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Different *MET* gene alterations in lung adenocarcinoma patients

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ABSTRACT

Introduction. In this study, we attempted to detect selected abnormalities in the *MET* gene using various molecular techniques.

Material and methods. Twenty-six lung adenocarcinoma patients had a diagnosis of abnormalities in the genes: *EGFR*, *ALK*, *ROS1*, *MET*, and *RET.* They were diagnosed using various techniques and assessment of PD-L1 expression using immunohistochemistry. Copy number variation of *MET* gene was assessed by qPCR and FISH techniques, *MET* exon 14 mutation by RT-PCR method, and *MET* mRNA expression by the RT-qPCR technique. Statistical analyses were performed using Statistica v. 13.1 and MedCalc 15.8.

Results. Most patients (57.7%) had a high *MET* gene copy number in the qPCR method, which was not confirmed by the FISH method. A significant positive correlation ($R = +0.573$, $p = 0.0022$) between the *MET* gene copy number assessed with the qPCR method and the relative *MET* mRNA expression was found.

Conclusions. The positive correlation between the *MET* mRNA expression and the *MET* gene copy number in the qPCR test indicates that these methods could complement each other. The performance of these two tests simultaneously increases the reliability of the *MET* gene assessment.

Key words: *MET* gene, adenocarcinoma, lung cancer

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Introduction

The *MET* gene is a proto-oncogene, and it encodes the hepatocyte growth factor receptor (HGFR). The binding of its ligand, hepatocyte growth factor, induces dimerization and activation of the receptor. This activates the downstream RAS/ERK/MAPK, PI3K/AKT, and Wnt/β-catenin signaling pathways, which play a role in cellular survival, embryogenesis, cellular migration, invasion, angiogenesis, and the epithelial to mesenchymal transition (EMT) [1].

Abnormalities of the *MET* gene are one of the most frequently identified genetic disorders in neoplastic diseases. Germline mutations in the *MET* gene have been found in hereditary papillary renal carcinoma (HPRC). Somatic *MET* mutations have been observed in sporadic papillary renal cell carcinoma, head and neck squamous cell carcinoma, and childhood hepatocellular carcinoma. Amplification and overexpression of this gene are also associated with multiple human cancers. The *MET* gene is altered in 5% of non-small cell lung cancer (NSCLC) patients. Alterations in exon 14 of the *MET* gene are detected in 3–4% of lung adenocarcinoma patients. The prevalence of *de novo MET* amplification in NSCLC ranges from 1% to 5% of patients, depending on the assay and the positivity cut-point used. Several agents have been developed to target MET or HGF. They are divided into small molecule inhibitors and monoclonal

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antibodies. Currently, two tyrosine kinase inhibitors (TKIs), i.e. tepotinib and capmatinib, have been approved by the Food and Drug Administration (FDA) for the treatment of NSCLC patients with a splice site mutation in exon 14 of the *MET* gene. Response to therapy occurs in 46–68% of patients, depending on the mutation testing method (liquid biopsy *vs.* tissue) and the treatment line. However, it has also been shown that MET inhibitors (crizotinib, cabozantinib, and capmatinib) may be effective in NSCLC patients with *MET* gene amplification [2–5].

Splice site mutations in exon 14 of the *MET* gene are currently examined with the next-generation sequencing (NGS) technique. In turn, with the use of fluorescence *in situ* hybridization (FISH), the ratio of MET to the centromeric portion of chromosome 7 (*CEP7*) can be used to distinguish between chromosome polysomy and gene amplification. However, there are other cheaper, simpler, and faster methods for the examination of *MET* exon 14 mutations and the *MET* gene copy number, e.g. quantitative PCR (qPCR), including one using reverse transcription (RT-qPCR). However, PCR-based methods have numerous limitations, for example, low sensitivity and specificity. In this study, we attempted to detect selected abnormalities in the *MET* gene using various molecular techniques.

Material and methods

Patients

The study group consisted of 26 patients with lung adenocarcinoma (median age: 67.2 ± 8.5 years, 8 women, 18 men) diagnosed and treated in the Department of Pneumonology, Oncology, and Allergology from 2014 to 2019. All enrolled patients had a diagnosis of abnormalities in the *EGFR* gene (real-time PCR method), the *ALK* gene (IHC, FISH, and RT-qPCR methods), the *ROS1* gene (FISH and RT-qPCR methods), the *MET* gene (FISH, qPCR, RT-qPCR methods), and the *RET* gene (qRT-PCR method), as well as assessment of PD-L1 protein expression (IHC method). We enrolled one patient with *ALK* gene rearrangement, one patient with *RET* gene rearrangement, and 6 patients with *EGFR* gene mutations (three with deletions in exon 19 and three with substitution Leu858Arg). Seven patients had PD-L1 expression on ≥ 50% of tumor cells. The *ALK*-positive patient was treated with crizotinib in first-line therapy and alectinib in second-line therapy. Three patients with *EGFR* gene mutations received erlotinib, and other 3 patients with these mutations were administered afatinib. In two patients with progression after erlotinib or afatinib, a Thr790Met mutation was detected and osimertinib was administered. Two *EGFR*-positive patients received chemotherapy with cipslatin and pemetrexed. Only one patient with PD-L1 expression on ≥ 50% of tumor cells was treated with pembrolizumab. The other patients received first-line platinum-based chemotherapy (13 patients — cisplatin plus pemetrexed, 4 patients — cisplatin plus vinorelbine, and one patient — cisplatin plus gemcitabine). In second-line therapy applied in chemotherapy-resistant patients, atezolizumab was used in 9 patients, nivolumab in 2 patients, and docetaxel plus nintedanib in one patient. The median overall survival of our patients was 48 months (95% CI: 16.3–48.0). The demographic and clinical-pathological characteristics of the patients are presented in Table 1.

All aspects of the work covered in this manuscript were approved by the Ethics Committee of the Medical University of Lublin, Poland (No. KE-0254/169/2014).

Table 1. Characteristics of lung adenocarcinoma patients

Characteristics	Number of patients	Percentage of patients
Sex		
Female	8	31
Male	18	69
Stage of disease		
IIIB	8	31
IV	18	69
Smoking status		
Non-smokers	9	35
Former smokers	11	42
Current smokers	6	23
***EGFR* gene mutations**	6	23
Deletion in exon 19	3	11.5
Substitution Leu858Arg	3	11.5
PD-L1 expression on ≥ 50% of tumor cells		
Yes	7	27
No	19	73
First-line treatment	**26**	**100**
Chemotherapy (cisplatin plus pemetrexed or vinorelbine or gemcytabine)	18	69
Pembrolizumab	1	4
Crizotinib	1	4
EGFR TKIs (erlotinib or afatinib)	6	23
Second-line treatment	**17**	**65**
Chemotherapy (cisplatin plus pemetrexed or docetaxel plus nintedanib)	3	12
Immunotherapy (atezolizumab or nivolumab)	11	42
Alectinib	1	4
Osimertinib	2	7

Routine diagnosis of predictive factors in adenocarcinoma patients

DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissues or cytological specimens (cell blocks). DNA was extracted using the QIAamp DNA FFPE Tissue Kit (CE-IVD marked, Qiagen, Germany). Isolation was performed according to the manufacturer's instructions (the same isolated DNA was also used for examination of the *MET* gene copy number using the qPCR technique). The concentration and quality of isolated DNA were estimated by spectrophotometry. Mutations of the *EGFR* gene were identified using the EntroGen *EGFR* Mutations Analysis Kit (CE-IVD marked, EntroGen, Woodland Hills, Canada) in the Cobas Z 480 real-time PCR system (Roche Diagnostics, USA). We examined all the most common mutations in exons 18 to 21 [6].

Abnormal ALK protein and PD-L1 protein expression were examined using an immunohistochemistry test. ALK protein IHC staining was conducted on the Ventana Benchmark GX platform using CE-IVD approved anti-ALK Rabbit Monoclonal Primary Antibody (clone D5F3). The OptiView Amplification Kit and the OptiView DAB IHC Detection Kit were used as detection systems. Rabbit monoclonal negative control immunoglobulin was used as a negative control (Ventana Medical System, Tuscon, USA). CE-IVD approved Ventana SP263 antibody was used for PD-L1 protein IHC staining. The same equipment and detection systems were used for the examination of ALK expression. Rabbit monoclonal negative control immunoglobulin (Ventana Medical System, Tucson, AZ, USA) was used as a negative control. The slides were assessed by pathologists using an Olympus BX41 microscope [7–8].

All positive results of ALK expression obtained in IHC staining were re-evaluated with the FISH method to visualize the presence of *ALK* rearrangement using the Vysis *ALK* Break Apart FISH Probe Kit (Abbot Molecular, USA) and the paraffin-pretreatment IV and Post-Hybridization Wash Buffer Kit (Abbot Molecular, USA). In the diagnosis of *ROS1* gene rearrangement, we used the ZytoLight SPEC ROS1 DualColor Break Apart Probe (ZytoVision, Germany) and the Vysis Paraffin Pretreatment and Post-hybridization Wash Buffer Kit (Abbott, USA). Fluorescence signals were assessed using an Axio Scope microscope (Zeiss, Germany). Interpretation of FISH results was conducted in accordance with the American Food and Drug Administration (FDA) and International Association for the Study of Lung Cancer (IASLC) guidelines [8].

Reverse transcriptase PCR analysis of *ALK*, *ROS1*, and *RET* gene rearrangements and *MET* gene skipping mutations

Total RNA was extracted from FFPE tissues with the miRNeasy FFPE Kit (Qiagen Inc., Germany) according to the manufacturer's instructions. The RNA concentration was measured with Qubit 4 fluorometers (Invitrogen, Thermo Fisher Scientific, Waltham, USA). RNA samples were stored at –80°C until RT-qPCR (reverse transcriptase-quantitative PCR) was performed. The same isolated RNA was also used for the examination of the *MET* mRNA expression.

To detect *ALK*, *ROS1*, and *RET* gene fusions, as well as *MET* exon 14 skipping mutations, we used the Lung Cancer RNA Panel kit (EntroGen, Woodland Hills, Canada) according to the manufacturer's instructions. There were 8 reactions of twenty microliters in the volume of one-step RT-qPCR for one patient. Every reaction mixture contained 10 μL of One-Step RT-qPCR Reaction Mix, 1 μL of RT Enzyme Mix, 4 μL of Reaction Detection Primer Mix (one from eight), and 5 μL of RNA (concentration 16 ng/μL). The RT-qPCR reaction was performed on the Illumina Eco real-time PCR platform (Illumina, San Diego, USA) in the following conditions: 55°C for 10 minutes, 95°C for 1 minute, and next 40 cycles: 95°C for 10 seconds and 60°C for 45 seconds. Ct values were obtained, and analysis was performed according to the manufacturer's instructions.

MET gene amplification assessment with the FISH technique

The *MET* gene amplification status was assessed using the ZytoLight SPEC MET/CEN 7 Dual Color Probe (CE, ZytoLight, Germany). The Paraffin-Pretreatment and Post-Hybridization Wash Buffer Kit (Abbot Molecular, USA) was also used for the pre-staining procedure. Three to five μm thick paraffin sections were cut and mounted on the positively charged glass slides. All procedures were carried out according to the manufacturer's procedure.

The SPEC MET/CEN 7 Dual Color Probe is a combination of a probe with an orange fluorochrome direct labeled specific for the alpha satellite centromeric region of chromosome 7 (D7Z1) and a probe with a green fluorochrome direct labeled targeted at the *locus* in the *MET* gene located at 7q31.2. In a normal interphase nucleus, two orange and two green signals are expected. In cells with amplification of the *MET* gene *locus*, multiple copies of the green signal or green signal clusters will be observed. The fluorescence signals were assessed using an Axio Scope microscope (Zeiss, Germany).

We classified the cases into two categories: *MET*-positive (with amplification) and *MET*-negative (without amplification). The cutoff of the *MET*/*CEP7* ratio was 2.0. A sample was considered to have *MET* amplification if the mean *MET*/*CEP7* ratio was ≥ 2.0 or if the *MET*/CEP7 ratio was < 2.0, but the *MET*-copy number was ≥ 5 copies per nucleus or *MET* signal clusters were seen in more than 10% of tumor cell nuclei. According to a different classification, *MET* amplification was also classified using the low ratio (≥ 1.8 to ≤ 2.2 *MET*/*CEP7*), intermediate ratio (> 2.2 to < 5 *MET*/*CEP7*), and high ratio (≥ 5 *MET*/*CEP7*) [9–10].

MET gene copy number assessment with the qPCR technique

The *MET* gene copy number was assessed using a TaqMan primer set and probe (TaqMan Copy Number Assays, Hs00305306_cn, Thermo Fisher Scientific, Waltham, Massachusetts, USA). RNazeP (TaqMan Copy Number Reference Assay, human, RNaseP, Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used as an internal control. The 10-microlitre qPCR reaction mixture contained 5 μL of Genotyping Master Mix, 0.5 μL of TaqMan Copy Number Assays or TaqMan Copy Number Reference Assay, and 4.5 μL of DNA (concentration 5 ng/μL). The real-time PCR was performed in Illumina Eco (Illumina Inc., San Diego, California, USA). The temperature conditions of qPCR were as follows: enzyme activation at 95°C for 10 minutes followed by 40 cycles of two-stage PCR at 95°C for 15 seconds and next at 62°C for 90 seconds. Method $2^{-\Delta\delta Ct}$ was used for the calculations. The calibrator, which was a mixture of DNA obtained from lymphocytes of healthy subjects, was used for the calculations. According to the literature data, we assumed that more than 3 copies of the *MET* gene allowed us to find a high copy number of this gene.

MET mRNA expression analysis using RT-qPCR

RNA reverse transcription was performed using the High Capacity cDNA Reverse Transcription Kits (Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to the manufacturer's instructions. cDNA was stored at –20° C until qPCR was performed.

The expression of *MET* mRNA (cDNA) was assessed using primers and the TaqMan probe kit (TaqMan™ Gene Expression Assay Hs01565584_m1, Thermo Fisher Scientific, Waltham, Massachusetts, USA). GAPDH (Gene Expression Reference Assay Hs03929097_g1, Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used as an internal control. The composition of the reaction mixture (10 μL) was as follows: 5 μL of TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 0.5 μL of TaqMan Gene Expression Assay or GAPDH reference assay, 3.5 μL of nuclease-free water, and 1 μL of cDNA (RT-PCR reaction product). The real-time PCR was performed in Illumina Eco (Illumina Inc., San Diego, California, USA). The temperature conditions of the qPCR reaction were as follows: 50°C for 2 minutes (UNG, uracil-N-glycosylase activation), 95°C for 20 minutes (polymerase activation) followed by 40 cycles of two-stage PCR (95°C for 3 seconds then 62°C for 45 seconds). Method $2^{-\Delta Ct}$ was used for the calculations.

Statistical analysis

The U-Mann Whitney test was used for testing the equality of population medians among groups differing in clinical and demographic factors. The Spearman test was used to calculate the correlation between countable variables. Data were expressed as a percentage (for the categorized variable), median, and standard deviation (for continuous variables). These tests were performed with Statistica v. 13.1 (Tibco Software, USA). Survival analyses were performed using the Kaplan-Meier estimation method in MedCalc 15.8 (MedCalc Software, Ostend, Belgium) with a calculation of the 95% confidence interval (CI). We considered p values below 0.05 to be statistically significant.

Results

In our patients, we did not find *MET* exon 14 skipping mutations with the use of the RT-qPCR technique. We also did not demonstrate the presence of *MET* gene amplification or chromosome 7 polysomy with the FISH technique. The median *MET*/*CEP7* ratio was 1.04 with a standard deviation of 0.145, and the median number of chromosome 7 was 2.5 ± 0,788. In the FISH method, the median *MET* gene copy number was 2.6 ± 0.457. In contrast, the median *MET* gene copy number was 3.43 ± 1.539 in the qPCR study. Fifteen patients had more than 3 copies of the *MET* gene detected by the qPCR technique. According to the criteria adopted by other authors, we may conclude that 57.7% of the patients had a high *MET* gene copy number. The relative *MET* mRNA expression was low, and its median was 0.01 with a standard deviation of 0.045.

The age (division into two groups according to the median), stage of disease (IIIB versus IV), and smoking status did not affect the assessed parameters (*MET*/*CEP7* ratio, *CEP7* number, *MET* gene copy number in the FISH, and qPCR methods, and *MET* mRNA expression). However, the *MET*/*CEP7* ratio and *MET* mRNA expression were slightly higher in

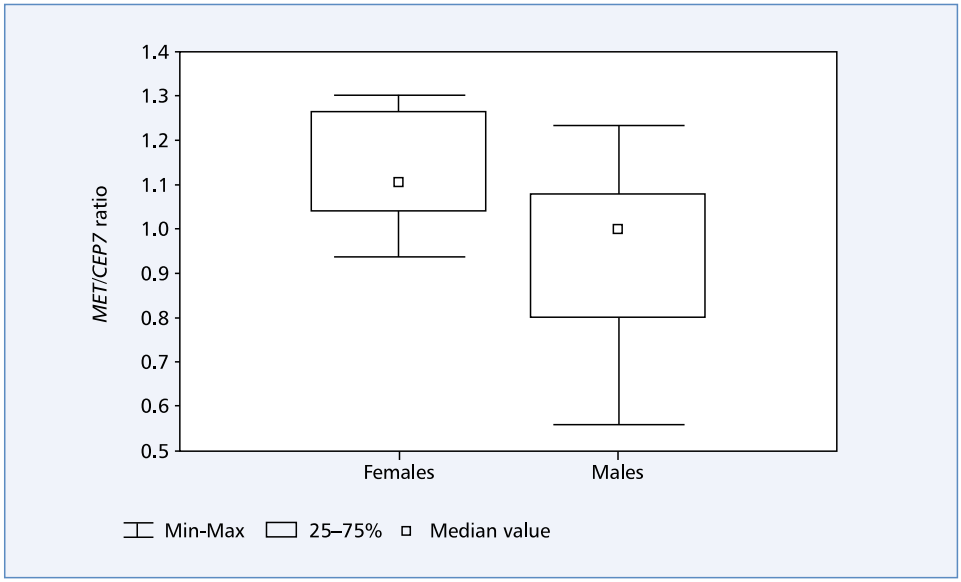

Figure 1. Value of the *MET/CEP7* ratio assessed with the FISH technique depending on the sex of the studied lung adenocarcinoma patients

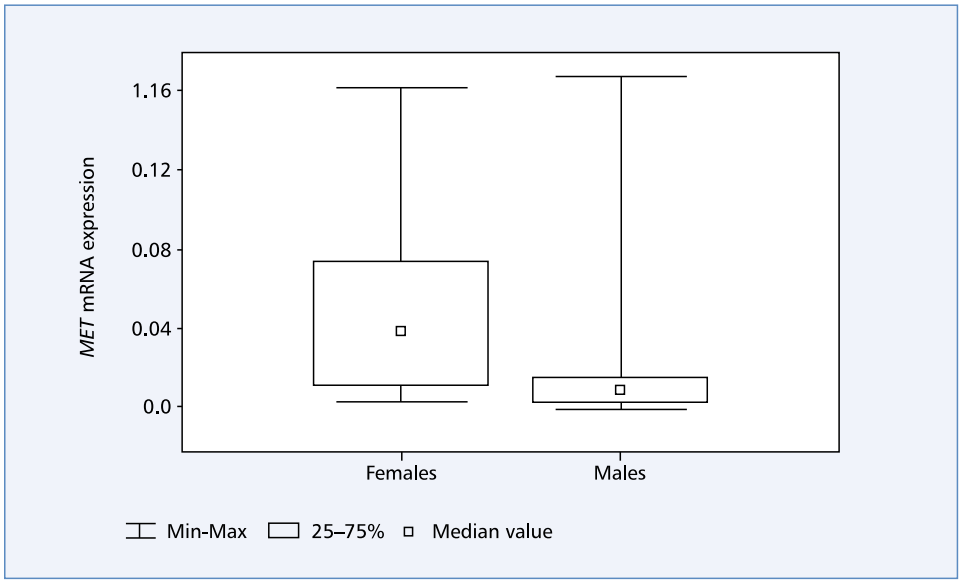

Figure 2. Relative *MET* mRNA expression assessed with the RT-qPCR method depending on the sex of the studied lung adenocarcinoma patients

the women than in the men ($p = 0.054$ and $p = 0.055$, respectively, Fig. 1 and 2). Moreover, the *MET* gene copy number in the FISH method was insignificantly higher in patients with genetic driver alterations (*EGFR* gene mutations, *ALK* and *RET* gene rearrangements) than in patients without these abnormalities ($p = 0.077$, Fig. 3).

We found a significant positive correlation ($R = +0.573$, $p = 0.0022$) between the *MET* gene copy number assessed with the qPCR method and the relative *MET* mRNA expression (Fig. 4). We did not detect a correlation between the *MET/CEP7* ratio and the *MET* gene copy number assessed with the qPCR method, as well as the *MET* mRNA expression. The *MET* gene copy number in the FISH technique did not significantly correlate with the *MET* gene copy number assessed with the qPCR technique and with the relative *MET* mRNA expression.

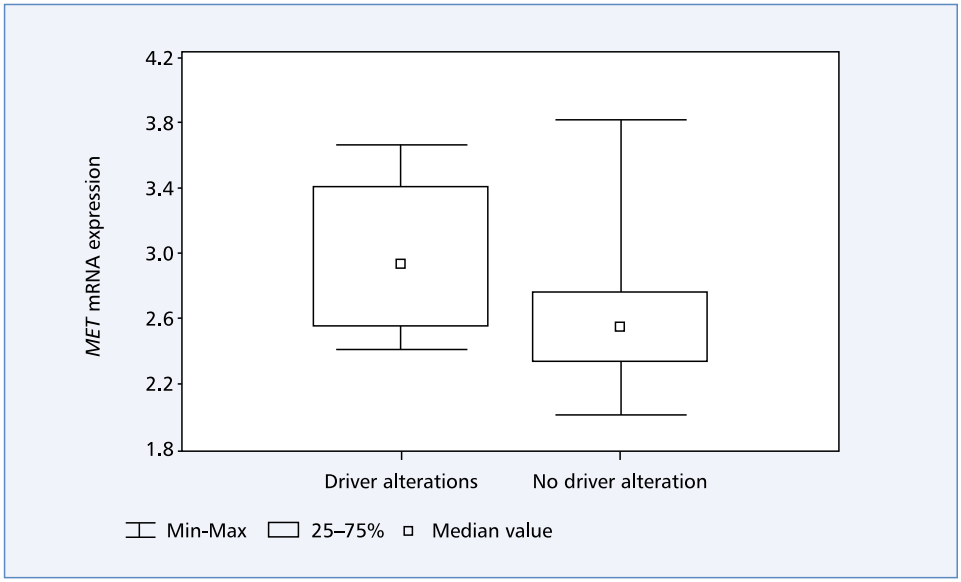

Figure 3. *MET* gene copy number assessed with the FISH method in patients with and without genetic driver alterations (*EGFR* gene mutations, *ALK* and *RET* gene rearrangements)

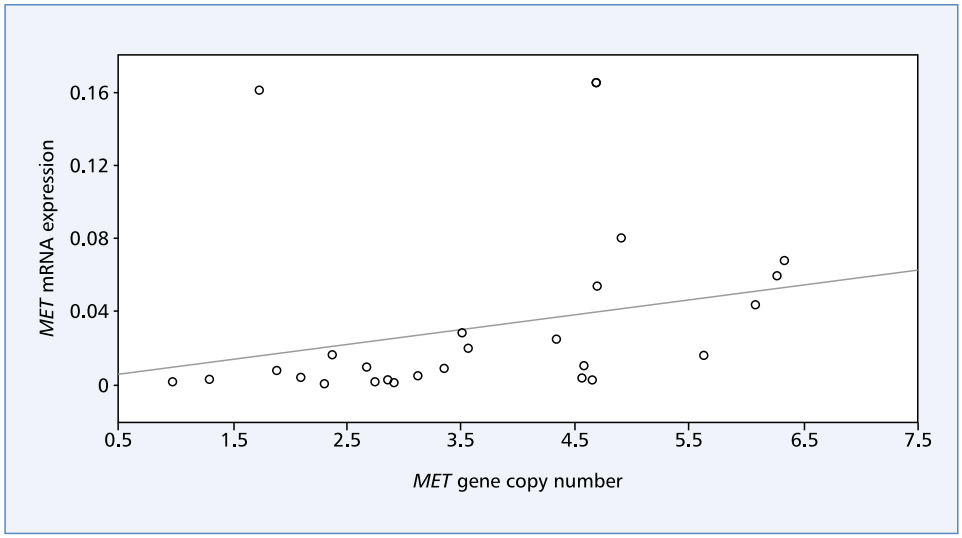

Figure 4. Significant positive correlation between the *MET* gene copy number assessed with the qPCR method and the relative *MET* mRNA expression in lung adenocarcinoma patients

Discussion

Most studies on the CNV of *MET* gene have been conducted in Asian populations. However, two studies on Caucasian NSCLC patients need to be mentioned. Capuzzo et al. [9], studied the CNV of the *MET* gene using the FISH technique in 435 Italian NSCLC patients, and Bubendorf et al. [11] studied abnormalities in the *MET* gene using the silver in situ hybridization (SISH) technique in the European population. Capuzzo et al. were the first to introduce a *MET* gene amplification evaluation system using the FISH technique. They found a high *MET* gene copy number (mean ≥ 5 copies/cell) in 48 cases (11.1%), including 18 cases with true gene amplification (4.1%). The high *MET* gene copy number was associated with an advanced stage ($p = 0.01$), a low grade of tumor differentiation ($p = 0.016$), and amplification of the *EGFR* gene in tumor cells ($p < 0.0001$). The authors found no relationship between patients' sex and the *MET* gene copy number in the cancer cells. No patient with an *EGFR* activating mutation showed a high *MET* gene copy number. *MET*-positive patients had

shorter survival than *MET*-negative patients (p = 0.005) [9]. These results differ from those presented in our study in which we showed that the women and patients with genetic driver alterations had a slightly higher *MET* gene copy number than the men and patients without other genetic abnormalities. On the other hand, we did not find patients with true *MET* gene amplification with an oncogenic character (responsible for tumor growth). This is likely to be related to the high percentage of patients with other genetic driver alterations included in our study. Driver abnormalities do not usually coexist in one patient.

Bubendorf et al. published results from the European Thoracic Oncology Platform (ETOP) Lungscape Project, which involved 1572 patients with surgically resected NSCLC. *MET* gene amplification was defined as a *MET*/*CEP7* ratio ≥ 2 and a high *MET* gene copy number as ≥ 5, as well as high MET protein expression in the IHC test as ≥ 2 + intensity in ≥ 50% of tumor cells. One hundred and eighty-two patients with MET protein expression and without mutations in the *EGFR* and *KRAS* genes were analyzed for the *MET* exon 14 skipping mutation. The high expression of the MET protein was significantly associated with the female sex and small tumor size. *MET* amplification occurred in 4.6% of patients, and a high *MET* gene copy number was detected in 4.1% of patients. The *MET* gene abnormalities were not significantly associated with the clinical and demographic characteristics of the patients. The *MET* exon 14 skipping mutation was detected in 5 of the 182 (2.7%) patients, including 4 adenocarcinoma patients (4.5%). The authors emphasized that the large inter-laboratory variability in the *MET* status assessment highlights the challenge of these analyses in routine practice [11].

Dziadziuszko R et al. [12] assessed the *MET* gene copy number using the SISH technique in 140 Polish NSCLC patients. The median value of the *MET* gene copy number per cell was 3.12 (from 1.74 to 11.84). Three patients (2.1%) showed gene amplification (*MET* gene clusters) and 14 (10%) had tumors with 5 or more gene copies per nucleus. There was a significant correlation between the *MET* copy number and the protein expression. The authors found no association between the *MET* gene copy number and the demographic or clinical features, including sex (p = 0.54), disease stage (p = 0.21), tumor grade (p = 0.86), and histology (p = 0.84), or smoking status (p = 0.47). They showed no associations between the *MET* copy number and disease-free survival or overall survival. In our study, we found that the median *MET* gene copy number tested with the qPCR technique exceeded 3, which is consistent with the observations reported by Dziadziuszko et al. [12], who used the FISH technique. It is debatable whether 3 or more copies of the gene should be used as a cutoff point for recognition of a high *MET* gene copy number in NSCLC patients [12].

In another study on the Polish NSCLC population, Kowalczuk et al. [13] used the qPCR technique to study the *MET* gene copy number and mRNA *MET* expression. In total, 151 patients with paired surgical samples of tumor and tumor-distant normal lung tissues were enrolled in the study. A high *MET* gene copy number (more than 3.0 copies per cell) was found in 18.5% of patients and occurred more frequently in adenocarcinoma with an increased *EGFR* and *HER2* gene copy number and with *EGFR* activating mutations (p = 0.051). The *MET* mRNA expression was 1.76-fold higher in the tumor compared to unaffected lung tissue, and it was associated significantly with the *MET* gene copy number. The results of this study are partially consistent with our results [13].

Aguado et al. [14] examined 422 NSCLC patients and identified 13 patients (3%) with *MET* exon 14 mutations and 15 patients (3.5%) with very high *MET* mRNA expression, which was analyzed using the quantitative transcript-based hybridization technology. These two subgroups of patients were mutually exclusive, displayed distinct phenotypes, and did not generally coexist with other genetic driver alterations. Ninety-two percent of patients with very high *MET* mRNA expression had *MET* gene amplification detected by FISH and/or NGS. However, FISH failed to identify three patients with very high *MET* mRNA expression, among whom one received MET tyrosine kinase inhibitors and obtained clinical benefit. These results indicated that *MET* mRNA expression assessment could improve the selection of patients for MET TKIs [14].

Kim JH et al. [15] performed a meta-analysis to evaluate the prognostic value of a high *MET* gene copy number in NSCLC patients. From 21 studies, 7647 patients were included in the pooled analysis of hazard ratios for disease-free survival or overall survival. Patients with a high *MET* gene copy number showed significantly worse survival than patients with a low *MET* gene copy number. The method used for *MET* CNV analysis included FISH, SISH, bright field in situ hybridization (BISH), and qPCR. The FISH technique was mostly used, but various cutoff criteria were adopted. A high *MET* gene copy number occurred in 1–38.9% of patients, depending on the technique used and the cut-point for positivity. Using the qPCR technique, more than 3 *MET* gene copies were considered a high *MET* gene copy number in most studies. A high *MET* gene copy number was detected in 5.6%, 18%, and 18.5% of NSCLC patients. In one study, the cutoff was the mean *MET* gene copy number, which was 1.31. In this study, a high *MET* gene copy number was found in 4.8% of patients. Surprisingly, there is a large discrepancy in the results and the fact that as many as 57.7% of patients

in our study had over 3 *MET* gene copies detected by the qPCR test [15].

Conclusions

Our study has several inherent limitations that need to be discussed. The group of adenocarcinoma patients was very small. Moreover, the group was heterogeneous regarding the occurrence of somatic driver alterations. Therefore, we did not detect any rare abnormalities in the *MET* gene. The 3 gene copy number cutoff for our qPCR test appears to be understated. This produces incorrect high *MET* gene copy number results in several patients. Nevertheless, the agreement of the *MET* mRNA expression and FISH results, as well as the positive correlation between the *MET* mRNA expression and the *MET* gene copy number in the qPCT test, indicate that these methods could complement each other. Performance of these two tests simultaneously (e.g. determination of mRNA expression and gene copy number) increases the reliability of *MET* gene assessment. Such a tool allows correct qualification of our patients for molecularly targeted therapies.

Conflict of interest

Authors declare no conflict of interest.

References

1. Organ SL, Tsao MS. An overview of the c-MET signaling pathway. Ther Adv Med Oncol. 2011; 3(1 Suppl): S7–SS19, doi: 10.1177/1758834011422556, indexed in Pubmed: 22128289.
2. AACR Project GENIE Consortium. AACR Project GENIE: Powering Precision Medicine through an International Consortium. Cancer Discov. 2017; 7(8): 818–831, doi: 10.1158/2159-8290.CD-17-0151, indexed in Pubmed: 28572459.
3. Drilon A, Cappuzzo F, Ou SHI, et al. Targeting MET in Lung Cancer: Will Expectations Finally Be MET? J Thorac Oncol. 2017; 12(1): 15–26, doi: 10.1016/j.jtho.2016.10.014, indexed in Pubmed: 27794501.
4. Paik PK, Felip E, Veillon R, et al. Tepotinib in Non-Small-Cell Lung Cancer with Exon 14 Skipping Mutations. N Engl J Med. 2020; 383(10): 931–943, doi: 10.1056/NEJMoa2004407, indexed in Pubmed: 32469185.
5. Wolf J, Seto T, Han JY, et al. GEOMETRY mono-1 Investigators. Capmatinib in Exon 14-Mutated or -Amplified Non-Small-Cell Lung Cancer. N Engl J Med. 2020; 383(10): 944–957, doi: 10.1056/NEJMoa2002787, indexed in Pubmed: 32877583.
6. Mok T, Carbone D, Hirsch F. IASLC atlas of EGFR testing in lung cancer. 2017. https://www.iaslc.org/research-education/publications-resources-guidelines/iaslc-atlas-egfr-testing-lung-cancer-guidebook.
7. Tsao MS, Kerr KM, Dacic S et al. The IASLC Atlas of PD-L1 Testing in Lung Cancer. 2017. https://www.iaslc.org/research-education/publications-resources-guidelines/iaslc-atlas-pd-l1-testing-lung-cancer.
8. Tsao MS, Hirsch FR, Yatabe Y. IASLC atlas of ALK and ROS1 testing in lung cancer. 2016. https://www.iaslc.org/research-education/publications-resources-guidelines/iaslc-atlas-alk-and-ros1-testing-lung-cancer.
9. Cappuzzo F, Marchetti A, Skokan M, et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. J Clin Oncol. 2009; 27(10): 1667–1674, doi: 10.1200/JCO.2008.19.1635, indexed in Pubmed: 19255323.
10. de Melo Gagliato D, Jardim DL, Falchook G, et al. Analysis of MET genetic aberrations in patients with breast cancer at MD Anderson Phase I unit. Clin Breast Cancer. 2014; 14(6): 468–474, doi: 10.1016/j.clbc.2014.06.001, indexed in Pubmed: 25065564.
11. Kerr KM, Dafni U, Schulze K, et al. ETOP Lungscape Consortium, Lungscape Consortium. Prevalence and clinical association of MET gene overexpression and amplification in patients with NSCLC: Results from the European Thoracic Oncology Platform (ETOP) Lungscape project. Lung Cancer. 2017; 111(1): 143–149, doi: 10.1016/j.lungcan.2017.07.021, indexed in Pubmed: 28838386.
12. Dziadziuszko R, Wynes MW, Singh S, et al. Correlation between MET gene copy number by silver in situ hybridization and protein expression by immunohistochemistry in non-small cell lung cancer. J Thorac Oncol. 2012; 7(2): 340–347, doi: 10.1097/JTO.0b013e318240ca0d, indexed in Pubmed: 22237262.
13. Kowalczuk O, Kozlowski M, Niklinska W, et al. Increased MET Gene Copy Number but Not mRNA Level Predicts Postoperative Recurrence in Patients with Non-Small Cell Lung Cancer. Transl Oncol. 2014; 7(5): 605–612, doi: 10.1016/j.tranon.2014.08.002, indexed in Pubmed: 25389455.
14. Aguado C, Teixido C, Román R, et al. Multiplex RNA-based detection of clinically relevant MET alterations in advanced non-small cell lung cancer. Mol Oncol. 2021; 15(2): 350–363, doi: 10.1002/1878-0261.12861, indexed in Pubmed: 33236532.
15. Kim JH, Kim HSu, Kim BJ. Prognostic value of MET copy number gain in non-small-cell lung cancer: an updated meta-analysis. J Cancer. 2018; 9(10): 1836–1845, doi: 10.7150/jca.24980, indexed in Pubmed: 29805710.