Emerging Targeted Therapies in the Treatment of Advanced or Metastatic Esophagogastric Cancer (EGC)

Emerging Targeted Therapies in the Treatment of Advanced or Metastatic Esophagogastric Cancer (EGC)

By

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Background

Esophagogastric cancer (EGC) is the 2nd leading cause of cancer-related death worldwide(1). Surgical resection remains the most effective and mainstay curative treatment (2).Most patients at the time of diagnosis present with locally advanced or metastatic disease and hence not amenable for surgical resection . Five year overall survival in advanced or metastatic disease is only 5-10%(3).

Platinum, 5-FU and Taxane based chemotherapy regimens are used as back bone of therapy for EGC. However, this leads to improvement in response rate of only ~35%, with a median overall survival (OS) of approximately 1 year(4). Therapy directed at molecular targets in EGC is one potential approach to improving treatment of these patients. For example, for patients with tumors overexpressing the HER2 protein, incorporation of the anti-ErBB2/HER2 antibody (Trastuzumab) with Cisplatin+5FU led to improvement in median overall survival to 13.8 vs 11.1 months with chemotherapy alone(3). The anti-angiogenic (anti VEGFR2) agent Ramucirumab, either alone or in combination with Paclitaxel, is approved for treatment of EGC in the 2nd line(5, 6). More recently, immunotherapy (Pembrolizumab) is approved for treatment of advanced or metastatic EGC positive for PD-L1 in 3rd line(7). However, even with improvements utilizing molecular targeted therapies, significant improvements are needed in the treatment of these patients.

Heat Shock proteins (HSP) are a family of highly conserved ubiquitously expressed proteins which function as molecular chaperones playing important roles in ensuring essential protein function including proper folding and transport. They prevent degradation of the proteins by the ubiquitin-protein pathway and are thus essential for protein homeostasis. They protect their client proteins from various cellular stresses (e.g heat shock,

hypoxia, genotoxic agents, and nutrition starvation) (8). Given the important functions of their client proteins, they play important roles in a number of essential cellular processes including differentiation, proliferation, and survival. One family of HSP proteins (HSP90) has been shown to be important for maintaining the stability of a number of oncoproteins important for various cancer cell functions including survival, proliferation, induction of angiogenesis and metastasis(9). Cancer cells have significant proteotoxic stress due to the number of genetic mutations including producing abnormal and misfolded proteins as well as their increased rate of proliferation. One of cancer cell's mechanisms of protecting against cell death from this increased stress is the production of increased levels of HSP90. Dysregulated HSP90 leads to various steps of carcinogenesis including proliferation, angiogenesis and metastasis by maintain various client proteins including CRAF, BRAF, EGFR,MET,VEGFR, AKT, HIF, and various proteins important in controlling the cell cycle. Given the important role of HSP90 in protecting the function of a number of oncoproteins in cancer cells, inhibitors of HSP90 have been synthesized and evaluated as anticancer agents. Inhibition of HSP90 produces degradation of these client proteins leading to inhibition of oncogenic signaling pathways responsible for various steps important for cancer cell survival and proliferation. Preclinical studies have demonstrated significant activity against cancer cells leading to clinical trials of these agents. Early drugs had significant toxicity leading to development of second generation inhibitors. One of these is Ganetespib which binds to N-terminal ATP binding pocket of HSP90 thus inhibiting it (10).This has been evaluated in clinical trials for a number of cancers.

The Mesenchymal epithelial transition (MET) protein is a growth factor receptor for the hepatocyte growth factor (HGF). HGF binding to MET stimulates a variety of cellular

signaling pathways important for maintaining tissue homeostasis. It plays an important role in normal physiological processes including embryogenesis, wound healing, tissue repair, and limiting fibrosis(11).Under normal conditions, these are self-limiting processes. But, dysregulated MET in cancer cells, either by overexpression or due to mutation, promotes an invasive growth program which leads to enhanced cell motility, invasion and reduced apoptosis. Targeting the HGF-MET axis in cancer cells that have dysregulation of this pathway has been shown to lead to cancer cell death in preclinical studies. Thus, it has been a therapeutic target for drug development in those cancer which have MET amplification or mutation(12). A variety of MET inhibitors have been developed and evaluated in clinical trials to treat these cancers. However, none of these have yet been approved for this indication so better understanding of the biologic characteristics of MET amplified tumors would be helpful in development of therapeutic approaches for these patients.

In an attempt to identify additional potential molecular targets that might lead to improved therapy for patients with EGC, we performed two studies. In the first study we investigated the efficacy and safety of the Heat Shock Protein (HSP 90) inhibitor Ganetespib in a Phase II clinical trial . In the 2nd study we explored the clinicopathological features and outcome of patients with MET amplified EGC.

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Project-1

Study Title

A Phase 2 Clinical Trial of the Heat Shock Protein 90 (HSP 90) inhibitor Ganetespib in Patients with Refractory Advanced Esophagogastric Cancer

Investigational New Drugs
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PHASE II STUDIES

A phase 2 clinical trial of the heat shock protein 90 (HSP 90) inhibitor ganetespib in patients with refractory advanced esophagogastric cancer

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Summary
Subsets of esophagogastric (EG) cancers harbor genetic abnormalities, including amplification of HER2, MET, or FGFR2 or mutations in PIK3CA, EGFR, or BRAF. Ganetespib which is a novel triazolone heterocyclic inhibitor of HSP90, is a potentially biologically rational treatment strategy for advanced EG cancers with these gene amplification. This multicenter, single-arm phase 2 trial enrolled patients with histologically confirmed advanced EG cancer with progression on at least one line of systemic therapy. Patients received Ganetespib 200 mg/m2 IV on Days 1, 8, and 15 of a 28-day cycle. The primary endpoint was overall response rate (ORR). Secondary endpoints included: Progression Free Survival (PFS); to correlate the presence of HSP clients with ORR and PFS; evaluating the safety, tolerability and adverse events profile. In this study 26 eligible patients mainly: male 77%, median age 64 years were enrolled. The most common drug-related adverse events were diarrhea (77%), fatigue (65%), elevated ALKP (42%), and elevated AST (38%). The most common grade 3/4 AEs included: leucopenia (12%), fatigue (12%), diarrhea (8%), and elevated ALKP (8%). The ORR of 4% reflects the single patient of 26 who had a complete response and stayed on treatment for more than seventy (70) months. Median PFS and OS was 61 days (2.0 months), 94 days (3.1 months) respectively. Ganetespib showed manageable toxicity. While the study was terminated early due to insufficient evidence of single-agent activity, the durable CR and 2 minor responses suggest that there may be a subset of EG patients who could benefit from this drug.

Keywords Heat shock protein · Esophagogastric cancer · Phase II · Clinical trial · HER2

Introduction

Esophagogastric cancer is the 2nd leading cause of cancer-related death worldwide [1]. Surgical resection remains the most effective and mainstay curative treatment [2]. Only a limited number of patients are amenable for surgical resection, and most patients present with locally advanced or metastatic disease. For advanced disease, the median overall survival is approximately 1 year [3]. Although survival has improved in the last few decades, overall prognosis remains poor because of high rates of recurrence and modest efficacy of systemic therapy. Exploring and confirming the efficacy and safety of novel treatment options is critical to improve outcomes in this disease.

Heat shock protein 90 (HSP90) is an adenosine triphosphate (ATP) dependent molecular chaperone that helps promote the maturation and stability of multiple cellular proteins known as "clients" including RAF, KIT, EGFR, HER2, PDGFRα, FGFRs and VEGFR2 [4]. Many of these clients are oncoproteins that have the potential to disrupt multiple key survival pathways involved in proliferation, angiogenesis, and

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metastasis associated with malignant cells [5] . Abnormally high expression of HSP90 is associated with oncogenesis and resistance to various chemotherapeutic agents in cancer cells [6]. Inhibition of HSP 90 leads to the degradation of these proteins, and hence stands as a potentially promising strategy for cancer therapy.

A second generation HSP 90 inhibitor, ganetespib (5-[2,4-dihydroxy-5-(1 methyl-ethyl)phenyl]-2,4 dihydro-4-(1-methyl-1*H* indol-5 yl)-3*H*-1,2,4 triazole-3-one), is a novel resorcinol-containing triazolone heterocyclic HSP90 inhibitor [7, 8] that is structurally unrelated to first generation, geldanamycin-derived inhibitors such as 17-AAG, 17-DMAG, and IPI-504 [7, 9, 10]. An advantage of the second generation HSP90 inhibitors is that due to the absence of the benzoquinone moiety, they lack the significant ocular and hepatotoxicity seen with the first-generation inhibitors. The decreased hepatotoxicity has been demonstrated with ganetespib in vivo when compared to 17-DMAG [7, 8] . Additionally, ganetespib showed a 20-fold greater potency compared to 17-AAG in vitro in hematologic and solid tumor cell lines [8].

Ganetespib has specifically shown significant activity against a number of gastric cancer cell lines. It induces G2/M cell cycle arrest in the AGS and N87 gastric cancer cell lines. There was a significant reduction in cells in the G0/G1 and S phase, indicating decreased proliferation [11]. It reduced the growth of gastric cancer cell lines MGC-803, SGC-7901 and MKN-28 in a dose-dependent manner and induced G2/M cell cycle arrest and apoptosis. Ganetespib demonstrated potent in vitro cytotoxic activity in a panel of 22 gastric cancer cell lines and promoted the stabilization of multiple HSP 90 clients protein and effectors, including HER2,EGFR,IGF-IR,cMET,cKit,P-AKT,P-ERK1/2, with durable response (i.e. kinetic analysis was suggestive of suppressed client activity for over 48 h following 1 h of drug exposure) [12]. Ganetespib significantly inhibited the growth of xenograft gastric tumors in vivo as a single agent or in combination with cisplatin [13].

Single agent ganetespib has shown evidence of antitumor activity in early phase trials in other treatment refractory solid tumors including metastatic breast cancer (MBC) [10] and advanced non-small cell lung cancer (NSCLC) [14]; of note the two partial responses in the MBC trial occurred in patients with HER2 positive disease, and the four partial responses in the NSCLC trial occurred in patients with ALK rearrangements. It has also shown an acceptable toxicity profile in patients with solid tumors with the main adverse events being diarrhea, fatigue, nausea, hypersensitivity reaction, and anorexia [10, 14, 15].

Based on the preclinical evidence of significant activity against gastric cell lines and models, and prior evidence of clinical tolerability and antitumor activity in clinical trials, this phase II study was designed and conducted for the first time in patients with advanced refractory esophagogastric cancer.

Patients and method

Patient selection

Patients with histologically or cytologically confirmed metastatic esophageal, gastroesophageal, or gastric cancer ≥18 years old who had progressed on one to two lines of cancer therapy were eligible. Prior neo-adjuvant chemoradiotherapy or perioperative chemotherapy could be considered as one line of therapy. Other inclusion criteria included: measurable disease by RECIST version 1.1 criteria, ANC ≥1500/μL, platelets ≥100,000/μL, hemoglobin ≥9, creatinine ≤2.0 mg/dL, total bilirubin ≤1.5x ULN, ALT, and AST ≤ 3x ULN or ≤ 5x ULN if liver metastases present, and Eastern Cooperative Oncology Group (ECOG) performance status ≤1. Patients with CNS disease were allowed to participate provided that whole brain radiotherapy had been received not less than 4 weeks prior to starting the study drug and the stability of the brain metastasis had been demonstrated. Patients with LVEF of less than 50% and other significant cardiac comorbidities were excluded. Pregnant and lactating women were excluded because ganetespib has potential teratogenic and abortifacients effects. Patients with underlying active infection and HIV positive individuals on combination antiretroviral therapy were ineligible because of the potential for pharmacokinetic interactions with ganetespib.

The trial was registered in clinicaltrials.gov (NCT01167114).

Study design

This was a single-arm, multicenter phase II study in patients with advanced refractory gastroesophageal cancer. Ganetespib at a dose of 200 mg/m^2 was administered intravenously by peripheral line once daily on day1, 8, and 15 of 28-day cycle. The ganetespib dose of 200 mg/m^2 used in this protocol was established as the maximum tolerated dose in the previous phase I trials in solid tumors [9, 15] . In the case of grade 3 and 4 hematological and non-hematological toxicity, on the first occurrence, ganetespib dose was reduced to 175 mg/m^2, and on the second occurrence, the dose was reduced to 150 mg/m^2. Study drug was continued until disease progression, unacceptable toxicity, or withdrawal of consent.

Safety monitoring

Patients were monitored and assessed for toxicity prior to and during every cycle for adverse events according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) ver 4.0. Other safety assessments performed during the study visits included vital signs, physical examination, complete blood counts, blood chemistries, serum amylase, and lipase. The trial was monitored and assessed

by the DFCI/Harvard Cancer Center Data and Safety Monitoring Committee (DSMC), composed of clinical specialists with experience in oncology and has no direct relationship with the study. All patients were evaluated for their compliance to study drug and visits. They were also monitored for any dose reduction or modification.

Tumour response evaluation

All patients were assessed for tumor response as per the RECIST version 1.1 criteria. Tumour.

response was performed by Contrast Enhanced Computed Tomography (CECT) during the study visit on alternate cycles in addition to the baseline evaluation. Response evaluation was performed by independent radiological review. Those study participants who received at least one dose of study drug were eligible for response evaluation. Response evaluation was performed by the independent DFCI/Harvard Cancer Center radiological review (tumor imaging metrics core, TIMC).

Statistical methods and analysis

The primary endpoint was overall response rate (ORR) as per RECIST version 1.1 criteria. Secondary endpoints included safety, tolerability, progression-free survival (PFS), and median overall survival (OS); additionally, ORR and PFS were correlated with the presence of an altered HSP-90 client. PFS which was calculated from date of first dose of STA 9090 to date of progression or death due to any cause. Median Overall survival (OS) was calculated from date of first dose of STA 9090 to date of death due to any cause.

This phase II study design was based on a two-stage Simon 'minimax" design, in order to minimize the number of patients exposed to an ineffective treatment regimen [16, 17]. In this study, 3 of 20 patients were needed to respond in order to move onto Stage 2. A total of 41 Patients who received at least one dose of study drug were to be included in the analysis if the maximum number of patients were accrued. If the total number responding out of the 41 patients was less than or equal to 11, the drug was rejected.

Molecular analysis for HSP90 clients

Molecular analysis was carried out on archived formalin-fixed, paraffin-embedded tissue of all the study participants. A tumor genotyping assay based on the SNaPshot multiplex platform system (Applied Biosystems, Carlsbad, CA, http://www.appliedbiosystems.com), was used to simultaneously query more than 150 previously described hotspot mutations across 16 cancer genes, as previously reported [18]. The SNaPshot genotyping assay is a fast, high-throughput, multiplex mutational profiling method that has the advantage over conventional dideoxynucleotide (Sanger) sequencing in that mutations can be detected when mutant DNA composes as little as 5% of the total DNA. Gene amplification of EGFR, HER2, and MET were assessed by FISH.

Results

Patient characteristics

Twenty-six patients with a median age 64 years (37–80 years) were enrolled in the study from July 2010 to May 2011, and all received at least one dose of drug. The last dose of study drug was given to a patient in May 2016, and the patient continued to maintain a complete response at the time of the last follow-up visit in July 2018. Two patients were registered for the study but did not receive drug because one patient developed obstructive jaundice and another patient withdrew consent. The majority of the patients were Caucasian (92%), male (77%), had a primary tumor at the GE junction (42%) and had metastatic disease at initial presentation (62%) (Table 1). Five patients (19%) presented with recurrent disease after curative resection. The most common sites of metastases among all enrolled patients were the lymph nodes (50%) and liver (42%). Histologically, the majority of the patients (88%) had adenocarcinoma with poorly differentiated (46%) or moderately differentiated (38%) histology. Twenty-three patients (88%) received prior systemic therapy for metastatic disease with the other 12% receiving either neoadjuvant or adjuvant therapy previously. A platinum-based agent (92%), fluoropyrimidine (80%) and taxane (30%) were the most commonly received classes of systemic chemotherapy for metastatic disease. Four (15%) patients had received targeted anti-Her2 (Trastuzumab) therapy prior to entering into the study.

Toxicity assessment

All the enrolled patients who have received at least one dose of ganetespib were evaluable for toxicity (Tables 2 and 3). The most common hematological toxicities included anemia (35%), leukopenia (19%), and neutropenia (8%) while diarrhea (77%), fatigue (65%), nausea (31%) and anorexia (31%) were the most common non-hematological toxicities. Two (8%) patients developed grade 1 blurry vision, and 1 (4%) developed grade 1 QTc prolongation which was possibly related to study drug. Increased alkaline phosphatase and AST were reported in 11(42%) and 10 (30%) patients respectively. The most common grade 3/4 toxicities included leukopenia (12%), fatigue (12%), neutropenia (8%),alkaline phosphatase (8%) and diarrhea (8%). One patient developed a serious adverse event (SAE), non-neutropenic grade 5 septic shock, and the patient subsequently died within 20 days of the last dose of drug; the death was determined to be unrelated to ganetespib. Out of 26 enrolled patients, 16 (61.5%) required

Table 1 Baseline patient characterstics (mITT: $n = 26$)

Characterstics	Value
Median Age,y (range)	64(37–80)
Age,(N,%)	
≤ 50	3(12%)
50–69	16(62%)
≥70	7(23%)
Sex,(N,%)	
Male	20(77%)
Female	6(23%)
Race,(N,%)	
White	24(92%)
Black	1(4%)
Hispanic	0(0%)
Asian	1(4%)
Others	0(0%)
ECOG Performance Status (N,%)	
0	11(42%)
1	15(58%)
Primary tumor site (N,%)	
Esophagus	7(27%)
Gastroesophageal Junction	11(42%)
Gastric	8(31%)
Disease status (N,%)	
Primary Metastatic	16(62%)
Recurrent after curative intent of surgery	05(19%)
Recurrent after curative intent of other treatment	05(31%)
Grade (N,%)	
Well differentiated	2(8%)
Moderately differentiated	10(38%)
Poorly differentiated	12(46%)
Histology (N,%)	
Squamous cell carcinoma	3(12%)
Adenocarcinoma	23(88%)
Sites of metastatic disease (N,%)	
Liver	11(42%)
Lymph node	13(50%)
Pancreas	1(4%)
Lung	6(23%)
Others	5(5%)
Prior anticancer therapy (N,%)	
Curative resection	5(19%)
Neoadjuvant radiation	4(15%)
Neoadjuvant chemotherapy	9(35%)
Adjuvant therapy	3(12%)
Prior systemic therapy for metastatic disease	23(88%)
Palliative Radiation	4(15%)
Prior systemic therapy (N,%)	
Platinum	24(92%)
Fluoropyrimidine	23(88%)
Taxane	10(38%)
Trastuzumab	4(15%)

mITT Modified Intent to Treat

ECOG Eastern Cooperative Oncology Group

Table 2 Drug related all grade adverse events >5% ($n = 26$)

Adverse event	All Grades N(%)	Grade 3 and 4 N(%)
Hematological		
Hemoglobin	9(35%)	1(4%)
Leukopenia	5(19%)	3(12%)
Neutropenia	2(8%)	2(8%)
Non hematological		
Diarrhea	20(77%)	2(8%)
Fatigue	17(65%)	3(12%)
Anorexia	8(31%)	0
Nausea	8(31%)	0
Vomitting	5(19%)	0
Dizziness	4(15%)	0
Allergy	3(12%)	0
Abdomen pain	3(12%)	0
Dehydration	2(8%)	0
Cough	2(8%)	0
Muscle pain	2(8%)	0
Blurry vision	2(8%)	0
Dyspnea	2(8%)	0
Neutropenic fever	2(8%)	0
Cardiac Arrythmia*	1(4%)	0
Laboratory Abnormalities		
Serum ALK Phosphatase increase	11(42%)	2(8%)
AST increase	10(38%)	1(4%)
ALT increase	5(19%)	0
Hyponatremia	5(19%)	1(4%)
Hyperglycemia	5(19%)	0
Hyperkalemia	3(12%)	0
Creatinine increase	3(12%)	0
Hypomagnesemia	3(12%)	0

AST Aspartate aminotransferase, *ALT* Alanine transferase

*less than 5% Cardiac Arrhythmia

dose reduction and dose modification of study drug. The mean number of drug infusions received was 13 (just over 5 mean number of cycles).

Tumour response and survival

Twenty-six patients were eligible for efficacy evaluation. One patient (3.8%) achieved a complete response, one patient (3.8%) achieved stable disease, and two patients (7.7%) achieved minor responses i.e. a 20%–29% reduction in sum of longest diameter of the target lesions from the baseline (Table 4). Nine (35%) of patients did not have radiographic reassessment after baseline and thus did not meet criteria to be evaluable for response assessment; 7 of the patients had clinical disease progression, 1 patient had grade 3 diarrhea and grade 3 neutropenia, and 1 patient had grade 3 diarrhea. Three out of 20 patients needed to respond in order to move onto stage 2 of the trial. The study was terminated early as only one patient responded. The one patient who achieved the complete response received 74 cycles (70.0 months) of Ganetespib. Among the two minor disease responders, one completed two cycles (2 months) and seven cycles (7 months) of study drug.

Table 3 Grade 3–5 treatment emergent adverse events

Adverse event	Grade 3 N(%)	Grade 4 N(%)	Grade 5 N(%)
Anemia	2(15%)		
Neutropenia	1(8%)	1(8%)	
Elevated lipase	2(15%)		
Elevated ALK phosphatase	2(15%)		
Hyperglycemia	2(15%)		
Hyperbilirubinemia	1(8%)		
Elevated Amylase	1(8%)		
Lymphopenia		1(8%)	
Septic Shock			1(8%)

Median PFS was 61.5 days (2.0 months)[95% CI 53–68 days], median TTP was 50 days (1.7 months) [95% CI 46–65 days] and median OS was 94 days (3.1 months) [95% CI 71–143 days] (Figs. 1 and 2).

Biomarker assessment

Biomarker analysis of archival tumor tissue was performed to evaluate for molecular abnormalities (Table 4). A total 15 patients underwent HER2 expression testing by immunohistochemistry, and 7 tested 2+ or 3+ positive, and the remainder tested negative. The 2 patients with minor responses to ganetespib had 3+ HER2 positive tumors. SNaPshot profiling for hotspot mutations was performed in a minority of patients ($n = 7$), largely due to inadequate tissue in many cases. One of 7 patients were found to have a KRAS G12D mutation in codon 12 and another patient was found to have EGFR amplification concurrently with 2+ HER2 overexpression. The first patient had a complete response and the second patient had progressive disease as the best response.

Complete responder

The single patient who achieved a complete response was a 54-year-old man who initially presented with progressive dysphagia. Upper endoscopy revealed a partially obstructive mass in his distal part of the esophagus, and biopsy of the mass showed poorly differentiated adenocarcinoma. He received neoadjuvant chemoradiation with 5FU and Cisplatin followed by Ivor-Lewis esophagectomy. Pathological staging showed pT3N0 disease. Seven months later, he presented with a biopsy-proven local recurrence in a mediastinal and hilar lymph node. Aside from the KRAS mutation in codon 12, no other concurrent mutations were identified on SNaPshot. The tumor was negative for HER2 amplification by FISH and not tested for EGFR and MET amplification. This patient received 2 lines of therapy for metastatic disease: Docetaxel+ Cisplatin+Irinotican (3 months) and 5-FU + Leucovorin (15 months). He subsequently enrolled in the current study. He completed 74 cycles (70.0 months). Ganetespib was discontinued as the company stopped manufacturing the drug. The patient was last seen in clinic in July 2018 and maintained complete response at that time.

Discussion

HSP 90 has been a target of interest for esophagogastric cancers given the frequency of activated molecular pathways involving HSP90 client proteins in these cancers. However, despite extensive preclinical evidence suggesting that HSP90 inhibitors might be potentially attractive anticancer agents, no HSP90 inhibitor has yet been approved for the treatment of esophagogastric cancer or any other cancer [19]. The largest trial of this class of agents, the phase III GALAXY-2 Trial of ganetespib and docetaxel compared to docetaxel alone in the second line treatment of NSCLC, was terminated due to

Table 4 Best overall response ($n = 26$)

Response by RECIST v1.1	Number of patients (%)	# of Cycles completed	Molecular abnormalities
Complete response	1(4%)	74	KRAS G12D(1)
Partial response	0(0%)		
Minor response	2(8%)	2,7	HER2 + (2)
Stable disease	1(4%)	2	HER2-(1)
Progressive DISEASE	13(50%)	≤2	HER2 + (2)
			EGFR+/HER2 + (1)
			HER2-(8)
			Unknown(2)
Not evaluable	9(35%)	≤1	HER2 + (2)
			HER2-(5)
			Unknown(2)

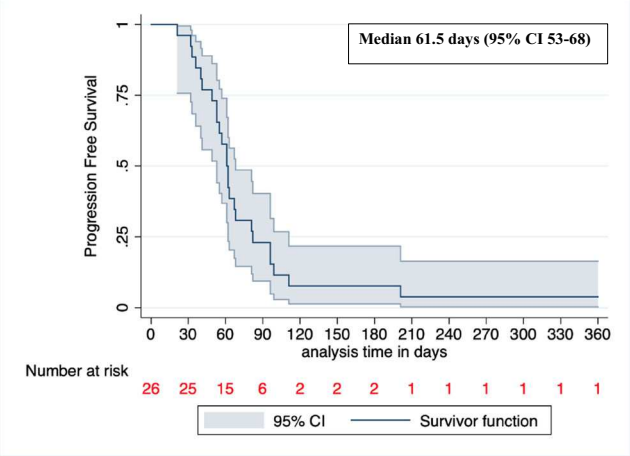

Fig. 1 Median progression free survival(PFS) in patients with advanced esophagogastric cancer treated with Ganetespib

futility [20]. Several reasons for the lack of clinical antitumor activity have been identified. HSP90 is essential for many normal cellular functions, and this may lower the ceiling for dosing drug to maintain patient safety. A retrospective analysis of 15 phase II clinical trials of HSP90 inhibitors found that treatment-related toxicities frequently led to dose interruptions and modifications, an issue that may lead to insufficient drug deliver to effectively target the protein [21]. A second reason for modest efficacy is that HSP90 inhibitors can induce the transcription of other heat shock proteins – such as HSP70, HSP40, or HSP27— which act as anti-apoptotic chaperones that protect proteins from degradation. This mechanism of resistance is called the heat shock protein response and potentially weakens the impact of HSP90 inhibition [22]. Both de novo and acquired resistance to HSP90 inhibitors via activation of various cellular signaling pathways such as the JAK-STAT pathway may also limit efficacy [23].

In the current study, the HSP90 inhibitor ganetespib failed to show a sufficient therapeutic response in this phase II study to warrant further single agent investigation in advanced refractory esophagogastric cancer. The Simon 2 stage design allowed for early termination of the study to avoid unnecessarily subjecting patients to ineffective therapy. The main limitation of this trial was the lack of biomarker selection and the limited biomarker assessment in the enrolled patients. Several patients had limited tissue available, and after prioritizing confirmation of diagnosis and HER2 testing, insufficient tissue often remained for further testing. Albeit a limited number of patients, the two minor responses in HER2+ disease in this study, and prior reports of partial responses of patients with HER2+ metastatic breast cancer or ALK+ NSCLC [10, 14], suggest that specific molecular subsets of patients may benefit from ganetespib [24].

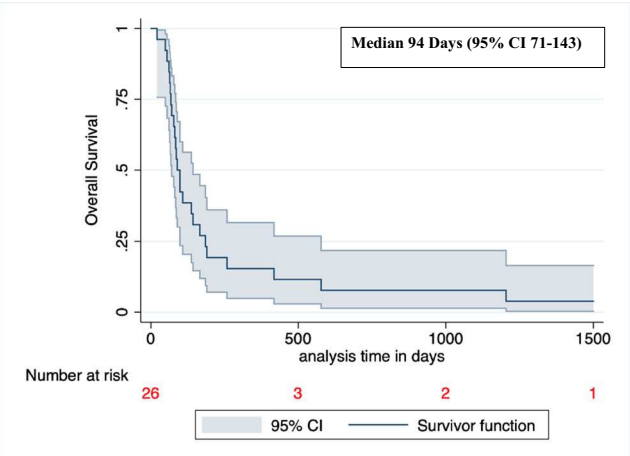

Fig. 2 Median Overall Survival(OS) in patients with advanced esophagogastric cancer treated with Ganetespib

Conclusion

While HSP90 inhibition remains a scientifically rational approach in treating advanced cancers, this promise has not been converted to improved outcomes for patients in the clinic. A better understanding of the impact of HSP90 inhibition at the cellular level in both normal and malignant cells is necessary in order to develop approaches with enhanced efficacy and decreased toxicity. This is critical for helping to define potential predictive biomarkers for HSP90 response as well as toxicity to aid in the selection of patients most likely to benefit from HSP90 inhibition.

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Compliance with ethical standards

Conflict of interest L.G. is a consultant or advisory board member for Debiopharm, H3 Biomedicine, Agios Pharmaceuticals, Taiho Pharmaceuticals, Klus Pharmaceuticals, QED, and Pieris Pharmaceuticals.B.M.W has received Grant support: Celgene, Eli Lilly and Consulting: BioLineRx, Celgene, G1 Therapeutics, GRAIL.JWC partially funded by P30CA06516 (Benz) 09/01/2009–08/31/2011 (Role: Investigator) and NCI-ASCO Clinical Investigator Team Leadership Supplemental Award. The rest of the authors declares no conflict of interest.

Ethical approvals The protocol was approved by the Institutional Review Board at the Dana Farber/Harvard Cancer Center. The study was conducted in accordance with International Conference on Harmonization and Good Clinical Practice (ICH-GCP) and Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent All patients provided written informed consent before study participation.

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Project-2

Study Title

Revisiting *MET*: Clinical characteristics and treatment outcomes of patients with locally advanced or metastatic, *MET*-amplified esophagogastric cancers

Revisiting *MET*: Clinical characteristics and treatment outcomes of patients with locally advanced or metastatic, *MET*-amplified esophagogastric cancers

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ABSTRACT

Background:

Metastatic esophagogastric cancers (EGC) have a poor prognosis with an approximately 5% five-year survival. Additional treatment approaches are needed. c-MET gene-amplified tumors (METamp) are an uncommon but potentially targetable subset of EGC. Clinical characteristics and outcomes were evaluated in patients with *MET* amplified EGC and compared to those without *MET* amplification to facilitate identification of these patients and possible treatment approaches.

Patients and Methods:

Patients with locally advanced or metastatic *MET*-amplified EGC at Massachusetts General Hospital (MGH) were identified using fluorescent in situ hybridization (FISH) analysis, with a gene to control ratio of $\geq$ 2.2 defined as positive. Non-*MET*-amplified patients identified during the same time period who had undergone tumor genotyping and treatment at MGH were evaluated as a comparison group.

Results:

We identified 233 patients evaluated for MET amplification from 2002 to 2019. METamp was seen in 28 (12%) of patients versus 205 (88%) patients without amplification. Most *MET*-amplified tumors occurred in either the distal esophagus (n=9; 32%) or GE junction (n=10; 36%). Of *MET*-amplified patients, 7 (25%) had a TP53 mutation, 5 (18%) had HER2 co-amplification, and 1 (3.6%) had a *PIK3CA* mutation. *MET*-amplified tumors more frequently had poorly differentiated histology [19 (68.0%) versus 66 (32%),p=0.0017]. Progression-free survival (PFS) to initial treatment was substantially shorter for *MET*-amplified patients (5.6 vs 9.1 months, p=0.013) and for those with metastatic disease at

presentation (4.4 vs 7.9 months, p=0.035). Overall, patients with *MET* amplification had shorter overall survival (15.3 vs 24.6 months, p=0.014). No difference in survival was seen between low MET amplified tumors (≥2.2 and <25) compared to highly amplified tumors (≥25) MET copy number.

Conclusion

MET-amplified EGC represents a distinct clinical entity characterized by rapid progression and short survival. Ideally, the identification of these patients will provide opportunities to participate in clinical trials in an attempt to improve outcomes.

Keywords: Esophagogastric cancer, MET amplification, targeted therapy, survival, progression

Introduction:

Esophageal, gastric and gastroesophageal (GE) junction cancers, collectively referred to as esophagogastric cancers (EGCs), have been increasing rapidly in incidence over the past three decades (1). EGC is the third leading cause of cancer-related death worldwide (2). Survival with metastatic disease remains limited, with a median survival of approximately 12-14 months and a 5-year overall survival rate of approximately 5% (3). The backbone of frontline therapy for advanced disease remains 5-fluorouracil in combination with a platinum agent, most commonly oxaliplatin in US patients.(4) Taxane-based cytotoxic chemotherapy, either alone or in combination with the anti-angiogenic agent ramucirumab as second-line therapy is additionally supported by level 1 evidence though treatment heterogeneity is common in Western patients (4-6).

Following a common paradigm in multiple tumor types, large molecular profiling efforts such as the TCGA and ACRG have identified recurrent molecular alterations in EGC(7, 8). Among molecular biomarkers, HER2 overexpression and/or amplification (15-20%), microsatellite instability (MSI-H, 3-5%), and PD-L1 positivity (50-70%) are now linked with US FDA approvals in patients with advanced disease (8, 9). Other potentially actionable recurrent alterations include EGFR amplification, FGFR2b overexpression, and CLDN18.2 overexpression(10). Despite preclinical rationale, none have yet had sufficient evidence of clinical activity to be FDA approved.

The cell surface receptor MET and its ligand, hepatocyte growth factor (HGF), play important roles in normal cell migration, development, growth, and survival (11). The HGF and MET receptor tyrosine kinase (RTK) pathway is normally tightly regulated. Physiologic MET

tyrosine kinase receptor activation is mediated by HGF binding, which leads to MET $\propto$ and β chain dimerization, in turn causing autophosphorylation at intracellular amino acid residues Y1234 and Y1235 within the enzymatic kinase site. Subsequent receptor phosphorylation mediates the recruitment of signal transducers and adaptors that lead to multiple potential downstream pathways, including those involving RAS, phosphatidylinositol (3,4,5)-triphosphate (PI3K)-mTOR, signal transducer and activator of transcription 3 (STAT3), and nuclear factor- κB (NF κB)(11, 12). MET signaling, however, can be subverted by inappropriate autocrine loops, ligand/receptor overexpression/gene amplification, or activating mutations in *MET*, which are found in a number of human cancers (13, 14). A number of cancers have been found to have *MET* gene amplification or mutations, including hereditary and sporadic papillary renal carcinoma, non-small cell lung cancer (NSCLC), esophagogastric cancer, hepatocellular cancer, head and neck cancer, ovarian carcinoma, small cell lung cancer, and glioma (13, 15, 16). *MET* gene amplification is a driver in a subset of esophagogastric cancers and NSCLCs (17-20). Increased MET protein expression has been correlated with advanced disease stage in several cancers, including colorectal cancer(21). In addition to the direct effects of HGF and MET in tumor cells, the HGF/MET axis can stimulate tumor endothelial cells, thereby affecting the tumor microenvironment and promoting angiogenesis (13, 22). The HGF/MET pathway is increasingly implicated in drug resistance, particularly in EGFR-mutant NSCLCs that possess de novo or acquired resistance to small-molecule inhibitors of the epidermal growth factor receptor (EGFR) (18, 23, 24).

Due to the widespread role that dysregulated MET plays in a variety of cancers, MET has been an attractive target for the development of inhibitors. In vitro, gastric cancer cell lines

possessing *MET* amplification demonstrated dramatic sensitivity to the effects of a preclinical inhibitor of MET(17). This in-vitro observation was recapitulated in a phase 1 clinical trial of crizotinib, a potent inhibitor of MET as well as ALK and ROS1 tyrosine kinases, when several patients with *MET*-amplified EGC experienced disease shrinkage upon treatment with crizotinib (3, 25).

The optimal methods to assess MET in EGC continues to evolve and IHC and/or FISH remain the most common approaches(26, 27). We previously published a small heterogenous series of METamp EGC highlighting the frequency and feasibility of FISH testing (3). Subsequent clinical efforts to target MET in EGC have been met with largely negative results, though factors ranging from patient selection, MET overexpression cutoffs, and optimal therapeutic agent are likely confounders(26-29). We sought to build upon existing data and look more granularly at the METamp population at our tertiary center where routine MET testing began in 2002.

Methods

Study population: We retrospectively identified cases of locally advanced or metastatic esophagogastric adenocarcinoma or squamous cell carcinoma at the Massachusetts General Hospital (MGH) from 2002 to 2019 for analysis. Key inclusion was prior MET testing performed by FISH at MGH. All work was conducted under an IRB-approved protocol.

Data collection and survival analysis. Medical records for patients whose tumors were *MET*-amplified and not amplified on the basis of MGH testing were reviewed to extract data on clinicopathologic characteristics and outcomes. Patients were de-identified and baseline

clinicopathologic characteristics, concurrent molecular abnormalities, sites of disease, first-line chemotherapy regimens, and survival outcomes were recorded.

Pathology and Molecular analyses: Hematoxylin and eosin-stained, formalin-fixed, paraffin-embedded tumor tissues obtained from surgical specimens and/or diagnostic biopsies of either metastatic or primary cancer sites were evaluated by gastrointestinal pathologists at MGH in order to establish the diagnosis of esophagogastric adenocarcinoma or squamous cell carcinoma.

MET gene copy number (CN), FISH was performed on formalin-fixed paraffin-embedded tissue by hybridization for *MET* and centromere 7 (CEP7), as previously reported (30). Where possible, amplification of HER2 and/or EGFR was performed on the same tissue sample using two separate hybridizations for *MET*/*EGFR*/centromere 7 (CEP7) and *HER2*/CEP17. Details of hybridization have been described previously (3). METamp was defined as a gene-to-CN control probe ratio G:CN of greater than or equal to 2.2 scored in 50 tumor nuclei as utilized, which was extrapolated from established *HER2* criteria and has been used previously. Specifically, polysomy, high polysomy, or equivocal (G:CN) ratio (i.e., 1.8 to less than 2.2) were scored as negative for amplification (3). *MET*- amplified tumors were defined as those with *MET/CEP7* ratios ranging from $\geq$2.2 to <25 (low amplification), and $\geq$ 25 (high amplification). These lower cutoff of 2.2 was chosen by direct extrapolation from the standard definition of HER2 positivity used in clinical practice(31, 32).

The lower bound for those who had high level expression was chosen at 25 to determine if very high level expression distinguished a group of patients with different biological characteristics, given that previous studies had not suggested differences when > 5 was used as the cutoff.

For most patients, additional tumor gene analyses were available using the same tissue specimens that had been subjected to *MET* FISH. The methodology for genotyping at MGH has been previously described by Dias-Santagata (30).

Statistical analysis*:* The distribution of continuous variables was assessed using the Shapiro-Wilk test and due to non-normality, summarized using medians and interquartile ranges. Comparisons were made using the Wilcoxon rank-sum test. Categorical variables are summarized with frequencies and proportions and compared using either the Chi-Square test or Fisher's exact test. Regression analysis was performed to find independent association with outcome. In the first stage, the univariate cox regression model was used to identify the eligible factors which had a marginal association of 10% with the outcome (progression and death). In the second stage, a backward stepwise multiple cox proportional hazard model was used to identify the independent factors of mortality with entry probability of 0.1 and exit probability of 0.05.

Survival estimates were generated using Kaplan-Meier methodology and compared among the various group using a log-rank test. Overall survival (OS) was defined as the time from initial diagnosis to death. Patients who were alive at last contact were censored at this date. Progression-free survival (PFS) was defined as the time from initial treatment (surgery,

chemotherapy and /or radiotherapy) to the earliest date of progression or death. Patients who were alive and progression-free at last contact were censored at this date.

Two-tailed p-values less than 0.05 were considered statistically significant. All analyses were conducted using Stata 15.1 (StataCorp, USA).

Results

We identified 28 patients with *MET*-amplified tumors and 205 patients with non-MET amplified tumors from the year 2002 to 2019. Baseline demographics and clinical characteristics are shown in **Table 1**. For the entire cohort, the majority of patients were male (n=175, 75.1%). The mean age at diagnosis was 59.9 years. There was no difference seen in baseline characteristics between patients with or without *MET* amplification except anemia which was more common in MET negative (non-amplified) patients (43% vs 31%,p=0.026). The most common tumor site evaluated for MET-amplification was the primary disease site 211 (90.5%). This reflects the tendency to establish diagnosis through endoscopic biopsy of the primary site of disease, and therefore this tissue is more available for MET testing, rather than reflecting the incidence of MET amplification at primary versus metastatic disease sites. The location of the primary tumor was most commonly in the gastroesophageal junction or distal esophagus (**Table 2).** *MET*-amplified tumors were more likely to be intestinal type (95.5% vs 68.0%, p=0.029) and poorly differentiated compared to non-*MET*-amplified tumors (68.0% vs. 32.0%, p=0.017) (**Table 2**). Patients with MET amplification were more likely to present with bulky metastasis measuring > 5 cm (n=7, 25% vs n=22, 11%) than non-MET-amplified patients (p=.05) **(Table 3)**. There were no differences in sites of metastatic disease or stage at presentation.

Most patients with metastatic disease received FOLFOX chemotherapy as first-line treatment (50.21%), followed by Carboplatin/paclitaxel (9.0%) and ECF/EOX (8.0%), with 5.6% of patients participating in clinical trials. Chemoradiation (with Carboplatin/Paclitaxel) was primarily given in the neoadjuvant setting to patients with esophageal and gastroesophageal cancers who did not present with metastatic disease. Overall, the primary site was the most common site of radiation (88.5%), followed by bone (2.9%), chest (2.9%), lymph node (2.9%), and others (2.9%) (see **Table 4).**

Common Genetic Amplifications and Co-amplifications with MET

Of the 233 patients who were tested for gene amplification, HER2 was the most common (13.0%) amplification found, followed by MET (12%) and EGFR (5.5%). Other genetic mutations included TP53 (39.0%), CDH1 (6.4%), CDKN2A (5.0%), PIK3CA (5.0%), and KRAS (4.0%). Mutations in BRAF (1.0%) and ALK (0.5%) were uncommonly detected using our panel based NGS platform.

HER2 (n=5, 18.0%) was the most common gene co-amplified with MET. Four of the 5 patients (14%) had co-amplification based on initial biopsy whereas the fifth had amplification seen on a biopsy post trastuzumab therapy (insufficient tissue was available from the initial biopsy for testing). EGFR amplification was seen in (n=2, 7.0%) patients with METamp. Mutations of TP53, CDKN2A, PIK3CA and KRAS were seen in patients with MET amplification in 7 (25%), 3 (11.0%), 1 (3.5%) and 1 (3.5%) patients, respectively **(see Table 5).**

Univariate and Multivariate Analyses

Univariate Cox proportional hazard analysis showed that family history of GI cancer (HR 1.5, p=0.045), ECOG performance status (PS) (HR 1.3, p=0.05), history of gastroesophageal reflux disease (HR 0.69, p=0.034), metastasis at presentation (HR 1.8, p=0.004), and metastases to bone (HR 1.5, p=0.005), liver (HR 1.2, p=0.002), and lymph nodes (HR 1.1, p=0.02) were significant prognostic factors for OS. MET amplification and MET copy number >25 were also poor prognostic factors for OS (HR 1.8, p=0.016) and (HR 1.4, p=0.024), respectively.

On multivariate Cox proportional hazard analysis, a family history of GI cancer (HR 1.5, 95% CI 1.0-2.3, p=0.027), ECOG PS>2 (HR 1.5, 95% CI 1.1-2.0, p=0.002), presentation with pain (HR 1.45, 95% CI 1.0-2.0, p=0.03), MET-amplification (HR 1.6, 95% CI 1.0-2.6, p=0.047), and metastasis at presentation (HR 2.0, 95%CI 1.3-3.1, p=0.001) were all significantly associated with poor OS. Patients who were MET-amplified (HR 5.1,95%CI 2.0-12,p=<0.001) and received radiation for primary or metastatic disease (HR 1.6, 95% CI 1.1-2.4, p=0.008) were at higher risk of disease progression. In the univariate analysis for progression free survival (PFS) for METamplified patients the HR was 1.65(95%CI 1.0-2.5,p=0.017) which is consistent with Kaplan Meier analysis and long rank ,HR 1.68(95% CI 1.0-2.5,p=0.013) Figure-1. However, in the multivariate analysis a more pronounced effect was seen HR 5.1(95%CI 2.0-12,p=0.001) due to adjustment of outcome factors in the multivariable analysis. The small total sample size of n=233 and METamp size n=28 was one of the limitations of this study and potential reason for this effect. This conclusion needs to be tested and validated on a larger dataset in future study. **(see Table 6).**

Genotype-Specific Treatment Outcomes

Progression-free survival (PFS) was substantially shorter for patients with *MET* amplification *compared to* no *MET* amplification for all patients (5.6 vs 9.1 months, HR 1.68 [95% CI, 1.1-2.5], p=0.014) **(Figure 1)** and for those with metastatic disease at presentation (PFS 4.4 vs 7.9 months, HR 1.63, 95% CI 1.0-2.5, p=0.038) (**Figure 3)**. When all patients were analyzed, *MET*-amplified patients had a shorter overall survival (OS) than non-*MET*-amplified patients (15.3 vs 24.6 months, HR 1.82, 95% CI 1.0-2.9, p=0.016), while for those who presented with metastatic disease, there was a trend towards shorter survival in the *MET*-amplified patients (15.3 vs 22.1 months, HR 1.44, 95% CI 0.8-2.5, p=0.21) **(Figure 2 and Figure 4).**

There was no significant difference in PFS (4.0 months vs 5.8 months, HR 1.0, 95%CI 0.45 - 2.8, p=0.95) or OS (13.4 months vs 15.3 months, HR 0.98 ,95% CI 0.38-2.55, p=0.97) between patients with lower level *MET*-amplified tumors ($\geq$2.2 and <25) versus higher amplified tumors ($\geq$25 MET copy number) **(Figure 5 and Figure 6).** *MET*- amplified tumors with co-expression of EGFR(n=2) amplification had worse overall survival (OS) (0.2 vs 31.9 months, HR 6.9, 95% CI 0.96-50.5, p=0.05), compared with non-*MET* amplified tumors with these amplifications. MET- amplified tumors with initial co-expression of HER2 (n=4) also had a trend towards worse overall survival (OS) (5.6 vs 26.2 months, HR 2.9, 95%CI 0.7-10.7, p=0.1) compared with non-MET amplified HER2 expressing tumors although this did not reach significance. However, these numbers should be interpreted cautiously due to the small sample size of HER2 and EGFR co-amplified patients.

Survival among patients receiving MET-directed therapy

Among the 28 patients with MET amplification, 10 patients received MET inhibitors at some point in their treatment. Six patients received the MET inhibitor AMG337, and four patients received Crizotinib. A statistically significant improvement in median overall survival was seen among those patients receiving MET inhibitors compared with those who did not receive MET-directed therapy (33.0 months vs 7.1 months, HR 0.31, 95% CI 0.1-0.9, p=0.032). However, there was not a statistically significant improvement in median PFS (8.5 vs 4.0 months, HR 0.67, 95% CI 1.0-2.9, p=0.34) (**Figure 7 and Figure 8).**

Discussion

In this retrospective study, we evaluated the clinicopathological features and outcomes of patients with *MET*-amplified and non-*MET*-amplified EGCs.

The difference in first line PFS between patients with non-*MET*-amplified versus *MET*-amplified EGCs was statistically significant, confirming previous observations that *MET*-amplified EGCs represent a particularly aggressive subtype of an inherently aggressive disease (3, 33, 34). Interestingly, the difference in OS between the groups is less striking than the difference in PFS. This may reflect the inclusion in this analysis of several patients with *MET*-amplified cancers who participated in a phase 1 clinical trial of AMG337, a highly selective and potent small-molecule inhibitor of MET receptor signalling. As previously reported, the response rate of *MET*-amplified patients on this early phase trial was 29.6%, and several of these patients received AMG337 on protocol for over 2 years, which may have skewed the OS survival analysis for the *MET*-amplified cohort (35). No difference in OS was

seen between those with high *MET* amplification $\geq$25 vs lower level amplification $\geq$2.2 - <25. This is consistent with the findings from the RILOMET-1 study (36). There are a number of potential explanations for this, including that there is a threshold level for MET amplification that determines overall biology, or possibly that the highly amplified tumors were the ones more likely to have been treated on one of the clinical trials (crizotinib or AMG337) or to have responded to anti-MET therapy. The numbers of patients are too small to distinguish between these possibilities.

MET gene amplification in EGC is a relatively uncommon event. Most series report MET amplification in 2% to 10% of tumors, although the frequency depends on the testing methodology, specific population tested, the histology and the proximity of the primary tumor to the GE junction (3,35,36). In the current study, MET amplification was observed in 12% (28 of 233) of patients. This slightly higher percentage may possibly be due to a number of factors, including defining the lower cutoff of MET amplification at a MET/CEP7 ratio of 2.2 or higher and the fact that there may have been some bias in which patients were screened for MET amplification, which was a requirement for inclusion in this analysis, especially in the earlier years of testing.

We observed co-occurrence of a number of other amplified or mutated genes important for cell signaling or DNA processing (e.g., p53, CDKN2A, EGFR, HER2, KRAS, and PIK3CA) with MET amplification, with HER2 and/or EGFR co-amplification seen in approximately 20% of patients on pretreatment biopsies. Co-amplified patients tended to have aggressive disease with short survival times. Since tumors with co-amplified or mutated genes may be resistant to inhibition of signaling through just one of the pathways, combinations of anti-*MET*-

directed therapy and targeting the other amplified (especially HER2 or EGFR) or mutated gene(s) could be investigated for this subgroup of patients in future clinical trials(37, 38).

An important observation is the median OS of 24.6 months in the MET negative population observed in our cohort. While this may be influenced by several factors it is likely impacted by the availability of ancillary services (nutrition, palliative care, etc.) and multi-disciplinary management in dedicated EGC programs at tertiary centers(39).

Summary

Here we further refine the understanding of METamp EGC and highlight treatment implications. Therapeutically exploiting molecular biomarkers, including MET, in EGC has been plagued by multiple barriers, among the most important of which is tumoral heterogeneity. Despite, encouraging phase II studies of c-MET antibodies (Rilotumumab, Onartuzumab) larger phase III EGC studies using the same screening criteria for c-MET positivity revealed no survival benefits, regardless of c-MET staining (40). Larger efforts to assess for concordance between tissue (including both primary and metastatic sites) and blood (ctDNA) as well as other molecular features, such as the presence of receptor tyrosine kinase (RTK) co-amplification, may aid in refining the population who are most likely to benefit from MET-directed therapies, either alone or in combination. Small trials with novel MET inhibitors and combination strategies with robust correlative work are likely the best path forward in re-visiting MET as a therapeutic target in EGC(41).

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Conflict of interest:

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Table 1. Patient and disease characteristics for MET and non-MET patients

	MET Negative (N,%) 205(88.0%)	**MET Positive** (N,%) 28(12.0%)	**P value**
Age (mean± SD)	59.9 ±12.2	59.8±13.2	0.96
Sex			0.64
Male	155(75.0%)	20(71.0%	
Female	50(24.0%)	8(28.0%)	
ECOG			0.60
1	107(52.0%)	12(43.0%)	
2	86(42.0%)	14(50.0%)	
3	12(6.0%)	2(7.0%)	
Race			0.18
Caucasians	170(83.0%)	22(78.0%)	
Asians	6(3.0%)	2(7.0%)	
African Americans	7(3.0%)	0(0%)	
Hispanic & Latinos	2(1.0%)	2(7.0%)	
Others	10(5.0%)	1(3.5%)	
Unknown	10(5.0%)	1(3.5%)	
Family History of GI cancer			0.07
Yes	35(17.0%)	9(32.1%)	
No	170(83.0%)	19(68.0%)	
Weight loss in lbs. (mean, SD)	8.8±14.6	11.9±19.65	0.84
Smoking			0.21
Current	21(10.0%)	5(18.0%)	
Past	87(42.0%)	14(50.0%)	
Non smoker	97(47.0%)	9(32.0%)	
Alcohol			0.67
Current/heavy	45(22.0%)	4(14.0%)	
Remote/heavy	13(6.0%)	2(7.0%)	
Non alcoholic	147(72.0%)	22(78.0%)	
H.Pylori			0.11
Yes	21(10.0%)	1(3.5%)	
No	31(15.0%)	1(3.5%)	
Unknown	153(74.6%)	26(93.0%)	
GERD			0.84
Yes	116(43.5%)	15(53.5%)	
No	89(43.5%)	13(46.5%)	
PPI			0.42
Yes	114(55.6%)	13(46.4%)	
No	91(44.3%)	15(53.5%)	
On Blood thinner			0.77
Yes	31(15.0%)	3(11.0%)	

No	174(85.0)	25(89.0%)	
Dysphagia			0.22
Yes	83(40.5%)	15(53.5%)	
No	122(59.5%)	13(46.4%)	
Anemia			
Yes	89(43.4%	6(21.4%)	0.026
Pain at presentation			1.0
Yes	99(48.0%)	13(46.4%)	
No	106(52.0%)	15(53.5%)	
Able to swallow			0.33
Yes	156(76.0%)	24(86.0%)	
No	49(24.0%)	4(14.0%)	

Table 2. Tumor characteristics for MET and non-MET patients

	MET Negative (N,%) 205(88.0%)	**MET Positive** (N,%) 28(12.0%)	**P value**
Tumor site			0.14
Esophagus	83(40.5%)	10(36.0%)	
GE junction	39(19.0%)	10(36.0%)	
Gastric	83(40.5%)	8(28.5%)	
Location of Primary Tumor			0.35
Mid esophagus	21(10.0%)	1(3.5%)	
Distal Esophagus	57(28.0%)	9(32.0%)	
GE Junction	36(17.5%)	10(36.0%)	
Cardia	31(15.0%)	2(7.0%)	
Fundus	7(3.4%)	1(3.5%)	
Body	27(13.0%)	2(7.0%)	
Antrum	26(13.0%)	3(11.0%)	
Histology			0.017
Well differentiated	30(15.0%)	2(7.0%)	
Moderately Differentiated	39(19.0%)	1(3.5%)	
Poorly differentiated	66(32.0%)	19(68.0%)	
Signet ring cell	15(7.0%)	1(3.5%)	
adenocarcinoma	17(8.0%)	0(0%)	
Mod to poorly differentiated	25(12.0%)	2(7.0%)	
Poor with signet ring cell	3(1.5%)	2(7.0%)	
Squamous Cell Carcinoma	3(1.5%)	2(7.0%)	
Unknown	1(0.5%)	1(3.5%)	
Neuroendocrine Features	6(3.0%)	1(3.5%	0.59
Diffuse	19(20.0%)	1(4.0%)	0.029
Intestinal	64(68.0%)	22(95.5%)	

Table-3: Metastatic Sites MET and non-MET

	MET Negative (N,%) 205(88.0%)	**MET Positive** (N,%) 28(12.0%)	**P value**
Bulky metastasis (>5 cm)			
Yes	22(11.0%)	7(25.0%)	0.05
no	183(89.0%)	21(75.0%)	
Metastatic at Presentation			1.00
Yes	158(77.0%)	22(78.5%)	
Metastatic site			0.12
Nodal	161(79.0%)	20(71.0%)	
Peritoneal	80(39.0%)	8(28.5%)	
Liver	50(24.0%)	9(32.0%)	
Lung	22(11.0%)	5(18.0%)	
Bone	10(5.0%)	3(11.0%)	
Brain	4(2.0%)	0	
MET amplification site			0.47
Primary	186(91.0%)	25(89.0%)	
Peritoneum	4(2.0%)	0	
Brain	1(0.5%)	0(0%)	
Nodal	3(2.5%)	2(7.0%)	
Bone	2(1.0%)	0	
Lung	4(2.0%)	0	
liver	5(2.5%)	0	
Common Metastatic Site on Progression			0.007
Local	19(9.0%)	0	
Liver	23(11.2%)	3(10.7%)	
Lung	14(7.0%)	0	
Nodal	41(20.0%)	4(14.0%)	
Bone	5(2.5%)	3(11.0%)	
Brain	5(2.5%)	1(3.5%)	
Peritoneum	16(8.0%)	2(7.0%)	
Others/Death	17(8.0%)	9(32.0%)	

Table-4: Systemic Chemotherapy and Radiotherapy Site in MET and non-MET

	MET Negative (N,%) 205(88.0%)	**MET Positive** (N,%) 28(12.0%)	**P value**
First line Chemo regimen			0.27
FOLFOX	102(50.0%)	15(53.5%)	
Paclitaxel/Carboplatin	20(10.0%)	1(3.5%)	
ECX/EOX	17(8.0%)	1(3.5%)	
Clinical Trial	12(6.0%)	10(3.5%)	
CDDP+5FU	10(5.0%)	1(3.5%)	
FLOT	8(4.0%)	0	
FOLFIRINOX	9(4.4%)	0	
Number of 1st Line cycles	7.0	7.5	0.54
Radio Therapy Site			0.26
Primary	24(92.0%)	7(78.0%)	
Bone	0	1(100%)	
Chest	1(100%)	0	
Nodal	0	1(100%)	
Others	0	1(100%)	

Table-5: Common molecular abnormalities and co-expression with MET amplification

	MET Negative (N,%) 205(88.0%)	**MET Positive** (N,%) 28(12.0%)	**Overall** N(%)
Other Genetic abnormalities			
TP53	83(40.0%)	7(25%)	90(39.0%)
HER2 amp	25(12.0%)	5(18.0%) *	30(13.0%)
CDH1	15(7.3%)	0	15(6.5%)
EGFR amp	11(5.4%)	2(7.0%)	13(5.5%)
CDKN2	8(4.0%)	3(11.0%)	11(5.0%)
PIK3CA	10(5.0%)	1(3.5%)	11(5.0%)
KRAS	8(4.0%)	1(3.5%)	9(4.0%)
FGFR	6(3.0%)	0	6(2.5%)
PTEN	4(2.0%)	0	4(2.0%)
PDGFR	4(2.0%)	0	4(2.0%)
ARIDIA	4(2.0%)	0	4(2.0%)
BRAF	2(1.0%)	0	2(1.0%)
CTNNB	2(1.0%)	0	2(1.0%)
ALK	1(0.5%)	0	1(0.5%)

* 4 were MET+/Her2+ on initial biopsy and one was on biopsy post trastuzumab

Table-6: Multivariate Regression Model

	Progression Free Survival		Overall Survival	
Variable	**HR(95% CI)**	**P value**	**HR(95%CI)**	**P value**
Family History of GI Cancer	-	-	1.58(1.0-2.3)	0.027
Presentation with pain	-	-	1.45(1.0-2.0)	0.03
ECOG >2 Status	-	-	1.58(1.1-2.1)	0.002
Metastasis at presentation	-	-	2.0(1.3-3.1)	0.001
RT site Pri/Met	1.6(1.1-2.4)	0.008	-	-
MET amplified	5.1(2.0-12.0)	0.000	1.6(1.0-2.6)	0.047

Figure-1

Median Progression Free Survival among MET vs non-MET amplified EGC in whole cohort

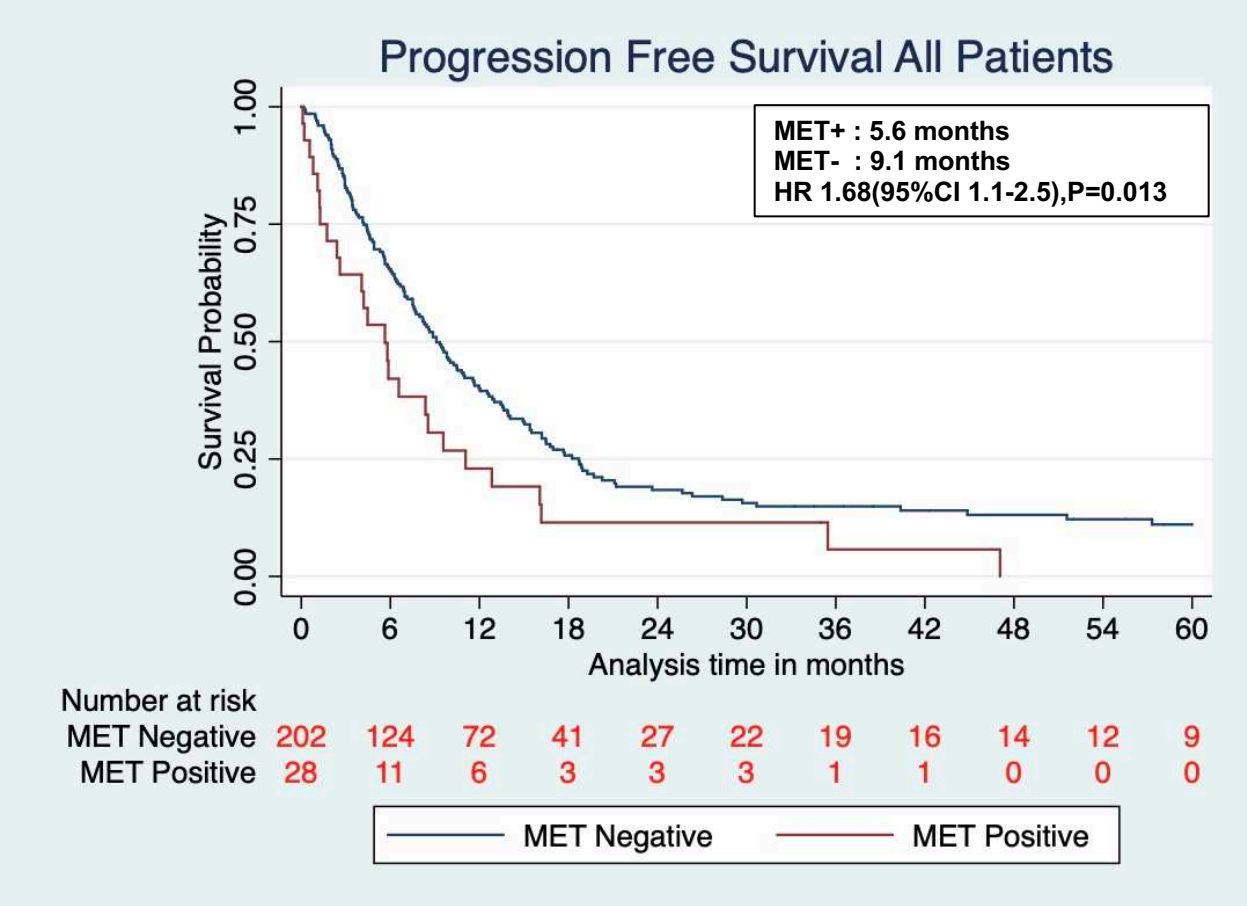


Figure-2

Median Overall Survival among MET vs non-MET amplified EGC in whole cohort

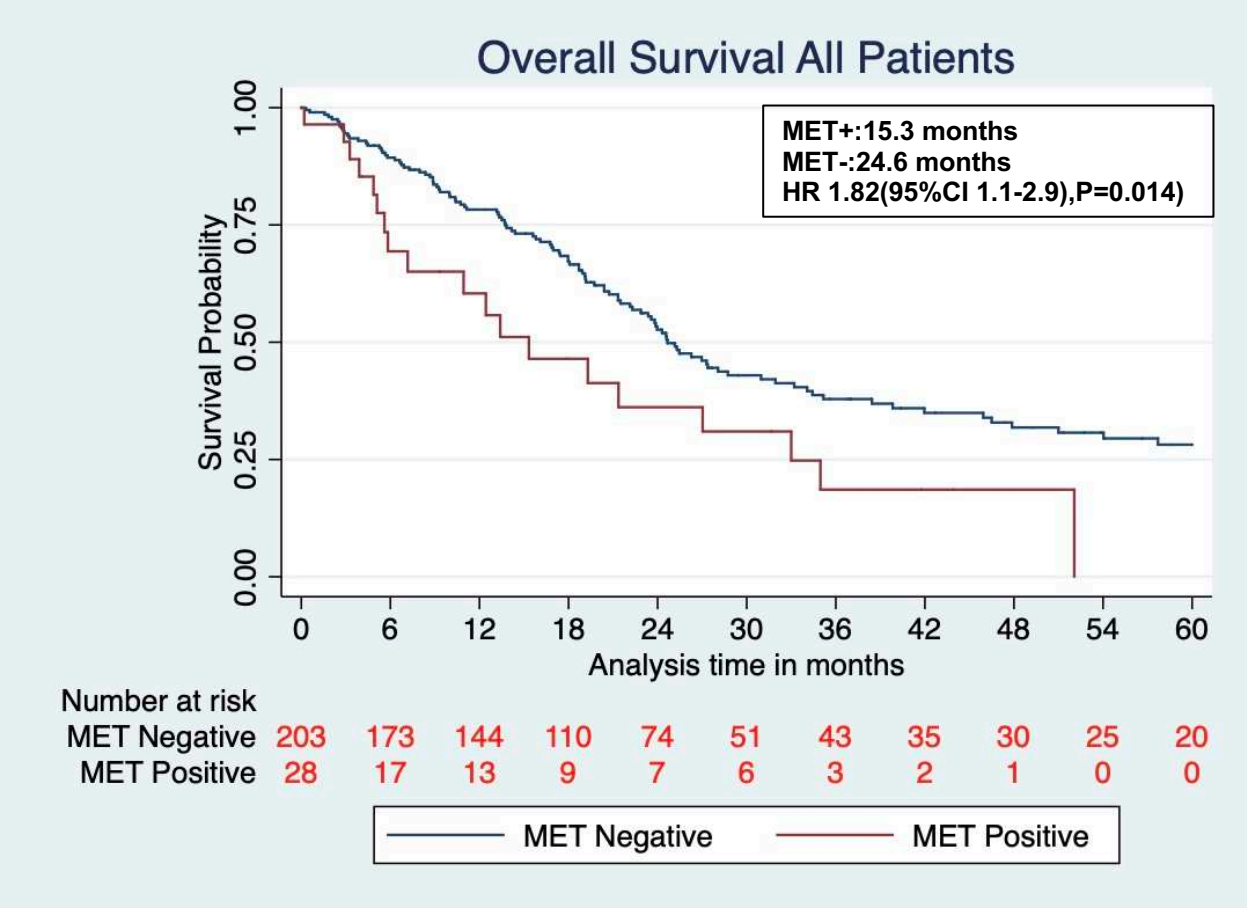


Figure-3

Median Progression Free Survival among MET vs non-MET amplified EGC in metastatic at presentation

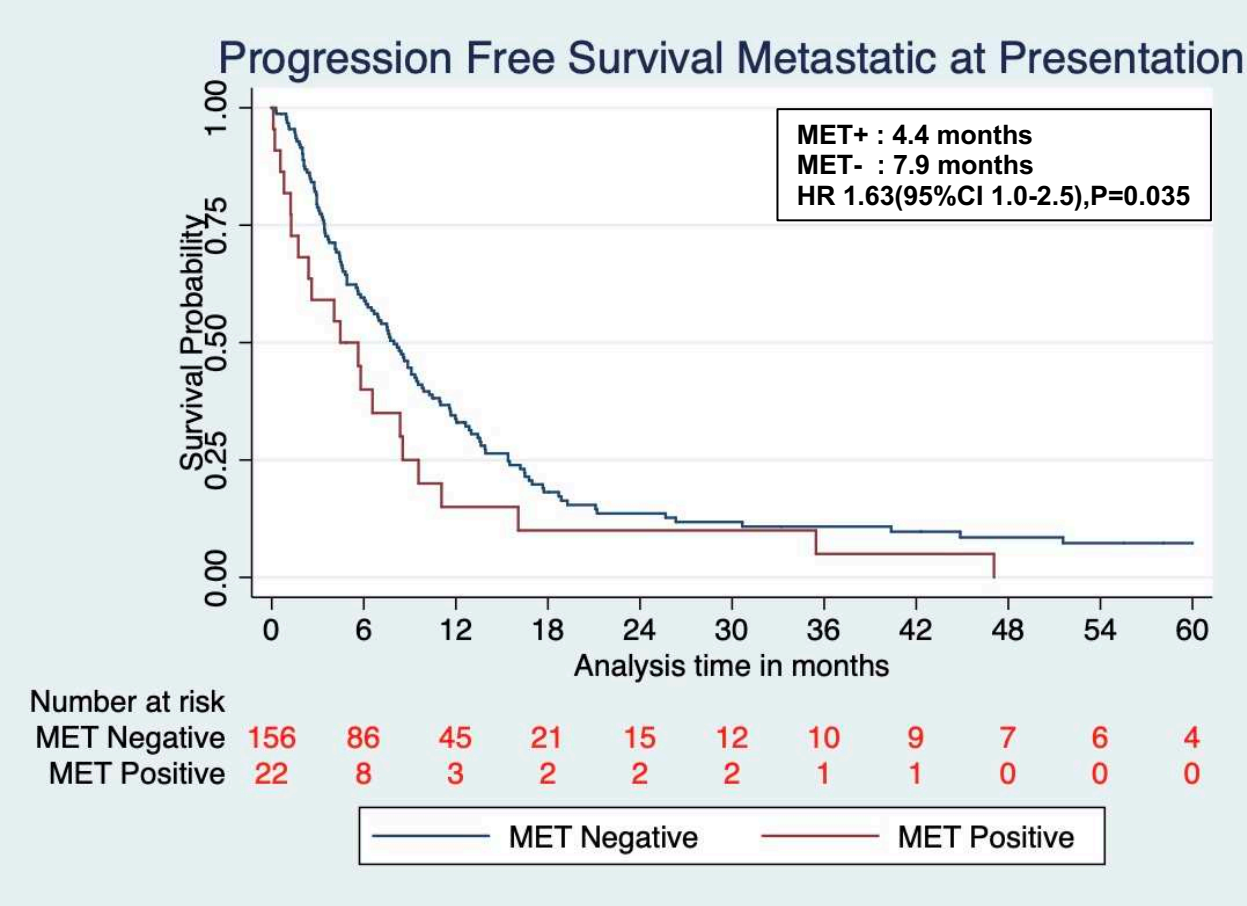


Figure-4

Median overall Survival among MET vs non-MET amplified EGC in metastatic at presentation

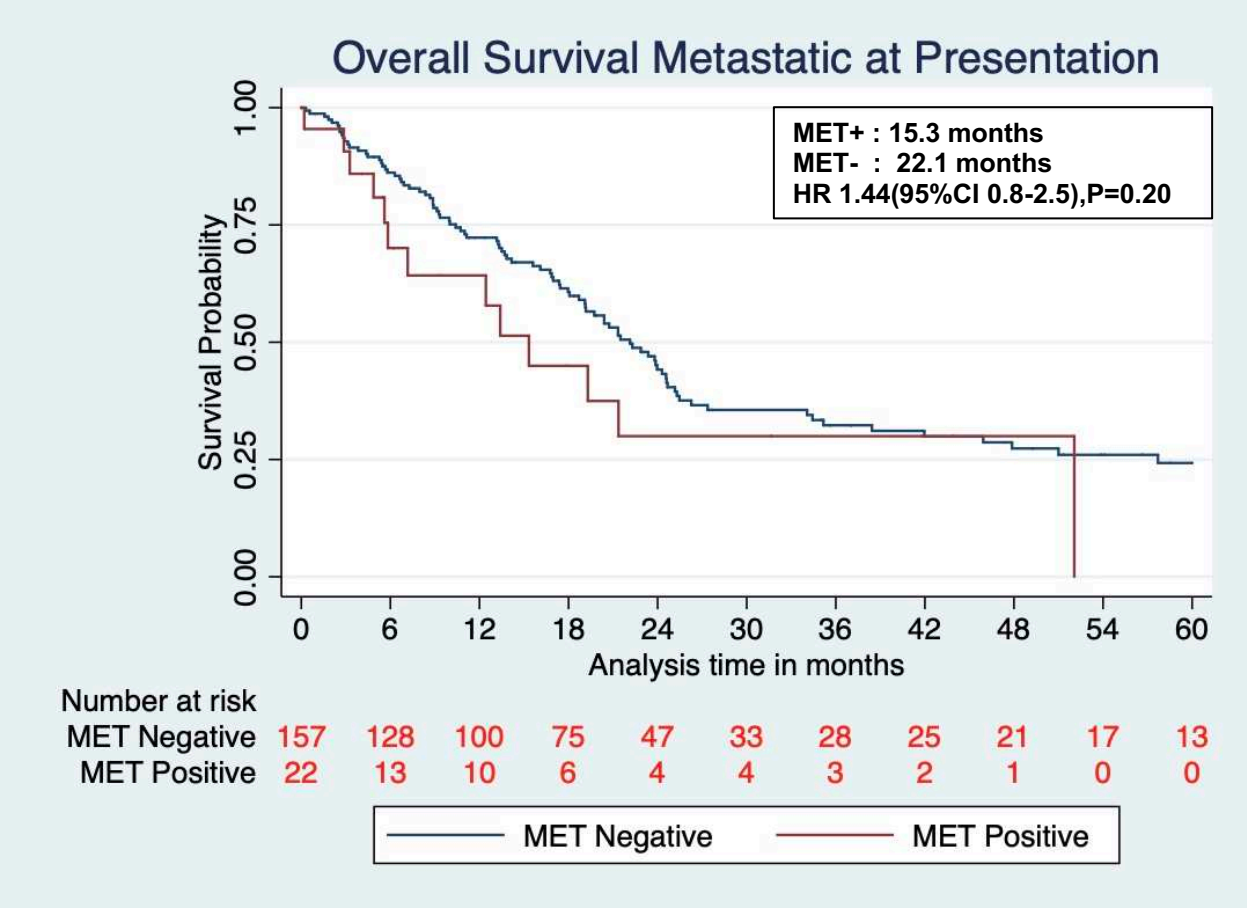


Figure-5

Median Progression Free Survival among MET >2.2-<25 vs >25

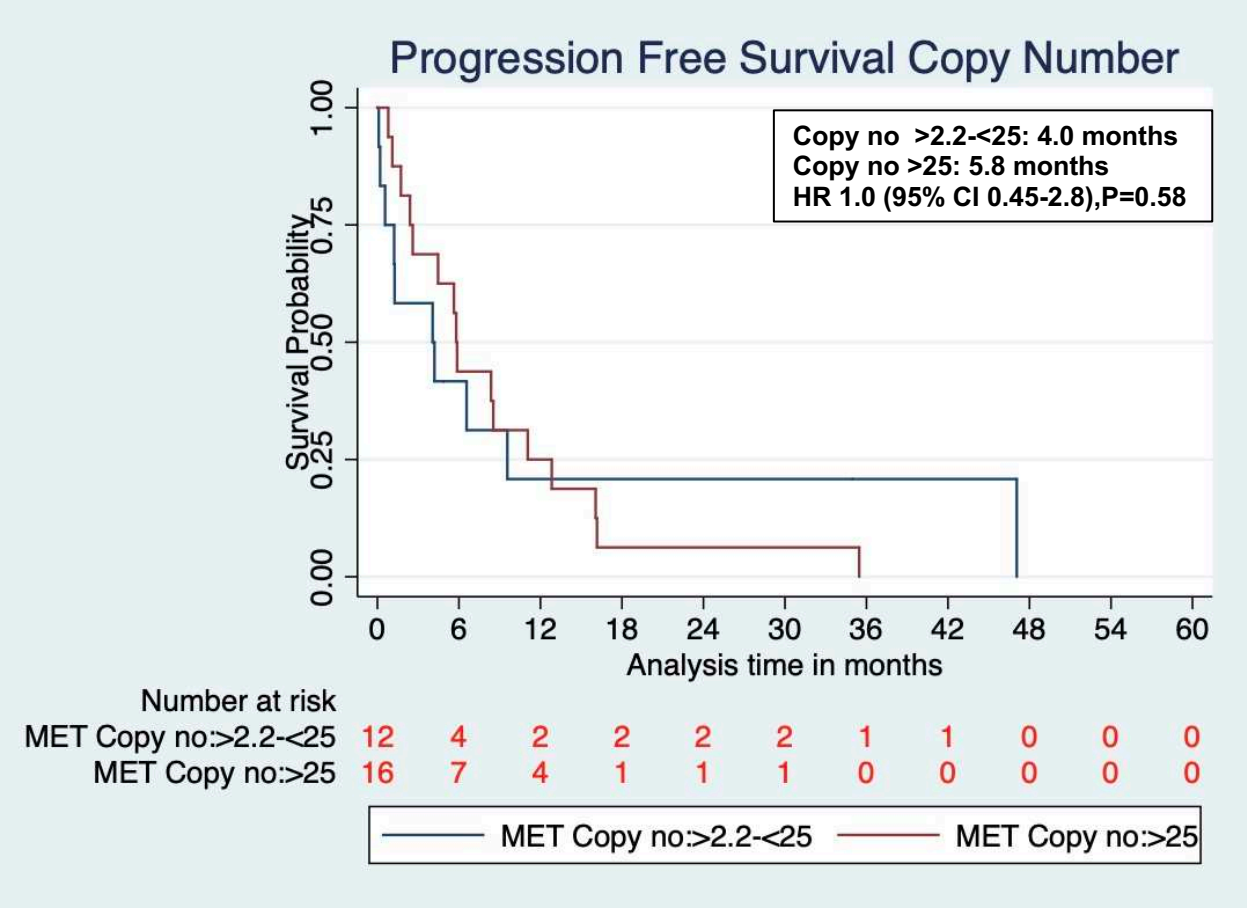


Figure-6

Median Overall Survival among MET >2.2-<25 vs >25

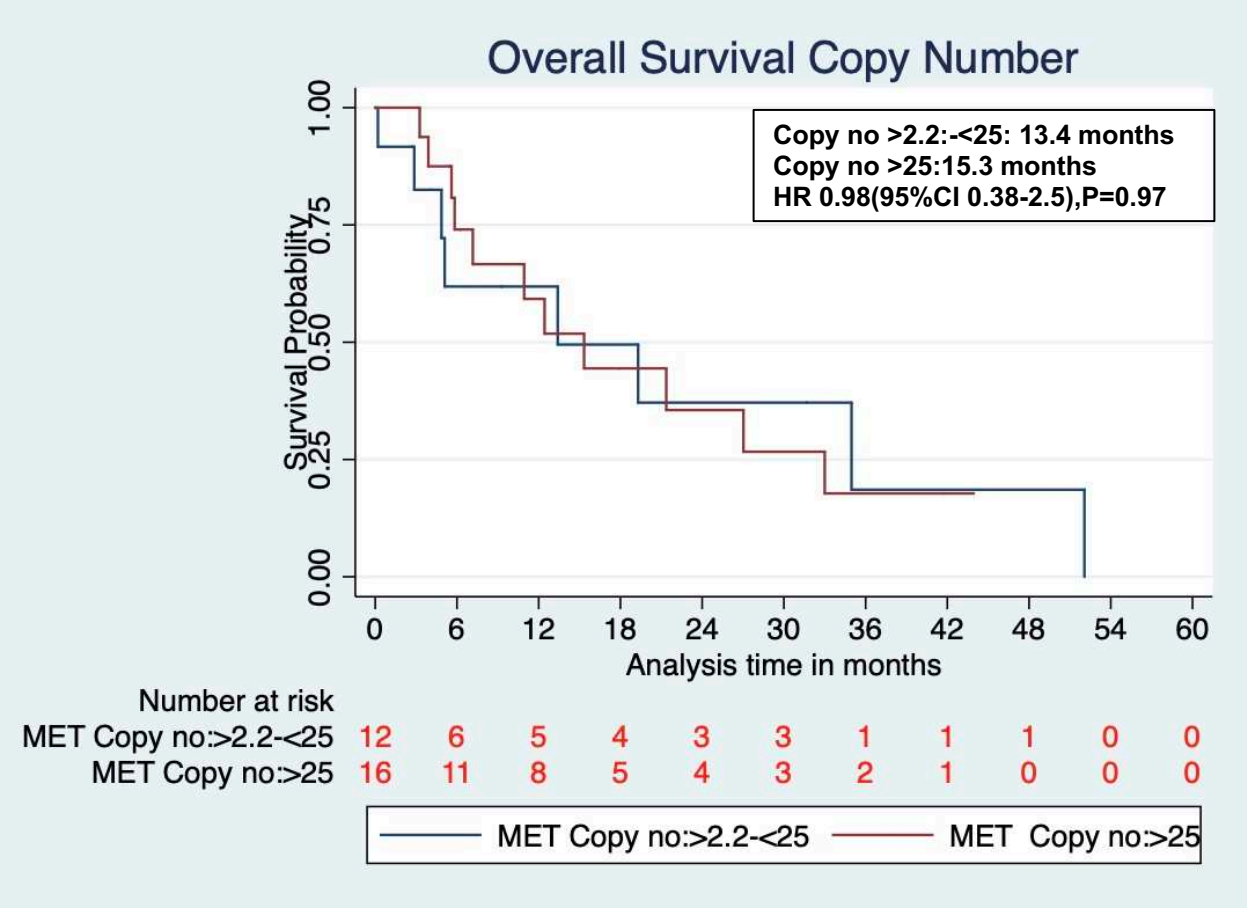


Figure-7

Median Progression Free Survival among MET directed therapy

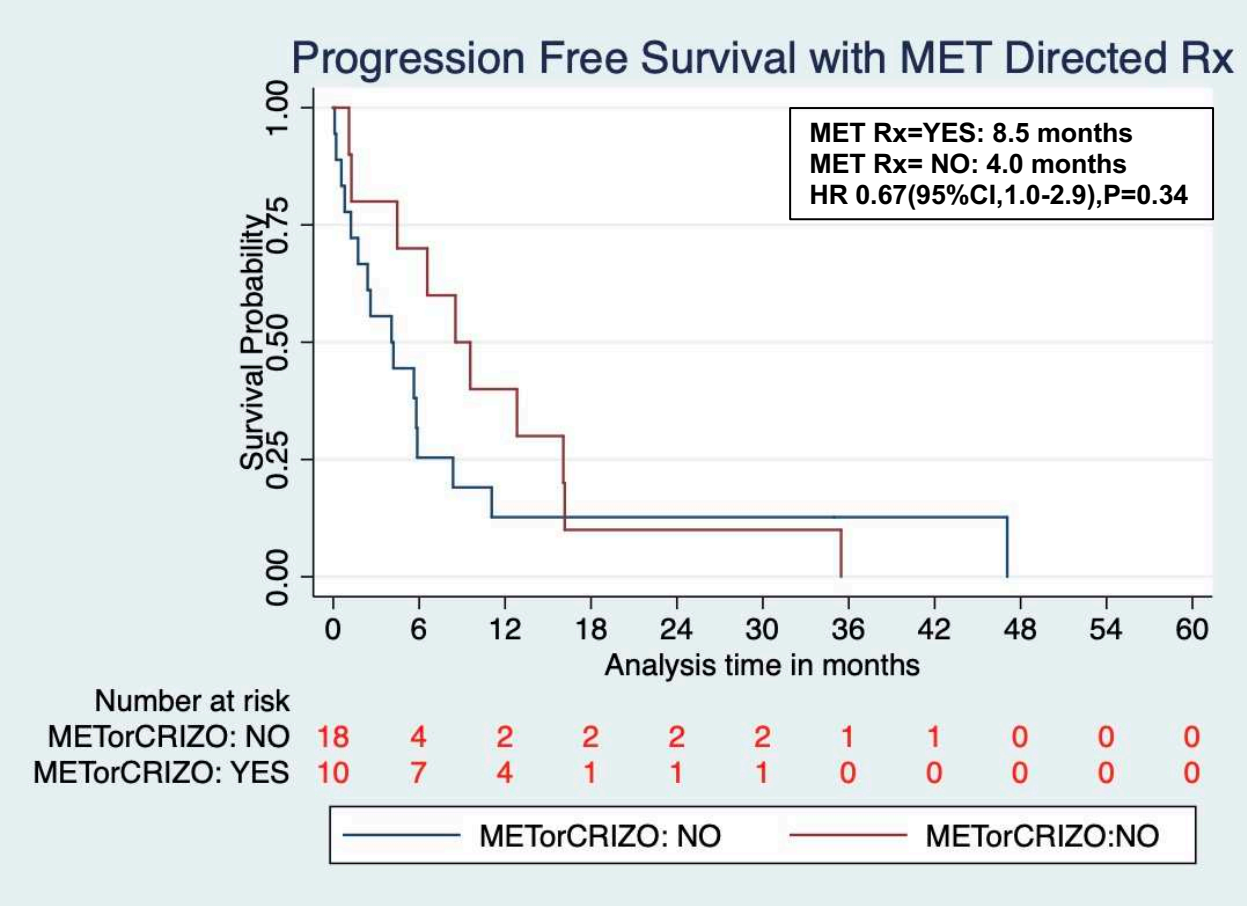
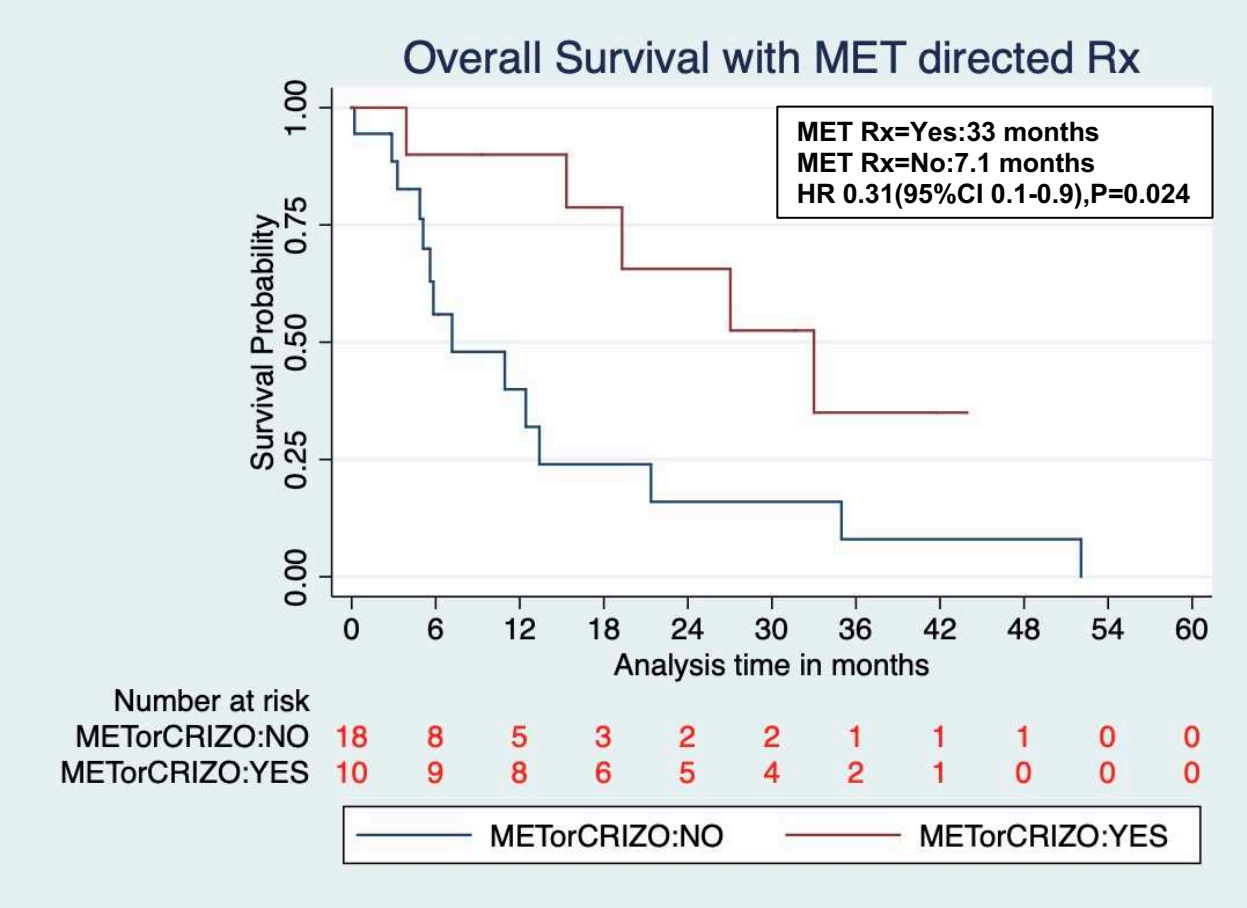


Figure-8

Median overall Survival among MET directed therapy



Discussion and future perspective

The Heat Shock Protein 90 (HSP90) is an important chaperone for maturation of a large number of proteins some of which are critical in controlling important processes in cancer cells including growth, invasion, and metastasis by inhibiting degradation of these oncogenic signaling proteins. Despite this, studies of HSP90 inhibitors in cancer have been disappointing to date. In our phase II study in patients with advanced or metastatic EGC, the HSP90 inhibitor Ganetespib failed to show significant efficacy overall in esophagogastric cancer (EGC). However, a complete response in one patient and minor responses in two other patients indicate potential activity in a subset of patients. The one patient who achieved a complete response(CR) had a mutation in the KRAS gene. It is possible that cancer cells which have a KRAS mutation may be more responsive to HSP 90 inhibitors because C-RAF may be particularly sensitive to destabilization by ganetespib in KRAS mutant cancers(1).Therefore, the degradation and loss of C-RAF protein afforded by ganetespib's inhibition of HSP90 may provide an effective means to uncouple aberrant KRAS signaling from downstream MEK and ERK activation which may be responsible for its effectiveness in this patient(2, 3).Overall this study showed that ganetespib has acceptable toxicity. Diarrhea and fatigue were the most common toxicity of all grades; and anemia, neutropenia and leucopenia were the most common hematological toxicities. One patient died by septic shock which was not determined to be drug related.

The protocol called for study enrollment to be stopped as soon as the requirement of the first stage couldn't be met (i.e overall response rate (ORR) of at least 3 patients to respond out of 20 by radiological assessment as per RECIST). Nine(35%) of the initial 20 patients enrolled

were not available for radiological response evaluation as these patients either came off study for clinical progression or died of disease progression before 8 weeks which was the first radiological assessment as per protocol. Thus, a total of 26 patients were enrolled in this study to account for this. With only one radiologic response in the first 17 evaluable patients, the decision was made by the sponsor to close the study for futility at that point.

Limitations to this study included the small sample size, the fact that it was non-randomized, and it was a monotherapy trial in an unselected population given the absence of any known predictive biomarker that might predict responsiveness to HSP 90 inhibitors.

The mesenchymal epithelial transition (MET) protein growth factor receptor and its ligand (hepatocyte growth factor or HGF) are involved in a number of critical biologic processes in normal cells. Overexpression of MET or mutations in the gene are present in a number of malignancies, including EGC, and have been shown to be important for survival and growth of these cells. A number of inhibitors of MET are in various phases of clinical development to treat cancer. While activity has been demonstrated in a proportion of patients, insufficient activity has yet been seen for any of these to be approved for this indication. A better understanding of the clinical and pathologic features of MET amplified tumors would hopefully provide insight into ways of potentially improving treatment approaches.

In order to better understand the clinical and pathologic correlates of MET amplified EGC as compared to patients without MET amplification, we performed a retrospective analysis of all EGC patients who had been tested for MET amplification by FISH analysis at MGH from 2002 to 2019. We identified 233 patients, 28(12%) of whom were MET amplified. A higher proportion of MET amplified tumors had features suggestive of a more aggressive biology including being poorly differentiated and having bulky metastases. This was consistent with

shorter progression free and overall survival in the patients with MET amplification. In a subset of patients, there was co-amplification of other proteins potentially critical for proliferation and metastasis of cancer cells which may partially explain why MET inhibitors have not been successful to date. Perhaps, combinations targeting both MET and the coamplified protein or MET inhibitors in combination with chemotherapy to overcome issues of heterogeneity in different cancer cells might be a strategy to improve therapy. Ultimately, better understand of the biology of EGC will help to design future clinical trials to try to develop MET inhibition as a clinically meaningful approach.

References

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