

## 2015 Advances in Gastric Cancer

# Clinical significance of MET in gastric cancer

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**Author contributions:** Inokuchi M and Kojima K contributed to writing this manuscript; Nakagawa M, Otsuki S, Fujimori Y and Sato Y contributed to gathering, reviewing, and analyzing the related literature.

**Conflict-of-interest statement:** The authors have declared that they have no competing financial interests.

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Received: February 24, 2015  
Peer-review started: February 26, 2015  
First decision: March 30, 2015  
Revised: April 6, 2015  
Accepted: August 25, 2015  
Article in press: August 28, 2015  
Published online: November 15, 2015

## Abstract

Chemotherapy has become the global standard treatment

for patients with metastatic or unresectable gastric cancer (GC), although outcomes remain unfavorable. Many molecular-targeted therapies inhibiting signaling pathways of various tyrosine kinase receptors have been developed, and monoclonal antibodies targeting human epidermal growth factor receptor 2 or vascular endothelial growth factor receptor 2 have become standard therapy for GC. Hepatocyte growth factor and its receptor, c-MET (MET), play key roles in tumor growth through activated signaling pathways from receptor in GC cells. Genomic amplification of *MET* leads to the aberrant activation found in GC tumors and is related to survival in patients with GC. This review discusses the clinical significance of MET in GC and examines MET as a potential therapeutic target in patients with GC. Preclinical studies in animal models have shown that MET antibodies or small-molecule MET inhibitors suppress tumor-cell proliferation and tumor progression in *MET*-amplified GC cells. These drugs are now being evaluated in clinical trials as treatments for metastatic or unresectable GC.

**Key words:** MET; Gastric cancer; Genomic amplification; Immunohistochemistry; Clinical trial

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**Core tip:** MET protein overexpression or *MET* gene amplification was associated with tumor progression and survival in gastric cancer (GC), although the definition of MET overexpression remains to be standardized. In preclinical studies, MET antibodies or small-molecule MET inhibitors suppressed cell proliferation and tumor progression in *MET*-amplified GC cells. Therefore, MET-targeting therapy is promising, and MET overexpression might be a useful biomarker of the response to chemotherapy inhibiting MET. Some clinical trials of MET inhibitors were conducted in metastatic GC, but sufficient benefits have not been demonstrated yet.

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K. Clinical significance of MET in gastric cancer. *World J Gastrointest Oncol* 2015; 7(11): 317-327 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v7/i11/317.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v7.i11.317>

## INTRODUCTION

Gastric cancer (GC) is the fourth most common cancer, with 989600 cases newly diagnosed in the world in 2008, accounting for about 8% of all newly diagnosed cancers<sup>[1]</sup>. The effectiveness of chemotherapy remains very limited in patients with unresectable or metastatic GC, and overall survival (OS) was 10 to 13 mo in patients who received combination chemotherapy with multiple cytotoxic agents<sup>[2,3]</sup>.

Receptor tyrosine kinases (RTKs) are growth factor receptors associated with various physiological responses to embryogenesis and homeostasis. RTK activity is strictly regulated in normal cells, although dysregulation or constitutive activation of RTKs has been found in various types of cancer cells<sup>[4]</sup>. Aberrant or oncogenic activation of RTKs augments tumor-cell proliferation, anti-apoptosis, vascularization, metastasis, and resistance to anticancer agents. RTKs are the most intensively pursued target molecules for anticancer drugs, because tumor cells with activated RTK signaling pathways are sensitive to appropriate RTK inhibitors<sup>[5]</sup>. Trastuzumab, a monoclonal antibody against p185 human epidermal growth factor receptor 2 (HER2), was first used clinically to treat GCs with HER2 overexpression. However, only 12% of patients who received trastuzumab had tumors that overexpressed HER2 in that trial<sup>[6]</sup>. Ramucirumab is a monoclonal antibody against vascular endothelial growth factor receptor 2 (VEGFR2). Second-line treatment with ramucirumab significantly prolonged survival in two phase III trials in GC<sup>[7,8]</sup>. Many inhibitors of RTKs have been investigated to identify potential targets for the treatment of GC.

Proto-oncogene c-MET (MET), a member of the RTK family, is a known hepatocyte growth factor (HGF) receptor that is encoded by the *MET* gene. MET has a primary single-chain precursor protein made of alpha and beta subunits, the latter of which contains a cytoplasmic kinase domain and a docking site<sup>[9]</sup>. Binding of HGF to the extracellular domain activates the kinase activity that phosphorylates the tyrosines at the carboxy terminal docking site. Phosphorylated MET (p-MET) can recruit a variety of proteins, including growth factor receptor-bound protein 2 (GRB2), GRB2-associated binding protein 1 (GAB1), phospholipase C (PLC)-gamma, SRC, and SHP2, and activates downstream signaling molecules such as phosphatidylinositol-3-kinase (PI3K)/AKT and extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathways<sup>[10,11]</sup>. Similar to other RTKs, MET plays key roles in tumor survival, growth, angiogenesis, and metastasis. The aberrant signaling of MET by overex-

pression or gene amplification has been detected and correlated with tumor progression or patients' survival in GC<sup>[12-15]</sup>. Alternative activation of the MET pathway is considered an important mechanism causing resistance to treatments targeting HER family members<sup>[16,17]</sup>. Unfortunately, a phase III study of rilotumumab, an HGF monoclonal antibody inhibiting MET pathway, has been recently discontinued because of high treatment-related mortality. However, inhibition of MET must undoubtedly be an important treatment for GC.

In this article, we reassess the clinical significance of MET in GC and summarize currently available results of preclinical studies and clinical trials of MET inhibitors.

## CLINICAL OUTCOMES OF MET EXPRESSION IN GC

### *Protein expression on immunohistochemistry*

Studies examining the relation between MET protein expression and clinical outcomes in GC specimens are summarized in Table 1. MET protein expression on immunohistochemistry (IHC) is predominantly detected in cytoplasm of tumor cells, but is also found in the cell membrane<sup>[12,18-20]</sup>. Lee *et al*<sup>[12]</sup> assessed membranous MET expression according to a standardized technique, similar to that used to evaluate HER2 expression. MET expression was observed even in stromal cells in tumors<sup>[18]</sup>. Moreover, MET overexpression was more frequently detected in dysplasia and precancerous gastric lesions than in intestinal metaplasia<sup>[21]</sup>.

MET overexpression has frequently been found in intestinal type or differentiated type cancers<sup>[12,14,22,23]</sup>, although one study reported a correlation with diffuse type<sup>[13]</sup>. Retterspitz reported that MET was overexpressed in 51% (45 of 88) of diffuse type tumors<sup>[24]</sup>. MET overexpression has been significantly associated with tumor invasion depth<sup>[12,13,23]</sup>, lymph-node metastasis<sup>[12,13,19,20,25,26]</sup>, distant metastasis<sup>[12,13,25]</sup>, tumor stage<sup>[12,20,23,26]</sup>, and recurrence<sup>[14]</sup>, although several studies found no relation to any clinicopathological factors<sup>[24,27,28]</sup>. MET overexpression correlated with liver metastasis only in stage IV disease<sup>[29]</sup>. Some studies showed that MET overexpression was an independent prognostic factor that was significantly related to poor survival<sup>[12-14,19,20,25,26,30-32]</sup>.

In one study, p-MET was detected in 59% (72 of 121) of GC tumors and was significantly associated with lymph-node metastasis, disease stage, and outcomes<sup>[20]</sup>. In another study, however, only 7% (2 of 30) of tumors overexpressed p-MET in spite of the fact that 63% (24 of 38) overexpressed MET<sup>[22]</sup>. In another study using a new technique, collaborative enzyme enhanced reactive-immunoassay, p-MET was detected in 24% (103 of 434) of GC tumors, including 31% of intestinal type, 24% of diffuse type, and 0% of mixed type<sup>[33]</sup>.

### *Gene expression*

Studies assessing *MET* gene expression are summarized

**Table 1** MET protein expressions on immunohistochemistry and clinical outcomes in gastric cancer

	<i>n</i>	Definition of overexpression	%	Relation to clinicopathological factors	Relation to survival	Ref.
Usual IHC	495	2+/3+, > 10%	22	Intestinal type, recurrence	Worse <sup>3</sup>	[14]
	170	Cytoplasmic, 2+/3+	13	ND	ND	[38]
	121	≥ 5%	66	N, stage	Worse	[20]
	114	> 30%	74	NA	Worse <sup>3</sup>	[30]
	98	Intensity and extensity scoring system	59	N, M	Worse	[25]
	50		78	NA	NA	[28]
	38	2+/3+, ≥ 25%	63	Intestinal type	ND	[22]
	94 <sup>1</sup>	≥ 50%	50	NA	NA	[24]
	121 <sup>2</sup>	Any staining	98	Liver metastasis	ND	[29]
	438	Membranous, 2+/3+, > 10%	24	T, N, M, stage, intestinal type	Worse	[12]
TMA	436	Intensity and extensity scoring system	44	T, N, M, diffuse type	Worse <sup>3,4</sup>	[13]
	215	Cytoplasmic, > 10%	69	NA	NA	[27]
	212	2+/3+	12	ND	Worse <sup>3</sup>	[32]
	182	Intensity and extensity scoring system	66	N, intestinal type, differentiated type	Worse	[19]
	163	Cytoplasmic 2+/3+ ≥ 10%, and positive > 75%	4	ND	Worse <sup>3</sup>	[31]
	124	Cytoplasmic, 3+	71	T, stage, intestinal type	ND	[23]
	114	Intensity and extensity scoring system	82	N, stage	Worse	[26]
	35		43	ND	Likely worse	[18]

<sup>1</sup>Limited to diffuse or mixed type; <sup>2</sup>Only stage IV; <sup>3</sup>An independent prognostic factor on multivariate analysis; <sup>4</sup>Only IHC3+. IHC: Immunohistochemistry; TMA: Tissue micro array; T: Tumor invasion depth; N: Lymph-node metastasis; M: Distant metastasis; ND: Not described; NA: Not associated.

**Table 2** MET mRNA expressions and clinical outcomes in gastric cancer

	<i>n</i>	Overexpression	%	Relation to clinicopathological factors	Relation to survival	Ref.
		Cut-off value				
Tumor	100	Value determined by nonparametric receiver operating characteristics	11	M	Worse	[34]
	100	ND	24	ND	ND	[43]
	45			N, stage, differentiated type	ND	[35]
	43	Value of mean + 2 SD in noncancerous tissue	70	NA	ND	[36]
	15			Intestinal type	ND	[22]
Serum	52	Detected	62	T, N, M, stage, recurrence, v	Worse	[37]

T: Depth of tumor invasion; N: Lymph-node metastasis; M: Distant metastasis; v: Venous invasion; ND: Not described; NA: Not associated.

in Table 2. *MET* mRNA expression in GC tissue has been reported to significantly correlate with lymph-node metastasis, distant metastasis, and disease stage<sup>[34,35]</sup>, although one study found no clinical significance<sup>[36]</sup>. Higher levels of *MET* mRNA expression were frequently detected in intestinal or differentiated type cancers<sup>[22,35]</sup>. Serum *MET* mRNA expression in peripheral blood has been detected and was significantly associated with tumor progression and short survival<sup>[37]</sup>.

Studies of *MET* gene alterations are summarized in Table 3. On fluorescence *in situ* hybridization (FISH) or silver *in situ* hybridization, *MET* gene amplification was detected in 3.4% to 7.1% of tumors<sup>[12,32,38]</sup>. In a study of esophagogastric adenocarcinoma, *MET* amplification was observed in 2.2% (10 of 460) of patients<sup>[39]</sup>. However, overexpression has been defined according to two patterns, *i.e.*, both amplification and high polysomy, or amplification alone. Gene amplification has been found to be significantly related to distant metastasis and tumor stage<sup>[12,39]</sup>. On copy number assay using reverse transcription polymerase chain reaction (RT-PCR), *MET* gene amplification was observed in 1.5% to 30% of

tumors, although the definition of *MET* amplification somewhat differed among studies<sup>[15,18,40-42]</sup>. In a study using single nucleotide polymorphism array, *MET* amplification was detected in 3% to 4% of patients<sup>[43,44]</sup>. Wang *et al.*<sup>[43]</sup> reported that *MET* amplification was found in 7% (3 of 41) of intestinal type cancers, but not in other types.

In many studies using FISH or RT-PCR, patients with *MET*-amplified tumors had significantly poorer survival than those with non-amplified tumors<sup>[12,15,18,32,39,41,42]</sup>. Only a Japanese study, with the lowest incidence of gene amplification, reported no relation of *MET* amplification to survival or any clinicopathological characteristic<sup>[40]</sup>.

### Gene mutation

A mutation of *MET* exon 14 coding for the juxta-membrane domain with a regulatory site was detected, and all other mutations were found in *MET* exons 16 to 20<sup>[45]</sup>. *MET* exon 2 skipping was found in 30% (82 of 272) of GC cases and was associated with increased *MET* gene expression. In addition, novel variants of *MET* exon 18 and/or 19 skipping were observed in 42% (47

**Table 3** *MET* gene alterations and clinical outcomes in gastric cancer

	<i>n</i>	Definition of positive expression	%	Relation to clinicopathological factors	Relation to survival	Ref.
FISH	460 <sup>1</sup>	GA	2.2	Stage	Worse	[39]
	196	GA	6.1	ND	Worse	[32]
	170	GA or HP	15 (GA7.1 HP7.6)	ND	ND	[38]
SISH	381	GA or HP	19 (GA3.4, HP16)	Intestinal (HP), M (GA), stage (GA)	Worse <sup>2</sup> (GA)	[12]
RT-PCR	472	> 4 copies	21	NA	Worse <sup>2</sup>	[33]
	266	> 4 copies	1.5	NA	NA	[40]
	216	≥ 5 copies	10	Unknown	Worse <sup>2</sup>	[41]
	128	≥ 4 copies	30	T, stage	Worse <sup>2</sup>	[42]
	45	≥ 7 copies	7	ND	Worse	[18]
SNP array	193	GA	4	ND	ND	[44]
	100	GA	3	ND	ND	[43]
Polymorphism analysis	34 (tumor)	Any alterations	59	T, N, M	ND	[47]
	34 (serum)	Any alterations	41	N, M	ND	[47]

<sup>1</sup>Esophagogastric adenocarcinoma; <sup>2</sup>An independent prognostic factor on multivariate analysis. FISH: Fluorescence *in situ* hybridization; SISH: Silver *in situ* hybridization; RT-PCR: Reverse transcription polymerase chain reaction; SNP: Single nucleotide polymorphism; GA: Gene amplification; HP: High polysomy; ND: Not described; NA: Not associated; T: Tumor invasion depth; N: Lymph-node metastasis; M: Distant metastasis.

of 272) of GC patients<sup>[46]</sup>. In another study, alterations of the *MET* gene were detected in both cancer tissue and peripheral blood of GC patients, and such alterations significantly correlated with tumor depth, lymph-node metastasis, and distant metastasis<sup>[47]</sup>. *MET* polymorphism (A/G or G/G genotype of *MET* rs40239) was significantly associated with favorable survival in a Japanese cohort, although no significant association was found in American or Austrian cohorts<sup>[48]</sup>.

## PRECLINICAL STUDIES OF MET INHIBITORS FOR GC

Several GC cell lines (Hs746T, GTL16, MKN45, SNU5, SNU620, HSC58, 58As9, and 58As1) have *MET* amplification and were used in preclinical studies of *MET* inhibition.

### Selective tyrosine kinase inhibitors for *MET*

Volitinib (HMPL-504/AZD6094) is a small, potent adenosine triphosphate (ATP)-competitive tyrosine kinase inhibitor (TKI) of *MET*. Volitinib showed higher anti-proliferative activity against GC cell lines with gains of *MET* gene copy number (SNU5, Hs746T, SNU620, GTL16, *etc.*) than against those without such gains (MKN1, MKN74, AZ521, KATO III, AGS, *etc.*). The expressions of p-MET, phosphorylated AKT (p-AKT), and phosphorylated ERK (p-ERK) were down-regulated by volitinib in Hs746T cells. In a GC patient-derived tumor xenograft model with *MET* amplification, volitinib inhibited tumor growth; furthermore, the antitumor activity of volitinib was enhanced by concurrent treatment with docetaxel<sup>[38]</sup>.

SU11274 is a small molecule TKI of *MET*. SU11274 blocked HGF-induced epithelial-mesenchymal transition, inducing down-regulation of Snail-2 and vimentin and up-regulation of E-cadherin in MKN45 cells, but not in non-amplified GC cells (MKN74). SU11274 suppressed

proliferation of tumor cells regardless of the presence of HGF and also inhibited migratory potential. In a mouse model of peritoneal dissemination established from MKN45, SU11274 reduced the numbers and sizes of peritoneal tumors<sup>[34]</sup>. SU11274 treatment combined with SN38 synergistically suppressed proliferation of GC cells (side population cells of OCUM-2M) and tumor volume in a xenograft model<sup>[49]</sup>.

PHA-665752 is a specific TKI for *MET*. In GTL16 cells, PHA-665752 inhibited growth in soft agar as well as cell proliferation and induced apoptosis regardless of the presence of HGF. PHA-665752 treatment decreased expression of *MET*-dependent signaling pathways, including p-MET, p-AKT, p-ERK, phosphorylated focal adhesion kinase (p-FAK), p-PLC-gamma, or phosphorylated signal transducer and activator of transcription, in GTL-16 or MKN45 cells<sup>[50,51]</sup>. Inhibition efficacy was higher in MKN45 cells than in non-amplified GC cells (MKN1, MKN28, and AGS)<sup>[51]</sup>. PHA-665752 significantly inhibited an increase in tumor volume in a GTL16 xenograft model<sup>[50]</sup>. PHA-665752 induced autophagy, and combined treatment with PHA-665752 and an autophagy inhibitor acted synergistically in GTL16 cells<sup>[52]</sup>. Furthermore, PHA-665752 restored growth inhibition in GC cells (SNU216) resistant to lapatinib (anti-EGFR and HER2)<sup>[16]</sup>.

SGX523 is a selective, ATP-competitive *MET* inhibitor. Tyr 1248 is essential for high-affinity binding of SGX523 to *MET*. SGX523 inhibited p-MET and downstream signal pathways (p-GAB1, p-AKT, and p-ERK) in GTL16 cells. SGX523 inhibited tumor growth in a GTL16 xenograft model<sup>[53]</sup>.

BAY-853474 is a highly selective, ATP-competitive *MET* inhibitor. It suppressed tumor growth in an Hs746T xenograft model and reduced plasma biomarkers, such as soluble *MET* ectodomain and IL-8<sup>[54]</sup>.

KRC-408 is a small-molecule TKI that inhibits *MET* by occupying the ATP binding site. KRC inhibited p-MET and its constitutive downstream effectors (p-AKT, p-MEK,



p-ERK, phosphorylated mammalian target of rapamycin (mTOR), and p-p70S6K in MKN45 cells. KRC-408 induced apoptosis as represented by increased levels of caspase-3 and PARP. MKN45 cells in G2/M phase accumulated and those in S phase decreased after KRC-408 treatment. KRC-408 significantly delayed tumor growth in an MKN45 xenograft model, accompanied by decreased expression of p-MET, p-AKT, p-ERK, and CD34<sup>[55]</sup>.

AMG 337 is a small-molecule ATP-competitive TKI of MET. Treatment with AMG 337 affected the viability of only two GC cell lines (SNU5 and Hs746T). Administration of AMG 337 resulted in dose-dependent antitumor efficacy in MET-amplified GC xenograft models<sup>[56]</sup>.

### Multikinase TKI

Crizotinib (PF-2341066) is an ATP-competitive, small-molecule TKI of MET and anaplastic lymphoma kinase. Crizotinib inhibited GTL16 cell growth and induced apoptosis in GTL16 cells. Crizotinib treatment reduced p-MET expression and inhibited tumor growth in a GTL16 xenograft model. These effects were accompanied by a decrease in tumor mitotic index (Ki67 expression), induction of apoptosis (caspase-3 expression), and a reduction in microvessel density (CD31 expression)<sup>[57]</sup>. Crizotinib induced apoptosis and reduced expression of p-AKT and p-ERK in *MET*-amplified GC cells (SNU5, HSC58, 58As9, and 58As1), but not in non-amplified GC cells (MKN28 and MKN1). Crizotinib treatment up-regulated the expression of a proapoptotic member of the Bcl-2 family (BIM), whereas it down-regulated the expression of members of the inhibitor of apoptosis protein (IAP) family, such as survivin, X-linked IAP, and c-IAP1. Crizotinib exhibited marked antitumor activity in 58As9 and SNU5 xenografts, but not in other xenografts derived from non-amplified GC cells (AZ521 and MKN28)<sup>[58]</sup>. In another study, crizotinib effectively inhibited the growth of *MET*-amplified GC cells (SNU620, SNU5, Hs746T, and GLT16) or *MET*-overexpressed GC cells (SNU638). *MET*-positive patient-derived GC xenografts responded to crizotinib and showed down-regulation of p-MET, p-AKT, and p-ERK<sup>[32]</sup>.

Foretinib (GSK1363089) is an ATP-competitive multikinase inhibitor of MET, RON, AXL, tunica internal endothelial cell kinase 2 (TIE2), and VEGFR2. Foretinib inhibited the growth of MKN45 cells and FGFR2-amplified GC cells (KATO-III) more strongly than that of non-amplified GC cells (MKN1, MKN7, and MKN74). Foretinib suppressed phosphorylation of EGFR, HER3, and FGFR3 *via* MET inhibition in MKN45 cells, while it inhibited phosphorylation of EGFR, HER3 and MET *via* FGFR2 inhibition in KATO-III cells<sup>[59]</sup>.

Cabozantinib (XL184) is an ATP-competitive, small-molecule multikinase inhibitor against MET, VEGFR2, and RET. SNU5 and Hs746T cells markedly responded to cabozantinib<sup>[60]</sup>.

S49076 is a potent ATP-competitive multikinase

inhibitor of MET, AXL/MER, and FGFR1-3. S49076 decreased p-MET expression and cell viability in GTL16 cells. S49076 down-regulated p-MET, p-AKT, and phosphorylated p70S6K and inhibited tumor growth in a GTL16 xenograft model<sup>[61]</sup>.

T-1840383 is a potent inhibitor that targets MET, VEGFR1-3, RET, RON, RSE, TIE2, and TRKA. T-1840383 inhibited tumor growth in association with reduced p-MET, p-AKT, and p-ERK expression in an MKN45 xenograft model. In a peritoneal dissemination model generated from GC cells (NUGC4 expressing luciferase), T-1840383 treatment significantly prolonged survival in mice<sup>[62]</sup>.

MK-2461, an ATP-competitive multitargeted inhibitor of activated MET, FGFR2, and platelet-derived growth factor receptor, potently inhibited the phosphorylation of three tyrosine residues of MET (Y1003 in the juxta-membrane domain, and Y1349 and Y1365 in the COOH-terminal docking site) in GTL16 cells. The anti-proliferative potencies of MK-2461 were higher in GC cells with amplification of *MET* or *FGFR2* (GTL16, SNU5, SNU16, KATO III) than in non-amplified GC cells (MKN74, AGS, SNU1, etc.). In GTL16 xenograft models, MK-2461 effectively suppressed MET signaling and tumor growth<sup>[63]</sup>.

### Other drugs

K252a is a potent small molecule inhibitor of the TRK family and reduced MET-driven proliferation in GTL16 cells. After K252a treatment, GTL16 cells lost the ability to form lung metastases in mice<sup>[64]</sup>.

Oridonin, a diterpenoid isolated from the plant *Rabdosia rubescens*, has been used in traditional Chinese medicine for the treatment of human cancer, such as esophageal and prostate carcinomas. Oridonin potently inhibited MET phosphorylation and MET-dependent cell proliferation in SNU5 cells. Oridonin inhibited tumor growth and down-regulated p-AKT, p-ERK, p-c-RAF in an SNU5 xenograft model. Expression levels of Ki67 and CD31 on IHC also decreased in that model<sup>[65]</sup>.

### Resistance to MET inhibitors

HER kinase activation has been shown to play a role in the acquisition of resistance to MET inhibitor in GC cells. Phosphorylation of EGFR and HER3, which are activated *via* MET-driven receptor cross-talk, were suppressed by a MET inhibitor (PHA-665752) in GTL-16 and MKN-45 cells. However, EGF or heregulin-beta1 (HRG) treatment activated MET-independent EGFR or HER3 and restimulated PI3K/AKT or MEK/MAPK pathway. EGF or HRG treatment increased expression of cyclin D1, which had been reduced by a MET inhibitor, and promoted the cell cycle from arrest phase to synthetic phase. Therefore, combined treatment with an MET inhibitor plus an MEK or AKT inhibitor suppressed cell proliferation that had been promoted by HER family activation<sup>[66]</sup>. In the other study, activation of HER family members induced resistance to MET inhibitor.

**Table 4** Development of MET-targeting agents for gastric cancer

Type	Agent	Other targets	Phase	Line	Combined therapy	Results or status	Ref.
MET selective non-ATP competitive TKI	Tivantinib (ARQ197)	None	II	2 <sup>nd</sup> /3 <sup>rd</sup>	None	No CR/PR Median PFS 1.4 mo	[72]
MET-selective ATP-competitive TKI	AMG 337	None	II	Any	None	Ongoing	[74]
			I	2 <sup>nd</sup> /3 <sup>rd</sup>	None	1 CR and 4 PR in 10 patients with MET -amplified tumor	[73]
Multitargeted ATP-competitive TKI	Foretinib (GSK1363089)	VEGFR2, RON, AXL, TIE2	II	1 <sup>st</sup> (95%)	Docetaxel, Cisplatin	No CR/PR Median OS 7.4	[75]
	Crizotinib (PF-2341066)	ALK	I			Tumor shrinkage in 2 patients with PFS 3.5 and 3.7 mo	[39]
MET mAb	Onartuzumab (MetMab)	None	III	1 <sup>st</sup>	mFOLFOX	Ongoing	[77]
HGF mAb	Rilotumumab (AMG 102)	None	III	1 <sup>st</sup>	ECX	Suspended	[79]
		None	III	1 <sup>st</sup>	CX	Suspended	[80]
		None	II	1 <sup>st</sup>	ECX	Median PFS 4.2 mo Median OS 5.6 mo	[78]

ATP: Adenosine triphosphate; TKI: Tyrosine kinase inhibitor; mAb: Monoclonal antibody; VEGFR: Vascular endothelial growth factor receptor; ALK: Anaplastic lymphoma kinase; TIE: Tunica internal endothelial cell kinase; CR: Complete response; PR: Partial response; RFS: Relapse-free survival; OS: Overall survival; FOLFOX: Folinic acid + fluorouracil + oxaliplatin; ECX: Epirubicin + oxaliplatin + capecitabine; CX: Oxaliplatin + capecitabine.

GTL16 cells that had acquired constitutive activation of EGFR by EGFR-L858R mutation did not respond to anti-MET treatment, such as MET silencing or MET inhibitor (PHA-665752). mRNA levels of HER family members significantly increased in the resistant GTL16 cells<sup>[67]</sup>. Qi *et al.*<sup>[68]</sup> reported two mechanisms of resistance to the MET inhibitors PHA-665752 and PF-2341066. One mechanism was the activation of EGFR signaling. In GC cells acquiring resistance to MET inhibitors, EGFR signaling (EGFR, AKT, and ERK) was activated *via* an increase in transforming growth factor alpha. The other mechanism involved a gene mutation in the MET activation loop (Y1230). That mutation destabilizes the autoinhibitory conformation of MET on structural analysis and abrogates interaction with the inhibitor<sup>[68]</sup>. Increased copy numbers of *MET* or *KRAS* and increased expression of p-ERK or p-AKT were detected in GTL16 cells resistant to the MET inhibitor PHA-665752<sup>[69]</sup>. In addition, a novel *SND1-BRAF* fusion was detected in GTL16 cells that were resistant to the MET inhibitor RF-04217903 and was proven to be responsible for the resistance<sup>[70]</sup>.

## CLINICAL STUDIES OF MET INHIBITORS IN GC

Published and ongoing clinical studies of MET inhibitors in GC are summarized in Table 4. Tivantinib (ARQ197) is a non-ATP-competitive, selective MET inhibitor. In a phase I trial in 51 patients with GC, 14 patients had stable disease (SD) for 4 mo or longer, and circulating endothelial cells decreased in 58% (25 of 43) of patients. Tivantinib decreased p-MET, MET, and phosphorylated focal adhesion kinase and increased terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling (TUNEL) staining in tumor biopsy specimens<sup>[71]</sup>. In a phase II study of tivantinib as second- or third-line therapy in GC, no

objective response was observed in the 30 patients enrolled; the disease control rate was 37%, and median progression-free survival (PFS) was only 43 d. Tivantinib seemed to have modest antitumor efficacy and mild toxicity. As for adverse effects, severe (grade 3 or higher) neutropenia and anemia were most common, each occurring in 13% (4 of 30) of the patients<sup>[72]</sup>.

Recently, favorable outcomes of treatment with ANG 337 have been reported in a phase I study in 10 patients with MET-amplified esophago GC<sup>[73]</sup>. One patient had a complete response, and 4 had partial responses, even when ANG 337 was given as second-line or subsequent chemotherapy. An ongoing phase II study is expected to explore whether the levels of MET amplification and expression or the presence of mutation in tumor specimens correlates with the response to AMG 337<sup>[74]</sup>.

Foretinib lacked efficacy against metastatic GC in a phase II study enrolling 74 patients. The best response was SD in 23% (10 of 44) of patients who received intermittent dosing and 20% (5 of 25) of those who received daily dosing. Only 4% (3 of 67) of the patients had *MET* amplification in tumor specimens, and one of them had SD. OS was 7.4 mo with intermittent dosing and 4.3 mo with daily dosing. Severe (grade 3 or higher) treatment-related adverse events occurred in 44% (21 of 48) of the patients who received intermittent dosing and 35% (9 of 26) of those who received daily dosing. Elevated aspartate aminotransferase levels (10%) and fatigue (15%) were the most frequent adverse events in patients who received intermittent dosing and daily dosing, respectively. Plasma levels of MET, HGF, VEGFR2, and VEGF-A were measured at baseline and during treatment, but these markers did not correlate with response<sup>[75]</sup>.

Crizotinib was administered to 4 patients with *MET*-amplified esophagogastric adenocarcinomas in part of a phase I study. Two patients had tumor shrinkage (16% and 30%) with PFS of 3.5 and 3.7 mo, respectively<sup>[39]</sup>.

Onartuzumab (formally called MetMab and PRO 143966) is an anti-MET receptor monoclonal antibody. In a phase I clinical trial, one patient with metastatic GC had a complete response for approximately 2 and a half years<sup>[76]</sup>. A phase III study of onartuzumab combined with modified FOLFOX (5-fluorouracil + leucovorin + oxaliplatin) is ongoing<sup>[77]</sup>.

Rilotumumab (AMG 102) is a monoclonal antibody against HGF. In a phase I b/II study of rilotumumab combined with epirubicin, cisplatin, and capecitabine (ECX) as first-line chemotherapy, 121 patients were randomly assigned to treatment (40 to rilotumumab 15 mg/kg; 42 to rilotumumab 7.5 mg/kg; 39 to placebo). Median PFS was significantly longer in both rilotumumab groups combined than in the placebo group (5.7 and 4.2 mo, respectively). The response rate was 39%, and the disease control rate was 80% in the combined rilotumumab group. MET status was evaluated on IHC in that study, and MET positivity was defined as at least 25% membrane staining of tumor cells at any intensity. In the MET-positive group, median OS was much longer in the combined rilotumumab group than in the placebo group (10.6 mo vs 5.7 mo). In the MET-negative group, patients had better survival than those in the MET-positive group, and rilotumumab was not significantly effective. As for adverse effects, severe (grade 3 or higher) venous thromboembolism occurred in 20% (16 of 81) of the patients<sup>[78]</sup>. However, the management of thromboembolism might be the most critical issue. Two phase III trials of rilotumumab plus ECX and rilotumumab plus cisplatin and capecitabine have been suspended because of increased treatment-related mortality<sup>[79,80]</sup>.

## CONCLUSION

Many studies have suggested that MET protein overexpression or *MET* amplification plays a critical role in the progression of GC and negatively affects survival in patients with GC. However, the criteria used to define overexpression of MET protein have differed among many studies, and the assessment of MET protein expression is unlikely to be standardized as strictly as that of HER2 or EGFR. It remains unclear whether staining intensity of the membrane or the cytoplasm of tumor cells should be assessed. Differences in staining intensity associated with the use of different antibodies and different IHC procedures used to assess MET expression remain a problem that must be solved before techniques for assessing MET status can be standardized. The use of different assessment techniques by different investigators is another problem. The evaluation of p-MET expression might provide the most objective measure of MET status; however, the fact that different antibodies recognize different phosphorylated sites might be a major obstacle to the standardization of techniques for assessing p-MET expression. On the other hand, *MET* amplification on FISH may be appropriate for standardized assessment,

similar to *HER2* amplification. Several studies have used consistent criteria to define *MET* amplification on FISH, and it is more objective assessment than that of protein expression on IHC, although the cost- and time-effectiveness of gene analysis may be poor. Deng *et al.*<sup>[44]</sup> reported that *MET* amplification was mutually exclusive from amplification of other genes, such as *EGFR*, *HER2*, *FGFR2*, and *KRAS*. Therefore, MET-targeting therapy is considered a promising treatment for GC with *MET*-amplification as well as GC with amplification of other RTKs.

Preclinical studies have suggested that MET inhibitors are most promising against *MET*-amplified or *MET*-overexpressed cancers. Various MET inhibitors have been developed and studied in clinical trials; however, several trials showed insufficient efficacy and unexpected outcomes. These results might have been caused by lack of identification of specific biomarkers. Methodological differences in the evaluation of MET status remain an important problem in conducting clinical trials. In an ongoing study of monoclonal antibodies of MET, patients with MET expression on IHC are being recruited<sup>[77]</sup>. As mentioned above, the assessment of MET protein expression on IHC remains to be standardized. The same procedure for assessment of MET status on IHC is needed for clinical studies. Many TKIs of MET have produced favorable results in *MET*-amplified GC in many preclinical studies, and AMG 337 and crizotinib were effective in some patients with *MET*-amplified GC in preliminary clinical studies<sup>[39,73]</sup>. MET TKIs thus may be a promising treatment for patients with *MET*-amplified GC.

Resistance to MET inhibitors is another critical issue. Several lines of evidence from preclinical studies suggest that activation of the HER family is involved in resistance to MET inhibitors, and treatment against HER family pathways may overcome this issue. Owing to the diversity of RTKs, treatment with a multitargeted TKI or combined therapy with single-targeted TKIs might be a promising approach to enhance efficacy. However, potential benefits of treatment with multiple inhibitors of RTKs have yet to be demonstrated in clinical trials in GC.

MET is considered a promising target in GC, although the results of phase III trials of rilotumumab have been disappointing. It is essential to identify specific subgroups of patients most likely to benefit from treatment with MET inhibitors. Future studies should attempt to define biomarkers that would optimize the selection of patients who respond to MET inhibitors.

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**P- Reviewer:** Alshehabi Z, Figura N, Goral V, Kim JJ, Maurel J

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