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c-MET as a biomarker in patients with surgically resected non-small cell lung cancer

Georgios Tsakonas[a,*], Johan Botling[b], Patrick Micke[b], Chris Rivard[c], Linnea LaFleur[b], Johanna Mattsson[b], Teresa Boyle[c], Fred R. Hirsch[c], Simon Ekman[a]

[a] Thoracic Oncology Center, Theme Cancer, Karolinska University Hospital/Department of Oncology- Pathology, Karolinska Institutet, Stockholm, Sweden
[b] Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden
[c] Division of Medical Oncology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

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ABSTRACT

Background: c-MET protein overexpression has been proposed as a biomarker in non-small cell lung cancer (NSCLC), albeit its role in the clinical setting has not been firmly established yet.

Patients and methods: We designed a retrospective cohort study, consisting of 725 patients with surgically removed NSCLC. Immunohistochemistry (IHC) was conducted in tissue microarrays (TMA) from lung tumors and healthy tissue. IHC staining was quantified using H-scores (range 0–300). Association between c-MET H-score and overall survival (OS) as well as progression-free survival (PFS) was explored.

Results: c-MET H-score ≥20 had a significant positive impact on OS in the multivariate analysis in the whole study population, HR = 0.79 (95%CI: 0.64 − 0.97). The prognostic effect of c-MET H-score ≥20 was even stronger in patients who received adjuvant treatment with a HR = 0.61 (95% CI: 0.40 − 0.93). In the subgroup of adenocarcinoma and squamous cell carcinoma patients with stage IIA-IIIB disease, the prognostic impact of c-MET was significant in the univariate analysis (HR = 0.60, 95% CI: 0.43 − 0.83).

Conclusion: c-MET H-score ≥20 is a positive prognostic biomarker for OS in early stage NSCLC. This benefit seems to be strongly correlated to adjuvant chemotherapy, therefore rendering c-MET H-score ≥20 a possible predictive biomarker for platinum-based adjuvant chemotherapy in early stage NSCLC.

1. Introduction

Hepatocyte Growth Factor (HGF) and its receptor cellular Mesenchymal Epithelial Transition factor (c-MET), a heterodimeric tyrosine kinase receptor, are frequently expressed in non-small cell lung cancer (NSCLC) and represents an oncogenic signaling pathway of major interest in NSCLC. c-MET protein overexpression, gene amplification and exon 14 splicing mutation (METex14) have been proposed as potential prognostic biomarkers as well as predictive biomarkers for targeted therapy, albeit their role in the clinical setting has not been firmly established yet [1,2].

c-MET protein is overexpressed in 13–36 % of primary lung cancer specimens and is often associated with poor prognosis [3–5]. Albeit, in the ETOP study by Bubendorf et al, neither c-MET overexpression nor MET gene amplification influenced prognosis in a large cohort of early stage NSCLC patients [6]. In the brain metastatic setting, c-MET overexpression has been reported to be as high as 44.4% [7]. The immunohistochemistry (IHC) methods used to define c-MET overexpression have varied in different publications, and up to date no consensus exists [2,6–10]. The role of c-MET as a predictive biomarker is still unclear. A recent surgical cohort study in Asian NSCLC patients (n = 311, of whom 151 received adjuvant chemotherapy), showed that overall survival (OS) was significantly prolonged in the c-MET positive cases who received platinum-based adjuvant chemotherapy, but this finding needs to be verified in larger cohorts [2]. However, in a study by Cappuzzo et al. in early stage NSCLC, MET gene amplification was a negative prognostic biomarker for OS [11]. Another study done in 689 patients with early stage disease who were treated with surgery, failed to show any prognostic value of MET amplification or cMET overexpression. In this study the prevalence of MET overexpression and MET amplification was 17% and 2.4% respectively [10].

An Asian randomized phase 3 trial with erlotinib with or without the c-MET inhibitor tivantinib (ARQ197) in EGFR wild-type non-squamous NSLCL failed to show any survival benefit with the combination, and was terminated prematurely due to higher incidence of interstitial lung disease in the tivantinib arm [12]. Another phase 3 study with a

* Corresponding author at: Department of Oncology, Karolinska University Hospital/Karolinska Institute, 17176, Stockholm, Sweden.
E-mail address: georgios.tsakonas@ki.se (G. Tsakonas).

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similar design in Caucasian population was terminated early at the interim analysis due to futility [13]. Both of these studies were done in an unselected population regarding c-MET expression. Onartuzumab, a fully humanized recombinant monoclonal antibody binding to the extracellular domain of c-MET, failed to show any survival benefit when combined with erlotinib compared to single erlotinib in a phase 3 randomized trial in previously treated stage IIIB or IV NSCLC determined to be c-MET positive (≥ 50% of tumor cells with IHC scores of 2+ [moderate] or 3+ [strong] levels of MET) [14]. Furthermore, no effect of onartuzumab/erlotinib combo was shown in exploratory analyses using MET FISH and gene expression. Although the above mentioned trials failed to show any benefit, several smaller trials and case series have shown efficacy of c-MET tyrosine kinase inhibitors or monoclonal antibodies targeting c-MET, mainly in MET exon 14 mutated NSCLC, but these findings need verification in larger randomized trials [9,15].

The aim of our study was to identify a clinically significant IHC cut-off for c-MET protein expression in patients with early stage NSCLC, and investigate its role as a potential prognostic or predictive (regarding adjuvant chemotherapy) biomarker.

2. Methods

2.1. Patient population

We designed a retrospective cohort study, consisting of 725 patients with surgically removed NSCLC. Patients received surgical treatment from 1/1/1995 to 30/12/2010 (Table 1)

Demographic data were collected retrospectively. We collected information about physician´s evaluation of performance status (PS) at the time of diagnosis, age at diagnosis, histology, received adjuvant treatment, surgical stage of disease, smoking status and gender. The TMA cohort was based on diagnostic tissue from NSCLC patients operated at the Uppsala University Hospital between 1995 and 2010 and histopathological data for parts of this cohort has been reported previously [16].

2.2. IHC analysis

Immunohistochemistry (IHC) was conducted in tissue microarrays (TMA) from lung tumors and healthy tissue adjacent to the tumor, using a specific antibody against human c-MET (MET PharmDx). The same surgical specimen was used for the preparation of healthy tissue and tumour TMA. Haematoxylin-eosin stained slides were reviewed by two pathologists (PM, JB) and tumor areas to be included in the TMA were marked. No major discrepancies regarding IHC scoring between the two pathologists were observed. The TMA was constructed using a manual tissue arrayer (MTA-1, Beecher Instruments, Sun Prairie, CA), essentially as previously described [17,18]. All tumours were included in duplicates (2 x 1 mm tissue cores). Four-micrometer sections were cut from the TMA blocks, mounted on adhesive slides and baked in 60 °C for 45 min. IHC staining was quantified using H-scores (range 0–300), which incorporate staining intensity (range 0–3) and the percentage of positively-stained tumor cells (range 0–100%). IHC analysis of c-MET was performed in the whole study population, and the average H-score of 2 separate core biopsies for each tumour was used. 682 patients had an average H-score and were included in the analysis.

For further characterization of c-MET we calculated inter-quartiles using the average H-score. Skewness was calculated and found to be 1.41, indicating that the distribution was not normal, with a median H-score of 20. The H-score for 25-, 50- and 75- quartiles was 0.875, 20 and 80, respectively (Suppl. Fig. 1). For the main purpose of the analysis, the median H-score of 20 was used.

Table 1
Study population.

	Inclusion	Exclusion	
All patients	725		
		3	Missing survival date
		43	Missing cMET
		2	Missing smoking status
		2	Missing ALK mutation status
		4	Missing histology and exclusion of SCLC
		18	Exclusion of stage IV at diagnosis
		72	Summary of all exclusions
Final study cohort	653		

cMET: cellular Mesenchymal Epithelial Transition factor, ALK: Anaplastic Lymphoma Kinase, SCLC: Small-Cell Lung Cancer.

2.3. Statistical analysis

Demographical and clinical characteristics were described by an H-score ≥ and < 20, including p-tests calculated using the Chi-square, and the t-test. The primary outcome of interest was overall survival defined as the time from diagnosis to the date of death by any cause, or last date of follow-up (for patients who received surgery from January 1995 to January 2005 last date of follow- up was 28/11/2010 and for patients who received surgery from February 2005 to December 2010 it was 10/10/2015). The secondary outcome was progression-free survival (PFS) which was determined from date of diagnosis to date of relapse or death, whichever occurred first, or to date of last planned visit to the oncology department without any signs of relapse radiologically or clinically. Overall survival was assessed by the Kaplan-Meier method. The relative risk of death of all-causes was expressed as hazard ratios (HR) with 95% confidence intervals (CI) using univariate and multivariate Cox regression analyses.

Univariate Cox regression analyses were undertaken with performance status (PS), age at diagnosis, histology, received adjuvant treatment, surgical stage of disease, smoking status, gender, ALK and c-MET H-score as independent variables. Univariate Cox regression analyses were done in the whole population and in the subgroup of lung adenocarcinoma and squamous cell carcinoma patients with stage IIA-IIIB.

The three different quartiles were examined separately with Cox regression multivariate analyses in the whole population. All the collected variables were included in these analyses, but we performed separate analyses with or without adjuvant treatment included, due to a larger number of missing data regarding the delivery of adjuvant treatment compared with all other variables.

The assumption of proportional hazards in the Cox Regression models was in a first step violated, which was verified visually and tested based on weighted residuals. Therefore, we included time-dependent variables in the model such as ALK rearrangement, treatment and age at diagnosis, and then the final models were not violated.

Multivariate OS and PFS analyses were done separately in subgroups stratified according to delivery of adjuvant treatment, as well as in the subgroup of lung adenocarcinoma and squamous cell carcinoma patients with stage IIA-IIIB.

All tests were two-sided and statistical significance was considered at a 5% level. Statistical analyses were performed using R 9.2.

3. Results

3.1. Patient characteristics

The total number of patients included in our analyses was 653 after patients with missing information were excluded (Table 1). The demographics of the whole cohort and stratified by c-MET H-score 20 are presented in Table 2. We had information about c-MET IHC and ALK FISH for the whole cohort, but KRAS and EGFR mutation status only for 360 patients, this being the reason for excluding KRAS and EGFR from

Table 2
Demographical and clinical characteristics by cMET score.

	cMET				p-value	Total	
	< 20		≥20				
	n	%	n	%		n	%
All	317	100	336	100	–	653	100
cMET, mean sd	3.1	4.2	83.9	48.9	< 0.001	44.7	53.6
Gender							
Male	162	51.1	175	52.1		337	51.6
Female	155	48.9	161	47.9	0.86	316	48.4
Age, mean sd	66	8.6	66.9	8.3	0.19	66.5	8.4
Histology							
Adenocarcinoma	153	48.3	211	62.8		364	55.7
Sqamous cell	133	42	84	25		217	33.2
LCLC	27	8.5	36	10.7		63	9.6
NOS	1	0.3	2	0.6		3	0.5
Adeno squamous	5	0.9	3	0.9	< 0.001	6	0.9
Stage at diagnosis							
IA	103	32.5	113	33.6		216	33.1
IB	106	33.4	108	32.1		214	32.8
IIA	22	6.9	29	8.6		51	7.8
IIB	41	12.9	34	10.1		75	11.5
IIIA	41	12.9	41	12.2		82	12.6
IIIB	4	1.3	11	3.3	0.439	15	2.3
Performance Status (PS)							
PS 0	170	53.6	197	58.6		367	56.2
PS 1	128	40.4	124	36.9		252	38.6
PS 2	16	5	12	3.6		28	4.3
PS 2-3	3	0.9	3	0.9	0.558	6	1
Smoking status							
Current smoker	164	51.7	161	47.9		325	49.8
Ex smoker	120	37.9	144	42.9		264	40.4
Never smoked	33	10.4	31	9.2	0.42	64	9.8
ALK mutation							
No	311	98.1	331	98.5		642	98.3
Yes	6	1.9	5	1.5	0.92	11	1.7
Adjuvant treatment							
No treatment	121	38.2	134	39.9		255	39.1
CT	68	21.5	93	27.7		161	24.7
CT/RT	9	2.8	9	2.7		18	2.8
RT	6	1.9	4	1.2		10	1.5
Missing	113	35.6	96	28.6	0.22	209	32
Relapse							
No	138	43.5	161	47.9		299	45.8
Yes	98	30.9	110	32.7		208	31.9
Missing	81	25.6	65	19.3	0.16	146	22.4

cMET: cellular Mesenchymal Epithelial Transition factor, ALK: Anaplastic Lymphoma Kinase, LCLC: Large-Cell Lung Cancer, NOS: Non Otherwise Specified, CT: Chemotherapy, RT: Radiotherapy, sd: standard deviation.

the present analysis. Regarding the adjuvant treatment regimen, data collection was feasible for 148 patients, of which 34.5% received Cisplatin/Vinorelbine, 54.7% Carboplatin/Vinorelbine, 9.4% Carboplatin/Gemcitabine and 1.4% Carboplatin/Paclitaxel.

3.2. Survival analyses

The OS and PFS analyses for all the c-MET H-score quartiles failed to show any statistical significance (data not shown for 25- and 75-quartiles). Regarding the 50-quartile (c-MET H-score 20), we performed separate Kaplan-Meier OS and PFS analyses in the whole study population, in the subgroup of patients who received adjuvant treatment and in the subgroup of patients who did not receive any adjuvant treatment (Figs. 1 and Suppl. 2). We observed a crossover of the curves at 7.5 years in the whole study population (Fig. 1a) and in patients not receiving adjuvant treatment (Fig. 1c), something which was not seen for the subgroup of patients who received adjuvant treatment (Fig. 1b). A similar pattern was observed in the PFS analyses (Suppl. Fig. 2).

The univariate survival analysis for the whole study population, presented in Table 3, showed that younger age at diagnosis, female gender, lower stage and better PS were associated with prolonged OS, whereas the remaining variables showed a trend towards statistical significance in a varying manner. We observed no significant impact on OS for the 25- and 75- c-MET H-score quartiles (data not shown).

The three different quartiles of c-MET H-score were examined separately with Cox-regression multivariate analyses (adjuvant treatment not initially included because of 209 patients with missing data, see Table 2). All other variables were included in these analyses. We observed no statistical significant impact on OS for the 25- and the 75-quartile (data not shown), but the 50-quartile (c-MET H-score 20) was an independent prognostic variable in the whole study population with a HR = 0.79 (95%CI: 0.64 − 0.97, p value = 0.022), together with gender, histology, stage and PS (Table 3).

There was a larger number of missing data regarding the delivery of adjuvant treatment (see Table 2), compared to all other variables; therefore we performed two additional Cox-regression multivariate analyses, one including adjuvant therapy as a co-variate and one in the subgroup of patients who received adjuvant therapy. In the first analysis, c-MET H-score ≥20 was an independent prognostic factor with a HR = 0.73 (95% CI: 0.57 − 0.94, p-value = 0.016), together with histology and stage with adjuvant treatment showing a strong trend in favour of longer OS (HR = 0.77 with 95% CI: 0.59–1.03, p-value = 0.081) (Table 3). In the multivariate subgroup analysis including only patients who received adjuvant therapy, we observed a stronger prognostic effect of c-MET H-score ≥20 with a HR = 0.61 (95% CI: 0.40 − 0.93, p-value = 0.022), with histology and stage retaining their independent prognostic value (Table 3). In all the above mentioned multivariate models, higher c-MET H-score as a continuous variable was an independent positive prognostic factor (Suppl. Table 1).

Univariate and multivariate Cox-regression PFS analyses were undertaken in a similar manner to the OS analyses mentioned above. Although these analyses failed to show any statistically significant result for c-MET as an independent prognostic variable, we observed a trend towards better PFS in patients with H-score ≥20. This trend was more profound in the multivariate analysis, including adjuvant treatment as a covariate (HR = 0.77, 95 %CI: 0.69–1.04, p-value = 0.085), and in the subgroup of patients who received adjuvant treatment (HR = 0.65, 95% CI: 0.41–1.04, p-value = 0.075) (Suppl. Table 1).

3.3. Subgroup OS analysis in lung adenocarcinoma and squamous cell carcinoma patients with stage IIA-IIIB

Descriptive analysis for the whole study population showed that 20.4% of stage IA and 40.6% of stage IB patients received adjuvant chemotherapy, whereas the majority of patients with stage IIA-IIIB received adjuvant therapy (Suppl. Table 2). This discrepancy between existing evidence regarding adjuvant therapy and the real life data in our cohort resulted in the subgroup analysis of patients with stage IIA–IIIB disease (n = 202). We included only patients with adenocarcinoma and squamous cell carcinoma in this analysis due to low/no representation of the remaining histological subtypes in patients with stage IIA-IIIB disease (Suppl. Table 2).

Univariate OS analysis showed a significant OS impact of c-MET H-score ≥20 in this subgroup (HR = 0.60, 95% CI: 0.43 − 0.83, p-value = 0.002). Multivariate Cox regression OS analyses done as previously described, showed that c-MET H-score ≥20 was a strong independent positive prognostic factor (Suppl. Table 4) in all three multivariate models. Univariate PFS analysis failed to show any significant effect of c-MET H- score, but the multivariate PFS analyses in the whole study population (adjuvant treatment not included) and in the analysis with adjuvant treatment included as a co-variate, showed a positive correlation between H-score ≥20 and longer PFS (Suppl. Table 1).

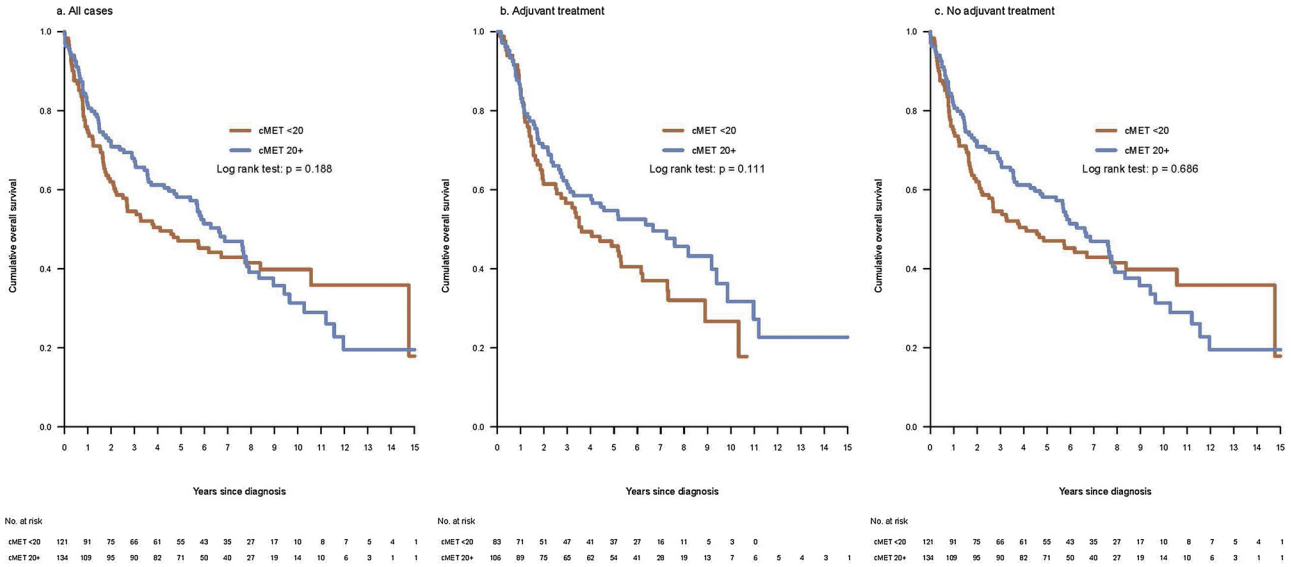

Fig. 1. Overall Survival curves. 1a: Whole study population, 1b: Patients who received adjuvant treatment, 1c: Patients who did not receive any adjuvant treatment.

Table 3
Univariate and multivariate Cox regression models (cMET < 20, ≥20 score).

	Univariate			Multivariate			Multivariate incl. Treatment			Multivariate, only treated patients		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
cMET												
< 20	1.00	ref.		1.00	ref.		1.00	ref.		1.00	ref.	
≥20	0.87	0.72-1.07	0.19	0.79	0.64-0.97	0.022	0.73	0.57-0.94	0.016	0.61	0.40-0.93	0.022
Gender												
Male	1.00	ref.		1.00	ref.		1.00	ref.		1.00	ref.	
Female	0.75	0.61-0.91	0.004	0.74	0.60-0.92	0.006	0.64	0.49-0.84	0.001	0.66	0.43-1.01	0.058
Age, in years	1.02	1.00-1.03	0.002	1.00	1.00-1.00	0.689	1.00	1.00-1.00	0.154	1.00	1.00-1.01	0.825
Histology												
Adenocarcinoma	1.00	ref.		1.00	ref.		1.00	ref.		1.00	ref.	
Squamous cell	1.00	0.81-1.24	0.97	0.74	0.59-0.94	0.013	0.76	0.57-1.00	0.052	0.52	0.32-0.83	0.006
LCLC	1.01	0.72-1.42	0.95	0.81	0.57-1.14	0.24	0.75	0.46-1.22	0.243	0.79	0.41-1.53	0.492
NOS	3.28	1.05-10.28	0.042	2.48	0.76-8.16	0.134	5.88	1.29-26.73	0.022	3.03	0.59-15.56	0.184
Adeno squamous	0.76	0.24-2.37	0.63	0.76	0.24-2.42	0.646	0.93	0.29-3.04	0.908	0.57	0.16-1.99	0.379
Stage												
IA	1.00	ref.		1.00	ref.		1.00	ref.		1.00	ref.	
IB	1.52	1.18-1.97	0.001	1.46	1.12-1.90	0.005	1.89	1.35-2.65	< 0.001	2.21	1.08-4.52	0.031
IIA	1.88	1.28-2.77	0.001	1.85	1.24-2.76	0.003	1.78	1.06-2.99	0.027	1.99	0.89-4.44	0.092
IIB	1.95	1.40-2.72	< 0.001	2.04	1.46-2.86	< 0.001	2.34	1.50-3.65	< 0.001	2.42	1.11-5.27	0.027
IIIA	2.33	1.69-3.20	< 0.001	2.37	1.72-3.27	< 0.001	3.17	2.10-4.78	< 0.001	2.84	1.37-5.85	0.005
IIIB	3.58	2.00-6.40	< 0.001	3.13	1.70-5.79	< 0.001	7.71	3.31-18.31	< 0.001	14.30	4.25-48.08	< 0.001
Performance status (PS)												
PS 0	1.00	ref.		1.00	ref.		1.00	ref.		1.00	ref.	
PS 1	1.67	1.36-2.04	< 0.001	1.64	1.33-2.03	< 0.001	1.94	0.50-2.51	< 0.001	1.19	0.78-1.83	0.41
PS 2	2.1	1.37-3.21	0.001	1.76	1.12-2.74	0.013	1.27	0.66-5.03	0.479	0.75	0.15-3.67	0.719
PS 2-3	1.5	0.62-3.67	0.36	1.7	0.69-4.18	0.251	1.82	0.66-2.45	0.248	1.62	0.36-7.30	0.528
Smoking status												
Current smoker	1.00	ref.		1.00	ref.		1.00	ref.		1.00	ref.	
Ex smoker	0.91	0.74-1.12	0.39	0.86	0.70-1.07	0.183	0.79	0.60-1.03	0.085	0.71	0.46-1.10	0.119
Never smoked	0.69	0.48-1.01	0.05	0.75	0.51-1.11	0.146	0.69	0.43-1.10	0.115	1.05	0.50-2.22	0.894
ALK												
No	1.00	ref.		1.00	ref.		1.00	ref.		1.00	ref.	
Yes	0.60	0.25-1.46	0.26	0.99	0.98-1.01	0.331	0.99	0.98-1.01	0.385	0.97	0.93-1.01	0.119
Adjuvant treatment												
No treatment	1.00	ref.		–	–	–	1.00	ref.		–	–	
Treatment	1.06	0.83-1.35	0.63	–	–	–	0.77	0.59-1.03	0.081	–	–	

cMET: cellular Mesenchymal Epithelial Transition factor, ALK: Anaplastic Lymphoma Kinase, LCLC: Large-Cell Lung Cancer, NOS: Not Otherwise Specified, HR: Hazard Ratio, Ref: Reference, CI: Confidence Interval.

4. Discussion

The role of c-MET protein overexpression as a prognostic and predictive biomarker in early stage NSCLC was the primary objective of our study. It is still unclear how c-MET protein expression, MET amplification and MET exon 14 mutation correlate with each other. The Cancer Genome Atlas study of lung adenocarcinoma and a Japanese study, both done in surgically resected tumors (early stage disease), showed that MET exon 14 mutation and MET amplification are mutually exclusive [19,20]. A recently published study with 28 MET exon 14 mutated cases showed a statistically significant co-existence of MET exon 14 skipping mutation and both MET gene amplification/c-MET protein overexpression in stage IV NSCLC [9]. In this study there was a significant association between MET amplification and stage IV disease, something which was not verified in a larger cohort with 298 MET exon 14 skipped patients [15]. Dziadziuszko et al showed that MET gene copy number determined by silver in situ hybridization (SISH) and protein expression evaluated by IHC correlated significantly in a cohort of 189 patients with surgically resected NSCLC [21]. This finding could not be confirmed in another surgical cohort (n = 222), where no significant association was observed between c-MET expression and gene copy number, but these two studies used different IHC scoring systems [8]. Bubendorf et al found a statistically significant correlation between MET gene amplification and c-MET protein overexpression in a large cohort of surgically resected NSCLC patients [6].

In the present surgical cohort, we aimed to evaluate c-MET as a prognostic and/or predictive biomarker and identify a c-MET H-score cut-off value with a prognostic or predictive clinical utility. The univariate analysis done in the whole study population failed to show any significant benefit for any of the c-MET quartiles, but this could be due to the crossover of the Kaplan-Meier curves after some years of follow-up. Because of this we used specific Cox regression analyses, taking time-dependent co-variates into consideration. These multivariate models showed a positive independent prognostic value for c-MET H-score ≥20 and for higher c-MET H-score, when c-MET was included as a continuous variable, which is in contrast with existing trial results [3–5,10], albeit in concordance with the Asian study discussed in the Introduction [2]. This generated the hypothesis that there may be an effect-modifying influence of adjuvant therapy in our models. The fact that the HR of c-MET H-score ≥20 was lower in the multivariate analysis done in the subgroup of patients who received adjuvant therapy, compared to the multivariate analysis including adjuvant treatment as a co-variate strengthened the hypothesis that c-MET H-score ≥20 may indicate a predictive value for patients receiving adjuvant chemotherapy. This finding is strengthened by the Korean study of Kim et al [2] which showed that adjuvant chemotherapy was a positive independent prognostic factor in c-MET positive but not in c-MET negative patients. In our analyses, it was not meaningful to use a control group with patients not receiving adjuvant therapy. It was due to comorbidity that some patients did not receive adjuvant therapy, though indicated. The OS in this group of patients is strongly influenced by comorbidity, rendering it a suboptimal control group in order to test the effect modifying hypothesis. There is no indication from the literature which cut-off value of c-MET best reflects the tumour biology and we have chosen the median value which is a usual cut-off used for different biomarkers in IHC studies. Furthermore, we tested different cut-off values with no further impact on prognosis. It is difficult to correlate expression level of a biomarker with a biological effect and it is possible that even low levels of c-MET may exert a biological effect.

An optimal cut-off value for c-MET protein expression with clinical impact on prognosis or prediction has not yet been defined. In our study we analysed different quartile cut-off values using the H-scoring system from 0 to 300 and identified H-score 20, being the 50-quartile, to be a clinically significant cut-off value. We believe that the H-scoring system is a good method taking into account both the intensity and extent of protein expression. Other studies have identified different clinically significant cut-off values, including H-score 60 in stage IV NSCLC patients [22] and ≥50% of cells with moderate or strong staining in the MetMAb trial [14]. These differences may be due to several factors that differ between the studies, including the use of different antibodies, scoring methods, disease stage and treatments. Regarding different antibodies, patient selection through immunohistochemical evaluation of c-MET expression in formalin-fixed and paraffin-embedded (FFPE) samples is problematic, because only a limited number of validated antibodies to c-MET that work in FFPE are available. Most studies in different types of cancer demonstrating a correlation between Met and disease progression have used antibodies directed against the COOH-terminus of Met. However, in one study on lymph node negative breast carcinoma, antibodies against the intracellular domain of Met were predictive of worse outcome whereas antibodies against the extracellular domain were not [23]. Furthermore, the cellular distribution of Met may vary and influence the IHC results; it has been shown that Met may be expressed not only in the cytoplasmic and membranous compartments but also in the nucleus, especially in the invasive front of tumors [24]. A harmonization between different studies would be needed to decide on an optimal cut-off value.

Adjuvant chemotherapy has proved to be of benefit in patients with stage IIA-IIIB NSCLC [25], and though its use in stage IB patients remains controversial, many centers offer adjuvant chemotherapy to patients with tumors ≥4 cm [26]. In our cohort, 20.4% with stage IA disease surprisingly received adjuvant chemotherapy while the majority of patients with stage IB disease did not receive any adjuvant therapy. A subgroup analysis in stage IIA-IIIB patients with adenocarcinoma or squamous cell carcinoma was undertaken and since the majority of NSCLC patients in our cohort had stage I disease, this decision resulted in less statistical power (n = 202). Nevertheless, univariate and multivariate Cox regression analysis showed a statistically significant positive impact on OS for c-MET H score ≥20. The PFS benefit was more profound in the multivariate analyses in this subgroup of patients. c-MET has been implicated in resistance to chemotherapy, including platinum [27], which would be in contrast to our finding that a higher c-MET score is beneficial in patients treated with adjuvant platinum-based chemotherapy. On the other hand, c-MET has been implicated in the process of metastasis and our results may be explained by the fact that patients with a higher c-MET expression derive a greater benefit from adjuvant chemotherapy than those with a lower expression.

The major limitation of our study is its retrospective nature making it prone to selection bias. There is also a risk of information bias regarding missing data mostly on patients receiving adjuvant therapy. Information bias regarding c-MET H-scoring is limited due to the methodology used (see methods), but can not be avoided Tumour fixation is important for the reliability of IHC staining, because overfixation or underfixation can cause false positive or false negative results, respectively.Interobserver variability, a major problem in interpreting IHC results, has been reduced in our study by considering the H-score as a continuous variable. Another consideration is that TMAs may not be representative of the entire tumour, because of intratumoral heterogeneity, this being a limitation in all published trials with IHC or H-scoring. Missing data regarding EGFR-mutation is not a major limitation since there is no evidence suggesting that it could be a confounder in this context [28]. However, there was a large number of patients included in our trial and we had available data regarding the use of adjuvant therapy for 444 patients. The real-life character of this cohort trial adds strength to the external validity of our results. Future trials should probably not be based on IHC alone. However, the use of MET kinase inhibitors for IHC positive or MET amplified tumors has not been fruitful. The exception might be patients with MET exon 14 skipping mutation positive tumors, where new treatments are currently under development [29]. The importance of MET amplification or MET exon 14 skipping mutation as oncogenic drivers is still unclear. The development of conjugate drugs (c-MET antibodies with cytotoxic drug

combinations) might be the solution for the successful treatment of c-MET positive patients, and in such a case, the IHC expression of c-MET is the most relevant biomarker which should be tested.

5. Conclusion

Despite all the limitations mentioned above, we conclude that c-MET H score ≥20 was a positive prognostic biomarker for OS in our cohort of surgically resected NSCLC patients. This conclusion is based mostly on the results of the multivariate analysis in the whole study population. There is enough statistical evidence from our analyses to support that this benefit of c-MET positivity is most likely correlated to adjuvant chemotherapy, therefore rendering c-MET H-score ≥20 a possible predictive biomarker for platinum-based adjuvant chemotherapy. This result should be verified in a randomized setting and generates the hypothesis that c-MET could be used in the future for a more individualized selection of patients who receive adjuvant chemotherapy.

Conflict of interest

None of the authors has any relevant conflict of interest to declare.

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Appendix A. Supplementary data

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