Published in final edited form as:

Lung Cancer. 2009 February ; 63(2): 169–179. doi:10.1016/j.lungcan.2008.06.011.

MET as a target for treatment of chest tumours

Nicole A. Cipriani¹, Oyewale O. Abidoye², Everett E. Vokes¹, and Ravi Salgia^{1,3}

¹Section of Hematology/Oncology, Department of Medicine, and University of Chicago Cancer Research Center, University of Chicago Medical Center, Chicago IL 60637

²Scripps Memorial Hospital, La Jolla, CA 92037

Abstract

The receptor tyrosine kinase MET has been studied of a large variety of human cancers, including lung and mesothelioma. The MET receptor and its ligand HGF (hepatocyte growth factor) play important roles in cell growth, survival and migration, and dysregulation of the HGF-MET pathway leads to oncogenic changes including tumor proliferation, angiogenesis and metastasis. In small cell lung cancer (SCLC), non small cell lung cancer (NSCLC), and malignant pleural mesothelioma (MPM), MET is dysregulated via overexpression, constitutive activation, gene amplification, ligand-dependent activation, mutation or epigenetic mechanisms. New drugs targeted against MET and HGF are currently being investigated in vitro and in vivo, with promising results. These drugs function at a variety of steps within the HGF-MET pathway, including MET expression at the RNA or protein level, the ligand-receptor interaction, and tyrosine kinase function. This paper will review the structure, function, mechanisms of tumorigenesis, and potential for therapeutic inhibition of the MET receptor in lung cancer and mesothelioma.

Introduction

Thoracic tumors, including those of the lung and bronchus, are estimated to account for 213,380 new cases and 160,390 deaths in the year 2007 in the US [1]. Furthermore, the incidence of malignant mesothelioma in the US is between 2,000 and 3,000 per year, with an estimated 250,000 deaths in Europe in the next three decades [2,3]. The receptor tyrosine kinase MET is mutated or overexpressed in a large variety of human cancers, including lung and mesothelioma. MET is expressed mainly on cells of epithelial origin, is activated by its ligand hepatocyte growth factor (HGF), and is responsible for triggering a number of intracellular signaling cascades [4-7]. MET and HGF are required for embryonic development, as Met and HGF-null mice display defects in epithelial-mesenchymal transition during organogenesis [8-10]. Furthermore, MET and HGF play important roles in cell proliferation and survival, angiogenesis, cell migration and invasion, morphogenic differentiation, and tissue organization [11,12].

The function of MET in human tumors can be enhanced by mutation or amplification of this receptor, leading to oncogenic changes including cell proliferation, reduced apoptosis, angiogenesis, altered cytoskeletal function and metastasis. This myriad of cellular changes induced by MET dysregulation deems it a powerful therapeutic target, and new drugs targeted against MET and HGF are currently being investigated in vitro and in vivo, with promising results. In this review, we will describe the basic biology of MET as well as its potential for therapeutic targeting. (See references [13-16] for other MET reviews)

³Address Correspondence: Ravi Salgia, M.D., PhD, Section of Hematology/Oncology, 5841 South Maryland Avenue, MC 2115, Chicago, IL 60637, Tel: (773) 702-4399, Fax: (773) 834-1798, Email: rsalgia@medicine.bsd.uchicago.edu

MET Structure and Function

The gene for MET is located on chromosome 7q21-q31, and it is 120 kb in length with 21 exons and 20 introns. The protein consists of a 50 kD extracellular alpha chain and a 140 kD transmembrane beta chain, which are linked by disulfide bonds. It bears similarity to the family of Ron and Sea kinases [17,18]. It contains the following domains: sema, PSI (in Plexins, Semaphorins, Integrins), four IPT repeats (in Immunoglobulins, Plexins, Transcription factors), TM (transmembrane), JM (juxtamembrane) and TK (tyrosine kinase). [see Figure 1a] The JM domain contains 2 protein phosphorylation sites: S985 and Y1003. Phosphorylation of S985 negatively regulates kinase activity [19] and phosphorylation of Y1003 recruits c-Cbl, which monoubiquitinates MET and interacts with endophilin, targeting MET for internalization and degradation [20-22]. The TK domain contains numerous tyrosine phosphorylation sites: Y1230, Y1234 and Y1235 are involved in autophosphorylation and initiation of tyrosine kinase activity [23]; Y1313 assists in activation of PI3K and recruitment of p85 [24]; Y1349 and Y1356 are multi-substrate docking sites for SH2 (Src homology-2), PTB (phosphotyrosine binding) and MDB (MET binding domain)-containing proteins [25,26]; Y1365 is involved in cell morphogenesis [27].

MET's ligand is HGF (hepatocyte growth factor) also known as scatter factor, which is secreted by fibroblasts and smooth muscle cells [28]. It binds MET's sema domain and induces MET dimerization, autophosphorylation, and activation of tyrosine kinase catalytic activity [29-31].

Tyrosine phosphorylation of JM, TK, and tail domains respectively regulate internalization, catalytic activity, and docking of substrates such as Gab-1, Grb2, Shc, c-Cbl, which subsequently activate signal transducers such as PI3Kinase, PLC-gamma, STAT, ERK1, ERK2, and FAK. [see Figure 2] MET's uniqueness as an RTK stems from its C-terminal multi-substrate docking site and its unique adaptor protein Gab-1, which mediates numerous MET-initiated signals [23,26,32-35]. Gab-1 activates both the Erk and PI3K pathways. The PI3K pathway mediates cell survival via the Akt/PKB pathway; the Erk pathway mediates mitogenesis; and both pathways mediate cell adhesion, motility, and invasion [36-40]. Cell migration and invasion are mediated by Ras, Crk, and c-src/FAK, and branching morphogenesis further requires the STAT3 and PLCgamma pathways [41-51]. Specifically, activation of Ras-Rac1/Cdc42-PAK and Gab1-Crk-C3G-Rap1 regulates cell adhesion and cytoskeletal proteins. Molecules involved in the regulation of MET-induced motility and migration include cadherins, integrins, focal adhesion kinase and paxillin [52]. Furthermore, the temporal and spatial dependence of MET-signaling and its interactions with non-kinase binding partners (such as focal adhesion complexes) deem it unique from other RTKs [53-55].

MET in Tumorigenesis

Because of their diverse biological functions, there are a variety of mechanisms by which MET and HGF influence tumorigenesis. Both MET and HGF have been shown to be overexpressed in neoplastic tissue relative to normal surrounding tissue, and the extent of overexpression correlates with disease severity and outcome. Mutation or amplification of the MET gene has been shown in various cancer types, and introduction of Met or HGF transgenes into murine germ cells induces neoplastic growth. Contrarily, inhibition of MET or HGF reverses cell proliferation, motility, invasion, and metastasis in vivo. Tumorigenesis is affected by mutation or alteration of the MET pathway in numerous ways.

First, the regulation of MET may be altered via overexpression, constitutive kinase activation, gene amplification, paracrine/autocrine activation via HGF, MET mutation or epigenetic mechanisms (tumor secreted growth factors, hypoxia, other oncogenes) [56-58]. [see Figure

3] In protein studies of human lung cancer tissues, Ma et al demonstrated MET expression in 100% of NSCLC cases studied, of which 61% stained strongly [59]. When subcategorized, 67% of adenocarcinomas, 60% of carcinoids, 57% of large cell carcinomas, 57% of squamous cell carcinomas and 25% of SCLCs strongly expressed MET. When assessing for functional activity with p-MET staining, 44% of adenocarcinomas, 86% of large cell, 71% of squamous cell, 40% of carcinoids and 100% of SCLCs demonstrated MET phosphorylation at the Y1003 c-Cbl binding site; 33% of adenocarcinomas, 57% of large cell and 50% of SCLCs demonstrated MET autophosphorylation at the Y1230/1234/1235 site [59]. In other studies of lung adenocarcinoma, 41-72% of patients showed MET expression and 25-67% showed MET overexpression in comparison to adjacent normal tissue [60-63]. MET mutation was detected in 8% of lung adenocarcinoma patients and 13% of small cell lung carcinoma patients [64]. In studies of human malignant pleural mesothelioma tissues, 82% demonstrated MET over-expression [65].

Second, the regulation of HGF may be altered via HGF upregulation and increased circulating HGF originating from tumor and stromal cells [12]. Third, the MET pathway may be activated in a ligand-independent manner, including activating Met mutations, overexpression and constitutive ligand-independent dimerization, truncation, translocation, rearrangement, hypoxic activation, transactivation and loss of inhibitory regulators [66-73]. Fourth, MET gene mutation or amplification may promote tumorigenesis, such as the germline missense mutation in hereditary papillary renal cell carcinoma, TK domain mutations, JM domain mutations, and sema domain mutations [64,74-79]. These may be present in both primary tumors and nodal, lung, and liver metastases [80-82]. Fifth, MET activation and constitutive phosphorylation also affects neoplastic growth, as MET is highly expressed in renal cell carcinomas, lung adenocarcinomas and colorectal carcinomas, and it is highly phosphorylated in lung adenocarcinoma, other adenocarcinomas, and head and neck cancer [13]. Finally, MET may act as a prognostic marker: in NSCLC, MET and HGF levels correlate with prognostic parameters and poor survival [60,83]. Patients with metastatic disease demonstrate higher MET expression at metastatic sites and higher plasma MET levels [84-91].

There have been over 20 mutations identified in MET, most frequently they are missense mutations located in the TK domain, although mutations in a number of other domains have been identified [77,92]. [see Figure 1b] Mutations in the Sema domain affect binding to HGF, those in the JM domain affect the actin cytoskeleton, cell motility and migration. They are also regulators of catalytic activity. Mutations in the TK domain facilitate oligomerization and activate c-Met in the absence of HGF [93].

MET in SCLC

Prior research has shown MET overexpression in SCLC and NSCLC [14,15,94]. The first activating mutations of MET in SCLC were published by Ma et al in 2003. They studied 10 SCLC cell lines and 32 paired tissues. Two unique JM domain missense mutations were identified: R988C was found in 2 cell lines with extensive stage SCLC, and T1010I was found in 1 cell line. These JM mutations affect MET signaling and the metastatic potential of cells [75]. They cause IL-3 independent proliferation, although lower than TPR-MET (which has constitutively activated MET) transfected BaF3 cells. The JM domain mutations also cause increased relative ratio of neurite-like projections per cell and increased neurite-like projection length as relative neurite-length index. Both R988C and T1010I cells showed less cell-spreading and less cell-adhesion than wild type MET cells. They also showed increased average speed of migration, focus formation, anchorage-independent growth, and constitutive tyrosine phosphorylation of cell proteins (including paxillin Y31). T1010I cells showed increased motility and migration and R988C cells had a higher percentage of migration. One unique Sema domain missense somatic mutation was also identified: E168D was found in 1 cell line

and may have a role in tumor progression via aberrant semaphorin signaling. Of note, there has been a previously reported homozygous deletion of 3p21.3 where sema 3B and 3F are located [95-97]. Ma et al also identified an alternatively spliced transcript that was missing exon 10 in the extracellular domain, present in 1 cell line. In 2 cell lines, they identified a 2 bp insertion in intron 13 in the pre-JM domain. This insertion can cause skipping of the entire JM domain [75].

Although variable expression of MET was present in the tumor lines, no correlation between expression and mutation was revealed [75].

In phosphoproteomics studies of SCLC cell lines, in addition to HGF-induced MET phosphorylation, HGF also induced phosphorylation of a number of downstream proteins. These include proteins involved in transcriptional control (STAT3 and CREB), the cell cycle G1-S checkpoint (RB and RB1), cell survival and apoptosis (AKT1 and JNK), cell proliferation and differentiation (MAKPP 1/2, ERK 1, ERK 2 and ERK 1/2), stress and the inflammatory response (MAPKK 3/6, p38 α -MAPK and JNK) and cytoskeletal functions (FAK, adducin- α and adducin- γ). On the other hand, HGF inhibited phosphorylation of PKC α , PKC α/β , PKC δ , PKR and CKD1 [98]. Activation of MET in SCLC subsequently activates a variety of downstream signaling pathways, and these wide-ranging effects help account for the important role that MET plays in tumorigenesis.

MET in NSCLC

MET mutations have also been documented in non-small cell lung cancer. Two years after the SCLC mutations, Ma et al reported their findings from 4 NSCLC cell lines and 127 adenocarcinoma tissues [59]. Three JM domain mutations were identified: R988C was found in 1 cell line, both R988C and T1010I were found in 1 tissue, and S1058P was found in 1 tissue. Four sema domain missense mutations were identified: E168D was found in 1 tissue, L229F was found in 1 tissue, S323G was found in 1 tissue, and N375S was found in 3 tissues. They also identified an alternative splice variant missing exon 14 in the JM domain, which was present in 1 tissue [59]. MET is not only mutated but is also amplified in NSCLC. Lutterbach et al studied 9 NSCLC cell lines, of which 22% demonstrated MET amplification, up to 2.5 fold greater than normal levels [99].

MET expression has been shown to be present in 100% of the NSCLC tissues and 89% of the cell lines. There was strong expression in 61% of NSCLC, 60% of carcinoid, and 25% of SCLC. Interestingly, preferential expression of activated p-MET was demonstrated in the invasive front of NSCLC tumor tissues. No MET staining was found in normal lung tissue. Increased MET mRNA expression was found in adenocarcinoma, carcinoid, and SCLC [59].

MET amplification has also been documented in lung cancer, especially after treatment with tyrosine kinase inhibitors. Engelman et al demonstrated the development of MET amplification in the HCC827 NSCLC cell line after exposure to increasing concentrations of the tyrosine kinase inhibitor gefitinib [100]. Cells lines that developed gefitinib resistance contained amplification of the MET-containing region 7q31.1 to 7q33.3. In an assessment of tumor tissue from 18 gefitinib-resistant NSCLC patients, 22% demonstrated MET amplification [100]. Bean et al also studied tissue from lung adenocarcinoma patients with gefitinib or erlotinib resistance, and found MET amplification in 21%. On the other hand, only 3% of patients who had not been treated with these drugs showed MET amplification [101]. Hence, amplification of the MET oncogene allows tumors to potentially overcome therapeutic inhibition of growth signals.

Amplification of focal adhesion signaling molecules such as paxillin has also been demonstrated in lung cancer. Increased expression and increased copy numbers of both paxillin

and MET were found in 38% of NSCLC cell lines tested; however, increased copy numbers of paxillin alone were found in 25% [102]. In analysis of tissue from 66 NSCLC patients, increased copy numbers of both paxillin and MET were found in 17% of large cell carcinomas, 8% of adenocarcinomas and 13% of squamous cell carcinomas. Furthermore, 21 paxillin mutations were found among 9.4% of lung cancers studied, more frequently in large cell carcinoma (18.4%) than adenocarcinoma (8.6%), squamous cell carcinoma (6%), or small cell carcinoma (0%) [102].

Activation of the HGF-MET signaling pathway in NSCLC induces similar cellular events as those seen in SCLC. HGF-induced stimulation of adenocarcinoma induced autophosphorylation, phosphorylation of the c-Cbl binding epitope in the JM domain, and downstream phosphorylation of PI3K, PDK-1, AKT, mTOR, and S6K. The viability of MET-expressing cells was decreased by small molecule inhibitor SU11274. It also inhibited HGF-induced phosphorylation of MET and downstream signaling [59].

MET in mesothelioma

Most recently, a similar role for MET in malignant pleural mesothelioma has been demonstrated. In a recent paper, Jagadeeswaran et al reported their finding from 6 malignant mesothelioma cell lines, 1 nonmalignant mesothelioma cell line, and 66 tissues [65]. Two JM domain mutations were identified: T1010I was present in 2 cell lines, and G1085X was present in 1 tissue. Of note, the 2 MPM cell lines with the T1010I mutation exhibited the most dramatic reduction of cell growth with the small molecule inhibitor SU11274. Three sema domain mutations were identified: N375S, M431V and N454I, each present in 1 tissue. They also found an alternative splicing isoform missing exon 10, present in 1 tissue [65].

Positive MET expression was found in 82% of malignant pleural mesothelioma microarray specimens. Robust expression was found in 33% of the MPM cell lines and minimal expression was found in the 1 nonmalignant cell line. Serum HGF and EGF levels were two times higher in MPM patients than controls [65].

In three MPM cell lines, HGF induced MET receptor internalization and increased growth. The growth of these three lines (plus another that was not sensitive to HGF) was inhibited by the small molecule SU11274. It also inhibited wound closure and caused decreased migrational velocity of H28 cells in vitro. It inhibited autophosphorylation of MET, phosphorylation of the c-Cbl site, and downstream phosphorylation of ERK1/2 and AKT. Similar to the effects of siRNA in NSCLC, siRNA in MPM also downregulated MET expression, with resulting inhibition of cell growth, viability and migration [65].

Therapeutics

As demonstrated, MET plays an important role in the tumorigenesis of numerous thoracic tumors, including small cell lung cancer, non-small cell lung cancer, and malignant pleural mesothelioma. Furthermore, various inhibitors of the HGF/c-Met pathway have been shown to abrogate tumor growth. Because of its prominent role in cell growth and motility, c-Met represents a powerful therapeutic target. There are a variety of steps in the c-Met pathway that may serve as potential targets, and there already exist a number of drugs currently in development in vitro and in vivo that antagonize this pathway. [see Figure 4]

- 1a. MET expression at the RNA level:

Small interference RNA (**siRNA**) acts to suppress expression of the MET protein by binding to ribosomes in place of MET RNA and effectively silencing MET RNA. Cassinelli et al investigated the effects of siRNA and the tyrosine kinase inhibitor RPI-1 in human lung cancer cell lines H460 and N592. Both the siRNA and RPI-1-

treated cells demonstrated down-regulation of MET activation and expression, inhibition of HGF-dependent downstream signaling (including AKT and paxillin), decreased expression of VEGF and B-FGF, and decreased growth, motility and invasiveness [103]. In non-small cell lung cancer, siRNA effectively silenced the MET gene by inhibiting MET protein expression. It also inhibited HGF-induced autophosphorylation, phosphorylation of the c-Cbl binding epitope, AKT phosphorylation, and cell growth and viability [59]. In small cell lung cancer, siRNA downregulated the MET receptor as well as downstream p-MET, p-AKT, p-ERK1/2 and p-S6 kinase [98]. siRNA has also been shown effective in mouse breast cancer as well as human prostate cancer, sarcoma, glioblastoma and gastric cancer. All cancers displayed decreased MET expression, decreased tumor cell growth and viability, decreased signaling (including AKT and p44/42 mitogen-activated protein kinase), decreased HGF-dependent scattering and invasion, and increased apoptosis [104].

Short hairpin RNA (**shRNA**) acts in a similar fashion to siRNA, but its hairpin structure must be cleaved after induction into cells. Lutterbach et al investigated the effects of shRNA on human lung cancer cell lines EBC-1 and H1993. The shRNA-treated cells demonstrated growth inhibition, G1-S cell cycle arrest, apoptosis and decreased tyrosine phosphorylation of δ -catenin, β -catenin, pAKT, pERK and Ras. Furthermore, these MET-amplified cells, which were initially rounded and non-adherent, became flattened and adherent after treatment with shRNA. Cell lines without MET amplification were not responsive to shRNA-mediated MET knockdown [99]. MET's role in cell growth, proliferation and motility could be effectively silenced by RNA interference.

Micro RNA (**miRNA**) is another form of single-stranded hairpin-forming RNA that is thought to regulate gene expression. Intracellularly, it is cleaved into partially double-stranded RNA and processed into mature miRNA strands of approximately 22 nucleotides. These miRNAs regulate gene expression in two ways: 1) by cleaving mRNA and 2) by pairing with target mRNAs to silence their translation [105,106]. The role of miRNA in human cancer was first discovered in CLL, in which two miRNA genes were deleted [107]. Reduced expression of let-7 miRNA in lung cancer was initially reported by Takamizawa in 2004 [108]. Other miRNAs implicated in lung cancer include mir-9 (which targets E-cadherin), mir-32 (which targets Integrin α V), mir-124a-3 (which targets Integrin β 1), mir-30a-5p (which targets Integrin β 3 along with let-7), mir-26a-1-prec (which targets Integrin α 5), mir-145 and mir-218-2 (which target Paxillin), mir181-c-prec (which targets VEGFR1), mir-155 (which targets HIF-1 α), mir-124a1 (which targets Angiopoietin 1), and mir-30a-5p (which targets PAI-1) [109]. Specific anti-MET miRNAs include mir-1/206, mir-199a, mir-34 and mir-23; anti-HGF miRNAs include mir-26 and mir-190 [109]. Micro RNAs can be overexpressed (as oncogenes) or underexpressed (as tumor suppressors) in human cancers, and treatment that either antagonizes or restores miRNA function can serve as anti-cancer therapy. Oligonucleotides called antagomirs have been designed to antagonize oncogenic miRNA; conversely, viral vectors or small molecule compounds could serve to enhance tumor suppressor miRNA [106]. For example, in lung cancer cell lines with reduced let-7 expression, overexpression of let-7 via expression constructs resulted in a 79% reduction in the number of colonies [108]. A relatively new concept in tumor biology, miRNA has already proven to be an important therapeutic target.

Ribozymes also silence MET protein expression by binding to and cleaving RNA based on sequence. They have proven effective in human glioblastoma, prostate cancer, and colorectal carcinoma. Glioblastoma cells demonstrated reduced

expression of HGF and MET, reduced HGF-dependent signal transduction, and decreased tumor cell clonogenicity, tumorigenicity and tumor growth in cells possessing an autocrine HGF-MET loop [110]. The prostate cancer cell line PC3-LN4, grown in mouse models, demonstrated decreased steady-state MET levels, decreased Src kinase activity, decreased VEGF expression, and decreased tumor growth, migration and invasion [111]. The colorectal carcinoma cell line KM20 demonstrated decreased cMET autophosphorylation and kinase activity, reduced in vitro growth rates and soft-agar colony-forming abilities, reduced in vivo growth, and decreased metastasis [112].

Recently, Stabile et al demonstrated that **anti-sense DNA** in the form of a MET antisense/U6 expression plasmid was able to block translation of mRNA into protein. This technique was effective in human non-small cell lung tumors in vitro and in mice xenografts. These cells showed decreased HGF-dependent phosphorylation of MET and MAP kinase, downregulation of MET protein, decreased tumor growth, and increased rates of apoptosis [113].

- 1b. MET expression at the protein level:

Geldanamycin or members of the anisomycin antibiotic family function by inhibiting the heat shock protein HSP90, which is activated in cancer cells and stabilizes cellular proteins including tyrosine kinases. In the SCLC cell line H69, geldanamycin inhibited maturation and functional expression of MET leading to reduced growth and viability of four of four SCLC cell lines and increased apoptosis [15]. In NIH3T3 cells expressing HGF or MET mutations, geldanamycin down-regulated MET protein expression, inhibited HGF-dependent motility and invasion, and blocked transformation of this cell line [114].

- 2. Ligand-receptor interaction: Once the mature MET protein is localized to the plasma membrane, its interactions with HGF can be mediated in a number of ways, including competitive antagonism of the MET receptor, antibody-mediated neutralizing of the HGF ligand, or prevention of the generation of a mature HGF ligand.

The **HGF/NK** (stands for N-terminal hairpin domain and kringle domain) is comprised of four variants of the alpha chain of HGF, containing one (NK1) to four (NK4) kringle domains. These proteins are weak MET agonists, and they act as competitive inhibitors of HGF for MET. They are effective in cells that possess paracrine or autocrine activation, but are not effective against intracellular MET-activating mutations [115-118]. Treatment of cancer cell lines with NK4 inhibited HGF-stimulated motility, migration and invasion in vitro. It inhibited tumor growth, invasion and dissemination in mouse models including Lewis lung carcinoma, GB-d1 gall bladder carcinoma, SUIT-2 orthotopic pancreas, MDA-MB-231 breast, and PC-3 prostate carcinoma models [117,119-123]. Furthermore, in Lewis lung carcinoma and Jyg-MC(A) mammary carcinoma mouse xenografts, it inhibited growth, metastasis and angiogenesis: Treated mice demonstrated increased numbers of apoptotic tumor cells, decreased tumor microvessel density, and decreased growth and migration of human microvascular endothelial cells induced by bFGF, VEGF, HGF [121]. Recently, cationized gelatin microspheres incorporating NK4 plasmid DNA were injected into the subcutaneous tissue of Lewis lung carcinoma mouse xenografts, which significantly prolonged the survival time period of the mice. This treatment also suppressed increases in the tumor volume and the number of lung metastatic nodules; it suppressed the number of blood vessels and increased the number of apoptotic cells [124]. NK4 was also shown effective in colorectal cancer, pancreatic cancer and glioblastoma cells [123,125,126].

Receptor-ligand interactions can also be modified non-competitively. **Soluble decoy receptors** bind HGF and, antagonize HGF binding to MET, and therefore inhibit MET dimerization and signaling. They have been shown to inhibit HGF-dependent tumor cell proliferation, survival, angiogenesis and metastasis and to induce tumor regression along with radiotherapy in mice [127].

Similarly, the **anti-HGF antibody** binds an epitope in the beta chain of HGF and prevents it from binding to MET. A combination of three mouse monoclonal antibodies (which bound three epitopes on HGF) blocked cell-scattering and morphogenesis in tumors dependent on an autocrine MET-HGF loop [128]. **AMG102** (Amgen, Inc) is a fully human IgG₂ monoclonal antibody that binds and neutralizes SF/HGF preventing its binding to c-MET and activation of this pathway [116]. Recent interim analysis from a phase I open label dose escalation clinical trial in solid tumors was reported by Gordon et al. At the time of report, 31 patients had been enrolled. The agent was administered intravenously every two weeks with a maximum tolerated dose of 20 mg/kg. Dose-limiting toxicities seen included grade 3 hypoxia and dyspnea seen in one patient with non-small cell lung cancer and chronic obstructive pulmonary disease, and grade 3 gastrointestinal bleeding observed in another patient with advanced pancreatic cancer. No responses (partial or complete) were observed, 13 patients had stable disease, 7 patients had progression of disease, and 11 were not available for evaluation [129].

AV-299 is an anti-HGF antibody that targets SF/HGF, and is currently in early development through a joint partnership of AVEO Pharmaceuticals and Schering-Plough Pharmaceuticals [130].

Another mechanism to prevent HGF from binding to the MET receptor is via **uncleavable pro-HGF**. HGF is usually cleaved from inactive pro-HGF to active HGF. Mazzone et al generated a non-cleavable form by substituting a single amino acid in the proteolytic site. They locally delivered the uncleavable pro-HGF using lentivirus vectors in mice and found that substitution of uncleavable pro-HGF for normal HGF inhibited HGF-mediated MET activation, tumor growth, angiogenesis and metastasis. Systemic expression inhibited the growth of transplanted tumors and the formation of metastases [131].

- 3. RTK inhibition: The inhibition of receptor tyrosine kinases by small molecules is already used in therapy of human tumors, and is epitomized by the following targeted therapies: imatinib for Bcr-Abl in CML and mutant c-Kit in GIST; trastuzumab in Her-2 overexpressing breast ca; bevacizumab in colorectal ca; gefitinib in certain NSCLCs [132]. These small molecule inhibitors can be tailored to receptor tyrosine kinases in order to specifically target tumor cells. Their effectiveness in treating human cancers has already been demonstrated, and development of new RTK inhibitor drugs seems promising.

One small molecule inhibitor currently undergoing in vitro testing is **SU11274** (Sugen, Inc.), and functions by blocking autophosphorylation of MET at sites required for downstream signaling including the P13K, AKT, mTOR pathways. It is effective in MET-transformed or MET-activated cell lines, but not in lines with activated ABL, JAK2, PDGFβR kinases [133]. In BaF3 cell lines, it induced G1 cell cycle arrest and apoptosis, decreased MET autophosphorylation and decreased phosphorylation of downstream targets including AKT, GSK-3β, and FKHR (a pro-apoptotic transcription factor) [133]. Specifically in non-small cell lung carcinoma cell lines, it inhibited MET autophosphorylation, phosphorylation of the c-Cbl binding epitope, and HGF-induced tyrosine phosphorylation of cell proteins including S6K, AKT, ERK1/2 [59]. In small cell lung cancer, it inhibited HGF-stimulated phosphorylation

of p-ERK2, p-ERK1/2, p-MEK1/2, p38 α , p-MAP kinase, p-AKT1, p-RB, p-adducin and p-CREB, and it inhibited HGF-mediated inhibition p-PKC α , p-PKC α / β and p-CDK1 [98]. In malignant pleural mesothelioma, it decreased cell growth, motility and migration and inhibited HGF-induced signal transduction [65].

A second promising small molecule in inhibitor, **PHA665752** (Pfizer, Inc.), has been shown effective *in vitro* in SCLC, NSCLC and MPM. It blocks phosphorylation of MET, AKT and p70-S6K [59]. Puri et al studied the effects of PHA665752 in mouse xenografts, and found that NCI-H69-derived SCLC tumor nodules treated with PHA665752 for three weeks became clinically undetectable in 50% of the mice, and tumor volume was reduced by 99%. NCI-HH41 NSCLC nodules were reduced by 75% and A549 NSCLC nodules by 59% [134]. Furthermore, this small molecule inhibited autophosphorylation of MET and phosphorylation at the c-Cbl binding site in NCI-H69 and A549 cells. An antiangiogenic effect was also demonstrated, as PHA665752 treatment resulted in greater than 85% reduction in CD31 staining, which corresponds to a reduction in vasculature. Similarly, VEGF (pro-angiogenic) was down-regulated and TSP-1 (thrombospondin-1, anti-angiogenic) was up-regulated [134]. PHA665752 was shown to cooperate with rapamycin by reducing growth, inducing apoptosis and cell cycle arrest, and inhibiting constitutive cell motility of BaF3 TPR-MET- and c-MET-expressing H441 NSCLC cells [135]. This small molecule inhibitor also proved effective in mesothelioma cell lines that exhibited a MET/HGF autocrine loop (H2461 and JMN-1B lines). It inhibited growth, decreased *in vitro* cell migration and invasion, induced cell cycle arrest at the G1-S boundary and induced a dose-dependent decrease in phosphorylation of MET, p70S6K, Akt, and ERK1/2 [136].

A third small molecule, **PF2341066** (Pfizer, Inc.), is an ATP-competitive inhibitor of MET kinase activity. In *in vitro* studies of lung cancer cell lines, NCI-H69 cells expressing the R988C mutation demonstrated decreased MET phosphorylation after treatment with PF2341066, and NCI-H441 cells demonstrated decreased HGF-stimulated migration and invasion [137]. In mouse xenografts of human NCI-H441 NSCLC cells, mean tumor volume decreased by 43% after 38 days of PF2341066 treatment. Similarly, in gastric carcinoma (GTL-16) xenografts, renal cell carcinoma (Caki-1) xenografts, glioblastoma (U87MG) xenografts and prostate cancer (PC-3) xenografts, tumor volume decreased by 60%, 53%, 97% and 84%, respectively. Further studies of PF2341066-treated gastric carcinoma GTL-16 cells demonstrated decreased mitosis, increased apoptosis, decreased angiogenesis and decreased phosphorylation of MET, Akt, Erk, PLC λ 1 and STAT5 [137].

A fourth small molecule, **ARQ197** (Arqule, Inc.) has demonstrated pre-clinical activity against several human xenografts, and is currently in clinical trials. It is an orally administered, selective small molecule inhibitor of MET. It underwent Phase I testing in 38 patients with advanced stage solid tumors who failed prior therapy. Administration was cyclic over 3 weeks: twice-daily dosing for two out of three weeks. Dosing was continued until the following endpoints: disease progression, unacceptable toxicity, or other. Doses were escalated from 10 to 360 mg/day. Thirty-three were evaluable for efficacy. Of these, two achieved a partial response and 19 achieved stable disease. Average time to progression was from 10 to 34 weeks. There was no dose limiting toxicity. Adverse events included fatigue (24%) and GI symptoms such as constipation (21%) and diarrhea (21%). Grade 3 toxicities seen included elevation in liver function enzymes seen in 3% of treated patients. The optimal dose was determined to be 120 mg PO BID. Phase II trials are now scheduled [138,139].

XL880 (Exelixis, Inc.) is another orally bioavailable small molecule targeted to multiple RTKs, including MET, VEGF, c-Kit, FLT3, PDGFR, Ron and Tie-2 [140]. Results of two phase I clinical trials were recently presented at the 2007 ASCO meeting in Chicago, Illinois [140,141]. The first trial of XL880 (XL880-001) involved an interrupted dosing schedule of five days on and nine days off of a 14-day cycle and the second (XL880-002) consisted of a fixed daily dose until disease progression [140,141]. In the XL880-001 study, the maximum tolerated dose was 3.6 mg/kg with a total of 40 patients dosed and evaluated for safety and tumor response [140]. In the 002 study, 15 patients were accrued, and the maximum tolerated dose was defined by the protocol to be 80 mg/day. Grade 3 toxicities seen in the 001 study included asymptomatic elevation in liver function enzymes, palmar-plantar erythrodysesthesia, and tumor hemorrhage. Partial response (by Recist criteria) was seen in five patients. Six patients had a minor response (less than 30%) while six patients had stable disease after 3 months [141]. The XL880-001 trial was recently updated at the 2007 AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics in San Francisco, California [142]. At this update, 33 patients were accrued with 24 and 22 patients being available for safety and tumor response, respectively. Grade 3 toxicities observed were hypertension, dehydration and confusion and a total of 10 out of 22 patients had stable disease after 3 months [142]. Interim data from an early phase II trial of XL880 in papillary renal cell carcinoma, gastric cancer, and head and neck cancer was also reported at the 2007 AACR-NCI-EORTC International Conference [143]. At the time of presentation, a total of 21 patients had been enrolled, five with activating MET mutations and 16 with wild type MET. Hypertension was the only reported grade 3 toxicity. 100% disease control rate was observed in 15 out of 19 patients and 12 out of 16 evaluable patients had stable disease after 6 months. In all three studies, no grade 4 or 5 toxicities have been reported [143].

The third orally bioavailable small molecule undergoing clinical trials is **XL184** (Exelixis, Inc.). It is also targeted to multiple RTKs including MET, VEGFR2/KDR, KIT, FLT3, and Tie-2, and has demonstrated preclinical inhibition of tumor growth and regression of tumor in human xenografts [144]. Results from a phase I dose-escalation study was reported at the 2007 ASCO meeting and later updated at the 2007 AACR-NCI-EORTC International Conference. At the time of update, 44 patients had been enrolled and maximum tolerated dose had not yet been defined. A total of 38 and 40 patients were evaluable for safety and efficacy reporting, respectively. Grade 3 toxicities seen included elevated liver function enzymes and palmar-plantar erythrodysesthesia. One serious adverse event included grade 4 pulmonary embolism. A total of 4 patients had a partial response (2 confirmed) and 15 patients demonstrated stable disease after 3 months of therapy. Dose escalation is ongoing [145].

Other agents undergoing early phase and preclinical testing include **SGX523**, (SGX, Inc.) an orally bioavailable small molecule inhibitor of MET in phase I dose escalation clinical trials and the multi-kinase inhibitor **MGCD265** (MethylGene, Inc.) that targets MET, VEGFR-1,2,3, Tie-2, and Ron RTKs. It has been investigated in cell culture and is scheduled for clinical studies [146,147]. Similarly, **HPK-56** (MP-470) (Supergen, Inc.) inhibits c-Kit, PDGF and MET kinase [148]. **K252a** is a staurosporine analog and an ATP-competitive MET kinase and serine/threonine kinase inhibitor that modulates wild type and mutant (M1268T) MET-dependent function and dissemination of tumor cells in vivo [149].

MET peptides are large molecules derived from the MET receptor carboxy-terminal tail region which inhibit downstream signaling by binding to MET and inhibiting HGF-mediated invasion, cell migration and branched morphogenesis [150]. The

monovalent **anti-MET antibody** “5D5” (as well as its single arm version) acts as an antagonist and inhibits MET phosphorylation, HGF-dependent proliferation and migration in vitro. The bivalent form acts as an agonist/antagonist [151-153].

Conclusion

The receptor tyrosine kinase MET is implicated in a wide variety of human cancers, including lung and mesothelioma. Overexpression or mutation of this receptor or its ligand promotes cell growth, survival, angiogenesis, and metastasis. Targeted therapy against other RTKs has already proven clinically beneficial, and current research into therapy against MET and its downstream pathways is similarly efficacious. By targeting the MET pathway in tumors of the thorax, we hope to decrease the morbidity and mortality associated with lung cancer and mesothelioma, and will continue to search for new pathways and mechanisms for anti-tumor therapy.

Acknowledgment

Supported in part by NIH/NCI, American Lung Association, V-Foundation (Guy Geleerd Memorial Foundation), Mesothelioma Applied Research Foundation (Jeffrey Hayes Memorial) to RS.

References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2007;57:43–66. [PubMed: 17237035]
2. Peto J, Decarli A, La Vecchia C, Levi F, Negri E. The European mesothelioma epidemic. *Br J Cancer* 1999;79:666–672. [PubMed: 10027347]
3. Weill H, Hughes JM, Churg AM. Changing trends in US mesothelioma incidence. *Occup Environ Med* 2004;61:438–441. [PubMed: 15090665]
4. Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF, Aaronson SA. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. *Science* 1991;251:802–804. [PubMed: 1846706]
5. Di Renzo MF, Narsimhan RP, Olivero M, Bretti S, Giordano S, Medico E, Gaglia P, Zara P, Comoglio PM. Expression of the Met/HGF receptor in normal and neoplastic human tissues. *Oncogene* 1991;6:1997–2003. [PubMed: 1719465]
6. Naldini L, Weidner KM, Vigna E, Gaudino G, Bardelli A, Ponzetto C, Narsimhan RP, Hartmann G, Zarnegar R, Michalopoulos GK, et al. Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. *Embo J* 1991;10:2867–2878. [PubMed: 1655405]
7. Sonnenberg E, Meyer D, Weidner KM, Birchmeier C. Scatter factor/hepatocyte growth factor and its receptor, the c-met tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development. *J Cell Biol* 1993;123:223–235. [PubMed: 8408200]
8. Bladt F, Riethmacher D, Isenmann S, Aguzzi A, Birchmeier C. Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. *Nature* 1995;376:768–771. [PubMed: 7651534]
9. Schmidt C, Bladt F, Goedecke S, Brinkmann V, Zschiesche W, Sharpe M, Gherardi E, Birchmeier C. Scatter factor/hepatocyte growth factor is essential for liver development. *Nature* 1995;373:699–702. [PubMed: 7854452]
10. Tsarfaty I, Rong S, Resau JH, Rulong S, da Silva PP, Vande Woude GF. The Met proto-oncogene mesenchymal to epithelial cell conversion. *Science* 1994;263:98–101. [PubMed: 7505952]
11. Comoglio PM, Boccaccio C. Scatter factors and invasive growth. *Semin Cancer Biol* 2001;11:153–165. [PubMed: 11322834]
12. Jiang W, Hiscox S, Matsumoto K, Nakamura T. Hepatocyte growth factor/scatter factor, its molecular, cellular and clinical implications in cancer. *Crit Rev Oncol Hematol* 1999;29:209–248. [PubMed: 10226727]

13. Christensen JG, Burrows J, Salgia R. c-Met as a target for human cancer and characterization of inhibitors for therapeutic intervention. *Cancer Lett* 2005;225:1–26. [PubMed: 15922853]
14. Ma PC, Maulik G, Christensen J, Salgia R. c-Met: structure, functions and potential for therapeutic inhibition. *Cancer Metastasis Rev* 2003;22:309–325. [PubMed: 12884908]
15. Maulik G, Shrikhande A, Kijima T, Ma PC, Morrison PT, Salgia R. Role of the hepatocyte growth factor receptor, c-Met, in oncogenesis and potential for therapeutic inhibition. *Cytokine Growth Factor Rev* 2002;13:41–59. [PubMed: 11750879]
16. Sattler M, Salgia R. c-Met and hepatocyte growth factor: potential as novel targets in cancer therapy. *Curr Oncol Rep* 2007;9:102–108. [PubMed: 17288874]
17. Comoglio PM, Boccaccio C. The HGF receptor family: unconventional signal transducers for invasive cell growth. *Genes Cells* 1996;1:347–354. [PubMed: 9135079]
18. Maestrini E, Tamagnone L, Longati P, Cremona O, Gulisano M, Bione S, Tamanini F, Neel BG, Toniolo D, Comoglio PM. A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor. *Proc Natl Acad Sci U S A* 1996;93:674–678. [PubMed: 8570614]
19. Gandino L, Longati P, Medico E, Prat M, Comoglio PM. Phosphorylation of serine 985 negatively regulates the hepatocyte growth factor receptor kinase. *J Biol Chem* 1994;269:1815–1820. [PubMed: 8294430]
20. Abella JV, Peschard P, Naujokas MA, Lin T, Saucier C, Urbe S, Park M. Met/Hepatocyte growth factor receptor ubiquitination suppresses transformation and is required for Hrs phosphorylation. *Mol Cell Biol* 2005;25:9632–9645. [PubMed: 16227611]
21. Petrelli A, Gilestro GF, Lanzardo S, Comoglio PM, Migone N, Giordano S. The endophilin-CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met. *Nature* 2002;416:187–190. [PubMed: 11894096]
22. Soubeyran P, Kowanetz K, Szymkiewicz I, Langdon WY, Dikic I. Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. *Nature* 2002;416:183–187. [PubMed: 11894095]
23. Rodrigues GA, Park M. Autophosphorylation modulates the kinase activity and oncogenic potential of the Met receptor tyrosine kinase. *Oncogene* 1994;9:2019–2027. [PubMed: 8208547]
24. Fournier TM, Kamikura D, Teng K, Park M. Branching tubulogenesis but not scatter of madin-darby canine kidney cells requires a functional Grb2 binding site in the Met receptor tyrosine kinase. *J Biol Chem* 1996;271:22211–22217. [PubMed: 8703035]
25. Furge KA, Zhang YW, Vande Woude GF. Met receptor tyrosine kinase: enhanced signaling through adapter proteins. *Oncogene* 2000;19:5582–5589. [PubMed: 11114738]
26. Ponzetto C, Bardelli A, Zhen Z, Maina F, dalla Zonca P, Giordano S, Graziani A, Panayotou G, Comoglio PM. A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family. *Cell* 1994;77:261–271. [PubMed: 7513258]
27. Weidner KM, Sachs M, Riethmacher D, Birchmeier W. Mutation of juxtamembrane tyrosine residue 1001 suppresses loss-of-function mutations of the met receptor in epithelial cells. *Proc Natl Acad Sci U S A* 1995;92:2597–2601. [PubMed: 7708691]
28. Stella MC, Comoglio PM. HGF: a multifunctional growth factor controlling cell scattering. *Int J Biochem Cell Biol* 1999;31:1357–1362. [PubMed: 10641789]
29. Hammond DE, Urbe S, Vande Woude GF, Clague MJ. Down-regulation of MET, the receptor for hepatocyte growth factor. *Oncogene* 2001;20:2761–2770. [PubMed: 11420688]
30. Peruzzi B, Bottaro DP. Targeting the c-Met signaling pathway in cancer. *Clin Cancer Res* 2006;12:3657–3660. [PubMed: 16778093]
31. Teis D, Huber LA. The odd couple: signal transduction and endocytosis. *Cell Mol Life Sci* 2003;60:2020–2033. [PubMed: 14618253]
32. Fournier TM, Lamorte L, Maroun CR, Lupher M, Band H, Langdon W, Park M. Cbl-transforming variants trigger a cascade of molecular alterations that lead to epithelial mesenchymal conversion. *Mol Biol Cell* 2000;11:3397–3410. [PubMed: 11029044]
33. Naldini L, Vigna E, Ferracini R, Longati P, Gandino L, Prat M, Comoglio PM. The tyrosine kinase encoded by the MET proto-oncogene is activated by autophosphorylation. *Mol Cell Biol* 1991;11:1793–1803. [PubMed: 2005882]

34. Sachs M, Brohmann H, Zechner D, Muller T, Hulsken J, Walther I, Schaeper U, Birchmeier C, Birchmeier W. Essential role of Gab1 for signaling by the c-Met receptor in vivo. *J Cell Biol* 2000;150:1375–1384. [PubMed: 10995442]
35. Weidner KM, Di Cesare S, Sachs M, Brinkmann V, Behrens J, Birchmeier W. Interaction between Gab1 and the c-Met receptor tyrosine kinase is responsible for epithelial morphogenesis. *Nature* 1996;384:173–176. [PubMed: 8906793]
36. Day RM, Cioce V, Breckenridge D, Castagnino P, Bottaro DP. Differential signaling by alternative HGF isoforms through c-Met: activation of both MAP kinase and PI 3-kinase pathways is insufficient for mitogenesis. *Oncogene* 1999;18:3399–3406. [PubMed: 10362361]
37. Fan S, Ma YX, Wang JA, Yuan RQ, Meng Q, Cao Y, Laterra JJ, Goldberg ID, Rosen EM. The cytokine hepatocyte growth factor/scatter factor inhibits apoptosis and enhances DNA repair by a common mechanism involving signaling through phosphatidylinositol 3' kinase. *Oncogene* 2000;19:2212–2223. [PubMed: 10822371]
38. Nakanishi K, Fujimoto J, Ueki T, Kishimoto K, Hashimoto-Tamaoki T, Furuyama J, Itoh T, Sasaki Y, Okamoto E. Hepatocyte growth factor promotes migration of human hepatocellular carcinoma via phosphatidylinositol 3-kinase. *Clin Exp Metastasis* 1999;17:507–514. [PubMed: 10763917]
39. Potempa S, Ridley AJ. Activation of both MAP kinase and phosphatidylinositide 3-kinase by Ras is required for hepatocyte growth factor/scatter factor-induced adherens junction disassembly. *Mol Biol Cell* 1998;9:2185–2200. [PubMed: 9693375]
40. Xiao GH, Jeffers M, Bellacosa A, Mitsuuchi Y, Vande Woude GF, Testa JR. Anti-apoptotic signaling by hepatocyte growth factor/Met via the phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase pathways. *Proc Natl Acad Sci U S A* 2001;98:247–252. [PubMed: 11134526]
41. Boccaccio C, Ando M, Tamagnone L, Bardelli A, Michieli P, Battistini C, Comoglio PM. Induction of epithelial tubules by growth factor HGF depends on the STAT pathway. *Nature* 1998;391:285–288. [PubMed: 9440692]
42. Chen HC, Chan PC, Tang MJ, Cheng CH, Chang TJ. Tyrosine phosphorylation of focal adhesion kinase stimulated by hepatocyte growth factor leads to mitogen-activated protein kinase activation. *J Biol Chem* 1998;273:25777–25782. [PubMed: 9748249]
43. Gual P, Giordano S, Williams TA, Rocchi S, Van Obberghen E, Comoglio PM. Sustained recruitment of phospholipase C-gamma to Gab1 is required for HGF-induced branching tubulogenesis. *Oncogene* 2000;19:1509–1518. [PubMed: 10734310]
44. Kodama A, Takaishi K, Nakano K, Nishioka H, Takai Y. Involvement of Cdc42 small G protein in cell-cell adhesion, migration and morphology of MDCK cells. *Oncogene* 1999;18:3996–4006. [PubMed: 10435623]
45. Lai JF, Kao SC, Jiang ST, Tang MJ, Chan PC, Chen HC. Involvement of focal adhesion kinase in hepatocyte growth factor-induced scatter of Madin-Darby canine kidney cells. *J Biol Chem* 2000;275:7474–7480. [PubMed: 10713050]
46. Lamorte L, Kamikura DM, Park M. A switch from p130Cas/Crk to Gab1/Crk signaling correlates with anchorage independent growth and JNK activation in cells transformed by the Met receptor oncoprotein. *Oncogene* 2000;19:5973–5981. [PubMed: 11146548]
47. Lamorte L, Rodrigues S, Sangwan V, Turner CE, Park M. Crk associates with a multimolecular Paxillin/GIT2/beta-PIX complex and promotes Rac-dependent relocalization of Paxillin to focal contacts. *Mol Biol Cell* 2003;14:2818–2831. [PubMed: 12857867]
48. Lamorte L, Royal I, Naujokas M, Park M. Crk adapter proteins promote an epithelial-mesenchymal-like transition and are required for HGF-mediated cell spreading and breakdown of epithelial adherens junctions. *Mol Biol Cell* 2002;13:1449–1461. [PubMed: 12006644]
49. Ridley AJ, Comoglio PM, Hall A. Regulation of scatter factor/hepatocyte growth factor responses by Ras, Rac, and Rho in MDCK cells. *Mol Cell Biol* 1995;15:1110–1122. [PubMed: 7823927]
50. Royal I, Lamarche-Vane N, Lamorte L, Kaibuchi K, Park M. Activation of cdc42, rac, PAK, and rho-kinase in response to hepatocyte growth factor differentially regulates epithelial cell colony spreading and dissociation. *Mol Biol Cell* 2000;11:1709–1725. [PubMed: 10793146]
51. Sakkab D, Lewitzky M, Posern G, Schaeper U, Sachs M, Birchmeier W, Feller SM. Signaling of hepatocyte growth factor/scatter factor (HGF) to the small GTPase Rap1 via the large docking protein Gab1 and the adapter protein CRKL. *J Biol Chem* 2000;275:10772–10778. [PubMed: 10753869]

52. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003;4:915–925. [PubMed: 14685170]
53. Giordano S, Corso S, Conrotto P, Artigiani S, Gilestro G, Barberis D, Tamagnone L, Comoglio PM. The semaphorin 4D receptor controls invasive growth by coupling with Met. *Nat Cell Biol* 2002;4:720–724. [PubMed: 12198496]
54. Trusolino L, Bertotti A, Comoglio PM. A signaling adapter function for alpha6beta4 integrin in the control of HGF-dependent invasive growth. *Cell* 2001;107:643–654. [PubMed: 11733063]
55. van der Voort R, Taher TE, Wielenga VJ, Spaargaren M, Prevo R, Smit L, David G, Hartmann G, Gherardi E, Pals ST. Heparan sulfate-modified CD44 promotes hepatocyte growth factor/scatter factor-induced signal transduction through the receptor tyrosine kinase c-Met. *J Biol Chem* 1999;274:6499–6506. [PubMed: 10037743]
56. Furge KA, Kiewlich D, Le P, Vo MN, Faure M, Howlett AR, Lipson KE, Woude GF, Webb CP. Suppression of Ras-mediated tumorigenicity and metastasis through inhibition of the Met receptor tyrosine kinase. *Proc Natl Acad Sci U S A* 2001;98:10722–10727. [PubMed: 11535809]
57. Moghul A, Lin L, Beedle A, Kanbour-Shakir A, DeFrances MC, Liu Y, Zarnegar R. Modulation of c-MET proto-oncogene (HGF receptor) mRNA abundance by cytokines and hormones: evidence for rapid decay of the 8 kb c-MET transcript. *Oncogene* 1994;9:2045–2052. [PubMed: 8208549]
58. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. *Cancer Cell* 2003;3:347–361. [PubMed: 12726861]
59. Ma PC, Jagadeeswaran R, Jagadeesh S, Tretiakova MS, Nallasura V, Fox EA, Hansen M, Schaefer E, Naoki K, Lader A, Richards W, Sugarbaker D, Husain AN, Christensen JG, Salgia R. Functional expression and mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer. *Cancer Res* 2005;65:1479–1488. [PubMed: 15735036]
60. Ichimura E, Maeshima A, Nakajima T, Nakamura T. Expression of c-met/HGF receptor in human non-small cell lung carcinomas in vitro and in vivo and its prognostic significance. *Jpn J Cancer Res* 1996;87:1063–1069. [PubMed: 8957065]
61. Olivero M, Rizzo M, Madeddu R, Casadio C, Pennacchietti S, Nicotra MR, Prat M, Maggi G, Arena N, Natali PG, Comoglio PM, Di Renzo MF. Overexpression and activation of hepatocyte growth factor/scatter factor in human non-small-cell lung carcinomas. *Br J Cancer* 1996;74:1862–1868. [PubMed: 8980383]
62. Takanami I, Tanana F, Hashizume T, Kikuchi K, Yamamoto Y, Yamamoto T, Kodaira S. Hepatocyte growth factor and c-Met/hepatocyte growth factor receptor in pulmonary adenocarcinomas: an evaluation of their expression as prognostic markers. *Oncology* 1996;53:392–397. [PubMed: 8784474]
63. Tsao MS, Liu N, Chen JR, Pappas J, Ho J, To C, Viallet J, Park M, Zhu H. Differential expression of Met/hepatocyte growth factor receptor in subtypes of non-small cell lung cancers. *Lung Cancer* 1998;20:1–16. [PubMed: 9699182]
64. Ma PC, Jagdeesh R, Jagadeeswaran EA, Fox JG, Christensen JG, Maulik G, et al. c-Met expression/activation, functions, and mutations in non-small cell lung cancer. *Proc Am Assoc Cancer Res* 2004;44:1875.
65. Jagadeeswaran R, Ma PC, Seiwert TY, Jagadeeswaran S, Zumba O, Nallasura V, Ahmed S, Filiberti R, Paganuzzi M, Puntoni R, Kratzke RA, Gordon GJ, Sugarbaker DJ, Bueno R, Janamanchi V, Bindokas VP, Kindler HL, Salgia R. Functional analysis of c-Met/hepatocyte growth factor pathway in malignant pleural mesothelioma. *Cancer Res* 2006;66:352–361. [PubMed: 16397249]
66. Follenzi A, Bakovic S, Gual P, Stella MC, Longati P, Comoglio PM. Cross-talk between the proto-oncogenes Met and Ron. *Oncogene* 2000;19:3041–3049. [PubMed: 10871856]
67. Jo M, Stolz DB, Esplen JE, Dorko K, Michalopoulos GK, Strom SC. Cross-talk between epidermal growth factor receptor and c-Met signal pathways in transformed cells. *J Biol Chem* 2000;275:8806–8811. [PubMed: 10722725]
68. Kuniyasu H, Yasui W, Kitadai Y, Yokozaki H, Ito H, Tahara E. Frequent amplification of the c-met gene in scirrhous type stomach cancer. *Biochem Biophys Res Commun* 1992;189:227–232. [PubMed: 1333188]

69. Oh RR, Park JY, Lee JH, Shin MS, Kim HS, Lee SK, Kim YS, Lee SH, Lee SN, Yang YM, Yoo NJ, Lee JY, Park WS. Expression of HGF/SF and Met protein is associated with genetic alterations of VHL gene in primary renal cell carcinomas. *Apmis* 2002;110:229–238. [PubMed: 12076276]
70. Park M, Dean M, Cooper CS, Schmidt M, O'Brien SJ, Blair DG, Vande Woude GF. Mechanism of met oncogene activation. *Cell* 1986;45:895–904. [PubMed: 2423252]
71. Prat M, Crepaldi T, Gandino L, Giordano S, Longati P, Comoglio P. C-terminal truncated forms of Met, the hepatocyte growth factor receptor. *Mol Cell Biol* 1991;11:5954–5962. [PubMed: 1944272]
72. Wallenius V, Hisaoka M, Helou K, Levan G, Mandahl N, Meis-Kindblom JM, Kindblom LG, Jansson JO. Overexpression of the hepatocyte growth factor (HGF) receptor (Met) and presence of a truncated and activated intracellular HGF receptor fragment in locally aggressive/malignant human musculoskeletal tumors. *Am J Pathol* 2000;156:821–829. [PubMed: 10702398]
73. Wang R, Ferrell LD, Faouzi S, Maher JJ, Bishop JM. Activation of the Met receptor by cell attachment induces and sustains hepatocellular carcinomas in transgenic mice. *J Cell Biol* 2001;153:1023–1034. [PubMed: 11381087]
74. Lee JH, Han SU, Cho H, Jennings B, Gerrard B, Dean M, Schmidt L, Zbar B, Vande Woude GF. A novel germ line juxtamembrane Met mutation in human gastric cancer. *Oncogene* 2000;19:4947–4953. [PubMed: 11042681]
75. Ma PC, Kijima T, Maulik G, Fox EA, Sattler M, Griffin JD, Johnson BE, Salgia R. c-MET mutational analysis in small cell lung cancer: novel juxtamembrane domain mutations regulating cytoskeletal functions. *Cancer Res* 2003;63:6272–6281. [PubMed: 14559814]
76. Park WS, Dong SM, Kim SY, Na EY, Shin MS, Pi JH, Kim BJ, Bae JH, Hong YK, Lee KS, Lee SH, Yoo NJ, Jang JJ, Pack S, Zhuang Z, Schmidt L, Zbar B, Lee JY. Somatic mutations in the kinase domain of the Met/hepatocyte growth factor receptor gene in childhood hepatocellular carcinomas. *Cancer Res* 1999;59:307–310. [PubMed: 9927037]
77. Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, Scherer SW, Zhuang Z, Lubensky I, Dean M, Allikmets R, Chidambaram A, Bergerheim UR, Feltis JT, Casadevall C, Zamarron A, Bernues M, Richard S, Lips CJ, Walther MM, Tsui LC, Geil L, Orcutt ML, Stackhouse T, Lipan J, Slife L, Brauch H, Decker J, Niehans G, Hughson MD, Moch H, Storkel S, Lerman MI, Linehan WM, Zbar B. Germline and somatic mutations in the tyrosine kinase domain of the MET protooncogene in papillary renal carcinomas. *Nat Genet* 1997;16:68–73. [PubMed: 9140397]
78. Schmidt L, Junker K, Weirich G, Glenn G, Choyke P, Lubensky I, Zhuang Z, Jeffers M, Vande Woude G, Neumann H, Walther M, Linehan WM, Zbar B. Two North American families with hereditary papillary renal carcinoma and identical novel mutations in the MET proto-oncogene. *Cancer Res* 1998;58:1719–1722. [PubMed: 9563489]
79. Tanyi J, Tory K, Rigo J Jr. Nagy B, Papp Z. Evaluation of the tyrosine kinase domain of the Met proto-oncogene in sporadic ovarian carcinomas*. *Pathol Oncol Res* 1999;5:187–191. [PubMed: 10491015]
80. Di Renzo MF, Olivero M, Martone T, Maffe A, Maggiora P, Stefani AD, Valente G, Giordano S, Cortesina G, Comoglio PM. Somatic mutations of the MET oncogene are selected during metastatic spread of human HNSC carcinomas. *Oncogene* 2000;19:1547–1555. [PubMed: 10734314]
81. Lorenzato A, Olivero M, Patane S, Rosso E, Oliaro A, Comoglio PM, Di Renzo MF. Novel somatic mutations of the MET oncogene in human carcinoma metastases activating cell motility and invasion. *Cancer Res* 2002;62:7025–7030. [PubMed: 12460923]
82. Zeng Z, Weiser MR, D'Alessio M, Grace A, Shia J, Paty PB. Immunoblot analysis of c-Met expression in human colorectal cancer: overexpression is associated with advanced stage cancer. *Clin Exp Metastasis* 2004;21:409–417. [PubMed: 15672865]
83. Siegfried JM, Weissfeld LA, Singh-Kaw P, Weyant RJ, Testa JR, Landreneau RJ. Association of immunoreactive hepatocyte growth factor with poor survival in resectable non-small cell lung cancer. *Cancer Res* 1997;57:433–439. [PubMed: 9012470]
84. Koochekpour S, Jeffers M, Rulong S, Taylor G, Klineberg E, Hudson EA, Resau JH, Vande Woude GF. Met and hepatocyte growth factor/scatter factor expression in human gliomas. *Cancer Res* 1997;57:5391–5398. [PubMed: 9393765]

85. Natali PG, Nicotra MR, Di Renzo MF, Prat M, Bigotti A, Cavaliere R, Comoglio PM. Expression of the c-Met/HGF receptor in human melanocytic neoplasms: demonstration of the relationship to malignant melanoma tumour progression. *Br J Cancer* 1993;68:746–750. [PubMed: 8104462]
86. Ng EH, Pey HB, Law HY, Ng I, Ji CY, Lin V, Soo KC. Loss of c-met protooncogene in primary and metastatic sites of breast cancer. *Ann Surg Oncol* 1997;4:499–502. [PubMed: 9309340]
87. Siegfried JM, Weissfeld LA, Luketich JD, Weyant RJ, Gubish CT, Landreneau RJ. The clinical significance of hepatocyte growth factor for non-small cell lung cancer. *Ann Thorac Surg* 1998;66:1915–1918. [PubMed: 9930468]
88. Taniguchi Y, Yorioka N, Yamashita K, Xue-Feng Y, Nishiki T, Ogawa T, Oda H, Yamakido M. Localization of hepatocyte growth factor and tubulointerstitial lesions in IgA nephropathy. *Am J Nephrol* 1997;17:413–416. [PubMed: 9382157]
89. Uchida D, Kawamata H, Omotehara F, Nakashiro K, Kimura-Yanagawa T, Hino S, Begum NM, Hoque MO, Yoshida H, Sato M, Fujimori T. Role of HGF/c-met system in invasion and metastasis of oral squamous cell carcinoma cells in vitro and its clinical significance. *Int J Cancer* 2001;93:489–496. [PubMed: 11477552]
90. Ueki T, Fujimoto J, Suzuki T, Yamamoto H, Okamoto E. Expression of hepatocyte growth factor and its receptor, the c-met proto-oncogene, in hepatocellular carcinoma. *Hepatology* 1997;25:619–623. [PubMed: 9049208]
91. Yamashita J, Ogawa M, Yamashita S, Nomura K, Kuramoto M, Saishoji T, Shin S. Immunoreactive hepatocyte growth factor is a strong and independent predictor of recurrence and survival in human breast cancer. *Cancer Res* 1994;54:1630–1633. [PubMed: 8137271]
92. Yi S, Tsao MS. Activation of hepatocyte growth factor-met autocrine loop enhances tumorigenicity in a human lung adenocarcinoma cell line. *Neoplasia* 2000;2:226–234. [PubMed: 10935508]
93. Cooper CS, Park M, Blair DG, Tainsky MA, Huebner K, Croce CM, Vande Woude GF. Molecular cloning of a new transforming gene from a chemically transformed human cell line. *Nature* 1984;311:29–33. [PubMed: 6590967]
94. Maulik G, Kijima T, Ma PC, Ghosh SK, Lin J, Shapiro GI, Schaefer E, Tibaldi E, Johnson BE, Salgia R. Modulation of the c-Met/hepatocyte growth factor pathway in small cell lung cancer. *Clin Cancer Res* 2002;8:620–627. [PubMed: 11839685]
95. Goshima Y, Ito T, Sasaki Y, Nakamura F. Semaphorins as signals for cell repulsion and invasion. *J Clin Invest* 2002;109:993–998. [PubMed: 11956234]
96. Kagoshima M, Ito T. Diverse gene expression and function of semaphorins in developing lung: positive and negative regulatory roles of semaphorins in lung branching morphogenesis. *Genes Cells* 2001;6:559–571. [PubMed: 11442635]
97. Roche J, Boldog F, Robinson M, Robinson L, Varella-Garcia M, Swanton M, Waggoner B, Fishel R, Franklin W, Gemmill R, Drabkin H. Distinct 3p21.3 deletions in lung cancer and identification of a new human semaphorin. *Oncogene* 1996;12:1289–1297. [PubMed: 8649831]
98. Ma PC, Tretiakova MS, Nallasura V, Jagadeeswaran R, Husain AN, Salgia R. Downstream signalling and specific inhibition of c-MET/HGF pathway in small cell lung cancer: implications for tumour invasion. *Br J Cancer* 2007;97:368–377. [PubMed: 17667909]
99. Lutterbach B, Zeng Q, Davis LJ, Hatch H, Hang G, Kohl NE, Gibbs JB, Pan BS. Lung cancer cell lines harboring MET gene amplification are dependent on Met for growth and survival. *Cancer Res* 2007;67:2081–2088. [PubMed: 17332337]
100. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale CM, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC, Janne PA. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007;316:1039–1043. [PubMed: 17463250]
101. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, Chitale D, Motoi N, Szoke J, Broderick S, Balak M, Chang WC, Yu CJ, Gazdar A, Pass H, Rusch V, Gerald W, Huang SF, Yang PC, Miller V, Ladanyi M, Yang CH, Pao W. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci U S A* 2007;104:20932–20937. [PubMed: 18093943]
102. Jagadeeswaran R, Surawska H, Krishnaswamy S, Janamanchi V, Mackinnon AC, Seiwert TY, Loganathan S, Kanteti R, Reichman T, Nallasura V, Schwartz S, Faoro L, Wang YC, Girard L,

- Tretiakova MS, Ahmed S, Zumba O, Soulii L, Bindokas VP, Szeto LL, Gordon GJ, Bueno R, Sugarbaker D, Lingen MW, Sattler M, Krausz T, Vigneswaran W, Natarajan V, Minna J, Vokes EE, Ferguson MK, Husain AN, Salgia R. Paxillin is a target for somatic mutations in lung cancer: implications for cell growth and invasion. *Cancer Res* 2008;68:132–142. [PubMed: 18172305]
103. Cassinelli G, Lanzi C, Petrangolini G, Tortoreto M, Pratesi G, Cuccuru G, Laccabue D, Supino R, Belluco S, Favini E, Poletti A, Zunino F. Inhibition of c-Met and prevention of spontaneous metastatic spreading by the 2-indolinone RPI-1. *Mol Cancer Ther* 2006;5:2388–2397. [PubMed: 16985073]
 104. Shinomiya N, Gao CF, Xie Q, Gustafson M, Waters DJ, Zhang YW, Vande Woude GF. RNA interference reveals that ligand-independent met activity is required for tumor cell signaling and survival. *Cancer Res* 2004;64:7962–7970. [PubMed: 15520203]
 105. Kuhn DE, Martin MM, Feldman DS, Terry AV Jr, Nuovo GJ, Elton TS. Experimental validation of miRNA targets. *Methods* 2008;44:47–54. [PubMed: 18158132]
 106. Ross JS, Carlson JA, Brock G. miRNA: the new gene silencer. *Am J Clin Pathol* 2007;128:830–836. [PubMed: 17951207]
 107. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A* 2002;99:15524–15529. [PubMed: 12434020]
 108. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, Mitsudomi T, Takahashi T. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res* 2004;64:3753–3756. [PubMed: 15172979]
 109. Dalmay T, Edwards DR. MicroRNAs and the hallmarks of cancer. *Oncogene* 2006;25:6170–6175. [PubMed: 17028596]
 110. Abounader R, Ranganathan S, Lal B, Fielding K, Book A, Dietz H, Burger P, Laterra J. Reversion of human glioblastoma malignancy by U1 small nuclear RNA/ribozyme targeting of scatter factor/hepatocyte growth factor and c-met expression. *J Natl Cancer Inst* 1999;91:1548–1556. [PubMed: 10491431]
 111. Kim SJ, Johnson M, Koterba K, Herynk MH, Uehara H, Gallick GE. Reduced c-Met expression by an adenovirus expressing a c-Met ribozyme inhibits tumorigenic growth and lymph node metastases of PC3-LN4 prostate tumor cells in an orthotopic nude mouse model. *Clin Cancer Res* 2003;9:5161–5170. [PubMed: 14613995]
 112. Herynk MH, Stoeltzing O, Reinmuth N, Parikh NU, Abounader R, Laterra J, Radinsky R, Ellis LM, Gallick GE. Down-regulation of c-Met inhibits growth in the liver of human colorectal carcinoma cells. *Cancer Res* 2003;63:2990–2996. [PubMed: 12782608]
 113. Stabile LP, Lyker JS, Huang L, Siegfried JM. Inhibition of human non-small cell lung tumors by a c-Met antisense/U6 expression plasmid strategy. *Gene Ther* 2004;11:325–335. [PubMed: 14737093]
 114. Webb CP, Hose CD, Koochekpour S, Jeffers M, Oskarsson M, Sausville E, Monks A, Vande Woude GF. The geldanamycins are potent inhibitors of the hepatocyte growth factor/scatter factor-met-urokinase plasminogen activator-plasmin proteolytic network. *Cancer Res* 2000;60:342–349. [PubMed: 10667586]
 115. Bowers DC, Fan S, Walter KA, Abounader R, Williams JA, Rosen EM, Laterra J. Scatter factor/hepatocyte growth factor protects against cytotoxic death in human glioblastoma via phosphatidylinositol 3-kinase- and AKT-dependent pathways. *Cancer Res* 2000;60:4277–4283. [PubMed: 10945642]
 116. Burgess T, Coxon A, Meyer S, Sun J, Rex K, Tsuruda T, Chen Q, Ho SY, Li L, Kaufman S, McDorman K, Cattley RC, Sun J, Elliott G, Zhang K, Feng X, Jia XC, Green L, Radinsky R, Kendall R. Fully human monoclonal antibodies to hepatocyte growth factor with therapeutic potential against hepatocyte growth factor/c-Met-dependent human tumors. *Cancer Res* 2006;66:1721–1729. [PubMed: 16452232]
 117. Matsumoto K, Nakamura T. NK4 (HGF-antagonist/angiogenesis inhibitor) in cancer biology and therapeutics. *Cancer Sci* 2003;94:321–327. [PubMed: 12824898]

118. Zhang YW, Vande Woude GF. HGF/SF-met signaling in the control of branching morphogenesis and invasion. *J Cell Biochem* 2003;88:408–417. [PubMed: 12520544]
119. Date K, Matsumoto K, Kuba K, Shimura H, Tanaka M, Nakamura T. Inhibition of tumor growth and invasion by a four-kringle antagonist (HGF/NK4) for hepatocyte growth factor. *Oncogene* 1998;17:3045–3054. [PubMed: 9881707]
120. Davies G, Mason MD, Martin TA, Parr C, Watkins G, Lane J, Matsumoto K, Nakamura T, Jiang WG. The HGF/SF antagonist NK4 reverses fibroblast- and HGF-induced prostate tumor growth and angiogenesis in vivo. *Int J Cancer* 2003;106:348–354. [PubMed: 12845672]
121. Kuba K, Matsumoto K, Date K, Shimura H, Tanaka M, Nakamura T. HGF/NK4, a four-kringle antagonist of hepatocyte growth factor, is an angiogenesis inhibitor that suppresses tumor growth and metastasis in mice. *Cancer Res* 2000;60:6737–6743. [PubMed: 11118060]
122. Martin TA, Parr C, Davies G, Watkins G, Lane J, Matsumoto K, Nakamura T, Mansel RE, Jiang WG. Growth and angiogenesis of human breast cancer in a nude mouse tumour model is reduced by NK4, a HGF/SF antagonist. *Carcinogenesis* 2003;24:1317–1323. [PubMed: 12807719]
123. Tomioka D, Maehara N, Kuba K, Mizumoto K, Tanaka M, Matsumoto K, Nakamura T. Inhibition of growth, invasion, and metastasis of human pancreatic carcinoma cells by NK4 in an orthotopic mouse model. *Cancer Res* 2001;61:7518–7524. [PubMed: 11606388]
124. Kushibiki T, Matsumoto K, Nakamura T, Tabata Y. Suppression of tumor metastasis by NK4 plasmid DNA released from cationized gelatin. *Gene Ther* 2004;11:1205–1214. [PubMed: 15103321]
125. Martens T, Schmidt NO, Eckerich C, Fillbrandt R, Merchant M, Schwall R, Westphal M, Lamszus K. A novel one-armed anti-c-Met antibody inhibits glioblastoma growth in vivo. *Clin Cancer Res* 2006;12:6144–6152. [PubMed: 17062691]
126. Parr C, Hiscox S, Nakamura T, Matsumoto K, Jiang WG. Nk4, a new HGF/SF variant, is an antagonist to the influence of HGF/SF on the motility and invasion of colon cancer cells. *Int J Cancer* 2000;85:563–570. [PubMed: 10699931]
127. Michieli P, Mazzone M, Basilico C, Cavassa S, Sottile A, Naldini L, Comoglio PM. Targeting the tumor and its microenvironment by a dual-function decoy Met receptor. *Cancer Cell* 2004;6:61–73. [PubMed: 15261142]
128. Cao B, Su Y, Oskarsson M, Zhao P, Kort EJ, Fisher RJ, Wang LM, Vande Woude GF. Neutralizing monoclonal antibodies to hepatocyte growth factor/scatter factor (HGF/SF) display antitumor activity in animal models. *Proc Natl Acad Sci U S A* 2001;98:7443–7448. [PubMed: 11416216]
129. Gordon MS, et al. Interim results from a first-in-human study with AMG102, a fully human monoclonal antibody that neutralizes hepatocyte growth factor (HGF), the ligand to c-Met receptor, in patients (pts) with advanced solid tumors. *J Clin Oncol* 2007;25:18Sa3551
130. AVEO Pharmaceuticals I. AVEO enters into worldwide license and development agreement with Schering-Plough for AV-299, AVEO's novel anti-HGF antibody.
131. Mazzone M, Basilico C, Cavassa S, Pennacchietti S, Risio M, Naldini L, Comoglio PM, Michieli P. An uncleavable form of pro-scatter factor suppresses tumor growth and dissemination in mice. *J Clin Invest* 2004;114:1418–1432. [PubMed: 15545993]
132. Laird AD, Cherrington JM. Small molecule tyrosine kinase inhibitors: clinical development of anticancer agents. *Expert Opin Investig Drugs* 2003;12:51–64.
133. Sattler M, Pride YB, Ma P, Gramlich JL, Chu SC, Quinnan LA, Shirazian S, Liang C, Podar K, Christensen JG, Salgia R. A novel small molecule met inhibitor induces apoptosis in cells transformed by the oncogenic TPR-MET tyrosine kinase. *Cancer Res* 2003;63:5462–5469. [PubMed: 14500382]
134. Puri N, Khramtsov A, Ahmed S, Nallasura V, Hetzel JT, Jagadeeswaran R, Karczmar G, Salgia R. A selective small molecule inhibitor of c-Met, PHA665752, inhibits tumorigenicity and angiogenesis in mouse lung cancer xenografts. *Cancer Res* 2007;67:3529–3534. [PubMed: 17440059]
135. Ma PC, Schaefer E, Christensen JG, Salgia R. A selective small molecule c-MET Inhibitor, PHA665752, cooperates with rapamycin. *Clin Cancer Res* 2005;11:2312–2319. [PubMed: 15788682]

136. Mukohara T, Civiello G, Davis IJ, Taffaro ML, Christensen J, Fisher DE, Johnson BE, Janne PA. Inhibition of the met receptor in mesothelioma. *Clin Cancer Res* 2005;11:8122–8130. [PubMed: 16299245]
137. Zou HY, Li Q, Lee JH, Arango ME, McDonnell SR, Yamazaki S, Koudriakova TB, Alton G, Cui JJ, Kung PP, Nambu MD, Los G, Bender SL, Mroczkowski B, Christensen JG. An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. *Cancer Res* 2007;67:4408–4417. [PubMed: 17483355]
138. Garcia A, et al. Phase I study of ARQ 197, a selective inhibitor of the c-Met RTK in patients with metastatic solid tumors reaches recommended phase 2 dose. *J Clin Oncol* 2007;25:18Sa3525
139. Rosen L, Garcia A, Mulay M, et al. A phase 1 dose escalation of ARQ 197, a selective inhibitor of the cMet receptor in patients with metastatic solid tumors. *Eur J Cancer* 2006;196.
140. Eder JP, et al. Phase I experience with c-MET inhibitor XL880 administered orally to patients (pts) with solid tumors. *J Clin Oncol* 2007;25:18Sa3526
141. Ross RW, et al. A phase II study of the c-Met RTK inhibitor XL880 in patients (pts) with papillary renal-cell carcinoma (PRC). *J Clin Oncol* 2007;25:18Sa15601
142. Shapiro, GI., et al. A phase I dose-escalation study of the safety, pharmacokinetics (PK), and pharmacodynamics of XL880, a VEGFR and MET kinase inhibitor, administered daily to patients (pts) with advanced malignancies; Presented at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics: Discovery, Biology, and Clinical Applications; San Francisco, CA. October 22-26, 2007; 2007.
143. Ross, RW., et al. A phase 2 study of the dual MET/VEGFR2 inhibitor XL880 in patients (pts) with papillary renal carcinoma (PRC); Presented at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics: Discovery, Biology, and Clinical Applications; San Francisco, CA. October 22-26, 2007; 2007.
144. Salgia R, et al. A phase I dose escalation study of the safety and pharmacokinetics (PK) of XL184, a VEGFR and MET kinase inhibitor, administered orally to patients (pts) with advanced malignancies. *J Clin Oncol* 2007;25:18Sa14031
145. Salgia, R., et al. A phase I dose escalation study of the safety and pharmacokinetics (PK) of XL184, a VEGFR and MET kinase inhibitor, administered orally to patients (pts) with advanced malignancies; Presented at the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics: Discovery, Biology, and Clinical Applications; San Francisco, CA. October 22-26, 2007; 2007.
146. Froning, KJ., et al. SGX523: a potent and highly selective small molecule inhibitor of the MET receptor tyrosine kinase; Presented at the AACR Centennial Annual Meeting; Los Angeles, CA. April 14-18, 2007; 2007.
147. Inc. SP. SGX initiates phase I trials for SGX523.
148. Welsh J, Mahadevan G, Dougherty G, Stea B. 2626 c-MET inhibition radiosensitizes melanoma by inhibiting double strand DNA repair. *International Journal of Radiation Oncology, Biology, Physics* 2006;66:S557–S558.
149. Morotti A, Mila S, Accornero P, Tagliabue E, Ponzetto C. K252a inhibits the oncogenic properties of Met, the HGF receptor. *Oncogene* 2002;21:4885–4893. [PubMed: 12118367]
150. Bardelli A, Longati P, Williams TA, Benvenuti S, Comoglio PM. A peptide representing the carboxyl-terminal tail of the met receptor inhibits kinase activity and invasive growth. *J Biol Chem* 1999;274:29274–29281. [PubMed: 10506185]
151. Morton PA, Joy WD, Bono CP, Arbuckle A, Evans ML, Huyng MS, et al. In vitro and in vivo activity of fully-human monoclonal antibody antagonists to c-Met protein tyrosine kinase. *Proc Am Assoc Cancer Res* 2003;43:5604.
152. Schwall R, Adams CW, Zheng Z, Romero M, Mai E, Moffat B, et al. Inhibition of cmet activation by a one-armed antibody. *Proc Am Assoc Cancer Res* 2004;44:1424.
153. Zheng Z, Adams C, Moffat B, Schwall R. A chimeric Fab antibody serves as an antagonist to the HGF/SF receptor c-Met. *Proc Am Assoc Cancer Res* 2003;43:5717.

Figure 1. MET Structure

SEMA (Semaphorin-like); PSI (found in Plexins, Semaphorins, and Integrins); IPT (found in Ig-like regions, Plexins and Transcription factors); TM (Trans-Membrane); JM (Juxta-Membrane); TK (Intracellular Tyrosine Kinase); S (Serine); Y (Tyrosine); NSCLC (non-small cell lung carcinoma); SCLC (small cell lung carcinoma); MPM (malignant pleural mesothelioma).

Figure 2. The MET Pathway

Figure 2: The MEK1 pathway
 PYK2 (protein tyrosine kinase 2); PXN (paxillin); FAK (focal adhesion kinase); SOS (son of sevenless); GRB2 (growth factor receptor-bound protein 2); GAB1 (GRB2-associated binding protein 1); RHO (Ras homolog gene family); RAC1 (ras-related C3 botulinum toxin substrate 1); CDC42 (cell division cycle 42); PI3K (phosphoinositol 3 kinase).

Figure 3. MET regulation

Figure 4. Anti-MET Therapeutics

RNA (ribonucleic acid); siRNA (small interference RNA); shRNA (short hairpin RNA); miRNA (micro RNA); DNA (deoxyribonucleic acid); HSP (heat shock protein); HGF (hepatocyte growth factor); Ab (antibody); RTK (receptor tyrosine kinase); P (phosphate).