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Abstract

Background: Mesenchymal-to-epithelial transition (MET) abnormalities like amplification and exon 14 mutations may be responsive to targeted therapies. They are prevalent in lung sarcomatoid carcinomas (LSCs) and must be diagnosed as efficiently as possible. Hypothetically, c-MET overexpression by immunohistochemistry (IHC) may prove effective as a screening test for MET abnormalities. **Material and methods**: Tissue samples were obtained from consecutive patients with a resected LSC, in four oncologic centers. IHC was performed using the SP44 antibody (Ventana) and evaluated using the MetMab score and H-score. Fluorescence in situ hybridization (FISH) was applied with the dual color probe set from Zytovision (Clinisciences). True *MET* amplification was diagnosed when *MET* gene copy number was ≥5 and the ratio between *MET* gene copy number and chromosome 7 number was >2. All *MET* exon 14 alterations including those affecting splice sites occurring within splice donor and acceptor sites were detected in the routine molecular testing on genetic platforms. **Results**: A total of 81 LSCs were included. 14 (17%) exhibited positive IHC using the MetMab score and 15 (18.5%) using the H-score. *MET* amplification was detected in six tumors (8.5%) and *MET* exon 14 mutation in five (6%). A weak positive correlation between IHC and FISH was found (r=0.27, p=0.0001). IHC sensitivity for *MET* amplification was 50%, with a specificity of 83%, positive predictive value of 21.4%, and negative predictive value of 94.7%. IHC sensitivity for *MET* exon 14 mutations was 20%, with a specificity of 83%, positive predictive value of 7%, and negative predictive value of 94%. **Conclusion**: IHC is not a relevant screening tool for MET abnormalities in LSC.

Keywords

MET, immunohistochemistry, amplification, exon 14 mutations, lung sarcomatoid carcinoma

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Author's disclosures of potential conflicts of interest

Dr MORO-SIBILOT reports personal fees from Pfizer, Novartis, Roche, Lilly, Boehringer, Astra Zeneca, Amgen, during the conduct of the study; personal fees from Abbvie, Takeda, BMS, Msd, outside the submitted work.

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Introduction

The mesenchymal-to-epithelial transition (MET) pathway includes the tyrosine kinase receptor (TKR) c-MET, its ligand, hepatocyte growth factor (HGF), as well as downstream pathways involved in tumor growth, cell survival, invasion, and cell migration (1). MET pathway activation involves different mechanisms, such as amplification of the *MET* gene, exon 14 splicing site mutations, activating point mutations, or HGF-dependent upregulation.

Amplification of the *MET* gene, characterized by an increased *MET* gene copy number measured by FISH (fluorescence in situ hybridization), is found in 1 to 4% of non-small-cell lung cancers (NSCLCs) (2).

Mutations in *MET* exon 14 and its flanking introns can induce exon 14 splicing that is thought to cause loss of the intracellular juxta-membrane domain, whilst increasing c-MET expression at the membrane. The diversity of *MET* exon 14 alterations, including base substitutions or indels that disrupt the branch point of intron 13, the 3' splice site of intron 13, or the 5' splice site of intron 14, requires improved technologies such as next-generation sequencing (NGS) to cover the whole c.2942-64 to c.3082+42 region (3).

Recently, MET tyrosine kinase inhibitors (TKIs), such as crizotinib and capmatinib, have proven their efficacy on tumors harboring *MET* mutations or amplifications, suggesting oncogene addiction, therefore rendering MET screening highly relevant (4), (5).

Lung sarcomatoid carcinomas (LSCs) are rare tumors, accounting for less than 3% of all NSCLCs. They are more prevalent among males and smokers and characterized by both high resistance to platinum-based chemotherapy and poor prognosis (6). A higher frequency of *MET* exon 14 mutations has been reported in LSCs (7), with a prevalence ranging between 4.9% and 31.8% (8).

Techniques for the diagnosis of *MET* amplification and exon 14 mutations are not readily accessible in routine practice. FISH is expensive and time-consuming, whereas NGS panels

require sufficient tumor material and high DNA quality, although samples usually are needle-biopsied formalin-fixed paraffin-embedded (FFPE) tumors.

IHC could prove effective as a screening test in molecular abnormalities such as ALK rearrangements. While FISH remains the gold standard, ALK IHC is highly sensitive (100%), FISH being only performed in ambiguous IHC cases (9).

The aim of this study was to compare MET IHC, FISH, and exon 14 mutation detection in a series of LSC in order to evaluate IHC as a screening test.

Patients and methods

Patients and tissue tumor collection

Tissue samples were obtained from surgical lung biopsies of all consecutive patients with LSC diagnosed between 2005 and 2012 in four referral thoracic oncology centers. Clinical data and tumor characteristics were recorded as previously described (8). Each patient signed an informed consent form as required by national guidelines and samples were collected in line with current legislation.

Immunohistochemistry (IHC)

In brief, c-MET protein expression was assessed by IHC on 3µm-thick sections of FFPE tumor samples, using the rabbit monoclonal primary antibody SP44 (Ventana, Arizona, USA). The MetMab score was calculated using the method detailed in Table 1 (10). The H-score was obtained by multiplying the intensity (from 0 to 3) by the percentage of positive cells (from 0 to 100%), thus obtaining a scale ranging between 0 and 300. The H-score positivity threshold was 150 (11). IHC was conducted by a referent pathologist (Dr. M. Antoine).

Fluorescence in situ hybridization (FISH)

MET gene amplification was assessed using the *MET*/CEP7 dual color probe set from Zytovision (Clinisciences, France), according to the manufacturer's instructions. *MET* gene amplification was defined as a *MET* gene copy number ≥5 and a *MET*/CEP7 ratio >2 (2), performed by the same referent pathologist.

Molecular testing for MET gene sequence abnormalities

Overall, 10µm-thick sections were cut from the paraffin blocks. Tumor enrichment was performed through selection and macro-dissection of areas with at least 50% tumor cells. Total DNA was extracted and purified after paraffin removal, as previously described (8). All *MET* alterations from c.2942-64 to c.3082+42 affecting exon 14 splice sites that are indels occurring within splice donor and acceptor sites were detected in the routine molecular testing on ISO15189 certified genetic platforms. Mutations analysis methods were previously reported (8). All tumor samples were tested by a combined strategy of High Resolution Melting (HRM) assay using Lightcycler 480 system (Roche Diagnostics) in order to screen all gene sequence abnormalities of exon 14 of the *MET* gene c3082, c3082+1, c3082+2, and c3082+3, confirmed using MassARRAY iPLEX technology (Agena Bioscience). Samples for which material was available (*n=40*) were also tested by NGS using the solid tumor solution by Sophia Genetics® based on the xGen Lockdown IDT® probe-based capture technology.

Molecular screening for other mutations

Mass spectrometry was employed to test 214 mutations affecting 26 oncogenes and tumor suppressor genes (Panel Lungcarta©MassARRAY iPLEX genotyping technology [Agena Bioscience, San Diego, USA]), as previously described (8).

Statistical analyses

Continuous variables were expressed as medians with [min, max] intervals. Categorical variables were expressed as percentages. Comparisons between non-parametric continuous variables were conducted via Mann-Whitney test, and those between categorical variables with the Chi-squared test, or the Fisher's exact test when $n < 5$. Correlation between non-normally distributed continuous variables was calculated using Spearman's correlation coefficient. All the tests were two-sided, with results considered significant when $p < 0.05$. Analyses were performed using GraphPad Prism (GraphPad Software Inc, California, USA).

Results

General characteristics

Between 2005 and 2012, 81 patients with LSC were included, of whom 60 (74%) were male, and 75 (94%) were smokers (Table 2). The median age was 62 years. At diagnosis, 37 patients (46%) were at stage I-II disease, and 42 (52%) Stage III-IV disease. The most common histological subtype consisted of pleomorphic carcinoma (*n=63*, 77.5%). The most commonly detected mutations were *KRAS* (*n=21*, 26%), and *EGFR* (*n=11*, 13.5%). Positive c-MET IHC was observed in 15 (18.5%) tumors using the H-score (median 30 [0 - 260]), and in 14 (17%) using the MetMab score (median 0 [0 - 3]). True *MET* amplification was found in six patients (8.5%) and *MET* exon 14 mutation in five (6%). High *MET* amplification (*MET*/CEP7 ratio $\geq$5) was found in two patients.

Characteristics of patients with true MET amplification (MET gene copy number ≥5 and ratio MET/CEP7 >2)

Men represented 100% of patients with *MET* amplification, *vs* 71% (*n=46*) of patients without amplification (p=0.18, Table SI). Tumors with *MET* amplification all displayed a pleomorphic histological subtype, *vs* 50 (77%) without amplification (p=0.33). Patients with *MET* amplification exhibited less KRAS mutations, yet the difference did not reach statistical significance. Patients with polysomy were not considered (*n=8*, 11%).

Characteristics of patients with MET exon 14 mutations

MET exon 14 mutations were detected in five patients (6%). They were more often women (80% vs 22.5%, p=0.02). No other significant differences were found according to *MET* mutation status (Table SII).

Correlation between IHC and MET FISH / MET exon 14 mutation

In total, 15 patients (18.5%) had a positive IHC using the H-score and 14 (17%) using the MetMab score. Among *MET* amplifications (*n*=6), three (50%) had a positive c-MET IHC. No *MET* exon 14 mutations were associated with *MET* amplification. One tumor sample out of five (20%) with exon 14 mutation exhibited c-MET positive IHC (Figure 1).

Among chromosome 7 polysomies (*n=8*), one (12.5%) had a positive IHC, with no significant association (p=1.0). Two (25%) had a *MET* exon 14 mutation (versus 3% *MET* exon 14 mutations in patients without polysomy) with a significant association (p=0.02).

The correlation between IHC H-score and FISH proved weakly positive (R coefficient between 0 and 0.5) (*MET* gene copy number ≥5 regardless of *MET*/CEP7 ratio, i.e. including polysomies, in Figure 2, then *MET* gene copy number ≥5 and *MET*/CEP7 >2, i.e. without polysomies, in Figure 3) .

Considering FISH as the gold standard for *MET* amplification, IHC sensitivity was 50%, specificity 83%, positive predictive value 21.4%, and negative predictive value 94.7%. For high *MET* amplifications, IHC sensitivity was also 50%. IHC sensitivity for *MET* exon 14 mutations was 20%, with a specificity of 83%, positive predictive value of 7%, and negative predictive value of 94%.

Discussion

In this cohort of surgically resected LSCs, c-MET IHC overexpression, as well as *MET* amplification and *MET* exon 14 mutations were analyzed, to determine whether IHC could be a screening test for amplification or mutation. Altogether, 14 tumors (17%) had positive c-MET IHC, six had a *MET* amplification (8.5%), and five a *MET* exon 14 mutation (6%).

As *MET* amplification and exon 14 mutations are potential targets for TKIs (4) (5) in NSCLCs, and particularly in LSCs, these abnormalities must be diagnosed as efficiently as possible.

In this study, c-MET IHC could not be considered as a screening test either for *MET* amplification or *MET* exon 14 mutations, as sensitivity (50% and 20% respectively) and correlation (r=0.27) proved poor, with similar results found for MetMab and H-score. Current data regarding correlation between IHC and MET molecular alterations are discordant. Watermann *et al.* found a weak correlation (r=0.06, p >0.05) between MET IHC and FISH in 214 NSCLC samples (12). Casadevall *et al.* showed no association between IHC and FISH in a non-squamous NSCLC cohort (13). Conversely, Park *et al.* reported a significant association between IHC score and *MET* amplification (p<0.001) using the Chi-squared test on a large series of 316 adenocarcinomas, with neither sensitivity nor correlation reported (14). Additionally, Tong *et al.* revealed in 687 NSCLCs a significant association between IHC and amplification, and between IHC and exon 14 mutation (p <0.001), based on Chi-squared test analyses. Nevertheless, the correlation using Spearman's test proved to be weak (3). Furthermore, whether significant association between IHC and amplification/mutation does exist or not, sensitivity is a better parameter for diagnosis purposes.

For several other molecular abnormalities, such as ALK rearrangement, IHC proves to be an effective screening option. ALK IHC and FISH are highly correlated, with a sensitivity for IHC approaching 100% (9). FISH is performed only in ambiguous IHC cases. For ROS1 rear-

rangements, IHC sensitivity compared to FISH is reported close to 100% with a specificity of 97% (15).

As *MET* exon 14 mutations induce loss of ubiquitination and increased c-MET membrane presence, their lack of association with c-MET overexpression remains to understand. One explanation is that the oncogenic properties of *MET* exon 14 mutations involve other mechanisms (loss of serine 985 with increased kinase activity, loss of aspartate 1002 resulting in loss of pro-apoptotic signals) (1). Another explanation is that LSCs have a different genomic background compared to NSCLCs, which might influence these results. Indeed, *MET* gene abnormalities occur in a context of other oncogenic and tumor suppressive genes abnormalities involving *TP53*, *LKB1* or *EGFR* point mutations, or gene copy number variations that can influence the c-MET protein expression.

Regarding *MET* exon 14 mutation detection, we used a combined technique (HRM + MassARRAY) for all tumor samples (n=81). This allows detection of point mutations, and indels that are deleterious with a higher sensitivity than NGS. Nevertheless, the MassARRAY technology can miss some large deletions. Therefore, NGS was performed on tumor samples with sufficient material (n=40) to increase detection sensitivity. It is worth noticing that NGS allowed detection of one exon 14 mutation that was not diagnosed by HRM + MassARRAY, but also that MassARRAY allowed diagnosis of one more exon 14 mutation, undetected by NGS (data not shown).

Few studies focus specifically on LSC. The main cohorts investigating MET pathway abnormalities in LSCs are summed up in Table SIII. Discrepancies exist in the prevalence of positive IHC, amplification or exon 14 mutations, between those cohorts. Our results tend to be on the low end of each one of those abnormalities. It is however worth noticing that patients with exon 14 mutations and negative IHC exist in other series. These discrepancies can be explained by the small number of positive cases, which also reduces the power of the given sensitivity and specificity values.

Our findings suggest that MET IHC may not be employed as a screening tool. *MET* FISH and molecular biology techniques (NGS, WES, and fragment analysis) are recommended for the detection of amplifications or exon 14 mutations in NSCLC in routine practice.

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Figures and tables

Table 1: IHC results according to the MetMab score

≥50% strong intensity	**3+**	**Positive IHC**
≥50% strong or moderate intensity, but **<50% strong intensity**	**2+**	
≥50% strong or moderate or weak intensity, but **<50% moderate or weak intensity**	**1+**	**Negative IHC**
No staining or <50% of any intensity	**0**	

Table 2: Characteristics of overall population (*n=81*)

Variable	**All patients *(n = 81)***
Gender	
Male	60 (74)
Female	21 (26)
Age	
Median (Range)	62 (44 – 81)
Ethnicity	
Caucasian	65 (80)
Asian	0 (0)
Northern African	10 (12.5)
Sub-Saharan African	1 (1.5)
Smoking history	
Smoker	75 (94)
Non-smoker	5 (6)
Clinical stage	
Stage I – II	37 (46)
Stage III – IV	42 (52)
Histological subtype	
Pleomorphic carcinoma	63 (77.5)
Spindle cell carcinoma	4 (5)
Giant cell carcinoma	6 (7.5)
Other	8 (10)
Mutations	
MET exon 14	5 (6)
EGFR (TKI sensitizing)	11 (13.5)
KRAS	21 (26)
BRAF	2 (2.5)
PI3KCA	4 (5)
Positive IHC	
MetMab	14 (17)
H-score	15 (18.5)
Positive FISH	14 (20)
Polysomy	8 (11)
True *MET* amplification	6 (8.5)

MET = Mesenchymal to Epithelial Transition; *EGFR* = Epidermal Growth Factor Receptor; TKI = tyrosine kinase inhibitor; *KRAS* = Kirsten rat sarcoma oncogene; *BRAF* = B-raf proto-oncogene; *PI3KCA* = Phosphatidyl-inositol-3-kinase Catalytic Subunit Alpha; IHC = immunohistochemistry; FISH = Fluorescence in situ hybridization.

Table SI: Characteristics according to *MET* amplification status

Variable	***MET* amplification (*n = 6*) *n (%)* or median (range)**	**No *MET* amplification (*n = 65*) *n (%)* or median (range)**	***p*-value**
Gender			
Male	6 (100)	46 (71)	***p* = 0.18**
Female	0 (0)	19 (29)	
Age			
Median (Range)	61.5 (57 – 80)	61.5 (44 – 81)	*p* = 0.52
Ethnicity			
Caucasian	5 (83)	53 (81)	*p* = 0.66
Asian	0 (0)	0 (0)	
Northern African	0 (0)	8 (12)	
Sub-Saharan African	0 (0)	1 (1.5)	
Smoking history			
Smoker	6 (100)	60 (92)	*p* = 1.0
Non-smoker	0 (0)	4 (6)	
Tobacco use (pack-years)			
Median (Range)	37.5 (12 – 40)	36 (0 – 100)	*p* = 0.62
Occupational exposure	0 (0)	8 (12)	*p* = 1.0
Clinical stage			
Stage I – II	2 (33)	30 (46)	*p* = 0.68
Stage III – IV	4 (67)	34 (52)	
Histological subtype			
Pleomorphic carcinoma	6 (100)	50 (77)	***p* = 0.33**
Spindle cell carcinoma	0 (0)	3 (4.5)	
Giant cell carcinoma	0 (0)	5 (8)	
Other	0 (0)	7 (10.5)	
Mutations			
MET exon 14	0 (0)	5 (7.5)	*p* = 0.51
EGFR (TKI sensitizing)	1 (17)	7 (11)	*p* = 0.52
KRAS	0 (0)	21 (32)	***p* = 0.17**
BRAF	0 (0)	2 (3)	*p* = 1.0
PI3KCA	0 (0)	4 (6)	*p* = 1.0
TP53	3 (50)	10 (15)	***p* = 0.07**
NRAS	0 (0)	1 (1.5)	*p* = 1.0

Table SII: Characteristics according to *MET* exon 14 mutation status

Variable	***MET* exon 14 mutation (*n = 5*)** ***n (%)* or median (range)**	**No *MET* exon 14 mutation (*n = 76*)** ***n (%)* or median (range)**	***p*-value**
Gender			
Male	1 (20)	59 (77.5)	***p* = 0.02**
Female	4 (80)	17 (22.5)	
Age			
Median (range)	66 (65 – 74)	61 (44 – 81)	*p* = 0.21
Smoking history			
Smoker	4 (80)	71 (94)	*p* = 0.4
Non-smoker	1 (20)	4 (6)	
Tobacco use (pack-years)			
Median (range)	18.5 (17 – 56)	35.5 (0 – 100)	*p* = 0.18
Clinical stage			
Stage I – II	2 (40)	35 (46)	*p* = 0.88
Stage III – IV	3 (60)	39 (51.5)	
Histological subtype			
Pleomorphic carcinoma	3 (60)	60 (79)	*p* = 0.33
Spindle cell carcinoma	0 (0)	4 (5)	
Giant cell carcinoma	1 (20)	5 (6.5)	
Other	1 (20)	7 (9)	
Mutations			
EGFR (TKI sensitizing)	1 (25)	10 (13)	*p* = 0.88
KRAS	1 (25)	20 (26)	*p* = 0.9
BRAF	0 (0)	2 (2.5)	*p* = 0.9
PI3KCA	0 (0)	4 (5)	*p* = 0.84

Study	LSC (n)	cMET IHC+ n (%)	Primary antibody	*MET* Amp+ n (%)	*MET* ex 14+ n (%)	*MET* ex 14 technique	*MET* ex 14 mutations and c-MET IHC+ n (%)*	*MET* ex 14 mutations and *MET* Amp+ n (%)**
Vieira et al, Lung Cancer, 2014	77	26 (34%)	SP44	NA	2 (3%)	Sizing analysis of PCR products (only 3'-splice site of *MET* ex 14 deletions)	1 (50%)	NA
Liu et al, JCO 2015	36	NA	NA	NA	8 (22.2%)	Whole exome sequencing	NA	NA
Awad et al, JCO 2016	15	NA	SP44	NA	4 (26.6%)	NGS	NA	NA
Schrock et al, JTO 2016	104	NA	NA	NA	8 (7.7%)	NGS	NA	1 (NA)
Tong et al, Clin Can Res, 2016	22	9 (40.9%)	SP44	3 (13.6%)	7 (31.8%)	Whole ex 14 + flanking intronic regions Sanger sequencing	7 (100%)	2 (28.5%)
Kwon et al, Lung Cancer 2017	45	NA	SP44	NA	9 (20%)	qRT-PCR	3 (33%)	NA
Schrock et al, JTO 2017	125	NA	NA	n=1	15 (12%)	CGP	NA	NA
Mignard et al, 2018	81	15 (18.5%)	SP44	6 (8.5%)	4 (4.9%)	HRM + MassArray + NGS	1 (20%)	0 (0%)

Table SIII: Comparison of MET pathway abnormalities prevalence among different cohorts of patients with LSC

Caption: LSC = Lung Sarcomatoid Carcinoma ; IHC+ = positive immunohistochemistry ; Amp+ = positive amplification ; ex 14+ = positive exon 14 mutation ; PCR = Polymerase chain reaction ; NGS = Next-Generation Sequencing ; CGP = Comprehensive genomic profiling ; HRM = High resolution melting ; NA = Not applicable ; * Percentage of patients harboring a *MET* exon 14 mutation, exhibiting positive c-MET IHC ; ** Percentage of patients harboring a *MET* exon 14 mutation, exhibiting positive *MET* amplification

Figure 1

Title: Distribution of MET expression positivity by IHC, FISH and exon 14 mutations in the overall population

Caption: Positive c-MET IHC was found in 15 (18.5%) tumors using the H-score and in 14 (17%) using the MetMab score. Six patients (8.5%) had a true *MET* amplification (*MET* gene copy number ≥5 and ratio *MET*/CEP7 >2). Five patients (6%) had a *MET* exon 14 mutation.

Figure 2

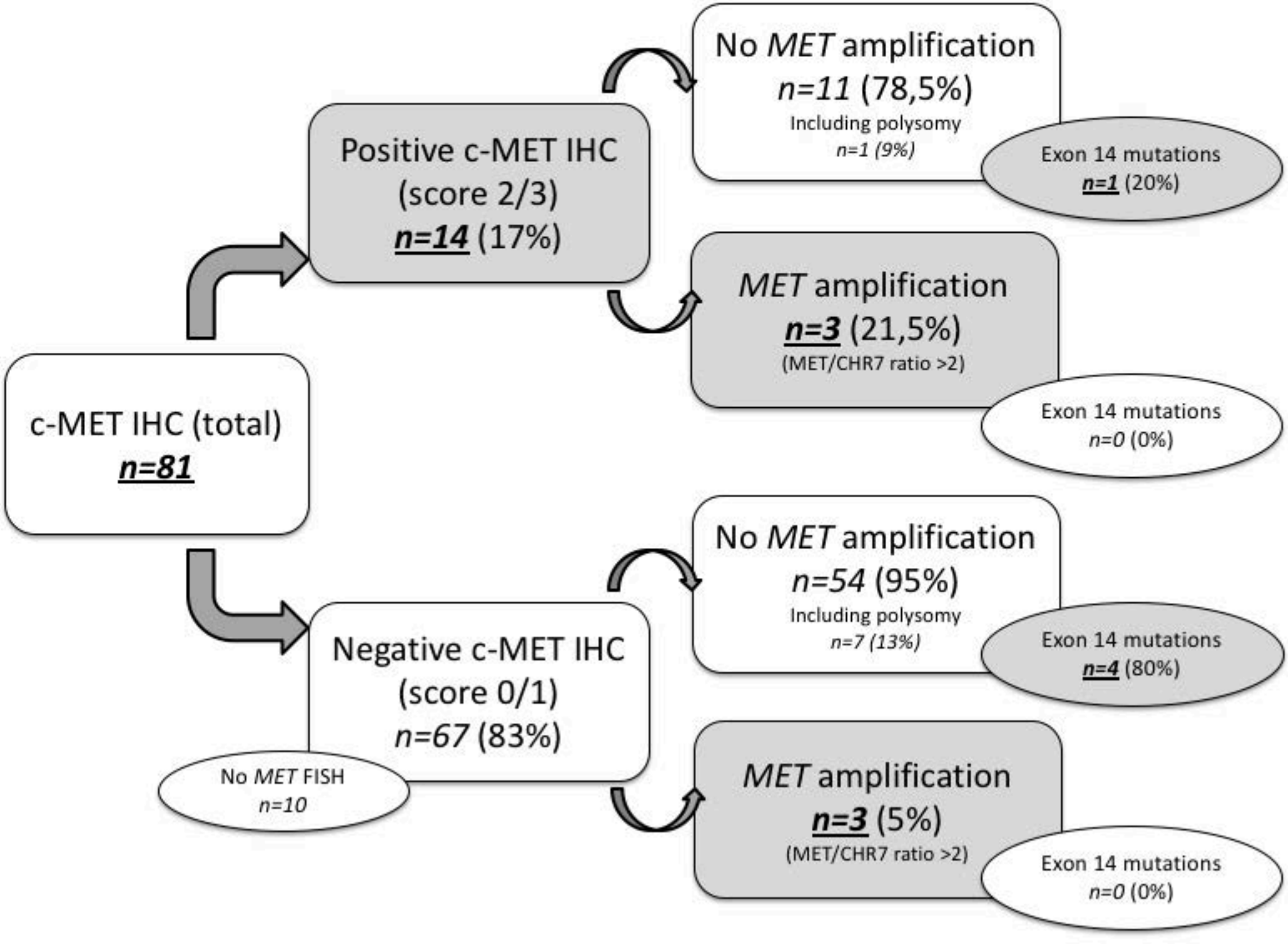
Title: Correlation between H-score based IHC and *MET* gene copy number by FISH

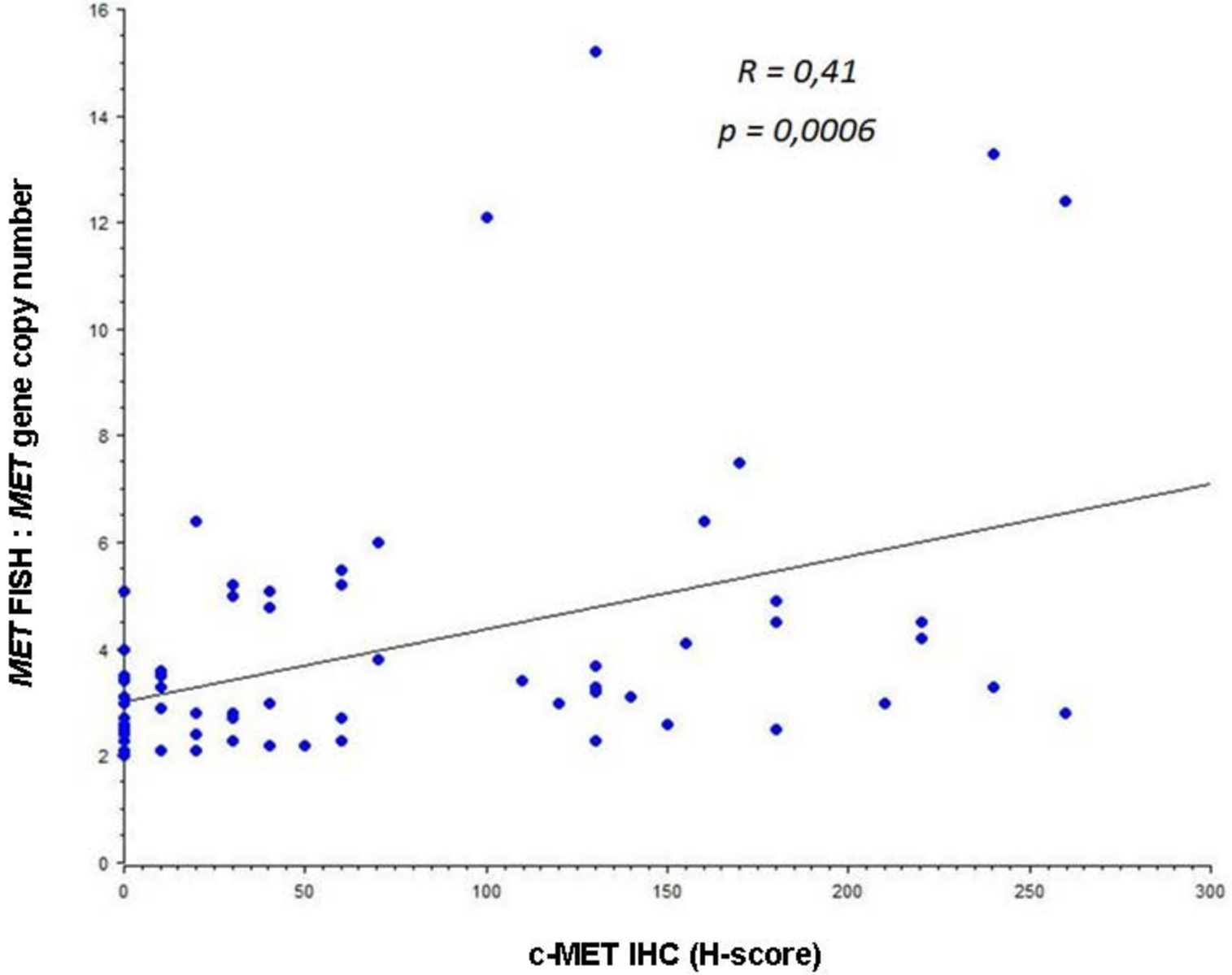
Caption: The horizontal axe represents the H-score value (between 0 and 300) for c-MET IHC and the vertical axe represents the *MET* gene copy number measured by FISH. Spearman's rho coefficient was calculated (R=0.41, p=0.0006).

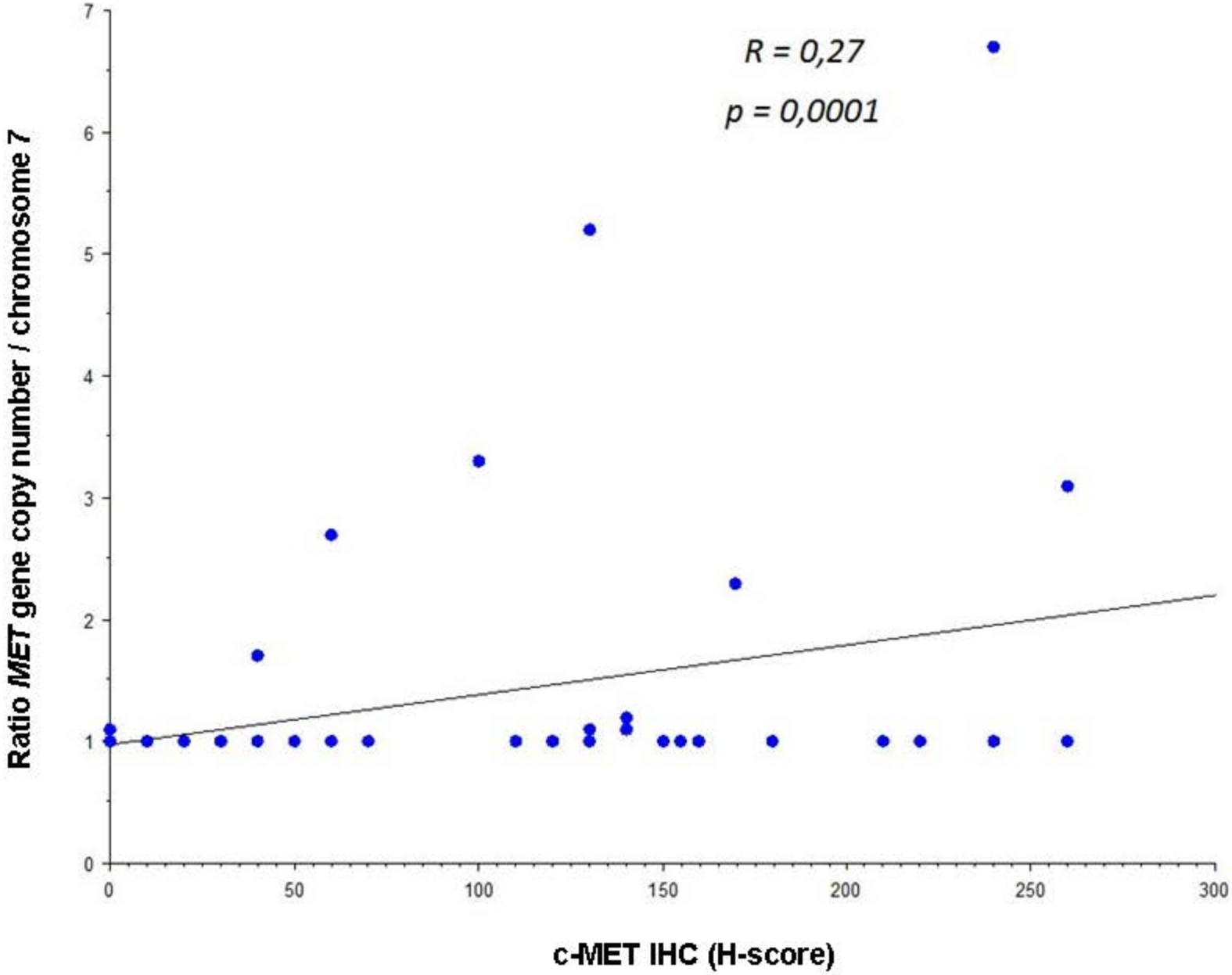
Figure 3

Title: Correlation between H-score based IHC and *MET* true amplification by FISH

Caption: The horizontal axe represents the H-score value (between 0 and 300) for c-MET IHC and the vertical axe represents the *MET* gene copy number/chromosome 7 ratio measured by FISH. Spearman's rho coefficient was calculated (R=0.27, p=0.0001).


c-MET IHC (total)
n=81
Positive c-MET IHC
(score 2/3)
n=14 (17%)
No MET amplification
n=11 (78,5%)
Including polysomy
n=1 (9%)
Exon 14 mutations
n=1 (20%)
MET amplification
n=3 (21,5%)
(MET/CHR7 ratio >2)
Exon 14 mutations
n=0 (0%)
Negative c-MET IHC
(score 0/1)
n=67 (83%)
No MET FISH
n=10
No MET amplification
n=54 (95%)
Including polysomy
n=7 (13%)
Exon 14 mutations
n=4 (80%)
MET amplification
n=3 (5%)
(MET/CHR7 ratio >2)
Exon 14 mutations
n=0 (0%)


R = 0,41
p = 0,0006
MET FISH : MET gene copy number
c-MET IHC (H-score)
0
2
4
6
8
10
12
14
16
0
50
100
150
200
250
300


R = 0,27
p = 0,0001
Ratio MET gene copy number / chromosome 7
c-MET IHC (H-score)
0
1
2
3
4
5
6
7
0
50
100
150
200
250
300