Review Article: The Role of cMet in Non-Small Cell Lung Cancer Resistant to EGFR-Inhibitors: Did we Really Find the Target?

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Abstract: The advent of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) represented the most important innovation in NSCLC treatment over the last years. However, despite a great initial activity, secondary mutations in the same target, or different alterations in other molecular pathways, inevitably occur, leading to the emergence of acquired resistance, in median within the first year of treatment. In this scenario, the mesenchymal–epidermal transition (cMET) tyrosine kinase receptor and its natural ligand, the hepatocyte growth factor (HGF), seem to play an important role. Indeed either the overexpression or the amplification of cMET, as well as the overexpression of the HGF, have been reported in a substantial subgroup of NSCLC patients resistant to EGFR-TKIs. Several cMET-inhibitors have been developed as potential therapeutic candidates, and are currently under investigation in clinical trials. These compounds include both monoclonal antibodies and TKIs, and most of them have been investigated as dual combinations including an anti-EGFR TKI, to improve the efficacy of the available treatments, and ultimately overcome acquired resistance to EGFR-inhibitors.

Keywords: cMET, cMET-Inhibitors, EGFR-TKIs resistance, HGF, NSCLC, targeted therapies.

INTRODUCTION

In the last years, the discovery of oncogene driver mutations and the subsequent advent of targeted therapies have revolutionized the treatment paradigm of Non-Small Cell Lung Cancer (NSCLC). We have witnessed a gradual shift of tumor classification from a histological to a molecular basis profile, which is now becoming crucial for the selection of patients candidate to the treatment with a new tailored agent [1, 2]. The inhibition of EGFR has led to unexpected and clinically significant results, becoming a very important strategy in the treatment of NSCLC [3]. Eight different randomized clinical studies compared EGFR TKI with standard platinum-based chemotherapy in first-line treatment of EGFR-mutated, NSCLC patients. All these trials have shown that targeted therapy is better than the standard cytotoxic treatment in a population selected according to the tumour's molecular profile, leading to a significant improvement of the progression free survival (PFS), with median survival (OS) reaching today a plateau of 24-30 months [4-11]. However, despite tailoring therapeutic approach may optimize patients' survival-outcomes, acquired resistance to TKIs inevitably occurs [12], usually within the first year of treatment. This leads to the clinical progression of the disease, often followed by the interruption of current targeted treatment and switch to a standard platinum-doublet chemotherapy. In 2010 Jackman et al., first, provided a clinical definition of acquired resistance to EGFR TKIs, proposing some clinical criteria in order to favour a more precisely identification and classification in NSCLC patients [13]. Several studies have recently elucidated the molecular basis of the acquired resistance to EGFR-TKIs, in order to favour innovative genotype-guided approaches to the therapy. In fact, the results of the Lung Cancer Mutation Consortium (LCMC) study, have recently shown that the treatment with the new tailored agents improves survival outcomes in patients with advanced NSCLC, whose
tumour harbour specific oncogene drivers [14]. Therefore re-biopsy on progression and re-genotyping to facilitate personalized therapy are both a valid research and therapeutic strategy. Amplification of the mesenchymal–epidermal transition (cMET) gene [15], as well as the over-expression of the hepatocyte growth factor (HGF) [16-18], has been reported in approximately 5%–22% of NSCLC resistant to EGFR-TKIs, suggesting that activation of the HGF–cMET pathway may be involved in the occurrence of acquired resistance. This review will briefly analyze the biology of the HGF-cMet pathway, focusing on its role in the development of resistance to EGFR-TKIs in NSCLC, and is intended to provide an overview of the multiple cMET inhibitors, currently under clinical development.

THE CMET-HGF PATHWAY

The cMET receptor is a tyrosine kinase receptor [19] encoded by the cMET proto-oncogene. This gene is located at locus q31 of chromosome 7 and was first isolated from a human osteosarcoma-derived cell line in 1984 [20]. cMET is mainly expressed in epithelial cells, but also in endothelial cells and neurons [21, 22]. Its ligand, hepatocyte growth factor (HGF), is secreted by mesenchymal cells [23].

CMET plays several biological roles like causing cell scattering by epithelial mesenchymal transition, increasing cell motility and promoting cell survival. During embryogenesis cMET signaling is needed for the correct proliferation, development and survival of the placenta and liver [24]. Later, it is necessary to induce cell migration of skeletal muscle progenitor cells [25]. Also, cMET signaling is needed during branching morphogenesis, which is needed for the correct formation of several organs like placenta, liver, kidney, pancreas, mammary gland, testis and lung [26]. In adults, cMET is important in wound healing, liver regeneration and angiogenesis [27].

The ligand HGF is secreted by mesenchymal cells as an inactive pro-HGF that shows many resemblance with plasminogen. In the tissues pro-HGF is bound to heparin-like proteoglycans of the extracellular matrix, thus limiting the diffusion range of pro-HGF as well as the free circulating amount [28]. Pro-HGF is cleaved by urokinase plasminogen activator (uPA), tissue plasminogen activator (tPA) or coagulation factors X, XI and XII. After cleavage it forms an active dimer with an α- and β-subunit that is connected by a disulfide bond [29].

In contrast to HGF, cMET is mainly secreted by epithelial cells. It is formed as pro-cMET that forms a heterodimer after cleavage with an extracellular α- and a membrane-spanning β-subunit [30]. After binding with mature HGF, cMET homodimerizes and cross-phosphorylation at Tyr1234 en Tyr1235 takes place. This phosphorylation ultimately leads to phosphorylation of Tyr1349 and Tyr1236 in the intracellular C-terminus of the receptor. These phosphorylated tyrosines are part of the docking site of the adaptor proteins growth-factor-receptor-bound protein 2 (GRB2) [31] and Grb2-associated-binding-protein 1 (GAB1) [32].

There are five main signaling pathways that are activated by cMET: mitogen-activated-protein-kinase (MAPK), phosphoinositide-3-kinase (PI3K-Akt), STAT (signal-transducer-and-activator-of-transcription), nuclear-factor-κB (NF-κB) en focal-adhesion-kinase (FAK) [33] (Fig. 1).

The MAPK cascade consists of a series of three protein kinases: MAPKKK, MAPKK and MAPK. These kinases phosphorylate and activate each other. cMET activates two MAPK family cascades: the MEK-ERK (MAPK-ERK - extracellular signal regulated kinase) and the MEK-JNK (c-Jun terminal kinase) cascade. These MAPK cascades lead to a stimulation of cell cycle progression, cell differentiation, cell transformation and play a role in apoptosis [34].

cMET activation also triggers PI3K-Akt signaling. PI3K signaling has an anti-apoptotic activity through the inhibition of Bcl 2-associated-death-promotor (BAD) as well as stimulation of mouse-double-minute-2 homologue (MDM2), which ultimately leads to p53 inactivation. It also stimulates cell growth by inactivation of glycogen-synthase-kinase-3β (GSK3β) [35].

A third signaling pathway starts with the phosphorylation of STAT3 by cMET binding. After phosphorylation, STAT3 dissociates from the receptor, dimerizes and translocates to the nucleus. There, it functions as a transcription factor, activating several genes that are involved in both cell proliferation and cell differentiation [36].

NF-κB is a transcription factor able to activate a group of genes that are involved in apoptosis. Inhibitor of NF-κB (IκB) sequesters and inhibits NF-κB. cMET signaling leads to phosphorylation and degradation of IκB and thus releases NF-κB [37].

Finally FAK is recruited by phosphorylated cMET. FAK plays a crucial role in both cell adhesion and motility. Phosphorylation of FAK is necessary for cell invasion and migration [38].

After activation of the receptor, cMET is internalized. Next there are two options: cMET can be dephosphorylated and recycled back to the membrane in its inactive form. The other possibility is that c-CBL ubiquitinizes cMET and that the receptor is broken down in the 26S proteasome [39].

CMET IN NSCLC

There are several possible mechanisms by how cMET signaling can be deregulated in cancer (Fig. 2). A first mechanism is cMET amplification. Definition of MET amplification differed among clinical studies. In the study performed by our group in surgically resected NSCLC, cMET amplification was defined as a mean cMET gene copy number ≥ 5 and this event was detectable in 11.1% of cases. Nevertheless, by using more stringent criteria, i.e. ratio cMET/centromere ≥ 2.2 similarly to what is used for human epidermal growth factor receptor 2 (HER2) testing in breast cancer, cMET amplification is detectable in less than 4% of cases [40]. In addition, high levels of cMET amplification, often with a ratio gene/centromere >5 is a known resistance mechanism against epidermal-growth-factor-receptor tyrosine kinase inhibitors (EGFR-TKI) [15, 40]. cMET amplification can lead to an over-expression of the cMET receptor on the cell membrane. When EGFR-TKI’s block the EGFR-signaling pathway these cells can rely on cMET signaling to...
sustain cell survival and growth. In EGFR-TKI resistant NSCLC patients, the reported percentages of cMET amplification lie around 20% [15].

Another change at the genetic level are mutations in the cMET gene. Germline mutations in cMET are not associated with NSCLC but do play a role in other cancers like renal papillary carcinoma. Somatic mutations in cMET can be found in the juxtamembrane, sema and the kinase domain. The juxtamembrane domain of the receptor has an auto-inhibition loop, which upon phosphorylation folds back to allow activity of the receptor. Mutations in this loop (e.g. R988C or T1010I) can cause phosphorylation of this loop, and thus activation of the receptor without ligand binding. Ma et al. showed that these mutations increase the tumorigenicity of cells [30].

Mutations in cMET are also reported in the extracellular sema- and tyrosine kinase domain. It is possible that these mutations respectively influence ligand binding and receptor clustering or the activity of the receptor. However, the exact biological effect of the reported mutations has not been unraveled yet, it has been shown that these mutations are enriched in metastatic lesions [41]. Mutations in cMET are not limited to NSCLC alone, but also to other solid tumors like renal papillary carcinoma, gastric carcinoma or hepatocellular carcinoma. However, the kind of mutations found seems to depend on the type of tumor. In NSCLC, the majority of mutations seem to be in the juxtamembrane and sema domain [42].

Apart from gene amplification, cMET overexpression can be due to post-transcriptional upregulation. This includes a higher transcriptional and/or translational rate of the mRNA, or changes in protein half-life of the receptor by reduced destruction or more recycling of the receptor [39]. Alternative splicing variants have been reported [43], however, the biological function of these splicing variants and whether they play a role in cancer growth is still largely unknown.

Finally, changes in the present amount of HGF can change the signaling activity of cMET [44]. This can either take place in a paracrine or autocrine manner. It has been shown that lung cancer cells recruit fibroblasts to produce HGF that activates cMET in a paracrine way [45]. This illustrates the role of the tumor microenvironment in the development of resistance against EGFR-TKI. It is also possible...
**Fig. (2). The different mechanisms of cMET-based cancer growth and resistance.** Panel A) The wild-type cMET signalling. Panel B) The cMET gene amplification, which results in overexpression of the cMET receptor on the cell membrane. Panel C) The 2 different possible results of cMET mutation: In the left situation the mutation affects ligand binding or receptor clustering which results in cMET signalling in the absence of hepatocyte growth factor (HGF). The right situation affects the kinase domain of the receptor. Panel D) The post-transcriptional modification results in an increased translation of the cMET mRNA by increasing the translational speed, increasing the half-life of the mRNA of preventing the destruction of the mRNA. Panel E) The increased production of the HGF results in an aberrant cMET signalling.

that the tumor cells produce their own HGF, so autocrine stimulation can take place. This might be the case in hypoxic regions of the tumor, since hypoxia upregulates HGF production and thus provokes a more invasive and metastatic phenotype of cancer cells [46]. An elevated level of HGF has also been detected in rebiopsies of EGFR-TKI resistant patients, were 61% showed high HGF expression and 9% bared cMET amplification [18, 47].

**cMET – Inhibitors**

Improved understanding of cMET-HGF molecular pathway in the pathophysiology of NSCLC, and increasing evidences on the role of cMET in the development of resistance to EGFR-TKIs, has led to the advent of several cMET-inhibitors, as candidate for clinical setting (Table 1). Such molecules include both Monoclonal Antibodies (MAbs), and small-molecules, TKIs, which have shown a great antitumor activity in NSCLC pre-clinical models [48-51]. Such pre-clinical data have suggested a possible synergistic activity of anti-EGFR/cMET combinations, providing a strong rationale for the design of clinical studies which explore both the activity and safety of dual EGFR-cMET inhibition in NSCLC, in order to improve the efficacy of the available treatments and ultimately overcome the acquired resistance to EGFR-TKIs.

**Anti HGF-cMET Monoclonal Antibodies**

**Onartuzumab**

Is an engineered, humanized, MAb, that was designed to block the ligand binding site of the hepatocyte growth factor receptor (HGF-R), thereby inhibiting HGF-induced activation and the downstream signaling pathways [52]. Among the new anti-cMET MAbs under development in NSCLC, Onartuzumab represents the compound in the most advanced phase of clinical investigation, capturing the attentions and the expectations of both oncologic scientific community and drug developers. A randomized, phase II trial, compared Erlotinib plus Onartuzumab combination to Erlotinib plus Placebo, in 128 NSCLC patients, who have previously received one or more lines of chemotherapy. The results of such trial have shown a significant survival benefit in favour of the experimental arm, limited to the subgroup of patients (n.66) whose tumors reported the overexpression of cMET, defined as “50% or more tumor cells showing moderate or strong expression by immunohistochemistry (IHC)” [53]. In contrast, the outcomes were worse for those patients with low levels of cMET tumor expression treated with Onartuzumab, suggesting cMET overexpression, detected by immunohistochemical analysis, as a positive predictive biomarker for METMAb activity [54]. The treatment was very well tolerated, with a significant increase of peripheral edema in
Table 1. Clinical Trials with HGF/cMET Inhibitors in NSCLC.

<table>
<thead>
<tr>
<th>Agents</th>
<th>Phase</th>
<th>Patients Included</th>
<th>ORR</th>
<th>OS/PFS</th>
<th>Author</th>
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<tbody>
<tr>
<td>Onartuzumab(mAb)+Erlotinib</td>
<td>Phase II</td>
<td>137 pre-treated pt 66 pt (MET IHC 2+/3+)</td>
<td>N.A</td>
<td>OS (HR: 0.80, p=0.34)</td>
<td>Spigel 2013 (53)</td>
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<td>PFS (HR: 1.09, p=0.69)</td>
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<td>OS (HR: 0.37, p=0.002)</td>
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<td>PFS (HR: 0.53, p=0.04)</td>
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<td>Phase III</td>
<td>499 pre-treated pt (MET IHC 2+/3+)</td>
<td>8.4% vs 9.6% p=0.63</td>
<td>OS: 6.8 vs 9.1 month</td>
<td>Spigel 2014 (55)</td>
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<td>(HR: 1.27, p=0.068)</td>
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<td>PFS: 2.7 vs 2.6 month</td>
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<td>(HR: 0.99, p=0.92)</td>
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<td>Ficlatuzumab(mAb)+Gefitinib</td>
<td>Phase II</td>
<td>188 naïve-Asian pt (not selected)</td>
<td>43% vs 40% (p: N.A)</td>
<td>PFS: 5.6 vs 4.7 month</td>
<td>Mok 2012 (59)</td>
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<td>(HR: N.A)</td>
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<td>Rilotumumab(mAb)+Erlotinib</td>
<td>Phase I/II</td>
<td>Pre-treated pt (Recruiting pt)</td>
<td>Ongoing (N.A)</td>
<td>PFS: 3.8 vs 2.3 month</td>
<td>Sequist 2011 (64)</td>
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<td>(HR: 0.81, p=0.24)</td>
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<td>OS: 0.46, p=0.21</td>
<td>Rodig 2012 (65)</td>
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<td>PFS: 0.58, p=0.28</td>
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<td>Tivantinib(TKI)+Erlotinib</td>
<td>Phase II</td>
<td>167 pre-treated pt 27 pt (MET IHC 2+/3+)</td>
<td>10% vs 7% (p: N.A)</td>
<td>OS: 8.5 vs 7.8 month</td>
<td>Scagliotti 2013 (67)</td>
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<td>(HR: 0.98, p=0.81)</td>
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<td>PFS: 3.6 vs 1.9 month</td>
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<td>(HR: 0.74; p &lt; 0.0001)</td>
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<td>OS: 9.3 vs 5.9 month</td>
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<td>(HR: 0.70, p=0.03)</td>
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<td>Cabozantinib(TKI)+Erlotinib</td>
<td>Phase II</td>
<td>37 pre-treated pt Progression to Erlotinib (EGFR +)</td>
<td>8%</td>
<td>N.A</td>
<td>Reckamp 2014 (68)</td>
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<tr>
<td>Cabozantinib(TKI)</td>
<td>Phase II</td>
<td>Pre-treated pt Progression to TKI and anti-VEGF agent</td>
<td>10%</td>
<td>(4.2 vs 4.2 month) (HR:1.00)</td>
<td>Hellerstedt 2012 (69)</td>
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<td>Crizotinib(TKI)</td>
<td>Phase I</td>
<td>16 pre-treated pt (MET-amplified)</td>
<td>33%</td>
<td>N.A</td>
<td>Camidge 2014 (76)</td>
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<tr>
<td>Foretinib(TKI)+Erlotinib</td>
<td>Phase I/II</td>
<td>Pre-treated pt</td>
<td>Ongoing (N.A)</td>
<td>Ongoing (N.A)</td>
<td>(NCT01068587)</td>
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ORR: objective response rate; OS: overall survival; PFS: progression free survival; mAb: monoclonal antibody; TKI: tyrosine kinase inhibitor; pt: patients; HR: hazard ratio; N.A: not available

Onartuzumab-treated patients. On the basis of these evidences, the phase III, confirmatory, METLung trial, of Erlotinib plus Onartuzumab versus Erlotinib plus placebo in patients with MET-overexpressed NSCLC (2+/3+ IHC), has been initiated. The results of such trials have been recently reported, showing that the addiction of Onartuzumab to Erlotinib does not confer any improvement in OS, PFS, and overall response rate (ORR), regardless of cMET Gene Copy Number changes, detected by Fluorescent in situ hybridization (FISH), or EGFR-mutational status [55]. The main reason explaining the failure of this study is that probably IHC is not the right method for selecting patients eligible for anti-cMET treatments. Preclinical studies [56] showed that anti-cMET agents are more effective in presence of high levels of cMET amplification and IHC is not able to properly detect such cases [57].

Ficlatuzumab

Is a humanized anti-HGF IgG1 MAb which specifically binds the HGF, inhibiting the ligand-dependent, c-MET activation. A phase Ib study investigating the activity and safety of Ficlatuzumab plus Gefitinib combination in Asiatic patients with unresectable NSCLC, showed a good tolerability profile (no DLT observed in the dose-escalation cohort), and a great activity in the 20 mg/kg cohort. Among 12 patients, 5 partial responses, 4 stabilizations and 3 progressions were observed. All 5 responders were EGFR-TKI naïve patients. The most common side effects reported for the combination treatment were were skin rash and diarrhea [58]. The results of a randomized phase II trial, comparing Ficlatuzumab plus Gefitinib versus Gefitinib alone in previously untreated Asian NSCLC patients, not selected for EGFR status, have been recently published. Not significant survival differences
were observed in the overall population, while the subgroup analysis favored those patients with low levels of cMet tumor-expression treated with Ficlatuzumab, [59], suggesting that the dual inhibition of EGFR/HGF-cMET pathways may delay the occurrence of resistance to EGFR-TKIs.

**Rilotumumab and TAK 701**

Are two other humanized anti-HGF neutralizing monoclonal antibodies in the early phase of clinical investigation in NSCLC. Phase I studies exploring the safety profile of both compounds in advanced, refractory, solid tumors have shown that both the drugs were well tolerated and the maximum tolerated dose (MTD) has not been achieved [60, 61]. A phase I/II study of Erlotinib and Rilotumumab in NSCLC patients that have been treated with at least one and a maximum of two prior chemotherapy regimens is currently recruiting participants (NCT01233687), while this drug is in a more advanced phase of clinical development for gastrointestinal tumors. The combination of TAK 701 with gefitinib has shown a great antitumoral activity in NSCLC cell lines [62], but further clinical trials should investigate its potential benefit also in NSCLC patients.

**HGF-cMET Tyrosine Kinase Inhibitors**

**Tivantinib**

Is an oral, selective, non-ATP competitive TKI, which specifically binds the unphosphorylated form of cMET outside of the ATP binding site, stabilizing the inactive conformation by allosteric mechanism, and inhibiting both ligand-dependent/independent kinase activation and downstream signaling pathways [63]. A randomized phase II trial compared Tivantinib plus Erlotinib combination to Erlotinib plus Placebo in pre-treated, NSCLC patients, who have not received any EGFR-TKIs. The primary endpoint of PFS was not reached in the overall population. Tivantinib showed a good safety profile, and not significant differences in side effects have been reported [64]. Moreover, a subsequent biomarker analysis of 50 tumor samples reported an higher percentage of c-MET expression in non-squamous NSCLC compared to squamous tumors. Furthermore more pronounced survival improvements were found among 27 patients treated with Tivantinib plus Erlotinib, whose tumors reported IHC high cMET expression [65]. These encouraging data have led to the design of the randomized, phase III, MARQUEE trial, comparing Tivantinib plus Erlotinib to Erlotinib plus Placebo in advanced non-squamous NSCLC patients, who had previously received one or more lines of treatment [66]. However such trial has been early interrupted because the primary end-point (OS) was not achieved in the intent to treat analysis, even if a significant clinical benefit was demonstrated in the experimental arm both in the PFS and ORR. One year later, the results of a molecular subgroup analysis were presented at the 32nd ESTRO European Cancer Congress, revealing that Tivantinib improved OS in the subgroup of tumors with high cMET expression [67]. Tivantinib/Erlotinib combination has shown a good tolerability profile, similar to Placebo/Erlotinib. The major side effects were low-grade rash, diarrhea, fatigue, nausea, vomiting, dyspnea, and anemia. Only the percentage of grade 3/4 neutropenia was higher with the combination treatment. Currently, the phase 3, AT-TENTION trial (NCT01377376) is investigating the combination of Tivantinib plus Erlotinib in EGFR wild-type, non-squamous, NSCLC patients.

**Cabozantinib**

Is an oral, non-selective, multi-target TKI, which potentially inhibits cMET, VEGFR2, cKIT, RET, AXL and FLT3, that was recently approved by FDA for metastatic medullary thyroid cancer. A phase II trial of Cabozantinib plus Erlotinib in EGFR mutant NSCLC patients, previously treated with an EGFR-TKI resulted in 68% DCR, and 22/26 patients (85%) achieving a significant growth-rate reduction in their tumors, corresponding to a >30% increase in the time to tumor size doubling. The combination treatment was generally well tolerated. Diarrhea was the most frequent grade 3-4 adverse event, reported in 11/37 (30%) of the patients [68]. A randomized phase II study of Erlotinib, Cabozantinib or Erlotinib plus Cabozantinib in previously treated, EGFR wild type, NSCLC patients is currently recruiting patients (NCT01708954). Another phase II, randomized discontinuation trial, investigated both the tolerability and activity of Cabozantinib in NSCLC, including patients who received prior anti-EGFR(50%) and VEGF (32%) targeted therapy. Cabozantinib treatment resulted in a 10% RR, 40% DCR, and 64% of objective tumor regression rate, while no differences were seen in median PFS between treatment arms [69].

**Crizotinib**

Is an oral, selective, ATP-competitive, small molecule, originally developed as cMET inhibitor, recently approved for clinical use as the best treatment for NSCLC patients whose tumors harbor EML-ALK chromosome rearrangements [70]. In pre-clinical studies, Crizotinib showed a great activity against both cMET-positive and ALK-negative cell lines [71]. However the dramatic responses and symptoms improvement observed in a subgroup of patients with ALK-rearranged NSCLC, during the dose escalation phase I study [72], oriented the clinical development of this compound for this molecular setting of NSCLC. Evidence of both preclinical [73] and clinical activity of Crizotinib (extended stabilization or tumor shrinkage) was observed also in patients with cMET-amplification [74, 75]. Recently, the first study evaluating Crizotinib in cMET-amplified NSCLC was presented by Camidge et al. at the American Society of Clinical Oncology (ASCO) meeting, showing very encouraging results, especially for patients with highly-amplified tumors, that reported an ORR of 67% and a median duration of treatment of 52 weeks. The treatment was very well tolerated. Most commonly side effects were “diarrhea (50%), nausea (31%), vomiting (31%), peripheral edema (25%) and visual impairment (25%)” [76]. Therefore clinical trials are currently investigating the combination of Crizotinib with therapies targeting EGFR (Erlotinib, NCT00965731; Dacomitinib, NCT01121575) to overcome EGFR-TKI acquired resistance, in cMET-amplified, advanced NSCLC.

**Foretinib and Golvatinib**

Are two ATP-competitive dual cMET/VEGFR-TKIs. On the basis of encouraging pre-clinical data showing a synergistic activity with Erlotinib and other anti-EGFR targeted agents in tumor cell-lines [77], a phase I-II trial (NCT01068587) comparing Erlotinib plus Foretinib versus
Erilotinib plus Placebo in pre-treated NSCLC has been started, but results are not yet available. Golvatinib has shown to prevent the development of HGF-induced resistance to EGFR-TKIs, in EGFR-mutant, NSCLC cell lines [16], but further clinical trials should investigate the potential benefit of this new compound in clinical setting.

DISCUSSION

The HGF-cMET pathway has emerged to play an important role in NSCLC, especially as a relevant mechanism involved in the development of secondary resistance to EGFR-TKIs. Increasing understanding of tumor biology, and emerging evidences about a potential synergistic activity between EGFR and cMET inhibitors, have led to the advent of several anti-cMET agents, but none of these are yet available for clinical use. Several randomized studies are evaluating new treatment strategies, including the dual inhibition of cMET and EGFR pathways, in order to overcome EGFR-TKIs acquired resistance, but preliminary results were very disappointing. Indeed theMARQUEE trial, investigating the combination of Tivantinib plus Erlotinib, in pre-treated, advanced, non-squamous NSCLC, has been early stopped in 2012, and the results of the METLung study, of Erlotinib plus Onartuzumab versus Erlotinib plus Placebo in patients with cMET-overexpressed NSCLC (2+/3+ IHC), have shown no survival benefit from the addiction of an anti-cMET MAb to the EGFR-TKI [55].

What are the reasons of such failure? A critical issue concerns the lack of a strong and validated, potential predictive biomarker for the selection of patients eligible to the targeted anti-cMET therapies. In fact the selection of patients included in the MARQUEE trial was based on clinical criteria [66], while a subgroup analysis of the phase II study by Spigel et al. suggested high levels of cMET-expression, detected by a IHC test, as a good predictive biomarker for METMab activity [53]. However, cMET-overexpression evaluated by IHC is a semi-quantitative, subjective, and not perfectly reliable biomarker, and the very limited sample size (n.66) of MET-positive patients in the phase II trial [53], may have negatively influenced trial results. On the other hand, Cappuzzo et al. in 2009, showed that high levels of cMET gene amplification (ratio cMET/CEP7>2.2) drive resistance to Gefitinib in approximately 3% of NSCLC patients, suggesting that “cMET-inhibitors may only be effective in a very small number of NSCLC patients who develop resistance to EGFR-TKIs” [78]. These data have been recently confirmed by Camidge et al., who reported the results of a small clinical study at the ASCO meeting, showing clinical activity of the anti-cMET Crizotinib in patients with intermediate-high (ratio cMET/CEP7>2.2) cMET-amplified NSCLC [76]. Both these studies suggest the use of cMET-amplification and cMET/CEP7 ratio as a novel, more reliable, predictive biomarker for anti-cMET treatments in NSCLC patients with acquired resistance to EGFR-TKIs, irrespective of histological subtype [78]. Further studies need to define the best cut-off of the cMET/CEP7 ratio (>2.2 versus >5) and to prospectively validate the role of cMET-amplification in the selection of patients that are potentially sensible to anti-cMET treatments, while cMET overexpression (IHC), and gene copy number (GCN), analysis may not be considered as optimal companion diagnostic tests for selecting patients to treat with cMET-inhibitors. Another interesting issue regards the best timing of treatment with an anti-cMET agent in order to overcome acquired resistance to EGFR-TKIs. On the basis of pre-clinical evidences, suggesting a synergism with EGFR-TKIs, most of the clinical trials have investigated cMET inhibitors in combination with Gefitinib or Erlotinib, in either EGFR-TKI or chemotherapy pre-treated patients, with variable results. However the positive data of the study of Ficlatuzumab plus Gefitinib [59] in the subgroup of previously untreated, Asiatic, NSCLC patients, with low cMET expression, suggest that the early inhibition of the HGF-cMET pathway may delay the development of acquired resistance to EGFR-TKIs, but these data need to be confirmed in further prospective studies.

A deeper understanding of the cMET/HGF molecular pathway in NSCLC and the subsequent development of active cMET inhibitors suitable for clinical setting, represent a fascinating and open challenge for lung cancer translational research. Although there are several compounds under investigation in clinical trials, none of these has already been approved for clinical use, missing a great opportunity to benefit a significant subgroup of patients who developed resistance to EGFR-TKIs. We have recently learned an important lessons derived from the METLung trial, and we are now moving on the prospective validation of stronger and more reliable predictive biomarkers. New chances of success may be offered by the new cMET-inhibitors, currently in the early phases of clinical development. Only a very close collaboration between clinical oncologists, molecular pathologists, biologists, and pharmacologists will help to find the right answers to the unsolved questions, in order to optimize the treatment of NSCLC patients resistant to EGFR-TKIs.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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