MET Signaling: Novel Targeted Inhibition and Its Clinical Development in Lung Cancer

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Abstract: MET is a versatile receptor tyrosine kinase within the human kinome which is activated by its specific natural ligand hepatocyte growth factor (HGF). MET signaling plays an important physiologic role in embryogenesis and early development, whereas its deregulation from an otherwise quiescent signaling state in mature adult tissues can lead to upregulated cell proliferation, survival, scattering, motility and migration, angiogenesis, invasion, and metastasis in tumorigenesis and tumor progression. Studies have shown that MET pathway is activated in many solid and hematological malignancies, including lung cancer, and can be altered through ligand or receptor overexpression, genomic amplification, *MET* mutations, and alternative splicing. The MET signaling pathway is known to be an important novel target for therapeutic intervention in human cancer. A number of novel therapeutic agents that target the MET/HGF pathway have been tested in early-phase clinical studies with promising results. Phase 3 studies of MET targeting agents have just been initiated. We will review the MET signaling pathway and biology in lung cancer and the recent clinical development and advances of MET/HGF targeting agents with emphasis on discussion of issues and strategies needed to optimize the personalized therapy and further clinical development.

Key Words: MET, HGF, Targeted therapy, Lung cancer.

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Molecular targeted therapy has been successfully applied in the treatment of many human cancers, including lung cancer.[1] Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib, have been approved for advanced non-small cell lung cancer (NSCLC) treatment in second- and third lines, and most recent data providing strong rationale for first-line use in patients harboring EGFR-TKI sensitizing mutations (L858R and exon 19 deletions).[2–6] Nonetheless, development of other novel molecular targeted therapy is still urgently needed to impact lung cancer and improve survival. We now recognize that histologically similar NSCLC indeed should not be considered as one disease but a collection of heterogeneous molecular disease subgroups with different underlying genetic/genomic alterations. Hence, the quest for more novel targets to be inhibited in lung cancer continues beyond EGFR. MET receptor kinase has been under extensive basic and preclinical investigation for over 25 years. MET is now known to be a "druggable" target within the human kinome, with promising results of early phase clinical investigations of MET targeting agents emerging. This review will provide a concise summary of our current understanding of the biology, signaling, targeted therapeutic agents, and the updated status of MET-targeted clinical trial studies.

MET: STRUCTURE, FUNCTION, AND SIGNALING

MET is a receptor tyrosine kinase (RTK) composed of the α-chain (50 kDa) and the transmembrane β-chain (140 kDa) subunit linked by a disulphide bond. The cytoplasmic tyrosine kinase domain contains a number of key serine and tyrosine phosphorylation sites important in the recruitment of SRC-homology-2-domain (SH2) containing signaling transducers and intermediaries. The representative functional structures and domains of MET and its signaling cascades are shown in Figure 1. The natural ligand for MET receptor is hepatocyte growth factor (HGF), also called scatter factor (SF), which is produced by stromal and mesenchymal cells and acts primarily on MET-expressing epithelial cells in an endocrine and/or paracrine fashion.[7] It was originally identified as a mitogen for hepatocytes and motogen for epithelial cells. HGF/MET autocrine activation in *HGF*-transgenic mice or *MET*-transgenic mice in vivo with promotion of hepatocarcinogenesis has also been reported.[8–10] Upon ligand binding to HGF, MET is phosphorylated at multiple residues with subsequent catalytic activation of a multitude of signaling cascade involved in cell proliferation, survival, angiogenesis, morphogenesis, cell scattering, motility, migration, and invasion. Importantly, it is known to have pivotal functions in embryogenesis and organogenesis under its physiological genetic invasive programming.[11] The phosphorylation of the major autophosphorylation sites Y1230, Y1234, and Y1235, located within the activation loop of the tyrosine kinase domain, activates the intrinsic catalytic kinase activity of MET. As a result, an activated docking site in the kinase

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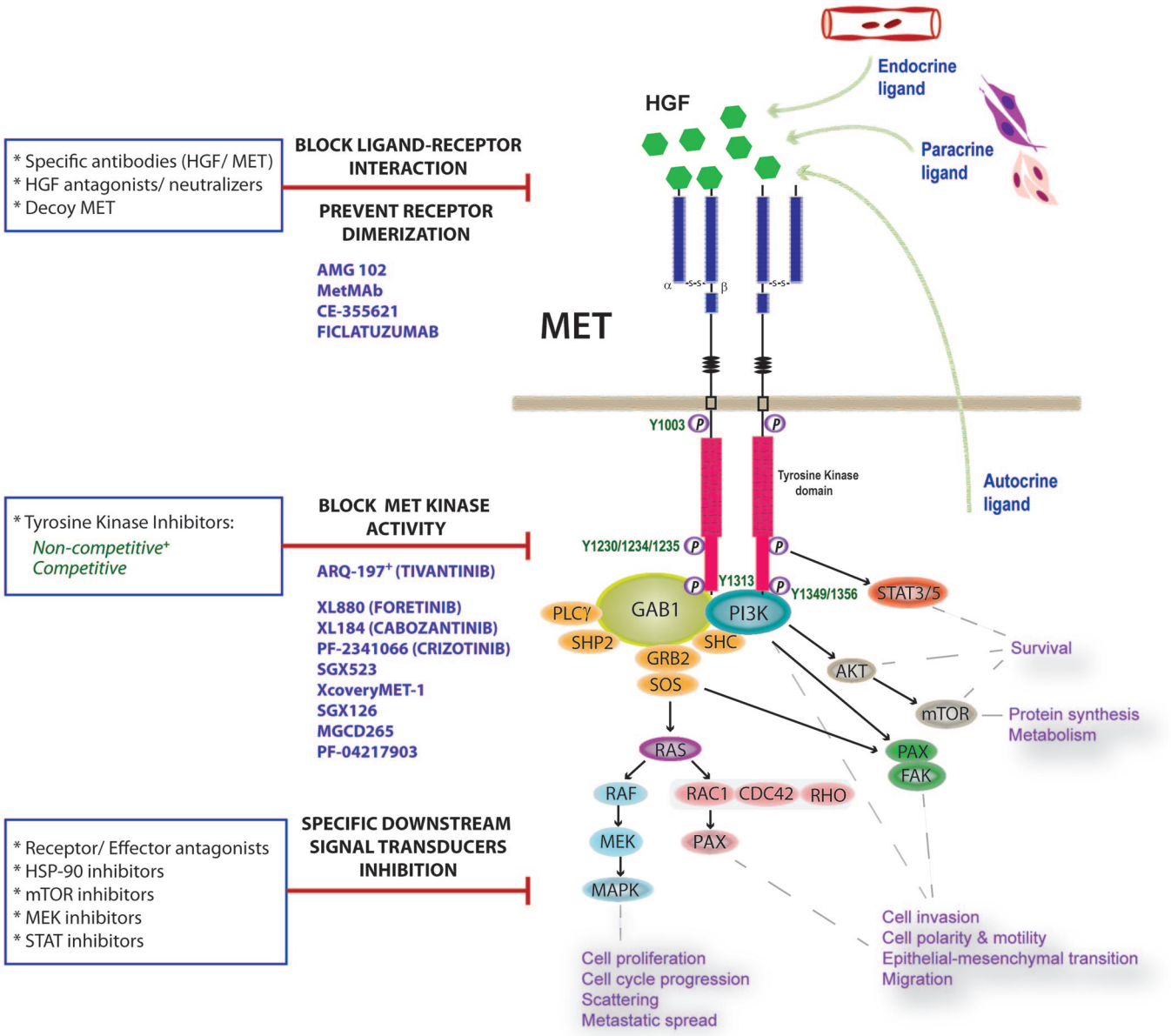

FIGURE 1. MET receptor signaling and strategies of therapeutic inhibition in lung cancer. The natural ligand for MET is hepatocyte growth factor (HGF), also called scatter factor (SF). Aberrant HGF stimulation of MET in human cancer can occur by aberrant autocrine (intratumoral), paracrine (mircoenvironmental), or endocrine (circulatory) loop signal activation. Upon HGF binding to the Sema domain, MET dimerizes leading to autophosphorylation of intracellular tyrosine residues Y1230/Y1234/Y1235 followed by the phosphorylation of Y1349 and Y1356 near the carboxyl terminal portion. MET activation results in the recruitment and activation of downstream adaptor proteins and kinase targets resulting in a multitude of effects such as increased cell proliferation, cell cycle progression, scattering, motility, survival, extracellular matrix remodeling, and changes in metabolism. Thus, MET signaling contributes to tumor growth, scattering, motility, invasiveness, and metastasis, thereby playing important roles in mediating tumor addiction/dependence and tumor expedience. Therapeutic intervention strategies to block and inhibit MET receptor oncogenic signaling cascade include blocking ligand-receptor interaction, preventing receptor dimerization, blocking MET kinase intrinsic activity, and inhibiting specific downstream signal transducers. PI3K, phosphatidylinositol 3-kinase; GRB2, growth factor receptor-bound protein 2; GAB1, Grb2-associated adaptor protein1; STAT 3/5, signal transducer and activator of transcription 3/5; mTOR, mammalian target of rapamycin; SOS, son of sevenless; SHP2, SRC homology protein tyrosine phosphatase 2; SHC, SRC homology domain; PLC-γ, phospholipase c-γ; RAS, rat sarcoma oncogene homolog; MAPK, mitogen-activated protein kinase; RAC1, Ras-related C3 botulinum toxin substrate 1; RHO, Ras homologos; PAX, paxillin; FAK, focal adhesion kinase.

domain further recruits intracellular adaptor molecules through the SH2 domains and other recognition motifs, such as GAB1 (key coordinator of the cellular responses to MET). Downstream signaling of the GRB2-mitogen-activated protein kinase (MAPK) cascade, the PI3K-mTOR pathway, and the STAT pathway are eventually activated, mediating various cellular functions.[12,13] MET is also known to cross-talk with various signaling pathways.[14] The cross-talk between MET and EGFR/HER family receptors is particularly important in lung cancer.[15,16] The notion of cross-talk between MET and KRAS signaling is also emerging with both preclinical and clinical findings of high interest.[17,18] Matsubara et al.,[19] in a study to determine molecular predictors of sensitivity to the MET inhibitor PHA-665752 in lung carcinoma cells, identified that high P-MET and dependence of the AKT and ERK signaling pathways on MET activation may predict drug sensitivity, especially in KRAS-mutated cell lines. Furthermore, PHA-665752 has been shown to reverse lung premalignancy induced by mutant-KRAS (KrasLA1 mice) in vivo.[20]

MET AMPLIFICATION, OVEREXPRESSION, ALTERNATIVE SPLICING AND MUTATION, AND PHOSPHORYLATION IN LUNG CANCER

MET/HGF axis is essential in embryogenic development of placenta, liver, kidney, neurons, and muscle.[11] In vivo, the MET receptor triggers a unique biological program leading to "invasive growth, " a phenomena including cell proliferation, scattering, survival, motility and invasion, epithelial-mesenchymal transition (EMT), and branched morphogenesis.[21,22] Studies have shown that MET alterations occur in numerous human solid and hematological malignancies (http://www.vai.org/met). MET pathway has been found to be activated in a number of human malignancies, including lung cancer. MET can be altered through receptor overexpression, genomic amplification, mutations, or alternative splicing. These alterations lead to signaling deregulation which can be mediated through ligand (HGF)-independent receptor activation or through its ligand (HGF)-dependent activation via autocrine (intratumoral HGF), paracrine (mesenchymal or microenvironmental HGF), or endocrine (circulatory HGF) loop signaling cascades (Fig. 1).

MET receptor is overexpresssed in both SCLC and NSCLC (particularly in nonsquamous NSCLC).[23–25] Recent tumor microarray expression analysis of MET/HGF in human cancers demonstrated that both MET and HGF are commonly expressed in human solid cancers, including lung cancer. Seventy-two percent (29 of 40) of lung cancer tissue was found to express MET, and 40% (16 of 40) have MET receptor overexpression.[26] Moreover, phospho-MET expression is found to be the highest in lung cancer, followed by ovarian, breast, renal, and colon cancers.[26] Using a phosphoproteomic approach, Rikova et al.[27] characterized tyrosine kinase signaling across 41 NSCLC cell lines and more than 150 NSCLC tumor samples and established that MET is the topmost highly tyrosine-phosphorylated RTK in NSCLC tumor samples (ranked third in cell lines). The study findings lend further support to the role of MET as a primary "driver" oncogenic kinase in NSCLC.

MET gene mutations and copy number variations have been reported in a variety of human tumor tissues, especially in lung cancer.[22,25,26,28–32] The MET receptor mutations in lung cancer were mainly found clustered in the nontyrosine kinase domain, namely in the juxtamembrane (JM) domain and sema domain. MET kinase domain mutations have been found to be somatically selected in the metastatic tissues, compared with the primary solid cancers.[33] Previous studies characterizing the JM domain mutations (R988C, T1010I, alternative spliced JM-deleting variant) demonstrated that these are oncogenic activating variants with enhanced oncogenic signaling, tumorigenecity, cell motility, and migration.[30,34] Both somatic and germline variants of MET have been reported in various human cancers, including renal cell carcinoma (in which MET kinase mutations were first identified both in hereditary and sporadic diseases) and thoracic malignancies.[30,33,34] Their relative significance and relevance in lung cancer biology and progression remain to be further defined. By using quantitative polymerase chain reaction (QPCR) assay for *MET* amplification, multiple studies have reported primary *MET* amplification to be in the wide range of 2 to 21%, in NSCLC lung adenocarcinomas, particularly in TKI-naive cohorts.[28,29,31,32] By using the fluorescence in situ hybridization (FISH) assay, the Lung Cancer Mutation Consortium reported that 4.1% of adenocarcinoma ($n = 295$) have *MET* amplification >2.2 (defined by MET/CEP7), while 9.6% ALK 2p23 translocation was identified in ALK break-apart FISH assay.[35] Cappuzzo et al.[36] tested *MET* gene copy number in 447 NSCLC patients and found that high *MET* gene copy number (≥5 copies/cell) was negatively associated with survival (hazard ratio [HR] = 0.66, $p = 0.04$). On the other hand, conflicting result was reported by Kanteti et al.[29] who demonstrated that the *MET* gene copy number >4 in lung adenocarcinoma tissue samples was associated with a trend of better prognosis (median survival: 39 versus 16 months, $p = 0.06$), albeit with a small sample size in the study. Of note, this study was performed using QPCR method, but not FISH, on DNA samples extracted from formalin-fixed paraffin-embedded (FFPE) archival tumor tissues.

MET EXPRESSION: STEM/PROGENITOR CELLS AND TUMOR HYPOXIA

HGF and MET are highly expressed in various stem and progenitor cells, but only expressed in low levels in their mature cells, indicating their important physiologic role in cell differentiation and normal embryonic development.[11] The concept of cancer stem cell (CSC) has been strengthened in several instances through the identification and isolation of CSCs from leukemia, breast and lung carcinomas, and tumors of the central nervous system.[37] MET has been postulated to be a potential marker of an expanding cell population that is undergoing an aberrant differentiation program and that retains stem cell properties.[37,38] MET expression was found to co-localize at the lung bronchioalveolar duct junction where the bronchioalveolar stem cells were identified.[26,39] Recently, De Bacco et al.[40] reported an increased MET expression found

in response to ionizing radiation through the ATM-NFκB signaling pathway, leading to radioresistance and cancer invasion.[40] Hence, it is of high interest to ascertain whether MET expression indeed is elevated in CSCs and contributes to CSC-mediated therapeutic resistance and invasion.[41]

Hypoxia in tissue favors both stemness and invasive growth in normal embryonic development and also in tumor growth. It has been shown that hypoxia-induced MET expression and activation via hypoxia inducible factor-1α mediated transcriptional upregulation and amplified HGF signaling, resulting in MET-HGF-synergized induction of invasion. The inhibition of MET expression prevented hypoxia-induced invasion growth.[42]

ROLE OF MET-HGF IN EGFR-TKI RESISTANCE IN LUNG CANCER

The EGFR-TKIs gefitinib and erlotinib have demonstrated efficacies in NSCLC, particularly with EGFR activation mutations. Nonetheless, invariable acquired resistance remains a formidable challenge that ultimately leads to patient's mortality. Using in vitro cell line model, Engelman et al.[43] demonstrated the emergence of *MET* gene amplification in EGFR-TKI-resistant cell clones (HCC827-GR) derived upon chronic escalating gefitinib exposure. *MET* amplification was observed in 4 of 18 (22%) lung cancer specimens with acquired EGFR-TKI resistance,[43] a result that was confirmed in another study by Cappuzzo et al. also.[44] Bean et al.[45] reported a similar *MET* amplification rate (9/43, 21%) in patients with acquired EGFR-TKI resistance, compared with only 3% (2/62) in untreated EGFR-mutant patients. Nonetheless, 44% (4/9) patients found with *MET* amplification also had concurrent T790M-EGFR-resistant mutation, raising queries about the relative role of the two alterations in mediating EGFR-TKI resistance. Of note, two most recent tissue rebiopsy studies on genetic analysis of acquired EGFR-TKI-resistant NSCLC reported 11% (4/37) and 5% (2/37) of samples found with *MET* high gene copy number, frequencies that are somewhat lower than anticipated based on earlier data.[46,47] Rho et al.[48] demonstrated that *MET* activation, instead of *MET* gene amplification, in PC-9 EGFR-TKI-resistant cells, and sustained activation of MET by HGF-enhanced tumor cell migration and invasion abilities. However, their work showed that the MET activation was secondary to increasing passage number without exposure to EGFR-TKI and was not related to EGFR-TKI resistance.[48] Our previous study demonstrated that in erlotinib-resistant H1975 lung adenocarcinoma cells (T790M/L858R-EGFR) expressing wild-type *MET* without genomic amplification, SU11274 (MET inhibitor) alone and even better when in combination with erlotinib effectively inhibited H1975 cells with enhanced abrogation of cytoskeletal functions and regression of the xenograft growth.[16] Together, the studies above provide a rationale to develop clinical studies of MET inhibitor, alone and in combination with EGFR-TKI in NSCLC (especially TKI-resistant patients), both as primary or secondary strategies to prevent or overcome EGFR-TKI resistance.

With in vitro and in vivo evidence, we recently showed that drug-sensitive cells with mutant EGFR (HCC827, PC-9 [with deletion exon 19] against erlotinib and H1975 [with L858R/T790M] against CL-387,785) exhibited a very early, within the first 6 to 9 days of drug exposure, "adaptive" tumor resistance to escape the EGFR-TKIs, implicating a model of "minimal residual disease."[49] Most interestingly, these cells that existed in a quiescence state evaded the TKIs through MET-independent survival mechanism with newly addicted dependence on the mitochondrial BCL-2/BCL-xL antiapoptotic pathway, which can be further targeted by BH3-mimetic agents to inhibit the BCL-2/BCL-xL signal path.[49] Hence, the role of MET in acquired EGFR-TKI resistance might be more relevant in the late stages of resistance development but not necessarily so in the early emergence of adaptive drug resistance state.

CURRENT CLINICAL DATA OF MET INHIBITORS IN LUNG CANCER

Preclinical MET Inhibitors

Early preclinical MET inhibitors that have been studied include geldanamycin, K252a, SU11274, and PHA-665752.[18,25,50,51] It has been demonstrated that small molecule MET inhibitors SU11274 and PHA-665752 inhibited cell viability and growth and induced apoptosis by inhibiting MET/HGF signaling on various NSCLC cell lines.[16,18,20,25]

Tivantinib (ARQ197; ArQule/Daiichi-Sankyo)

Currently, there are many MET-targeting agents, including small molecules and antibodies that are under development (Table 1). Seven of them have been approved for human clinical trials. Among them, tivantinib is the only one currently in phase 3 trial. It is uniquely the first non-ATP–competitive small molecule that selectively targets the MET RTK, with the mechanism of action as locking the kinase in a "closed" and "inactive" conformation when bound to the drug. In vivo study demonstrated its antitumor activity in various cancers (colon, gastric, and breast cancers).[52] Tivantinib is mainly metabolized by CYP2C19. The ratio of the poor metabolizer (PM) of CYP2C19 in Asians is reported to be around 20%, while it is very low in Caucasians. A phase I trial in Japanese found that tivantinib was well tolerated in PM patients as well as extensive metabolizer (EM) patients. CYP2C19 genotypes clearly affected the exposure to tivantinib, a finding that led to different recommended phase 2 doses: 360 mg twice a day for EM patients and 240 mg twice a day for PM patients.[53] A number of phase 2 trials have been launched to investigate the effects of ARQ197 in various malignancies, including lung cancer. ARQ197-209 is a global randomized placebo-controlled phase 2 clinical trial comparing erlotinib (150 mg) plus ARQ197 (360 mg, twice a day) (E + A) versus erlotinib plus placebo (E + P) in advanced NSCLC patients. Primary end point is progression-free survival (PFS), and secondary end points are overall response rate, overall survival, and subset analysis. The trial result was presented in the 2010 American Society of Clinical Oncology (ASCO) meeting. The trial enrolled 167 patients who were randomized to E + A (84 patients) or E + P (83 patients).

TABLE 1. Examples of c-MET Inhibitory Agents

	Targets	Company	Phase
Tivantinib (ARQ 197)	MET (non-ATP competitive)	ArQule/Daiichi-Sankyo	Phase 1/2–3
Crizotinib (PF-2341066)	MET, ALK	Pfizer	Phase 1/2–3
MetMAb	MET (one-arm MAb)	Genentech/Roche	Phase 2
Ficlatuzumab (AV-299)	HGF Ab	AVEO	Phase 2
Foretinib (XL880, EXEL-2880, GSK1363089	MET, VEGFR2, AXL	Exelixis/GSK	Phase 1/2
Cabozantinib (XL184, BMS-907351)	MET, RET, VEGFR2	Exelixis/BMS	Phase 1/2
PF-04217903	MET	Pfizer	Phase 1
SGX523	MET	SGX	Phase 1
AMG 102	HGF (Hu MAb)	Amgen	Phase 1
CE-355621	MET (MAb)	Pfizer	Preclinical
Xcovery MET-I	MET	Xcovery	Preclinical
SGX126	MET	SGX	Preclinical
MGCD265	MET	MethylGene	Preclinical

Final PFS was prolonged with E + A versus E + P (median: 16.1 versus 9.7 weeks, HR 0.81, $p = 0.23$). Planned multivariable Cox regression model adjusting for prognostic factors yielded PFS HR 0.68 (95% CI 0.47–0.98, $p < 0.05$). The subgroup analyses showed that PFS was significantly better in ARQ197 arm in patients with KRAS mutant (median PFS: 2.3 versus 1.0 months, HR = 0.18, $p < 0.05$), and similar trend (not statistically significant) in patients with nonsquamous cell and EGFR wild type. The side effects, including rash, diarrhea, fatigue, and anemia, were similar in two groups.[54] Interestingly, ARQ197 combined with erlotinib was also found to inhibit tumor metastases in this phase 2 study, consistent with the notion of the *MET* "oncogene expedience" that can be targeted.

A global randomized phase 3 trial (MARQUEE) of ARQ197 in combination with erlotinib, versus erlotinib plus placebo, for advanced nonsquamous NSCLC who have received one or two prior systemic anticancer therapies is now ongoing. There is also a parallel randomized phase 3 study in Asia (Japan, Korea, Taiwan) (ARQ197-006) for wild-type EGFR advanced nonsquamous NSCLC with E + A versus E + P arms which doses ARQ197 according to the CYP2C19 polymorphism (ATTENTION, NCT 01377376), already started recruiting since June 2011. In addition, a phase 2 randomized open-label study of erlotinib plus ARQ197 (E + A) versus single-agent chemotherapy in previously treated KRAS mutation-positive patients with locally advanced or metastatic NSCLC (ARQ197-218) has just been started in July 2011 (NCT01395758). This trial has the primary objective to evaluate median PFS among subjects with KRAS mutation-positive NSCLC (intent-to-treat population) treated with E + A compared with single-agent chemotherapy.

Cabozantinib (XL184/BMS-907351; Exelixis/Bristol Myer Squibb)

Cabozantinib, a multitargeted inhibitor with activities against MET/VEGFR2/RET, has been studied in a phase 1b/2 trial with and without erlotinib (E) in patients with NSCLC in patients with acquired erlotinib resistance.[55] The trial was previously reported in 2010 ASCO. The overall safety and tolerability profile of cabozantinib and erlotinib appear acceptable without evidence of a cabozantinib/erlotinib drug-drug interaction. Six of 36 patients assessable for response including at least 3 with prior erlotinib therapy had ≥30% reduction in tumor measurements on at least 1 postbaseline scan, including 3 with a complete or partial response (PR) (1 with *MET* amplification). Prolonged stable disease (SD) ≥4 months has been observed in some patients including one for more than 9 months and one with EGFR T790M.[55]

The phase 2 randomized discontinuation trial of cabozantinib (XL184) in patients with advanced solid tumors has been completed and the interim result was recently presented in ASCO 2011 Annual Meeting.[56] 398 of 483 enrolled patients with nine different types solid tumors were evaluable, and evidence of soft tissue tumor regression was observed in all tumor types. Most common related adverse events (grade ≥3) were fatigue (9%), hand-foot syndrome (8%), and HTN (5%). Dose reductions for adverse events occurred in 41% patients. Rate of overall disease control (PR or SD) at week 12 of 40% or higher were observed in six different solid tumors, including NSCLC (40%, PR 6/47, 13%). Interestingly, all the patients with *EGFR* (4/23) or *KRAS* (3/23) mutations had PR or SD, and none of the nonresponders had *EGFR* or *KRAS* mutations. However, the sample size is too small to draw any conclusion between the response rate and *EGFR*/*KRAS* mutation status. The *MET* amplification status was not reported in this interim report. Quite interestingly, there were soft tissue and visceral tumor regression and resolution of bone lesions on bone scan observed across multiple tumor types, supporting the notion of targeting MET pathway as the player of "oncogene addiction/dependence" and "oncogene expedience."[56]

Foretinib (XL880, EXEL-2880, GSK1363089; Exelixis/GSK)

Foretinib is a multitargeted small-molecule kinase inhibitor that targets MET and members of the VEGF receptor kinase families, with additional inhibitory activity toward KIT, FLT-3, PDGFR β, Tie-2, RON, and AXL.[57] In vivo, these effects produce significant dose-dependent inhibition of tumor burden in an experimental model of lung metastasis.[57] A phase1 study of foretinib has been conducted in metastatic

or unresectable solid tumor patients.[58] A phase 1/2 clinical trial of GSK1363089 versus GSK1363089 and erlotinib in locally advanced or metastatic NSCLC is currently ongoing.

MetMAb (Genentech-Roche)

Several MET/HGF antibodies have also been developed for targeting the pathway. MetMAb is a recombinant, humanized, monovalent (one-armed) monoclonal antibody antagonist of the MET receptor to achieve targeted inhibition of the ligand HGF-induced MET signaling. Its safety and recommended dose has been established in patients with solid tumor in a phase1b trial.[59] Recently, a global randomized, double-blind phase 2 study comparing MetMAb + erlotinib (ME) to placebo + erlotinib (PE) in second-/third-line NSCLC (OAM4558g) has been completed. The updated efficacy results were presented in ASCO 2011 Annual Meeting. One hundred twenty-eight NSCLC patients were equally randomized to receive ME or PE. Fifty-four percent (54%) of patients had MET immunohistochemistry (IHC) expression (2+ or 3+) NSCLC, which was associated with a worse outcome (OS HR = 2.52, PE cohort). In the METIHC 2+ or 3+ group ($N = 65$), ME resulted in a statistically and clinically significant improvement in both PFS (median 3.0 versus 1.5 months, HR = 0.47, $p = 0.01$) and OS (median 12.6 versus 4.6 months, HR = 0.37, $p = 0.002$).[60] In MET-IHC low/negative group, the OS was worse in ME than PE group (HR = 3.02, $p = 0.021$). E-related toxicities were comparable between treatment arms. The design of MetMAb phase 2 trial incorporating the development of a companion diagnostic test for assaying MET receptor expression level by IHC is worthy of merits. In the MetMAb trial, according to the trial investigators, the overall survival benefit (or the trend of OS benefit) was not exclusive to EGFR mutations or *MET* FISH+ (≥5 copies)($p = 0.19$) and was observed in FISH−/IHC+ ($p = 0.09$) patients, suggesting that IHC is a more sensitive predictor of benefit from MetMAb. Removing patients with EGFR mutations did not alter results, albeit with the p value of survival benefit becoming statistically insignificant ($p = 0.29$) in the analysis likely due to a small sample size.[60]

These results lend support for further investigation of MetMAb as a potential personalized MET-targeting cancer therapeutics for NSCLC patients, and a phase 3 study is being planned currently. Nonetheless, in light of the negative survival data seen in the ME arm in patients with low MET expression in the phase 2 study, and despite a lack of obvious and satisfactory mechanistic explanation for the observation, the phase 3 study would likely be only limiting to the inclusion of MET-expressing patients.

Crizotinib (PF-2341066, Pfizer)

Crizotinib has very recently been approved by the Food and Drug Administration in the United States for patients with NSCLC whose tumor harbors *EML4-ALK* gene fusion as determined by the parallelly approved Vysis LSI dual color probe ALK 2p23 break-apart rearrangement FISH assay (Abbott). Crizotinib has known MET kinase inhibitory activity in addition to that of ALK. Interestingly, the drug was initially developed intending as a MET inhibitor preclinically and subsequently in a phase 1 trial.[61,62] It potently inhibited MET phosphorylation and MET-dependent proliferation, migration, or invasion of human tumor cell in vitro. In addition, it also potently inhibited HGF-stimulated endothelial cell survival or invasion and serum-stimulated tubulogenesis in vitro, indicating that its cytoreductive antitumor efficacy may be mediated by direct effects on tumor cell growth or survival as well as antiangiogenic mechanisms.[63] Subsequently, the clinical development of crizotinib was steered toward focusing primarily on ALK-rearranged NSCLC, ultimately leading to the drug's recent Food and Drug Administration approval in August 2011. However, in a recent case report, a NSCLC patient with de novo MET amplification but no ALK rearrangement achieved a rapid and durable response to crizotinib, indicating that it is also a bona fide MET inhibitor clinically.[64] Dramatic clinical improvement and radiographic regression were also observed in patients with *MET*-amplified esophagogastric adenocarcinoma[65] and glioblastoma multiforme[66] upon treatment with crizotinib.

Others

A growing list of other MET or HGF targeting agents continues to emerge, and some of the agents are currently still under preclinical and early clinical development, as listed in Table 1 and Figure 1.

STRATEGIES FOR OPTIMIZED MET-TARGETED PERSONALIZED LUNG CANCER THERAPY

The role of MET/HGF in cancer growth and invasion has been extensively studied, and the recent clinical studies on MET inhibitor or antibody in malignancy including NSCLC are quite promising. However, a number of important questions remain to be fully answered in the optimization of MET target therapy as outlined below. Addressing these critical areas of MET inhibition strategies in lung cancer would help to bring MET-targeted agents to full clinical fruition to meaningfully impact patient outcomes.

1. Should MET targeted inhibition be developed as primary or secondary strategies in the context of mutated EGFR and wild-type EGFR lung cancer patients? Current studies testing MET agents have both been conducted in primary inhibition strategy (tivantinib, MetMAb) and in secondary strategy (cabozantinib) to overcome acquired EGFR TKI resistance, in combination with erlotinib. Emerging data implicate that MET inhibition either alone or in combination with EGFR-TKI may indeed have a role in primary therapy for EGFR-TKI-naive NSCLC patients.
2. Can we identify biomarker of response to MET inhibition? The optimal patient subgroup that would benefit from single-agent MET inhibitor treatment remains to be determined. Further effort to better define the molecular determinants of MET therapy response would be needed to unleash the full impact of the targeted therapeutics. Beyond *MET* genomic amplification as potential predictive biomarker, MET overexpression, HGF status, *MET* mutations, and cross-talk partners, e.g., EGFR and KRAS, would all be worthy of further investigations here. To this end, newer venues of bioin-

formatics and pathway analysis may be of benefit to achieve these goals, especially when incorporated into upcoming future MET agents clinical studies.

3. Can we inhibit MET to target oncogene expedience? It is attractive to further test the notion of inhibiting MET with targeting inhibitors in human cancers to abrogate or delay MET-driven cancer invasion and metastasis in disease progression. Emerging clinical trial data from the tivantinib phase 2 study in combination with erlotinib[54] and also the cabozantinib randomized discontinuation phase 2 trial study[56] highlighted the potential in inhibiting MET-driven oncogene expedience in impacting on tumor progression and metastasis. Further preclinical modeling and clinical trial design incorporating this question for validation would be warranted.
4. Can we optimize combinational MET therapy with other targeted agents? A number of potential opportunities exist in this regard which require further testing and validation. Examples for rational combination therapy include HGF targeting agents, EGFR TKIs, other targeted kinases agents, or downstream signaling effector inhibitors, e.g., PI3K-I, MEK-I, BCL-2/BCL-xL inhibitor (BH3 mimetic).
5. What are the potential acquired resistance mechanisms against MET targeting inhibition? Prior experience in other targeted therapeutics, such as erlotinib against mutant EGFR and crizotinib against EML4-ALK, suggests that acquired resistance is thus far unavoidable despite initial response. Proactive efforts in identifying potential resistance mechanisms in MET-targeted inhibition would be needed to accelerate the discovery of newer cotargeting strategies to dampen drug resistance. Efforts to derive a deeper understanding of tumor biology and molecular pathways in progressing disease from rebiopsy tissues in sites of resistant disease could have profound impact in advancing our rational therapeutic strategies in MET-targeting therapy.

FUTURE PERSPECTIVES

The important role of MET pathway in various malignancies including lung cancer has been demonstrated in extensive preclinical studies. Studies also support MET as an attractive druggable target, as seen in various recent early-phase clinical trial studies. The clinical efficacy of MET-targeting agents in lung cancer will need be ultimately tested in phase 3 randomized studies, some of which are already ongoing (tivantinib, MetMAb). However, to fully impact the clinical outcome in lung cancer in using MET targeting therapeutics, a number of questions remain to be further addressed. What constitutes MET-driven/dependent lung cancer, in terms of addiction/dependence and expedience? Is there a role for MET monotargeting therapy, and when, versus combination therapy with EGFR TKI and others? What are the molecular determinants of response, as predictive biomarkers, to MET agents (might differ in different drugs with varying mechanisms of action)?

To this end, studies on the biomarkers to predict MET inhibitor sensitivities are beginning to show promise. *MET* amplification has been shown to correlate with drug sensitivity in preclinical models.[67] MET overexpression as determined by IHC staining is showing great promise as illustrated by the MetMAb phase 2 study results, and further work in developing this as a companion diagnostic test is only certain. Optimizing and standardizing MET expression and amplification analysis, whether FISH, QPCR, FFPE, or IHC, would be crucial in the future clinical development. More novel technological platforms might be useful in this regard, as in the example of multiplexed expression assays and FFPE-LCM mass spectrometry-based quantitative tissue proteomics. Better and more sophisticated bioinformatics algorithm would be highly helpful in defining MET activation and thus molecular candidate patient selection. Moreover, noninvasive in vivo MET expression in murine xenograft model by using [C^{11}]SU11274-PET imaging[68] also shows promise in developing an imaging platform of clinical utilities in molecular-targeting patient selection and in monitoring therapeutic response and progression under MET inhibitor therapy. With concerted efforts in translational and clinical development of the MET-targeting agents to continue, it will not be unrealistic to expect MET-targeting therapies to finally come to full clinical fruition in the foreseeable future to impact on lung cancer clinical outcome.

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