Single-Agent and Combination Therapeutic Strategies to Inhibit Hepatocyte Growth Factor/MET Signaling in Cancer

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Abstract Receptor tyrosine kinases are often aberrantly activated in human malignancies and contribute to cancer development and progression. Specific receptor tyrosine kinase inhibitors have been shown to be clinically effective therapies in subsets of cancer patients with either hematologic or solid tumors. Activation of the hepatocyte growth factor (HGF)/MET signaling pathway has been found to play a critical role in oncogenesis, cancer metastasis, and drug resistance. These observations have led to the development of agents that can effectively inhibit HGF/MET signaling through direct inhibition of the receptor (anti-MET antibodies), through inactivation of its ligand HGF (AMG102, L2G7), by interfering with HGF binding to MET (NK4), or by inhibiting MET kinase activity (PHA-665752 and SU11274). Moreover, the combination of anti-MET therapeutic agents with either signal transduction inhibitors (ERBB family or mTOR inhibitors) or with cytotoxic chemotherapy has been evaluated in preclinical models. These studies provide insight into the rational development of combination therapeutic strategies that can be evaluated in clinical trials. This review will discuss different strategies of MET inhibition with a specific focus on combination therapeutic approaches.

Background

Receptor tyrosine kinases are often deregulated in human malignancies, contributing to cancer development and progression. Deregulation leads to aberrant receptor activity resulting in increased cell proliferation, inhibition of apoptosis, invasion, and enhanced tumor metastases. Because receptor tyrosine kinases are often selectively altered on malignant cells, they represent attractive targets for cancer therapy, with a number of agents already approved for clinical use.

The *MET* gene encodes a high-affinity receptor for the hepatocyte growth factor (HGF), also known as scatter factor, and consists of an extracellular α-chain disulfide-bonded to a membrane-spanning β-chain (Fig. 1; ref. 1). The transforming properties of *MET* were initially described in a human osteosarcoma cell line following chemically induced mutagenesis (2). In this *in vitro* model, MET was found to be constitutively activated as a consequence of a t(1;7) translocation, which fused sequences of the *MET* gene on chromosome 7q31 to the translocated promoter region on chromosome 1q25 (3). Whereas HGF is mostly secreted by mesenchymal cells, MET is widely expressed on the surface of epithelial cancer cells (4). HGF binds to MET, induces receptor homodimerization, and leads to phosphorylation of the cytoplasmic tyrosine kinase domain at two specific sites (Y1234 and Y1235) and activation of MET-mediated signaling (5). These events are essential during embryogenesis and also play a critical role in normal adult tissues, as addressed by a number of studies on hepatocytes, renal tubule cells, and myoblasts (6).

The phosphorylation of two tyrosine residues within the COOH terminus (Y1349 and Y1356) has been shown to be necessary and sufficient to mediate all the biological effects induced by activation of the MET pathway (7). These two residues can directly recruit a number of adapter proteins, including Gab1, Grb2, Shc, and the p85 subunit of the phosphatidylinositol 3-kinase (6). The involvement of such a diverse number of effectors allows the activation of different downstream pathways, including the phosphatidylinositol 3-kinase-Akt signaling and Ras-mitogen-activated protein kinase pathways (6).

Role of MET in Cancer

Deregulation of the MET pathway has been observed in many human malignancies, and the effects of sustained MET activation have been extensively characterized in preclinical models (8). Wild-type *MET* is transforming in NIH 3T3 cells, and in several cancer types the receptor can be aberrantly activated as a result of gene mutations or overexpression with or without gene amplification (9). *In vivo* studies have shown that activation of the HGF/MET signaling promotes cell invasiveness and triggers metastases through direct involvement of angiogenic pathways (10). More specifically, HGF can stimulate endothelial cell proliferation and migration through

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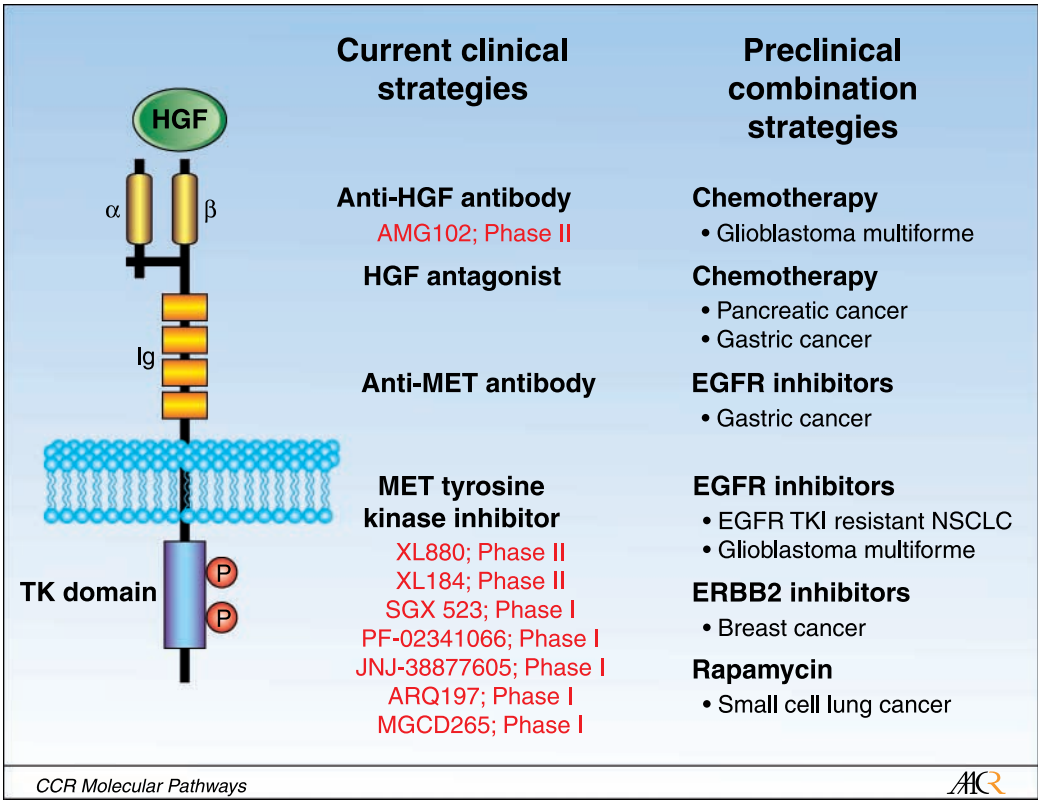

Fig. 1. The MET receptor and therapeutic strategies to inhibit HGF/MET signaling. Following HGF binding to the extracellular domain, composed of α and β subunits linked by a disulfide bond, the MET receptor undergoes phosphorylation on intracellular tyrosine residues, which leads to activation of downstream signaling. Different therapeutic strategies aimed at inhibiting HGF/MET signaling, including anti-HGF antibodies, HGF antagonists, anti-MET antibodies, and MET tyrosine kinase inhibitors, have been developed. In red are listed agents from these four categories and the status of their clinical development. HGF/MET inhibitors have also been examined in combination in a variety of preclinical models. The specific combination strategies and the setting in which they have been examined are shown on the right. Ig, immunoglobulin-like domains.

induction of vascular endothelial growth factor expression and down-regulation of thrombospondin-1, resulting in new blood vessel formation (10).

A variety of mechanisms that lead to aberrant MET signaling have been characterized, and these include overexpression of HGF and/or MET, *MET* gene amplification, mutations, or structural rearrangements. Importantly, missense germ-line mutations in the tyrosine kinase domain have been described in patients with hereditary papillary renal carcinoma (11), whereas sporadic mutations involving the tyrosine kinase, juxtamembrane, or semaphorin domains have been detected in several human cancers (12–14). However, only some of these mutant alleles have been shown to have a role in malignant transformation as a result of constitutive receptor activation, thus offering the potential for therapeutic inhibition (12, 15). *MET* amplification has been detected in patients with gastric, esophageal, and colorectal cancers and glioblastomas and in non–small-cell lung cancer patients that have developed acquired resistance to gefitinib or erlotinib (16–21).

Altered HGF secretion has been reported in both solid and hematologic malignancies. Particularly, both tumor and mesenchymal cells can be responsible for increased HGF production, leading to paracrine and/or autocrine mechanisms for receptor activation (22). This mechanism of enhanced MET signaling has been shown to be tumorigenic and metastatic in athymic nude mice (23). The prognostic role of HGF and/or MET has also been examined in several studies. Whereas the majority of these studies show a poor outcome for individuals in whom tumor HGF and/or MET overexpression was detected (24–26), a few studies failed to show a prognostic role for MET overexpression (27, 28).

Clinical-Translational Advances

Strategies for inhibiting HGF/MET signaling

Given the important role of aberrant HGF/MET signaling in cancer, several different therapeutic strategies, aimed at inhibiting HGF/MET signaling, have been developed and are currently being evaluated in clinical trials (Fig. 1). These include agents that directly inhibit HGF and or its binding to MET, antibodies targeted at MET, and small-molecule MET tyrosine kinase inhibitors. The mechanism of how the HGF/MET signaling pathway is aberrantly activated in a particular cancer is likely to influence the potential efficacy of each of these different therapeutic approaches. Recent preclinical studies highlight the use of these diverse therapeutic approaches alone or in combination with other agents. These preclinical studies are likely to lead to rationally designed treatments that will need to be evaluated in future clinical trials.

Single agent studies

Inhibitors of HGF and/or HGF binding. Given the association between HGF overexpression and tumorigenesis in xenograft models (29), attempts to therapeutically neutralize the autocrine/paracrine interaction of HGF with MET have been pursued. Promising results were initially observed in the preclinical setting with NK4, an internal fragment of HGF composed of the NH_2-terminal domain and four kringle domains. The agent acts as a full competitive antagonist, binding to MET without inducing receptor activation, thus preventing HGF-mediated MET signaling (30). Additionally, NK4 inhibits angiogenesis induced by vascular endothelial growth factor and basic fibroblast growth factor (31). The compound has been shown to be effective at inhibiting

angiogenesis, tumor growth, and tumor metastases in colorectal and pancreatic cancers *in vivo* through both locoregional and systemic administration (32). Similar results were observed with the use of an noncleavable form of pro-HGF in lung, colorectal, and breast cancers with HGF-induced MET activation (33). Like NK4, this molecule binds to MET without inducing receptor activation. Most of the studies, however, have shown that the agent is particularly active when it is delivered through gene therapy approaches, which may limit its potential clinical development.

An alternative approach has been to develop antibodies that directly inhibit HGF (34–36). Burgess et al. (34) reported on a panel of five anti-HGF antibodies that inhibit tumor growth in an autocrine HGF/MET–driven xenograft model of glioblastoma. These agents have been shown to prevent the interaction between HGF and MET, thus inhibiting MET activation and downstream signaling. The furthest along in clinical development is AMG102, a fully humanized anti-HGF antibody, which is currently undergoing phase II trial testing as single agent in patients with renal cell carcinoma and glioblastoma multiforme (Fig. 1). The rationale for testing systemically delivered monoclonal antibodies against brain tumors has recently been supported by a preclinical study showing that L2G7, an anti-HGF antibody, can effectively inhibit the growth of central nervous system cancers although it was administered into the systemic circulation (35).

HGF-mediated activation of MET can also be prevented with antibodies directed against the extracellular domain of MET, thus interfering with HGF binding. This approach was initially found to be particularly challenging because most of the antibodies that were first characterized also showed agonistic activity, thus also activating MET signaling (37). The agonistic activity was mainly associated with the use of bivalent compounds and thus led to the development of monovalent antibodies. A one-armed variant of the MET antibody 5D5 (38, 39), CE-355621 (40), and DN30 (41) have yielded promising activity in preclinical models of glioblastomas, pancreatic, gastric, and breast cancers where tumor growth was sustained by HGF/MET autocrine or paracrine signaling.

These therapeutic strategies may be clinically effective in cancers in which MET is activated by HGF through either an autocrine- or paracrine-mediated mechanism. However, in many cancers MET activation occurs through HGF-independent mechanisms (such as by amplification and/or mutation), and thus strategies aimed solely at inhibiting HGF and/or its binding might be clinically ineffective. For example, NK4 does not inhibit MET phosphorylation in models where receptor activation was due to overexpression or by activating mutations in *MET* (42).

MET tyrosine kinase inhibitors. A significant focus has been on the development of small tyrosine kinase inhibitors that are competitors for the ATP binding site in the tyrosine kinase domain of MET. Tyrosine kinase inhibitors have been proved to be particularly successful in the treatment of several human malignancies, and several agents including imatinib, erlotinib, lapatinib, sunitinib, and sorafenib are approved therapies by the Food and Drug Administration. For MET, this strategy may also be effective especially because MET tyrosine kinase inhibitors should be effective in tumors where MET signaling is activated by both ligand-dependent and ligand-independent mechanisms. The MET kinase inhibitors PHA-665752 and SU11274 have been most extensively evaluated in preclinical models (9).

PHA665752 has been found to be effective in experimental models of HGF-driven MET activation (43, 44) and also in cells with constitutive MET phosphorylation, particularly in those with gene amplification (16, 44). Smolen and colleagues (16) screened a panel of cancer cell lines for sensitivity to PHA-665752 and identified that 5 of 17 gastric cancer cell lines, which contained *MET* amplification, were particularly sensitive (IC_{50} ≤100 nmol/L). Importantly, whereas treatment with an anti-HGF antibody in *MET*-amplified gastric cancer cell lines did not result in down-regulation of MET phosphorylation, these cells showed dramatic *in vitro* sensitivity and associated inhibition of MET phosphorylation following PHA-665752 treatment. These findings suggest that *MET* amplification can result in ligand-independent activation of the receptor, which may be therapeutically inhibited by a MET tyrosine kinase inhibitor. Based on these data, clinical development of MET tyrosine kinase inhibitors in gastric cancer patients with *MET* amplification has started, offering the potential for effective targeted treatment for this subset of gastric cancers.

A relevant issue for novel tyrosine kinase inhibitors is to determine whether mutated variants of the target are associated with a differential sensitivity compared with a wild-type receptor, as has been the case for gefitinib and erlotinib in epidermal growth factor receptor (*EGFR*) mutant lung cancer (45). Recent data indicate that PF-02341066, a novel MET and anaplastic lymphoma kinase inhibitor, and SU11274 have differential activity against different mutant forms of *MET*, which have been detected in hereditary or sporadic papillary renal cancer, lung, head and neck, and gastric cancers and childhood hepatocellular carcinoma (46, 47). PF-02341066 was more effective at inhibiting MET and AKT phosphorylation in models harboring the mutant *MET* receptor [located at the ATP binding pocket (V1092I and H1094R) and P-loop (M1250T)], compared with those with wild-type *MET* receptor (46). In a study aimed at assessing MET inhibition by SU11274 in four mutated variants of the receptor, which have been reported in hereditary and sporadic papillary renal cancer (H1112Y, ATP-binding site; L1213V, hinge region; Y1248H, activation loop; and M1268T, *p*+1 loop), two of the mutant forms of *MET* (H1112Y and M1268T) were associated with sensitivity to the drug, whereas the two other variants (L1213V and Y1248H) were resistant (47). Furthermore, AM7, a novel MET tyrosine kinase inhibitor, was effective against the latter two variants and has a different MET binding modality compared with SU11274, suggesting that specific mutant forms of MET may be effectively targeted by different types of MET kinase inhibitors (48). PF-02341066 and PHA-665752 have also shown activity against mice xenografts derived from the small cell lung cancer cell line H69, which contains a point mutation in the juxtamembrane domain (46, 49).

Combination therapeutic studies with agents targeting HGF/MET signaling

Cancer therapies using agents aimed at a single target have thus far been successful when the target controls the majority, if not all, of the critical signaling pathways for cell survival. Compelling clinical examples of such "oncogene-addicted" cancers include *EGFR*-mutant lung cancer treated with EGFR tyrosine kinase inhibitors (45), chronic myeloid leukemia, and

gastrointestinal stromal tumors treated with imatinib (50, 51). However, the vast majority of human malignancies are more complex, and thus inhibiting a single target alone is likely to be therapeutically ineffective. To this extent, a number of studies have addressed the possible role of inhibiting HGF/MET signaling in combination with other signal transduction inhibitors or with conventional cytotoxic agents.

MET inhibitors in combination with other signal transduction inhibitors. Recent studies have shown that the growth of subsets of cancers can be effectively inhibited using a combination of anti HGF/MET agent with other signal transduction inhibitors. Several studies have focused on the combination of MET inhibitors and agents targeting ERBB family members. The rationale for exploring these combination strategies derives from evidence of a cross talk between MET and other EGFR family members described in several preclinical studies (52, 53). For instance, Jo et al. (52) showed that in human hepatoma cell lines and A431, MET was phosphorylated as a consequence of transforming growth factor-α–driven EGFR activation. MET phosphorylation was inhibited as a consequence of inhibiting transforming growth factor-α or EGFR, suggesting that MET can be activated even in the absence of HGF. Additional data show that HGF-driven MET activation can lead to increased EGFR phosphorylation as a result of enhanced transforming growth factor-α and heparin-binding EGF-like growth factor expression, suggesting that the cross talk between the two receptors can occur in both directions (54). Furthermore, EGFR, ERBB2, and ERBB3 are phosphorylated in the *MET*-amplified gastric cancer cell line MKN45, and phosphorylation of these ERBB family members can be inhibited with the MET inhibitor SU11274 (55).

These models provide examples of cross talk between the EGFR and MET signaling pathways. More recently, examples of cancers that are codependent on both MET and EGFR signaling have been identified. In these models, inhibition of both EGFR *and* MET is necessary to inhibit cell growth and/or to down-regulate phosphatidylinositol 3-kinase-Akt signaling. In one such example, MET amplification was identified in an *EGFR*-mutant non–small-cell lung cancer cell line, which was selected *in vitro* for resistance to the EGFR inhibitor gefitinib (20). In this model, both EGFR and MET independently activate ERBB3/PI3K/Akt signaling, and inhibition of *both* EGFR and MET is necessary to inhibit cell growth. *MET* amplification has also been detected in NSCLC patients that have clinically developed resistance to the EGFR inhibitors gefitinib or erlotinib (20, 21). Clinical trials are currently under way combining EGFR and MET tyrosine kinase inhibitors and will determine whether this is also a clinically effective therapeutic approach.

Activation of MET can also occur in the presence of the EGFR vIII mutant in glioblastoma cell lines (53, 56). Huang et al. (56) showed that MET activation increases as a function of increased EGFR vIII expression. At least two different studies have reported that combined treatment of EGFR vIII–expressing U87 H and U87 MG glioblastoma cells with EGFR and MET inhibitors given concurrently led to a significant increase in cell death when compared with either agent alone, addressing the collaborative role of MET in EGFR vIII tumors. These findings suggest that subsets of patients with glioblastoma might also clinically benefit from this combination therapeutic approach (53, 56).

The combination of the EGFR inhibitor gefitinib and the HGF antagonist NK4 has also been tested in a preclinical model of gastric cancer (57). *In vitro*, NUGC-4 gastric cancer cells were sensitive to gefitinib, but *in vivo*, NUGC-4 cells were resistant to gefitinib treatment when co-injected with gastric fibroblasts. This effect was presumably due to stromal production of HGF. The combination of gefitinib and NK4 was significantly more effective in this model than either agent alone (57).

The aberrant overexpression of MET may also play a role in resistance to the anti-HER2 antibody trastuzumab in breast cancer (24). HER2-expressing breast cancer cell lines and primary breast cancers have been found to commonly co-overexpress MET. In addition, the proliferation of *HER2*-amplified breast cancer cell lines can be enhanced *in vitro* by HGF (24). Shattuck and colleagues (24) showed that concomitant MET inhibition with SU11274 and trastuzumab in HER2-overexpressing cell lines could significantly increase the sensitivity to trastuzumab. Additionally, prolonged treatment of HER2-overexpressing cell lines with trastuzumab resulted in up-regulation of MET expression, suggesting a role for MET signaling in trastuzumab resistance (24). These studies further highlight the need to evaluate combination therapeutic strategies against MET and ERBB family members in preclinical models and in clinical trials.

Because MET activation leads to increased downstream signaling through a variety of different pathways, enhanced therapeutic efficacy could potentially be achieved through a combined approach by inhibiting MET and its known downstream signaling intermediates. This approach may also be effective in cancers where multiple receptors are concurrently activated (such as by EGFR or HER2 as discussed above) because these receptors often activate the same downstream signaling proteins. This hypothesis has been tested in a preclinical study exploring a combination of PHA665752 and rapamycin, which is an inhibitor of mTOR (58). Treatment of Ba/F3 cells engineered to overexpress the *TRP-MET* fusion gene with rapamycin resulted in increased growth suppression in the presence of PHA665752 as compared with rapamycin alone, suggesting that this combination may be therapeutically efficacious. Similar findings have been observed in the PHA665752-sensitive H441 small-cell lung cancer cell line (58).

MET inhibition in combination with chemotherapy. Although the recent advances in the molecular knowledge of cancer shed new light on the development of selective targeted compounds, chemotherapy remains the mainstay of treatment for several malignancies. However, the use of conventional chemotherapeutic agents is frequently limited by *de novo* or acquired resistance, which often results from increased growth factor receptor signaling (59, 60). These observations have prompted the evaluation of growth factor receptor inhibitors in combination with chemotherapy. Successful clinically validated examples of this approach include cetuximab, an anti EGFR antibody, in colorectal cancer (61) and trastuzumab in patients with *ERBB2*-amplified breast cancer (62). Emerging preclinical data suggest that inhibitors of the HGF/MET signaling pathway may also be effective in combination with chemotherapy (63, 64).

In models of gliobastoma using the U87 MG cell line, which contains an autocrine HGF/MET loop, the anti-HGF antibody AMG102 synergistically inhibited growth in combination with either temozolomide or docetaxel both *in vitro* and *in vivo* when compared with either AMG102 or chemotherapy alone

(36). Similarly, treatment of U87 H cells, which show EGFR vIII–dependent phosphorylation of MET, with SU11274 reverted cisplatin resistance (56). Analogous findings were observed when NK4 was combined with either cisplatin or gemcitabine in gastric and pancreatic cancer models *in vivo*, with NK4 enhancing the antitumor activity of both chemotherapeutic agents (65, 66). Intriguingly, activation of HGF/MET signaling has also been found to lead to an increase in sensitivity to cisplatin and paclitaxel in ovarian cancer cell lines both *in vitro* and *in vivo* (67, 68). In a recent study, Bardella et al. (69) engineered ovarian cancer cell lines with MET expression to continuously produce HGF in an autocrine fashion. Although the growth of the resulting xenograft tumors was increased by HGF, the tumors were unexpectedly more sensitive to cisplatin and paclitaxel *in vivo* than those that did not produce HGF. In fact, chemotherapy doses that were ineffective against non–HGF-producing cells effectively inhibited the growth of HGF-producing ovarian cancer xenografts. These data highlight the need to conduct preclinical studies using a variety of different tumor types as there may be tumor-specific observations. In addition, these studies might explain the observation that in some cancers MET expression is associated with a more favorable prognosis (70, 71).

Conclusions

Inhibition of HGF/MET signaling represents a promising strategy for cancer treatment either alone or as part of a combination therapeutic approach. Clinical trials with agents targeting HGF/MET signaling are currently under way and will hopefully validate clinically the observations from the preclinical studies. A key issue in the current and planned clinical studies will be appropriate patient selection strategies. It will be important to understand the specific mechanism of MET activation in different tumor types because that will affect the choice of HGF/MET–targeted agents. Furthermore, the optimal methods for the assessment of HGF/MET overexpression or *MET* amplification have yet to be determined. Finally, understanding the other key activated signaling pathways that occur concurrently with HGF/MET activation will be critical and will aid in the rational development of combination therapeutic strategies.

Disclosure of Potential Conflicts of Interest

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