Review Article

Role of mesenchymal-epithelial transition amplification in resistance to anti-epidermal growth factor receptor agents

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Abstract: All patients with epidermal growth factor receptor (EGFR) mutant advanced non-small cell lung cancer (NSCLC) treated with an EGFR-tyrosine kinase inhibitor (EGFR-TKI) such as gefitinib, erlotinib or afatinib will progress after a median of 9-12 months. So far, development of a secondary T790M mutation represents the most common (approximately 60%) mechanism of resistance to these drugs. The relative rarity of mesenchymal-epithelial transition (MET) amplification in NSCLC suggests that this event plays a limited role in primary resistance to EGFR-TKI. In contrast, MET gene amplification has been detected as a secondary event representing one of the most relevant mechanisms involved in the acquired resistance to EGFR-TKIs both in preclinical and clinical studies. The aim of this review is to discuss the role of MET amplification as a mechanism of resistance to anti-EGFR therapies and to review strategies which aim at overcoming this mechanism of resistance, including studies assessing drug combinations targeting both EGFR and MET pathways.

Keywords: Epidermal growth factor receptor (EGFR); mesenchymal-epithelial transition (MET); tivantinib; onartuzumab; crizotinib

Submitted Jan 20, 2015. Accepted for publication Feb 06, 2015.
doi: 10.3978/j.issn.2305-5839.2015.03.44
View this article at: http://dx.doi.org/10.3978/j.issn.2305-5839.2015.03.44

Introduction

Lung cancer remains the leading cause of cancer-related death and one of the most difficult diseases to treat (1). Over the last decade, there has been a lot of progress in the knowledge of molecular biology of non-small cell lung cancer (NSCLC) and it is now clear that this is a heterogeneous disease with several biological events driving tumour growth and progression.

A major breakthrough has been identification of activating epidermal growth factor receptor (*EGFR*) gene mutations which are associated with durable and dramatic clinical benefit from EGFR-tyrosine kinase inhibitors (EGFR-TKIs) such as gefitinib, erlotinib and afatinib (2-4).

These mutations are identifiable in 10-35% of NSCLC patients and are more frequent in patients with specific characteristics such as never/light smokers, women, adenocarcinoma histology and East Asian ethnicity. Several randomized phase III trials have consistently shown that these EGFR-TKIs are more effective in terms of response rate (RR) and progression free survival (PFS), less toxic and better tolerated than standard platinum-based doublet chemotherapy when given to untreated advanced NSCLC patients with tumors harbouring an activating EGFR mutation (5-13). For this reason, these drugs currently represent the standard of care as first-line treatment for EGFR-mutant advanced NSCLC (14,15).

Despite disease control can be achieved in about 80% of the patients, resistance will inevitably develop after a median of 10-12 months of treatment. The most common

biological mechanism of resistance is the development of a secondary EGFR mutation, T790M, which is identifiable in about 50-60% of cases (16,17). Other mechanisms of resistance could be related to activation of bypass tracks that make ongoing inhibition of the target alone insufficient to control tumour proliferation. Presence of bypass tracks accounts for about 20% of mechanisms of resistance and these are represented by mesenchymal-epithelial transition (MET) amplification (18,19) and activation of MET through its ligand hepatocyte growth factor (HGF) (about 5%) (20) HER2 amplification (about 8-13%), PIK3CA mutation (about 2%) and BRAF mutation (about 1%) together with epidermal-to-mesenchymal transition (about 2%) and transformation into small cell carcinoma (SCLC) (about 10%) (21).

For these reasons and due to new emerging therapies, repeating biopsy at progression can help guiding the right treatment or participation in the most appropriate molecularly driven clinical trial. At present, there are no guidelines to suggest which patient should be re-biopsied and which molecular test should be performed. A small prospective multicenter French study (GFPC study 12-01) evaluated the success rate of re-biopsy on 100 patients from 18 centres (22). Authors demonstrated that only 82 patients were fit to undergo a re-biopsy and, of these, about a quarter retrieved insufficient material for molecular analysis. For the patients where re-biopsy was adequate, molecular testing influenced subsequent treatment choice in about 30% of cases. A prospective study conducted at Memorial Sloan-Kettering Cancer Centre in 155 adenocarcinoma patients with acquired resistance to erlotinib or gefitinib showed that about 92% of patients were able to undergo another biopsy, and sufficient material for biological analysis was obtained in 80% of cases (23). Samples were tested for mutations in EGFR, AKT1, BRAF, ERBB2, KRAS, MEK1, NRAS and PIK3CA, and FISH for MET and HER2. A secondary EGFR T790M mutation was identified in 63% of patients and 3% had small cell transformation. MET amplification and HER2 amplification were found in 4/75 (5%) and 3/24 (13%) of analyzed patients, respectively. No acquired mutations in PIK3CA, AKT1, BRAF, ERBB2, KRAS, MEK1, or NRAS were detected and overlap among mechanisms of acquired resistance was seen in 4% of patients.

The aim of this review is to discuss the role of MET amplification as a mechanism of resistance to anti-EGFR therapies and to review strategies which aim at overcoming this mechanism of resistance, including studies assessing drug combinations targeting both EGFR and MET pathways.

Mechanisms of aberrant met signalling in NSCLC

MET normal function

The MET factor gene (c-MET) encodes for a membrane-bound receptor tyrosine kinase (RTK) expressed predominantly by epithelial cells. The natural ligand for this receptor is the HGF, produced by stromal and mesenchymal cells, that acts primarily on MET-expressing epithelial cells in an endocrine and/or paracrine fashion (24,25). Ligand-binding dependent MET activation results in receptor phosphorylation and subsequent activation of multiple intracellular signaling cascades involved in cell proliferation, survival, angiogenesis, morphogenesis, cell scattering, motility, migration and invasion, all processes involved in a unique biological program leading to "invasive growth". MET pathway has been found to be activated in several human malignancies, including lung cancer through receptor over-expression, genomic amplification, mutations, or alternative splicing.

MET expression in NSCLC

MET receptor has been reported as over-expressed in both small cell lung cancer (SCLC) and NSCLC, particularly in non-squamous histotype. In early reports, MET was 2- to 10-fold increased in 25% of primary NSCLC samples comparing to adjacent normal tissue along with the expression of its ligand (26). More recently tumour tissue microarray expression analysis from human cancers demonstrated that both MET and HGF are commonly expressed, including in lung cancer (27). In particular MET was expressed in 72% of human lung cancer tissues and in 40% of these samples the receptor resulted over-expressed. In addition, phospho-MET expression was found to be at the highest levels in lung cancer (73%), followed by ovarian (33%), breast (23%), and renal (18%) cancer. Phospho-proteomic analysis across 41 NSCLC cell lines and more than 150 NSCLC tumour samples established that MET is the highest tyrosine-phosphorylated receptor in NSCLC tumor samples (and the third in cancer cell lines) further supporting the role of MET as a primary "driver" oncogenic kinase in NSCLC (28).

MET gene amplification

MET gene amplification has been reported in 2-21% of NSCLC adenocarcinomas, particularly in EGFR-TKI naïve cohorts, acting as 'oncogenic driver' (29-32). Using

the fluorescence in situ hybridization (FISH) assay, the Lung Cancer Mutation Consortium reported that 4.1% of adenocarcinoma (n=295) has MET amplification (defined by MET/CEP7 >2.2, see below).

MET gene amplification can guide the dependency of lung cancer cell survival and proliferation upon the MET signalling. In these cells, the blockage of MET causes significant growth inhibition, G1-S arrest and apoptosis (33). The only study to demonstrate an association between MET FISH status and clinical characteristics was from Okuda and colleagues, where amplification or high gene copy number of MET were related to male gender and smoking status (34). In the same series, both FISH positive and gene-amplified cases had a worse prognosis, although the difference was not statistically significant. Notably, among the MET FISH-positive NSCLC, patients with gene amplification did not have a shorter OS compared to those with high polysomy. Cappuzzo *et al*. evaluated *MET* gene copy number in 447 patients with resected early stage NSCLC and found that high MET gene copy number (>5 copies/cell) was negatively associated with survival [hazard ratio (HR) =0.66, P=0.04] (35). Similarly, Beau-Faller and colleagues found a trend toward shorter event-free survival in adenocarcinoma patients with increased *MET* gene copy number (29). Differently from previous studies, Kanteti and colleagues demonstrated that the high *MET* gene copy number was associated with longer survival (39 *vs.* 16 months, P=0.06), which did not reach statistical significance, possibly due to the small sample size in the study (30). It is worth noting that one possible limitation of this study is represented by the use of qPCR method, and not FISH, on DNA samples extracted from formalin-fixed paraffin-embedded (FFPE) archival tumor.

The relative rarity of MET amplification in NSCLC suggested that this event plays a limited role in primary resistance to EGFR-TKI. In contrast, *MET* gene amplification has been detected as a secondary event representing one of the most relevant mechanisms involved in the acquired resistance to EGFR-TKIs both in preclinical and clinical studies, evaluating EGFR-TKI-resistant NSCLC after exposure to gefitinib or erlotinib (18). The cross talk of *MET* with the HER family receptors is indeed particularly relevant in lung cancer (36-39). MET and EGF family receptors are often described as co-expressed in tumours and are bi-directionally interplaying: trans-activation of MET often depends on the elevated expression of EGFR in many human tumours and conversely, HGF stimulation promotes trans-activation of EGFR in multiple cell lines, including NSCLC (40,41). Cooperation between MET and EGFR occurs also indirectly where activation of Src by MET leads to the EGFR phosphorylation and the creation of docking sites for EGFR interactors involved in downstream signalling (42).

Using *in vitro* cell line models, Engelman *et al*. reported that the amplification of MET in the adenocarcinoma NSCLC cell line, HCC827, which harbours the activating mutation of the *EGFR* gene, mediates resistance to EGFR-TKIs and activates HER3 and consequently of the PI3K-AKT cell survival pathway (43). Inhibition of MET in EGFR-inhibitors resistant cells, either *in vitro* or *in vivo*, promotes apoptosis, tumor growth reduction and significant necrosis and the use of both MET and EGFR inhibitors cooperatively abrogate the ErbB3 signalling activation (39,43). In xenograft models, the combined treatment strategy has been proven to overcome primary EGFR-TKI resistance (44). In another study, the use of golvatinib, a multitarget small-molecule inhibitor, has been shown to restore sensitivity to EGFR-TK inhibition and to prevent the emergence of resistant cell clones after continuous HGF exposure *in vitro* (45). Indeed, another mechanism of both primary and acquired resistance to EGFR-TKIs is represented by HGF overexpression (46). Furthermore, gefitinib-resistant MET-amplified NSCLC (HCC827 GR) cells showed an increased activation of the tyrosine kinase Src, and the use of Src inhibitors resulted in tumor-cell inhibition and apoptosis (47,48).

Preclinical data also support that MET cross-talks and cooperates with other members of the EGF receptor family, including HER2, to enhance cell invasion and this lead to the possibility to explore therapeutic activity of dual MET and HER2 therapies (49,50).

Definition of overexpression: methods

The oncogene MET can be studied both at the protein and gene levels. In order to evaluate the protein expression level, immunohistochemistry (IHC) represents the standard technique. The selection of the appropriate antibody is based on the specific aim of the investigation: some antibodies recognize only the non-phosphorylated domain of the protein and are therefore indicated to measure the amount of total protein, while others match the phosphorylated form and are used to measure the activation rate of the receptor. In addition, different antibodies recognize different residues located at the N- or C-terminus.

A consensus on the evaluation criteria of the IHC results has not been reached yet. The semi-quantitative H-score system combines staining intensity (scored from 0 to 4) with the percentage of positive cells (scored 0-100%) (51). Each single intensity level is multiplied by the percentage of cells, and all values are summed up to obtain the final IHC score, which ranges from 0 to 400. Scores <200 are considered to be associated with negative/low expression, while scores from 201 to 400 are considered to show positive/high expression (52,53). For the modified H-score system, three staining intensity levels (scored from 0 to 3) are considered and a total score ranging from 0 to 300 is provided; cases are classified as negative (score 0-50), weakly positive [51-100], moderately positive [101-200], or strongly positive [201-300] (54). Another method used to evaluate the expression of c-MET is similar to that applied to HER2 evaluation, according to the intensity and location of staining. Samples are classified as negative (0, 1+), when no staining or faint staining is present in <10% of cells; ambiguous (2+) when moderate staining is present in >10% of cells; positive (3+), when a circumferential, basolateral, or lateral signal for c-MET over-expression of protein with strong intensity is present in >10% of the cells (55). Although IHC is the most commonly used method for MET expression evaluation, it cannot establish whether the receptor over-expression is due to gene amplification or to other mechanisms, such as transcriptional activation or hypoxia (56). To assess the amplification of MET, in situ hybridization techniques have to be performed. Both FISH and single or double silver in situ hybridization (SISH) provide the measurement of the number of gene copies and of the chromosome 7 centromere (CEP7) copy number. MET amplification can be defined as a gene-to-centromere ratio (MET/CEP7) ≥2.2 or MET copy number ≥6.

Alternatively, the method described for EGFR (52) is still used to evaluate the MET status. Amplification of MET is classified into six groups as follows: (I) disomy (≤2 copies in ≥90% of cells); (II) low trisomy (≤2 copies in ≥40% of cells, 3 copies in 10-40% of cells, ≥4 copies in <10% of cells); (III) high trisomy (≤2 copies in ≥40% of cells, 3 copies in ≥40% of cells, ≥4 copies in <10% of cells); (IV) low polysomy (≥4 copies in 10-40% of cells); (V) high polysomy (≥4 copies in ≥40% of cells); and (VI) gene amplification (defined by the presence of tight MET clusters and a ratio of MET/CEP7 ≥2, or ≥15 copies of MET/cell in ≥10% of analyzed cells). High polysomy and gene amplification are considered as a positive SISH result, while the others represent negative results.

MET gene mutations

MET gene mutations and copy number variations have been reported in a variety of human tumour tissues, especially in lung cancer. The highest frequency of MET mutations among NSCLC patients has been found in East Asians. The majority of MET receptor mutations in lung cancer has been described as clustered in the non-tyrosine kinase domain, namely in the juxtamembrane (JM) domain and semaphorin (Sema) extracellular domain (57). Interestingly, the mutations found in the JM domain, encoded by exons 14-15, seem to be involved in tumorigenesis (58-61). Previous studies characterizing the JM domain mutations (R988C, T1010I, alternative spliced JM-deleting variant) demonstrated that these alterations result in oncogenic variants with enhanced proliferating signaling, tumorigenicity, cell motility, and migration (62). For example, a point mutation in the tyrosine residue Tyr1003 located in the MET JM region may prevent the binding with the c-Cbl protein thus inhibiting receptor polyubiquitination and degradation leading to MET oncogenic activity (63). MET kinase domain mutations have been found to be somatically selected in the head and neck squamous cell carcinoma metastatic tissues, compared with the primary solid cancers (64,65) whereas no data report occurrence of MET mutations in NSCLC as a secondary event resulting from exposure to prior therapies.

Strategies to overcome resistance to EGFR-TKIs

Since MET amplification represents a major mechanism of acquired resistance to EGFR-TKIs, a number of strategies have been developed in an effort to inhibit MET signalling, alongside with EGFR inhibition. The two main categories of molecular agents that target the MET protein include monoclonal antibodies against the membrane receptor and TKIs of the intracellular domain of the receptor. Tivantinib, initially thought to work as a MET-TKI, has been investigated in a randomized phase II trial comparing tivantinib plus erlotinib (ET) versus placebo plus erlotinib (EP) in 167 pre-treated patients with advanced NSCLC (66). The primary endpoint of the trial was not MET: PFS was 3.8 and 2.3 months (HR =0.81; 95% CI, 0.57-1.16; P=0.24) for the ET group and EP group, respectively. Overall RRs (ORR) were 10% *vs.* 7% for the ET and EP groups, respectively. The most common all grades toxicities with erlotinib were diarrhoea (47.6%) and rash (65.5%). The addition of tivantinib to erlotinib did not result in additive

toxicity, with the most common adverse events being fatigue (33.3% *vs.* 37.3%), anorexia (28.6% *vs.* 33.7%), nausea (26.2% *vs.* 27.7%), vomiting (26.2% *vs.* 14.5%), and dyspnea (22.6% *vs.* 26.5%) for the ET versus the EP arm, respectively. Although the trial was negative, a pre-planned subgroup analyses showed that among patients with non-squamous histology (n=117), there was a trend toward benefit from ET in both PFS (HR =0.71; 95% CI, 0.46-1.10; P=0.12) and OS (HR =0.72; 95% CI, 0.44-1.17; P=0.18). Furthermore, among the small number of patients with KRAS mutations (n=15), there was a significant benefit in PFS (HR =0.18; 95% CI, 0.05-0.70; P<0.01; interaction P=0.006) and a corresponding trend in OS (HR =0.43; 95% CI, 0.12-1.50; P=0.17). On the basis of this suggestion of activity from the subgroup analysis, a phase III trial was conducted. The MARQUEE study was a randomized, double blind trial evaluating the tivantinib/erlotinib (T + E) versus placebo/erlotinib (E + P) in pre-treated advanced non-squamous NSCLC patients without any molecular selection (67). The study randomized 1,048 patients (34% had received two prior regimens), the primary endpoint was OS and secondary endpoints included PFS, OS in molecular subgroups and safety. EGFR mutant (10.4%), and KRAS mutant (27.1%) patients were well balanced between the two study arms. In September 2012, the independent data monitoring committee recommended trial discontinuation because the pre-planned interim analysis crossed the futility boundary. Median OS was 8.5 months (95% CI, 7.1-9.3) in the T + E arm and 7.8 months (95% CI, 7.0-9.0) in the P + E arm (HR =0.98; 95% CI, 0.84-1.15; P=0.81). Median PFS was 3.6 months (95% CI, 2.8-3.7) in the T + E arm and 1.9 months (95% CI, 1.9-2.0) in the P + E arm (HR =0.74; 95% CI, 0.62-0.89; P<0.001). ORR was 10.3% in the T + E arm, as compared with 6.5% in the P + E arm (P<0.05). Biomarker analysis did not show any difference in efficacy in the subgroup of patients with KRAS mutant tumors; Nevertheless, MET HC status of moderate or increased expression (2+/3+) was predictive for the efficacy of the E + T combination.

Onartuzumab is a monoclonal antibody against the MET proto-oncogene product. A randomized phase II trial compared onartuzumab/erlotinib to erlotinib/placebo in patients with pre-treated advanced NSCLC (68). In the intention to treat (ITT) population there was no difference in PFS (2.6 *vs.* 2.2 months; HR =1.09; P=0.69) or OS (7.4 *vs.* 8.9 months; HR =0.80; P=0.34) for the placebo and onartuzumab arm, respectively. Nevertheless, a retrospective analysis showed that MET-positive patients (defined as a score of 2+ or 3+ as per the MET IHC scoring system) treated with erlotinib/onartuzumab had longer PFS (2.9 *vs.* 1.5 months, HR =0.53; P=0.04) and longer OS (12.6 *vs.* 3.8 months, HR =0.37; P=0.002) than MET-negative patients.

These results observed in the subgroup of MET-positive patients prompted a large phase III, double-blind trial evaluating the erlotinib/onartuzumab combination in pre-treated MET positive (IHC 2+/3+) patients with advanced NSCLC (69). The study was powered to assess the effect of MET IHC expression, which was used as a surrogate of MET activation, as an independent stratifying factor. Patients were randomized in a 1:1 fashion and the primary endpoint was OS.

A pre-planned interim analysis on 244 events led to the decision to discontinue the trial for futility. There was no significant difference in OS, PFS or RR between the two arms. The study withstood criticism during the ASCO 2014 meeting, mainly due to the fact that MET IHC expression was used as a biomarker of MET activation for the selection of "MET-positive" patients. It is likely that use of MET gene amplification (with MET/CEP7 ratio ≥2.2), would have been more appropriate to select the target population. MET IHC expression is quite frequent (50-67%) in advanced NSCLC (18) and varies according to clinical stage, histological type (less frequent in squamous histology) and ethnicity, whereas true MET amplification occurs only in 1-7% of cases (70).

Cabozantinib (XL184) is a small-molecule kinase inhibitor with potent activity against MET and VEGF receptor 2 (VEGFR2)-mediated signalling, as well as a number of other RTKs, including RET, KIT, AXL, and FLT3. Preliminary clinical data on the first three patients treated with cabozantinib within a phase II trial for patients with RET fusion-positive NSCLC (NCT01639508) showed confirmed partial responses in two patients (including one harbouring a novel TRIM33-RET fusion) and a prolonged disease stabilization in a third patient (71). Two additional trials assessing the combination of cabozantinib with erlotinib in patients with previously treated NSCLC are currently ongoing (NCT01866410 and NCT01708954). Foretinib, on the other hand, is principally developed as a potent ROS1 inhibitor, but also possesses capacity of c-MET inhibition (72). A randomized phase I/II trial of erlotinib with or without foretinib in previously treated patients with advanced NSCLC has recently completed accrual (NCT01068587) and results are awaited.

Crizotinib, was initially developed as a highly active and selective MET inhibitor and then was found to be

also a ALK and ROS1 inhibitor. Camidge *et al*. recently reported on a cohort of MET-amplified patients treated with crizotinib as part of a phase I trial (73). In the archival tissue, MET amplification was determined by FISH and patients were divided in three categories of amplification according to MET/CEP7 ratio: ≥1.8 and ≤2.2 (low), >2.2 and <5 (intermediate) and ≥5 (high). Thirteen patients with c-MET-amplified NSCLC [low (n=1), intermediate (n=6) and high (n=6)] were enrolled, with 12 being evaluable for response. Notably, 77% of the patients were ex-smokers. ORR was 0%, 17% and 67% for low, intermediate and high MET/CEP7 ratio, respectively, suggesting a preferential activity of the drug in patients with MET-amplified tumors. Median duration of response was 16 and 73.6 weeks in the intermediate and high MET group, respectively. Median treatment duration was 15.7 weeks and six patients experienced continued response at data cut-off. Altogether these data are encouraging and warrant further evaluation of crizotinib in patients with advanced NSCLC whose tumours harbour MET amplifications. Notably this evidence clarifies that MET positive patients by FISH (GCN >5) are a different population than the MET amplified using MET/CEP7 ratio ≥2.2, and only the latter is likely to derive clinical benefit from MET inhibition.

Conclusions and future directions

Despite the initial clinical benefit from an EGFR-TKI, all patients will eventually progress and, unless enrolled in a clinical trial, will go on to receive standard chemotherapy. Development of a secondary EGFR T790M mutation represents the most common acquired mechanism of resistance (about 60% of patients) to an EGFR-TKI. There are no approved treatments for patients with T790M+ NSCLC and a number of molecules specifically aiming at overcoming this mechanism are in different stages of clinical development.

AZD9291 is a mutant selective, irreversible inhibitor of EGFR that has shown an effect in preclinical tumour models with both EGFR-TKI-sensitizing and T790M resistance mutations while maintaining a margin of selectivity against wild-type EGFR. This drug was evaluated in a phase I trial (AURA) enrolling patients with advanced NSCLC who had progressed on EGFR-TKI (74). The trial had an escalation cohort of 31 patients and an expansion cohort of 201 patients (total n=232). No dose limiting toxicities were identified at any dose evaluated, and the maximum tolerated dose (MTD) has not been defined. In the overall population, RR was 53% (no difference in RR was detected by ethnicity) and disease control rate (DCR) was 83%. In patients harbouring T790M mutation, RR was 64% with a DCR of 94%. In 56 pts with EGFR T790M+ tumors and confirmed response: longest duration of response was ongoing at approx 7.5 months with 12% of patients responding for at least 6 months. AZD9291 is currently being evaluated in a phase III trial versus standard platinum-based chemotherapy in patients who progressed after a first/second generation EGFR-TKI (NCT02151981) or against gefitinib or erlotinib as first-line treatment for EGFR mutant locally advanced or metastatic NSCLC (NCT02296125).

Rociletinib (CO-1686) is an oral irreversible EGFR-TKI that selectively targets mutant forms of the EGFR while sparing wild-type EGFR. Rociletinib was tested in a phase I/II trial evaluating patients with advanced NSCLC and activating EGFR mutation who progressed after an EGFR-TKI (75). A total of 625 mg BD of optimized oral formulation (fed state) was identified as the optimal dose and schedule but 500 mg BD remains under investigation as a step-down dose. Early evidence of activity was observed with durable RECIST responses, particularly in T790M positive patients. Updated results of the expansion phase in T790M positive patients (TIGER-X) were recently presented (76). A total of 56 patients were evaluable and the median number of previous lines of treatment was 3. RR was 67%, DCR was 89% and median PFS was 10.4 months. Treatment was well tolerated with no cutaneous toxicity of note. Most common toxicities (all grades) observed were hyperglycaemia (32%), diarrhoea and nausea (25%), appetite loss (20%) and fatigue (14%). Rociletinib will be evaluated in a number of trials as part of the TIGER trials programme (NCT01526928, NCT02322281, NCT02186301 and NCT02147990).

Having recognised MET amplification as a potential 'druggable' mechanism of resistance there is a number of efforts to incorporate the MET pathway inhibition to backbone treatments for advanced NSCLC. Onartuzumab has been tested in combination with paclitaxel plus cisplatin or carboplatin as first-line treatment for patients with stage IIIB or IV squamous NSCLC (NCT01519804) and in combination with either bevacizumab/platinum/paclitaxel or pemetrexed/platinum as first-line treatment for patients with stage IIIB-IV nonsquamous NSCLC (NCT01496742). Both trials have recently completed accrual and results are pending. Onartuzumab is also being tested in combination with erlotinib in patients with MET-positive stage IIIB-

IV NSCLC carrying an activating EGFR mutation (NCT01887886) and the trial is currently recruiting patients. Tivantinib, on the other hand, is currently being evaluated in combination with erlotinib versus single agent chemotherapy in patients with pre-treated KRAS mutation positive advanced NSCLC in a randomized phase II trial (NCT01395758) and with the same combination in patients with advanced NSCLC harbouring activating EGFR mutations as first-line treatment (NCT01580735).

Development of targeted therapies for patients with advanced NSCLC resistant to first generation EGFR-TKI raise some important issues, such as the possibility of use novel personalized biomarkers and also the combination of novel agents with immune-checkpoint blockade. Appropriate development of these strategies could help overcoming the tumour's 'oncogene addiction' and 'improve patients' outcome in the near future.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

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Cite this article as: Califano R, Morgillo F, De Mello RA, Mountzios G. Role of mesenchymal-epithelial transition amplification in resistance to anti-epidermal growth factor receptor agents. Ann Transl Med 2015;3(6):81. doi: 10.3978/j.issn.2305-5839.2015.03.44

