comparison between the groups. Cox proportional hazard model was used to evaluate the effect of clinicopathologic and molecular variables on OS and DFS. P values less than .05 were considered as significant. All the statistical analyses in this study were performed using STATA/SE 11.1 software.

Results

Patient Characteristics
A total of 151 patients with NSCLC aged from 39 to 82 years (median age, 63.0 years) was included in the study. The majority of the patients were males (78.8%) and current or former smokers (90.7%). According to the TNM classification, pathologic staging were given as follows: stage I in 58 (38.4%) patients, stage II in 62 (47.0%) patients, and stage IIIA in 22 (14.6%) patients. About 40% of the patients had mediastinal lymph node metastases at the time of surgery, classified as stage N1 and stage N2 in 43 (28.5%) and 62 (47.0%) patients, and stage IIIA in 22 (14.6%) patients. About 11.9% of the cases with 3.0 to 3.99 MET copies per cell and the remaining 13 samples containing from 4.0 to 7.7 copies (Table 1).

In our cohort of patients with NSCLC, MET CNG was observed approximately 2.7- and 2.0-fold more frequently in the tumors with increased EGFR and HER2 CN compared to the tumors without the increase (P = .002 and .049 for EGFR and HER2, respectively) and about 2.4-fold more frequently in tumors harboring EGFR mutations compared to tumors with wild-type EGFR (P = .071). However, subgroup analysis for particular tumor histologic types revealed that statistically significant associations between MET CNG and EGFR or HER2 gene alterations occurred only in the ADC group but not in the LCC or SCC group. No associations between MET CN and KRAS gene mutations or copy gain were found in particular histologic types of cancer (Table 2).

MET mRNA Expression
We were unable to determine MET cDNA in 16 analyzed tumor and/or normal lung tissue specimens and these paired samples were excluded from the assay. The MET mRNA level was significantly higher in tumor tissue as compared to unaffected tissue (relative quantity (RQ) geometric mean, 1.76; 95% confidence interval (CI), 1.29-2.40; P < .001). However, with respect to tumor histologic types, a statistically significant alteration was obtained only in ADCs (RQ geometric mean, 2.14; 95% CI, 1.33-3.45; P < .001). No significant associations between MET mRNA expression and patients’ characteristics were found (Table 1).

Table 1. Relationship between MET CN, MET mRNA Level, and Patients’ Clinicopathologic Characteristics

| Variable                          | Calculated MET CN in Tumor Tissue | Patient Number with Calculated MET CN | MET mRNA Level in Tumor Tissue (ΔCt Value) | Differences in MET mRNA Levels between Tumor and Unaffected Lung Tissues |
|-----------------------------------|-----------------------------------|---------------------------------------|-------------------------------------------|------------------------------------------------------------------------|
|                                   | n (%)                            | Median PValue                         | n (%) Mean ± SD PValue                    | n Mean ± SD PValue                                                      |
|                                   |                                  |                                       |                                           |                          Log(FC) Mean ± SD PValue Mean 95% CI                                   |
| Total Age (years)                 | 151                              | 2.05                                  | 123 (81.5) 28 (18.5)                      | 142 15.63 ± 2.20                                                       | 135 0.82 ± 2.63 1.76 1.29-2.40                                      |
| <63                               | 75                               | 2.09                                  | 59 (78.7) 16 (21.3)                       | 71 15.60 ± 2.12                                                        | 67 0.95 ± 2.33 1.93 1.30-2.86                                     |
| ≥63                               | 76                               | 1.98                                  | 64 (82.4) 12 (15.8)                       | 38 (11.9) 6.12 ± 2.47                                                 | 68 0.69 ± 2.92 1.61 0.99-2.63                                     |
| Gender                            |                                  |                                       |                                           |                          Log(FC) Mean ± SD PValue Mean 95% CI                                   |
| Male                              | 119 (78.8)                       | 2.05                                  | 96 (80.7) 23 (19.3)                       | 112 15.77 ± 2.23                                                       | 105 0.82 ± 2.56 1.77 1.25-2.49                                    |
| Female                            | 32 (21.2)                        | 2.04                                  | 27 (84.4) 5 (15.6)                        | 30 15.11 ± 2.32                                                        | 30 0.81 ± 2.93 1.75 0.82-3.73                                     |
| Smoking                           |                                  |                                       |                                           |                          Log(FC) Mean ± SD PValue Mean 95% CI                                   |
| Never                             | 14 (9.3)                         | 2.08                                  | 12 (85.7) 2 (14.3)                        | 13 15.23 ± 2.70                                                        | 12 0.45 ± 3.81 1.36 0.26-7.30                                     |
| Ever                              | 137 (90.7)                       | 2.04                                  | 111 (81.0) 26 (19.0)                      | 129 15.67 ± 2.22                                                       | 123 0.85 ± 2.51 1.81 1.32-2.46                                    |
| Histology                         |                                  |                                       |                                           |                          Log(FC) Mean ± SD PValue Mean 95% CI                                   |
| ADC                               | 64 (42.4)                        | 1.98                                  | 51 (79.7) 13 (20.3)                       | 60 15.38 ± 2.15                                                        | 59 1.10 ± 2.62 2.14 1.33-3.45                                     |
| LCC                               | 35 (23.2)                        | 2.05                                  | 27 (77.1) 8 (22.9)                        | 33 16.24 ± 2.59                                                        | 32 0.47 ± 2.36 1.39 0.77-2.51                                     |
| SCC                               | 52 (34.4)                        | 1.94                                  | 45 (86.5) 7 (13.5)                        | 49 15.54 ± 2.13                                                       | 44 0.69 ± 2.84 1.61 0.88-2.93                                    |
| pTNM                              |                                  |                                       |                                           |                          Log(FC) Mean ± SD PValue Mean 95% CI                                   |
| I                                 | 58 (38.4)                        | 2.04                                  | 48 (84.5) 9 (15.5)                        | 57 15.64 ± 2.50                                                        | 54 0.95 ± 2.31 1.93 1.25-3.00                                    |
| II                                | 71 (47.0)                        | 2.05                                  | 58 (81.7) 13 (18.3)                       | 66 16.61 ± 2.23                                                       | 62 0.66 ± 3.03 1.91 0.93-2.70                                    |
| IIIA                              | 22 (14.6)                        | 1.94                                  | 26 (81.8) 6 (18.2)                        | 22 15.69 ± 1.65                                                       | 19 0.93 ± 2.14 0.84 1.91 0.93-3.91                                |
| Lymph node metastases             |                                  |                                       |                                           |                          Log(FC) Mean ± SD PValue Mean 95% CI                                   |
| No                                | 90 (59.6)                        | 2.05                                  | 74 (82.2) 16 (17.8)                       | 85 15.81 ± 2.35                                                       | 81 0.77 ± 2.72 1.70 1.12-2.58                                    |
| N1 to N2                          | 61 (40.4)                        | 2.04                                  | 49 (80.3) 12 (19.7)                       | 57 15.38 ± 2.12                                                       | 54 0.89 ± 2.52 0.79 1.85 1.15-2.99                                |
| Lymph node pathologic status      |                                  |                                       |                                           |                          Log(FC) Mean ± SD PValue Mean 95% CI                                   |
| N0                                | 90 (59.6)                        | 2.05                                  | 74 (82.2) 16 (17.8)                       | 85 15.81 ± 2.35                                                       | 81 0.77 ± 2.72 1.70 1.12-2.58                                    |
| N1                                | 43 (28.5)                        | 2.04                                  | 36 (89.7) 7 (16.3)                        | 41                                                                       | 38 0.75 ± 2.68 1.68 0.91-3.10                                     |
| N2                                | 18 (11.9)                        | 1.94                                  | 13 (72.2) 5 (27.8)                        | 16 1.527 **                                                               | 16 1.22 ± 2.12 0.808 ** 2.33 1.07-5.11                                |

* Geometric mean.  
† 95% CI for geometric mean.  
‡ Wilcoxon rank-sum test.  
§ Pearson chi-squared test.  
¶ Two-sample t test with Welch’s correction for unequal variances.  
§§ Kruskal-Wallis rank test.  
** One-way analysis of variance test with Bonferroni multiple comparison.
The Association between MET CN and MET mRNA Expression

Linear regression model revealed a statistically significant link between MET CN and mRNA expression in lung tumor tissue (Figure 1). Gain of an additional gene copy resulted in a 1.51-fold increase in the expression level (95% CI, 1.22-1.87; P < .001).

Patient Survival

During the follow-up period, 34.4% of the patients showed disease recurrence and most of them (31.8%) died. The median OS was 30 months (ranged from 2 to 86 months), and the DFS was 33 months (ranged from 2 to 85 months). In Kaplan-Meier curve analysis, neither MET CN alterations nor MET mRNA expression level influenced patients' OS or DFS (Figure 2, A and B). However, when the analysis was restricted to patients with ADC histology, both DFS and OS were shorter in the cases with an increased MET CN, although only DFS difference was statistically significant (log-rank test, P = .044 and P = .071 for DFS and OS, respectively; Figure 2, C and D). In contrast, in patients with SCC, MET copy gain was associated with a better outcome in terms of both DFS and OS in Kaplan-Meier analysis (log-rank test, P = .03 and P = .05 for DFS and OS, respectively; Figure 2, E and F). In patients with LCC, no effect of MET CNG on DFS or OS was found (data not shown).

For the whole cohort of the patients, in both univariate and multivariate proportional hazards models including patients' age, gender, smoking habit, TNM stage of the disease (I vs II + IIIA), lymph node metastases, MET CN, MET mRNA level in tumor, and tumor-associated alteration in MET mRNA, only the disease stage was an independent prognostic factor in terms of OS and DFS (hazard ratio [HR], 12.95 and 2.66; 95% CI, 4.36-38.46 and 1.13-6.23; P < .001 and P = .024 for OS and DFS, respectively; Table 3). However, in the univariate model, patients with ADC harboring increased MET CN had a 1.58-fold higher risk of disease relapse than those without a CNG (HR, 1.58; 95% CI, 1.10-2.27; P = .013). The significance also remained in the simplified multivariate model after age, TNM stage, lymph node metastases, MET mRNA level in tumor, and tumor-associated alteration in MET mRNA only the disease stage was an independent prognostic factor in terms of OS and DFS (hazard ratio [HR], 12.95 and 2.66; 95% CI, 4.36-38.46 and 1.13-6.23; P < .001 and P = .024 for OS and DFS, respectively; Table 3). No effect of analyzed parameters on DFS or OS in patients with LCC or SCC was found (Tables 4 and 5).

Discussion

In the current study, we showed a gain in MET CN in 18.5% of the analyzed tumors and a 1.76-fold tumor-associated increase in MET mRNA expression level. The observed proportion of MET copy gain was about two-fold higher than those in most previously reported studies, possibly due to different methods and scoring criteria used. In most investigations, the fluorescence in situ hybridization (FISH) or a similar (like silver or bright-field in situ hybridization) method was used and about 10% of NSCLCs were defined as MET FISH-
Figure 2. Kaplan-Meier survival curves of OS and DFS for the 151 patients with NSCLC in relation to \(MET\) CN (solid line in the case of \(MET\) CN < 3.0; dashed line in the case of \(MET\) CN \(\geq\) 3.0) in lung tumor tissue: (a and b) for the overall NSCLC patient group, (c and d) for the ADC patients only, and (e and f) for the SCC patients only.

Table 3. Univariate and Multivariate Analyses of Prognostic Factors for DFS and OS of Patients with NSCLC (Cox Proportional Hazards Model)

| Variable                        | DFS                  |                      | OS                    |                      |
|---------------------------------|----------------------|----------------------|-----------------------|----------------------|
|                                 | Univariate           | Multivariate         | Univariate            | Multivariate         |
|                                 | HR                   | 95% CI *             | P Value               | HR                   | 95% CI *             | P Value               |
| Age                             | 1.00                 | 0.97-1.04            | .999                  | 0.99                 | 0.95-1.02            | .458                  |
| Gender (female/male)            | 0.82                 | 0.43-1.57            | .549                  | 0.67                 | 0.32-1.43            | .303                  |
| Smoking (never/ever)            | 2.15                 | 0.67-6.91            | .199                  | 1.96                 | 0.50-7.74            | .335                  |
| \(pTNM\) (I/II + IIIA)          | 2.41                 | 1.30-4.46            | .005                  | 2.66                 | 1.13-6.23            | .026                  |
| Lymph node metastases (no/yes) | 1.55                 | 0.90-2.68            | .115                  | 1.14                 | 0.53-2.43            | .745                  |
| \(MET\) CN calculated          | 1.13                 | 0.91-1.41            | .279                  | 1.21                 | 0.92-1.59            | .175                  |
| \(MET\) mRNA in tumor (\(\Delta\text{Ct}\) value) | 1.00                 | 0.98-1.04            | .749                  | 1.04                 | 0.86-1.27            | .687                  |
| \(\log_{2}(MET\text{ mRNA RQ})\) | 1.10                 | 0.91-1.13            | .845                  | 1.10                 | 0.92-1.32            | .309                  |
| \(MET\text{ mRNA RQ}\)         | 1.00                 | 0.99-1.01            | .481                  | 0.99                 | 0.98-1.00            | .204                  |

* 95% CI for HR.
positive [6,8,9,16,18,20], although the results strongly depended on the cutoff criteria applied [8,9]. Very recently, Jin et al. found MET gene CNG by silver in situ hybridization in 24.1% of Korean NSCLC patients, although only stage I ADCs had been included in the study [17]. In our study, we used a qPCR method with a commercially available assay for MET CN evaluation and defined the cutoff value for copy gain as 3.0. Our results are similar to those obtained by Beau-Faller et al. [21] who also applied the qPCR technique. However, when we followed the cutoff definition by Beau-Faller as a mean CN in the corresponding normal lung tissues plus two SDs (equal to 3.99; data not shown), only 8.6% of the tumor samples analyzed in our study demonstrated an increased gene dosage, similar to the data reported by others [16,22].

According to our study, MET dosage status was not associated with the analyzed clinicopathologic features like age, gender, smoking history, histology, or pathologic stage. These results are in line with most previously reported data [8,15,16], although in a number of studies an increased MET CN was found to be more common in ADCs [18], women [7], smokers [7,17,22], and in larger [17] and poorly differentiated tumors [6,18]. A higher prevalence of MET amplification was also shown in advanced (pTNM III-IV) NSCLCs compared to early-stage (pTNM I-II) cases [6,9,22] and in stage IA ADCs compared to stage IB ones [17], as well as in lymph node stage 2 metastases compared to primary tumors [23].

We also found a statistically significant association between MET copy gain and an increase in MET mRNA level in tumor tissue. The association between MET dosage status and the expression at protein level by immunohistochemistry has been explored in a number of studies and a strong correlation has invariably been shown [7,16,17]. However, to our best knowledge, the present study is the first investigation where this association was demonstrated at mRNA level, suggesting that MET overexpression in the cells with an increased gene CN at least partly results from an enhanced transcription level.

According to the present study, the rate of MET copy gain was found to be higher in the tumors harboring increased EGFR or HER2 CN and/or EGFR activating mutations as compared to the tumors without these alterations. However, these associations were statistically significant only in ADC cases (with the exception of the association with EGFR mutations that did not reach the statistical significance) but not in LCC or SCC tumors. However, no correlation between MET copy gain and KRAS dosage or mutational status was found. The association between EGFR and MET copy gains had been demonstrated

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Table 4. Multivariate Analysis of Prognostic Factors for OS by Histologic Type (Cox Proportional Hazards Model)

| Variable                              | ADC (N = 64) | SCC (N = 52) | LCC (N = 35) |
|---------------------------------------|--------------|--------------|--------------|
|                                       | HR 95% CI    | P Value      | HR 95% CI    | P Value      | HR 95% CI    | P Value      |
| Age                                   | 0.97         | 0.92-1.02    | 1.00         | 0.93-1.06    | 1.08         | 0.96-1.22    | 2.14         |
| Gender (female/male)                  | 0.24         | 0.06-0.89    | 0.56         | 0.14-2.20    | 0.24         | 0.03-2.14    | 2.02         |
| Smoking (never/ever) *                | 40.97        | 2.04-823.49  | 0.15         | 1.97         | 0.46-8.48    | 0.361        | 11.63        | 113.19-33.3  | 0.39         |
| pTNM (I/II + IIIA)                    | 3.64         | 0.68-19.48   | 0.131        | 0.70         | 0.23-2.17    | 0.540        | 2.42         | 0.45-12.88   | 0.301        |
| Lymph node metastases (no/yes)       | 0.65         | 0.13-3.15    | 0.592        | 0.58         | 0.29-1.19    | 0.136        | 1.44         | 0.80-2.61    | 0.225        |
| MET CN calculated §                   | 1.56         | 0.91-2.37    | 0.118        | 1.73         | 0.87-1.58    | 0.800        | 0.68         | 0.35-1.32    | 0.255        |
| MET mRNA in tumor (DCR value)         | 0.76         | 0.49-1.18    | 0.218        | 1.03         | 0.85-1.36    | 0.538        | 0.83         | 0.44-1.57    | 0.569        |
| Log(MET mRNA FC)                      | 0.88         | 0.59-1.31    | 0.529        | 0.38         | 0.15-0.94    | 0.038        | 19.62        | 2.03-189.77  | 0.010        |
| Gender (female/male)                  | 0.29         | 0.09-0.94    | 0.038        | 1.76         | 1.20-2.57    | 0.004        | 1.33         | 0.86-2.06    | 0.199        |
| Smoking (never/ever)                  | 19.62        | 2.03-189.77  | 0.010        | 0.88         | 0.59-1.31    | 0.529        | 5.77         | 1.46-22.90   | 0.013        |
| MET CN calculated §                   | 0.60         | 0.35-1.04    | 0.068        | 0.58         | 0.29-1.19    | 0.136        | 1.42         | 0.91-2.21    | 0.118        |

* There were no nonsmokers among patients with SCC included in the study.

Table 5. Multivariate Analysis of Prognostic Factors for OS by Histologic Type (Cox Proportional Hazards Model)

| Variable                              | ADC (N = 64) | SCC (N = 52) | LCC (N = 35) |
|---------------------------------------|--------------|--------------|--------------|
|                                       | HR 95% CI    | P Value      | HR 95% CI    | P Value      | HR 95% CI    | P Value      |
| Age                                   | 0.96         | 0.92-1.00    | 1.00         | 0.92-1.07    | 1.04         | 0.94-1.38    | 0.192        |
| Gender (female/male)                  | 1.04         | 0.21-5.24    | 0.61         | 0.11-3.31    | 0.04         | 0.01-0.68    | 0.026        |
| Smoking (never/ever) *                | 3.30         | 0.12-87.46   | 0.475        | 9.35         | 1.03-84.78   | 0.047        | 605.46       | 10.50-34901  | 0.002        |
| pTNM (I/II + IIIA)                    | 12.37        | 1.95-78.50   | 0.008        | 0.63         | 0.13-3.07    | 0.567        | 0.21         | 0.02-2.69    | 0.235        |
| Lymph node metastases (no/yes)       | 0.80         | 0.50-1.29    | 0.563        | 1.24         | 0.88-1.74    | 0.214        | 1.99         | 0.84-4.70    | 0.116        |
| MET CN calculated §                   | 1.00         | 0.57-1.75    | 0.993        | 1.08         | 0.71-1.64    | 0.708        | 0.66         | 0.38-1.44    | 0.293        |
| MET mRNA in tumor (DCR value)         | 0.60         | 0.35-1.20    | 0.169        | 1.17         | 0.79-1.47    | 0.429        | 8.37         | 1.05-66.98   | 0.045        |
| Log(MET mRNA FC)                      | 1.08         | 0.71-1.64    | 0.325        | 1.13         | 0.88-1.46    | 0.325        | 0.69         | 0.22-2.26    | 0.550        |
| MET CN calculated §                   | 1.17         | 0.79-1.47    | 0.214        | 6.44         | 1.81-22.99   | 0.004        | 1.20         | 0.79-1.82    | 0.393        |

* There were no nonsmokers among patients with SCC included in the study.

† Simplified analysis after age, gender, smoking, lymph node metastases, MET mRNA level in tumor, and log(MET mRNA FC) removal from the model at the last step of the multivariable analysis.

‡ Simplified analysis after age, gender, smoking, lymph node metastases, MET mRNA level in tumor, and log(MET mRNA FC) removal from the model at the last step of the multivariable analysis.

§ Univariate analysis.

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Invasive growth and tumor progression, we are not able to explain the reason why FISH positivity was associated with a poor survival in our study. However, no association between MET protein expression level and prognosis was found in a study by Chyczewski et al. [16].

The relations between MET and EGFR alterations are of a great clinical importance in the light of the hypothesis that increased MET dosage might lead to the primary resistance of NSCLCs with EGFR mutations to EGFR TKIs [12], as has been demonstrated for the acquired resistance in approximately 20% of patients with NSCLC [10,11]. Recent investigations on cell cultures and clinical studies revealed that only a high level of MET amplification develops under EGFR TKI treatment and very rarely found in untreated tumors could result in TKI resistance [10,13,27], rather contradicting the impact of MET gene dosage on the primary response [15]. Only a moderate increase in MET CN was found in our study. However, the mean gene CN value for all the cells of the sample is defined by qPCR, not excluding a high level of gene amplification in a subset of cells due to tumor heterogeneity, as has been recently demonstrated for KRAS [28]. A more detailed analysis of tumor samples with MET alterations established with FISH method should clarify the issue.

Another important aspect concerning MET status is its possible significance as a prognostic factor in NSCLC. Most of the studies reported thus far consistently indicated a negative impact of MET abnormalities on the survival of patients with NSCLC [6,8,17,22], although contradictory results have also been reported [16]. According to the present study, ADC patients with an increased MET CN had a significantly shorter DFS, and the effect was independent of other clinopathologic variables in the multivariate analysis. Similar results had been obtained in a number of previous investigations where different methods for MET gene dosage evaluation were used [9,17,18,21]. To our surprise and in contrast to Beau-Faller results [21], an increased MET CN correlated significantly with a better outcome of our SCC patients in terms of both DFS and OS but was not an independent prognostic factor in the multivariate analysis. The prognostic impact of MET FISH status in patients with SCC had been reported previously by Go et al. [8], although in their study FISH positivity was associated with a poor survival of the patients. In the light of the current state of knowledge on the role of hepatocyte growth factor (HGF)/MET signaling in cellular invasive growth and tumor progression, we are not able to explain the beneficial influence of an increased MET CN on SCC patients’ outcome. Interestingly, the elevated MET CN correlated positively with a better prognosis in patients with NSCLC in the retrospective analysis by Kanteti et al. [29]. Further investigations on a larger patient cohort are needed to validate these observations. We also demonstrated a lack of correlation between MET mRNA expression and the clinical outcome in the whole patient cohort as well as, respectively, to a particular histologic type of tumor. Contradictory results have been reported by others, although the prognostic implications of MET protein expression by immunohistochemistry (IHC) instead of gene transcription level have been examined [6,9,29]. However, no association between MET protein expression level and survival was found in Dzidzioiuszko investigation, which was performed on a similar cohort of Polish NSCLC patients [16].

Conclusion
In conclusion, the obtained results demonstrate an increase in MET CN in a subset of untreated stage I to IIIA NSCLCs that occurs more frequently in tumors with EGFR and/or HER2 copy gain and EGFR activating mutations. An association between MET CN and MET mRNA expression level in tumor tissue also exists. An increased MET CN determined by qPCR with a commercially available assay might be a prognostic factor in patients with ADC after a curative surgery.

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