c-MET as a potential therapeutic target and biomarker in cancer

J. Rafael Sierra and Ming-Sound Tsao

Abstract: The receptor tyrosine kinase c-MET and its ligand, hepatocyte growth factor (HGF), regulate multiple cellular processes that stimulate cell proliferation, invasion and angiogenesis. This review provides an overview of the evidence to support c-MET or the HGF/c-MET signaling pathway as relevant targets for personalized cancer treatment based on high frequencies of c-MET and/or HGF overexpression, activation, amplification in non-small cell lung carcinoma (NSCLC), gastric, ovarian, pancreatic, thyroid, breast, head and neck, colon and kidney carcinomas. Additionally, the current knowledge of small molecule inhibitors (tivantinib [ARQ 197]), c-MET/HGF antibodies (rilotumumab and MetMAb) and mechanisms of resistance to c-MET-targeted therapies are discussed.

Keywords: cancer, c-MET, hepatocyte growth factor, personalized medicine, targeted therapy

Introduction

The *MET* proto-oncogene encodes for the receptor tyrosine kinase (RTK), c-MET. Cells of epithelial–endothelial origin widely express c-MET, where it is essential for embryonic development [Brand-Saberi *et al.* 1996; Heymann *et al.* 1996; Bladt *et al.* 1995] and tissue repair [Borowiak *et al.* 2004; Huh *et al.* 2004]. Hepatocyte growth factor (HGF) is the only known ligand for the c-MET receptor and is expressed mainly in cells of mesenchymal origin, although some epithelial cancer cells appear to express both HGF and c-MET [Ma *et al.* 2008; Tsao *et al.* 2001, 1998; To and Tsao, 1998; Tuck *et al.* 1996; Furukawa *et al.* 1995]. Under normal conditions c-MET dimerizes and autophosphorylates upon ligand binding, which in turn creates active docking sites for proteins that mediate downstream signaling leading to the activation of the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)-AKT, v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (SRC), signal transducer and activator of transcription (STAT) signaling pathways [Organ *et al.* 2011; Trusolino *et al.* 2010; Seiden-Long *et al.* 2008; Peschard and Park, 2007]. Such activation evokes a variety of pleiotropic biological responses leading to increased cell growth, scattering and motility, invasion, protection from apoptosis, branching morphogenesis, and angiogenesis [Sierra *et al.* 2008; Conrotto *et al.* 2005; Yi and Tsao, 2000;

Silvagno *et al.* 1995]. However, under pathological conditions improper activation of c-MET may confer proliferative, survival and invasive/metastatic abilities of cancer cells [Benvenuti and Comoglio, 2007; Danilkovitch-Miagkova and Zbar, 2002]. This review article discusses the evidence for the potential role of c-MET as a biomarker and therapeutic target in cancer, especially non-small cell lung carcinoma (NSCLC).

Role of c-MET in cancer

c-MET was first identified in the early 1980s as the product of a chromosomal rearrangement after treatment with the carcinogen *N*-methyl-*N*'-nitro-N-nitrosoguanidine [Cooper *et al.* 1984]. This rearrangement results in a constitutively fused oncogene, *TPR-MET*, which translates into an oncoprotein following dimerization by a leucine-zipper motif located in the TPR moiety. This provides the structural requirement for c-MET kinase to be constitutively active. *TPR-MET* has the ability to transform epithelial cells [Rodrigues and Park, 1993; Park *et al.* 1986], and to induce spontaneous mammary tumors when ubiquitously overexpressed in transgenic mice [Liang *et al.* 1996]. These findings set the starting point for a currently ongoing effort to unveil all oncogenic abilities of c-MET. It took more than a decade to provide the proof of concept for the role of c-MET in human cancers, which became evident following the

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identification of activating point mutations in the germline of patients affected by hereditary papillary renal carcinomas [Olivero *et al.* 1999; Schmidt *et al.* 1999]. However, spontaneously occurring oncogenic *MET* mutations remain rare at 2–3% [Schmidt *et al.* 1999, 1997].

A large number of reports have shown that an altered level of RTK activation can play an important role in the pathophysiology of cancer [Lemmon and Schlessinger, 2010]. Deregulation and the consequent aberrant signaling of c-MET may occur by different mechanisms including gene amplification, overexpression, activating mutations, increased autocrine or paracrine ligand-mediated stimulation, and interaction with other active cell-surface receptors. Many studies have reported that c-MET is overexpressed in a variety of carcinomas including lung, breast, ovary, kidney, colon, thyroid, liver, and gastric carcinomas [Knowles *et al.* 2009; Lengyel *et al.* 2005; Tokunou *et al.* 2001; Ramirez *et al.* 2000; Tsao *et al.* 1998; Koochekpour *et al.* 1997; Olivero *et al.* 1996; Tuck *et al.* 1996; Di Renzo *et al.* 1995c; Furukawa *et al.* 1995; Liu *et al.* 1992; Soman *et al.* 1991; Houldsworth *et al.* 1990], (Table 1). Such overexpression could be the result of transcriptional activation, hypoxia-induced overexpression [Pennacchietti *et al.* 2003], or as a result of *MET* amplification, which is notably observed only in a small subset of cancers [Cappuzzo *et al.* 2009a, 2009b; Beau-Faller *et al.* 2008; Miller *et al.* 2006; Han *et al.* 2003; Tsugawa *et al.* 1998; Kuniyasu *et al.* 1992]. Transgenic mice overexpressing c-MET have been reported to spontaneously develop hepatocellular carcinoma, and when the transgene was inactivated, tumor regression was reported even in large tumors [Wang *et al.* 2001].

MET gene mutation and amplification

As mentioned, somatic mutations on the *MET* gene are rarely found in patients with nonhereditary cancer. To date, missense mutations and single nucleotide polymorphisms (SNPs) have been found in the SEMA and juxtamembrane domain of *MET*, whereas, activating mutations have been described mainly in NSCLC, hereditary and spontaneous renal carcinomas, hepatocellular carcinomas, gliomas, gastric, squamous cell carcinoma of the head and neck, and breast carcinomas [Stella *et al.* 2011; Seiwert *et al.* 2009; Ma *et al.* 2008; Giordano *et al.* 2000; Lee *et al.* 2000; Park *et al.* 1999]. Potentially oncogenic mutations involve mainly point mutations that generate an alternative splicing encoding a shorter protein that lacks exon 14, which encodes for the juxtamembrane domain of c-MET [Ma *et al.* 2008; Lutterbach *et al.* 2007]; point mutations in the kinase domain that render the enzyme constitutively active [Giordano *et al.* 2000]; and Y1003 mutations that inactivate the Cbl binding site leading to constitutive c-MET expression [Kong-Beltran *et al.* 2006; Peschard *et al.* 2001; Vigna *et al.* 1999]. In contrast, several other point mutations (i.e. N375S, R988C and T1010I) have been reported as SNPs since they have been found to lack transforming abilities [John *et al.* 2011; Tyner *et al.* 2010; Tengs *et al.* 2006].

The most frequent genetic alteration is gene amplification, and as a consequence high

Table 1. c-MET and hepatocyte growth factor (HGF) are highly expressed in a variety of carcinomas.

Cancer site	c-MET (%)	HGF (%)	References
Lung	40	50	[Ma *et al.* 2008; Tokunou *et al.* 2001; Tsao *et al.* 2001, 1998; Siegfried *et al.* 1997; Olivero *et al.* 1996; Takanami *et al.* 1996; Liu and Tsao, 1993a, 1993b; Wang *et al.* 1992]
Breast	17	91	[Ma *et al.* 2008; Lengyel *et al.* 2005; Edakuni *et al.* 2001; Tuck *et al.* 1996]
Ovary	31	42	[Aune *et al.* 2011; Yamamoto *et al.* 2011; Ma *et al.* 2008]
Kidney	70	60	[Ma *et al.* 2008; Tanimoto *et al.* 2008]
Colon	78	95	[Ma *et al.* 2008; Kammula *et al.* 2007; Seiden-Long *et al.* 2006; Di Renzo *et al.* 1995a; Liu *et al.* 1992]
Gastric	>95	87	[Ma *et al.* 2008; Nakajima *et al.* 1999; Tsugawa *et al.* 1998; Houldsworth *et al.* 1990]
Thyroid	>90		[Ma *et al.* 2008; Ramirez *et al.* 2000; Di Renzo *et al.* 1995b]
Pancreas	>70	>35	[Liu *et al.* 1998; Di Renzo *et al.* 1995c; Furukawa *et al.* 1995]
Head and neck	>58		[Chau *et al.* 2011]

c-MET protein expression and activation which has been reported as associated with a poor prognosis in NSCLC, colorectal and gastric cancers (Table 2) [Go *et al.* 2010; Cappuzzo *et al.* 2009a, 2009b; Zeng *et al.* 2008; Nakajima *et al.* 1999; Di Renzo *et al.* 1995a]. There were also reports that *MET* is more frequently amplified in metastatic tumors, suggesting a role in the late phases of malignant progression [Zeng *et al.* 2008; Tsugawa *et al.* 1998; Di Renzo *et al.* 1995a].

c-MET protein overexpression

Over the years many groups have established that c-MET and HGF are highly expressed in a large number of solid and soft tumors (for a comprehensive list, see www.vai.org/met). The list of tumors in which c-MET is expressed is large, and it has been shown that high levels of c-MET can lead to the constitutive activation of the enzyme, as well as rendering cells sensitive to subthreshold amounts of HGF. Although many of these studies have not identified the level of c-MET receptor activity/phosphorylation or compared the expression level with its normal counterpart, it could be speculated that it is expressed with autocrine loops of HGF/c-MET which increase cell proliferation and metastases [Navab *et al.* 2009; Yi and Tsao, 2000; Yi *et al.* 1998; Rong *et al.* 1994; Tsao *et al.* 1993]. Furthermore, independent studies have shown that HGF is expressed ubiquitously throughout the body, showing this growth factor to be a systemically available cytokine as well as coming from the tumor stroma [Vuononvirta *et al.* 2009; Parr *et al.* 2004; Aguirre Ghiso *et al.* 1999]. A positive paracrine and autocrine loop

of c-MET activation can therefore lead to further *MET* expression [Boccaccio *et al.* 1994].

c-MET as prognostic markers

High levels of c-MET and/or HGF expression have been associated with poor patient outcome. Nearly half of lung adenocarcinomas demonstrate high expression of c-MET and HGF [Tsao *et al.* 2001; Siegfried *et al.* 1997; Takanami *et al.* 1996]. Such high expression patterns have been reported to correlate with increased tumor growth rate and metastasis, poor prognosis and resistance to radiotherapy [De Bacco *et al.* 2011; Matsui *et al.* 2010; Navab *et al.* 2009]. High levels of c-MET/HGF in breast carcinoma have been correlated with histological grade, poor prognosis and high proliferative cell index [Garcia *et al.* 2007a; Edakuni *et al.* 2001; Yamashita *et al.* 1994], and even with a greater incidence of metastases [Chen *et al.* 2007; Garcia *et al.* 2007b]. In these reports, c-MET overexpression was observed in hypoxic areas and correlated directly with poorer overall survival. Table 3 provides an overview of several reports that have described c-MET and/or HGF as a prognostic marker.

Plexins: modulators of c-MET activation

In the last decade several groups have reported that Plexin B family members (Plexin-B1, Plexin-B2, and Plexin-B3) can bind to both HGF RTK family members: c-MET and RON [Conrotto *et al.* 2004; Giordano *et al.* 2002]. B-Plexins are single-pass transmembrane receptors shown to share structural similarities with c-MET [Tamagnone and Comoglio, 2000; Tamagnone *et al.* 1999]. Giordano and colleagues have

Table 2. Types of cancers where c-MET/hepatocyte growth factor (HGF) has been reported to be important in primary tumor or metastatic progression.

	Primary	Metastases	Clinical association
NSCLC	4.1% (18/435)	—	Poor prognosis [Cappuzzo *et al.* 2009b]
	3.9% (7/180)	—	[Go *et al.* 2010]
	3.8% (2/53)	—	[Chen *et al.* 2009]
	7% (12/183)	—	[Onitsuka *et al.* 2010b]
Colorectal	9% (3/32)	89% (8/9)	[Di Renzo *et al.* 1995a]
	3.6% (9/247)	18% (25/147)	[Zeng *et al.* 2008]
Gastric	9.4% (13/138)	Poorer prognosis	[Nakajima *et al.* 1999]
	21.2% (100/472)	Poorer prognosis	[Lee *et al.* 2011]
	23% (15/64)	Advanced stage and poor prognosis	[Kuniyasu *et al.* 1992]
Ovarian	5.6% (5/89)	Poor prognosis	[Yamamoto *et al.* 2011]

NSCLC, non-small cell lung carcinoma.

Table 3. Reports that have considered c-MET and hepatocyte growth factor (HGF) as prognostic markers.

	Type of cancer	Number of patients studied	c-MET	HGF
[Ichimura *et al.* 1996]	NSCLC	107	High c-MET protein, worse prognosis	
[Nakamura *et al.* 2007]	NSCLC	130	High c-MET associated with tumor differentiation	High HGF associated with tumor differentiation
[Takanami *et al.* 1996]	NSCLC		c-MET-positive patients, worse prognosis	HGF-positive patients, worse prognosis
[Siegfried *et al.* 1997]	NSCLC	56		HGF-positive patients, worse prognosis
[Masuya *et al.* 2004]	NSCLC	88	c-MET-positive patients, worse prognosis	
[Iwasaki *et al.* 2004]	NSCLC	71		No correlation
[Aune *et al.* 2011]	Ovarian	123		Patients with high HGF, poor prognosis
[Barakat *et al.* 2010]	Gastrointestinal	118		High HGF discriminates malignant from benign
[Skoldenberg *et al.* 2009]	Neuroblastoma	73		High HGF, higher disease stage
[Toiyama *et al.* 2009]	CRC	184		Patients with high HGF, poor prognosis
[Tanimoto *et al.* 2008]	RCC	45		Patients with high HGF, poor prognosis
[Kammula *et al.* 2007]	CRC	60		Patients with high HGF, poor prognosis

CRC, colorectal cancer; NSCLC, non-small cell lung carcinoma; RCC, renal cell cancer.

demonstrated that activation of Plexin-B1 by its high-affinity ligand, Sema4D, can transactivate c-MET's invasive growth program, thus promoting tumor growth, invasion, migration and angiogenesis [Sierra *et al.* 2008; Conrotto *et al.* 2005, 2004; Giordano *et al.* 2002]. Similarly, Swiercz and colleagues [2008, 2004] observed that the Sema4D/Plexin-B1 complex can transactivate ERBB2 promoting tumor growth [Swiercz *et al.* 2008, 2004].

As with other semaphorins and plexins, Plexin-B1's roles in cancer have been contradictory [Ch'ng and Kumanogoh, 2010; Capparuccia and Tamagnone, 2009]. Sema4D/Plexin-B1 complex has been associated with potentiating tumor progression in gastric [Schroeder, 1992], breast [Sierra *et al.* 2008; Swiercz *et al.* 2008], lung [Basile *et al.* 2006], head and neck [Basile *et al.* 2006, 2005], cervical [Qiang *et al.* 2011], ovary [Valente *et al.* 2009] and pancreatic [Kato *et al.* 2011] cancers and tumor-associated macrophage proangiogenic effects [Sierra *et al.* 2008].

Furthermore, Plexin-B1 point mutations found in prostate cancer bone metastases and lymph node metastases of other primary cancers render cells more motile with an increase in invasion, adhesion, and lamellipodia extension [Wong *et al.* 2007]. Conversely, Plexin-B1 has been shown to play a tumor suppressor role in melanomas. Two different groups have shown that Plexin-B1 expression in melanomas reduces BRAF signaling pathways [Argast *et al.* 2009] and decreases c-MET expression levels [Stevens *et al.* 2010]. These results challenge the previously published murine model lacking Plexin-B1, which demonstrated that tumor growth or angiogenesis of human melanoma cells growing in Plexin-B1-lacking environments are not affected [Fazzari *et al.* 2007].

c-MET as a mechanism of resistance to epidermal growth factor receptor therapies
During the past 5 years, c-MET has gained considerable interest following the report that lung adenocarcinoma cell line HCC827 (bearing the

sensitizing mutation in the epidermal growth factor gene) developed resistance by amplification of the *MET* gene when exposed to increasing concentrations of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), erlotinib, for long periods of time [Engelman *et al.* 2007]. This finding was further supported by clinical evidence showing lung tumors from four EGFR TKI refractory patients displayed the *MET* amplification [Engelman *et al.* 2007]. Engelman's report was also the first to demonstrate that patients could bear two different mechanisms of resistance to an EGFR TKI, as one of the four patients harbored both the *MET* amplification and the commonly found gatekeeper mutation in EGFR (T790M). The latter has been shown to decrease the sensitivity of the EGFR kinase to the reversible TKI [Yun *et al.* 2008]. Furthermore, cells with amplified *MET* were now sensitive to a dual treatment with EGFR and c-MET TKI, suggesting that inhibition of both receptors could result in disease stabilization.

Several investigators have since reported that a large cohort of patients with lung cancer who were treated with EGFR TKI and relapsed (approximately 18%) displayed *MET* amplification [Turke *et al.* 2010; Cappuzzo *et al.* 2009a; Chen *et al.* 2009; Bean *et al.* 2007] or high HGF levels [Onitsuka *et al.* 2010a]. It is important to note that the prevalence of *MET* amplification in patients with NSCLC who are untreated has been reported to be as low as 4–7% (Table 4). Turke and colleagues have suggested that EGFR TKI-resistant tumors with *MET* amplification arise in tumors with pre-existing clones of *MET*-amplified cells, which survive TKI therapy [Turke *et al.* 2010].

c-MET activation may bypass EGFR TKIs in sensitive cells by two putative mechanisms: c-MET autophosphorylation creates docking sites where downstream signaling proteins can transduce prosurvival signals via the MAPK and PI3K/AKT signaling pathways; and transphosphorylation of other ERBB receptors may amplify the protumorigenic invasive program of c-MET. The latter mechanism is also known as 'cross talk' between RTKs. For all these properties, c-MET is believed to play a significant role in tumor progression and metastasis.

c-MET as a therapeutic target

Because of its pleotrophic role in cellular processes important in oncogenesis and cancer progression, c-MET is considered to be an important target in anticancer therapy [Trusolino *et al.* 2010; Migliore and Giordano, 2008; Peschard and Park, 2007; Corso *et al.* 2005]. Several molecules targeting c-MET have recently been evaluated in early phase clinical trials. Most of them are small kinase inhibitors, while some are biological antagonists and monoclonal antibodies targeting either the ligand or the receptor [Eder *et al.* 2009; Comoglio *et al.* 2008; Toschi and Janne, 2008].

Preclinical studies have shown that in animal models, the inhibition of c-MET or neutralization of its ligand impairs tumorigenic and metastatic properties of cancer cells [Corso *et al.* 2008; McDermott *et al.* 2007; Zou *et al.* 2007; Petrelli *et al.* 2006; Smolen *et al.* 2006; Michieli *et al.* 2004]. Based on this evidence, compounds that abrogate the kinase activity of c-MET have been developed [Porter, 2010]. An important issue relevant to the development of c-MET inhibitors is the identification of a molecular profile predictive

Table 4. Reports that analyzed *MET* gene copy number (GCN) in non-small cell lung carcinoma (NSCLC).

	Number of patients analyzed	Number of patients with high *MET* GCN
[Engelman *et al.* 2007]	8	0
[Bean *et al.* 2007]	62	2 (3%)
[Okuda *et al.* 2008]	213	12 (5%)
[Beau-Faller *et al.* 2008]	106	22 (21%)
[Cappuzzo *et al.* 2009a]	206	12 (6%)
[Cappuzzo *et al.* 2009b]	435	18 (4%)
[Chen *et al.* 2009]	53	2 (4%)
[Onitsuka *et al.* 2010b]	183	12 (7%)
[Go *et al.* 2010]	180	7 (4%)
Total	1446	87 (6%)

of tumors that would benefit from this targeted therapy. Several studies have shown that certain gastric cancer and NSCLC cell lines display an exquisite sensitivity to c-MET inhibitors. Collectively, these studies have analyzed large panels of cell lines with known *MET* gene copy number and mutation variations. These studies demonstrated that cell lines with activated HGF/c-MET autocrine loop or *MET* amplification upon treatment with a c-MET TKI undergo apoptosis both *in vitro* and *in vivo* [Pan *et al.* 2010; Corso *et al.* 2008; Vigna *et al.* 2008; Lutterbach *et al.* 2007; Petrelli *et al.* 2006; Smolen *et al.* 2006]. The results have identified a subset of tumors based on genetic alterations that appear to be dependent on sustained c-MET activity for their growth and survival, and treatment with a single agent may inhibit tumor growth and induce cell death.

To date, only a few c-MET inhibitors have entered clinical trials. The non-ATP competitive c-MET inhibitor, tivantinib (ARQ 197), has recently completed a phase II clinical trial and shown to produce an increased response rate and overall survival when combined with erlotinib [Schiller *et al.* 2010]. Prior to this study, a phase I trial showed that 27% (14 out of 51 patients) of patients had stable disease for over 4 months [Yap *et al.* 2011]. Based on these results, tivantinib has entered a randomized, double-blind, placebo-controlled phase III study in previously treated patients with metastatic NSCLC [DeJager, 2010]. Cabozantinib (XL184), a multikinase inhibitor that targets c-MET, VEGFR2, AXL, KIT, TIE2, FLT3, and RET, has reached phase II/III trials showing reduction of tumor mass in almost 60% of patients treated with glioblastoma and an overall disease control rate of almost 50% in all of the patients who received this inhibitor in phase II studies [Wen, 2010; Salgia *et al.* 2008]. Cabozantinib has entered a combination regimen with erlotinib as a phase Ib/II trial. Very little is known about the results of this trial, with the exception of a partial response by patients who have *MET* amplification or EGFR T790M mutation [Wakelee *et al.* 2010]. Foretinib (XL880) is a multikinase inhibitor that targets c-MET and VEGFR2 at nanomolar concentrations [Qian *et al.* 2009]. It was found to stabilize the disease in 55% of the patients treated in a phase I trial [Eder *et al.* 2010] and, similar to other c-MET inhibitors, it recently started a phase II trial that considers the combination of this inhibitor with

erlotinib [Seymour, 2010]. Lastly, the dual c-MET and ALK inhibitor, crizotinib (PF02341066), is being studied in a phase III randomized, double-blind, placebo-controlled study following several articles that demonstrated its tumor and metastasis inhibitory effects in both c-MET and ALK-positive patients [Kijima *et al.* 2011; Kimura *et al.* 2011; Ou *et al.* 2011]. This drug has also started a combination phase I study that considers the combination of this multikinase c-MET inhibitor with the irreversible pan-HER inhibitor PF00299804. Other compounds such as JNJ38877605 are in early phase clinical trials, but little is known regarding their results. Several reviews have been published on the subject of c-MET TKI in clinical trials [Eder *et al.* 2009; Comoglio *et al.* 2008; Toschi and Janne, 2008].

Another type of c-MET-targeted agent is monoclonal antibodies that have displayed promising results in tumors with high HGF/c-MET levels. Rilotumumab (AMG102) is an anti-HGF monoclonal antibody that interferes with c-MET's activation by HGF [Giordano, 2009]. Rilotumumab is currently evaluated in clinical phase I/II studies alone or in combination with the EGFR-blocking antibody panitumumab [Amgen, 2008]. Previous studies have shown that rilotumumab decreases c-MET phosphorylation and can stabilize the progression of certain solid tumors [Wen *et al.* 2011; Gordon *et al.* 2010].

MetMAb (OA-5D5), a human, monovalent antagonistic anti-MET antibody [Jin *et al.* 2008], in preclinical studies was able to inhibit glioblastoma U87 and pancreatic BxPC3 and KP4 tumor xenografts followed by a decrease in cellular proliferation and motility [Jin *et al.* 2008; Martens *et al.* 2006]. A recent phase II clinical trial using MetMAb in combination with erlotinib to treat patients with NSCLC resulted in a doubling of patient survival from 6.4 to 12.4 months [Spigel *et al.* 2010; Zhou *et al.* 2010]. In this trial, the authors point out that 'c-MET diagnostic negative tumors' when treated with MetMAb and erlotinib had a worse overall survival when compared with the erlotinib plus placebo arm [hazard ratio (HR) = 2.52], pointing out that only c-MET-diagnostic positive tumors benefited from the combinational treatment (HR = 0.56) [Khachatryan *et al.* 2010]. So far, monoclonal antibodies in preclinical and clinical studies have only demonstrated partial or complete response in patients (or cell lines) with high

c-MET levels or an HGF/c-MET autocrine loop [Wen *et al.* 2011; Gordon *et al.* 2010; Khachatryan *et al.* 2010; Jin *et al.* 2008; Vigna *et al.* 2008; Martens *et al.* 2006].

In recent years, the therapeutic aim of finding drugs that selectively target a molecule expressed on neoplastic cells has shifted to finding drugs or combinations of drugs that are able to inhibit multiple pathways both in cancer cells and cells of the microenvironment [Sierra *et al.* 2010]. We have described multiple clinical trials in which c-MET-specific inhibitors are being combined with other RTK inhibitors. This rationale has gained momentum after clinical experience shows that patients who undergo a single-targeted therapy develop drug resistance and relapse. In addition, we have become more knowledgeable with regard to the notion that the tumor microenvironment plays an important role in maintaining the tumor niche; therefore, combination therapies attempt to inhibit neoplastic cells, the vessels and supporting cells (cancer-associated fibroblasts or tumor-associated macrophages) that transport and/or provide nutrients and growth factors to them [Petrelli and Valabrega, 2009]. Finally, the use of multikinase inhibitors has the potential of delaying the development of resistance, since it is known that neoplastic cells are able to undergo an 'oncogenic switch' by which the cell that was originally dependant on a single oncogene can rely on the activation of alternative(s) oncogenes [Cepero *et al.* 2010b; Sierra *et al.* 2010].

Blocking HGF or c-MET antibodies and TKI appears promising and it is anticipated that many studies will be initiated in the years to come, since c-MET is highly expressed in a wide variety of tumors. All of the c-MET-targeted therapies previously discussed exhibit the potential to enter a phase III study either alone or in combination with other kinase inhibitors and reach the approval of the US Food and Drug Administration in the future.

Potential resistant factors to c-MET inhibitors
As with other TKI inhibitors, sensitive cells/ tumors treated with c-MET inhibitors develop resistance [Cepero *et al.* 2010b; Sierra *et al.* 2010]. Since no clinical specimens are still available, only preclinical *in vitro* systems have been able to predict the possible mechanisms of resistance that patients will develop when exposed to anti-MET treatments. So far, three mechanisms of resistance to c-MET inhibitors have been described. First, cells treated with c-MET TKI at high fixed doses develop a dependency on EGFRs [Corso *et al.* 2010; McDermott *et al.* 2010]. Both reports conclude that cells harboring high *MET* copy number will undergo an oncogenic switch that will create an ERBB tyrosine kinase dependency, similar to the oncogenic switch from EGFR to c-MET in NSCLC cells. The second known mechanism of resistance reported by Cepero *et al.* [2010a] is that when c-MET-dependent NSCLC and gastric cell lines are exposed to increasing doses of c-MET inhibitors amplify wild-type *MET* and *KRAS*, it enables cells to overcome the inhibitory threshold of the compound and still sustain high MAPK and PI3K/AKT activity. The third reported mechanism of resistance is the acquisition of a point mutation in the activation loop of c-MET (Y1230H) [Qi *et al.* 2011]. This mutation had previously been described as a somatic mutation in hereditary and sporadic renal carcinomas [Giordano *et al.* 2000]; here it has been shown to overcome the inhibitory effect of any c-MET kinase inhibitor.

Summary
With just over 25 years since its first discovery, the c-MET receptor is emerging as an important target for personalized cancer therapy. Inhibition of c-MET receptor activity *in vivo* has shown promising results in inhibition of tumor cell growth and in overcoming resistance to anti-EGFR therapy, which has now becoming a standard therapy for patients with advanced NSCLC. Results from early phase clinical trials are starting to demonstrate the importance of c-MET/HGF signaling in cancer biology. The challenge will be to explore and discover further other important crosstalk mechanisms involving this pathway, which could lead to further improvement in the efficacy of novel anticancer therapies and improve patient survival.

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