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REVIEW

MET Inhibitors for the Treatment of Gastric Cancer: What's Their Potential?

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Haidar El Darsa[1]
Rola El Sayed[2]
Omar Abdel-Rahman[1]

[1]Division of Medical Oncology, Department of Oncology, Cross Cancer Institute, University of Alberta, Edmonton, Alberta, Canada; [2]Division of Hematology-Oncology, Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Abstract: Gastric cancer remains a disease with a dismal prognosis. Extensive efforts to find targetable disease drivers in gastric cancer were implemented to improve patient outcomes. Beyond anti-*HER2* therapy, *MET* pathway seems to be culprit of cancer invasiveness with *MET*-overexpressing tumors having poorer prognosis. Tyrosine kinase inhibitors targeting the *HGF/MET* pathway were studied in *MET*-positive gastric cancer, but no substantial benefit was proven. Some patients responded in early phase trials but later developed resistance. Others failed to show any benefit at all. Etiologies of resistance may entail inappropriate patient selection with a lack of *MET* detection standardization, tumor alternative pathways, variable *MET* amplification, and genetic variation. Optimizing *MET* detection techniques and better understanding the *MET* pathway, as well as tumor bypass mechanisms, are an absolute need to devise means to overcome resistance using targeted therapy alone, or in combination with other synergistic agents to improve outcomes of patients with *MET*-positive GC.

Keywords: gastric cancer, *MET* over-expression, *MET* amplification, *HGF*, tyrosine kinase inhibitors, monoclonal antibodies

Introduction

Globally, gastric cancer (GC) represents a significant healthcare burden. It constitutes the fifth most common malignancy worldwide (5.7%), and the third most common cause of cancer-related mortality (8.2%).[1] It is responsible for over 1,000,000 new cases and estimated 783,000 deaths in 2018. Its incidence rate is significantly elevated in Eastern Asia reaching (32/100,000), whereas in regions like Northern America and Europe, the incidence rate is generally low, reaching (5–6/100,000).[1] Although rates of non-cardia gastric cancer have been steadily declining in western countries, the frequency of gastric cardia cancers has been soaring.[2,3]

GC is quite heterogeneous. It is categorized into several subtypes based on anatomy (cardia vs non-cardia), histology (diffuse vs intestinal) and molecular characteristics (microsatellite instability (MSI), Epstein–Barr virus (EBV) positivity, genomic stability (GS), and chromosomal instability (CIN)).[4] Different tumor characteristics have different outcomes. Nevertheless, the prognosis of patients with advanced GC with standard therapy remains dismal worldwide, with a median overall survival (OS) of 8 to 12 months,[5,6] and a 5-year OS reaching 5%.[7,8] Therefore, alternative means were sought to improve outcomes.

The millennial advances in cell biology and genetic assays led scientists to scrutinize cell machinery responsible for oncogenesis, and attempt targeting driver

Correspondence: Omar Abdel-Rahman
Division of Medical Oncology, Department of Oncology, Cross Cancer Institute, University of Alberta, Edmonton, Alberta, Canada
Email Omar.Abdelsalam@ahs.ca

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mutations has shown to improve outcomes in a number of tumors. In GC, a variable genomic profile including variations in *HER2, EGFR, FGFR2, KRAS and c-MET* constitutes about 37% of cases that can be potentially targeted.[9] For example, trastuzumab, an inhibitor of human epidermal growth factor receptor 2 (*HER2*), was shown to improve survival when added to chemotherapy in *HER2* positive GC, becoming the first targeted treatment to be approved.[10] However, as only 20% of cases of advanced GC overexpress *HER2*, it was imperative to look for alternative options.

Proto-oncogene *c-Mesenchymal-Epithelial Transition (MET)* signaling pathway plays an integral role in GC. An aberrant, over-activated *MET* pathway promotes disease progression, and serves as a common mechanism of resistance to *HER* targeted therapy.[11] Therefore, the rationale for investigating *c-MET* targeting in GC was warranted. In this article, we will summarize the clinical significance of *MET* in GC onco-pathogenesis, elucidating the available results of trials including multiple *MET* inhibitors as single or combination therapy. We will also tackle the mechanisms of resistance to *MET* inhibitors, as well as the possible means to overcome it.

Role of *MET* in GC Onco-Pathogenesis

Mesenchymal-Epithelial Transition (MET) Pathway (Figure 1)

MET gene is located on chromosome 7q21-q31. It encodes the *Hepatocyte Growth Factor (HGF)* receptor, which is a member of the Receptor Tyrosine Kinase (RTK) family. RTKs are growth factors responsible for physiological responses such as embryogenesis, tissue regeneration, homeostasis, and wound healing.[11] RTK activity is strictly regulated in normal cells while erratic activation in malignancy activates multiple downstream molecular signaling pathways,[12] leading to tumorigenesis, cell survival, angiogenesis, metastasis, and resistance to anticancer agents.[13,14]

MET is a disulfide heterodimer made of alpha and beta subunits. The alpha subunit is solely extracellular whereas the beta subunit contains a membrane-spanning segment, an intracellular cytoplasmic kinase domain, and a docking site

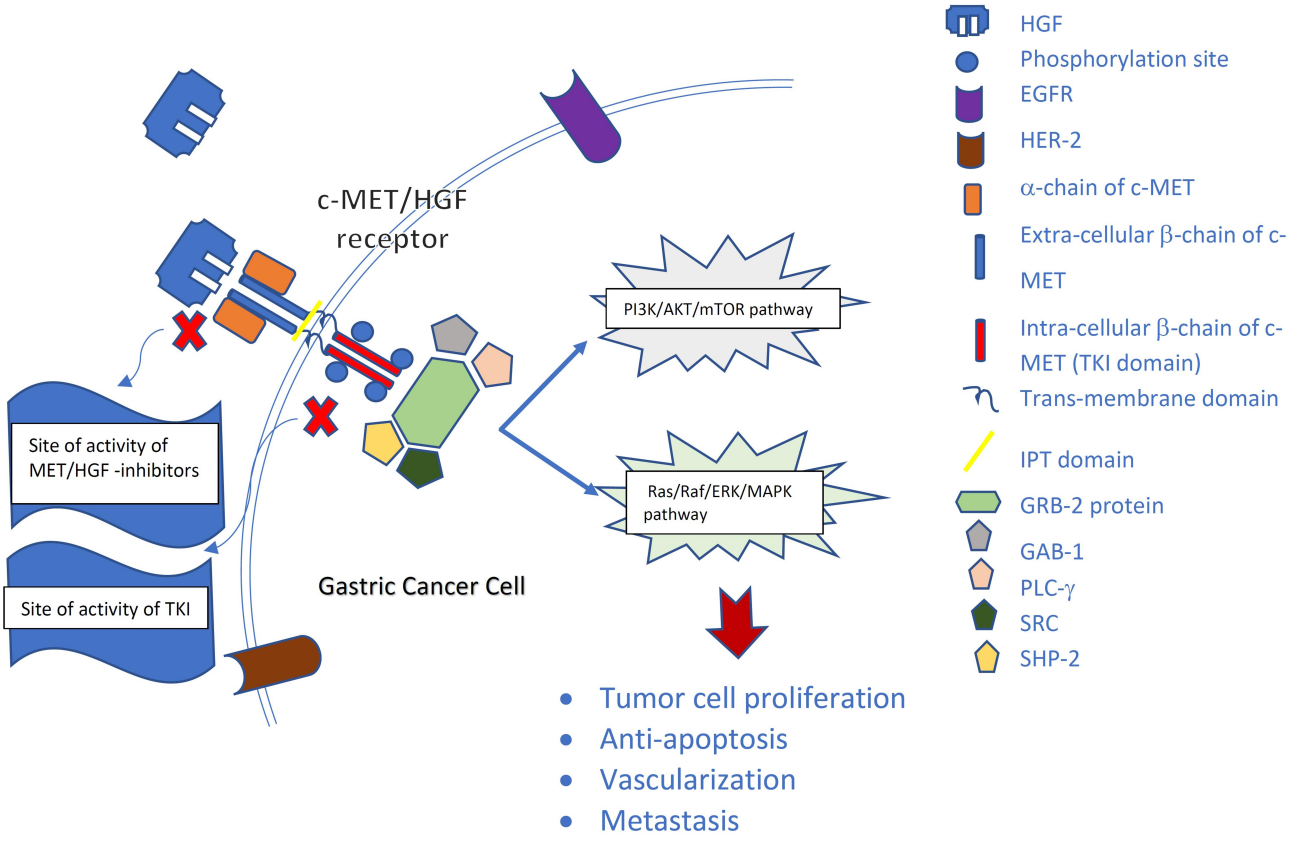

Figure 1 *c-MET/HGF* pathway in gastric cancer pathogenesis. *Hepatocyte growth factor (HGF)* binds to *c-MET*, causing phosphorylation and activation of tyrosine kinase domain with consequent triggering of down-stream signaling via *PI3K/AKT/mTOR* pathway as well as *RAS/RAF/ERK/MAPK* pathway, eventually leading to tumor cell proliferation, tumor survival, angiogenesis and metastasis.

in addition to the extracellular domain.[15,16] *HGF* is a cytokine released by mesenchymal cells with a very high affinity to *MET* receptors. Binding of *HGF* to the extracellular domain of *MET* leads to receptor dimerization, tyrosine phosphorylation at the carboxy-terminal docking site, and finally kinase activation.[16,17] This facilitates the binding of *SRC HOMOLOGY-2 domain (SH2)*-containing proteins and recruitment of proteins such as *growth factor receptor-bound protein 2 (GRB2), GRB2-associated binding protein 1* (*GAB1), Phospholipase C (PLC)- gamma, SRC, and SHP2*. Ultimately, multiple downstream signaling pathways are activated, including *Phosphatidyl-Inositol-3- Kinase (PI3K)/AKT, Extracellular signal-Regulated Kinase (ERK)/ Mitogen-Activated Protein Kinase (MAPK) also known as (RAS-RAF-MEK-ERK) pathway, Signal Transducer and Activator of Transcription 3 (STAT3)*, and *Nuclear Factor-κB (NF-κB)*.[18,19] This pathway cascade activates *CYCLIN D-CDK4/6* and phosphorylates *Retinoblastoma Rb*, releasing the *E2F-1* transcription, which is essential in cell-cycle regulation. Moreover, several downstream genes mediating the phase G1-phase S transition are produced, enhancing cellular proliferation.[20,21] In normal conditions, *MET* receptor is regulated through 26S proteasome-dependent ubiquitination, destruction, internalization, endocytosis, and eventual lysosomal degradation, all while retaining signaling capacity.[22] However, aberrant *MET* signaling disrupts the process and promotes cell invasiveness, growth, angiogenesis, and metastasis, even in hypoxic conditions where excessive *HGF* is released.[14,23]

MET Alteration in GC (Table 1)

Aberrant *c-MET* pathway activation plays an important role in tumorigenesis. It can occur by protein overexpression, gene amplification, increased *HGF* ligand autocrine expression, enhanced paracrine ligand-mediated stimulation, inadequate c-*MET* degradation, ligand-independent activation and rarely gene mutation,[19] in addition to the role of environmental conditions such as inflammation and hypoxia.[24] The most common mechanism of *MET* pathway abnormal activation in GC is via protein overexpression with resultant excessive kinase activation. *MET* protein expression on immunohistochemistry (IHC) is predominantly detected in 50–65% of GC.[25] Mainly, it is expressed in cancer cell cytoplasm, cell membrane and in stromal cells of tumors.[26,27] Moreover, *MET* overexpression is mostly noted in dysplasia and precancerous intestinal metaplasia illustrating its critical role in the early phase of oncogenesis of GC.[26,27] It is frequently encountered in well-differentiated tubular adenocarcinoma (67%), intestinal-type tumors (35%) and to a lesser extent in diffuse-type GC tumors (15–51%).[25,28] *MET* overexpression has been linked to aggressiveness, tumor invasion depth, lymph node metastasis, distant metastasis, advanced tumor stage, recurrence, and poor survival.[25–29]

Table 1 MET Alteration in GC

Met Dysregulation In Gastric Cancer	
MET Protein over-expression	Most common, detected by IHC, 50% of GC
MET gene amplification	Mutually exclusive with other amplifications, 4% of GC
Variable different MET gene mutations	Identified in multiple malignancies, 1–2% in GC
Enhanced paracrine ligand-mediated stimulation	Other factors at play including involvement and cross-talk with other pathways
Increased HGF ligand autocrine expression	

Notes: This table displays the different mechanisms by which MET expression and MET pathway activation is enhanced in gastric cancer.
Abbreviations: MET, mesenchymal-epithelial transition; GC, gastric cancer; IHC, immunohistochemistry; HGF, hepatocyte growth factor.

Another mechanism of aberrant *MET* pathway activation is through *MET* gene amplification, usually mutually exclusive with different other genes. Nevertheless, co-amplification can occur in around 4% (3.4%-7%) of GC, commonly intestinal sub-types,[26,30] leading to *de novo* or secondary treatment resistance.[9,31] Other activating genetic mutations of *MET* remain exceedingly rare in GC reaching only 1–2% of patients.[4,32]

Cross-Talk Between Pathways

MET co-expression and pathway activation exhibit significant cross talk with *ERBB2 (HER2), Vascular Endothelial Growth Factor (VEGF)*, and its receptor *(VEGFR)* signaling pathways, which may cause resistance to targeted therapy and *MET* inhibitors.[11,33,34] One example is the induction of *HGF*-independent c-*MET* activation in some cancer cellular models via *Epidermal Growth Factor Receptor* (*EGFR)* phosphorylation.[35] *RON (receptor originated from nantes)* is another example of an RTK occasionally co-expressed with *c-MET*, where studies showed receptor interaction, one phosphorylating the other, and knockdown of one leading to compensation by the other.[36]

Moreover, c-*MET* expression in human GC specimens was found to be positively correlated to *Jagged1* expression, responsible for activating the *Notch1* signaling pathway that leads to *COX-2* expression, elevation of *prostaglandin E2*, and resultant increased proliferation and migration ability of GC cells. However, constitutive activation of *Notch1* limited *HGF* activity, repressed the c-*MET* oncogene, suppressed c-*MET* expression and decreased *HGF* sensitivity. It can be concluded that *COX-2* and *Notch* knockdown or inhibitors may play a role in the therapeutic strategy against *HGF/c-MET* pathway.[37,38]

Targeted Therapy Against *MET* Pathway in GC

The development of inhibitors targeting *MET/HGF* or downstream signaling proteins has become an attractive goal for GC drug development, including variable tyrosine kinase inhibitors (TKIs), selective or multi-kinase inhibitors, and monoclonal antibodies.

Tyrosine Kinase Inhibitors of *MET* Pathway (Table 2)

Selective Inhibitors

Tivantinib

Tivantinib (ARQ197) is a *non-adenosine triphosphate (ATP)* competitive, selective *MET* inhibitor. It was first studied in a Phase I trial including 51 patients with GC, showing good tolerance with a recommended dose of 360 mg twice daily (BID). The best response was stable disease (SD) for ≥4 months in 14 patients. Tivantinib decreased intra-tumoral *phosphorylated MET (p-MET)*, total c-*MET* levels, and *phosphorylated Focal Adhesion Kinase (FAK)*. However, it increased *terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling*

Table 2 MET Inhibitors

Type	Target	Name	Mechanism of Action	Trial and Reference	Phase	Results
Tyrosine Kinase Inhibitors		Tinvatinib	Selective MET TKI inhibition	Kang Y. et al[40]	II	Modest activity 36% DCR, no ORR
				Pant S. et al[83]	II	ORR 41%, not much improvement over chemotherapy alone
		AMG 337		Van Cutsem et al[41]	II	ORR 18%
		Volitinib		Gavine et al[43]	preclinical	Favorable pre-clinical outcomes and acceptable safety in xenografts
		Savolitinib		VIKTORY Umbrella Trial[44]	II	ORR 50%
		Foretinib	Multi-kinase Inhibition	Shah M et al[48]	II	Minimal efficacy in unselected GC patients, best response SD 23%
		Crizotinib		Lee J et al[50]	Expanded Cohort	4 patients with MET+ GC, 2 had tumor shrinkage
Monoclonal Antibody	HGF	Rilotumumab	Blocks HGF	RILOMET-1[52]	III	Stopped early for non-response
				RILOMET-2[53]	III	Stopped early for non-response
	MET	Onartuzumab	Blocks MET	METGastric[57]	III	No difference in survival
	Bivalent MET inhibitor	Emibetuzumab	Blocks HGF binding and causes MET internalization	Sakai et al[58]	II	Well-tolerated, limited single agent activity, PFS only 47% at 8 weeks

Notes: The table above elaborates the different available MET/HGF inhibitors, whether targeting MET/HGF receptors or the RTK domain, including their mechanism of action, studies in which they were evaluated and the corresponding study results.

Abbreviations: MET, mesenchymal-epithelial transition; HGF, hepatocyte growth factor; ORR, overall response rate; DCR, disease control rate; PFS, progression-free survival; TKI, tyrosine-kinase inhibitor; SD, stable disease; GC, gastric cancer; RTK, receptor tyrosine kinase.

(TUNEL) staining in tumor biopsy, which was correlated with apoptosis. Furthermore, c-*MET* blockade decreased circulating endothelial cells (CEC) in 58% (25 of 43) of patients.[39]

In a Phase II study of 31 patients with advanced GC receiving tivantinib as second- or third-line therapy, modest activity with no objective response (OR) was observed. Disease control rate (DCR) was 36.7%, and median progression-free survival (PFS) was only 43 days. There was no clear correlation between efficacy and biomarkers including gene amplification of c-*MET*, c-*MET*, p-*MET*, and *HGF* expression. Concerning adverse effects (AEs), neutropenia and anemia were the most frequent (grade 3 or higher), each occurring in 13% of cases (4 of 30).[40]

AMG 337

AMG337 is a highly selective and potent small-molecule *MET* inhibitor. In one human phase I trial, 111 patients with solid tumors, 21% of gastro-esophageal origin, received *AMG337* as second-line or later therapy, at a dose of 300 mg orally daily (QD). *AMG337* showed a higher ORR of 29.6% among *MET*-amplified patients compared to 9.9% in all patients, regardless of *MET*-amplification status. The most common AEs were headache, fatigue, nausea, and vomiting.[41] In a Phase II, single-arm study, 45 adults with *MET*-amplified GC and gastroesophageal junction (GEJ) adenocarcinoma, *AMG337* showed promising anti-tumor activity with an ORR of 18% (8 partial responses). The median PFS and OS as well as the duration of response (DOR) were 3.4, 7.9, and 6.0 months, respectively. The most frequent AEs were headache (60%), nausea (38%), vomiting (38%), and abdominal pain (33%). However, 71% had grade ≥3 AEs and 59% had serious AEs.[42]

Volitinib

Volitinib is a potent, highly selective, *ATP*-competitive c-*MET* small-molecule TKI that showed favorable preclinical outcomes and an acceptable safety profile in xeno-graft models. In one Chinese study, volitinib was tested in 3 out of 34 GC models after proving *MET* gene amplification and *c-MET* overexpression, and it resulted in a significant reduction in tumor p-*MET* with tumor growth inhibition.[43]

Savolitinib

Savolitinib is a first-in-class potent, highly selective *MET* inhibitor that showed clinical efficacy and safety in multiple tumor types as monotherapy or in combination in phase Ib/II trials. As part of the phase II VIKTORY trial (targeted agent eValuation In gastric cancer basKeT KORea studY), 25 patients with *MET*-amplified GC were recognized out of 772 patients (3.5%), 20 of whom received savolitinib as monotherapy, attaining the highest ORR reaching 50% (10/20, 95% CI: 28.0–71.9) and meeting the pre-specified PFS endpoint of 6 weeks.[44]

KRC-00715

KRC-00715 is an exclusively selective c-*MET* inhibitor tested among 18 GC cell lines with *c-MET* overexpression. It significantly suppressed the growth of *c-MET* overexpressed cell lines, inducing G1/S arrest, reducing downstream signals, and impairing c-*MET* activity. In vivo, KRC-00715 proved activity in xenograft models with significant tumor size regression.[45]

Multi-Kinase Inhibitors

Foretinib

Foretinib (*GSK1363089*) is an oral multi-kinase inhibitor that targets *MET, RON, AXL, TIE-2, and VEGFR2* receptors. In preclinical and xenograft GC models, foretinib exhibited significant *c-MET* inhibition, preventing cancer stemness,[46] and strongly enhancing the antitumor effect of chemotherapy.[47] In a phase II study enrolling 74 patients with metastatic GC, foretinib was given as intermittent dosing (240 mg/day for 5 consecutive days every 2 weeks) in 48 patients, and daily dosing (80 mg/day during 2-week cycles) in 26 patients. Minimal efficacy was noted in unselected patients, best response being SD in 23% (10 of 44) in those receiving intermittent dosing and 20% (5 of 25) in those on daily dosing. Only 4% (3 of 67) had *MET* amplification in tumor specimens, one of whom had SD. OS was 7.4 months with intermittent dosing and 4.3 months with daily dosing. Most treatment-related AEs were mild, encompassing fatigue, hypertension, nausea, and diarrhea, plus asymptomatic transaminase elevation. Grade 3 or higher treatment-related AEs occurred in 44% of patients on intermittent dosing and 35% of those on daily dosing.[48]

Crizotinib

Crizotinib is an *ATP*-competitive, small-molecule TKI of *MET* and *Anaplastic Lymphoma Kinase (ALK)*. In an expanded study cohort, four patients with *MET*-amplified GC received crizotinib, only two had tumor shrinkage (16% and 30%) with a PFS of 3.5 and 3.7 months, respectively.[49] An ongoing pilot study is testing crizotinib

in patients with c-*MET*-positive GC in third-line setting after chemotherapy failure; pending results.[50]

Monoclonal Antibodies Targeting HGF and c-MET

Rilotumumab (AMG 102)

Rilotumumab (AMG 102) is a fully humanized IgG2 monoclonal antibody that targets *HGF*, preventing receptor binding and consequently inhibiting c-*MET* activation. Its safety and efficacy were evaluated in a dose de-escalation phase Ib study and a double-blind randomized phase II study, in combination with epirubicin, cisplatin, and capecitabine (ECX) as first-line treatment. One hundred and twenty-one patients were randomized equally to receive rilotumumab at a dose of 15 mg/kg (n=40); a dose of 7.5 mg/kg (n=42), or placebo (n=39). Rilotumumab improved clinical outcomes with a median PFS in combined rilotumumab arms reaching 5.7 months versus 4.2 months with placebo. The ORR was 39%, and the DCR was 80% in the combined rilotumumab group. Subgroup analysis showed response in 50% of patients with high *MET*-expression, treated with rilotumumab. This sub-category had a statistically significant OS advantage with a median OS of 10.6 months compared to patients with low *MET* expression having a median OS of 5.7 months (Hazard Ratio (HR)=0.29; p = 0.012). In the *MET* negative subgroup, median OS was similar between rilotumumab and placebo groups. *MET* negative patients had better survival than those in the *MET*-positive group in the placebo arm with a median OS of 11.5 vs 5.7 months, respectively.[51]

Based on these results, two phase-III studies of rilotumumab were started including only IHC-selected *MET* overexpressers: RILOMET-1 and RILOMET-2. Both studies recruited patients with advanced untreated *MET*-positive (IHC ≥1+, ≥25% cells) GC or GEJ adenocarcinoma. In RILOMET-1 study, 690 patients were randomized to rilotumumab 15mg/kg or placebo in combination with ECX chemotherapy. In RILOMET-2, 450 Asian patients were randomized to rilotumumab 15mg/kg or placebo plus cisplatin and capecitabine (CX) chemotherapy.[52,53] The primary endpoint in RILOMET-1 was OS, whereas in RILOMET-2 PFS and OS were co-primary endpoints. Unfortunately, the studies were stopped prematurely because of an increased death rate due to disease progression in the rilotumumab plus chemotherapy arm compared to chemotherapy alone (R vs P: 128 vs 107 deaths) in RILOMET-1. Rilotumumab was strikingly ineffective, with a median OS of 9.6 months compared to 11.5 months with chemotherapy alone (HR=1.37; p = 0.016). All clinical outcomes in all subgroups were statistically worse with rilotumumab.[52] Therefore, RILOMET-2 trial was terminated shortly after.[53] The most common AEs in the rilotumumab groups were neutropenia, anemia, peripheral edema, thromboembolism and fatigue.[51,52]

Another phase II 3-arm trial studied mFOLFOX6 (oxaliplatin, folinic acid, and fluorouracil) monotherapy or in combination with panitumumab or rilotumumab in *HER2*-negative, *MET*-positive advanced GC. The MEGA French study revealed that the addition of panitumumab or rilotumumab was not effective. The 4-month PFS was 71% with chemotherapy alone, 57% with panitumumab, and 61% with rilotumumab. There were more side effects in combination arms; grade 3 or more AEs occurring in 62% with chemotherapy alone, 83% with panitumumab and 89% with rilotumumab.[54]

Onartuzumab

Onartuzumab is a recombinant, fully humanized, monovalent monoclonal antibody that binds the extracellular domain of *MET*; prevents binding with HGF and blocks subsequent *MET* pathway signaling. In a Phase 2 study, onartuzumab was combined with erlotinib in the second or third-line setting, resulting in improved PFS and OS compared to placebo plus erlotinib.[55] Another phase 2 trial also recruited *HER2* negative advanced GC patients to test onartuzumab-mFOLFOX6 combination. There was no survival advantage over chemotherapy alone; neither in the general study population nor in *MET*-positive patients, only more toxicity.[56] Hereafter, a Phase III MetGastric study investigating FOLFOX6 ± onartuzumab in *HER2* negative, *MET*-positive advanced GC patients was prematurely halted. The addition of onartuzumab to first-line mFOLFOX6 did not improve OS, PFS, or ORR, irrespective of *MET* expression status. Grade 3 and above AEs were more frequently observed with onartuzumab including neutropenia, hypoalbuminemia, peripheral edema, thrombocytopenia, pulmonary embolism, and gastric perforation.[57]

Emibetuzumab

Emibetuzumab is a humanized immunoglobulin monoclonal bivalent anti-*MET* antibody. It blocks *HGF-MET* receptor interaction, and causes receptor internalization and degradation; therefore, suppressing ligand-independent *MET* activation. It was evaluated in a non-randomized, single-arm, phase 2 study including 15 Asian patients with *MET*-positive advanced GC, defined by IHC as ≥60% tumor-cell staining

at >2 + intensity, having received ≥2 prior lines of chemotherapy. Emibetuzumab proved to be well tolerated with limited single-agent activity. PFS reached 47% at the 8-week landmark. Grade ≥ 3 AEs were mainly electrolyte imbalances: hyperkalemia, hyponatremia, and hyperuricemia.[58]

Agents Under Investigation

Several other monoclonal antibodies targeting *HGF* or *MET* in GC are being developed. For example, ficlatuzumab and TAK-701 are humanized monoclonal antibodies that specifically target soluble *HGF*, blocking its binding to *c-MET*. Phase I/II clinical studies are ongoing to evaluate their tolerability, safety, pharmacodynamics, and pharmacokinetics.[59,60] ABT-700 is another humanized anti-c-*MET* monoclonal antibody which has interestingly revealed strong single-agent activity in *MET*-amplified GC patients based on results of a phase I study.[61]

Resistance to *MET* Targeted Therapy (Figure 2)

Unfortunately, preclinical and clinical data support the development of acquired resistance to *HGF/c-MET* inhibitors. Numerous mechanisms of resistance to anti-*HGF /c-MET* therapies need to be overcome to improve anticancer effects including: poor MET-status recognition, alternative signaling pathways, emergence of new mutations, and heterogeneity of *MET* expression.

Poor *MET*-Status Recognition

Therapeutic decisions regarding *MET* targeted therapy use require reliable *MET*-status identification. Over many years, techniques for *MET*-status recognition varied from *MET* protein expression on IHC (protein level), to *MET* amplification via *Fluorescent/Silver In-situ Hybridization*

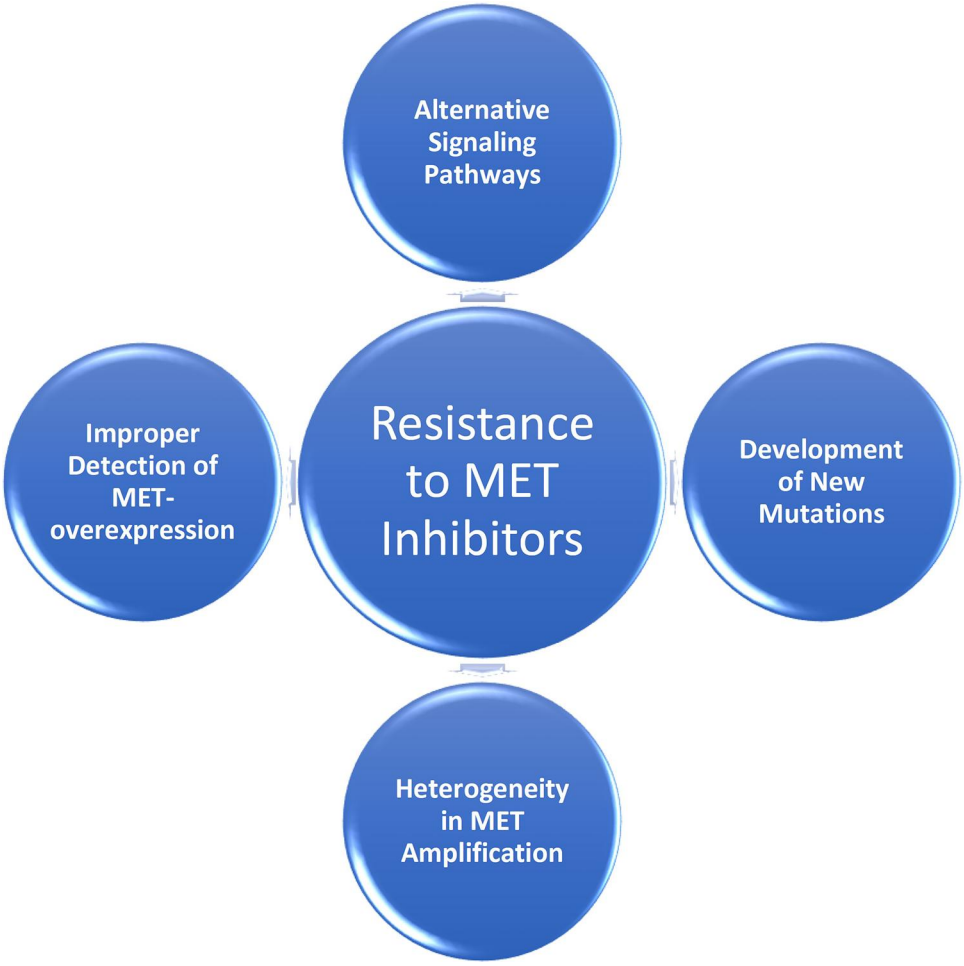

Figure 2 Mechanisms of Resistance to MET inhibitors.

(FISH/SISH), or even genome sequencing assays (gene level). Major discrepancies were noted and no consensus was reached. Moreover, IHC overexpression may not truly reflect gene/pathway driver status, as it does not always correlate with gene amplification, transcriptional activation, or hypoxia. Given this variability in *MET*-status determination with different diagnostic criteria, *MET* targeting may become somewhat challenging.[62,63]

Alternative Signaling Pathways

Alternative signaling pathways may be a rescue mechanism for cancer cells to overcome the effect of *MET* inhibitors. Interestingly, in one study, 40–50% of patients having *MET*-amplified GC were characterized to have *HER2* and/or *EGFR* co-amplification that compromised c-*MET* inhibitor anti-tumoral effects.[31] This predominantly occurs in CIN subtypes characterized by frequent somatic copy number alterations.[4]

Furthermore, pathway crosstalk and *MET's* ability to heterodimerize with *HER* family members, including *EGFR* and *HER2-3*, with their subsequent activation, driven by overexpressed ligands (*TGFα/EGF, heregulin)*, leads to reactivation of downstream *PI3K/AKT/MEK/MAPK* pathways;[31,64] thereby overcoming *MET* inhibition, and enhancing tumor aggressiveness.[65,66] Such co-amplification of *MET* and *HER2* was translated into a reduction of the antitumor capacity with monotherapies such as lapatinib in *HER2* amplified GC.[11]

Emergence of New Mutations

Acquired resistance can be driven by the emergence of new mutations within or outside the *MET* gene. Point mutations within the c-*MET* activation loop, which constitutes the drug target, destabilize the receptor, decrease its binding capacity and cause resistance to *MET* inhibitors while maintaining downstream *MEK-ERK* and *PI3K-AKT* signaling.[66,67] In the VIKTORY trial, three patients treated with Savolitinib developed resistance through emerging mutations, particularly *MET D1228H, MET D1228N, MET D1228V* and *MET Y1230C*.[67]

Resistant mutations include point mutations, increased copy number; skip mutations or alterations in *exon 14* splicing site.[68,69] Examples include: *MET Y1248H* and *MET D1246N*.[70]

Other mutations such as *KRAS* and *RON* mutations have been noted to bypass pathway suppression by *MET* inhibitors.[31]

Heterogeneity in MET Amplification

Another mechanism of resistance to *MET* inhibitors is the dramatic heterogeneity in *MET*-amplification, which may differ within the same tumor as well as between different metastatic lesions and the primary tumor in GC. This leads to mixed responses to *MET* inhibition, and treatment failure due to the outgrowth of non-*MET*-amplified clones.[9] Even upregulation of *MET* gene amplification may confer resistance to *MET* Inhibitors through association with *E-cadherin* and *epithelial to mesenchymal transition* (*EMT*).[65]

Microenvironment Interference and Immune Regulation

The tumor microenvironment (TME) is an important factor in resistance to targeted therapies. In *MET*-positive GC models, tumor-associated fibroblasts within the microenvironment oversecrete *HGF*, activating downstream signals, promoting cancer colony formation, and causing tumor resistance. *HGF* secretion is enhanced by lactate within the TME, in addition to paracrine *HGF* provided by the extra-cellular matrix. Hypoxia, another characteristic of the TME, significantly reduces *MET* phosphorylation, while maintaining downstream signaling.[69]

Furthermore, *HGF/MET* signaling affects immune cells within the TME, such as mast cell activation by *MET-α2β1 integrin*,[71] and dendritic cell (DC) impairment by diminished antigen presentation through *matrix-metalloproteinase MMP2-MMP9*.[72] *MET* inhibition also limits the activity of anti-tumor neutrophils, aiding in tumor growth.[73] More recent studies showed that *MET* inhibition, in general, upregulated *Programmed death-ligand 1* (*PDL-1)* expression, compromising the killing effect of *MET* inhibitors.[74,75]

V-Combination Therapy and Future Perspectives to Bypass Resistance

Synergism with Other Targeted Therapies

Combinatorial targeting of multiple pathways such as *EGFR, HER2*, and *HGF/c-MET* axis could potentially maximize the anti-tumorigenic effect for certain *MET*-addicted GC patients.[11,31,55,76]

Combination with *VEGF* inhibitors is another possibility given the proven benefit of ramucirumab in advanced GC.[77,78] In colorectal models, volitinib, a selective *MET* inhibitor, plus apatinib, a *VEGF* inhibitor, have shown synergy with significant tumor suppression and apoptosis.[79]

Use of Autophagy Inhibitors

After the use of *MET*-TKIs, some cells were noted to resort to protective autophagy through the *MET/mTOR/ULK1* cascade, where cancer cells become less sensitive to further therapies.[80,81] Autophagy inhibitors such as the immunomodulatory hydroxychloroquine, or *mammalian target of rapamycin (mTOR)* inhibitor everolimus can be used in addition to *MET* inhibitors to overcome resistance. Preclinical models in *MET*-amplified tumors showed that autophagy blockade helped *MET*-TKIs better control tumor growth, making the combination a promising therapeutic option to explore.[82]

Combination with Chemotherapy

Multiple studies tested chemotherapy combined with *MET* inhibitors. One phase I/II study included 32 patients with previously untreated advanced GC and GEJ cancers, to receive FOLFOX (day 1) plus Tivantinib (360 mg PO BID, days 1–14 in 2-week cycles) for a median of eight cycles. ORR was 41%. Treatment-related toxicities were mainly hematological, gastrointestinal, and peripheral neuropathy. Median PFS and OS were 6.1 and 9.6 months, respectively, similar to FOLFOX alone. Patients with high c-*MET* expression had inferior PFS and OS, proving the correlation of c-*MET* expression with poorer outcomes.[83] Another ongoing Phase I–II trial is testing the combination of AMG-337 with mFOLFOX6 in *c-MET*-positive advanced-stage GC patients.[84] Volitinib was also tested in combination with docetaxel, and results showed good tolerance and superior anti-tumor efficacy than either agent alone.[43] Furthermore, *MET*-targeting monoclonal antibodies: rilotumumab, onartuzumab and emibetuzumab, were tested in combination with chemotherapy.[52,53,56–58] However, results were disappointing.

Combination with Radiation Therapy

Radiation therapy has been found the culprit of up-regulation of c-*MET* expression and activity, mainly through the *ATM* and *NF-κB* signaling pathways,[85] where levels of c-*MET* increased with radiation time and dose.[86] In GBM models, radiotherapy enhanced HGF secretion, and radio-resistance was related to high *HGF* levels.[87] Irradiation also showed to increase c-*MET* phosphorylation leading to *HGF*- independent increased downstream signaling.[88]

Moreover, the role c-*MET/HGF* pathway has proven to play in DNA repair with consequent radiation resistance,[89] inclined researchers to test c-*MET* inhibitors with radiation therapy, with proof of capability to sensitize cancer cells to radiation in vitro and in vivo.[90] For example, crizotinib sensitized cetuximab-resistant *KRAS* mutant colorectal cancer cells to radiation and improved outcomes in patients undergoing chemo-radiation.[91] Similarly in breast cancer, a study of 208 pre-menopausal patients with breast cancer suggested that adding a *MET* inhibitor to radiotherapy might be an option for patients with c-*MET* overexpression.[92] In esophageal cancer, foretinib increased radio-sensitivity via c-*MET* modulation, where it prompted cell apoptosis, and induced cell-cycle arrest by irradiation, thus diminishing the tumor burden, and improving patient outcomes.[93]

Use of Immunotherapy

Future perspectives should better explore combining *HGF/MET* inhibitors with immunotherapy. *HGF/MET* signaling pathway is intertwined with tumor immunity affecting DCs, mast cells, and neutrophils plus causing up-regulation of *PD-1*/*PDL-1* expression. Researchers have attempted *MET* inhibition as an immunologic stimulant, and a bi-specific *MET/PD-1* dual-acting monoclonal antibody was created and tested with good pre-clinical results.[94] Moreover, *NK1*-targeted chimeric antigen receptors (CAR-T cell immunotherapy) were also developed to mediate *MET*-dependent T-cell activation against malignant cells.[95]

MET Knockdown and Molecular Therapy

Knockdown of c-*MET* not only significantly diminished tumor cell growth, migration and invasion, but also induced apoptosis, and enhanced activity of chemotherapy.[96] At the level of mRNA, *lentivirus*-mediated RNA silencing of c-*MET* markedly suppressed the peritoneal dissemination of gastric cancer in vitro and in vivo.[97]

A number of non-coding RNAs (*ncRNAs*) were discovered, including microRNAs (*miRNAs*) and long noncoding RNAs (*lncRNAs*) that have been involved in *HGF/MET* pathway, responsible for tumor aggressiveness and metastasis.[98] Some *lncRNAs, such as lncRNA-TUG1*, are overexpressed in GC, particularly in diffuse-type, and cause significant proliferation of GC through indirect activation of c-*MET*, whose knockdown remarkably impairs migration, invasion and metastasis both in vitro and in vivo.[99] Other *miRNAs* such as *miR-206, miR-1* and *miR-34a* on the other hand, act as tumor suppressors, down-regulating *c-MET* in vitro as well as in xenograft models.[100,101] More studies should be performed to better characterize the relationships

among *lncRNAs, miRNAs*, and *c-MET*, and perhaps devise new therapies.

Finally, knockdown of *N-acetylgalactosaminyltransferase 2 (GALNT2)*, an enzyme that mediates glycosylation and suppresses malignant phenotypes in GCs through *MET/HGF* activity, enhanced *MET* phosphorylation and decreased *MET* expression, thereby suppressing aggressive tumor phenotypes.[102]

Conclusion

Deciphering the underlying molecular heterogeneity of cancer could have significant clinical utilization by developing specified targeted therapies. Despite the success of targeting *HER2* in advanced GC, research failed to find other targeted therapies with substantial proven benefit. Low 5-year GC survival rates emphasize the need for novel techniques to develop effective targeted therapies. The role of *HGF/MET* pathway in tumor prognosis has driven attention towards this entity as a target of inhibition to improve outcomes. Several selective/non-selective c-*MET* TKIs and monoclonal antibodies were tested; yet, none proved substantial clinical benefit.

The complexity of *MET* signaling pathway, the lack of consensus and poor biomarker determination as well as the diverse resistance mechanisms (cross-talk, new mutations, upregulated gene amplification), all resulted in the limitation of clinical efficacy of *MET* inhibition. Scientists have attempted multiple means to overcome resistance and render these tumors more sensitive to treatment via combination with other targeted treatment, chemotherapy, radiation therapy, immunotherapy or molecular approaches.

As a conclusion, it is vital to standardize *MET*-status determination, so as to properly select patients for trials. Furthermore, a profound understanding of the coexistence of genetic alterations, the complex cross-talk between pathways, and resistance mechanisms will provide guidance for the innovation and validation of effective combination strategies that may improve patient outcomes in GC.

Author Contributions

All authors, HE, RS, and OA, contributed equally to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no potential conflicts of interest for this work.

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