

## Review

# Potential therapeutics specific to c-MET/RON receptor tyrosine kinases for molecular targeting in cancer therapy

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Products of proto-oncogenes c-MET and RON belong to a subfamily of receptor tyrosine kinases that contribute significantly to tumorigenic progression. In primary tumors, altered c-MET/RON expression transduces signals regulating invasive growth that is characterized by cell migration and matrix invasion. These pathogenic features provide the basis for targeting c-MET/RON in cancer therapy. In the last decade, various approaches have been investigated to suppress c-MET/RON-transduced oncogenesis. Among the therapeutics developed, monoclonal antibodies (mAbs) and small-molecule inhibitors (SMIs) have emerged as promising candidates. The mechanism of these therapeutic candidates is the disruption of tumor dependency on c-MET/RON signals for survival. The mAbs specific to hepatocyte growth factor (AMG102) and c-MET (MetMAb) are both humanized and able to block c-MET signaling, leading to inhibition of tumor cell proliferation *in vitro* and inhibition of tumor growth in xenograft models. The mAb AMG102 neutralizes hepatocyte growth factor and enhances the cytotoxicity of various chemotherapeutics to tumors *in vivo*. AMG102 is currently in phase II clinical trials for patients with advanced solid tumors. IMC-41A40 and Zt/f2 are RON-specific mAbs that down-regulate RON expression and inhibit ligand-induced phosphorylation. Both mAbs inhibit tumor growth in mice mediated by colon and pancreatic cancer cells. SMIs specific to c-MET (ARQ107 and PF-02341066) are in various phases of clinical trials. Therapeutic efficacy has also been observed with dual inhibitors such as Compound I, which is specific to c-MET/RON. However, a potential issue is the emergence of acquired resistance to these inhibitors. Clearly, development of c-MET/RON therapeutics provides opportunities and challenges for combating cancer in the future.

**Keywords:** receptor tyrosine kinase; therapeutic antibody; tyrosine kinase inhibitor; targeted cancer therapy; cellular mechanism; acquired resistance

Acta Pharmacologica Sinica (2010) 31: 1181–1188; doi: 10.1038/aps.2010.106; published online 9 Aug 2010

## Introduction

Molecular-targeted cancer therapy is an advanced anti-neoplastic strategy used in clinical practice. By acting on particular tumorigenic molecules, targeted therapeutics directly inhibit cellular growth and survival machinery to eradicate tumor cells and thereby achieve clinical significance. Applications of monoclonal antibodies (mAbs) and small-molecule inhibitors (SMIs) such as trastuzumab and lapatinib in the treatment of various solid tumors are ideal examples<sup>[1]</sup>. These drugs target tumors via cell surface proteins known as recep-

tor tyrosine kinases (RTKs) and their connate ligands<sup>[2]</sup>. Epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), VEGF receptors, and other ligands and receptors are currently being targeted with mAbs and SMI for clinical cancer treatment<sup>[1, 2]</sup>. Other RTKs and their specific ligands such as c-MET and hepatocyte growth factor (HGF) are also targets that are under intensive clinical evaluation<sup>[3, 4]</sup>.

RTKs have unique structural and biochemical features that transduce extracellular signals into intracellular compartments<sup>[5]</sup>. The evidence of RTK involvement in cancer progression is well documented<sup>[6, 7]</sup>. Aberrant RTK expression and activity are directly linked to various stages of cancer development, from cell transformation *in situ* to distant metastasis in remote organs<sup>[8, 9]</sup>. These findings led to the establishment of the oncogene addiction theory<sup>[10]</sup>, which provides the theoretic

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Received 2010-05-25 Accepted 2010-07-01

cal basis for the development of molecular-based therapeutics for targeted cancer therapy.

This review focuses on the progress of potential therapeutics that target a unique subfamily of RTKs known as the c-MET proto-oncogene family, including two of its members, c-MET and RON<sup>[11, 12]</sup>. Genetic and biological studies have revealed that altered c-MET/RON expression contributes to the pathogenesis of various epithelial cancers<sup>[11, 12]</sup>. Oncogenic addition of tumor cells to c-MET/RON signaling for survival and growth has also been demonstrated<sup>[13]</sup>. Moreover, pharmacological inhibition of c-MET/RON pathways has achieved therapeutic benefits in various animal xenograft models and in human cancer patients<sup>[3, 14, 15]</sup>. Thus, the use of therapeutics targeting c-MET/RON signaling is a promising approach for the treatment of malignant cancers.

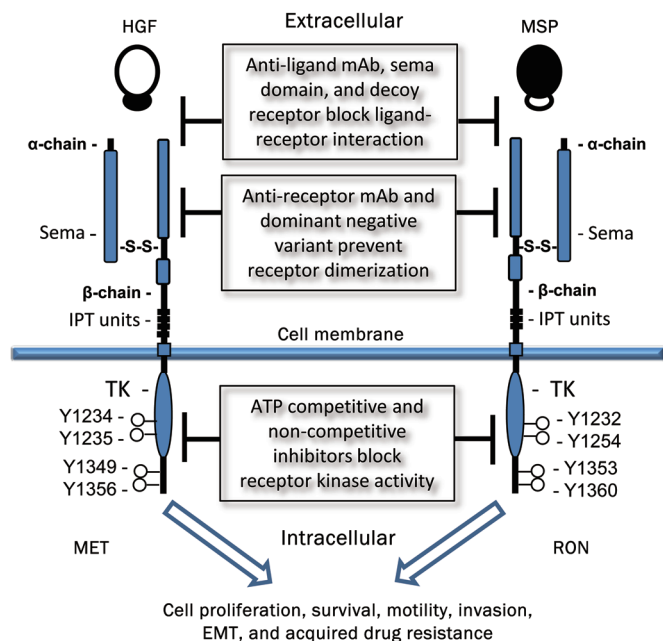
### c-MET/RON in tumor pathogenesis and signaling addiction

c-MET and RON share similar structural and biochemical properties (Figure 1)<sup>[16, 17]</sup>. Both proteins are heterodimers composed of a ~40-kDa extracellular  $\alpha$ -chain and a ~150-kDa transmembrane  $\beta$ -chain with intrinsic tyrosine kinase activ-

ity<sup>[16, 17]</sup>. The extracellular sequences of c-MET/RON contain functional domains such as sema that regulate ligand binding, receptor dimerization, and phosphorylation<sup>[18]</sup>. c-MET is recognized by HGF, also known as scatter factor<sup>[19]</sup>. The specific ligand for RON is macrophage-stimulating protein (MSP), also known as HGF-like protein<sup>[12, 20]</sup>. c-MET and HGF are distributed and expressed in various types of cells and tissues<sup>[21]</sup>. In contrast, RON is highly restricted in cells of epithelial origin, and MSP is produced mainly by liver cells<sup>[22, 23]</sup>.

Ligand-dependent or independent activation of c-MET/RON results in cell proliferation, migration, and matrix invasion, collectively known as invasive growth<sup>[11, 12]</sup>. These activities facilitate epithelial cell transformation and malignant progression. The roles of c-MET/RON in cancer pathogenesis are supported by the following evidence. First, oncogenic mutations in the c-MET gene occur during the early stages of tumorigenesis in certain types of cancers<sup>[24]</sup>, suggesting that aberrant c-MET activation contributes to tumor initiation. Mutations in the RON gene have not been reported in primary tumors; however, aberrant splicing, resulting in formation of oncogenic RON variants, is frequently observed in primary tumors such as colon and breast cancers<sup>[25]</sup>. Second, c-MET/RON overexpression exists in various types of primary and metastatic tumors<sup>[21, 22]</sup>, indicating that c-MET/RON overexpression is involved in tumorigenic progression. Moreover, increased c-MET/RON expression is a validated prognostic factor for predicting disease progression and survival rate in certain cancer patients<sup>[26, 27]</sup>. Third, c-MET/RON activation promotes a malignant phenotype in cancer cells. In tumor cells overexpressing c-MET/RON, cells undergo epithelial to mesenchymal transition (EMT), featuring spindle-like morphology, diminished E-cadherin expression, and increased vimentin expression<sup>[28, 29]</sup>. EMT is a unique phenotype observed in cancer stem cells and a critical process required for cancer metastasis<sup>[30]</sup>. Fourth, altered c-MET/RON expression results in increased survival and pro-apoptotic activity of tumor cells<sup>[11, 12]</sup>, which sustains tumor growth under hostile conditions such as hypoxia. Fifth, abnormality in c-MET/RON expression contributes to the acquired resistance to conventional chemoagents<sup>[31, 32]</sup>. Recently, acquired resistance by lung cancers treated with SMIs was attributed to amplification of the c-MET gene and protein expression<sup>[33, 34]</sup>. We have recently observed that down-regulation of c-MET/RON expression under chronic hypoxia is a mechanism that contributes to the insensitivity of tumor cells toward SMI-induced inhibitory or cytotoxic activity<sup>[35]</sup>. Given that hypoxia selectively advances tumor cells with malignant phenotypes<sup>[36]</sup>, our observation provides a mechanistic insight into the development of acquired resistance in hypoxic tumor cells. Clearly, aberrant c-MET/RON expression participates in tumor formation and malignant progression. Such activities also provide the molecular basis of targeting c-MET/RON for potential therapeutic intervention.

The principle of targeted cancer therapy is to aim at oncogenic molecules that dictate survival and growth of tumor cells, a process known as oncogene addiction<sup>[10]</sup>. Oncogene



**Figure 1.** Schematic representation of the structures of human c-MET, RON, and potential signaling inhibition strategies. Mature c-Met/RON composed of an extracellular  $\alpha$ -chain and a transmembrane  $\beta$ -chain with intrinsic tyrosine kinase (TK) activity. The extracellular sequences of c-MET/RON contain several functional domains, including sema, PSI and immunoglobulin-like plexin transcription (IPT) units. Binding of HGF or MSP results in the c-MET/RON auto-phosphorylation of several tyrosine residues in the kinase activation loop or in the C-terminal tail, which increases enzymatic activities. These activities stimulate intracellular signaling cascades and lead to increased cellular activities. Different strategies using various candidate therapeutic agents were applied to block c-MET/RON signaling pathways.

addiction such as dependence of breast and colon cancers on aberrant EGFR signaling is the rationale for the clinical use of mAbs or SMIs specific to EGFR family members. The c-MET-addicted phenotype has consistently been observed in some established cell lines from gastric and lung carcinomas<sup>[13]</sup>. Amplification of the c-MET gene seems to be required for establishment of such addiction<sup>[13]</sup>. Cell lines from colon, breast, and pancreatic cancers, which are addicted to RON signaling at variable levels, have also been reported<sup>[12]</sup>. One study even showed the death of pancreatic cancer BxPC-3 cells after RON gene expression was silenced by specific siRNA<sup>[32]</sup>. Thus, oncogenic addiction of c-MET/RON signaling occurs in a fraction of tumor cells. Inhibition of tumor growth and induction of tumor cell apoptosis by specific mAbs or SMIs clearly indicates that c-MET/RON signaling is integrated into the cell survival or growth machinery<sup>[37, 38]</sup>. Recent data from clinical trials using specific c-MET/RON inhibitors further confirm this observation<sup>[14, 15]</sup>.

### Strategies and mechanism of blocking c-MET/RON pathways

Various strategies have been reported to block c-MET/RON pathways (Figure 1). These studies establish pharmaceutical feasibility, technological capability, and mechanistic understanding for blocking c-MET/RON signaling for targeted cancer treatment. Studies of blocking ligand-receptor interaction using decoy receptors<sup>[39, 40]</sup>, soluble variants<sup>[41, 42]</sup>, or ligand-specific mAbs<sup>[43]</sup> have shown that such approaches are capable of impairing c-MET/RON-mediated tumorigenic activity both *in vitro* and *in vivo*. The soluble c-MET sema domain is a potent antagonist that inhibits c-MET-mediated tumor growth *in vivo*<sup>[41]</sup>. A splicing variant of RON comprising the sema domain also inhibits MSP binding to RON and blocks RON-mediated signaling and cell migration<sup>[42]</sup>. Anti-HGF antibodies AMG102 are able to inhibit HGF binding to c-MET and prevent c-MET activation<sup>[43]</sup>. Given that c-MET overexpression often coexists with HGF synthesis in stromal tissues around tumor masses as HGF:c-MET autocrine loops<sup>[44]</sup>, inhibition of the HGF:c-MET axis is also a rational approach for cancer treatment. However, these methods have limitations due to ligand-independent c-MET/RON activation caused by genetic mutation, gene amplification, protein overexpression, and generation of constitutively active variants.

Reduction of c-MET/RON density on the cell surface by mAb-induced receptor ectodomain shedding and internalization is an effective method of impairing c-MET-RON signaling<sup>[45, 46]</sup>. Treatment of tumor cells with mAb DN30 specific to c-MET efficiently down-regulates c-MET expression through a mechanism involving proteolytic cleavage, leading to c-MET ectodomain shedding and intracellular degradation<sup>[45]</sup>. We have recently shown that the mAbs Zt/g4 and Zt/f2 specifically down-regulate RON expression by various colon cancer cells through a proteasomal pathway<sup>[46]</sup>. This reduction leads to diminished tumorigenic activity *in vitro*<sup>[46]</sup>, which could be a mechanism that impairs tumor growth.

The siRNA-mediated silencing of c-MET/RON mRNA

expression has therapeutic value<sup>[47, 48]</sup>. In lung cancer cells, silencing c-MET expression results in significant growth inhibition, G<sub>1</sub>-S arrest, and apoptosis<sup>[48]</sup>. Similar observations were made in colon cancer cells overexpressing RON after siRNA treatment<sup>[47]</sup>. Moreover, silencing RON expression by specific siRNA inhibits tumor growth in animal models<sup>[49]</sup>. Nevertheless, the inhibitory effect of siRNA was not observed in cancer cells without c-MET/RON gene amplification or protein overexpression<sup>[47, 48]</sup>. Thus, a precondition is required for the success of the siRNA-mediated therapy. These observations also suggest that selection of cancers with gene amplification or protein overexpression could lead to therapeutic success.

Several SMIs highly selective to c-MET or RON, or both, have been synthesized, and their effects are under preclinical or clinical evaluation<sup>[3, 13]</sup>. The mechanism of SMI consists of the following categories<sup>[50]</sup>: a) competitive binding to an ATP binding site when the kinase is in its active conformation, b) binding to non-active conformation of an ATP binding site of the kinase, c) allosteric inhibition by binding to a site other than an ATP binding pocket, and d) covalent inhibition by irreversible binding to an ATP binding pocket. The action of c-MET/RON SMI is mediated either by non-ATP-competitive means (such as ARQ197 from ArQule/Daiichi)<sup>[38]</sup> or by an ATP-competitive mechanism (such as PF-2341066 from Pfizer)<sup>[3, 13]</sup>. Because of the high degree of similarity in the kinase domains of the c-MET and the RON receptors, these SMIs often show a dual inhibitory effect, with slight differences in IC<sub>50</sub> values. SMIs highly specific to c-MET are available<sup>[3, 13, 38]</sup>, and dual SMIs to both MET and RON have also been synthesized<sup>[51]</sup>. Moreover, SMIs highly specific to RON with only residual activity to c-MET have been reported<sup>[52]</sup>. The availability of such highly specific SMIs could help to define the pathogenic roles of c-MET or RON in certain types of cancer.

### Specific c-MET/RON therapeutics in clinical trials

The evaluation of various c-MET/RON candidate drugs that are currently in phase I or phase II trials is detailed in several recent reviews<sup>[4, 13, 44]</sup>. Owing to space constraints, this section focuses on the most recently available information from a selected group of specific c-MET/RON mAbs and SMIs.

#### Therapeutic mAbs specific to HGF or c-MET

Several anti-HGF mAbs, including AMG102, SCH900105, and TAK-701, are currently undergoing clinical trials (Table 1)<sup>[13-15, 44]</sup>. AMG102, which will be described here in detail, is a fully human IgG2 neutralizing mAb developed by Amgen<sup>[43]</sup>. AMG102 specifically binds to the HGF β-chain with a K<sub>d</sub> of 0.22 nmol/L and blocks the HGF-c-MET interaction with an IC<sub>50</sub> of 2.1 nmol/L<sup>[43]</sup>. In preclinical paracrine HGF models, AMG102 potently inhibits c-MET-dependent tumor growth<sup>[44]</sup>. Phase I trials of AMG102 have been completed, yielding a favorable pharmacokinetic profile<sup>[44]</sup>. The mean half-life of AMG102 is about 15.4 h<sup>[14, 15]</sup>. The dose of AMG102 is well tolerated up to the planned maximum dose of 20 mg/kg. Treatment-related adverse events, including fatigue, constipa-

**Table 1.** Potential Therapeutic Monoclonal Antibodies Specific to HGF, c-MET, and RON\*.

Products	Manufacturer	Target	Status	Descriptions in clinical trials	Ref
AMG102 (rilotumumab)	Amgen	HGF	Phase II	SCLC, CRC, Gliomas, PSC, RCC, GC, EC, Mesothelioma, and OC	43
SCH 900105	Schering/Aveo	HGF	Phase I	Advanced solid tumors, lymphomas or multiple Myeloma	70
TAK-701	Millennium	HGF	Phase I	Advanced non-hematological malignancies	44
L2G7	Galaxy Biotech	HGF	Preclinical	N/A	69
MetMab	Genentech	c-MET	Phase II	Locally advanced or metastatic solid tumors; advanced NSCLC	54
DN30	Methersis	c-MET	Preclinical	N/A	68
IMC-41A10	Imclone	RON	Preclinical	N/A	37
Zt/f2	TTUHSC	RON	Preclinical	N/A	57

\* All information about individual antibodies in clinical trials are from the website: <http://clinicaltrials.gov>, a service of the US National Institutes of Health. BC, breast cancer; EC, esophagus cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer; OC, ovarian cancer; and PSC, prostate cancer. RCC, renal cell carcinoma; and SCLC, small-cell lung cancer.

tion, anorexia, nausea, and vomiting, are generally mild<sup>[14, 15]</sup>. In phase II trials involving 23 patients with advanced solid tumors, 70% of patients had a best response of stable disease, with progression-free survival ranging from 8 to 40 weeks<sup>[14]</sup>. In another study, AMG102 was used in combination with bevacizumab to treat patients with advanced solid tumors such as colon and breast cancers<sup>[15]</sup>. Bevacizumab is a humanized mAb that recognizes and blocks VEGF-A, leading to inhibition of VEGFR activation<sup>[53]</sup>. In 14 evaluated patients, the combination of AMG102 with bevacizumab appeared to have an acceptable profile without dose-limiting toxicities. Treatment-emergent adverse events among patients receiving AMG102 plus bevacizumab are generally mild and include fatigue, nausea, constipation, and peripheral edema<sup>[15]</sup>. Bevacizumab does not seem to affect the pharmacokinetics of AMG102. More importantly, AMG102 in combination with bevacizumab leads to a best result of no tumor progression (stable disease) in 9 of 10 evaluable patients. Eight of 10 evaluable patients showed a reduction in tumor dimension, although no partial or complete responses occurred. Stable diseases with a duration of  $\geq 8$  and  $\geq 16$  weeks were noted in nine and seven patients, respectively, and four patients maintained stable disease for  $\geq 24$  weeks<sup>[15]</sup>. These data suggest that AMG102-mediated c-MET inhibition synergizes with anti-VEGF-mediated angiogenic activity to contain tumor cell growth. Clearly, investigation of AMG102 in combination with other anti-cancer agents should also be pursued to enhance the therapeutic efficacy.

The single-armed MetMab is a humanized anti-c-MET antibody from Genentech<sup>[54]</sup>. MetMab binds to c-MET with an IC<sub>50</sub> of 2.6–8.7 nmol/L in intact cells and diminishes c-MET density on the cell surface<sup>[54]</sup>. Phase I clinical trials have revealed that MetMab is safe and well tolerated as a single agent at doses up to 30 mg/mL<sup>[55]</sup>. Phase II clinical trials evaluating MetMab in combination with erlotinib for second- and third-line metastatic non-small-cell lung cancer are ongoing<sup>[56]</sup>. Given that c-MET over-expression is known to diminish EGFR-targeted therapy<sup>[33, 34]</sup>, MetMab-mediated c-MET inhibition could show a clinical benefit in these tumors.

### Candidate mAbs specific to RON

Potential anti-RON mAbs are IMC-41A10, a fully human IgG1 mAb (Imclone Inc)<sup>[37]</sup>, and Zt/f2, a mouse IgG2a mAb (from our laboratory)<sup>[57]</sup>. Both mAbs are still at the preclinical stage. IMC-41A10 binds to RON with an ED<sub>50</sub> of 0.15 nmol/L and blocks MSP interaction with RON. In colon, breast and pancreatic xenograft tumor models, IMC-41A40 inhibits tumor growth by 50%–60% as a single agent. These effects seem to be linked to IMC-41A40-induced inhibition of RON activation and its downstream signaling pathways<sup>[37]</sup>.

Zt/f2 binds to RON and its oncogenic variants such as RON160 with an ED<sub>50</sub> of 2.3 nmol/L. Zt/f2 interacts with an epitope(s) on the RON extracellular domain essential for RON maturation and activation<sup>[57]</sup>. Binding of Zt/f2 effectively induces RON internalization, which diminishes RON expression and impairs downstream signal activation. Administration of Zt/f2 as a single agent into Balb/c mice results in partial inhibition of tumor growth caused by transformed NIH-3T3 cells expressing oncogenic RON160. Colon cancer HT-29 cell-induced tumor growth in athymic nude mice was also attenuated following Zt/f2 treatment. In both cases, an inhibition of  $\sim 50\%$  of tumor growth was achieved<sup>[57]</sup>. Moreover, Zt/f2 in combination with 5-fluorouracil showed a synergistic effect on HT-29 cell-induced tumor growth *in vivo* (our unpublished data). Another strategy that enhances therapeutic efficacy of Zt/f2 is conjugating cytotoxic drugs for increased cancer cell killing. Studies using Zt/f2-directed immunoliposome loaded with doxorubicin have shown increased cytotoxic activities against various cancer cells *in vitro*<sup>[58]</sup>. Thus, Zt/f2 is a potential therapeutic mAb capable of inhibiting RON-mediated oncogenesis by colon cancer cells in animal models.

### Specific c-MET/RON SMIs in clinical studies

More than 10 SMIs relevant to c-MET/RON inhibition are at various stages of clinical trials (Table 2)<sup>[4, 13, 44]</sup>. The majority of these SMIs are specific to c-MET. The representatives are ARQ197 (ArQule/Daiichi Sankyo) and PF-02341066 (Pfizer)<sup>[4, 38, 44]</sup>. ARQ197 is a c-MET-selective and non-ATP-

**Table 2.** Selective Small Molecule Tyrosine Kinase Inhibitors Specific to c-MET and RON\*.

Compound	Manufacturer	Targeted RTKs	Status	Descriptions in clinical trials	Ref
ARQ197	ArQule/Daiichi Sankyo	c-MET	Phase II	Advanced solid (prostate) tumor; HCC; locally advanced, inoperable or metastatic primary solid tumors; advanced HCC, RCC, BC, NSCLC and melanoma	38
XL184	Exelixis	c-MET	Phase I/II/III	MTC, PCAC, PSC, HCC, GE(GE)JC; Melanoma, SCLC, OC, PFTC, BC, NSCLC, and GM	74
PF-02341066 (crizotinib)	Pfizer	c-MET/ALK	Phase I/II	Advanced NSCLC; anaplastic LCL; relapsed/refractory solid tumors, primary CNS Tumors	44
EMD 1214063/ EMD 1204831	EMD Serono	c-MET	Phase I/II	Advanced, refractory solid tumors	71
GSK1363089 (Foretinib)	GlaxoSmithKline	c-MET/KDR/RON	Phase I/II	PRCC, AMCC, DEAGC; GE(GE)JC	72
MGCD265	MethylGene Inc	c-MET/VEGFR/ RON/Tie-2	Phase I/II	Advanced metastatic/ unresectable malignancies	73
JNJ-38877605	Johnson & Johnson	c-MET	Phase I	Advanced/refractory solid tumors	13
PHA665752	Pfizer	c-MET/RON	Preclinical	N/A	13
Compound I	Amgen	c-MET/RON	Preclinical	N/A	51

\* All information about individual inhibitors in clinical trials are derived from the website: <http://clinicaltrials.gov>, a service of the US National Institutes of Health. AMCC, advanced/metastatic gastric carcinoma; BC, breast cancer; DEAGC, distal esophageal adenocarcinoma gastric cancer; GE(GE)JC, gastro esophageal (GE) junction cancer; GM, glioblastoma multiforme; HCC, hepatocellular carcinoma, LCL, large-cell lymphoma; MTC, medullary thyroid cancer; NSCLC, non-small cell lung cancer; PFTC, peritoneal or fallopian tube carcinoma; PRCC, papillary renal-cell carcinoma; and RCC, renal cell carcinoma.

competitive SMI. Preclinical data have demonstrated that ARQ197 inhibits c-MET activation in various human tumor cell lines and shows anti-tumor activity against several human tumor xenografts<sup>[38]</sup>. A phase I dose-escalation study in patients with metastatic cancers shows that ARQ197 is well tolerated and has resulted in tumor responses and prolonged stable disease across broad ranges of tumors and doses<sup>[4, 38, 44, 59]</sup>. ARQ197 is currently in phase II trials as a single agent for germ cell tumors (GCT), including testicular and non-central nervous system (non-CNS) tumors<sup>[59]</sup>. Also under way is a phase I/II clinical trial designed to evaluate the safety of ARQ197 administered in combination with irinotecan and cetuximab in patients with metastatic colorectal cancer who possess the wild-type KRAS gene<sup>[59]</sup>.

PF-02341066 (crizotinib) is an ATP-competitive SMI highly specific to c-MET and anaplastic lymphoma kinase (ALK)<sup>[60]</sup>. The features of PF-02341066 include a) suppression of c-MET-dependent proliferation, migration and invasion of various cancer cells; b) inhibition of HGF-induced endothelial cell survival or serum-stimulated tubulogenesis; and c) high potency against a variety of c-MET mutants compared to wild-type c-MET<sup>[60]</sup>. These data suggest that PF-02341066 has a broad antitumor profile in terms of regulating proliferation, angio-

genesis, and abnormalities in c-MET mutants. PF-02341066 is currently in various stages of clinical trials as a single agent or in combination with chemoagents for patients with advanced tumors, including non-small-cell lung cancer and anaplastic large cell lymphoma<sup>[61]</sup>. A recent report found that PF-02341066 was highly effective in 82 patients with non-small-cell lung cancer harboring aberrant ALK expression<sup>[62]</sup>. Given that PF-02341066 is a dual c-MET/ALK SMI, it would be interesting to see whether it displays similar efficacy in cancers with a c-MET abnormality.

Development of RON-specific SMIs is still in progress. Compound I (Amgen), a dual inhibitor of c-MET/RON, was identified in 2008<sup>[51]</sup>. It selectively inhibits the kinase activities of c-MET and RON with IC<sub>50</sub> values of 4 and 9 nmol/L, respectively. Compound I inhibits c-MET/RON-mediated cell migration *in vitro* and causes partial inhibition of tumor growth mediated by an oncogenic RON160 variant<sup>[51]</sup>. Recently, a novel series of potent RON SMIs was identified through chemical designing and synthesis<sup>[52]</sup>. The most attractive products, Compounds 4 and 13, selectively inhibited RON kinase activity with IC<sub>50</sub> values of 0.05 and 0.06 μmol/L, respectively, in a cell-based assay and showed only residual activity against c-MET and no significant inhibitory activity

against VEGFR and other RTKs<sup>[52]</sup>. Clearly, the synthesis of RON-specific SMIs provides a platform for the development of more efficient RON-specific therapeutics for clinical evaluation. However, altered expression of c-MET and RON often coexist in various cancers, and the feasibility of developing an SMI specific to RON still needs to be investigated in terms of technological feasibility and clinical benefits.

### Acquired resistance to SMIs specific to c-MET/RON

Acquired resistance by advanced cancers to SMIs such as gefitinib and erlotinib, which are specific to EGFR, is a serious challenge in the treatment of cancer patients<sup>[63]</sup>. One of the mechanisms recently discovered in gefitinib insensitivity is c-MET gene amplification in resistant cells, which leads to activation of ERBB3 signaling<sup>[63,64]</sup>. Increased HGF production by tumor cells also induces gefitinib resistance of lung cancer cells harboring EGFR-activating mutations<sup>[65,66]</sup>. In this case, HGF-mediated hyposensitivity acts as a novel mechanism of resistance to both reversible and irreversible EGFR SMIs<sup>[65,66]</sup>. Although c-MET/RON-specific SMIs are still in clinical trials, it is predicted that acquired resistance to these SMIs will emerge. This notion is supported by a recent *in vitro* study showing that tumor cells acquire resistance to c-MET-specific SMIs such as PF-02341066<sup>[67]</sup>. Under such conditions, a cellular switch by tumor cells to EGFR signaling dependence leads to the resistance in these tumor cells<sup>[67]</sup>. These results suggest that crosstalk between c-MET and EGFR family members is the mechanistic cause that results in the escape of cancer cells from SMI-mediated cytotoxic or inhibitory activity. It is believed that as more SMIs are used clinically, additional novel mechanisms relevant to acquired resistance will be discovered, which should stimulate more research in this field.

We recently uncovered another novel mechanism by which cancer cells acquire resistance to a dual c-MET/RON-specific SMI<sup>[35]</sup>. It was previously observed that hypoxia-induced down-regulation of c-MET/RON expression contributes to acquisition of resistance to c-MET/RON dual inhibitor Compound I<sup>[51]</sup>. Diminished c-MET/RON expression under chronic hypoxia results in an insensitivity of tumor cells to Compound I-induced growth inhibition. These findings have clinical relevance because hypoxia affects therapeutic efficiency of SMIs in treatment of c-MET/RON-expressing tumors. Studies are currently under way to determine the mechanisms by which hypoxia regulates acquired resistance to specific c-MET/RON inhibitors.

### Future directions

The evidence that c-MET/RON plays a critical role in cancer development is overwhelming, which provides the rationale to target these two receptors for cancer therapy. Over the past several years, specific c-MET/RON inhibitors have advanced from the laboratory to the clinic with promising outcomes. However, these achievements are only beginning to unveil the clinical significance of c-MET/RON-targeted therapy. The goals we must address in the near future include: a) selection of the most suitable cancer types or patient population;

b) assessment of immediate clinical benefit and long-term effectiveness; c) evaluation of an agent both alone and in combination with chemotherapy or radiation; d) development of acquired drug resistance and the underlying mechanisms; e) improvement of the next generation of SMI or mAb; and f) potential mechanisms of the therapeutic activity. The progress in the study of c-MET/RON-specific therapeutics certainly provides the opportunity to meet these challenges.

### Acknowledgements

This work was supported in part by grant R01 CA91980 from the National Cancer Institute of the US National Institutes of Health and the Amarillo Area Foundation of Texas (to Dr Ming-Hai WANG). We apologize to the authors of many publications relevant to the topic covered in this review that we were unable to cite because of space constraints. We are grateful to Ms K BOHN (Texas Tech University, Amarillo, TX) for editing the manuscript.

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