Prognostic and predictive value of *MET* deregulation in non-small cell lung cancer

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Abstract: Recent progress in cancer biology has led to the discovery of increasing number of oncogene alterations that have dramatically changed the paradigm of lung cancer treatment. MET is a tyrosine kinase receptor for the hepatocyte growth factor (HGF) that is deregulated in several malignancies, including non-small cell lung cancer (NSCLC). Abnormal MET-HGF signaling pathway activation can occur via different mechanisms, including HGF and/or MET overexpression, MET gene amplification, mutations or rearrangements. MET protein overexpression and increased *MET* gene number have been identified as poor prognostic factors in several series of surgically resected NSCLC making this receptor an attractive target for cancer treatment. Several clinical trials have recently evaluated the activity of a variety of anti-MET strategies in NSCLC patients with or without molecular selection with a variable degree of success, underscoring the need of establishing the best predictive biomarker for the identification of responding patients.

Keywords: MET protein expression; *MET* gene copy number (GCN); prognostic; predictive; non-small cell lung cancer (NSCLC)

Introduction

The paradigm of non-small cell lung cancer (NSCLC) treatment has changed since the discovery of sensitizing mutations in the tyrosine kinase domain of the epidermal growth factor receptor (*EGFR*) and translocations involving the anaplastic lymphoma kinase (*ALK*), which confer exquisite sensitivity to EGFR and ALK tyrosine kinase inhibitors (TKIs), respectively (1,2). In fact, treatment of *EGFR*-mutant patients with gefitinib, erlotinib or afatinib and *ALK*-rearranged tumors with crizotinib significantly improves progression free survival (PFS) in metastatic NSCLC patients compared with standard platinum-based chemotherapy (3-6) representing successful examples of targeted therapies. However, despite these advances, the prognosis of NSCLC patients remains poor, highlighting the need of implementing prognostic and predictive factors to identify patient with high risk of relapse after surgery, to better select patients

for cancer treatment and improve outcome.

MET is a receptor for the hepatocyte growth factor/scatter factor (HGF/SF) with tyrosine kinase activity that is codified by a gene located on chromosome 7q31 (7). MET is normally activated by the binding to its natural ligand, HGF, leading to receptor dimerization and trans-phosphorylation which triggers conformational changes that activate MET tyrosine kinase activity (8,9). MET signalling pathway has been found to be often deregulated in solid malignancies, including lung cancer, as a result of several mechanisms such as autocrine/paracrine stimulation, MET overexpression, genomic amplification, translocations, point mutations and alternative splicing (10). These events generally lead to increased signaling of the MET pathway, which in turn promotes tumour growth, progression and invasion (11,12), suggesting that the receptor and its ligand could represent potential prognostic markers and therapeutic targets. In addition, MET activation has been shown to be implicated in resistance to selected targeted

agents, including EGFR inhibitors.

The aim of the present review is to give an overview of the relevance of MET as a prognostic marker and discusses its role as a predictor of sensitivity or resistance to anti-EGFR and MET strategies in NSCLC.

MET abnormalities in NSCLC and tools for their assessment

HGF/MET protein overexpression

HGF is the natural ligand of MET and can be secreted either by stromal, epithelial or tumor cells, resulting in increased signaling of the MET pathway by paracrine or autocrine loops. Activation of the HGF/MET axis has been observed in many types of cancers, including breast carcinomas, gliomas, osteosarcomas, melanomas and NSCLC, and it has been suggested to play a role in driving cell motility and metastasis (13-17).

The most common mechanism responsible for MET protein overexpression is transcriptional upregulation of the receptor without gene amplification, which can be triggered by a number of different events including MET crosstalk or synergies in downstream pathways. In fact, crosstalk between MET and other signaling systems, such as vascular endothelial growth factor (VEGF), EGFR, and developmental signaling pathways, like WNT-β-catenin and transforming growth factor-β-bone morphogenetic protein (TGFβ), ERBB2 or insulin-like growth factor 1 receptor (IGF1R) and others, has been reported in several models and has emerged as a major mechanism for cancer progression and resistance to treatments (10,18,19).

The most widely used tool to assess the prevalence of HGF/MET protein expression in lung cancer patients has been immunohistochemistry (IHC) performed on paraffin-embedded formalin-fixed (FFPE) samples (20-26), while fewer studies assessed circulating HGF protein levels in blood samples with ELISA assays (27-29). To date, IHC procedures and scoring methods for HGF and MET assessment have not been extensively validated, as indicated by the high degree of variability in prevalence of overexpression in unselected NSCLC series, ranging from 20% to 70% for both markers (22-26,30,31). In fact, monoclonal antibodies with different sensitivity and specificity have been used, and several different scoring systems have been investigated with a generally retrospective approach. Among several commercially available antibodies for detecting MET overexpression,

the CONFIRM anti-total MET (SP44) rabbit monoclonal primary antibody (Ventana Medical Systems, Tucson, AZ, USA) is one of the most widely used, particularly in clinical trials (30,32,33). SP44 antibody is directed against a membranous and cytoplasmic epitope of MET and has been shown to have increased sensitivity when compared to other antibodies (34). More recently, a mass spectrometry approach has been proposed to accurately quantify absolute MET levels in gastroesophageal cancer FFPE tissues with high precision, although the reproducibility of the technique requires further investigation (30,33,35-37).

A key consideration when discussing HGF/MET assessment at the protein level is that the association between increased HGF levels or total MET protein expression and activation of the receptor has not been clearly demonstrated yet. In fact, in a recent study conducted in a cohort of 906 surgically resected NSCLC, IHC expression of MET and Tyr1234/1235 phospho-MET, which should indicate receptor activation, was observed in 22.2% and 5.6% of cases, respectively (30). Similarly, in a smaller Japanese study limited to resected lung adenocarcinomas a large discrepancy was observed between HGF protein expression and Tyr1234/1235 phospho-MET (57% *vs.* 7%) (24). While it remains unclear whether investigation of additional MET phosphorylated tyrosine residues might have increased the prevalence of cases with MET activation, these studies suggest that overexpression of HGF or MET protein does not necessarily implicate that the receptor is actually active. On the other hand, in a preclinical model of HGF-overexpressing lung adenocarcinoma cell line, total MET protein expression was reduced as a result of increased HGF-induced receptor ubiquitination and degradation (38), highlighting the notion that MET can signal regardless of its total protein levels. As of today, it remains unclear whether HGF or total MET protein overexpression represents a reliable indicator of MET activation and whether phospho-MET assessment should be preferred.

MET gene copy number (GCN)

The interest in assessing *MET* GCN in lung cancer has increasingly grown since 2007, when *MET* gene amplification was identified as a druggable mechanism of acquired resistance to EGFR TKIs in patients with EGFR-mutant NSCLC (39) and *MET*-amplified NSCLC cell lines exhibited exquisite sensitivity to MET inhibition (40).

The most commonly used technique to study *MET* gene amplification has been fluorescence in situ hybridization

Table 1 Studies of *MET* GCN in surgically resected NSCLC

Study	Histology	Technique	Criteria or cut-off for test+	*MET* GCN+, N (%)
Schildhaus (41)	NSCLC	FISH	High, intermediate, low level amplification[§]	Total: 227 (33.0) high: 22 (3.2) intermediate: 43 (6.2) low: 162 (23.4)
Kowalczuk (42)	NSCLC	RT-PCR	*MET* gene copies ≥3	28 (18.5)
Sun (21)	NSCLC	RT-PCR	*MET* gene copies >3	11 (18.0)
Dziadziuszko (33)	NSCLC	SISH	*MET* gene copies ≥5	14 (10.0)
Park (20)	NSCLC	FISH	*MET* gene copies ≥5	42 (11.1)
			UCCC criteria[#]	27 (7.1)
Tanaka (43)	ADC	FISH	*MET* gene copies ≥5	21 (15.0)
			MET/CEP7 ≥2.0	6 (4.0)
Tsuta (30)	NSCLC	BISH	UCCC criteria	92 (10.9)
Chen (44)	NSCLC	FISH	*MET* gene copies ≥3	22 (10.6)
Go (45)	NSCLC	FISH	*MET* gene copies ≥5	12 (6.7)
			UCCC criteria[#]	30 (16.7)
Onitsuka (24)	ADC	RT-PCR	*MET* gene copies >1.31	8 (4.4)
Cappuzzo (46)	NSCLC	FISH	*MET* gene copies ≥5	Total: 48 (11.1) High: 18 (4.1)
Okuda (47)	NSCLC	RT-PCR	*MET* gene copies >3	12 (5.6)

[§], high-level amplification: (I) *MET/CEP7* ratio ≥2.0 or (II) an average *MET* gene copy number per cell of ≥6.0 or (III) ≥10% of tumor cells containing ≥15 *MET* signals; intermediate level of gene copy number gain: (I) ≥50% of cells containing ≥5 *MET* signals and (II) criteria for high-level amplification are not fulfilled; low level of gene copy number gain: (I) ≥40% of tumor cells showing ≥4 *MET* signals and (II) criteria for high-level amplification or intermediate-level of gene copy number gain are not fulfilled. [#], ISH-positive: ≥4 gene copies in ≥40% of cells or presence of gene clusters and a ratio of MET/CEP7 ≥2 or ≥15 gene copies per cell in ≥10% of analysed cells. Abbreviations: GCN, gene copy number; NSCLC, non-small cell lung cancer; FISH, fluorescence in situ hybridization; RT-PCR, real time polymerase chain reaction; SISH, silver in situ hybridization; ADC, adenocarcinoma; BISH, bright field in situ hybridization; UCCC, University of Colorado Cancer Center.

(FISH), a widely available tool to assess copy number changes of a selected gene in the clinical setting (i.e., *HER2* gene amplification in breast and gastric cancer). Other techniques used to assess *MET* GCN have been reverse transcriptase polymerase chain reaction (RT PCR) and different in situ hybridization techniques, including chromogenic, silver, bright-field and dual probe in situ hybridization (CISH, SISH, BISH, DISH), which should present some advantages compared to FISH (30,33,35,37).

Several different scoring systems have been explored to evaluate MET GCN in NSCLC, including the assessment of *MET/CEP*7 ratio (for ISH techniques) and total *MET* gene copies. Depending on the goal and lung cancer populations of the several studies conducted, a number of presumably useful cut-offs have been proposed, either predefined or retrospectively identified, resulting in a great difficulty in understanding the prevalence and relevance of *MET* GCN gain in lung cancer (*Table 1*). Indeed, *MET* GCN can be influenced by treatments received and change over disease course. For example, several reports suggested that *MET* amplification is a rare event in NSCLC patients who have never received EGFR TKIs occurring only in 3-4% of cases (41,46,48), while it has been reported in up to 20% of the cases of EGFR-mutant lung adenocarcinoma with acquired resistance to EGFR TKIs (39).

The vast majority of the studies showed no association between *MET* GCN and histology, gender, *EGFR* or *KRAS* gene mutations (42,46,49), while some authors observed that increased *MET* GCN was more frequently found in patients with advanced stage (21,44), suggesting that *MET*

gene gain could be a late event in lung carcinogenesis. Additionally, several studies have sought to compare *MET* GCN assessed by different techniques and MET protein expression evaluated by IHC with conflicting results, highlighting once again the lack of standardization of the methodologies used for MET assessment (21,49,50).

MET gene mutations

MET gene activating mutations have been described in different tumor types such as hereditary and sporadic renal papillary carcinoma as germline mutations (51) and head/ neck squamous cell carcinoma as somatic variants (52). Somatic mutations in NSCLC represent a rare event and have been found to occur in the juxtamembrane (53) and semaphorin extracellular domain (54), while no mutations in the tyrosine kinase domain have been reported.

Interesting data emerged from a study conducted by Krishnaswamy *et al.* regarding ethnic differences in the distribution of *MET* mutations in lung cancer patients (55). In this study, Asian, Caucasian and African American subjects with lung cancer were screened for mutations in exons coding for semaphorin, juxtamembrane and tyrosine kinase domains of *MET* gene. The authors found that East Asian patients had the highest frequency of mutations, the majority of which were germline. Of note *MET* mutations were associated with squamous cell histology and had the highest prevalence in male smokers, suggesting a possible relationship between tobacco smoking exposure, *MET* mutations and potential risk of developing lung cancer.

Due to the lack of a comprehensive functional characterization of *MET* mutations, with the exception of a few variants (51) and in absence of data supporting their prognostic or predictive role, *MET* mutations will not be further discussed in the present review.

Prognostic value of HGF/MET

The impact of the activation of the MET pathway on survival of lung cancer patients has been mostly studied in terms of *MET* GCN and HGF/MET overexpression in surgically resected patients, while the prognostic impact of HGF/MET aberrations in an unselected metastatic setting remains largely unexplored.

MET GCN

The possible prognostic value of increased *MET* GCN was suggested for the first time by Okuda *et al.* (47) in a Japanese cohort of radically resected NSCLC. *MET* gene gain was analyzed by quantitative real time PCR in 213 surgical samples of NSCLC. Increased *MET* GCN (more than 3 copies per cell) was identified in 12 patients, all of whom were male and smokers (5.6%) and resulted in a statistically significant worse prognosis in terms of overall survival (OS; P=0.041) although this finding was not confirmed by multivariate analysis.

Subsequently, Cappuzzo *et al.* conducted a larger retrospective analysis (46) evaluating the prognostic effect of *MET/EGFR* gene status by FISH in 447 cases of surgical NSCLC. FISH analysis was positive (≥5 copies per cell) for *MET* in 48 cases (11.1%) and associated with advanced stage and grade 3. *MET* FISH positive patients had a significantly shorter survival in comparison with *MET* FISH negative patients (25.8 *vs.* 47.5 months; P=0.005). This observation was confirmed in the multivariable model, providing the first evidence that *MET* gene gain assessed with the abovementioned FISH criteria could represent an independent unfavorable prognostic factor, regardless of clinico-pathological characteristics. A further interesting finding of this study was the statistically significant association described between increased GCN of *MET* and *EGFR*, both located on chromosome 7, suggesting that this chromosomic area is generally interested by broad genomic gain events or polisomy rather than focal amplifications.

Similar data have been reported in later studies employing different technologies for *MET* assessment (20,30,44,45,49), although conflicting results regarding the impact of histology on the prognostic relevance of *MET* GCN have emerged. In fact, while some authors reported that the negative survival impact for *MET* gene gain was limited to patients with squamous cell cancer (45), other investigators observed a prognostic role for *MET* GCN only in lung adenocarcinoma (20,43).

A recent meta-analysis by Dimou *et al.* (56) combined results from nine studies that investigated the association of *MET* GCN with survival in NSCLC patients who received surgery. When all studies were analyzed in a random-effect model, the net HR (HR, 1.78) indicated a worse OS for patients with higher *MET* GCN. When grouped according to histology only studies that had at least 50% of adenocarcinoma had a significantly poor prognosis, suggesting that the prognostic impact of *MET* GCN may be adenocarcinoma histology specific. Similarly, a meta-analysis by Guo *et al.* including 18 retrospective trials, confirmed the independent negative prognostic impact for increased *MET* GCN in surgically resected NSCLC, with a more

Table 2 Studies of MET assessed by IHC in surgically resected NSCLC

Study	Histology	Cut-off for IHC+	MET IHC+ patients, N (%)
Sun (21)	NSCLC	>3§	36 (59.0)
Dziadziuszko (33)	NSCLC	>60 (median, range 0-400)	83 (44.0)
		MetMAb trial score#	44 (25.0)
Tsuta (30)	NSCLC	≥10% stained cells	196 (22.2)
Park (20)	NSCLC	≥4 (range, 0-12)	52 (13.7)

§, score based on the sum of intensity of staining (from 0 to 3) and the percentage of positively stained tumor cells (from 0 to 4); #, 3+ (≥50% of tumor cells staining with strong intensity); 2+ (≥50% of tumor cells with moderate or higher staining but <50% with strong intensity); 1+ (≥50% of tumor cells with weak or higher staining but <50% with moderate or higher intensity); or 0 (no staining or <50% of tumor cells with any intensity). Abbreviations: IHC, immunohistochemistry; IHC+, 2+ and 3+; NSCLC, non-small cell lung cancer.

pronounced effect in lung adenocarcinoma as compared with squamous cell cancer (57).

Finally, it should be noted that from a methodological point of view the majority of the suggested cut-offs for increased *MET* GCN have been functional to the identification of groups of patients with a different prognosis but do not necessarily contribute to understand which tumors are MET-dependent.

MET protein expression

MET protein overexpression assessed by IHC has been shown to retain a negative prognostic value in surgically resected NSCLC cohorts (20,21,58). Park *et al*. observed a MET IHC-positive rate of 13.7% in a cohort of 380 Korean NSCLC patients treated with surgery. MET-overexpressing cases had a statistically significantly shorter OS than patients with no MET protein expression (P=0.01), and this finding was confirmed by multivariate analysis (HR, 1.618; 95% confidence interval, 1.066-2.456; P=0.024).

Other studies conducted in unselected NSCLC or specifically in lung adenocarcinoma produced non-conclusive results. Tsuta *et al*. assessed MET and phospho-MET protein expression by IHC, as well as *MET* GCN by BISH, in tissue microarrays from a large cohort of resected Japanese NSCLCs (n=906) (30). MET/phospho-MET expression had no impact on survival in the whole cohort nor when grouping patients according to histology (*Table 2*).

The discrepancy between the findings of the several studies performed in this setting led to a meta-analysis (57) with the aim of better understanding the prognostic impact of MET protein expression in resected patients. Importantly, in the multivariate model which included 8 retrospective studies, MET expression was found to independently double the risk of mortality, even when excluding those studies responsible for heterogeneity (HR, 2.00, P<0.001).

Circulating and intratumoral HGF protein levels

Japanese investigators have explored the prognostic impact of a number of circulating factors, including HGF, in a cohort of 109 patients undergoing preoperative chemotherapy and surgery (27). When using the median serum HGF value measured with ELISA as a cut off, patients with low HGF levels had a significantly better OS than those with high HGF levels (P=0.019), and this finding was confirmed by multivariate analysis in the subset of stage III patients. Similar studies have been conducted in smaller cohorts of surgically resected patients with contrasting results (59,60), likely due to the small sample size.

Some efforts have been carried out to test whether intratumoral HGF protein expression could affect survival in surgically resected NSCLC patients. The first evidence supporting an independent negative prognostic role for HGF overexpression at the tumor level came from a small study conducted in 56 resectable NSCLC patients (61) where HGF protein was quantified by Western Blot. When using median HGF levels as a cutoff, patients with increased HGF protein expression had a shorter OS than patients with HGF below the median value (P=0.03), and this observation was confirmed in the multivariate analysis (P=0.0001). Other studies have sought to explore the survival impact of intratumoral protein levels of HGF with IHC (24,62,63). One of the largest studies was performed in 183 resected lung adenocarcinomas and detected HGF overexpression in 57% of cases. HGF-positive status was associated with

MET phosphorylation (Tyr 1234/1235) and predicted worse OS in univariate and multivariate analysis.

Predictive value of MET

The predictive role of MET activation has been explored in different settings, including NSCLC patients treated with EGFR TKIs and those receiving anti-MET based strategies.

EGFR TKIs

In preclinical studies conducted in lung adenocarcinoma cells harboring EGFR mutations high levels of HGF immunoreactivity were detected as a potential mechanisms of primary resistance mediated by the MET/PI3K signaling axis (64,65). This hypothesis has been confirmed in a later study by the same group which detected high levels of HGF expression in 61% non-responders patients with intrinsic or acquired resistance to EGFR-TKIs, suggesting a role of HGF in contributing to EGFR TKIs resistance (66,67). In fact, as shown by Turke *et al.*, high levels of HGF could mediate the EGFR-TKI resistance either by stimulating *MET* amplification or autocrine HGF production (68).

Engelman *et al.* for the first time demonstrated that *MET* amplification represents one of the main mechanisms of EGFR TKI acquired resistance by driving the ERBB3-dependent activation of PI3K occurring under drug pressure (39). However, primary resistance to EGFR TKI in patients harboring *MET* gene copy gain before treatment is a rare event (48). On the other hand, Benedettini *et al.* reported that MET protein expression and phosphorylation evaluated before EGFR TKI treatment correlated with shorter time to progression suggesting a potential role of MET activation in *de novo* resistance to EGFR inhibitors (69). These results are consistent with the data from Zucali *et al.* showing that MET protein expression was significantly associated with primary resistance to gefitinib in advanced NSCLC. Moreover in order to test the role of MET in resistance mechanisms, they evaluated the combination of the MET antibody DN-30 with gefitinb in a panel of NSCLC cell lines showing that the addition of DN-30 to gefitinb enhance the growth inhibition of cell lines with positive MET expression (70).

More recently preclinical data have shown that MET receptor can induce gefitinib resistance through regulation of expression of specific miRNAs (71) suggesting that modulation of specific miRNAs may provide a new therapeutic approach to lung cancer treatment.

Anti-MET strategies

Since preclinical evidence has shown that cell lines with *MET* overexpression or *MET* gene amplification made tumor cells addicted to this oncogene (72-74), several anti-MET strategies have been explored in the clinical setting. Inhibition of MET can be achieved with monoclonal antibodies targeting the receptor or its ligand or small TKIs exerting their activity at the intracellular level by inhibition of the kinase domain of the receptor.

HGF/MET monoclonal antibodies

A number of monoclonal antibodies aimed at disrupting MET signaling have been evaluated in the clinical setting, including agents that bind to HGF preventing its interaction with MET (rilotumumab, ficlatuzumab), and molecules that bind to the extracellular domain of MET (onartuzumab) (75-77).

Rilotumumab (AMG 102; Amgen, Thousand Oaks, CA, USA) has been evaluated in a phase I study in unselected solid tumors in combination with VEGF pathway inhibitors bevacizumab or motesanib and showed acceptable toxicity in patients receiving rilotumumab plus bevacizumab with mild fatigue, nausea, constipation and peripheral edema (78). An early phase II study of rilotumumab failed to demonstrate a benefit in patients with glioblastoma (79). To date, a phase I/II study evaluating a combination of rilotumumab and erlotinib in unselected pretreated metastatic NSCLC is ongoing (ClinTrial.gov, NCT01233687).

Fliclatuzumab (AV-299; AVEO, Cambridge, MA, USA) has been evaluated in some phase I trials alone or in combination with EGFR inhibitors erlotinib or gefitinib in patients with solid tumors, including NSCLC, with a favorable toxicity profile (80). More recently, Mok *et al.* presented the results of a randomized phase II trial comparing gefitinib alone versus gefitinib plus fliclatuzumab in Asian patients with lung adenocarcinoma with clinical predictors of presence of *EGFR* activating mutations. The study, whose primary end point was to compare overall response rate between treatment arms, was conceived due to the preclinical evidence that HGF upregulation can be responsible for acquired resistance to EGFR TKIs in NSCLC with EGFR mutations, as previously discussed (64,66,68). In the intent to treat (ITT) population there was neither statistical difference in response rate (40% for gefitinib arm versus 43% for combination arm) nor PFS (4.7 months in gefitinib arm *vs.* 5.6 months in the combination arm). However, in subgroup analyses it emerged that patients with *EGFR*

mutations and low MET expression benefited more from the combination treatment arm in terms of PFS suggesting that inhibition of HGF/MET could delay resistance to EGFR TKIs in EGFR-mutant patients (80,81). To better clarify the activity of a two-drug approach in this setting phase II trials evaluating fliclatuzumab in combination with erlotinib in *EGFR* mutant patients are ongoing (clinicaltrials. gov, NCT 02318368).

Onartuzumab (MetMab; Genentech-Roche) is a monoclonal antibody directed against the semaphorin domain of MET thus blocking the ligand-induced dimerization and activation of tyrosine kinase activity of the receptor (82). Efficacy data from onartuzumab derived from a global randomized double-blind phase II trial comparing erlotinib plus onartuzumab or placebo as second or third line treatment in advanced NSCLC patients. The co-primary end points were PFS in the whole population and in MET expression positive patients evaluated by IHC. Importantly, MET status was determined after random assignment and before unblinding and IHC scoring were predefined as follows: 3+ (≥50% of tumor cells staining with strong intensity); 2+ (≥50% of tumor cells with moderate or higher staining but <–50% with strong intensity); 1+ (≥50% of tumor cells with weak or higher staining but <-50% with moderate or higher intensity); or 0 (no staining or <50% of tumor cells with any intensity). Patients with 2+ or 3+ score were considered MET-positive. Patients were stratified according to smoking history and histology. In the ITT population (n=137) there was no difference in terms of PFS between treatment arms (median 2.9 months for placebo plus erlotinib *vs.* 2.2 months for onartuzumab plus erlotinib; HR, 1.09; P=0.69) or OS (median 7.4 months for placebo plus erlotinib versus 8.9 months in the experimental arm; HR, 0.80; P=0.34). However, when considering only the 66 MET-positive patients (52%) the experimental arm resulted in both clinically and statistically significant improved PFS (HR, 0.53; P=0.04) and OS (HR, 0.37; P=0.002) which translated in twofold reduction in the risk of disease progression and threefold reduction in the risk of death compared with erlotinib alone (32,83). Noteworthy, the benefit in MET-positive patients was independent of FISH analysis for *MET* GCN although the small number of patients (n=19) with *MET* gene gain (≥5 copies) precluded any conclusion (83). Interestingly, the outcome was worse in the MET-negative population treated with onartuzumab plus erlotinib in terms of PFS as well as OS (HR, 2.01 and 3.02 respectively) (32) highlighting the relevance of proper patient selection for such combination

therapy. Additional biomarker analyses failed to show any role for MET, EGFR, amphiregulin, epiregulin, or HGF mRNA expression to predict a benefit from onartuzumab plus erlotinib, suggesting that MET IHC could be the best predictor of onartuzumab activity (83).

On the wave of the promising results of the phase II trial, a global double-blind phase III study comparing onartuzumab plus erlotinib *vs.* placebo plus erlotinib in pretreated MET positive (2+/3+) metastatic NSCLC patients (MetLung study) was performed (84). The primary end point was OS. A total of 480 patients worldwide have been enrolled, but unfortunately the trial closed prematurely after an interim analysis showing no difference in OS (6.8 *vs.* 9.1 months for onartuzumab + erlotinib *vs.* erlotinib; HR, 1.27; P=0.07) and PFS (2.7 *vs.* 2.6 months for the experimental *vs.* control arm; HR, 0.99, P=0.92). Surprisingly, in the subgroup analysis, patients harboring *EGFR* mutations allocated in the experimental arm seemed to have a detrimental effect from the combination treatment. Such disappointing results could be related to the low predictive value of MET protein expression as a tool to detect MET activation and to select patients for anti-MET strategies. In fact, we still do not know whether MET overexpression, which can be triggered from different biological events, reflects tumor cells dependency on the MET pathway. Additionally, it remains unclear whether onartuzumab is active against tumors with *MET*-amplification, which has been shown to represent a model of oncogene addiction *in vitro* (39,40,73). Indeed, as recently showed by Arriola *et al.*, MET expression assessed by IHC with different scoring criteria, including the onartuzumab scoring system, did not correlate with *MET* gene amplification in small NSCLC biopsies (50), highlighting the challenges in the identification of MET-driven tumors.

MET TKIs

Several small molecules directed against the intracellular tyrosine kinase domain of MET have been characterized preclinically and some of them have reached the clinical trial setting with variable results. As compared with monoclonal antibodies these agents are administered orally and act at the intracellular level, regardless of ligand-receptor interactions.

Tivantinib (ARQ 197; ArQule Woburn, MA, USA; Daiichi Sankyo, Tokyo, Japan) is a non-adenosine-triphosphate competitive small molecule selective for MET receptor. The compound has been shown to act as

a potent MET inhibitor but at the same time preclinical data suggested that tivantinib exerts citotoxic activity independently of MET status through disruption of microtubules polymerization (85-87).

Due to the role of MET in contributing to intrinsic and acquired resistance to EGFR TKIs, a phase II randomized, double blind, placebo-controlled trial of erlotinib in combination with tivantinib or placebo in 167 pretreated, TKIs naïve, metastatic NSCLC patients was performed (88). The primary end point was PFS in the ITT population. Patients were unselected for molecular characteristic, but a biomarker analysis was performed after study entry. In the ITT population there was a modest trend for improved PFS in favor of the experimental arm, although the difference did not reach statistical significance (3.8 *vs*. 2.3 months for the combination *vs*. control arm, respectively; HR, 0.81; P=0.24). The PFS advantage became significant after adjustment for known prognostic factors (HR, 0.68, P=0.04), but subgroup analyses revealed that only nonsquamous patients seemed to benefit from the combination arm, both in terms of PFS (HR, 0.61, P=0.04) and OS (HR, 0.58, P=0.04). Of note, in the preplanned exploratory analysis of PFS and OS according to molecular characteristics it emerged that patients with increased *MET* GCN had a trend toward a benefit for the tivantinib plus erlotinib arm and the benefit grew in magnitude as the copy number cutoff rose, although not statistically significant. Importantly, in patients with low *MET* GCN (<2 or <3) there was no difference in efficacy between the two treatment arms (88). Additionally, despite small patient numbers, a significant improvement in PFS (HR, 0.18, P<0.01) was observed in KRAS-*mutant* patients, while a trend for longer PFS was observed in patients with wild type EGFR (HR, 0.70; P=0.12).

Based on these results the confirmatory phase III MARQUEE trial (Met inhibitor ARQ 197 plus erlotinib *vs*. erlotinib plus placebo) in previously treated, TKIs naïve, metastatic non-squamous NSCLC patients, was conducted and enrolled 998 unselected patients stratified according to *EGFR* and *KRAS* gene status (89). The primary end point was OS but unfortunately the trial was halted after an interim analysis showing that the study would not meet its primary end point of improved OS (HR, 0.98). Of note, the interim analysis did show a statistically significant improvement in PFS but this benefit did not translate into OS prolongation. Although the trial was negative it is noteworthy that in approximately 40% of enrolled patients for which tumor tissue was available MET was evaluated by IHC showing a substantial improvement in both PFS and OS in MET high (2+/3+ in >50% of cells) patients treated with tivantinib plus erlotinib. The final results of biomarker analysis will further clarify whether the combination of erlotinib and tivantinib can prove active in molecularly selected NSCLC patients.

Crizotinib (PF 02341006, Xalkori®) is a potent *ALK* and *ROS1* inhibitor, approved by FDA in 2011 for patients harboring *ALK* translocations, that was initially developed as a MET inhibitor. In a preclinical study crizotinib showed a marked antiproliferative effect in *MET*-amplified cell (90), confirming previous evidence that *MET* amplification results in constitutive activation of the receptor and represents a model of oncogene addiction. Ou *et al*. reported a case of a patient with *de novo* high level MET amplification assessed by FISH (MET/CEP7 ratio >5.0) but without *ALK* translocation who achieved a rapid and durable response to crizotinib (91), confirming that the drug is a potent MET inhibitor and that *MET* amplification could be a good predictor of response.

More recently, the activity and safety of crizotinib in *MET*-amplified metastatic NSCLC patients has been investigated in a phase I study (92). Patients were selected according to *MET* amplification assessed by FISH and divided into three categories according to *MET/CEP*7 ratio (low: 1.8-2.2; intermediate 2.2-5.0; high: ≥5.0). The preliminary results in 12 patients showed remarkable tumor shrinkage in the group with intermediate and high levels of *MET*-amplification, with response rates of 17% and 67%, respectively. Interestingly, the subjects who achieved a greater response were mainly smokers and, although the small number of patients preclude any conclusion, this is consistent with several reports (45,47) suggesting a possible relationship between *MET* gene amplification and squamous cell carcinoma's oncogenesis. Exploratory analyses to identify the optimal *MET/CEP*7 ratio to predict clinical benefit from crizotinib are ongoing.

Cabozantinib (XL184/BMS-907351; Exelixis South San Francisco, CA, USA/Bristol-Meyers Squibb) is an oral multikinase inhibitor targeting MET, VEGFR, FLT3, RET and KIT that has recently received FDA approval for metastatic medullary thyroid cancer (93). Cabozantinib has been evaluated in a phase I/II study in combination with erlotinib in NSCLC patients with acquired resistance to erlotinib. The study enrolled 54 patients showing encouraging clinical activity of cabozantinib plus erlotinib in erlotinib-pretreated population including patients with *MET* amplification and T790M *EGFR* mutation (94). Clinical activity of cabozantinib was further investigated in

a larger phase II study involving several solid tumors where MET signaling plays an important role, including NSCLC (n=60), where partial responses were observed in six patients (13%). Interestingly, some of the responders harbored driver mutations (*EGFR* or *KRAS* mutations) at baseline while non-responders did not (54,95).

Conclusions

The HGF/MET pathway represents an extremely appealing therapeutic target in NSCLC, although clinical trials with anti-HGF/MET strategies in patients with advanced disease have produced mixed results. While it is becoming increasingly clear that *MET* gene amplification confers dependency on MET for survival of tumor cells and is susceptible of drug inhibition with MET TKIs in the clinical setting, the role of MET IHC to select patients for anti-MET agents remains unclear.

In the setting of surgically resected NSCLC, increased *MET* GCN or protein levels of HGF/MET could help to identify subjects with a higher risk of death, highlighting the need for effective adjuvant approaches for these patients.

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