Targeting MET in cancer: rationale and progress

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Abstract | Uncontrolled cell survival, growth, angiogenesis and metastasis are essential hallmarks of cancer. Genetic and biochemical data have demonstrated that the growth and motility factor hepatocyte growth factor/scatter factor (HGF/SF) and its receptor, the tyrosine kinase MET, have a causal role in all of these processes, thus providing a strong rationale for targeting these molecules in cancer. Parallel progress in understanding the structure and function of HGF/SF, MET and associated signalling components has led to the successful development of blocking antibodies and a large number of small-molecule MET kinase inhibitors. In this Review, we discuss these advances, as well as results from recent clinical studies that demonstrate that inhibiting MET signalling in several types of solid human tumours has major therapeutic value.

NATURE REVIEWS | CANCER

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Coagulation cascade
An ordered sequence of chemical reactions triggered by tissue components after tissue damage and catalysed by enzymes present in serum that ultimately causes the formation of a blood clot.

At a glance
- The growth and motility factor hepatocyte growth factor/scatter factor (HGF/SF) and its receptor tyrosine kinase MET, the product of the MET proto-oncogene, provide essential signals for survival and long-distance migration of epithelial and myogenic precursor cells during embryogenesis. Cancer cells hijack HGF/SF–MET for invasion and metastasis, hence these molecules have emerged as key targets for cancer therapy.
- Aberrant MET activation occurs in many types of cancer, and results from multiple mechanisms. Many carcinomas overexpress MET and the surrounding stroma overexpresses HGF/SF. Furthermore, certain patients with renal papillary, hepatocellular or gastric carcinomas carry point mutations in MET. These mutations have proved important in demonstrating a causal role of aberrant MET signaling in human cancer.
- The intracellular signalling cascades activated by MET include the PI3K–AKT, RAC1–cell division control protein 42 (CDC42), RAP1 and RAS–MAPK pathways. An intricate network of cross-signalling involving the MET–epidermal growth factor receptor (EGFR), MET–vascular endothelial growth factor receptor (VEGFR) and MET–WNT pathways has also emerged in the past few years. This signalling network has major implications for therapy.
- Structural studies of HGF/SF, the MET ectodomain and the pathways involved in activation of the precursor form of HGF/SF (pro-HGF/SF) have yielded important results and new opportunities for therapeutic intervention, namely specific inhibitors of the major HGF/SF activators, HGF/SF fragments with antagonistic activity — such as NK4 — and HGF/SF and MET antibodies.
- Parallel efforts in the structural analysis of the MET kinase have led to extensive progress in the development of MET kinase inhibitors for cancer therapy, and three major classes of inhibitors have emerged from this work that differ in their binding mode, activity on MET kinase mutants and enzyme specificity.
- A number of recent clinical trials have demonstrated strong activity of MET inhibitors in patients with a variety of advanced or metastatic tumours, including non-small-cell lung cancer (NSCLC), and breast, prostate, liver and renal cancer. MET inhibitors have also displayed clinical benefits in patients with NSCLC and patients with breast cancer who had developed resistance to EGFR therapy. These recent data clearly indicate that HGF/SF–MET therapeutics may have potential in several groups of cancer patients either alone or in combination with inhibitors of other signalling pathways.

The oncogene TPR–MET is isolated from a chemically transformed cell line
[1987–1989] SF, which disrupts epithelial junctions, promotes migration of epithelial cells and induces EMT, is isolated from embryo and 3T3 fibroblast cultures

Cloning of the full-length MET cDNA reveals that the encoded protein is an RTK

The first sequences of HGF cDNAs reveals homology to plasminogen

[1990–1991] Protein and DNA sequencing reveal that SF and HGF are identical (HGF/SF)

HGF/SF induces invasion of epithelial cells in a 3D culture assay

[1992–1994] MET is shown to be a potent oncogene, and 3T3 cells that co-express MET and HGF/SF metastasize in an animal model

HGF/SF and MET have essential roles in the development of the placenta and liver, and they control EMT of the epithelial dermomyosist and migration of myogenic precursor cells

Activating mutations of MET are found in hereditary papillary renal carcinomas and sporadic renal cancers


[1987–1989] A potent mitogen for liver and renal parenchymal cells (HGF) is purified from rat platelets and human plasma

MET is identified as the receptor of HGF/SF

A bidentate docking site that binds multiple SH2 domain-containing proteins is identified in MET

HGF/SF overexpression induces growth, abnormal development and tumour formation in the liver of transgenic mice

GAB1, a new adaptor molecule that binds to activated MET, is identified

Discoveries related to cancer are outlined in red: 3D, three-dimensional; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; GAB1, GRB2-associated-binding protein 1; HGF, hepatocyte growth factor; InlB, Internalin B; MACC1, metastasis-associated gene in colon cancer 1; NK1, N-terminal and kringle domain 1; NSCLC, non-small-cell lung cancer; RTK, receptor tyrosine kinase; SF, scatter factor; SH2, SRC homology 2; SHP2, SRC homology 2 domain-containing phosphatase 2; SPP1, serine proteinase homology; TPR, translocated promoter region.
Basic mechanisms of MET signalling. Ligand-induced MET dimerization activates the tyrosine kinase by phosphorylation of tyrosine residues (Tyr1230, Tyr1234 and Tyr1235) in the kinase domain, which leads to autophosphorylation of the carboxy-terminal bidenate substrate-binding site (Tyr1349 and Tyr1356) of MET. Various cytoplasmic effector proteins, including GAB1, GRB2, phospholipase C (PLC) and SRC are directly recruited to this site, and these proteins become frequently phosphorylated on tyrosine residues (reviewed in refs 16,18). Phosphorylated GAB1 bound to MET at the plasma membrane can attract further docking molecules and enzymes such as SRC homology 2 domain-containing phosphatase 2 (SHP2; also known as PTEN11), PI3K, CRK-like protein (CRKL) and others that together activate various downstream signalling cascades. MET signalling, which is mainly mediated by the RAS–MAPK and PI3K–AKT pathways, affects gene expression and cell cycle progression through the binding of transcription factors, such as the ETS family (TIMELINE). In humans, activating mutations of MET, which encodes SHP2, are associated with a number of malignancies, most prominently juvenile myelomonocytic leukaemia (JMML).

Several types of signal cooperation and crosstalk between MET and other receptor pathways have been unveiled in recent years, and a recent study has demonstrated cooperative signalling between MET and the epidermal growth factor receptor (EGFR) during kidney development (TIMELINE), providing genetic evidence for the role of HGF/SF in kidney–branching morphogenesis that was inferred from earlier experiments with cell and organ cultures. The absence of MET during renal development caused reduced branching of the ureteric bud and a decreased number of nephrons, and the defect was particularly severe in mice in which both MET and...
EGFR signalling was impaired\(^{43}\). Crosstalk between MET and other signalling systems (such as transforming growth factor-β (TGFβ), WNT and others) has emerged as a major mechanism in human cancer and is discussed below.

**MET ubiquitylation, endocytosis and shedding.** On ligand activation, MET — like other RTKs — is internalized through endocytosis. The internalized receptor is then either degraded or recycled to the plasma membrane. Derailed receptor trafficking and degradation, as well as unbalanced recycling, can cause sustained signalling and can contribute to cell transformation, tumorigenesis and metastasis\(^{46}\).

MET endocytosis and degradation are initiated by ligand-dependent phosphorylation of the receptor, which is then internalized primarily by clathrin-coated pits and vesicles. The internalized receptor is delivered to endosomal compartments and remains capable of signalling during vesicle trafficking. Subsequently, the receptor is either degraded or recycled. MET mutations that increase endocytosis and/or recycling activity and that decrease degradation result in enhanced anchorage-independent growth, tumorigenesis and experimental metastasis *in vivo*\(^{47}\). The ubiquitin E3 ligase CBL contains a phosphotyrosine-binding module that recognizes the phosphorylated Tyr1003 residue in the juxtamembrane domain of MET\(^{48}\) and induces degradation (TIMELINE). CBL also contains a RING finger domain that engages E2 protein ubiquitin ligases to mediate ubiquitylation of MET, which might occur at the cell membrane or in the early endocytic compartment. Ubiquitylated MET is degraded in a late endosomal or lysosomal compartment in a proteasome-dependent manner\(^{49,50}\). Mutation or deletion of the CBL-binding site converts MET into a transforming protein. Such receptor variants are still internalized on ligand activation, but they escape degradation owing to a change in endosomal sorting\(^{51}\). Endosomal sorting is regulated by adaptors that recognize and bind ubiquitylated proteins, among them hepatocyte growth factor-regulated tyrosine kinase substrate (HRS; also known as HGS), which is a MET substrate that is involved in endosomal sorting of ubiquitylated MET\(^{51}\). Several mechanisms that affect RTK internalization and trafficking have been reported to be altered in cancer cells (see REF 46 for a recent review) but their role in aberrant MET activity requires further analysis.

Certain cancer cells express alternatively spliced MET mRNAs that encode a receptor without the juxtamembrane CBL-binding site or with mutations in the juxtamembrane domain that interfere with CBL binding\(^{52}\). Owing to the inability to recruit the CBL E3 ubiquitin ligase, receptor downregulation is impaired\(^{46,53}\). Aberrant endocytosis in cancer cells and the resulting escape from degradation results in MET overexpression\(^{46}\).

Another mechanism that leads to downregulation of MET is regulated proteolysis and shedding of the extracellular domain. Shedding is mediated by members of the a disintegrin and metalloproteinase (ADAM) family and results in the formation of a soluble MET ectodomain and a membrane-anchored cytoplasmic tail. The surface-associated cytoplasmic tail undergoes proteolysis by γ-secretase and is rapidly cleared by proteasome-mediated degradation\(^{44}\).

**Physiology of HGF/SF and MET**

Recent genetic studies in mice have demonstrated that HGF/SF–MET signalling is essential for regeneration in liver and skin. These normal functions have served as a paradigm for understanding the roles of HGF/SF and MET in cancer and are discussed below.
**HGF/SF and MET in EMT and cell migration during embryogenesis.** During development, MET controls the epithelial-to-mesenchymal transition (EMT) of myogenic progenitor cells that are released from the epithelial dermomyotome. These cells migrate in a MET- and GAB1-dependent manner over long distances in the embryo. MET-dependent EMT and long-distance migration of tumour cells also have major roles in invasion and metastasis.

**MET in organ regeneration.** Distinct cellular mechanisms are used for regeneration. Stem cells can provide a source, but, in certain tissues, terminally differentiated cells can re-enter the cell cycle and sustain regeneration. It has been argued that cancer resembles a persistent regeneration process that is unable to define its end point (tumours have been referred to as “wounds that do not heal” [REF 56]). Loss of liver mass can be induced in rodents by administering hepatotoxic chemicals or by surgical removal of up to two-thirds of the liver. Shortly after partial hepatectomy, HGF/SF is mobilized from the extracellular matrix, resulting in MET activation in hepatocytes, which leads to DNA synthesis and cytokinesis. Conditional ablation of Met in hepatocytes in mice interferes with hepatocyte re-entry into S phase and cell cycle progression after partial hepatectomy, resulting in impaired proliferation and incomplete liver regeneration.

In the skin, stem cell populations generate different epidermal cell types during normal turnover and wound repair, and a recent study showed that MET...
Keratinocytes
Epithelial cells of the skin and its appendages, such as hair and skin glands.

Autocrine signalling
A type of cell signalling in which the same cell produces both the chemical messenger (a hormone, growth factor or cytokine) and the membrane receptor that triggers the biological response to the messenger.

is essential for wound repair\(^6\). In mice with conditional ablation of Met in keratinocytes, only cells that had escaped recombination and that continued to express a functional MET\(^7\) could contribute to regeneration. This result was unexpected because growth factors of the EGF and fibroblast growth factor (FGF) families are also involved in re-epithelialization, but cannot compensate for a lack of HGF/SF–MET signalling in the skin in vivo. During the repair of skin wounds HGF/SF and MET are co-expressed in keratinocytes, which implies that autocrine signalling occurs. Upregulated HGF/SF levels were also reported after injury to other epithelial organs, such as the kidney and lung, as well as skeletal muscle and heart\(^8\).

How HGF/SF and MET can cause cancer
The tight regulation of HGF/SF and MET signalling that is observed in development and regeneration is lost in cancer at multiple levels. These changes often involve transcriptional deregulation, but a number of other mechanisms, including inadequate degradation, receptor crosstalk or synergies in downstream signalling, have also been observed. The association of HGF/SF–MET alterations with different types of cancer can be found in a searchable, fully referenced table (see the HGF/SF - MET and cancer online table; see Further information).

Genetic abnormalities that cause aberrant HGF/SF–MET signalling. Activating point mutations of MET occur in sporadic and inherited human renal carcinomas, hepatocellular carcinomas and several other cancer types\(^20,64\). Most of these mutations are located in the kinase domain and are homologous to cancer-inducing mutations that occur in other RTKs (such as EGFR, RET and KIT). When used to replace endogenous Met in the mouse germ line, these mutations cause a variety of tumours, including sarcomas, lymphomas and carcinomas. When expressed in the mammary gland, they induce basel-like breast carcinomas\(^56,66\). Thus, these studies constitute proof of concept that aberrant MET signalling can cause human cancer. Activating mutations of MET are clonally selected for during the metastasis of human head and neck cancers\(^8\), as their frequency increased from 2% in the primary tumours to 50% in the metastases, and this constitutes additional proof of principle that, at least in this type of tumour, aberrant MET is associated with progression and metastasis. Finally, in certain human gastric and colorectal carcinomas, as well as in other tumours\(^48–51\), amplification of MET (on chromosome 7q31) can occur.

Crosstalk between MET and other signalling pathways. Functional crosstalk of MET with EGFR, ERBB2 or insulin-like growth factor 1 receptor (IGF1R) has been reported in several systems\(^22,73\) and has emerged as a major mechanism for cancer progression and resistance to therapy. Even in cells that express moderate levels of EGFR and MET, EGFR stimulation results in MET phosphorylation and activation\(^74\). Conversely, MET amplification can activate ERBB3–PI3K–AKT signalling in lung cancer cells that carry EGFR mutants that are resistant to EGFR kinase inhibitors\(^35\), but resistance can be prevented by combined inhibition of EGFR and MET, as shown in human lung, pancreatic and breast tumour xenografts (see, for example, REF.S 76,77). The semaphorin 4D receptor also controls cell migration by coupling with MET\(^29,79\).

Crosstalk between MET and developmental signalling pathways, such as WNT–β-catenin and TGFβ–bone morphogenetic protein (BMP), has also been demonstrated. Mutation or overexpression of key components of the WNT–β-catenin pathway can cause cancer\(^86\). MET is a direct transcriptional target of WNT–β-catenin in colon cancer cell lines and other tissues\(^81\) (FIG. 3); conversely, MET and integrin α3β1 signalling regulate the transcription of WNT7B in the kidney\(^85\). Moreover, HGF/SF induces the nuclear translocation of β-catenin–TCF and the transcription of their target genes in liver and bladder cancer cells\(^34\), as well as the tyrosine phosphorylation of BCL52 (also known as BCL9L) in colon cancer cells\(^84\).

An intricate interaction between TGFβ and MET signalling has been discovered by genetic experiments in mice\(^86\). Following mutation of TGFβ receptor II (Tgfbr2) in mesenchymal cells, epithelial tumours developed in the fore-stomach and the mammary gland as a result of the upregulation of stromal HGF/SF and MET activation in epithelial cells. Further functional links of MET have been uncovered with tetraspanins and the tumour suppressors INK4A and ARF, which are encoded by the CDKN2A locus. The tetraspanins kanga 1 (KA1; also known as CD82) and CD151 can attenuate integrin-mediated adhesion and MET signalling in cancer cells by inhibiting SRC or by preventing the binding of GRB2 and PI3K to MET\(^86,87\). Finally, genetic and biochemical experiments have defined a functional link between MET, INK4A and ARF in the origin of rhabdomyosarcoma, a tumour that occurs with high penetrance and short latency in Cdkn2a\(^–/–\) mice that overexpress HGF/SF\(^86\).

HGF/SF and MET in angiogenesis. Angiogenesis and lymphangiogenesis are important processes in tumour formation and metastasis. The vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) families have a prime role in both processes but HGF/SF–MET signalling is a potent inducer of endothelial cell growth and promotes angiogenesis and lymphangiogenesis in vitro and in vivo\(^80–91\). Furthermore, MET signalling can induce VEGFA expression and angiogenesis through common signalling intermediates such as SRC homology 2 domain-containing proteins (SHCs). Thrombospondin 1 (TSP1; also known as THBS1) is a negative regulator of angiogenesis that is suppressed by HGF/SF; by ‘turning on’ VEGFA and ‘turning off’ TSP1, HGF/SF–MET functions as a potent regulator of the angiogenic switch\(^82\). HGF/SF–MET and VEGF–VEGFR2 cooperate in inducing angiogenesis in vitro and in vivo. MET and VEGFR do not physically associate or trans-phosphorylate each other but they synergistically activate common signalling intermediates: ERK–MAPK, AKT and focal adhesion kinase (FAK)\(^81\). In line with these results, MET kinase inhibitors and a peptide
that contained the MET bidentate docking site blocked cancer growth and decreased the number of blood vessels in experimental tumours.64,65

A further interesting aspect of MET biology in tumours that has emerged in recent years is the regulation of MET expression and activity by hypoxia. Hypoxia induces the expression of the transcription factor hypoxia-inducible factor 1α (HIF1α), and HIF (which is comprised, in this case, of HIF1α and HIF1β (also known as ARNT)) by inhibition of the β-catenin degradation complex. The presence of these cells, therefore, has implications for tumour therapy, although their actual role in progression and metastasis is debated.66

HGF/SF and MET signalling in metastasis. The ability of HGF/SF–MET to induce metastasis in different organs has been shown experimentally with xenografts of tumour cells that are transfected with HGF/SF or MET, as well as in transgenic mice that overexpress HGF/SF or MET. A role of MET in metastatic progression has also been established in patients with head and neck cancer.67 The bidentate docking site of MET is essential for the role of MET in metastasis, because single point mutations at this site have been shown to prevent experimental metastases induced by TPR–MET in vivo.68 RAS–MAPK and RAC1 signalling are important in the early steps of metastasis. The presence of these cells, therefore, has implications for tumour therapy, although their actual role in progression and metastasis is debated.

Developmental signalling pathways have crucial roles in the generation and maintenance of stem cells and cancer stem cells, and a role for HGF/SF and MET in mesenchymal and haematopoietic progenitor and stem cells has recently been demonstrated. HGF/SF is involved in the mobilization of cardiac stem cells after myocardial infarction and it has been implicated in the activation of satellite cells, which are the stem cells of adult muscle. The developing liver harbours bipotent hepatic stem or progenitor cells, which could be enriched in the presence of HGF/SF. Further, HGF/SF–MET signalling also has important roles in stem or progenitor cell functions in both the developing and the adult pancreas.

A recent report has demonstrated that HGF/SF–MET signalling is essential for the maintenance of colon cancer stem cells, as these cells depend on mesenchyme (myofibroblast)-derived HGF/SF for cooperation between the HGF and WNT–β-catenin pathways. A recent report has shown that interaction of stroma-derived hepatocyte growth factor/scatter factor (HGF/SF) controls the maintenance of stem cell-like properties of colon cancer cells, which is a function of WNT–β-catenin signalling. A stem cell niche (top of the figure) contains epithelial (cancer) stem cells (shown in yellow) that are surrounded by mesenchymal (myofibroblast) niche cells (shown in blue), which secrete HGF/SF. Multiple mechanisms have been reported to allow interactions between MET and WNT–β-catenin signalling in epithelial cells, of which a few are shown here. MET can contribute to the transcriptional activation of WNT ligands, such as WNT7B. MET can also induce the activation of β-catenin–TCF–LEF-target genes; for example, through direct or indirect (SRC) tyrosine phosphorylation (P) and nuclear targeting of β-catenin, or by inhibition of β-catenin degradation complex by AKT phosphorylation of glycogen synthase kinase-3β (GSK3β), β-TrCP, β-transducin repeat-containing protein; APC, adenomatous polyposis coli; CBP, CREB-binding protein; CK1, casein kinase 1; DSH, dishevelled; FRZ, frizzled; GAB1, GRB2-associated-binding protein 1; GRB2, growth factor receptor-bound protein 2; LRP, low-density lipoprotein receptor-related protein; PLC, phospholipase C; PYGO, pygopus.
REVIEWS

WNT–β-catenin-dependent transcription and stemness\(^{111}\) (FIG. 5). These findings are important because, although the role of WNT–β-catenin signalling in cancer stem cells is well known, that of HGF/SF–MET has only just emerged\(^{127,130}\). There may be additional tumour pathways in which the WNT and MET pathways cooperate in maintaining a cancer stem cell compartment. For example, in human breast cancer metastases to the bone, bone-derived or autocrine HGF/SF activates MET–SRC–WNT signalling\(^{126}\). Intratumoral MET and stromal HGF/SF affect growth and the prognosis of patients with non-small-cell lung cancer (NSCLC)\(^{127}\). In multiple myeloma, HGF/SF–MET and WNT are aberrantly activated and interference with either pathway inhibits the growth of multiple myeloma cells\(^{128,129}\).

**HGF/SF and MET inhibitors for cancer therapy**

An increased understanding of the structure–function relationship of ligand, receptor and activators has enabled considerable progress in the development of HGF/SF–MET inhibitors for cancer therapy. The first three classes of inhibitors are discussed below. Inhibitors of HGF/SF activators, HGF/SF inhibitors and MET antagonists are protein therapeutics that function outside target cells. The kinase inhibitors function inside the cell and have constituted, so far, the largest effort within the pharmaceutical industry towards MET-based therapies. Inhibitors of the downstream components of MET signalling, such as inhibitors of RAS, RAF1, SRC, SHP2, MAPKs, AKT and others, are not discussed in this Review.

**Inhibitors of HGF/SF activators and HGF/SF.** Crystallographic and functional studies by scientists at Genentech, USA, have shown that the first Kunitz domain (KD1) of HAI1 inhibits the activity of the catalytic domain of HGF/A, blocking access to pro-HGF/SF\(^{130}\). The specificity of KD1 mirrors that of the HAI1 ecto-domain, and pegylated KD1 was shown to inhibit the invasion and metastasis of human prostatic cancer cells that overexpress hepsin in severe combined immunodeficient (SCID) mice\(^{131}\). HGF antibodies that bind either the substrate-binding domain (Fab58 (REF. 132)) or outside the catalytic cleft (Fab40 (REF. 133)), as well as anti-matriptase antibodies (FabE2 (REF. 134)), have also been developed (FIG. 4a).

In a separate approach, Vande Woude and colleagues\(^{135}\) have demonstrated that antibodies directed towards HGF/SF inhibit the growth of cancer cell lines that are dependent on HGF/SF–MET signalling\(^{136}\). These early studies relied on mixtures of antibodies directed against two or more epitopes, but subsequent work has led to the isolation of individual monoclonal antibodies that can block HGF/SF binding to MET. One of these, AMG102, binds the SPH domain\(^{138}\) (FIG. 4b).

**MET antagonists.** Truncated splice variants of HGF/SF have formed a strong basis for developing MET antagonists (FIG. 4c). NK1, the shortest splice variant of HGF/SF, has agonistic activity \(\text{in vivo}^{137}\) and dimerizes in solution in the presence of heparin, but it can be converted into a receptor antagonist by the mutation of residues at the dimer interface, such as Tyr124 and Asn127 (REFS 138,139). NK2, the most abundant alternative splice variant of HGF/SF, has partial agonistic and antagonistic activity \(\text{in vivo}^{140}\), and mutation of an unpaired cysteine (Cys214) yields a variant with receptor antagonistic activity\(^{134}\). Finally, NK4, a fragment that corresponds to the α-chain of HGF/SF (FIG. 1a), has been extensively characterized as a receptor antagonist by Nakamura, Matsumoto and colleagues\(^{141}\). In addition to MET antagonistic activity, NK4 has broad anti-angiogenic activities against HGF/SF–, VEGF– or bFGF (also known as FGF2)-induced angiogenesis\(^{142}\); however, NK4 is more difficult to produce than NK1 and NK2 and may require delivery via gene therapy.

Several MET antibodies with antagonistic activity are now available (FIG. 4d). METMab (also known as onartuzumab) is a monovalent antibody developed at Genentech that binds the SEMA domain of MET\(^{143}\) and that displays potent antagonistic activity\(^{144}\). METMab may act as a classic receptor antagonist by competing for the binding of HGF/SF to MET. The MET antibody DN-30 causes MET activation and shedding through ADAM10 (REFS 145,146). Conversion of the intact IgG into a monovalent form abolished agonistic activity and yielded a bona fide antagonist\(^{145}\). A MET antibody with antagonistic activity in a bivalent format (11E1) has also been described\(^{146}\). The mechanism for the antagonistic activity of 11E1 is unclear but is probably different from those of METMab and DN-30.

**MET kinase inhibitors.** Impressive numbers of MET kinase inhibitors have been developed over the past 10 years. Compounds for which structural data have been made available (see the MET inhibitors online table; see Further information) are discussed below (see REF. 149 for a recent review). The catalytic domain of MET has the typical architecture of other protein kinases in which an N-terminal domain (N) with a predominant β-structure is connected by a short linker to an α-helical C-terminal domain (C). Structures of the auto-inhibited\(^{150}\) and catalytically active form of the MET kinase\(^{111}\) are available and, in line with results from studies on other protein kinases, the switch from the inactive to the active conformation is associated with major structural rearrangements (FIG. 5a). In the inactive conformation, the activation loop blocks access of ATP to the enzyme but on activation the loop is extruded (FIG. 5b). ATP binds in a pocket between the N and C lobes and makes extensive hydrogen and ion bonds with residues in the linker (Pro1158 and Met1160), the nucleotide-binding loop (Gly1087 and His1088), the catalytic loop (Asp1204, Arg1208 and Asn1209) and the activation loop (Asp1225)\(^{152}\) (FIG. 5c).

Superimposed structures of the ATP–MET kinase complex and 25 crystal structures of inhibitor–MET kinase complexes show that inhibitors can be clustered in three groups that differ in their mode of binding (FIG. 5d) (see the MET inhibitors online table; see Further information). Type I compounds and the binding of one of these (PF-02341066 (also known as crizotinib);
Met1160 and Asp1222, and ππ stacking interactions with Tyr1230 of the activation loop. Most type I compounds display preferential binding to the inactive conformation of the enzyme and have limited activity against the Tyr1230H mutation that is present in certain human tumours. However, there are type I compounds, for example MK-2461, that have a different set of contacts and a strong binding preference for the active form of the kinase.

Type II inhibitors (FIG. 5d) also occupy the ATP-binding pocket but also extend into a second pocket that is formed when the side chain of Asp1222, a residue of the activation loop that coordinates a Mg2+ ion bound to ATP during catalysis, instead points away from the ATP-binding pocket. The resulting, inactive ‘DFG out’ conformation that is adopted by residues Asp1222, Phe1223 and Gly1224 enables the binding of type II inhibitors (FIG. 5f) (such as compound BMS-777607 (PDB ID: 3F82)153). A smaller number of type III compounds (FIG. 5d) occupy the ATP-binding pocket and extend into a hydrophobic cavity that is formed by the displacement of the αC helix rather than into the pocket that is formed by the DFG out conformation (such as compound MT3 (PDB ID: 3EFJ)155) (FIG. 5g). Whereas the majority of MET kinase inhibitors that are under development can be clustered into these three main types, there are exceptions. For example, ARQ 197 (also known as tivantinib), like type I inhibitors, inhibits ATP binding to the MET kinase in a non-competitive manner158, binds the ATP binding cleft and makes canonical contacts with Met1160 but it also occupies a small hydrophobic pocket located between Phe1089 of the glycine-rich loop and Phe1223 (REF. 157).

The different binding modes of the available MET inhibitors have implications for specificity and activity. Although the body of data available is very large, it remains incomplete. As a result, the activity of the MET inhibitors against kinases other than in vitro kinase platforms or a limited set of kinase mutants has not been extensively studied and the activity profile of individual inhibitors may change as additional data are obtained. It is also clear that even small modifications can have a profound effect on both potency and/or specificity and, as a great deal of work on the MET kinase inhibitors is still in progress, the activity of several final drugs might display significantly improved profiles compared with those of the lead compounds that have been initially reported.

For example, type I inhibitors have generally been described as specific for the MET kinase (see the MET inhibitors online table; see Further information). However, PF-02341066 has strong activity against anaplastic lymphoma kinase (ALK) and has recently demonstrated impressive therapeutic activity in a group of patients with NSCLC carrying EML4–ALK fusions158. Among the type II inhibitors are compounds that have been described as specific (for example, L8V) but also others that inhibit multiple RTKs. For example, BMS-777607 has strong activity against MET, RON (also known as MST1R), AXL (also known as UFO) and TYRO3, and XL880 (also known as GSK1363089 and foretinib) inhibits MET, AXL, VEGFR2, platelet-derived growth factor receptor-β (PDGFRβ) and TIE2 (also known as TEK) (see the MET inhibitors online database).
ous clinical trials that are in progress important component of study design in the numer ‑ needs further development and is not currently an HGF/SF–MET expression or MET phosphorylation and deployed. Thus, patient stratification according to (FIG. 6a,b) (see the Clinical Trials Involving HGF/SF–MET Inhibitors online table; see Further information). Here, we argue for patient stratification as an essential component for ther‑ sis of HGF/SF–MET expression levels160 and/or receptor activity have not been extensively validated and deployed. Thus, patient stratification according to HGF/SF–MET expression or MET phosphorylation needs further development and is not currently an important component of study design in the numerous clinical trials that are in progress (FIG. 6a,b) (see the Clinical Trials Involving HGF/SF–MET Inhibitors online table; see Further information). Here, we argue for patient stratification as an essential component for therapeutic success and suggest that antibody‑based analysis of HGF/SF–MET expression levels160 and/or receptor phosphorylation161 may constitute valid strategies.

**Targeting HGF/SF–MET in cancer**

**Patient stratification.** The most notable advances in cancer therapy that have occurred in the past decade — for example, with tumours carrying BCR–ABL or EML4–ALK fusion genes158,159 — have resulted from three crucial factors: a genetic defect yielding a single target for therapy and the availability of an effective inhibitor and effective methods for the identification of tumours carrying the relevant genetic defect. In the case of HGF/SF–MET, the role of aberrant signalling in cancer is clear, and effective therapeutics are now available, but methods for assessing the level of HGF/SF–MET expression and activity have not been extensively validated and deployed. Thus, patient stratification according to HGF/SF–MET expression or MET phosphorylation needs further development and is not currently an important component of study design in the numerous clinical trials that are in progress (FIG. 6a,b) (see the Clinical Trials Involving HGF/SF–MET Inhibitors online table; see Further information). Here, we argue for patient stratification as an essential component for therapeutic success and suggest that antibody‑based analysis of HGF/SF–MET expression levels160 and/or receptor phosphorylation161 may constitute valid strategies.

**MET signalling crosstalk and therapy.** In recent years signalling crosstalk has evolved from a loose biochemical concept to one with a rigorous genetic foundation83,85 and major clinical relevance, as demonstrated by the findings of studies with MET and EGFR in NSCLC75,76 (discussed above). This mechanism is also active in a subset of breast cancers162 and might be at work in other tumours, as revealed by preclinical studies with human tumour xenografts163. Conversely, the treatment of tumour cells with MET kinase inhibitors may lead to the selection of tumour cell populations that escape growth inhibition via the EGFR or SRC kinases164–166. The implications of these findings for therapy are clear and argue for a shift from monother‑apy to combination (multi‑target) therapies in which both the signalling pathway primarily responsible for the cancer phenotype and the ‘rescue pathways’ are targeted concurrently (FIG. 6c).

**Anti‑angiogenesis therapy and MET activation.** Anti‑angiogenesis therapies have been shown to impair the growth of a number of experimental and human tumours and are currently used in metastatic colon cancer and NSCLC; the rationale for combining anti‑angiogenesis therapies with inhibitors of MET is discussed above. Concurrent inhibition of VEGFR and MET can be achieved either by combining specific VEGF–VEGFR and MET inhibitors or by dual or multi‑specificity kinase inhibitors that inhibit both MET and VEGFR2, as has been shown in human tumour
Figure 6 | **Clinical trials with HGF/SF–MET inhibitors.** The figure illustrates data from 96 clinical studies involving antibodies to hepatocyte growth factor/scatter factor (HGF/SF) or MET and small-molecule inhibitors of the MET kinase listed in the US National Institutes of Health registry of Clinical Trials (see the ClinicalTrials.gov website and the Clinical Trials Involving HGF/SF–MET Inhibitors online table; see Further information).  

**a** | The distribution according to study type and stage (96 trials) is shown. 54% of these trials are Phase I studies primarily focusing on drug dosage and safety; whereas, 46% are Phase I/II, Phase II and Phase III trials addressing clinical efficacy.  

**b** | The distribution according to tumour type (84 trials) is shown. These 84 trials involve cancer patients, of which 41% had advanced stage multiple solid tumours; whereas, 59% had specific tumour types. Among the 59%, the studies on lung tumours constitute the largest group (21%), followed by brain tumours (7%) and tumours of the gastrointestinal (GI) tract (6%) and liver (6%).  

**c** | The distribution according to therapeutic strategy (monotherapy versus combined therapy) is shown. Of 44 efficacy studies (Phase I/II, Phase II and Phase III), 41% involve HGF/SF–MET monotherapies, 21% involve an HGF/SF–MET drug combined with chemotherapy, and 27% and 6% involve an HGF/SF–MET drug combined with inhibitors of epidermal growth factor receptor (EGFR) or vascular endothelial growth factor receptor (VEGFR), respectively. Note that monotherapy includes not only specific HGF/SF–MET inhibitors but also agents with multiple targets.

**Progression-free survival (PFS).** A statistical parameter that measures the time — for example, after diagnosis and/or treatment — in which the disease remains stable (progression free). It can also be expressed as the proportion of patients whose disease has remained stable after diagnosis and/or treatment at a specified time.

**Overall survival**

A statistical parameter that measures the survival time of a patient or a patient group after diagnosis and/or treatment, regardless of the cause of death. It can also be expressed as the proportion of patients who remain alive at a specified time.

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**Early results from clinical trials.** The vast majority of the clinical trials that aim to define the efficacy of HGF/SF–MET therapeutics are currently in progress but initial results from several studies have been made available. Striking results with the MET antibody METMab in combination with an EGFR inhibitor (erlotinib) have been reported in patients with NSCLC167. As determined retrospectively by immunohistochemistry, METMab increased progression-free survival (PFS) in patients with high levels of MET expression compared with the group receiving erlotinib alone; however, patients with low or no MET expression experienced decreased PFS167. Improvement in overall survival has also been reported in a different patient group — specifically, patients with advanced gastric adenocarcinoma in which treatment with the HGF/SF monoclonal antibody AMG102 (also known as rilotumumab) combined with chemotherapy was compared with chemotherapy alone168. Even in this study, the best response was observed in patients expressing a high level of MET in the tumour168. Thus, both the study with METMab in NSCLC and the study with AMG102 in gastric cancer highlight an essential requirement for patient stratification to ensure clinical benefit.

A large number of kinase inhibitors are now in clinical trials (FIG. 6c) (see the Clinical Trials Involving HGF/SF–MET Inhibitors online table; see Further information). A Phase II study with ARQ 197 in patients with NSCLC has shown a clear trend of improved PFS and overall survival in patients treated with the inhibitor plus erlotinib compared with patients who received erlotinib and a placebo169; a trial with this drug combination has now advanced to a Phase III study. In other clinical trials, treatment with ARQ 197 alone also inhibited the growth of hepatocellular and pancreatic carcinomas, as well as tumours driven by microphthalmia-associated transcription factor (MITF)170. A recent report has documented striking activity of the MET and ALK inhibitor PF-02341066 in patients with NSCLC carrying an EML4–ALK fusion158. The EML4–ALK fusion protein occurs in 2–7% of patients with NSCLC, and PF-02341066 induced a major therapeutic response in this patient group compared with standard chemotherapy175. The bulk of the therapeutic effect of PF-02341066 in patients with NSCLC carrying the EML4–ALK fusion protein is most probably due to inhibition of ALK158, but the drug is also a potent MET kinase inhibitor and it would be interesting, therefore, to further analyse the patient response on the basis of the level of MET expression in the tumour.

Studies with the multi-target MET inhibitor XL184 (also known as caboctinib) have shown significant activity against a number of solid tumours, including breast cancer, NSCLC, melanoma and liver cancer171. Ovarian cancer172 displayed notable responses to XL184, but the most remarkable response was seen in both soft tissue and bone metastatic lesions in patients with metastatic castration-resistant prostate cancer (CRPC)173. The success of XL184 against CRPC primary and metastatic tumours marks a turning point for MET kinase inhibitors and their power to change terminal cancer progress. XL184 also showed activity against medullary thyroid cancer174 and the range of applications for this drug may further expand.

Finally, the multi-target MET inhibitor XL880 has been reported to cause tumour reduction in patients with breast cancer with resistance to inhibitors of EGFR (such as erlotinib) or EGFR and ERBB2 (such as lapatinib)175, a result that mirrors those obtained with MET and EGFR inhibitors in NSCLC.

**Conclusions and perspectives**

The availability of a wealth of HGF/SF–MET inhibitors with a range of potencies and specificities has provided a strong basis for assessing the therapeutic value of HGF/SF–MET inhibition in human cancer, and initial results from clinical studies have demonstrated therapeutic benefits in patients with a variety of advanced or metastatic tumours, including NSCLC, and breast, prostate, liver and renal cancer. These results have enabled the progression of several compounds to Phase III trials, and larger studies and rigorous patient stratification procedures will further clarify the therapeutic value and long-term safety of HGF/SF–MET inhibitors in cancer patients.

With the exception of biological agents such as METMab, and the low-molecular-mass compound ARQ 197, the first group of therapeutics to reach Phase II and Phase III studies predominantly included inhibitors with multiple specificity that, in addition to MET,
target other RTKs involved in cancer, such as VEGFR and RET. This makes it currently difficult to define the contribution of MET inhibition to the overall therapeutic response but comparisons of results with more specific kinase inhibitors or biological molecules is likely to define the clinical benefit derived from targeting MET in the future. Whether the emergence of resistance to MET kinase inhibitors will constitute a serious limitation for this class of therapeutics is currently difficult to assess. Resistance can rapidly develop with cancer cell lines in culture through multiple mechanisms, but patient data are clearly required to define the potential effect on therapeutic outcome. Resistance to kinase inhibitors can also be circumvented by combining different types of inhibitors, as demonstrated recently for BCR–ABL, or by shifting to the use of MET antagonists.

Even at this early stage of clinical investigation, however, it is safe to conclude that inhibition of HGF/SF–MET signalling in cancer has evolved during the past decade from a concept built on strong experimental foundations (activity on cells and mouse models of disease) to one with considerable scope for the control of human cancer.

3. References 1 and 2 report a new transforming gene (MET) from a human osteosarcoma cell line treated with N-methyl-N-nitrosourea. Subsequent work established that it is the fusion of regulatory sequences from chromosome 1 (TPR) and sequences from chromosome 7 encoding a receptor tyrosine kinase (MET).
5. Reference 3 shows that cells made autocrine for HGF/SF–MET expression become highly metastatic in immunocompromised mice.
10. References 4–7 describe the isolation, cloning and characterization of a fibroblast growth factor/hepatocyte growth factor activator by thrombin.
11. Schmidt, C. et al. Scatter factor/hepatocyte growth factor by the transmembrane serine proteases known as EMT.
14. References 8 and 9 describe both the apo structure, as well as the structure of the kinase domain in complex with the Sema domain of the Met receptor.
16. Reference 21 describes the first crystal structures of the kinase domain of MET. The report describes both the apo structure, as well as the structure of the kinase domain in complex with the inhibitor K-252A. Reference 22 describes Cryo-EM and SAXS structures of HGF/MET complexes.
27. Reference 34 and 35 report on the first two crystal structures of fragments of the MET ectodomain in complex with the SHP domain of HGF/SF (reference 34) or the bacterial protein InhB (reference 35).
29. This report describes the bidendate docking site of MET (Y1349 and Y1356), which is essential in MET signalling and binding of metalloproteases.
The role of MET in liver regeneration and skin wound healing.

References 56, 57 and 60 describe an essential role for MET in hepatic regeneration and skin wound healing.


REVIEWS


121. Ubarbaker, K. et al. Cardiac stem cells possess growth factor-receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long-term survival. Circ. Res. 97, 663–672 (2000).


142. References 166 and 167 are the first reports to demonstrate that combined treatment of subgroups of patients with NSCLC with EGFR and MET inhibitors increases progression-free survival and overall survival.


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**Competing interests statement**
The authors declare no competing financial interests.

**DATABASES**
- AMG102 | ARQ 197 | BMS-777607 | erlotinib | lapatinib | METMab | PF-02341066 | XL184 | XL880

**FURTHER INFORMATION**
- Clinical Trials Involving HGF/SF-MET Inhibitors: [www.vai.org/metclinicaltrials](http://www.vai.org/metclinicaltrials)
- HGF/SF - MET and cancer: [http://www.vai.org/met/](http://www.vai.org/met/)
- MET inhibitors: [http://www.vai.org/metinhibitors/](http://www.vai.org/metinhibitors/)

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