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# Targeting the MET oncogene in cancer and metastases

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## Abstract

Importance of the field: ‘Invasive growth’ is a genetic program involved in embryonic development and adult organ regeneration and usurped by cancer cells. Although its control is complex, tumor- and context-specific and regulated by several cytokines and growth factors, the role played by the MET oncogene is well documented. In human cancers the contribution of MET to invasive growth is mainly through overexpression, driven by unfavorable microenvironmental conditions. MET activation confers a selective advantage to neoplastic cells in tumor progression and drug resistance. A subset of tumors feature alterations of the MET gene and a consequent MET-addicted phenotype. Areas covered in this review: The molecular basis and rationale of MET inhibition in cancer and metastases are discussed. A number of molecules designed to block MET signaling are under development and several Phase II trials are ongoing.

What the reader will gain: Knowledge of the state of the art of anti-MET targeted approaches and the molecular basis and strategies to select patients eligible for treatment with MET inhibitors.

Take home message: Due to its versatile functions MET is a promising candidate for cancer therapy. Understanding molecular mechanisms of sensitization and resistance to MET inhibitors is a priority to guide tailored therapies and select patients that are most likely to achieve a clinical benefit.

Keywords: gene amplification, ligand antagonists, neutralizing antibodies, oncogene addiction, small-molecule inhibitors, somatic mutation

## 1. Introduction

### 1.1 ‘Invasive growth’: a genetic program

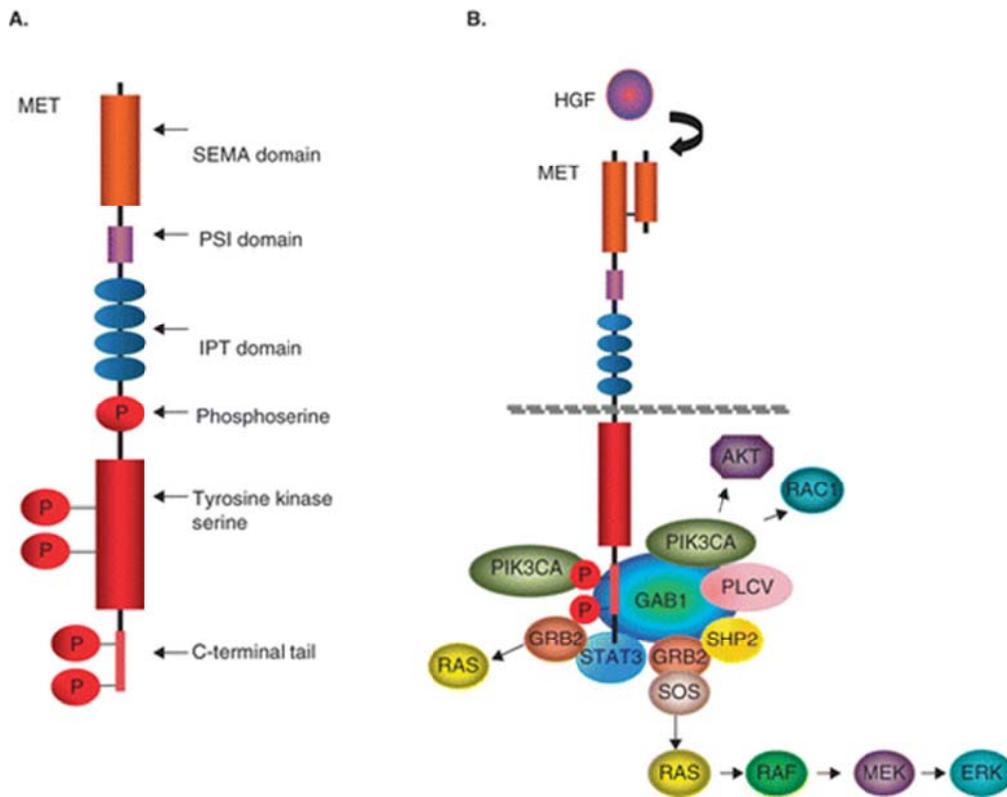
Cancer is a progressive process through which cells accumulate genetic lesions that are responsible either for oncogenic activation or inactivation of tumor suppressor genes [1]. These events lead to transformation of a normal cell into a malignant clone. Indeed a growing tumor is defined as malignant when transformed cells acquire the capability to disseminate from their primary context and to colonize other tissues and organs. Through tumor progression cells proliferate without control, loose contact-inhibition, detach from their primitive site and give rise to secondary macroscopic lesions. Metastases are generally poorly treatable and eventually lead to patient's death. The ability of neoplastic cells to spread involves the execution of a complex genetic program named ‘invasive growth’ [2]. This process involves integration of different biological activities: cellular proliferation, cell–cell dissociation (‘scattering’), migration, invasion and avoidance of apoptosis induced by inadequate or inappropriate cell-matrix interactions (anoikis). Indeed invasive growth does not only occur in cancer cells. It is essential in a wide variety of physiological and pathological settings. During embryogenesis it drives key events, such as gastrulation and nervous system development; in post-natal life it is involved in inflammatory response and tissue regeneration after injuries. The aberrant execution of the

invasive growth program is otherwise responsible of aggressive phenotype that defines malignant behavior and leads to metastatic progression.

Several cytokines and growth factors are involved in promoting proliferation, chemotaxis, migration and protection from apoptosis: among them are EGF, IGF-1, fibroblast growth factor (FGF) and TGF-beta. However, there is now firm evidence that the invasive growth program, as a whole, is controlled by a discrete family of soluble factors known as scatter factors [3], represented by hepatocyte growth factor (HGF) and Macrophage Stimulating Protein (MSP). Their receptors are the tyrosine-kinases encoded by MET and RON oncogenes. Moreover, two other families of molecules structurally related to MET are likely to be involved in this program: semaphorins, that act as ligands, and plexins that act as receptors [2].

The signaling pathway of HGF is mediated by its receptor encoded by the MET proto-oncogene [4,5], located on chromosome 7q31.1. It is constituted of 21 exons encoding a transmembrane tyrosine kinase made of a disulphide-linked heterodimer, which originates from the proteolytic cleavage of a single-chain precursor. The heterodimer is formed by a single-pass transmembrane 145 kDa  $\beta$ -chain and an extracellular 50 kDa  $\alpha$ -chain. The extracellular region contains a conserved region of 500 aminoacids, named the semaphorin (SEMA) domain, involved in ligand-receptor interaction; a cysteine-rich domain made of 80 amino acids known as MET-related sequence (MRS) and a protein-protein interaction domain made of four immunoglobulin-like structures (integrin, plexin, transcription factor (IPT) domain). In the intracellular portion the juxtamembrane region contains the residue Ser 985 which is essential for receptor downregulation and a tyrosine (Tyr 1003) that, upon phosphorylation, binds the E3-ubiquitin ligase Cbl, which finally promotes receptor ubiquitination and degradation. The catalytic site of MET contains two tyrosines (Tyr 1234 and Tyr 1235), regulating the enzymatic activity. Finally, in the C-terminal regulatory tail are located two tyrosine residues (Tyr 1349 and Tyr 356) that, when phosphorylated, create a unique docking site which is responsible for the recruitment of a large-spectrum of downstream signal transducers, such as the cytosolic tyrosine kinase SRC, the lipid kinase PI3K, the transcription factor signal transducer and activator of transcription3 (STAT3) and the adaptor proteins growth factor receptor bound protein 2 (GRB2), Src homology 2 domain containing) transforming protein (SHC) and GRB2-associated binder 1 (Gab1) (Figure 1) [2].

Figure 1. The MET receptor structure and signalling pathway. A. MET receptor schematic functional structure. MET is a single-pass disulphide-linked  $\alpha/\beta$  heterodimer that is formed through a proteolytic processing of a common precursor in the post-Golgi compartment. The extracellular portion of the receptor is composed of three domains. The semaphorin (SEMA) domain encompasses the whole  $\alpha$ -chain and a part of the  $\beta$ -subunit; a homologous region is present in plexins and semaphorins. The SEMA domain is followed by a PSI domain – also found in plexins, semaphorins and integrins – that spans 50 residues and contains four disulphide bonds. The following four integrin, plexin, transcription factor (IPT) domains display a immunoglobulin-like structure that is detectable also in the structure of plexins and growth factors. The intracellular region includes – in the juxtamembrane portion – the serine 975 which down-regulates the kinase activity. Indeed phosphorylation of the two tyrosines (Tyr 1234–Tyr1235) located in the tyrosine kinase site activates the enzymatic function of the receptor. The carboxy-terminal tails includes two critical tyrosine residues (Tyr 129 and Tyr1256) that upon phosphorylation induce the recruitment of several transducers. B. MET signaling pathway. Through the activation multifunctional docking site – located at the C-terminal tail – and the association with the multi-adaptor growth factor receptor bound protein 2-associated protein 1 (GAB1), MET phosphorylation induces recruitment of several SH2-domain containing transducers: growth factor receptor bound protein 2 (GRB 2)–son of sevenless (SOS)–RAS-RAF–MAP kinase-ERK kinase (MEK)–extracellular signal regulated kinase (ERK) pathway as well as the PIK3CA–AKT axis and the SCR, signal transducer and activator of transcription 3 (STAT3) cascade. Overall these transducers amplify the MET-driven intracellular signal leading to enhancement of cell proliferation, survival and motility.



When the receptor is in a quiescent status its conformation is inaccessible to ATP. HGF binding induces MET dimerization and activation by trans-phosphorylation of residues Tyr 1234 and Tyr 1235 [6]. Receptor activation leads to intramolecular phosphorylation of two tyrosines (Tyr 1349 and Tyr 1356) forming the docking site and activating the downstream signaling pathway cascade. In this way the MET receptor is able to trigger a wide spectrum of biological responses that eventually define the invasive growth program. While in physiological contexts MET activation is a transient event, during transformation/metastatic process MET is unleashed with consequent constitutive activation of its downstream transducers [7,8].

## 2. Targeting the MET oncogene

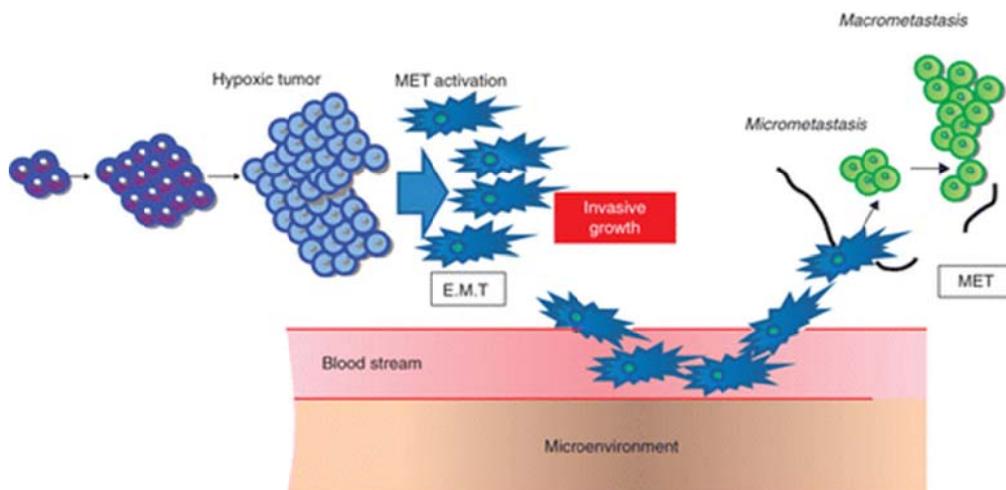
### 2.1 MET activation in cancer and metastases

Cancer cells inappropriately execute the MET-driven invasive growth program that is actively involved in tumor onset and progression. MET activation is implicated both as a primary (causative) event in neoplastic transformation as well as in malignant spread, as a consequence of its growth promoting activity on one hand, and enhancement of cell motility and anoikis avoidance on the other [9]. Receptor constitutive activation results from different mechanisms: i) HGF-dependent activation either in a paracrine or in autocrine manner. Autocrine activation occurs when tumor cells aberrantly express both HGF and its receptor. Paracrine mechanisms, typical of physiological conditions, can become pathological in the presence of abnormal HGF production by mesenchymal cells; ii) HGF-independent mechanisms. MET activation can indeed occur in an HGF-independent manner through transactivation by other membrane receptors such as CD44 and integrins and signal transducing receptor such as RON, EGFR family members, FAS and B plexins. iii) receptor overexpression, which facilitates receptor oligomerization and reciprocal activation, even in the absence of exogenous ligands; iv) activating point mutations, which results in kinase constitutive activation [10].

MET activation in human cancers is mainly driven by overexpression which is often a consequence of transcriptional upregulation induced by negative microenvironmental conditions. For example, low oxygen tension (hypoxia) in the stroma surrounding the tumor switches on the invasive growth program, which results in cell invasion and metastases. This event is likely to occur rather late during tumor progression, when blood supply becomes insufficient for the growing neoplastic mass. (Figure 2) Noticeably the cross-talk between cancer cells and tumor microenvironment plays a

significant role in MET signaling activation. Hypoxia is invariably followed by MET overexpression and consequent ligand-independent activation, mostly through upregulation of MET transcription [11]. Besides there is considerable evidence that MET activation could be driven by a paracrine loop mediated by HGF production by tumor invading fibroblast-like cells [12]. In some instances MET overexpression is consequent to gene amplification [13-15]. Growing evidence demonstrates that increased MET gene copy number correlates with both malignant progression and drug resistance [16]. Amplification of the MET gene has been reported in a number of human cancers including esophageal and gastric carcinomas [17-20]; NSCLC – with acquired resistance to EGFR inhibitors [21,22], medulloblastomas [23] as well as in 4% of glioblastomas (<http://cancergenome.nih.gov/objects/pdfs/nature07385.pdf>). Increased MET gene copy number can also occur late during neoplastic progression: in colorectal carcinoma (CRC) MET amplification is in fact detected in a high percentage of liver metastases but not in primary lesions [12].

Figure 2. Invasive growth program in cancer. MET activation mediates multiple steps in cancer onset and progression. Primary tumor proliferation leads to imbalance between tumor volume and blood supply. The oxygen deprivation in neoplastic tissue (hypoxia) triggers MET-driven invasive growth. This program results from integration of different biological processes, such as motility, invasion, anoikis avoidance and morphogenesis. Through a mechanism known as epithelial–mesenchymal transition (E.M.T.), cancer cells acquire a metastatic phenotype. Metastatic cells reach a secondary site via blood or lymphatic vessels; after extravasation and the arrest of tumor cells in distant organs, the EMT process could be reverted through a mesenchymal–epithelial transition (M.E.T.); this last step coincides with cell repolarization and terminal differentiation in a tissue pattern that usually resembles branching tubules. Micrometastases can die or can give rise to macroscopic secondary lesions.



Moreover, MET receptor might be activated as a consequence of structural alterations, such as mutations. Somatic mutations occur as a (relatively) rare event in unselected primary solid cancers; reported frequencies account for no more than 3 – 4% of cases ([www.sanger.ac.uk](http://www.sanger.ac.uk)) [24]. Activating point mutations have been described in hereditary and sporadic papillary renal cancer [25], childhood hepatocellular carcinoma [26] and gastric carcinoma [27]; more recent works have reported MET somatic mutations in thoracic neoplasms (lung cancer and malignant pleural mesothelioma) [28] as well as in melanoma [29]. Indeed it has been described that cells carrying mutated MET seem to be selected during progression of head and neck carcinomas as they are more represented in secondary lesions [30].

Finally HGF is able to transcriptionally induce MET, and can activate MET through an autocrine loop in glioblastomas [31], breast carcinomas [32], rhabdomyosarcomas and osteosarcomas [33,34]. It has been established that MET mutations affecting the tyrosine kinase domain require HGF to enhance the catalytic function [35].

## 2.2 MET activation, angiogenesis and blood coagulation

Well-documented experimental evidence demonstrates a role of the HGF/MET pair in angiogenesis and coagulation cascade. MET activation induces a pro-angiogenic effect – which eventually cooperates in sustaining tumor invasiveness [36] – and HGF itself acts as a potent stimulus for endothelial cell growth, migration and survival. These activities on tumor surrounding microenvironment promote neoplastic growth and distant embolization. These observations have relevant implications since malignant behavior of cancer cells is significantly increased by MET pro-angiogenic effect. Indeed blood deprivation leads to oxygen deficiency which, in turn, upregulates MET, thus enhancing cancer cells spreading. Therefore there is a strong rationale to combine MET inhibitors with anti-angiogenic drugs.

Importantly, it has been demonstrated that MET activation alters the coagulation cascade. In 1865 Armand Trousseau [37] investigated the close relationship between malignancies and perturbation of the blood coagulation cascade. The ‘Trousseau syndrome’ includes chronic disseminated intravascular coagulopathy associated with microangiopathy, verrucous endocarditis and arterial emboli in patients affected by carcinomas. Despite it is a well-known clinical entity, the molecular basis of Trousseau's sign has been clarified only in recent times. Boccaccio and colleagues [38,39] provided for the first time experimental evidence that cancer and haemostasis are functionally interconnected through MET activation: introduction of an activated MET oncogene in adult mice (by gene transfer) is able to cause a slowly progressing hepatocarcinogenesis, which is preceded and accompanied by a syndrome ‘manifesting first with blood hypercoagulation (venous thromboses), and then evolving toward fatal internal hemorrhages’. The molecular pathogenesis of Trousseau's sign is related to the MET-induced upregulation of the genes encoding plasminogen activator inhibitor type-1 (PAI-1) and COX-2. These two proteins, ultimately, create a rudimentary fibrin scaffold that further sustains neoangiogenesis and facilitates dissemination of neoplastic emboli.

The close relationship between pro-coagulant and pro-invasive properties of MET is further supported by the HGF structure itself. HGF belongs to the plasminogen protein family and, as in clotting factors, it requires proteolytic cleavage to become biologically active. It is conceivable that HGF and MET genes are themselves ancient members of the coagulation cascade and that they evolved to exploit fibrin polymerization as a scaffold for invasive growth.

### 2.3 Strategies in MET pharmacological targeting

Different strategies have been developed to inhibit MET. They target the different steps of MET signaling activation: i) interaction between MET and its ligand HGF; ii) receptor transphosphorylation and activation; iii) kinase activity and phosphorylation of the signal transducer docking site; iv) interference with the docking site and signal transducers. Moreover, v) the receptor can be wiped out from the cell (quantitatively deregulated) by antibody-induced release (shedding) from the cell surface (Figure 3 and Table 1) [40,41].

Figure 3. MET pharmacological inhibition. Schematic representation of the different levels at which MET pharmacological inhibition can be exploited.

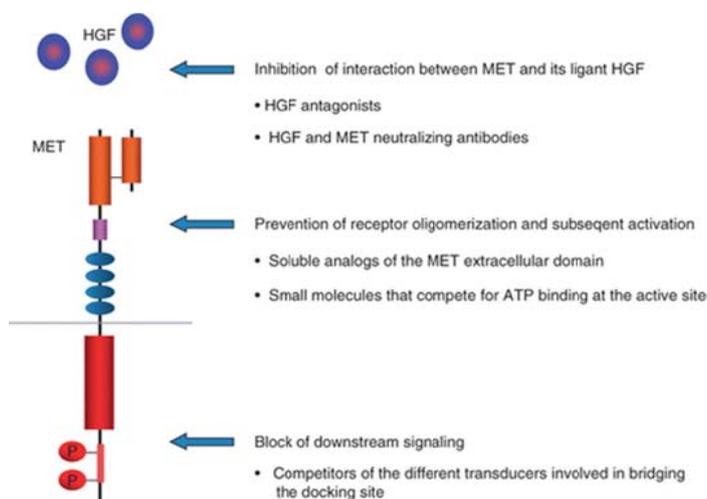


Table 1. MET and HGF inhibitors.

Class of compounds	Mechanism of action	Molecular targets	Molecule (Company)
<i>MET inhibitors</i>			
Small molecules	MET ATP-binding site competitors	MET	ARQ197 (ArQule) PHA665752 (Pfizer) SU11274 (Pfizer) JNJ-38877605 (Johnson & Johnson) SGH523 (SGX Pharmaceuticals) AMG 208 (Amgen) MK2461 (Merck) BMS777607 (BMS) INCB28060 (Incyte) EMD1214063 (EMD Serono)
	Multikinase inhibition	MET, VEGFR, TIE	XL880, XL184 (Exelixis) MGCD265 (Methylgene) E7050 (Eisai)
		MET, PDGFR, c-KIT, RET	MP470 (Supergene)
Antibodies	MET-specific	MET, RON, RET	(Amgen)
	HGF-Specific	MET, ALK	PF-2341066 (Pfizer) OA5D5 (Genentech) DN30 AMG 102 (Amgen) L2G7 (Galaxy) SCH 900105 (Aveo/Schering)
Biological antagonists	Inhibits HGF binding to MET Inhibits proteolytic HGF activation Inhibits HGF binding and MET dimerization		NK4 Uncleavable HGF Decoy MET and SEMA

Data from [10,40,41].

ALK: Anaplastic lymphoma kinase; HGF: Hepatocyte growth factor; PDGFR: Platelet-derived growth factor receptor; SEMA: Semaphorin domain; TIE: Tunica interna endothelial cell kinase.

### 2.3.1 Targeting HGF–MET interaction

HGF–MET interaction might be blocked using HGF antagonists, molecules that bind with high affinity the receptor without activating the downstream signal transducers. The precursor, inactive form, of HGF (pro-HGF) is present in almost all tissues, where it is retained in the extracellular matrix. Many molecules that are present in stroma that surrounds cancer cells display the enzymatic activity necessary to activate pro-HGF. This mechanism of activation has been described in different human tumors [42,43]. HGF contains two binding sites with different affinity for the MET receptor: a high-affinity site located within the  $\alpha$ -chain and a low-affinity site in the  $\beta$ -chain [44,45], which becomes accessible only after pro-HGF activation and which is essential for receptor dimerization and specifically interacts with the SEMA and the IPT domains, respectively [46-48]. Several molecules have been validated as MET antagonists. HGF has two natural splice variants, NK1 and NK2, which contain the N-terminal domain and the first two kringle domains of HGF. NK1 is a MET agonist, which is able to form a head-to-tail dimer complex in crystal structures; mutations in the NK1 interface convert NK1 to a MET antagonist. NK2, is a MET antagonist capable of inhibiting HGF's activity in cell proliferation without a clear mechanism. This HGF fragment has been shown to inhibit HGF-induced epithelial mitogenesis and morphogenesis in vitro [49]. Mutations that were designed to open up the NK2 closed conformation by disrupting the N/K2 interface convert NK2 into a MET agonist. [50]. It should be noted that, under certain conditions, these HGF fragments might behave as partial MET agonists, a property that may limit their therapeutic utility. NK4, a synthetic truncated form of HGF that contains only the  $\alpha$ -chain, can inhibit a number of MET-dependent responses [51]. Independent of its inhibition of HGF–MET, NK4 acts as an angiogenesis inhibitor. Uncleavable pro-HGF, structurally

designed to lock the molecule in its inactive conformation, is able to compete with active HGF for MET binding, thus inhibiting catalytic activity [52].

The silencing of MET expression might be also achieved by using antisense and ribozyme techniques, delivered in vivo through liposomes. The chimeric U1snRNA/ribozyme transgene, designed to inhibit HGF-MET expression, has shown successful results when administered subcutaneously in glioma xenografts [53].

Decoy MET is the soluble and enzymatically inactive extracellular domain of the receptor that can interact with both HGF and full-size MET, thus sequestering the ligand and making inactive dimers with the native receptor [54]. Notably the isolated SEMA domain retains the ligand-binding properties, interfering with HGF and blocking receptor dimerization [55].

As described above, phosphorylation of MET catalytic domain induces activation of the multifunctional docking site and the subsequent recruitment of several SH2-containing transducers [56]. MET signalling inhibition can be reached through peptides that compete – mimicking their SH2 domain – with MET-related mediators for the binding to the docking site at the C-terminal tail of the receptor.

### 2.3.2 Antibodies

Several monoclonal antibodies against HGF are currently in human clinical trials for various cancers. Amgen reported the generation of some fully human monoclonal antibodies against HGF that exhibit therapeutic potential in xenografts of human glioma featuring an HGF-dependent autocrine loop [57]. Systemic administration of another anti-HGF antibody, L2G7 (Galaxy Biotech), proved to be particularly effective in inducing regression of both subcutaneous and intracranial glioma xenografts, suggesting that the blood–brain barrier does not block its efficacy [58,59]. Amgen has also performed clinical trials with AMG102 a fully human antibody (Ig2) recombinantly produced in mammalian cells [60]. This molecule has been tested in advanced glioblastomas and kidney carcinomas and clinical trials are now ongoing against malignant pleural mesotheliomas and ovarian and primary peritoneal cancers ([www.clinicaltrials.gov](http://www.clinicaltrials.gov); NCT01105390, NCT01039207 studies).

It should be noted that the efforts to develop antibodies available for anti-MET therapy have been at the beginning largely unsuccessful due to their rather agonistic than antagonistic properties on receptor activation. This is mainly due to the bivalent structure of the immunoglobulins, which act as natural dimerizing agents for tyrosine kinase receptors.

The first promising results were reached with the design of a one-armed antibody (OA-5D5) by Genentech which consists of a monovalent Fab with murine variable domains for the heavy and light chains fused to human IgG1 constant domain. In preclinical studies this antibody showed a strong growth inhibition of HGF-expressing glioblastoma cells when delivered locally and it is now in the early stages of clinical development [61]. DN30 is a monoclonal antibody raised against MET extracellular domain; it is able to reduce anchorage-independent growth and xenograft development of gastric carcinoma cells displaying MET amplification as well as metastases formation of melanoma cells [62]. DN-30 works by inducing proteolytic cleavage of MET extracellular domain, thus decreasing on one side the number of receptors available for activation and, on the other generating a decoy effect, binding to HGF and preventing the interaction with the intact surface receptor [63,64]. Transfection of epithelial cancer cells with cDNA coding for the heavy and light DN-30 chains results in downregulation of MET receptor and inhibition of the invasive growth program. Transfer of the monoclonal antibody into live animals by systemic administration or local intratumor delivery results in significant reduction of tumor growth [60].

Very recently Aveo/Shering presented the SCH900195 molecule (AV-299), a highly potent monoclonal antibody featuring antagonistic properties against HGF. SCH900195 has been demonstrated to be efficacious in advanced solid cancers in a Phase I trial [65].

### 2.3.3 Targeting MET catalytic site: the role of small molecules

Catalytic activity and transphosphorylation might be prevented through small molecules that compete with ATP in binding the active site of the receptor. The first studies directed to design ATP-competitive MET inhibitors lead to development of K252a, a wide-spectrum kinase inhibitor, able to block MET kinase at sub-micromolar concentration [66]. Further studies allowed the development of more selective inhibitors, all defined by the indolin-2-one core structure: all compounds share the indolinone motif substituted at the 5-position of the indolinone core with 3-hlorobenzyl-sulphonamide groups (SU11274) or with 3,5-dimethyl pyrrole groups (PHA665752). In vitro assays assessed on various cancer cell lines showed that both compounds (from Pfizer) inhibit MET-dependent biochemical and biological responses, being PHA665752 at least tenfold more potent than SU11274 [67,68]. Interestingly, SU11274 displays a selective inhibition pattern towards the different MET mutants identified in papillary renal carcinomas [69], whereas PHA665752 is particularly effective in tumor cell lines and xenografts harboring amplification of the wild-type MET gene [13]. These molecules however do not show good pharmacokinetic properties and oral availability, so that their use is limited to studies in animal models. More recently a new molecule named PF-2341066 has been generated: it is structurally similar to PHA665752, it is orally available and selectively blocks MET and anaplastic lymphoma kinase (ALK). This molecule displays citoreductive and antiangiogenic properties; it is now under clinical evaluation and seems to be well tolerated at therapeutic doses [70]. Several other molecules have been developed and are now being tested. XL880 (Exelixis), one of the first orally bioavailable molecule, is a multikinase inhibitor targeting MET, VEGFR2 and to a lesser extent platelet-derived growth factor receptor (PDGFR), RON, KIT and tunica interna endothelial cell kinase (TIE)-2. The MTD is 3.6 mg/kg and common side effects include hypertension and fatigue. The agent is being evaluated in papillary renal cell carcinoma, gastric and head and neck cancers [71,72].

ARQ-197 (ArQule) is an analogous MET inhibitor that is in early-phase trials. The molecule acts as a non-ATP-competitive drug and has demonstrated clinical benefit (in terms of prolonged stable disease) in Phase II clinical testing among patients with several types of solid tumors, including NSCLC, sarcomas, pancreatic cancer, hepatocellular carcinoma, germ cell tumors and colorectal cancer (<http://www.arqule.com/cli/>). The recommended Phase II dose for ARQ-197 was determined to be 360 mg twice daily. Common side effects included fatigue, diarrhea and constipation. Grade 3 elevated liver enzymes were the more severe toxicity [73]. Main interest is now addressed towards association of selective MET inhibition through ARQ-197 and erlotinib in NSCLC: first reports demonstrate evaluable Response Evaluation Criteria In Solid Tumors (RECIST) responses in NSCLC patients treated with combinatorial approach versus those who received erlotinib alone [74]. In particular an improved benefit was seen among those patients with non-squamous histology, KRAS mutations and EGFR wild-type status [75].

Based on unpublished data available online, several other compounds are now under design and preclinical evaluation. Merck is developing the MK2461 inhibitor, a small molecule which is now undergoing Phase II study in patients with advanced solid cancers. JNJ-38877605 (Johnson & Johnson) is an orally bioavailable, small-molecule receptor tyrosine kinase inhibitor with potential antineoplastic activity which selectively inhibits [76].

Also SGX Pharmaceuticals includes MET inhibitors in its pilot products pipeline. The SGX523 [77], a novel orally bioavailable ATP-competitive molecule, has been reported to be the most selective inhibitor of MET catalytic activity. This drug has shown an efficient antitumor activity in vivo at nanomolar concentrations with no effects on other signaling dependent kinases, such as RON. Importantly, SGX523 has been recently tested through in vitro and in vivo experiments on gliomas: the molecule was able to inhibit brain tumor cell and stem cell malignancy thus representing one of the most promising approach to brain cancer therapy [78].

### 3. Conclusions

Tumorigenesis and neoplastic progression have multiple aetiology, associated with the combination of genetic and epigenetic lesions. The concept that cancer is essentially a genetic disease has now been (or will be) exploited by pathologists to set up a novel classification of tumors, based on the presence of defined genetic lesions. Classical histopathological diagnosis is (and will still be) important to evaluate the extent of malignant phenotype, but personalized

molecular diagnosis is needed to understand which specific genetic lesion is responsible for the tumor of a single patient and could be successfully targeted.

Furthermore the association of venous thrombosis and cancer is intriguing and has relevant clinical implications. Cancer cells interfere with blood clotting in three main ways: release of proteins directly involved in blood coagulation, release of cytokines modulating the activities of endothelial cells and monocytes, intravasation and endothelial injury and activation. It suggests that the ability to interfere with blood coagulation is an inherent property of cancer cells and/or their microenvironment, a property that can be functionally related to the onset of neoplastic transformation. Thus, even in the absence of overt coagulation disorders, there might be abnormalities in laboratory coagulation tests that could be exploited for screening of early cancer. Moreover therapeutic implications of MET procoagulant effect need to be carefully considered and pharmacological interference with haemostasis proteins may be useful for prevention or treatment of invasive cancer.

In the vast majority of tumors MET activation is a late event that exacerbates the malignant properties of transformed cells. In other words, complete neoplastic cells often usurp anti-apoptotic and pro-invasive activities of MET as an expedience [41] to gain a selective advantage and to become more proficient under adverse environmental conditions. Thus, due to its dual role as a necessary oncogene in some tumors and as adjuvant gene which facilitate metastatic process, MET is a versatile candidate in anticancer targeted therapy. Burgeoning evidence suggests cross-talk at a molecular level between MET and several receptor tyrosine kinases (RTKs) providing a rationale for combinatorial targeted therapies. This approach might be also useful in overcoming resistance phenomena. To decipher the identity of potentially responsive tumors the different roles that MET can play in neoplastic tumors should be considered before starting anti-MET therapy in order to avoid random selection of patients without prior genetic characterization.

## 4. Expert opinion

### 4.1 RTKs-based cancer therapies

The MET gene encodes a protein that belongs to the RTK family. The latter represents a subfamily of transmembrane receptors with an intrinsic, ligand-controlled tyrosine-kinase activity. Phosphorylation is the biochemical process which regulates, in a reversible way, protein activation: protein kinases and protein phosphatases are the main mediators of these reactions and their appropriate activity is required for cellular homeostasis; in contrast their aberrant activation is crucial in oncogenesis [79]. Indeed firm evidence has shown that the vast majority of human cancers carry a genetic alteration in the kinase (kinome) and/or phosphatase (phosphatome) gene families [80]. The characterization of both molecular structure and functions of RTKs and their ligands has opened the door to a new era in anticancer drugs development. In resting cells, RTKs' activity is tightly controlled, but when mutated or overexpressed RTKs act as dominant oncogenes [81]. More than twenty-years ago the structure of a RTK, the EGFR, was elucidated. An important challenge after RTK structure definition was the identification of the molecular mechanisms through which these receptors regulate signal transduction across the plasma membrane. The idea that oncogenesis is sustained by signal generation through tyrosine phosphorylation paved the way to the development of several RTK inhibitors. The success of RTK-targeted therapies is based on the fact that the receptor targeted by the drug is active – as a result of a genetic lesion – only in the tumor and not in the surrounding healthy tissue. Consequently the identification of genetic alterations responsible for RTK activation is a priority for successful RTK targeted therapy. At the same time the targeted therapy concept has significantly modified the principles of anticancer treatment. Classical cancer chemotherapeutic agents are designed either to kill cancer cells (cytotoxic effect) or to halt proliferation (cytostatic effect). Most cancer chemotherapy has selective toxicity based on the concept that tumor cells are dividing more rapidly than non-malignant host cells [82]. On the other hand, biologically targeted therapies have different endpoints with respect to conventional chemotherapy. In the cytotoxic drug paradigm, toxicity defines the surrogate endpoint of drug anticancer activity, meaning that the more side effect are induced by the drug, the more the therapeutic efficacy [83]. In molecularly tailored therapy, target inhibition becomes the surrogate endpoint of the biological activity, which in turn is the surrogate for antitumor activity.

The first successful employment of a tyrosine kinase inhibitor was reported with Imatinib mesilate (Gleevec) in chronic myeloid leukemia (CML) treatment. So far, small molecules (e.g., gefitinib, erlotinib) and monoclonal antibodies (e.g., trastuzumab, cetuximab, bevacizumab) have demonstrated the potential of molecularly targeted cancer therapeutics [84]. Several other RTK-based experimental anticancer strategies are now under preclinical and clinical evaluation. Among them MET, due to its versatile biological actions in cancers, is one of the most promising targets and anti-MET molecules are gaining growing interest in pharmacogenomics research and translational clinical trials. Importantly it should be noted that there is considerable experimental evidence suggesting that MET signaling activates pathways that make cancer cells resistant to cytotoxic agents such as chemotherapy and radiation therapy. This suggests that MET inhibitors may cooperate with cytotoxic therapies and should be kept in consideration in drug design and development.

#### 4.2 Rationale for targeting MET in cancer and metastases

Experiences derived from EGFR-inhibition have indicated that the targeted treatment approach is really effective only in a small subset of tumors, that ultimately are those carrying genetic alterations (point mutations or gene amplification) of the targeted receptor [85]. The same results have been confirmed by *in vitro*, where it has been shown that cell lines yielding the EGFR genetic lesions found in human cancers undergo cell cycle arrest or apoptosis upon EGFR inhibition [86]. This phenomenon is named 'oncogenic addiction' [87] and identifies the dependence of neoplastic clone – for survival and proliferation – on continued expression of a single signaling molecule pathway, which is aberrantly activated in consequence to a genetic lesion. Therefore, switching off the oncogenic activity by specific inhibitors will trigger an 'oncogenic shock' [75], which eventually will lead tumor cells to die. This model relies on the concept that the oncogenic pathway might induce both pro-apoptotic and proliferative signals [86] with an obvious prevalence of survival outputs. In contrast, acute inactivation of the oncogene (e.g., through RTKs inhibitors) will result in an early reduction of proliferation, followed by a significant enhancement of apoptosis. The latter identifies a vulnerability period which represents the effective therapeutic window. The MET-addicted phenotype has been described in several cell lines derived from gastric carcinomas [13] and NSCLC [14]. In these settings, addiction is related to gene amplification. Therefore, identification of increased MET gene copy number will strongly predict response to anti-MET therapy.

This result is coherent with previous experiences derived from anti-EGFR therapy in NSCLC [88] and sustains the rationale that only the subset of patients affected by tumors addicted to a specific gene could benefit from a specific targeted therapy. So far addiction to MET has been demonstrated in a restricted number of human tumors. This evidence theoretically justifies anti-MET therapy as front-line intervention in a limited subset of human cancers. However, experimental observations have demonstrated that many cell lines display a sensitivity to MET inhibition independently from the co-existence of a genetic lesion driving addiction. Thus in vast variety of tumors, MET is activated as a secondary event and exacerbates the malignant properties of already transformed cells [11,89]. These observations are strictly related to MET intrinsic biological properties. As discussed above, in the vast majority of cancers, invasive growth occurs as a late event in tumor progression and MET is activated in a context that is quite different from addiction. In these circumstances MET-driven invasive growth is rather the consequence than the cause of the transformed phenotype. In other words, activation of MET pathway facilitates cancer progression, conferring to cancer cells a powerful 'expedience' to sustain their metastatic potential [40]. This observation provides a strong rationale for anti MET therapy as combinatorial therapy to target progression of a wide spectrum of tumors.

#### 4.3 Cross-talk between MET and other receptor families

The MET receptor interacts with other membrane receptors and many different molecules act as MET partners such as integrin  $\alpha\beta 4$ , the adhesive molecule CD44, class B plexins, FAS and other RTKs such as RON, EGFR and ErbB2 [2,9,41].

The RON gene displays 25% homology with MET in the extracellular region and 63% homology in the TK domain. The RON ligand MSP shares a 45% homology with HGF [90]. It is well demonstrated that ligand-activated MET results in RON transphosphorylation and vice-versa. Transphosphorylation occurs in a direct way and does not require the C-terminal docking site of either receptor, whereas a TK inactive RON is sufficient to block MET transforming activity [91].

MET interacts with EGFR in several ways; which are discussed in the section below.

The MET receptor can be also activated in response to G-protein coupled receptor (GPCR) agonists. Both EGFR and GPCR ligands are known to increase the intracellular level of reactive oxygen species that inhibit phosphatases, hence the activation of MET [92].

Although a direct physical interaction between MET and ErbB2 has never been documented, the synergistic activity of the two receptors enhances the malignant invasive phenotype, mainly in cancer cells that display overexpression of ErbB2 and where HGF is normally detectable in the surrounding stroma [93]. Also, breast cancer cells that overexpress ErbB2 and are treated with trastuzumab upregulate Met expression. It is conceivable that these cells develop secondary resistance to trastuzumab through cross-talk activation of ErbB3 and downstream transducers [94].

#### 4.4 Combinatorial therapeutic strategies

Signaling by growth factors interacting with RTKs is enormously complex. Receptors activation involves different pathways that are part of complex and redundant molecular networks shared with other receptors.

In consequence the downstream cascades can be controlled at multiple levels with relevant implications for targeted therapies. Using its multifunctional docking site MET activates a number of intracellular transducers such the RAS–RAF–MEK and PI3K–AKT axis [95]. The combination of anti-MET therapeutic agents with either signal transduction inhibitors, or cytotoxic chemotherapy has been evaluated in preclinical models. An important example is the interactions between phosphatase and tensin homologue (PTEN) and MET in glioblastomas in which PTEN loss amplifies MET-induced malignancy. Deregulation of MET together with PTEN loss is a relatively common event in tumor such as malignant gliomas [31]. The loss of PTEN leads to transcriptional upregulation of an EGFR agonist (TGF- $\alpha$ ) which, in turn, induces EGFR signaling in an autocrine manner [96]. It suggests that EGFR signaling activation contributes to exacerbate the malignant phenotype in cells displaying a constitutive activated form of MET, thus providing a strong rationale for an anti-EGFR and anti-MET combinatorial therapeutic approach whenever concomitant loss of PTEN is detected. Moreover inactivation of PTEN is usually associated with resistance to RTK inhibitors, but combined inhibition of MET and EGFR in a glioblastoma model in which both receptors are constitutively overactivated can avoid PTEN deficiency and restore therapeutic responsiveness [97].

This concept is further supported by experimental observations in glioblastomas where combined anti-EGFR and anti-MET therapies overcome PTEN-loss associated resistance too.

As many kinase inhibitors exert their cytotoxic effects primarily by inhibiting a specific kinase, there is a strong selective pressure for cells to acquire (secondary) resistance through genetic lesions that activate parallel signaling pathways. Understanding the molecular mechanisms of sensitization and resistance to a specific drug is clearly required for tailoring the therapeutic regimen to the patients that are most likely to achieve a clinical benefit. Many efforts are now directed to the identification of genetic markers that can predict potential response to a certain drug. From this perspective MET amplification in cancer is an interesting hit, since growing evidence demonstrates that increased MET gene copy number is associated with acquired gefitinib resistance. In NSCLCs MET amplification leads to resistance to EGFR small inhibitors by driving ErbB3-dependent activation of PI3K, a pathway that was thought to be specific to EGFR signaling [15]. These findings have several clinical implications. MET amplification might explain why some NSCLC carrying sensitizing EGFR mutations failed to respond to erlotinb. Combinatorial therapy with MET inhibitors and irreversible anti EGFR small molecules – which are now under development – might be considered for NSCLC patients whose tumors have become resistant to erlotinb and gefitinib [98]. These observations are likely to imply a prognostic value; it has been recently shown that identification of MET amplification is an independent negative prognostic factor in surgically resected NSCLCs- whereas EGFR gain does not affect survival [99].

In summary, targeted therapies against MET should be effective, as first line treatment, in a restricted subset of MET-addicted cancers and, as a secondary approach, in a much wider spectrum of advanced tumors taking advantage (expedience) of MET for local invasion and distant spreading. Importantly MET inhibition is directed to block both the pro-invasive and the pro-angiogenic properties of this oncogene. Growing evidence suggests a relevant role of combinatorial multikinase inhibition. High-throughput molecular profiling and proteomics represent now a required approach to translate all the pre-clinical evidences into a full therapeutic platform.

#### Article highlights.

Malignant disease occurs when neoplastic cells abandon their primary growth site, cross tissue boundaries and penetrate the vasculature to colonize distant sites. This process – metastatic spreading – can be considered as the aberrant counterpart of a physiological programme for organ regeneration and maintenance. Scatter factors and semaphorins, together with their receptors, are involved in activating this programme.

The MET oncogene encodes the tyrosine kinase receptor for hepatocyte growth factor (HGF).

MET aberrant activation in cancer is mainly related to overexpression, which is often a consequence of transcriptional upregulation induced by negative microenvironmental conditions; somatic mutations are rarely found; gene amplification has been reported in a number of human cancers among which are gastric and lung tumors.

MET activation induces a pro-angiogenic effect which cooperates in sustaining tumor invasiveness and determines that coagulation cascade alteration that phenotypically defines the Trousseau syndrome.

Therapeutic interference with MET activation is thus a new and challenging approach to hamper tumorigenic and metastatic processes.

This box summarizes key points contained in the article.

#### Declaration of interest

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