Review

Understanding and Targeting MET Signaling in Solid Tumors – Are We There Yet?

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Abstract

The MET signaling pathway plays an important role in normal physiology and its deregulation has proved critical for development of numerous solid tumors. Different technologies have been used to investigate the genomic and proteomic status of MET in cancer patients and its association with disease prognosis. Moreover, with the development of targeted therapeutic drugs, there is an urgent need to identify potential biomarkers for selection of patients who are more likely to derive benefit from these agents. Unfortunately, the variety of technical platforms and analysis criteria for diagnosis has brought confusion to the field and a lack of agreement in the evaluation of MET status as a prognostic or predictive marker for targeted therapy agents. We review the molecular mechanisms involved in the deregulation of the MET signaling pathway in solid tumors, the different technologies used for diagnosis, and the main factors that affect the outcome, emphasizing the urge for completing analytical and clinical validation of these tests. We also review the current clinical studies with *MET* targeted agents, which mostly focus on lung cancer.

Key words: MET, HGF, FISH, IHC, molecular testing, targeted therapy.

Introduction

With the continuum of research on the biology of human neoplasms, the therapeutic options in cancer have dramatically progressed over the last decade from cytotoxic and cytostatic regimens to molecular targeted agents, which overall provide better efficacy and less toxicity. The discovery of activation mechanisms in receptor tyrosine kinases (RTKs), such as the epidermal growth factor receptor (*EGFR*) mutations and the anaplastic lymphoma kinase (*ALK*) fusions, led to the development of successful targeted therapeutic agents, which are the proof of the ongoing revolution in cancer treatment [1–3]. Among the genes coding numerous human RTKs, the MET proto-oncogene *(MET)* is considered one of the most critical regulators of an extensive signaling pathway relevant in normal development and carcinogenesis [4–7].

The human gene *MET* is located on chromosome 7q31.2. It was identified as a proto-oncogene in a human osteogenic sarcoma cell line in 1984 and in 1987 found to encode an RTK called MET or c-Met [4,5,8]. The gene encoding its ligand protein, the hepatocyte growth factor (*HGF*), is located on chromosome 7q21.1 and was discovered in two independent approaches, as HGF in 1989 and as the scatter factor (SF) in 1989, which later were found to be the same protein [9].

In general, receptor tyrosine kinases (RTKs) contain an N-terminal extracellular binding domain, a single transmembrane α helix, and a cytosolic C-terminal domain with tyrosine kinase activity. MET is a disulfide linked heteroimeric RTK consisting of an

extracellular α chain, a β chain that encompasses the remainder of extracellular domain, the juxtamembrane and the kinase domains. As illustrated in Figure 1A, the extracellular component groups several domains including a large N-terminal semaphorin (Sema) domain (exon 2), a plexin-semaphorin-integrin (PSI) domain, and a stalk structure consisting of four immunoglobulin-plexin-transcription factor (IPT) domains. The intracellular component contains a juxtamembrane region responsible for signal downregulation and receptor degradation, a catalytic region with the enzyme activity, and a C-terminal region acting as a docking site for adaptor proteins such as GRB2 and GAB1,

which leads to downstream signaling via PI3K, STAT and MAPK [10].

HGF, the only natural ligand of MET, is a large, disulfide, multidomain protein belonging to the plasminogen family. As shown in Figure 1B, HGF consists of six domains including an N-terminal domain, four kringle domains and a C-terminal domain which is a serine proteinase homology (SPH) domain [9,11]. HGF is secreted from mesenchymal cells as an inactive, single chain precursor and is converted to an active two-chain heterodimer by one of three serine proteinases: the soluble HGF activator, and the type II transmembrane enzymes matriptase and hepsin [12,13].
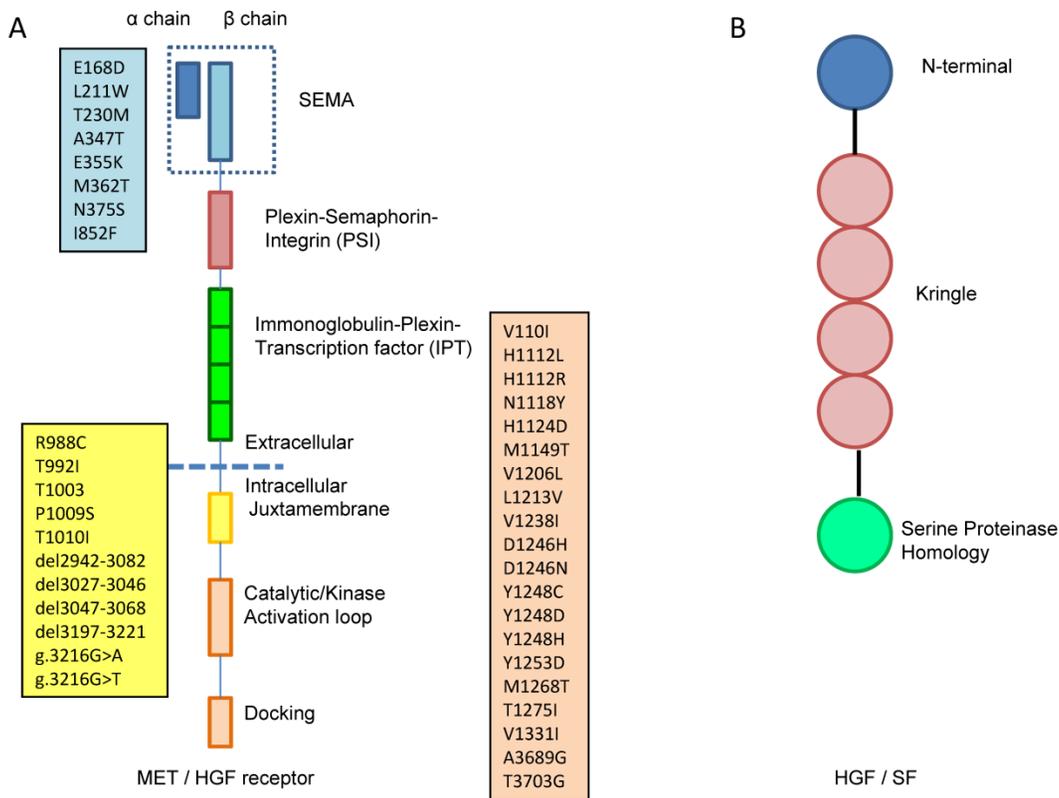
Figure 1. A. MET is a disulfide linked heteroimeric RTK consisting of an extracellular α chain, a β chain that encompasses the remainder of extracellular domain, the juxtamembrane and the kinase domains. The extracellular component groups several domains including a large N-terminal semaphorin (Sema) domain (exon 2), a plexin-semaphorin-integrin (PSI) domain, and a stalk structure consisting of four immunoglobulin-plexin-transcription factor (IPT) domains. The intracellular component contains a juxtamembrane region (exon 14), a catalytic region with the enzyme activity, and a C-terminal region (exon 15-21) acting as a docking site for adaptor proteins. The locations of gene mutation are shown in the boxes, the blue box lists the locations of mutation in sema domain (exon2), the yellow box lists the locations of mutation in juxtamembrane (exon 14) and the pink box lists the locations of mutation in tyrosine kinase domain (exon15-21). **B.** HGF consists of six domains including an N-terminal domain, four kringle domains and a C-terminal domain which is a serine proteinase homology (SPH) domain.
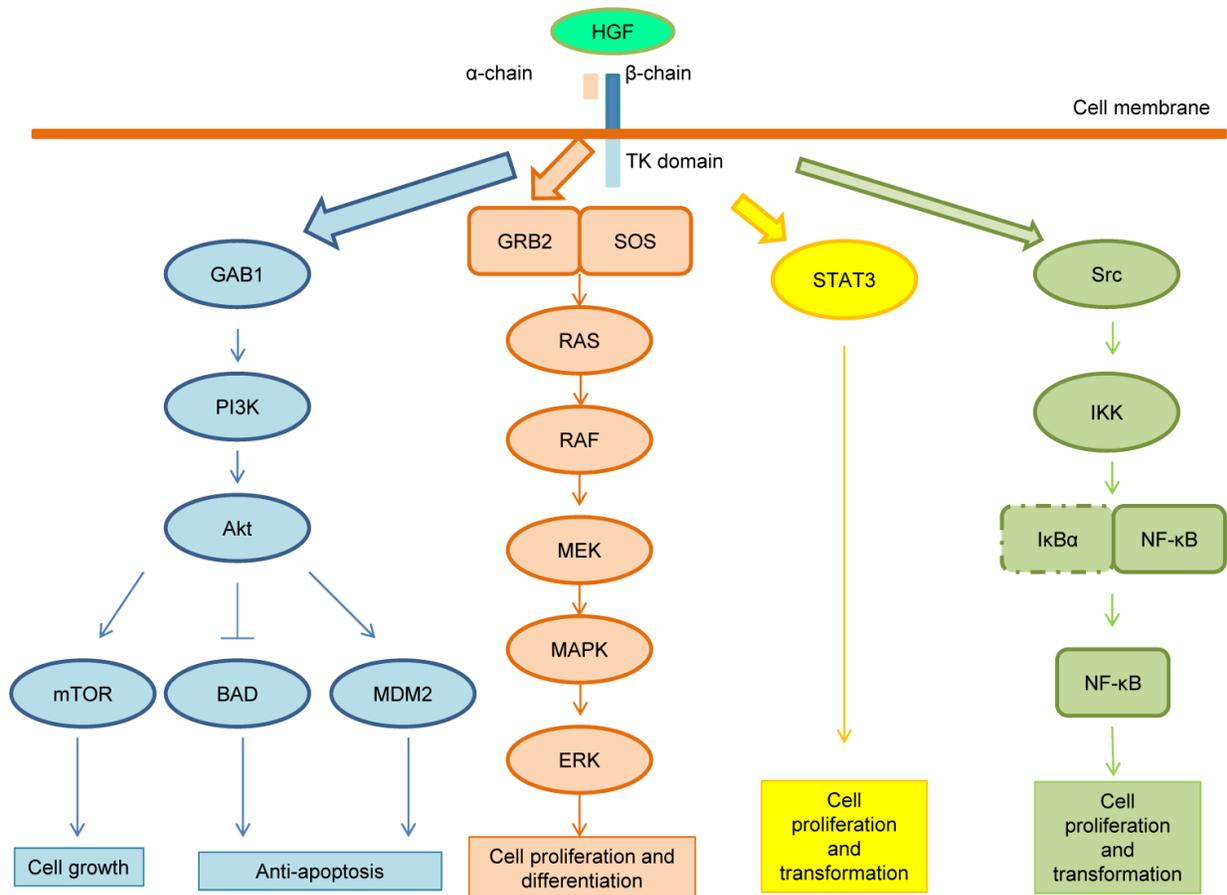
MET Signaling

Following the HGF binding, the kinase activity of MET is switched on by receptor dimerization and *trans*-phosphorylation of two catalytic tyrosine residues (Tyr1234 and Tyr1235) within the kinase activation loop, then by phosphorylation of two additional docking tyrosine kinases in the C-terminal tail (Tyr1349 and Tyr1356). These tyrosines recruit

signaling effectors including the adaptor proteins growth factor receptor-bound protein (GRB2), Src homology-2-containing (SHC), v-crk sarcoma virus CT10 oncogene homolog (CRK), and CRK-like (CRKL) [6,14]. Moreover, the adaptor protein GRB2-association binding protein 1 (GAB1) can bind either to MET directly or indirectly through GRB2 [6]. Phosphorylation of these different effectors initiate important downstream signaling pathways for cell

proliferation, morphogenesis, survival, migration and angiogenesis, such as the cascades related to PI3K-Akt, RAS-MAP kinase, STAT3 and nuclear factor-κB (NF-κB) complex [6,14,15]. These pathways are summarized in Figure 2. After MET activation, PI3K can bind either to MET directly or indirectly with GAB1 and signals through the AKT/protein kinase B axis. AKT can inactivate the pro-apoptotic protein BCL-2 antagonist of cell death (BAD) and activate the E3 ubiquitin-protein ligase MDM2, which results in apoptosis suppression and cell survival promotion [16]. Moreover, AKT can also activate mammalian target of rapamycin (mTOR), which stimulates protein synthesis and cell growth. MET activation also signals through the RAS-MAPK pathway. The nucleotide exchanger protein Son of Sevenless (SOS) activates RAS by binding with SHC and GRB2 (GRB2-SOS complex). This leads to activation of the v-raf murine sarcoma viral oncogene homolog B1 (RAF) kinases, which subsequently stimulates the MAPK effector kinase (MEK) and results in MAPK activation. MAPK phosphorylates,the final effector of the cascade, ERK. The RAS-MAPK pathway is responsible for cell proliferation, cell motility and cell cycle progression [17]. A third major downstream axis of MET signaling is the Signal transducer and activator of transcription 3 (STAT3) pathway. STAT3 can bind to MET directly resulting in STAT3 phosphorylation, which regulates cell transformation, tubulogenesis and invasion [10]. Finally, the fourth major cascade of MET activation is through the IκBα-NF-κB complex. NF-κB is bound to IκBα forming an inactive form. The phosphorylation of either PI3K-AKT or SRC activates IκB kinase (IKK) and results in degradation of IκBs. NF-κB is then released and translocated to the nucleus to stimulate gene transcription [15].

Figure 2. MET signaling pathway. After MET activation, PI3K can bind either to MET directly or indirectly with GAB1 and signals through the AKT/protein kinase B axis. AKT can inactivate the pro-apoptotic protein BCL-2 antagonist of cell death (BAD) and activate the E3 ubiquitin-protein ligase MDM2, which results in apoptosis suppression and cell survival promotion. Moreover, AKT can also activate mammalian target of rapamycin (mTOR), which stimulates protein synthesis and cell growth. MET activation also signals through the RAS-MAPK pathway. The nucleotide exchanger protein Son of Sevenless (SOS) activates RAS by binding with SHC and GRB2 (GRB2-SOS complex). This leads to activation of the v-raf murine sarcoma viral oncogene homolog B1 (RAF) kinases, which subsequently stimulates the MAPK effector kinase (MEK) and results in MAPK activation. MAPK phosphorylates ERK, which is the final effector of the cascade. The RAS-MAPK pathway is responsible for cell proliferation, cell motility and cell cycle progression. A third major downstream axis of MET signaling is the Signal transducer and activator of transcription 3 (STAT3) pathway. STAT3 can bind to MET directly resulting in STAT3 phosphorylation, which regulates cell transformation, tubulogenesis and invasion. Finally, the fourth major cascade of MET activation is through the IκBα-NF-κB complex. NF-κB is bound to IκBα forming an inactive form. The phosphorylation of either PI3K-AKT or SRC activates IκB kinase (IKK) and results in degradation of IκBs. NF-κB is then released and translocated to the nucleus to stimulate gene transcription.

Independently of the HGF-dependent pathway, MET may be upregulated and become overexpressed by growth factors and cytokines such as PAX5, PAX8 and hypoxia-inducible factor (HIFα) [18-20]. Conditions such as hypoxia induce overexpression of both HGF and MET [20]. The downregulation of MET is initiated after ligand activation by internalization of MET receptor through endocytosis. The internalized receptor is ubiquitinized by the binding of casitas B-lineage lymphoma (CBL) at the juxtamembrane domain and is then degraded in the lysosome. The ubiquitin E3 ligase CBL also initiates early endocytosis by interaction with CBL-interacting protein 85, which enhances the adaptation of cell membrane for early endocytosis [21]. Another mechanism of downregulation of MET is a two-step proteolytic cleavage. The first step is cleavage of the extracellular domain by a disintegrin and metalloprotease (ADAM)-like receptor protease, which in turn creates a decoy extracellular domain MET fragment to block activation of other MET ligands; the second step is cleavage of intracellular domain by γ-secretase into fragment subsequently degraded by proteasome [22].

Overall, the physiologic function of MET pathway involves cell proliferation, cell motility, and morphogenesis and organ regeneration. During embryonal development, *MET* has a crucial role in proliferation of hepatocytes and placental trophoblasts. Ablation of the *MET* gene causes impaired development of liver and placenta leading to death in utero [4]. During development, MET induces migration of progenitor cell to form the hypaxial muscle and neurons [4]. MET and HGF also play an important role in healing process. MET and HGF are upregulated in the response of inflammation and injury. Overexpression of MET and HGF is observed in organ injuries such as liver, kidneys, heart and skin from toxins or chemicals and damage. HGF is secreted from mesenchyme after hepatectomy and induces MET downstream signaling in hepatocytes and result in liver regeneration and an increase size of liver. MET ablational mice had impaired liver regeneration. MET pathway plays a protective role against tubular necrosis of kidneys [4], myocardial injury after ischemic or reperfusion injury, and administration of recombinant HGF can reduce the area of myocardial infarction [23]. When wound occurs, HGF and MET are expressed in keratinocytes and stimulate the wound healing [24].

Over-activation of MET pathway induces cell overgrowth and invasion. Several preclinical studies have provided evidence of MET deregulation in carcinogenesis. Invasive activity of HGF was shown by Rong et al. [25], with *MET*- transformed NIH 3TC cells displaying motility activity in the absence of HGF while *MET*-transfected (MT) cells, transformed by a non-autocrine mechanism and used as control, did not show migration activity. When HGF was introduced, MT cells showed dramatic migration. Transgenic mice with *MET* cDNA developed hepatocellular carcinoma (HCC) which regressed after the transgene was inactivated [26]. Transduced *MET* oncogene introduced to mouse liver progenitor cells induced phenotypic changes, characterized by increased proliferation rate, loss of contact inhibition and formation of transformation foci. Transplant of the *MET* transduced cells into the spleen of immune-deficient mice led to colonization of spleen and liver with tumors similar to HCC [27].

MET pathway also promotes angiogenesis, which has an important role in wound healing and tumor development, by upregulation of vascular endothelial growth factor (VEGF) and downregulation of thrombospondin-1 (TSP1) [28]. Crosstalk of MET with other cell surface proteins (CD44, α6β4Integrin, SEMA4D and Plexin B1) has been proposed as mechanism to promote cell motility, invasion and metastasis [29], crosstalk with G protein receptors such as EGFR and HER2 to promote downstream signaling [7] and crosstalk with FAS ligand to promote anti-apoptosis [30]. These aforementioned interactions between MET and other proteins have a demonstrated contribution to carcinogenesis and drug resistance in *in vitro* studies [31].

Molecular Mechanisms of MET Activation in Carcinogenesis

Deregulation of the HGF-MET cellular axis in cancer can be detected at different molecular levels such as by changes in extent of protein expression, by variation in gene copy number and by presence of gene mutations. Each of these levels may be explored by different technologies, including immunohistochemistry staining (IHC) and enzyme-linked immunosorbent assays (ELISA) for evaluation of protein expression, and SNP arrays, fluorescence *in situ* hybridization (FISH), and PCR-based or sequencing-based techniques for evaluation of genomic status.

HGF and MET Overexpression

HGF is secreted either by primary and metastatic tumors as an autocrine mechanism or by mesenchymal cells as a paracrine or combined auto-para mechanisms [32]. Olivera et al reported a 10 to 100 fold increase in HGF and a 2 to 10 fold increase of MET in NSCLC compared to normal lung [33]. As shown in Table 1, levels of HGF expression have been

sporadically accessed by either ELISA or IHC in bladder [34-36], breast [37-39], colorectal [40,41], head and neck [42,43], and lung cancers [31,32,44]. IHC was the common technique used to determine MET expression. Overexpression of the MET protein has been more commonly investigated in various solid tumors as seen in Table 2, with studies in bladder [45], breast [37-39, 46-51], colorectal [49,52-56], gastric [57], hepatobiliary [58], head and neck[42,43], lung [32,49,59–61], ovarian [49] and renal [49,62] cancers.

High levels of HGF and MET proteins have been reported in solid tumors, although in different frequencies in distinct tumor types. Tumor progression may be a differential factor in protein overexpression, but studies are not always concordant. Lee et al. [48] found MET overexpression in 45% of primary and in 81% of metastatic breast tumors while Voutsina et al. [54] found MET overexpression in 10% of primary and 18% of metastatic colorectal carcinomas (CRC).

Table 1. HGF protein expression in solid tumors.

Tumor Type	Diagnostic Method	Antibody - Clone; Manufacturer	Dilution	Interpretation for Positive	Positive/ Informative Cases (%)	Overexpression Prognosis	Reference
Bladder	IHC	H4, Otsuka Pharmaceutical	1:30	>10% of cells with cytoplasmic staining	nodular - 5/27 (18%) papillary - 33/33 (100%)	More frequent in papillary (more invasive) type (*p*<0.001)	Li [34]
	ELISA	Not reported	Not reported	Continuous analysis	Not reported	Higher level in muscle-invasive tumor (*p*<0.001)	Wang [35]
	ELISA	Not reported	Not reported	>1820 pg/mg protein	232/240 (97%)	Not reported	Eissa [36]
Breast	ELISA	Not reported	Not reported	Continuous analysis	Not reported	Shorter disease relapse (*p*<0.001) and OS (p<0.001)	Nagy [37]
	IHC	R & D Systems	1:50	≥10% of both cancer and stromal cells/matrix - no intensity specified	64/88 (73%)	Lower 10-year survival rate (*p* = 0.0096)	Edakuni [38]
	IHC	R & D Systems	Not reported	>10 % of tumor cells with moderate +2 and intense +3 staining	147/323 (46%)	NS for OS	Kang [39]
Colorectal	ELISA	Not reported	Not reported	Serum HGF > 0.37 ng/ml	67/184 (36%)	HR for OS 3.1 (*p*=0.0001)	Toiyama [40]
	ELISA	Not reported	Not reported	Serum HGF ≥1393.55 pg/ml	52/103 (50%)	PFS (*p*<0.001), OS (*p* = 0.002)	Takahashi [41]
HNSCC	IHC	H145, Santa Cruz Biotechnology	1:50	any tumor cell ≥+1 stain	58/68 (85%)	Not reported	Seiwert [43]
Hypopharynx	IHC	R&D Systems	50mcg/ml	>30% of cancer cells with moderate +2 and intense +3 staining	31/40 (78%)	OS (*p* = 0.07), Recurrence rate (*p*=0.22), Advanced stage (*p*=0.016)	Kim [42]
Lung Adenocarcinoma	IHC	Immuno Biological Laboratories	5mcg/ml	≥50% of tumor cells positively stained	5/16 (31%)	Not reported	Yano [31]
	IHC	SC-7949, Santa Cruz Biotechnology	1:100	Allred score : (positive 3-8 score)	104/183 (57%)	RR for OS 1.5 (*p* = 0.012)	Onitsuka [44]
Lung Non-Small Cell	IHC	SC-7949, Santa Cruz Biotechnology	1:100	≥50% of tumor cells positively stained	14/88 (16%)	NS for OS	Masuya [32]

ELISA; enzyme-linked immunosorbent assay, IHC; immunohistochemistry, NS; not significant, RR; risk ratio, OS; overall survival.

Table 2 . MET protein expression in solid tumors.

Tumor Type	Diagnostic Method	MET antibody - Clone, Manufacturer	Dilution	Interpretation for Positive	Positive/ Informative Cases (%)	Overexpression Prognosis	Reference
Bladder	IHC	AC, Zymed Laboratories Inc	Not reported	> 50% of tumor cells with strong staining intensity	37/133 (28%)	HR 3.76 (*p*=0.041)	Miyata [45]
Breast	Western blot	Not reported	Not reported	Any detectable protein	26/73 (36%)	Disease relapse (*p*=0.012)	Nagy [37]
	IHC	Santa Cruz Biotechenology Inc	1:250	>70-80% intense any reactivity	20/91 (22%)	HR for survival 3.27 (*p*=0.02)	Ghoussoub [46]
	IHC	Santa Cruz Biotechenology Inc	1:200	≥30% of area of slide	64/88 (73%)	Lower 10-year survival rate 61.5% vs 97.9% (*p*= 0.0096)	Edakuni [38]
	IHC	3D4; Zymed Laboratories	Not reported	moderate +2 and intense +3 staining	91/320 (28%)	Relative Risk 1.862 (*p*=0.0064)	Kang [39]
	IHC	AC, Zymed Laboratories	1:1	+3 staining	71/324 (22%)	Relative Risk 2.04 (*p*=0.0035)	Tolgay Ocal [47]
	IHC	Santa Cruz Biotechenology Inc	1:100	> 5% of tumor cells with intense cytoplasmic or membranous reactivity	Early stage - 23/51 (45%) Metastasis - 42/52 (81%)	More frequent in metastasis (*p*=0.0002), Lower 10 yr-DFS rate 35.4% vs 73.7% (*p* = 0.0004)	Lee [48]
	IHC	Zymed-Invitrogen	Not reported	+2 and +3 staining	6/37 (16%)	Not reported	Ma [49]
	IHC	Novocastra Laboratories	Not reported	IRS Remmele score : High (9-12 score)	80/302 (27%)	Better prognosis (*p*=0.0089)	Gisterek [50]
	IHC	SP44, Novus Biological	1:50	> 25% positive tumor cells (no intensity specified)	52/97 (54%)	More frequent in grade 3 tumor (*p*=0.004); OS (*p*=0.9)	Inanc [51]
Colorectal	IHC	Zymed-Invitrogen	Not reported	+2 and +3 staining	31/40 (78%)	Not reported	Ma [49]

Tumor	Method	Reagent	Dilution	Criteria	Frequency	Outcome	Reference
	IHC	Invitrogen	1:300	>200 based on H-score criteria	274/339 (81%)	OS (p=0.889)	Uddin [52]
	IHC	Novocastra Laboratories	1:30	>10% of cells positive with ≥+1 (0 - 3 intensity)	131/183 (72%)	More frequent in higher stage (p=0.02)	Garouniatis [53]
	IHC	C28, Santa Cruz Biotechenology	1:100	+3 (0 - 3 intensity)	Primary - 8/79 (10%) Metastasis - 14/76 (18%)	Shorter survival HR 4.6 (p<0.0001)	Voutsina [54]
	IHC	SP44, Spring Biosciences	Not reported	4 or greater score (intensity 0-3+ %of cells 1 (<25%) 2(25-50%) 3(>50%))	36/75 (48%)	Shorter PFS HR1.46 (p=0.018)	Kishiki [55]
	IHC	SP44 (CONFIRM), Ventana Medical Systems	Not reported	+2 and +3 staining	56/108 (52%)	Shorter RFS 9.7 vs 21.1 mo. (p = 0.013)	Shoji [56]
Gastric	IHC	SP44 (CONFIRM), Ventana Medical Systems	Prediluted	> 50% of tumor cells with moderate 2+ or strong 3+ intensity	22/229 (10%)	Shorter PFS (p <0.001) and OS (p <0.001)	An [57]
Hepatocellular	IHC				80/297 (28%)	Not significant for Survival	Lee [58]
HNSCC	IHC	CVD-12V	1:100	moderate +2 and intense +3 staining	84/97 (85%)	Not reported	Seiwert [43]
Hypopharynx	IHC	R&D Systems Inc	0.625 mcg/ml	> 30% of cells with moderate and strong intensity	28/40 (70%)	More frequent in lymphnode met and advanced stage, OS (p>0.05)	Kim [42]
Lung	IHC	Zymed-Invitrogen	Not reported	+2 and +3 staining	16/40 (40%)	Not reported	Ma [49]
Lung Adenocarcinoma	IHC	C28, Santa Cruz Biotechenology	1:100	> 50% of tumor cells with moderate 2+ or strong 3+ intensity	33/110 (30%)	Not reported	Xia [59]
Lung Non-Small Cell	IHC	SC-10, Santa Cruz Biotechenology	1:100	> 50% of tumor cells with > grade 1 intratumoral staining	36/88 (41%)	Relative Risk 2.64 (p=0.0029) for 5-yr survival	Masuya [32]
	IHC	SP44 (CONFIRM), Ventana Med Systems	Not reported	> 50% of tumor cells with moderate 2+ or strong 3+ intensity	210/1048 (20%)	HR for PFS 0.72 (p=0.014), HR for OS 0.70 (p=0.03)	Scagliotti [60]
	IHC	SP44 (CONFIRM), Ventana Med Systems	Not reported	> 50% of tumor cells with moderate 2+ or strong 3+ intensity	66/128 (52%)	Good predictive factor for onartuzumab plus erlotinib, OS HR 0.42 (p=0.01), PFS (p = 0.69)	Spigel [61]
Ovarian	IHC	Zymed-Invitrogen	Not reported	+2 and +3 staining	12/40 (30%)	Not reported	Ma [49]
Renal Cell	IHC	AC, Zymed Laboratories	1:350	>50% of cancer cells with higher staining than normal kidney	73/114 (64%)	OR for cause specific survival 2.94 (p=0.028)	Miyata [62]
	IHC	Zymed-Invitrogen	Not reported	+2 and +3 staining	28/40 (70%)	Not reported	Ma [49]

IHC; immunohistochemistry, HR; hazard ratio, OS; overall survival, PFS; progression-free survival, RFS; relapse-free survival, OR; odd ratio.

However, there is also a large variation within specific tumor types that may be due to technical features such as reagents and experimental conditions used in the assay as well as to the qualitative nature of the criteria considered for diagnosis [32,38,42,43,48,53,55,61,62]. As seen in Tables 1 and 2, consensus for qualitative diagnosis of overexpression of HGF and MET has not been established yet. Several studies did not provide enough technical details for a comparison and even for studies in which data are available it is not easy to reconcile the different results. For instance, both Toiyama et al. [40] and Takahashi et al. [41] derived the cut-off value for quantification in high or low HGF expressors in the ELISA assays from the HGF level in the serum of their Japanese CRC patients usingROC curves to determine the cut-off for normal serum level. However, Toiyama et al. reached the cut-off of 0.37 ng/ml, which was surpassed by 36% of patients, while Takahashi obtained the cut-off of 1.39 ng/ml, which was surpassed by 50% of patients. Despite these discrepancies, both studies similarly concluded that high level of serum HGF conferred poorer survival to the patients.

The effects of using different assay reagents and specific criteria for determination of positivity for overexpression are obvious in the breast cancer studies, in which frequencies of MET overexpression varied from 22%[46] to 73% [38]. Ghoussoub et al. [46] considered overexpressors (positive) the specimens displaying any reactivity in >70-80% of tumor cells whereas Edakuni et al. [38] considered any reactivity in ≥30% of the area. Therefore, it would be reasonable to expect that the frequency of cases with MET overexpression would be higher in the later (73% versus 22%). Similar effect occurred in CRC comparing Voutsina et al. [54], Ma et al. [49] and Shoji et al. [56] studies. In NSCLC, MET overexpression was reported by Spigel et al. [61] in 52% and by Masuya et al. [32] in 41% of the patients. These studies used different scoring criteria, however surprisingly the less stringent criteria (> grade 1 intensity in ≥50% of tumor cells) detected lower frequency of expressors than the more stringent (moderate 2+ or higher intensity in ≥50% of tumor cells). This could be due to the different reagents used, since some antibodies have higher level of efficacy for protein detection. Xia et al.[59] using similar criteria to Spigel et al. [61] but a different antibody detected lower frequency of MET

overexpression (30%) in Chinese patients with lung adenocarcinoma.

Few studies have applied semi-quantitative criteria to measure MET expression, such as the Allred score [63], the Immunoreactive-Remelle score (IRS) [64], or H-score [65], which are expected to perform better since they are less arbitrary in nature. Only one study [44] used Allred score to access HGF expression in lung adenocarcinoma and one study [50] has assessed MET expression level in breast cancer using IRS. The H-score has been widely employed to evaluate levels of hormone receptor in breast cancer [66] and EGFR in NSCLC [67] but has been applied to determine MET expression in only one study in CRC [52].

The variety of methodologies and classification criteria within the given methodologies used for definition of MET and HFG overexpression in the literature has created a set of circumstances that made it impossible to determine which molecular assay is best suitable to achieve this purpose. Consequently, the use of these variables as biomarkers for selecting cancer patients for novel MET targeted therapies has been significantly compromised as further discussed ahead.

MET Gene Mutation and Amplification

At least 30 distinct missense mutations in the *MET* gene have been found as somatic and germline events in cancer patients, and they are located mainly in three genomic areas: (a) the exon 2, which encodes the SEMA domain essential for dimerization [68], (b) the exon 14, which encodes the CBL-binding docking in the juxtamembrane domain that leads to ubiquitination and receptor degradation [69], and (c) the exons 15 to 21, which encompass the tyrosine kinase domain (Table 3, Figure 1A).

MET mutations in solid tumors were first reported by Schmidt in hereditary papillary renal cell carcinoma (RCC) [70]. The surge of high resolution technologies for mutation detection such as massive parallel and target next generation sequencing so far has not impacted the knowledge in this field but is expect to contribute in the near future. Nowadays these mutations have been reported in HNSCC [43,71,72], lung [68,73-76], papillary RCC [77-81], CRC [82], gastric [69,83,84], breast [83,85], ovarian [86] and prostate [81] cancers (Table 3). Highest frequencies of *MET* mutations were found in hereditary and sporadic papillary RCC (57%) and HNSCC (27%). In HNSCC, missense mutations in the MET TK domain have been reported more frequently in metastases, increased from 2-6% in primary tumors to 50% in metastatic lymph nodes, supporting the hypothesis that *MET* mutations occur during the progression of tumorigenesis [71]. The recently identified mutations in exon 14 caused by nucleic acid deletions lead to splicing of RNA transcription and abnormal CBL-binding [43,73-75,82,87]. Kong-Beltren et al. [87] found that lung tumors harboring exon 14 splicing mutations had strong MET expression by IHC, decreased ubiquination and degradation and prolonged cell signaling. *MET* gene splice mutation was reported in 1% [74] to 2.7% [75] of lung cancer patients.

Table 3. MET mutations in solid tumors.

Types of cancer	Specimen	Technique	Positive/Case (%)	Reference
Breast	tissue	PCR	1/30 (3%)	Lee [83]
	tissue	targeted NGS/Sequenom Mass ARRAY/PCR-based extension assay	8/88 (9%)	de Melo Gagliato [85]
Colorectal	tissue	PCR	0/20 (0%)	Schmidt [79]
	tissue	qRT-PCR	18/299 (6%)	Neklason [82]
Gastric	tissue	PCR	1/85 (1%)	Lee [83]
	cell lines	PCR	1/4 (25%)	Asaoka [69]
HNSCC	tissue	RT-PCR	4/15 (27%)	Di Renzo [71]
	tissue	qRT-PCR	9/66 (14%)	Seiwert [43]
	cell lines		1/8(13%)	
	All		10/74 (14%)	
Lung adeno	tissue	WES	16/230 (7%)	TCGA [76]
Lung non-small cell	tissue	PCR	10/127 (8%)	Ma [74]
	tissue	Multiplex PCR	27/283 (10%)	Krisnaswamy [68]
	cell lines		5/74 (7%)	
	All		32/357 (9%)	
	tissue	RT-PCR	7/262 (3%)	Onozato [75]
Lung small cell	tissue	PCR	4/32 (17%)	Ma [73]
Ovarian	tissue	targeted NGS/Sequenom Mass ARRAY/PCR-based extension assay	9/122 (7%)	Tang [86]
Prostate	tissue	targeted NGS/Sequenom Mass ARRAY/PCR-based extension assay	1/15 (7%)	Jardim [81]
Renal cell	tissue	targeted NGS/Sequenom Mass ARRAY/PCR-based extension assay	2/10 (10%)	Jardim [81]
Renal cell, papillary, hereditary	tissue	PCR	4/7 (57%)	Schmidt [70]
Renal cell, papillary, sporadic	tissue	PCR	17/129 (13%)	Schmidt [79]

WES; whole exome sequencing, PCR; polymerase chain reaction, RT-PCR; real time-polymerase chain reaction, qRT-PCR; quantitative real time-polymerase chain reaction.

The MET pathway may also be activated by gain in the *MET* gene copy number per cell or gene amplification. Amplification of the *MET* gene has been investigated, albeit scarcely, in tumor types such as adrenocortical, biliary tract, breast, colorectal, gastric, genitourinary and HNSCC, and much more extensively in NSCLC, as summarized in Table 4. The frequencies of *MET* amplification in the listed studies are largely variable and the main factors accounting for this variation are both biological and technical in nature. The relevant biological variables include patient ethnicity and cancer treatment received pre-molecular testing, and enrichment of the selected cohort for clinical, demographic and histological characteristics. The technical variables mainly include differences in the sensitivity and specificity of the technical platforms used and in the selected diagnostic criteria.

Table 4. MET gene amplification in solid tumors.

Tumor Type	Cohort Type	Diagnostic Method	Reagent	Interpretation for Positive	Positive/ Informative Cases (%)	Amplification Prognosis	Reference
Adrenocortical	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of *MET* in >10% of tumor cells	2/13 (15%)	NS in OS	Jardim [88]
Biliary tract	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of *MET* in >10% of tumor cells	2/21 (10%)	NS in OS	Jardim [88]
Bladder	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of *MET* in >10% of tumor cells	1/10 (10%)	NS in OS	Jardim [88]
Breast	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of *MET* in >10% of tumor cells	3/63 (5%)	NS in OS	de Melo Gagliato [85]
	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of *MET* in >10% of tumor cells	3/64 (5%)	NS in OS	Jardim [88]
Colorectal	Primary Metastasis	PCR		> mean + 2SD of normal tissue	21/67 (31%) 21/62 (34%)	Not reported	Voutsina [54]
	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of *MET* in >10% of tumor cells	4/208 (2%)	NS in OS	Jardim [88]
Gastric	Cell lines Tumor - advanced stage Tumor - early stage	Southern blot		≥3 fold increase of signal intensit than non-neoplastic mucosa	6/11 (55%) 15/64 (23%) 0/11 (0%)	Not reported	Kuniyasu [103]
	Treatment naïve	CGH	Not reported	*MET*/MTHFR >1.5	6/58 (10%)	Not reported	Sakakura [104]
	Pre chemo	FISH	MET/Cen 7, Kreatech Diagnostics	*MET*/CEP7 >2 or >20 copies of MET in >10% of tumor cells	19/227 (8%)	shorter PFS and OS (*p* <0.001)	An [57]
	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of MET in >10% of tumor cells	5/77 (6%)	NS in OS	Jardim [88]
Genitourinary	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of *MET* in >10% of tumor cells	7/97 (7%)	HR for OS 2.8 (*p* = 0.034)	Jardim [81]
HNSCC	Cell lines	FISH	LTD, RP11-163C9/LTD, RP11-144B2	copy number > 4	4/14 (29%)	Not reported	Seiwert [43]
	Recurrent/Metastasis	PCR		copy number ≥10	18/23 (78%)	Not reported	
Lung Adenocarcinoma	Pre EGFR TKI Acquired resistance to *EGFR* TKI	aCGH	LTD, RP11-163C9/CEP7 Abbott Molecular	*MET*/MTHFR >1.5	2/62 (3%) 9/43 (21%)	Not reported	Bean [99]
	EGFR TKI resistant cell lines	PCR		Not reported	4/18 (22%)	Not reported	Engelman [100]
	Pre EGFR TKI	PCR		> mean + 2SD of normal tissue	10/49 (20%)	NS in event-free survival (*p*=0.09)	Beau-Faller [89]
	Acquired resistance to *EGFR* TKI	FISH	LSI D7S522 /CEP7 Abbott Molecular	*MET*/CEP7 > 2 or tight *MET* gene cluster or > 15 copies of *MET*/cell in > 10% of cells	1/6 (17%)	Not reported	Jiang [93]
	Pre *EGFR* TKI Acquired resistance to *EGFR* TKI	PCR		Not reported	0/11 0/5	Not reported	Yano [31]
	Pre EGFR TKI Acquired resistance to *EGFR* TKI	qRT-PCR		> mean + 2SD of normal tissue	2/53 (4%) 5/29 (17%)	Not reported	Chen [94]
	Pre EGFR TKI Acquired resistance to *EGFR* TKI	qPCR		copy number > 3	0/8 (0%) 0/10 (0%)	Not reported	Onitsuka [91]
	Treatment naïve	qRT-PCR		> mean + 2SD of normal tissue	8/183 (4%)	NS in OS	Onitsuka [44]
	Acquired resistance to *EGFR* TKI	FISH	LDT, RP11-163C9; Kreatech MET probe/CEP7 Abbot Molecular	*MET*/CEP7 > 2	4/37 (11%)	Not reported	Arcila [95]
	Pre EGFR TKI	qPCR		*MET*/MTHFR >1.5	12/139 (9%)	shorter OS	Chen [101]

						(p=0.042)	
	Acquired resistance to *EGFR* TKI	FISH	LDT, CTB12N12 and EGFR probe (CTD-2113A18)	$\geq$4 copies in $\geq$40% of cells or presence of *MET* cluster and ratio of *MET*/CEP7 $\geq$ 2 or $\geq$ 15 copies of *MET*/cell in $\geq$ 10% of analyzed cells	2/37 (5%)	Not reported	Sequist [96]
	Pre EGFR TKI	qPCR		> mean + 2SD of normal tissue	6/110 (4%)	Not reported	Xia [59]
	Acquired resistance to *EGFR* TKI	FISH	LDT, RP11 163C9; Kreatech MET probe/CEP7 Abbott Molecular	*MET/CEP7* > 2	4/75 (5%)	Not reported	Yu [97]
	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of MET in >10% of tumor cells	1/18 (6%)	NS in OS	Jardim [88]
	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 > 2.2	13/733 (1.7%)	Not reported	Kris [98]
Lung Non-Small Cell	Treatment naïve	FISH	LDT, RP11-95I20 and CEP7 (Abbott Molecular)	copy number ≥5	48/435 (11%)	higher grade (p=0.016), advanced stage (p=0.01) and shorter survival (*p=0.005*)	Cappuzzo [90]
	Pre EGFR TKI	qPCR		copy number ≥5	2/100 (2%)	Not reported	Kubo [100]
	untreated and previously treated (with chemotherapy and/or TKI)	FISH	LTD, CTB12N13 and CEP7 (Abott Molecular)	$\geq$4 copies in $\geq$40% of cells or presence of *MET* cluster and ratio of *MET*/CEP7 $\geq$ 2 or $\geq$ 15 copies of *MET*/cell in $\geq$ 10% of analyzed cells	37/167 (26%)	NS in PFS with treatment of Tivantinib and Erlotinib	Sequist [92]
	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET/*CEP7 $\geq$1.8	not provided	improved ORR with Crizotinib in patients with intermediate and high *MET* level (ratio >2.2 - < 5.0 and $\geq$ 5)	Camidge [105]
Lung Squamous Cell	Pre EGFR TKI	PCR		> mean + 2SD of normal tissue	12/57 (21%)	NS in event-free survival	Beau-Faller [89]
	Pre EGFR TKI	qPCR		*MET/*MTHFR >1.5	7/59 (12%)	shorter OS p=0.042	Chen [102]
Melanoma	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of MET in >10% of tumor cells	2/61 (3%)	NS in OS	Jardim [88]
Ovarian	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET/*CEP7 $\geq$ 2	4/13 (4%)	NS in OS	Tang [86]
	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of MET in >10% of tumor cells	4/110 (4%)	NS in OS	Jardim [88]
Renal	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of MET in >10% of tumor cells	4/28 (14%)	NS in OS	Jardim [88]
Salivary gland	untreated and previously treated (with chemotherapy and/or TKI)	FISH	Not reported	*MET*/CEP7 >2 or >20 copies of MET in >10% of tumor cells	1/30 (3%)	NS in OS	Jardim [88]

aCGH; array comparative genomic hybridization, CGH; comparative genomic hybridization, FISH; fluorescent *in situ* hybridization, HR; hazard ratio, ORR; objective response rate, OS; overall survival, PCR; polymerase chain reaction, PFS; progression-free survival, qRT-PCR; quantitative real time-polymerase chain reaction, RT–PCR; real time-polymerase chain reaction.

In non-lung tumors, frequency of *MET* amplification ranged from ~5% in breast cancer [85,88] to ~78% in HNSCC [43]. In lung tumors, *MET* amplification ranged from 0% to 26% of patients, in non-comparative studies, there is no clear difference in patients never-treated with EGFR inhibitors from 0% to 26% [44,59,89–92] and EGFR TKI resistant patients from 1.7% to 21% [88,93–98]. Nevertheless, the difference became obvious in comparative studies of EGFR TKI naïve and resistant patients, with 3%-4% of *MET* amplification in EGFR TKI naïve patients and 17%-21% in EGFR TKI resistant patients [94,99]. These results corroborated the findings of Engelman et al. [100] that *MET* amplification is a mechanism of resistance to EGFR TKIs. Moreover, Chen et al. [101] also found that *MET* amplification was more frequent in chemotherapy-treated than chemotherapy-naïve lung adenocarcinoma patients (36% vs 7%,

respectively) in a subgroup analysis. In EGFR TKI naïve patients, MET amplification rate was higher in Caucasian, ranging from 11%-21%[89,90,99], than in Asian patients, ranging from 2%-17% [59,94,102]. However, lung tumor histology, adenocarcinoma or squamous cell carcinoma, did not show association with *MET* amplification [89,101].

MET amplification has been detected in a variety of technical platforms. Few early studies have used southern blotting [103] or comparative genomic hybridization (aCGH) [104], while more recent investigations have used PCR-based or FISH techniques. The PCR-based and aCGH assays evaluate extracted DNA, thus the high proportion of non-tumor cells in the tissue sample analyzed may compromise the measurement. Conversely, FISH is an *in situ* assay and is not impacted by presence of non-tumor cells. FISH measures gene amplification

usually in relation to an internal control; in the instance of MET amplification the internal control is the number of copies of chromosome 7 centromere. Both laboratory developed and commercially available DNA FISH probes have been used in the MET FISH assays, as shown in Table 4, but since these reagents should have been validated they would not influence the study conclusions. However, the criteria to classify a specimen as MET amplification positive by FISH were also highly variable. *MET* amplification has been defined by different cut-offs of the ratio *MET*/centromere 7 such as ≥1.8 [105], ≥2 [81,82][81,88] or ≥ 2.2 [105]. Other studies have classified tumors as *MET* positive not based on ratio against an internal control but based strictly on the mean *MET* copy number per cell. In that case, copy number gain has been defined as ≥4 copies in ≥40% of cells [106], ≥5 copies on average per tumor cell [90], ≥ 15 copies of *MET* in ≥ 10% of cells analyzed [106] or >20 copies of *MET* in >10% of cells analyzed [88]. Obviously, this variability may be responsible for a large proportion of the discordant results among studies. As noticeable from the above descriptions, *MET* copy number gain usually occurs at lower level than gene amplification. In the study conducted by Cappuzzo et al. [90], it was found that post-operative NSCLC had *MET* copy number gain rate of 11.1% but the true *MET* gene amplification rate was only 4.1%.

Prognostic and Predictive Value of the Aberrant MET Pathway

As summarized in Table 1, HGF overexpression was found in more invasive [34,35] or advanced stage [42] cancers and conferred poor prognosis [37,38,40,41]. Nagy et al. [37] measured serum HGF level in breast cancer patients using ELISA and found that high level of HGF was significantly related to shorter disease relapse time and shorter overall survival (OS). Li et al. [34] and Wang et al. [35] measured serum and urine HGF levels in bladder cancer patients and reported occurrence of highly invasive tumors in patients with higher HGF levels compared to those with lower HGF levels. Patients with high HGF overexpression detected by IHC also had significantly shorter progression-free survival (PFS) and OS in breast, CRC and hypopharynx cancers [38,40–42].

Similarly, high expression of MET was associated with advanced stage and poor prognosis in breast, bladder, colorectal , gastric, lung and RCC tumors [32,48,53,55] as listed in Table 2. Studies have shown that MET pathway was more often aberrant in advanced stage tumors and metastases rather than in early stage tumors [42,48,51,54,90] and aberrant MET pathway consistently associated with poorer patients'

prognosis [37,40–42,47,54,62]. In breast cancer, most investigations concluded that MET overexpression was associated to lower survival rate. Lee et al. [48] found higher rate of MET overexpression in breast cancer patients with advanced stage and patients with MET overexpressed tumors also had shorter 10-year disease free survival (DFS) compared to non-MET overexpressors (35.4% versus 73.7%; *p* = 0.0004). Similar findings were reported by Voutsina et al. [54] in CRC, MET overexpression was found at two fold higher rate in metastatic lesions compared to primary lesions (18% vs 10%) and conferred shorter survival (HR 4.6 *p*<0.0001).

The knowledge of prognostic impact of *MET* mutation is limited and its predictive impact remains undetermined. For *MET* amplification, despite the numerous studies suggesting trends toward poor prognosis this outcome was only confirmed in two lung cancer studies, as shown in Table 4. In resected NSCLC, *MET* copy number gain/amplification was associated with higher tumor grade (*p*=0.016), advanced clinical stage (*p*=0.01) and shorter OS (*p*=0.05) [90,101]. *MET* amplification was also associated with shorter PFS and OS in gastric cancer (*p*<0.001 for PFS and OS) [57] and with shorter OS in genitourinary cancer (HR for OS 2.8 *p*=0.034) [81].

In summary, tumors harboring MET pathway deregulation detected either as overexpression of HGF and MET or as amplification of *MET* were associated with higher pathological grade, advanced clinical stage and more invasive features [34,35,51,90] These findings significantly support the conclusion that increase in the MET signaling is one of the most common poor prognostic markers in lung carcinomas and maybe also in other carcinomas.

The predictive value of MET pathway has only been explored in lung cancer and in two major settings, in relation to resistance to EGFR-TKI inhibitors and sensitivity to MET inhibitors. With the discovery of the activating mutations in the exons 19 and 21 of *EGFR* gene, *EGFR*-TKIs have become a successful therapeutic option for advanced NSCLC patients harboring these mutations. The response rate of EGFR mutant patients to EGFR TKIs is very high at 65% to 82% [1,2,107], but these patients commonly became resistant to therapy over a short period of time. *MET* amplification is among the principal mechanisms driving this acquired resistance, found in 15%-20% of EGFR TKI resistant patients [94,99,100]. Interestingly, MET deregulation may have a less prominent role in primary resistance to TKIs. In a small cohort, Chen et al. [94] found no impact of *MET* copy number on *EFGR*-TKI sensitivity of TKI-naïve lung adenocarcinoma patients, that is the high *MET* gene copy number (*MET*≥3 by qRT-PCR) in the

diagnostic specimen has not affected the initial response to *EGFR* TKI treatment.

The efficiency of MET deregulation as biomarker for *MET* targeted therapies is less conclusive. Spigel et al. [61] conducted a phase II study in chemo-treated, *EGFR*-TKI naïve advanced NSCLC patients with the *MET* monoclonal antibody onartuzumab (METMab) in combination with erlotinib. The overexpression of MET was verified retrospectively by IHC. No difference in PFS and OS in the intention-to-treat (ITT) populations was found but addition of onartuzumab to erlotinib significantly prolonged PFS and OS in the MET IHC positive patients (moderate or strong intensity in ≥50% of tumor cells). Based on these results, MET high expression was assumed to be a predictive marker for response to the combined onartuzumab/erlotinib therapy and a randomized, placebo-controlled trial in previously treated stage IIIb or IV NSCLC with MET–positive (METLung) was conducted. Recent interim analysis in this phase 3 trial was disappointing and failed to show benefit of onartuzumab in OS. Final results are pending since the study is still in the follow up period [108].

Another relevant trial (A8081001) has been ongoing in NSCLC for several years with crizotinib, a small molecule kinase inhibitor of ALK, ROS1 and MET. This study was initiated as a phase I, expanded to a phase II for patients with *MET* amplification diagnosed by FISH, and more recently expanded to other patients such as with protein overexpression and exon 14 skipping mutation. By FISH, the patients are classified into three categories of amplification: high level (*MET*/CEP7 $\geq$5); intermediate level (*MET*/CEP7 >2.2 to <5); and low level (*MET*/CEP7 $\geq$1.8 to $\leq$2.2). In interim analyses, patients with high level *MET* amplification showed response rate of 67% (4 of 6 cases, including one complete response), while intermediate level of *MET* amplification showed response rate of 20% (1 of 5 cases) and no response was observed among the 2 low amplification cases [105]. As described, the METLung and A8001001 studies used different assays to classify patients' *MET* status. The METLung study measured MET protein expression by IHC using a low stringency cut-off that classified 52% of patients as positive. Conversely, the

A8001001 study measured *MET* copy number by FISH with stringent cut-offs that only classified as positive about 3% of patients tested [105]. The difference between these two assays was also detected in direct comparison in lung cancer by Arriola et al. [109], who found MET IHC positive (moderate and strong intensity in $\geq$50% of tumor cells) in 48% of patients while *MET* amplification by FISH (gene copy number $\geq$5) was only found in 3% of patients. From the clinical trial findings, expression of MET by IHC with the current cut-off does not seem to be a good test to identify patients who will derive benefits from *MET*-targeted therapy. The FISH assay looks more promising in terms of clinical outcome, but the frequency of eligible patients according to this technology is very low. This limitation is reducing significantly the fraction of patients who may benefit from MET inhibitors and even compromising the conclusion of the ongoing trial with crizotinib. In an attempt to address this limitation, the A8001001 trial has been recently amended to include patients with *MET* mutations and copy number gain detected by Next-Generation Sequencing (NGS).

In lung cancer, it is critical to continue exploring different platforms to evaluate the molecular profile of MET as a marker in a more comprehensive and standardized way to identify the best algorithms for identification of patients sensitive to MET inhibitors. Moreover, any successful test identified in lung cancer will need to be prospectively validated in other tumors in which MET deregulation occur in substantial frequency.

Clinical Applications of Deregulation in the MET Pathway

With the better understanding of the role of the MET signaling in carcinogenesis, its pathway has been targeted by newly developed therapeutic agents included into three major categories based on their specific targets (HGF and MET) and mechanisms of action (monoclonal antibodies and TKIs). These targeted agents have been clinically investigated in numerous solid tumors, but mostly in lung cancer as summarized in Table 5.

Table 5. Therapeutical agents targeting MET in lung cancer clinical trials.

HGF antagonist							
Drug	Chemical Name	Study Phase	Treatment Arms	Patient Population	Trial No.	Status	Primary outcome
AV-299	Ficlatuzumab	I / II	Ficlatuzumab plus Gefitinib	Advanced untreated Asian lung adenocarcinoma	NCT01039948	Active, not recruiting	Safety
		II	Arm A : Ficlatuzumab plus Erlotinib Arm B : Placebo plus Erlotinib	Previously untreated metastatic EGFR-mutated NSCLC and BDX004 positive	NCT02318368	Recruiting	PFS
AMG-102	Rilotumamab	I / II	Rilotumamab plus Erlotinib	Advanced pre-treated NSCLC	NCT01233687	Recruiting	Safety
		I / II	Rilotumamab plus Chemotherapy of choice	Extensive SCLC	NCT00791154	Completed	Safety

HGF antagonist							
Drug	Chemical Name	Study Phase	Treatment Arms	Patient Population	Trial No.	Status	Primary outcome
Anti-MET monoclonal antibody							
MetMab	Onartuzumab	II	Onartuzumab plus Erlotinib	Previously treated NSCLC	NCT00854308	Completed	PFS in ITT and MET-positive patients
		II	Arm A : Onartuzumab plus Bevacizumab/Platinum/Paclitaxel or platinum/Pemetrexed Arm B : placebo plus Bevacizumab/Platinum/Paclitaxel or platinum/Pemetrexed	Previously untreated non-squamous NSCLC	NCT01496742	Active, not recruiting	PFS in ITT and MET-positive patients
		II	Arm A : Onartuzumab plus Platinum + paclitaxel Arm B : Placebo plus Platinum + paclitaxel	Previously untreated squamous NSCLC	NCT01519804	Active, not recruiting	PFS in ITT and MET-positive patients
		III (METLung)	Arm A : Onartuzumab plus Erlotinib Arm B : placebo plus Erlotinib	Advanced MET-positive NSCLC	NCT01456325	Active, not recruiting	OS
		III	Arm A : Onartuzumab plus Erlotinib Arm B : placebo plus Erlotinib	Advanced previously treated MET-positive NSCLC	NCT02031744	Recruiting	OS
		III	Arm A : Onartuzumab plus Erlotinib Arm B : placebo plus Erlotinib	Advanced previously untreated MET-positive NSCLC carrying activation EGFR mutation	NCT01887886	Recruiting	PFS
MET tyrosine kinase inhibitor							
MP-470	Amuvatinib	II	Amuvatinib with Chemotherapy (Platinum and Etoposide)	Previously treated SCLC	NCT01357395	Active, not recruiting	ORR
XL 184	Cabozantinib	I/II	Arm A :Cabozatinib plus Erlotinib Arm B : placebo plus Erlotinib	Acquired resistant to Erlotinib	NCT00596648	Completed	Safety
		II	Cabozatinib	Previously untreated NSCLC positive for RET, ROS1, or NTRK fusion or increased MET activity	NCT01639508	Recruiting	ORR
		II	Arm A : Erlotinib Arm B : Cabozatinib Arm C : Combination	Previously treated NSCLC with wt-EGFR	NCT01708954	Active, not recruiting	PFS
INC 280	Capmatinib	Ib/II	Capmatinib plus Gefitinib vs Gefitinib alone	EGFR-TKI resistant *EGR-mt, c-MET* amplified NSCLC	NCT01610336	Recruiting	Safety
		I	Capmatinib plus Erlotinib vs Erlotinib alone	Erlotinib-treated NSCLC	NCT01911507	Recruiting	Safety
PF 02341066	Crizotinib	I	Crizotinib (A8081001 study)	Previously treated advanced malignancies that are known to be sensitive to PF-03241066 inhibition, e.g. ALK, c-MET and ROS	NCT00585195	Recruiting	Safety
		I/II	Arm A : Crizotinib plus Erlotinib Arm B : placebo plus Erlotinib	Previously treated lung adenocarcinoma	NCT00965731	Completed Phase I; Phase II withdrawn	Safety
		I	PF 00299804 (Sequential vs combination)	Previously treated NSCLC	NCT01121575	Completed	Safety
		I	Crizotinib plus PF 00299804	Previously treated NSCLC	NCT01441128	Completed	Safety
GSK 1363089	Foretinib	I/II	Foretinib plus Cabozatinib	Previously treated NSCLC	NCT01068587	Active, not recruiting	Safety
ARQ 197	Tivantinib	I	Tivantinib plus Erlotinib	Previously treated NSCLC	NCT01069757	Completed	Safety
		II	Arm A : Tivantinib plus Erlotinib Arm B : placebo plus Erlotinib	Previously treated NSCLC	NCT00777309	Completed	PFS
		III	Arm A : Tivantinib plus Erlotinib Arm B : placebo plus Erlotinib (ATTENTION study)	Previously treated Asian advanced non-squamous NSCLC with wild-type *EGFR*	NCT01377376	Discontinued due to high frequency of interstitial lung disease	OS
		III	Arm A : Tivantinib plus Erlotinib Arm B : placebo plus Erlotinib (MARQUEE study)	Previously treated advanced non-squamous NSCLC	NCT01244191	Discontinued due to failure to meet primary endpoint at planned intereim analysis	OS in ITT
		I/II	Tivantinib plus Carbolatin + Pemetrexed	Previously untreated non-squamous NSCLC	NCT02049060	Recruiting	Safety
		II	Arm A : Tivantinib plus Erlotinib Arm B : Single chemotherapy (Docetaxel, Pemetrexed, Gemcitabine)	Previously chemotherapy-treated, K-Ras mutant NSCLC	NCT01395758	Active, not recruiting	PFS

ITT; intentiion-to-treat, OS; overall survival, ORR; objective response rate, PFS; progression-free survival.

Anti-HGF Antibodies

Two antibodies against HGF, ficlatuzumab and rilotumumab, are being tested in lung cancer clinical trials. Ficlatuzumab (AV-299) is a humanized HGF IgG1 monoclonal antibody under investigation in Asian patients with previously untreated lung adenocarcinoma likely to harbor activating *EGFR*

mutations, e.g. never smoker with adenocarcinoma histology (NCT01039948). Patients were classified into two arms, one received ficlatuzumab 20mg/kg every 2 weeks plus gefitinib 250 mg/day and another received gefitinib 250mg/day alone. In preliminary analysis, there was no difference in PFS and OS in the ITT population. However, patients with high HGF expression who received the drug combination had better PFS (6.5 vs 2.7 mo, p=0.44) and OS (not reached vs 16 mo, p=0.03) in the subgroup analysis [110]. An ongoing phase II study is recruiting to compare ficlatuzumab plus erlotinib with erlotinib alone in treatment naïve, mutant *EGFR,* advanced stage IV NSCLC (NCT02318368). For this trial, mutant *EGFR* patients are selected based on the positive proteomic signature in the VeriStrat assay (Biodesix).

Rilotumumab (AMG 102) is a fully human monoclonal antibody against HGF, neutralizing HGF and preventing downstream METsignaling. Rilotumumab has been studied in a phase II trial of advanced gastric or esophagogastric cancer and has demonstrated efficacy in patients with MET[high] tumors, defined as harboring >50% of cells with intermediate and strong MET staining, addition of rilotumumab to chemotherapy increased median PFS from 4.4 to 6.9 months (HR 0.31; p=0.007) when compared with chemotherapy alone [111]. More data are anticipated soon from a IB/II trial of rilotumumab plus chemotherapy in extensive SCLC patients (NCT00791154) that was completed in February 2015 and from a phase l/ll trial of combination of rilotumumab and erlotinib in NSCLC patients (NCT01233687) that is still actively recruiting participants.

Anti-MET Antibody

The anti-MET antibody onartuzumab (MetMab) has been extensively studied in lung cancer. This humanized, monoclonal antibody binds to the Sema domain of MET receptor inhibiting the docking of the ligand HGF. As discussed previously, a phase II study of onartuzumab plus erlotinib in EGFR-TKI naïve showed benefit of adding onartuzumab in MET positive patients in terms of PFS which was not confirmed in the interim analysis of the subsequent phase III METLung study [108]. Another phase III study of onartuzumab plus erlotinib as second- or third-line treatment is recruiting patients with MET IHC-positive NSCLC patients (NCT02031744). Final results of the first trial after longer follow up and preliminary results of the second trial are keenly awaited.

MET Tyrosine Kinase Inhibitors

There are six oral MET small molecule TKIs in clinical trials, namely amuvatinib, cabozantinib, capmatinib, crizotinib, foretinib and tivantinib. Four of them (amuvatinib, cabozantinib, crizotinib and foretinib) are multi-kinase inhibitors whereas the other two (capmatinib and tivantinib) are selective MET inhibitors.

Amuvatinib (MP-470) acts on MET, KIT and platelet-derived growth factor receptor (PDGFR). In a phase IB trial in adult solid tumors, amuvatinib was well tolerated and showed 12% PR when combined with chemotherapy [112]. There is an ongoing phase II study of amuvatinib with chemotherapy in previously treated SCLC (NCT01357395).

Cabozantinib (XL184) inhibits MET, vascular endothelial growth factor receptor2 (VEGFR2), and RET.The agent is approved by the US FDA for treatment of aggressive, unresectable, locally advanced or metastasis medullary thyroid carcinoma [113]. Cabozantinib was also tested in phase ll randomized discontinuation cohort of different solid tumors e.g. melanoma, NSCLC, HCC and breast cancer, in which it reached objective tumor regression respectively in 60%, 64%, 5% and 64% of patients [114]. Cabozantinib is being tested as single agent in previously untreated NSCLC patients positive for RET, ROS1 or NTRK fusion or with increased MET activity (NTC01639508) or in combination with erlotinib in previously treated NSCLC patients with wild-type *EGFR* (NTC01708954). In these studies, MET activity was defined as overexpression, mutation or amplification. Moreover, there are several trials investigating the efficacy of cabozantinib in other conditions such as CRC and prostate cancer.

Capmatinib (INC 280) has effectively induced apoptosis in *in vitro* conditions and has shown significant negative effect in tumor cell proliferation and migration [115]. There are two phase I studies with this drug in NSCLC patients, one with capmatinib in combination with gefitinib in EGFR-TKI resistant EGFR-mutant, MET amplified (NTC01610336) and another in combination with erlotinib in NSCLC patients with progressive disease after erlotinib (NTC01911507). There are also ongoing phase I and II studies of Capmatinib in other solid tumors such as HCC, HNSCC, CRC and papillary RCC.

Crizotinib (PF02341066) inhibits ALK, ROS1 and MET and is a FDA approved TKI for ALK-positive NSCLC patients. Moreover, crizotinib showed a high efficacy in NSCLC patients with de novo *MET* amplification [116]. As discussed previously, crizotinib is under an expanded phase I/II trial (A80081001) in NSCLC patients with MET amplification detected by FISH, which have shown promising results. More recently, efficacy of crizotinib

was also detected in advanced NSCLC harboring splicing mutations in the *MET* exon 14 [117], which is relevant since these mutations have been found with relatively high frequency in NSCLC. Several other phaseI/II trials of crizotinib alone or in combination with other TKIs are ongoing in various types of cancers.

Foretinib (GSK1363089, XL880) inhibits MET, VEGF, RON, AXL, TIE-2 receptor and potentially also VEGFR2. Efficacy of foretinib was shown in a phase II study of papillary RCC with ORR of 13.5% [118]. Foretinib is under evaluation in phase I/II trial of previously treated NSCLC patients (NTC01068587).

Tivantinib (ARQ197) blocks both ligand-dependent and ligand-independent MET downstream signaling by stabilizing the inactive conformation. A randomized, phase II study of tivantinib plus erlotinib versus erlotinib alone in previously treated, EGFR TKI-naïve NSCLC patients showed improvement of median PFS in the drug combination arm (3.8 versus 2.3 months; adjusted HR 0.68; *p*=0.04) [92]. An exploratory molecular analyses in a subset of 50 patients with tissue available for molecular testing has not found significant difference in PFS and OS for MET-positive tumors between the combination arm and the erlotinib alone and no correlation was found between MET expression by IHC and *MET* copy number by FISH (r= 0.14, *p* = 0.35) [119]. A phase III study in previously treated, EGFR- and MET-TKI naïve, non-squamous lung cancer patients (MARQUEE trial) was discontinued prematurely because the interim analysis failed to show an improvement of OS in the ITT population. PFS improvement after addition of tivantinib to erlotinib was significant (HR 0.74; *p*<0.01) but this benefit was not carried on to OS (HR 0.89; *p*=0.81). However, OS was significantly improved in the combination treatment arm in patients with MET[high] tumors (HR 0.7; *p*=0.03) [60]. Another phase III study in previously treated, EGFR- and MET-TKI naïve, wild type *EGFR,* non-squamous cell lung cancer Asian patients (ATTENTION trial) was discontinued prematurely because of the higher rate of interstitial lung disease (0.8% in combination with erlotinib group vs 0.2% in erlotinib alone group). ITT analysis showed non-statistically significant improvement of OS (12.9 months vs 11.2 months HR 0.88 *p*=0.4) [120].

The adverse effects of these multi-kinase and selective MET inhibitors are quite similar. Fatigue, skin rash and gastrointestinal adverse effects including diarrhea, anorexia, nausea, vomiting, and constipation were commonly seen in both groups. Dysguesia was more common in cabozantinib and crizotinib which are multi-kinase inhibitors. Cabozantinib was reported to cause hypertension and mucositis and crizotinib was reported to cause visual disorder and edema [3,92,113]. Tivantinib had interstitial lung disease as serious adverse event which led to discontinuation of ATTENTION trial.

As noticeable, there are far more small molecule TKIs as MET inhibitors in clinical trials than antibodies, but favorable results have been observed in both drug categories. However, one of the major limiting factors for the success of these clinical studies is the lack of molecular markers validated to predict which patients are more likely to benefit from the novel targeted agents.

Conclusion

Extensive studies on the *MET* pathway have uncovered its important physiologic function in embryogenesis, organ development and tissue regeneration. Deregulation of *MET* pathway, including overexpression of HGF or MET, *MET* gene mutations and amplification, has shown to play an essential role in carcinogenesis progression and metastasis and, specifically in NSCLC, was also found to be important player in acquired resistance to targeted therapeutic agents such as the *EGFR*-TKIs.

This cellular pathway may be blocked by numerous targeted therapy agents, including HGF-antagonists and anti-MET antibodies and TKIs, which provide new venues for clinical benefit to patients whose tumors are significantly driven by this pathway. Many of those agents are currently in clinical investigation and some such as onartuzumab, tivantinib and crizotinib have shown promising preliminary results. However, molecular biomarkers for the MET pathway are not well defined. There is no proved technical platform for detection of activation, and no standardized methodology or criteria for diagnosis. Among the two more promising assays, MET expression by IHC failed to confirm its validation in randomized clinical trial and MET amplification by FISH still lacks standardization. Moreover, following the selection of the optimal biomarkers, prospective studies with larger number of patients are needed for their validation at the clinical level.

Acknowledgements

Competing Interests

The authors have declared that no competing interest exists.

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