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TITLE:
PANGEA-IMBBP: Personalized ANtibodies for Gastro-Esophageal Adenocarcinoma - A 1st Pilot Metastatic Trial of Biologics Beyond Progression

Trial Design: Expansion Platform Design Type II

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SCHEMA

PANGEA-IMBBP: Personalized ANtibodies for Gastro-Esophageal Adenocarcinoma- A 1st Pilot Metastatic Trial of Biologics Beyond Progression

Key Patient Eligibility Criteria:

See Section 3.1 for complete list of criteria.

1. Histologically confirmed metastatic gastric or esophagogastric junction (type I,II,III Siewert) adenocarcinoma
2. Newly-diagnosed chemo-naïve or recurrent after curative-intent surgery
   - ≥6 months after completion of adjuvant therapy (including chemotherapy and/or radiotherapy)
   - No prior treatment for stage IV disease with any targeted agent except trastuzumab/nivolumab
   - Patients who have started first line fluoropyrimidine/platinum therapy (+/-trastuzumab or nivolumab) may be considered for trial participation if they have received no more than 4 doses of therapy (5FU/LV bolus is optional with FOLFOX) at the time of consent and screening.-
     - These patients will be required to meet 'next cycle' parameters for eligibility before commencing treatment on trial (as per Section 6) rather than being required to meet parameters as indicated below in #12 which is for previously untreated metastatic/recurrent patients.
3. Measurable metastatic disease by RECIST 1.1 criteria,
   - Must be amenable to ultrasound or CT-guided biopsy of one metastatic lesion or:
   - Peritoneal disease as the sole site of occult metastasis or presenting as malignant ascites is acceptable if a cell block of tumor cells can be obtained showing >20% viable tumor cells.
4. No currently active second malignancy
5. No uncontrolled intercurrent illness or infection
6. ECOG PS 0-2
7. Age ≥ 18 years
8. No CVA within 6 months, no recent MI within 6 months
9. Patients must have normal organ and marrow function as defined below:
   - granulocytes ≥1,500/mcL
   - platelets ≥ 100,000/mcL
   - total bilirubin ≤ 1.5 x ULN, ≤1.8 x ULN with liver metastases and not amenable to biliary stent
   - AST(SGOT)/ALT(SGPT) ≤2.5 X upper limit of normal without liver metastases; ≤5 X institutional upper limit of normal with liver metastases
   - creatinine within normal institutional limits (<1.5)
     OR
   - creatinine clearance ≥50 mL/min/1.73m^2 for creatinine level above normal
   - INR ≤ 1.5
10. Consent to baseline metastatic biopsy and biopsy at time of each progressive disease (of metastatic/progressing lesion) for enabling biomarker assessment and treatment assignment (at each time point – baseline, PD1, PD2, PD3) as well as for correlative studies.
    - Consent to baseline and serial blood draws for plasma/serum/whole blood banking for correlative studies
11. Ability to understand and the willingness to sign a written informed consent document and consent to the serial nature of the proposed PANGEA treatment with first, second and third line therapy as tolerated.
12. Ability to comply with requirements of the protocol, as assessed by the investigator and signing the consent form.
13. If history of exposure to anthracyclines during perioperative treatment, the following cumulative doses of anthracyclines must be less than:
    Epirubicin < 720 mg/m2
    Doxorubicin or liposomal doxorubicin < 360 mg/m2
    Mitoxantrone > 120 mg/m2 and idarubicin > 90 mg/m2
    If more than one anthracycline has been used, then the cumulative dose must not exceed the equivalent of 360 mg/m2 of doxorubicin.
14. Cardiac Ejection Fraction >50% (for HER2+ patients) as assessed by echocardiogram, MUGA scan, or cardiac MRI
15. Willingness to use effective and reliable methods of contraception.
16. History of autoimmune disease (for MSI-H/EBV/PDL1≥10CPS/TMB>15mts/Mb patients) documented by rheumatologist/specialist

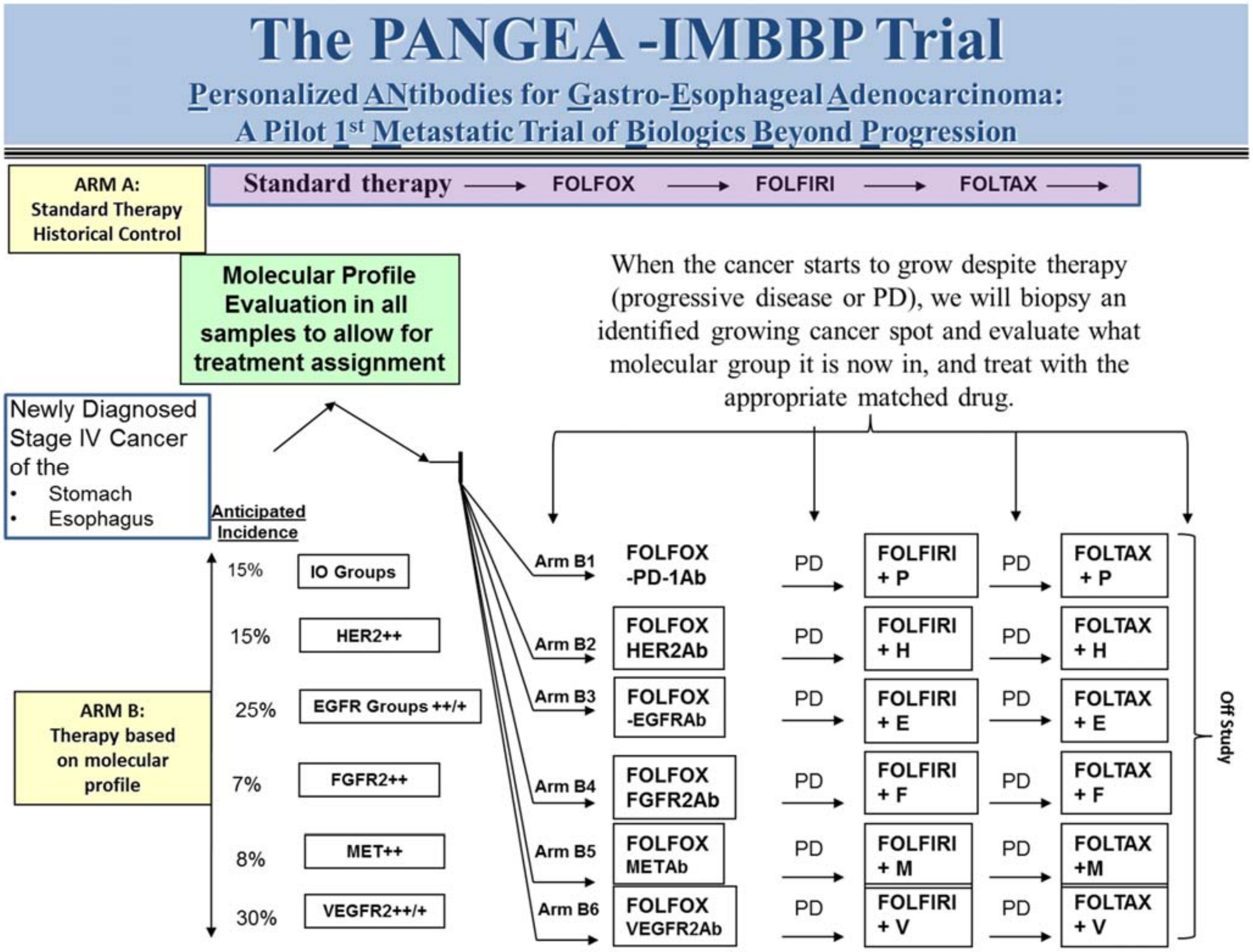

FIGURE 1: PANGEA Trial Schema.

1. Trastuzumab with 5FU plus a platinum is currently a standard treatment option for first line HER2+ metastatic disease, indicated in pink. Treatment for HER2+ patients will commence immediately upon obtaining results from routine clinical standard workup as well as biopsy of a metastatic site. (as in section 9.2), but this can be changed if the metastatic site is discordant or if a better treatment option (ie IO: MSI-High and/or EBV+ and/or TMB-high >15 mts/Mb and/or PDL1+ >10% CPS) is determined from their metastatic site
2. HER2-negative patients will commence FOLFOX therapy alone until appropriate investigational biologic agents are determined and (or become) available.
3. EGFRab = ABT806; VEGFR2ab = ramucirumab; **METab* = pending; FGFR2ab = pending***; PD1-Ab = nivolumab. *patients may be able to receive anti-MET/FGFR2 antibodies on other studies, and if so, they will be followed for survival outcomes and other translational correlatives as per the PANGEA study, but treated according to protocol of the other specific study with the anti-MET or anti-FGFR2 antibodies.
4. PD (progressive disease) will lead to a repeat biopsy of a progressing lesion, change of chemotherapy backbone, but continuation of assigned biologic.
   *However, if the tumor molecular category 'drifts' at any time point (PD1 or PD2) based on the new Biomarker Assessment and Treatment Algorithm (see **Figure 3**), then there will be a change of both i) chemotherapy backbone and ii) to the appropriate biologic, and the previous biologic, if any, will be discontinued.
   5. HER2++ incidence is ~15-20%, while MET++ incidence is ~8%. Approximately 20% will be EGFR++/+ (of which EGFR++ amp ~6%), and FGFR2++ 7%. The ImmunoOncologic (IO) group includes MSI-high, and/or EBV+ and/or PDL1+ CPS≥10% and/or High TMB ≥15mt/Mb tumors (together amounting to 15% of GEA) and the remainder will be in VEGFR2++/+ groups (See section 13.1 for statistical analysis for the primary efficacy endpoint). (see **Figure 3**)

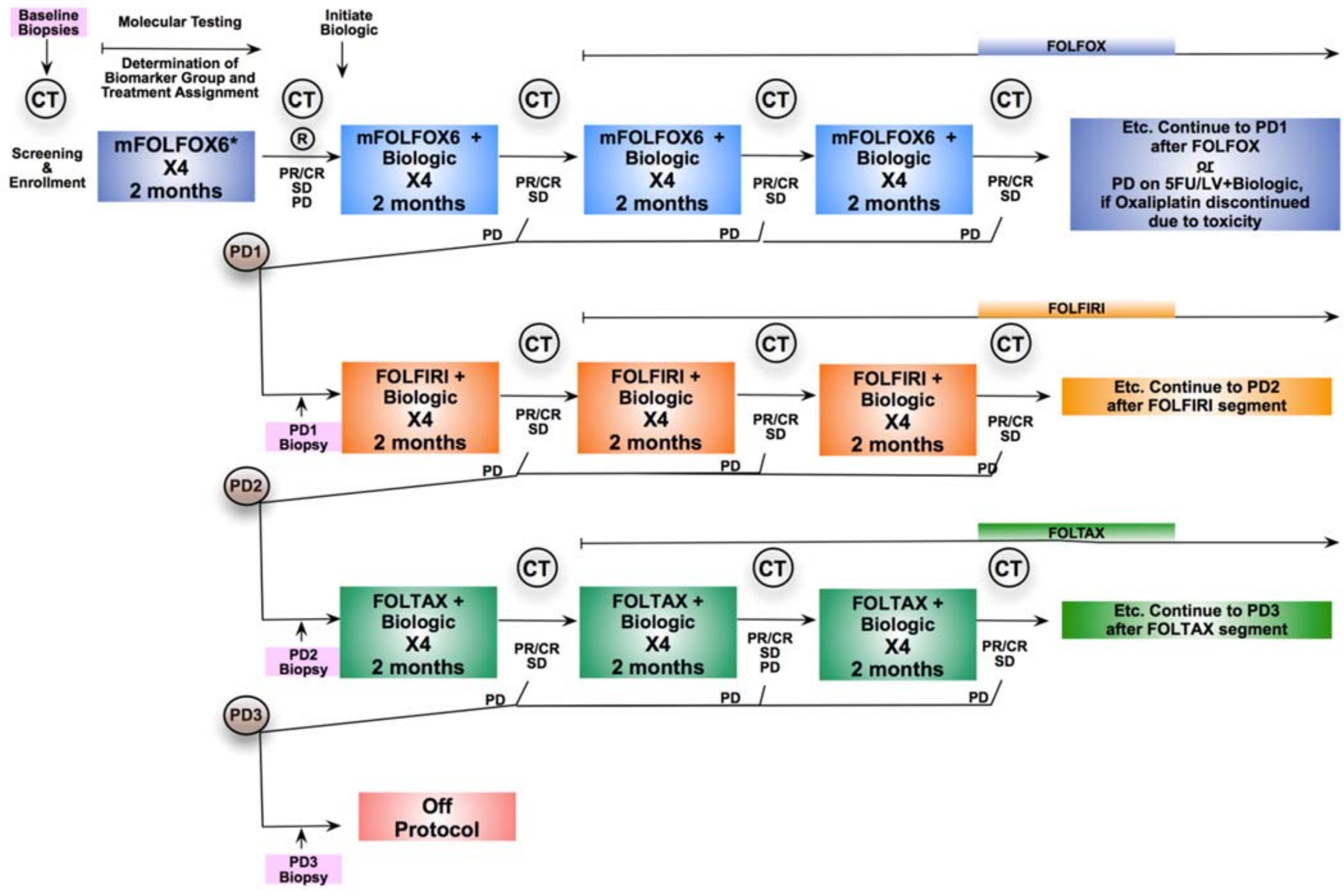

FIGURE 2: PANGEA TREATMENT STRATEGY

First Line: mFOLFOX6 – with appropriate Biologic
Second Line: *FOLFIRI – with appropriate Biologic
Third Line: *FOLTAX – with appropriate Biologic

*If the bolus 5FU is dropped for toxicity with any regimen, it should not be resumed with any subsequent regimen, and continuous 5FU doses once reduced are not to increase with subsequent regimens.

**** Patients will commence FOLFOX therapy alone until confirmation of their molecular profile. FOLFOX alone will be continued per routine while molecular testing is conducted to allow for all testing and treatment assignment to be performed. When the biologic group is identified, the molecularly targeted agent is added at the next due cycle.**

Treatment Assignment Algorithm‡

Biomarker Result Priority*	Treatment Arm	Anticipated HR
1. **MSI-H/EBV+/TMB≥15/PDL1+≥10%CPS****	**B1 – IO**	0.5
2. **HER2++** FISH+ (IHC 3/2+)***	**B2 - HER2++**	0.5
3. **EGFR++** amplified*** NGS/FISH	**B3 – EGFR++**	0.5
4. **FGFR2++** amplified*** NGS/FISH	**B4 – FGFR2++**	0.5
5. **MET++** amplified*** NGS/FISH	**B5 - MET++**	0.5
6. **RAF/RAS/MEK or PIK3CA/PTEN/AKT** NGS	**B6 – VEGFR2++**	0.8
- KDR/VEGFA amplified		
- RAS amplified/RAS mutant		
- RAF mutant		
- MEK/ERK mutant		
- PIK3CA mutant		
- PTEN/mTOR/AKT mutant		
- RAS/PIK3CA/AKT alteration		
- GNAS amplified/mutant		
7. All negative, **EGFR+** by MS	**B3 – EGFR+**	0.8
8. All negative, **EGFR-** by MS	**B6 – VEGR2+**	0.8
		Aggregate HR = 0.67

‡If Primary and Metastasis Sites are Discordant, Use Metastasis; if QNS use ctDNA; if ctDNA no alterations use primary tumor; if all non-diagnostic #8.
*Proceed to subsequent group # if negative for previous group.
** Only in ≥ second line therapy for HER2++ (#2) does IO (#1) take priority
*** Prioritize by highest Gene/Control ratio.

FIGURE 3: **PANGEA BIOMARKER ASSESSMENT & TREATMENT ALGORITHM**
The treatment assignment algorithm is derived as in Section 2.5. One notes various ‘layers’ of positivity for a given molecular group – eg. Steps 3 and 7 are classified as EGFR (++ or +), and Step 6 and 8 are classified to VEGFR2 (++ or +). This is an attempt to stratify and group tumors using a prioritized scheme, based on best currently available evidence regarding driver biology, as well as availability of targeted agents. If assigned to a biomarker where drug is currently not available, patients will be treated with standard of care until such targeted therapy becomes available – see protocol details.
Immunooncology (IO) therapy Group includes MSI-High and/or EBV+ and/or PDL1+ CPS>10% and/or High TMB >15mt/Mb tumors
Genomic aberrations in RAS/RAF/MEK/ERK and/or PI3K/PTEN/mTOR/AKT and/or GNAS pathways receive anti-angiogenesis with anti-VEGFR2 antibody.

Figure 4: PANGEA Practical Flow

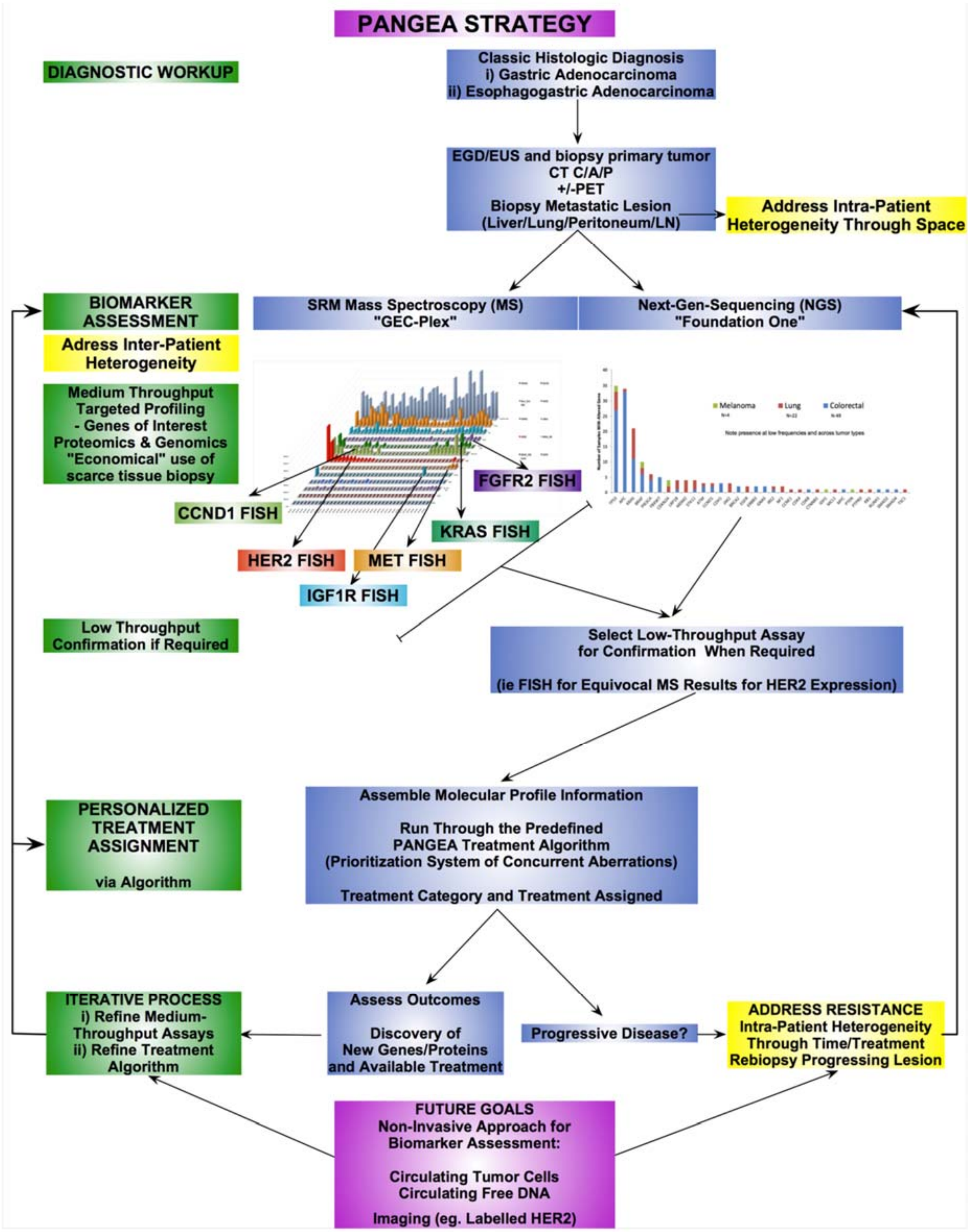

Figure 5: Demonstration of intra-patient and inter-patient tumor heterogeneity.
A) Intra-patient tumor heterogeneity in an individual patient through space (ordinate y-axis) from primary tumor to met ... d treatment selection pressures.
B) In A atie ... eterogeneity on z-axis.
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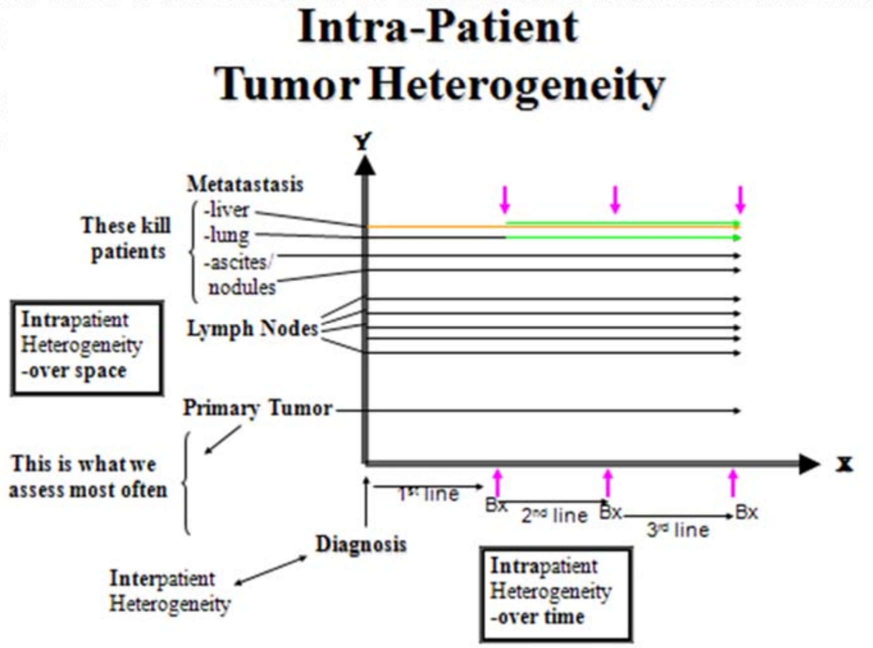

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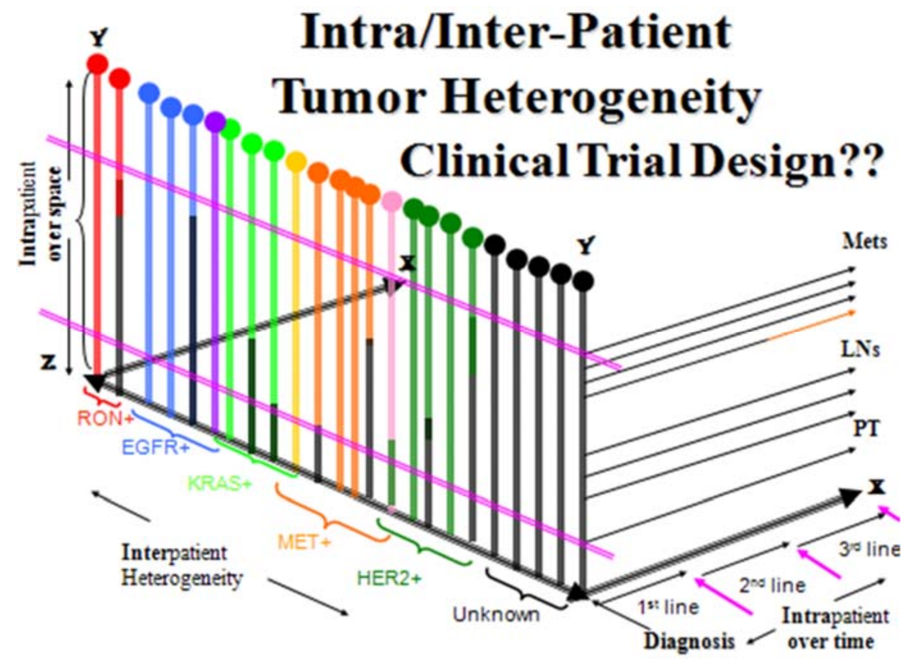
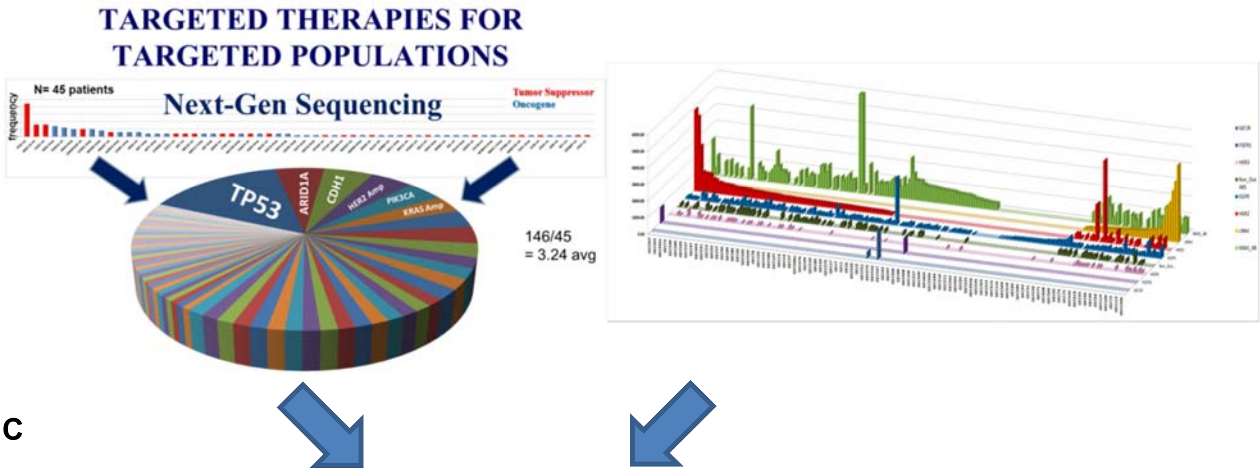


B Proteomics



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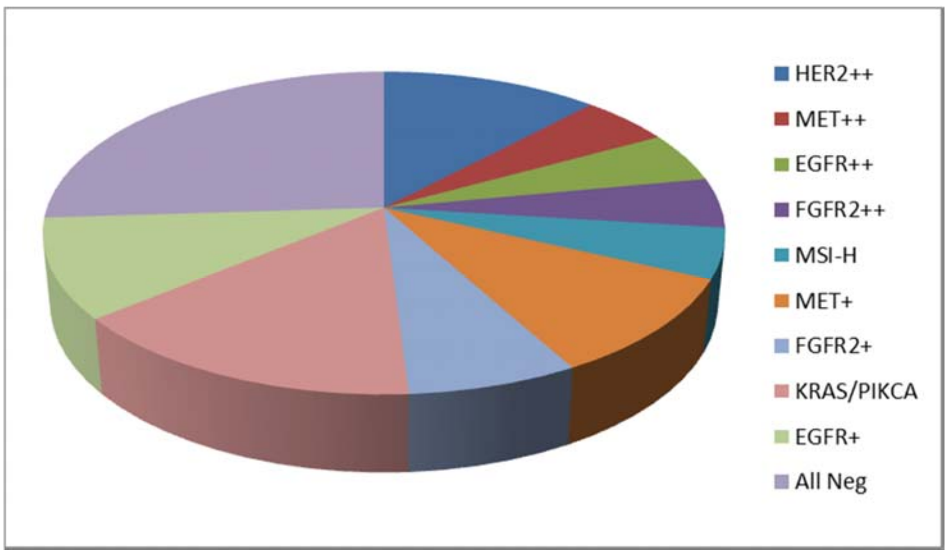

Figure 6. PANGEA classification into main categories using the Biomarker and Treatment Algorithm (Figure 3). Although essentially every patient has a unique profile, even when considering genomics (A) or proteomics (B) alone, to be practical, PANGEA incorporates both genomic and proteomic profiles as a compromise between the potential (infinite) number of treatment categories and actual feasibility of conducting such a trial, to derive a set of major molecular categories (using the prioritization scheme in **Figure** 3). Proportions (C) represent a preliminary cohort of approximately 50 patients' PANGEA classification. (++ = amplified, + = overexpressed non amplified)

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To determine the **safety and feasibility** of obtaining <u>baseline biopsies</u> of metastatic disease sites (liver, lung, lymph node, peritoneum/ carcinomatosis) for molecular testing, in order to proceed with the biomarker assessment and treatment assignment algorithm (Figure 3,4,6).

1.1.2 To determine the **safety and feasibility** of obtaining <u>serial biopsies</u> of progressing metastatic disease sites (liver, lung, lymph node, peritoneum/carcinomatosis) for molecular testing at each progression point ($PD_{1,2,3}$ – Figure 1,2,4,6), in order to proceed with the biomarker assessment and treatment assignment algorithm (Figure 3), as assessed by rate of successful treatment category assignment.

1.1.3 To determine the **median overall survival (mOS)** of the combined HER2++, MET++, EGFR++/+, MSI-H/EBV+/TMB-High≥15mt-Mb/PDL1+ CPS≥10%, FGFR2++, and VEGFR2++/+ groups (N=68 total treated per intented protocol with targeted therapies) treated with their respective targeted therapies per the treatment assignment algorithm (intention to treat), with each line of cytotoxic chemotherapy (up to three lines, Biologic Beyond Progression), compared to historical controls having an aggregate mOS of approximately 12 months.

1.2 Secondary Exploratory Objectives

1.2.1 To determine the median overall survival (mOS) collectively of all patients undergoing tumor molecular profiling with classification into one of six predefined gastroesophageal cancer (GEC) 'oncogenic driver' categories (HER2++, MET++, EGFR++/+, FGFR2++, MSI-H/EBV+/TMB-High≥15mt-Mb/PDL1+ CPS≥10%, VEGFR2++/+) with paired specific targeted therapy via the biomarker assessment and treatment algorithm (Figure 3), along with standard chemotherapy (up to 3 lines), compared to historical controls having an aggregate mOS of approximately 12 months. (Figure 1,2)

1.2.2 To determine the median progression free survival ($mPFS_1$) of first-line chemotherapy (mFOLFOX6) plus 'personalized' treatment of trastuzumab for HER2+ compared to historical controls of $mPFS_1$ of approximately 6 months. (Figure 1,2)

1.2.3 To determine the median progression free survival ($mPFS_1$) of standard care with first-line chemotherapy (mFOLFOX6) plus 'personalized' treatment, compared to historical controls of $mPFS_1$ of approximately 5 months for all patients in all five molecular categories. (Figure 1,2)

1.2.4 To determine the rate of continuing with a second-line and third-line treatment (chemotherapy backbone FOLFIRI, FOLTAX), compared to historical controls (50%, 25%); to determine the rate and type of further treatments off-protocol after completion of third line therapy. (Figure 1)

1.2.5 To determine the $mPFS_{2,3}$ of continuation of the re-targeted molecular therapy (Biologic Beyond Progression- BBP) along with the second and third line chemotherapy, compared to historical controls of $mPFS_2$ (of approximately 4 months) and $mPFS_3$ (of approximately 2 months) with chemotherapy alone. (Figure 1,2). ($mPFS_2$ denotes the median time to second disease progression after progressing on first-line therapy; $mPFS_3$ denotes the median time to third disease progression, after progressing on second-line therapy.)

1.2.6 To determine the 6 month, 12 month, 18 month and 24 month survival rate, compared to historical controls.

1.2.7 To determine the overall response rate (ORR) at each line of therapy ($ORR_{1,2,3}$), compared to historical controls (ORR_1 30%, ORR_2 20%, ORR_3 10%).

1.2.8 To determine the $mPFS_{1+2}$, $mPFS_{1+2+3}$.

1.2.9 To determine the disease control rate (DCR) at each line of therapy ($DCR_{1,2,3}$).

1.2.10 To determine the toxicity experienced by GEC patients treated with the combination of serial mFOLFOX6→ FOLFIRI → FOLTAX plus assigned biologic treatment with each line of therapy.

1.3 Laboratory/Translational Exploratory Correlatives Objectives

1.3.1 **To determine and refine our understanding of <u>inter-patient</u> GEC tumor heterogeneity by evaluating genomic and proteomic tumor profiles in all patients on trial.**

1.3.1.1 To determine the somatic <u>genomic changes</u> of 315 actionable cancer-related genes (mutation, amplification, and translocation), MSI-status/IO, and 27 genes with common translocations, using targeted deep next-generation sequencing (NGS), as well as fluorescence in situ hybridization (FISH) for *HER2*, *MET*, *FGFR2*, *EGFR, KRAS.*

1.3.1.2 To determine the <u>expression</u> of known oncogenic GEC drivers including HER2, MET, FGFR2, EGFR, KRAS using i) a novel GEC-multiplexed selected reaction monitoring mass spectrometry (containing 20 oncoproteins) and ii) immunohistochemistry (IHC).

1.3.1.3 To determine the relationship of these parameters in 1.3.1.(1-2) to clinical outcomes including mPFS, DCR, ORR, mOS, and clinical factors including ethnicity, tumor differentiation, histology, primary anatomical location, and stage.

1.3.2 **To determine and refine our understanding of <u>intra-patient</u> GEC tumor heterogeneity through space and time, by evaluating genomic and proteomic profiles of each tumor in all patients on trial.**

1.3.2.1 To determine the rate of baseline (prior to therapy) tumor molecular evolution from primary tumor to metastatic lesion (intra-patient heterogeneity through space) by comparing genomic changes (mutation, amplification, and translocation) using i) NGS (and FISH), ii) proteomic changes by GEC-plex mass spectrometry and IHC, and iii) kinase activity with PamGene iv) ctDNA sequencing results

1.3.2.2 To determine the rate of tumor molecular category migration at baseline within the Biomarker and Treatment Algorithm (6 category Classification, see Figure 6) comparing primary tumor to metastatic disease.

1.3.2.3 To determine tumor molecular evolution over time (intra-patient heterogeneity through time/treatment) from baseline to first progression (PD_1) and subsequent progressions (PD_2, PD_3) for those receiving second/third line therapy, assessing for genomic/proteomic evolution.

1.3.2.4 To determine the rate of new/loss of molecular aberrations at each progression point ($PD_{1,2,3}$)

1.3.2.5 To determine the rate of molecular category migration within the Biomarker and Treatment Algorithm (see Figure 6) at each progression point ($PD_{1,2,3}$).

2. BACKGROUND

2.1 Gastroesophageal cancer (GEC): Adenocarcinoma of the stomach and esophagogastric junction (EGJ)

2.1.1 Epidemiology of gastroesophageal adenocarcinoma (GEC)

Gastroesophageal cancer (GEC) represents a challenging global health problem. GEC is the fourth most common malignancy behind lung, breast, and colorectal cancers, with approximately 1 million cases per year occurring around the world. GEC is the second leading cause of cancer death globally and it is estimated that in excess of 700,000 patients will die from the disease annually.[1-4] There are significant geographic variations in the incidence of GEC; it is more common in East Asia, Eastern Europe, and parts of Central and South America than it is in the United States or Western Europe. Almost 70% of cases arise in developing countries with approximately 40% of cases occurring in China alone. There are clear epidemiologic differences between cancer localized to the proximal stomach (gastric cardia type III esophagogastric juntion (EGJ)) along with other EGJ (Type I,II) in contrast to those that are localized to the rest of the stomach (distal gastric cancer (GC)).[5] Cancer of the cardia accounts for 39% of GEC cases in Caucasian men in the United States but only in 4% of GEC in men in Japan. For reasons that are not clear, cancer of the gastric cardia and lower esophagus (EGJ) has increased rapidly in developed countries since the 1970s.[5]

2.1.2 Histologic Classification and Etiology of GEC

The histology of GEC falls into two broad subtypes based on microscopic features observed in gastric tumors, namely intestinal or diffuse, according to Lauren's classification.[6] Intestinal-type tumors tend to arise in the antrum or antral-corpus junction. Intestinal-type cancers are classically characterized by glandular differentiation on a background of gastric atrophy or intestinal metaplasia, whereas diffuse cancers typically appear as rows of single mononuclear "signet ring" cells with little cell adhesion.[5,7] These apparently distinct features, however, are not always discernible in clinical samples, where inter-observer variation and unclassifiable or "mixed" subtypes are not uncommonly reported. Intestinal-type tumors are significantly more common than the diffuse type and tend to be associated with intestinal metaplasia and chronic inflammation (e.g. atrophic gastritis), often as a result of chronic *Helicobacter pylori* infection. By contrast, diffuse tumors do not generally develop on a background of intestinal metaplasia and inflammation is characteristically absent.[7]

2.1.3 Prognosis

Survival rates from GEC have improved over the last few decades. Five-year overall survival (OS) in the Western world is estimated at ~20%.[8] In the West, fewer patients are referred for surgery compared with Asia, but those who undergo resection have a higher survival rate, which reaches 50%, possibly due to more accurate preoperative staging and improved imaging techniques. In large-scale screening programs in Asia, detection at earlier stages and more aggressive surgical approaches, including more frequent D2 lymph node resection, contribute to higher OS rates of ~60%. The median OS among patients with late-stage GEC is approximately 14 months in patients with locally advanced disease and 9-12 months in patients with metastatic disease.[9,10]

2.1.4 First Line Systemic Treatment for Advanced/Metastatic GEC

Active chemotherapeutics:

- **fluoropyrimidine, platinum, topoisomerase-1 inhibitor, taxane**

For patients with unresectable, metastatic disease, the main therapeutic option is chemotherapy. First line chemotherapy has been shown to increase survival and quality of life in patients with advanced GEC in several randomized trials and meta-analyses.[11,12] It is evident that combination therapy outperforms single-agent (mainly 5-fluorouracil [5-FU]) therapy (hazard ratio [HR] = 0.83; 95% CI: 0.74, 0.93).[13] Median survival for patients with metastatic disease treated with chemotherapy ranges, depending on the sited reference and the time period of the clinical trial, from 8–13.9 months. A recent clinical trial with mFOLFOX6 chemotherapy reported a median OS of 13.9 months in a placebo arm (N=64) of a 124 patient trial.[10] Increased estimates of mOS in recent trials compared to trials reported more than 5 years ago are likely due to improved best supportive care (BSC) as well as increasing numbers of patients proceeding to second and third line therapies (see Second and Third line Therapy, below). **However, given that these recent numbers are from smaller phase II trials, our most robust estimates for median OS is approximately 9-12 months based on large phase III trials, which is why we chose 12 months for our historical control arm.**

Despite intensive evaluation of multiple chemotherapy regimens, no international consensus exists regarding the optimal first-line regimen in advanced GEC. In Western countries and in Asia, the reference chemotherapy regimen for the first-line treatment of metastatic GEC is a fluoropyrimidine (5-FU or capecitabine) in combination with a platinum agent (either cisplatin or oxaliplatin) with or without a third cytotoxic drug (usually epirubicin or docetaxel).[8] Based on several Phase III studies and meta-analyses, oxaliplatin and capecitabine have both been shown to be non-inferior to cisplatin and 5-FU, respectively [8,14-16] National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology (ESMO) guidelines recommend that a fluoropyrimidine (either 5-FU or capecitabine) combined with either cisplatin or oxaliplatin are appropriate standards of care in the first-line setting. The use of irinotecan (topoisomerase-1 inhibitor) is usually reserved for second/third line, however, one first line trial evaluating S1/irinotecan showed a non-significantly improved mOS from 10.5 to 12.8 (p=0.23), but did have a significantly improved ORR_1 from 26.9% to 41.5%, compared to S1 oral chemotherapy alone.[17] Recently another trial with FOLFIRI showed no difference with ECX therapy with mOS: 9.7 vs 9.5, respectively, and therefore is an option for first line therapy.[18]

The use of three-drug “triplet” chemotherapy remains controversial, and usually is reserved for patients with very good performance status (PS – ECOG 0). The addition of a taxane or an anthracycline to a platinum-based doublet may be associated with an incremental improvement in survival of ~1 month for patients with metastatic GEC;[13] however, this marginal survival benefit is counter-balanced by significant treatment-associated toxicity[8]. Triplet regimens such as docetaxel, cisplatin, and 5-FU (DCF); epirubicin, cisplatin, and 5-FU (ECF); or epirubicin, oxaliplatin, and capecitabine (EOX) are considered appropriate for highly functioning patients with minimal comorbidities,[19] but their systematic use has not been widely recommended.[8] Additionally, clinical trials are evaluating mFOLFIRINOX (5FU, irinotecan, and oxaliplatin) in the first line setting at the University of Chicago(NCT01643499).

However, given the ease of use of mFOLFOX6 and the familiarity of this regimen in more common malignancies (ie colorectal cancer), mFOLFOX6 has become a standard first line treatment option for GEC both on or off clinical trials.[10] Moreover, this reserves other drug classes (including taxanes and topoisomerase-1 inhibitors) for second/third line treatments in a tandem strategy. Furthermore, it is easier from a toxicity standpoint to add an investigational targeted agent to a doublet cytotoxic regimen versus a triplet cytotoxic regimen. Dropping the 5FU/LV bolus can limit toxicity without detriment in efficacy, and treating physicians may opt to use or not depending on the clinical circumstance.

2.1.5 Second and Third Line Systemic Treatment for Advanced/Metastatic GEC

<u>Second Line:</u>

For patients who progress on front-line therapy, second-line therapy with irinotecan (or an irinotecan-containing regimen) or a taxane is recommended (NCCN category 2B).

Single agent irinotecan in the second line setting has been investigated in several small trials. One revealed a mOS of 4 months and mPFS of 2.6 months. The activity of irinotecan when used in combination with 5-fluorouracil and leucovorin (FOLFIRI) demonstrated a range of ORR_2 of 3-29%, median PFS_2 of 2.3-4 months and median OS_2 of 5.1-7.6 months and DCR_2 of 29-63%.[20-24]

Single agent taxane in the second line setting was evaluated in 49 patients and resulted in mOS 8.3 months, mPFS 2.5 months, and ORR 16.3%.[25] More recently, the relatively large (N=168 84:84) phase III Cougar-02 trial comparing docetaxel to best supportive care (BSC) resulted in improved mOS from 3.6 to 5.2 (p=0.01, HR 0.67), and RR_2 7% and disease stabilization (SD) rate of 46% for a total DCR_2 rate of 53%.[26]

Comparing irinotecan to docetaxel in the second line setting, a three-arm trial including a BSC arm revealed no difference between the two chemotherapy arms (6.5 versus 5.2 months, respectively), but both cytotoxics significantly improved over BSC of 3.8 months.[27]

Two studies evaluated the combination of docetaxel and irinotecan in the second line setting of GEC, one revealing 3 patients with complete response (CR) and 7 patients with partial response (PR) resulting in an ORR_2 of 20.4%.[28] Another, showed a mOS_2 of 4 months, and $mPFS_2$ of 2.8 months.[29] A meta-analysis of 34 second line therapy trials (monotherapy (8), combined (17), and targeted (6)) revealed a wide range of outcomes: mOS_2 4-11.4 months; $mPFS_2$, RR 0-63%.[30]

Third Line:

Few third line clinical trials have been performed, but a recent large 158 patient trial using FOLFIRI (after platinum, 5FU, taxane) in the third line revealed an ORR_3 9.6%, $mPFS_3$ 2.1 months and mOS 5.6 months and DCR_3 39.7%.[31]

Finally, a clinical trial evaluating the sequence of second/third line use of FOLFIRI vs docetaxel/5FU (DF=FOLTAX) after failure of first line mFOLFOX6, revealed no difference in mOS between the two sequences; mOS was 16 months.[32] Again, this is a smaller trial and done in Asia, where mOS is better than in Western countries; the 12 month mOS is more representative of our intended patient population.

Given the above, it is common practice for patients with preserved ECOG PS (<2) to continue with palliative second and third line therapy (irinotecan, docetaxel in either order) without or with the addition of continued 5-FU with the intention to achieve synergistic activity (FOLFIRI, FOLTAX = DF).

No immunotherapy checkpoint inhibitors have been approved to date for patients with GEC in first line of therapy, however promising results have been reported in PDL1+ patients by IHC and now approved for third line or higher monotherapy in PDL1+ patients, and most importantly MSI-H patients as monotherapy in second or higher, which accounts for <5% of stage IV GEC. However, when randomized to getting control chemotherapy versus monotherapy checkpoint inhitibitor, these studies have been negative in both the second and third line setting, making it quite reasonable to continue with PANGEA treatments FOLFOX→FOLFIRI→FOLTAX plus appropriate biologic currently. However, a recent study (Keynote 061) did demonstrate that patients with PDL1+ CPS (combined positivity score) ≥10% had survival benefit compared to chemotherapy in second line or higher (Fuchs et al. J Clin Oncol 36, 2018 (suppl; abstr 4062)).

2.1.6 Chemotherapy Regimens for GEC

In advanced GEC, 5-fluoruracil has been the cornerstone of chemotherapy regimens and as a single agent results in response rates (RR_1) of 15-30% alone.[33,34] Numerous studies have shown that when 5-fluorouracil is combined with other cytotoxics, there is a significant improvement in response rate and time to progression at the expense of mildly increased and acceptable toxicity.[34] Historically, the most commonly used agents in advanced gastric cancer are a combination of cisplatin and 5-fluorouracil. While epirubicin is often added to this combination in Europe, there is no convincing evidence of its additional value in terms of improved survival or quality of life.[35] The substitution of oxaliplatin for cisplatin has been shown to be non-inferior in terms of overall survival, with significantly less grade 3 and 4 neutropenia, nephrotoxicity, emetogenesis, and alopecia.[14,15] Studies using combined 5-fluorouracil and oxaliplatin have demonstrated similar outcomes when compared to other reference regimens in advanced GEC.[14,36,37] Notably, the addition of docetaxel to cisplatin and 5-flurouracil (DCF) has demonstrated a marginally improved overall survival in advanced gastric cancer patients (9.2 vs 8.6 months).[38] However, there is significantly more grade 3 and 4 neutropenia, complicated neutropenia, diarrhea and neurosensory toxicity, making this combination regimen suitable for only a carefully selected patient population treated by physicians familiar with its administration. Combination of 5FU with irinotecan (FOLFIRI) or docetaxel (DF=FOLTAX) are well established regimens, most often used for GEC in the second/third line settings, in any sequence.[32]

2.1.6.1 **5-Fluorouracil**

5-fluorouracil (5-FU) acts as false pyrimidine or antimetabolite to ultimately inhibit the formation of the DNA-specific nucleoside base thymidine. The agent appears to successfully compete for the enzyme thymidylate synthase by displacing natural substrate uracil deoxyribonucleotide. 5-fluorouracil, thus, is similarly handled as the normal uracil substrate for activity when it is first converted enzymatically through several steps to the ribonucleoside and ribonucleotide. This ultimate metabolite can then bind selectively with the target enzyme and inhibit the formation of thymidine, an essential substrate for DNA synthesis. RNA synthesis is also affected to a lesser degree by inhibiting the utilization of preformed uracil. 5-FU is cell cycle phase specific agent with cytotoxic effects seen mainly in S-phase. The two main routes of fluorouracil metabolism in man are intracellular activation to the active nucleoside and enzymatic destruction in the liver due to dihydro 5-FU dehydrogenase with ultimate conversion to inactive metabolites: CO2, urea, fluoro-*B*-alanine, and ammonia. The degradative enzymes are diffusely distributed and are found in the gastro-intestinal epithelium but apparently not in some colonic carcinoma. After a rapid intravenous injection of 15 mg/kg, peak plasma levels of 10^{-3} – 10^{-4} are achieved. Primary half-life is 8-14 minutes. Plasma levels then decrease rapidly and are undetectable after 1 hour. The distribution volume is 25-33% at body weight. Up to 80% of a dose is detoxified via metabolic degradation in the liver, with significant renal excretion occurring after the first few hours. Ultimately, from 60 to 80 of radio-labeled carbon from a given dose is excreted through the lungs as carbon dioxide, however, less than 10% of unchanged drug is excreted by the kidneys with 90% during the first hour. Depressed renal function then does not require dosage adjustment for 5-FU. Clarkson *et al* found that when given as a continuous infusion (40-60 mg/kg/24 hour)[39], stable plasma levels are obtained and less intact drug appears in the urine. This could indicate either more complete degradation to inactive metabolites or enhanced conversion to the active nucleoside. Fluorouracil distributes to all areas of body water apparently by simple diffusion. Thus significant quantities of drug may enter the CNS and after 15 mg/kg IV; cerebrospinal fluid levels of 6-8 x 10-6 M are obtained after 30 minutes. These levels persist for several hours and slowly subside. Although distribution to brain tissue is less rapid, abnormal areas such as those with neoplasms may take up the drug more readily. Intracarotid and intrathecal administration have led to augmented formation of neurotoxic metabolites fluoroacetate and fluorocitrate, and are therefore not recommended. Fluorouracil achieves high and persistent levels in effusions after intravenous administration.

The spectrum of toxicity associated with 5-FU varies according to dose, schedule, and route of administration. On a particular schedule, considerable variation in the incidence and severity of these toxicities among patients is observed. The IV bolus schedule may result in severe gastrointestinal toxicity and may be life threatening. These toxicities include mainly mucositis and diarrhea. In some cases, disruption of the integrity of the gut lining may permit access of enteric organisms into the bloodstream and potential overwhelming sepsis, particularly if the nadir coincides with diarrhea. In addition to dose limiting mucositis and diarrhea, IV bolus daily for 5 days can also result in granulocytopenia. With continuous infusion 5-FU, mucositis is usually dose limiting although diarrhea and dermatitis occur. Myelosuppression is generally mild to moderate in severity. High doses of 5-FU over a 24-hour period may have dose limiting gastrointestinal toxicity and neurotoxicity. A prolonged continuous infusion of low dose 5-FU can potentially have dose limiting mucositis and palmo-plantar erythrodysesthsia while diarrhea is less common. 5-FU is the most extensively studied single agent in gastric carcinoma. An overall response rate of 21% has been reported. Infusional 5-FU is of interest given the drug's short plasma half-life and because a much higher dose density can be achieved compared with bolus injections. 5-FU has an established efficacy in the treatment of advanced colorectal cancer for the last 40 years. A wide variety of regimens of 5-FU with or without folinic acid (FA) are in current use.

The use of FA (folinic acid – leucovorin (LV)) bolus (400mg/m2 over 2 hours) followed by 5-FU with both bolus (400mg/m2 over) and continuous infusion (2.4-3g/m2 over 46 hours) has become a common regimen, either alone or in combination with other cytotoxics oxaliplatin, irinotecan and docetaxel (mFOLFOX6, FOLFIRI, or FOLTAX).[40]

2.1.6.2 **Oxaliplatin**

Oxaliplatin (Eloxatin) (trans-l-1,2-diaminocyclohexane oxalatoplatinum) is an antineoplastic platinum derivative with a 1,2-diaminocyclohexane [DACH] carrier ligand. Although the precise mechanism of action is unknown, platinum compounds are thought to exert their cytotoxic effects through the formation of DNA adducts that block both DNA replication and transcription. Like cisplatin, oxaliplatin reacts with DNA, forming mainly platinated intra-strand links with two adjacent guanines or a guanine adjacent to an adenine.[41-43] However, DACH-platinum adducts formed by oxaliplatin are apparently more effective at inhibiting DNA synthesis and are more cytotoxic than cis-diamine-platinum adducts formed from cisplatin and carboplatin.[43-45]

The safety profile of oxaliplatin was evaluated in a phase I intra-patient escalation study of 44 patients with advanced cancer, who received 116 courses of oxaliplatin through seven levels, from 45 mg/m2 to 200 mg/m2 every four weeks. Oxaliplatin was administered without pre-hydration or post-hydration. Initially, all patients experienced nausea and vomiting. As a result, systematic pretreatment with antiemetics was given to all patients receiving >90 mg/m2 oxaliplatin, which reduced Grade 3 or 4 nausea and vomiting to 11%. Diarrhea was mild (primarily Grade 1 or 2 in 24% of therapy courses). Hematologic toxicity was moderate. Grade 1 or 2 thrombocytopenia was dose related and occurred in 13% of patients receiving from 135 to 150 mg/m2, and 28.5% of patients receiving 175 to 200 mg/m2. Similarly, only Grade 1 or 2 neutropenia was observed, and hemoglobin levels remained unchanged. The dose-limiting side-effect of oxaliplatin therapy was a transient peripheral neuropathy. This toxicity usually appeared at doses >90 mg/m2 and affected up to 75% of patients treated with 200 mg/m2. The recommended phase II dose was 135 mg/m2 administered over at least 1 hour every four weeks.

A review of several studies to evaluate overall safety in 682 patients who had received oxaliplatin either as a single agent or in combination with 5-FU was done to delineate the character and severity of oxaliplatin-induced neurotoxicity.[46] Grade 3 neurotoxicty presenting as fine movement disturbance or moderate sensitive ataxia was observed in12% of patients at a median cumulative dose of 900 mg/m2 oxaliplatin. Total cumulative doses of 780, 1170, and 1560 mg/m2 were correlated with an incidence of 10%, 50%, and 75% neurotoxicity, respectively. The total cumulative dose of oxaliplatin was the most significant prognostic factor for the occurrence of neurotoxicity and inversely related to the likelihood of recovery from toxicity. Symptoms resulting from Grade 1 and 2 neuropathy regressed in 82% of patients within 4 to 6 months, and disappeared entirely in 41% of patients within 6 to 8 months. In summary, all studies reported to date support that the neurotoxicty resulting from oxaliplatin treatment was specific, cumulative, and, unlike cisplatin-induced neuropathy, reversible in most patients.

2.1.6.2.1 **Combination 5FU/Oxaliplatin: mFOLFOX6**

5-FU and oxaliplatin combinations have proven activity in many tumor types including GEC, colon, breast, and lung.[47,48] The tolerability and safety of this combination is well established, and has been administered in various forms including FOLFOX4, FOLFOX6 and FOLFOX7.[40] Modified FOLFOX (mFOLFOX6) treatment, which entails bolus 5FU/LV and continuous infusion 5FU over 48 hours, along with 85mg/m2 of oxaliplatin every other week, has become a standard administration schedule of the two chemotherapies.[10,40] Neutropenia, diarrhea and peripheral sensory neuropathy are the most significant toxicities encountered.[48]

2.1.6.3 **Irinotecan**

Irinotecan (CPT-11, Camptothecan) prevents DNA from unwinding by inhibition of topoisomerase I. It is a semisynthetic analogue of the natural alkaloid camptothecin. Initially showing benefit in metastatic colorectal cancer, in particular, in combination with other chemotherapy agents, it has become a common drug used in various malignancies, including GEC, pancreatic cancer and other gastrointestinal malignancies. Irinotecan is activated by hydrolysis to SN-38, an inhibitor of topoisomerase I. This is then inactivated by glucuronidation by uridine diphosphate glucoronosyltransferase 1A1 (UGT1A1). The inhibition of topoisomerase I by the active metabolite SN-38 eventually leads to inhibition of both DNA replication and transcription.[49]
The most significant adverse effects of irinotecan are severe diarrhea and bone marrow suppression, particularly neutropenia. Patients homozygous for the *28 allele of UGT1A1 have been reported to be more susceptible to irinotecan toxicities, including neutropenia.[50,51]

2.1.6.3.1 **Combination 5FU/Irinotecan: FOLFIRI**

Administration of FOLFIRI consists of the same bolus/infusional 5-FU/LV backbone schedule as within mFOLFOX6, with the replacement of oxaliplatin with180 mg/m2 of irinotecan. Randomized trials in colorectal cancer showed improvements in clinical efficacy based on overall response rates, time to tumor progression, and median overall survival when irinotecan has been added to either infusional (FOLFIRI)[52] or bolus (IFL).[53] There is an abundance of preclinical and clinical evidence that there is synergistic effect of 5FU in combination with irinotecan, despite developed resistance to 5FU monotherapy or combination with prior platinum in various tumor types, including GEC.[54-56]

2.1.6.4 **Docetaxel**

Docetaxel (Taxotere) is a clinically well-established anti-mitotic chemotherapy medication that interferes with cell division. It is used in various malignancies, including GEC. Docetaxel may be administered IV weekly (25mg/m2, every other week (50mg/mg2) or every three weeks (75mg/m2) with relatively equal efficacy in the palliative setting.[57]

Docetaxel is of the chemotherapy drug class of taxanes, and is a semi-synthetic analogue of paclitaxel (Taxol), an extract from the bark of the rare Pacific yew tree Taxus brevifolia.[58] Due to scarcity of paclitaxel, extensive research was carried out leading to the formulation of docetaxel – an esterified product of 10-deacetyl baccatin III, which is extracted from the renewable and readily available European yew tree. Docetaxel differs from paclitaxel at two positions in its chemical structure. It has a hydroxyl functional group on carbon 10, whereas paclitaxel has an acetate ester, and a tert-butyl carbamate ester exists on the phenylpropionate side chain instead of the benzyl amide in paclitaxel. The carbon 10 functional group change causes docetaxel to be more water soluble than paclitaxel.[58]

A model based on electron crystallographic density and nuclear magnetic resonance deconvolution has been proposed to explain the binding of docetaxel to β-tubulin.[59] In this T-shaped/butterfly model, a deep hydrophobic cleft exists near the surface of the β-tubulin where three potential hydrogen bonds and multiple hydrophobic contacts bind to docetaxel. The hydrophobic pocket walls contain helices H1, H6, H7 and a loop between H6 and H7 that form hydrophobic interactions with the 3'-benzamido phenyl, 3'-phenyl, and the 2-benzoyl phenyl of docetaxel. 3'-phenyl also has contact with β-sheets B8 and B10. The C-8 methyl of docetaxel has Van der Waals interactions with two residues, Thr-276 and Gln-281 near the C-terminal end of β-tubulin. Docetaxel's O-21 experiences electrostatic attraction to Thr-276 and the C-12 methyl has proximity with Leu-371 on the loop between B9 and B10.

Docetaxel exhibits cytotoxic activity on GEC, and various other malignancies.[60] Docetaxel does not block disassembly of interphase microtubules and so does not prevent entry into the mitotic cycle, but does block mitosis by inhibiting mitotic spindle assembly.[61] Resistance to other chemotherapies including paclitaxel, the topoisomerase inhibitors doxorubicin or irinotecan, or platinum drugs, does not necessarily indicate resistance to docetaxel.[32,60] Microtubules formed in the presence of docetaxel are of a larger size than those formed in the presence of paclitaxel, which may result in improved cytotoxic efficacy.[62] Abundant formation of microtubules and the prevention to replicate caused by the presence of docetaxel leads to apoptosis of tumor cells and is the basis of docetaxel use as a cancer treatment. Common adverse effects include alopecia, peripheral neuropathy and cytopenias.

2.1.6.4.1 **Combination 5FU/Docetaxel: FOLTAX**

The combination of 5FU/LV and docetaxel has evidence of significant synergistic effects, and lack of cross resistance to prior chemotherapies, including monotherapy or combination with 5FU and platinum, irinotecan, and anthracyclines in GEC.[62-65]

The regimen combining docetaxel and 5-FU/LV (DF or FOLTAX) is administered similar to the 5FU/LV bolus/continuous infusion backbones of mFOLFOX6 and FOLFIRI, with the replacement of oxaliplatin/irinotecan with docetaxel.[66] A phase II comparison of docetaxel combined with continuous-infusion FU (DF) with ECF, using a 21-day infusion schedule of FU, in 90 patients with metastatic gastric cancer was conducted.[65] Response rates (36% to 38%), time to progression (5.3 to 5.5 months), and overall survival (9.5 to 9.7 months) were comparable for DF and ECF. The only GI toxicity that exceeded a rate of 10% was stomatitis (13%) for the DF regimen (compared with a rate of 2% with ECF). Although grade 3 or 4 neutropenia was substantial for DF (42%), neutropenic fever was uncommon (4%). The results for DF compare favorably with DC or DCF and suggest that DF is a more tolerable alternative to DC or DCF, with lower rates of neutropenia and neutropenic fever.[65] Moreover, consensus is that the use of weekly or biweekly infusion of FU is preferable given the superior toxicity profile.[14,56,66]

The use of 5FU/LV bolus/infusion along with oxaliplatin and docetaxel (FLOT) administered IV biweekly has a favorable safety profile. FOLTAX is a modified FLOT regimen without the oxaliplatin, and a slightly different 5FU/LV administration which is identical to 5FU/LV administration within the mFOLFOX6 and FOLFIRI regimens.[67]

2.1.7 **HER2 positive GEC**

2.1.7.1 **HER2 Positivity in Advanced Gastric/EGJ Cancer**

Human epidermal growth factor receptor 2 (HER2) (*ERBB2*) positivity, defined as overexpression of the cell surface receptor or amplification of its encoding gene, has been reported in a number of solid tumors and has been shown to confer adverse clinical prognosis particularly in breast cancer.[68-70]

In a literature survey of studies of HER2 overexpression in GEC tumors, 16 studies reported HER2 positivity assessed by immunohistochemistry (IHC).[71] In these studies, 3264 tumor specimens were tested, with a mean overall HER2 positivity rate of 17.6% (range, 6.8%–34.0%), with a mean rate of 14.0% in studies from Asian institutions and a mean rate of 19.8% in studies from non-Asian institutions. In another nine studies, HER2 positivity was assessed by in situ hybridization (ISH) methods. In 1232 tumor specimens, the mean overall HER2 positivity rate was 19.2% (range, 7.1%–42.6%), including mean rates of 14.9% in studies from Asian institutions and 18.6% in studies from non-Asian institutions.[71] The high variability in the HER2-positivity rates can be partly explained by the fact that initial HER2 data were generated using the breast cancer HER2 testing or scoring principles (or both), or were performed with non-validated tests.

As part of eligibility screening for a large randomized trial (Study BO18255, ToGA) that tested the safety and efficacy of adding anti-HER2 therapy to standard fluoropyrimidine/cisplatin chemotherapy for the treatment of advanced GEC, 3667 evaluable tumor samples (representing the largest cohort assessed in a central laboratory with the same assays and a standardized read-out) were tested by IHC and ISH methods, with HER2 positivity requiring either IHC 3+ (any FISH score) or ISH+ (any IHC score 0-3+) results. The overall rate of HER2 positivity in the screened population for the ToGA trial was 22.1% using both criteria.[72] Using current clinical standards, only ~15% of those patients on the ToGA trial would be considered HER2 positive (ICH2+ and FISH+, IC3+ irrespective of FISH).

2.1.7.2 **Trastuzumab in the Treatment of Advanced GEC**

The antitumor effect of trastuzumab (Herceptin®) against HER2-positive human GC cell lines of Western or Asian origin was demonstrated in nonclinical studies.[73,74] Synergistic antitumor effects of trastuzumab plus chemotherapy agents such as platinum-based drugs and fluoropyrimidines were also reported in nonclinical models of HER2-positive human GC lines.[74-76]

Based on nonclinical results and the proven clinical benefit of adding trastuzumab therapy to combination chemotherapy in the treatment of HER2-positive breast cancer in both the metastatic disease and adjuvant treatment settings, a randomized Phase III study was undertaken to investigate the efficacy and safety of adding trastuzumab to combination chemotherapy with a fluoropyrimidine (capecitabine or 5-FU) plus cisplatin (FP) for the treatment of HER2-positive AGC (Study BO18255, ToGA).[72] The study enrolled 594 patients in 24 countries. Patients were randomized in a 1:1 ratio to receive either the FP regimen alone or in combination with trastuzumab (TFP). The primary endpoint was OS. Treatment was six 3-weekly cycles of FP chemotherapy for all patients, with capecitabine or 5-FU chosen by the investigator for each patient. Patients randomized to the TFP regimen received trastuzumab every 3 weeks (Q3W) concurrently with chemotherapy; trastuzumab was then continued as a single agent until disease progression was documented by the investigator.

Results showed that treatment with TFP was superior to FP alone, with a median OS of 13.8 months for patients in the TFP arm compared with 11.1 months for those in the FP arm in the intent-to-treat (ITT) population (hazard ratio [HR] = 0.74; 95% confidence interval [CI]: 0.60, 0.91). In a post-hoc analysis among patients whose tumors highly overexpressed HER2 (IHC 3+ (the majority of which were ISH+) and IHC 2+/ISH+ -- the clinical criteria now used currently to determine positivity), a reduction of 35% in the risk of death (HR= 0.65; 95% CI: 0.51, 0.83) was observed with a median OS of 16.0 months in the TFP arm compared with 11.8 months in the FP arm. The most common adverse events (AEs) (nausea, vomiting, neutropenia), Grade 3–4 AEs, and cardiac AEs occurred at similar or identical frequencies in both treatment groups.[72]

The results of Study BO18255 (ToGA) have led to worldwide regulatory approvals of the cisplatin/5FU/trastuzumab regimen, and this regimen is a standard treatment for metastatic HER2-positive adenocarcinoma of the stomach and EGJ in the first line setting. (NCCN Clinical Practice Guidelines in Oncology, Gastric Cancer 2012). Given the known inter-changeability of cisplatin and oxaliplatin in various regimens, FOLFOX-trastuzumab combination is considered to be within treatment standards.[14,15]

2.2 Molecular Heterogeneity of GEC: Inter-Patient

Regrettably, there are a number of recent examples of failed clinical trials in GEC that assessed the addition of novel biologic agents to standard chemotherapy treatment regimens.[2] Failure to improve patient outcomes was likely due to a 'one-size-fits-all' treatment approach, and not taking into account inter-patient tumor molecular heterogeneity (inter-PH) at diagnosis - in other words, using 'targeted therapies for un-targeted patient populations'. Notable examples include (but not limited to) the REAL-3 trial (panitumumab), for previously untreated advanced GEC using chemotherapy with/without an anti-EGFR antibody.[77] Others include the negative AVAGAST (bevacizumab)[78] and EXPAND (cetuximab)[79] trials in the metastatic setting. Another example is the large GRANITE-1 study using everolimus (mTOR inhibitor) versus BSC in the second-line setting.[80] On the other hand, better outcomes have ensued when clinical trials attempted to select patients based on prospective molecular profiling of the tumors, based on sound preclinical evidence of predictive markers enriching for those patients most likely to benefit from the targeted agent.[81] This provides rationale to 'match' a targeted biologic agent with a molecular target that has undergone genomic activation ('oncogenic driver') via either an activating mutation, amplification, or translocation, or somewhat less so with proteomic aberration, namely protein overexpression.

The recent ToGA trial, as above in Section 2.1.7.2, evaluated the addition of anti-HER2 therapy to standard chemotherapy, for stage IV GEC, which improved mOS, albeit modestly from 11.1 months to 13.8 months.[72] In the subgroup of extremely high HER2 expression/gene amplification (defined as immunohistochemistry (IHC) 3+/ fluorescence in situ hybridization (FISH) positive) representing 44% of patients on trial, the mOS improvement was more pronounced, increasing from 12.3 to 17.9 months. This supports the notion that profiling tumors to identify a true oncogenic driver, and coupling this with specific therapeutic inhibition, will lead to better outcomes for patients.[82]

Another encouraging example of improved outcomes based on tumor inter-PH profiling and patient selection for treatment is with anti-MET kinase treatment. A recent phase II trial using anti-HGF antibody treatment (the growth factor for MET) improved mOS of stage IV GEC patients from 8.9 to 11.1 months for all (unselected) patients.[83] However, in a retrospective analysis using an arbitrary cut-off of $\geq$50% MET expression ($\geq$ 1+) by IHC in tumor cells as a predictive biomarker (~42% of patients on trial), it was observed that mOS was improved from 5.7 to 11.1 months in this sub-population. This confirmed MET high expression to be a poor prognostic marker and positive predictive marker to MET signaling pathway inhibition, as previously described. Importantly, those patients with low MET expression (~58% of patients) did slightly worse than in the placebo arm, strengthening the argument and importance of patient selection for treatment. See section 2.5.1.2 for updated phase III results.

In addition to HER2[84] and MET[85,86], we and others have identified a number of other potential molecular oncogenic drivers contributing to inter-PH at relatively low frequencies within this disease[87] with therapeutic potential, including RON[88], FGFR2[89-91], EGFR[92-94], HER3[95], IGF1R[96,97], KRAS[98,99], BRAF[100], PIK3CA[101,102] and CCND1/CCNDE in human GEC samples and cell lines.[103] A clearer understanding of the degree of inter-PH and the patterns of genomic profiles within GEC is desperately needed (**Figure 4,5**). An approach that acknowledges tumor heterogeneity between patients, in terms of best known oncogenic driver categories within GEC,[7,87] is discussed in more detail below in Sections 2.4 and 2.5.

2.3 Molecular Heterogeneity of GEC: Intra-Patient

In addition to molecular heterogeneity between individuals (inter-PH) with GEC, there is an increasing recognition of molecular heterogeneity within any given individual (intra-PH) with GEC and other tumors (**Figure 4,5**). This can be observed at any given time point (snap shot) of multiple locations of disease within the patient through space. Examples include: i) within the primary tumor, ii) from primary tumor to lymph node, iii) from primary tumor to metastatic lesion(s) and/or iv) from one metastatic lesion to another. In addition to intra-PH through space, it is invariable that intra-PH occurs over time (particularly with treatment) as the tumor evolves and treatment selects for resistant clones. The nature of intra-PH, including through space *and* time, in GEC is not well characterized.[5,104,105]

2.3.1 **Molecular Heterogeneity Through Space**

Intra-PH *through space*, both within the primary site, and from primary site to metastatic disease locations has been described in several tumor types (**Figure 4,5,6**). Discordance of a tumor molecular profile, due to intra-PH (from primary to metastatic lesion), was estimated to occur in colon cancer in up to 10-15% of cases.[106,107] In our preliminary data, we have observed, in general, tumor evolution such that there is acquisition of additional genomic abberations (eg. MET, HER2, KRAS amplification in metastatic lesions not present in the primary tumor). Clearly, trials evaluating biologic agents that specify enrollment criteria based on primary tumor molecular profiles would therefore be susceptible to mis-classification, which may lead to confusing results, biasing towards the null if true positive patients are classified as negative for a given biomarker, explained further in Section 2.4.3 (**Figure 5**).

2.3.2 **Molecular Heterogeneity Through Time**

Ultimately, however, even considering examples of highly selected patients for therapy based on inter-PH (eg. HER2, MET), tumor resistance and progression occurs despite the ‘personalized’ therapeutic approach, a phenomenon that is eventually invariable (**Figure 4,5B X-axis**). This highlights the increasingly recognized phenomenon of intra-patient tumor heterogeneity (intra-PH) and tumor selection/evolution *through time* (ie. after treatment), which results in resistant clones.[104,105,108]

2.4 Rationale of Mandatory Biopsies and Integral Biomarkers

The promise of 'personalized' cancer care with therapies targeted toward specific molecular aberrations has great potential to improve clinical outcomes. However, there is emerging understanding of profound molecular heterogeneity within GEC (inter-PH), and within an individual (intra-PH) through space (primary tumor to metastasis) and time (resistance to treatment). This heterogeneity is a hurdle to advancing GEC treatment.[1,2] Current clinical trial design paradigms are challenged by heterogeneity, as they are unable to test targeted therapeutics against low frequency genomic aberrations with adequate power.[81,109] Accrual difficulties to GEC trials are exacerbated by low frequencies of molecular 'oncogenic drivers'. Oncogenic drivers of GEC including MET amplification (not mere over-expression), FGFR2, and others, have even less frequent genomic activation (<10%) than HER2 (15-20%). The ToGA trial screened ~4000 patients in order to accrue adequate numbers for the primary endpoint. For biomarkers with lower incidence (<10%) this would require impossibly high screening numbers to attain adequate statistical power. Moreover, as we learn of more and more predictive biomarkers, there is limited tissue to test/screen for each of these using "à la carte" assays. To address this recognized challenge, there is need for novel clinical trial designs/strategies implementing medium throughput technologies in order to account for inter-patient molecular diversity and tissue economy. Importantly, there is also need for predefined treatment algorithms given multiple aberrations observed within any one individual.[1] Finally, access to multiple therapeutic agents are required to be available for treatment. Intra-PH may be addressed by post-treatment biopsy and repeating the 'biomarker and treatment assignment algorithm'. This innovative clinical trial design, PANGEA (Personalized Anti-Neoplastics for Gastro-Esophageal Adenocarcinoma), integrates novel medium throughput proteomic and genomic technologies with a practical 'biomarker assay and treatment algorithm'.[2] Attempts to understand tumor evolution through time via less-invasive approaches will also be important, potentially by evaluating circulating tumors cells and/or circulating DNA (**Figure 4**).

2.4.1 Rationale of Biopsies and Personalized Approach at Baseline

Given the recognition of profound tumor molecular heterogeneity from one patient to the next, tissue screening within clinical trials use diagnostic biopsies for molecular profiling, in addition to establishing a diagnosis by standard pathological methods. The baseline diagnostic biopsy is usually performed on the primary tumor via upper endoscopy. However, on occasion given various scenarios, biopsy of metastatic lesions for diagnostic purposes is also performed. In our experience, there is often enough tissue remaining after diagnostic testing. However, it is not uncommon that repeat biopsy is required when little diagnostic tissue remains. In fact, in the standard clinical setting, up to 25% of patients require repeat biopsy for HER2 testing that could not be performed on the original diagnostic sample.[72] As discussed in section 2.6, in order to appreciate the individual's tumor and the oncogenic drivers present within it, as well as molecular evolution from primary site to metastatic site, one must evaluate it.

However, mandatory biopsy of a metastatic lesion for patients with a known diagnosis poses its own challenges. The same is true for a proposed serial biopsy at each progression point. On the other hand, prospective biopsy has been deemed safe in such a setting,[110] and we believe that more accurate biomarker assignment (and treatment allocation) can be achieved by limiting false negative findings based on primary tissue, which in our experience may be as high as 10-15%.[1,2,106,107] This clearly has the potential to benefit each patient individually, as the knowledge gained would apply directly to their treatment strategy at baseline, and over time with each subsequent line of therapy. Moreover, this strategy has great potential to enhance our understanding of both inter-PH and intra-PH via the proposed biopsy schedule (**Figure 2,3,4,5**). Recently, there is a trend for clinical trial biomarker screening to require 'up-to-date' tumor biopsy to get the most relevant 'snap-shot' of the tumor biology.

2.4.2 **Addressing Inter-Patient Heterogeneity**

The understanding of inter-PH is one of extreme importance in the era of molecularly targeted therapies. Our understanding of redundant signaling as well as our success (limited) with inhibiting oncogenic drivers across tumor types including GEC (HER2+ and trastuzumab), brings the hypothesis of whether identifying an oncogenic driver at diagnosis and matching with a specific therapy that inhibits this driver directly will benefit patients.[1,2,82] Molecular profiling at diagnosis attempts to stratify patients into 'oncogenic driver' categories (see **Figure 1, 3, 6** and Section 2.5). Although, ultimately, each individual tumor profile is quite unique, we have determined that molecular patterns are prevalent and patients can be categorized into 'dominant' oncogenic driver categories as a compromise – the hypothesis tested by PANGEA is whether this algorithm of categorization and consequent matched therapy, like with HER2+ tumors, will result in improved clinical outcome versus current standard cytotoxic therapy for HER2 negative tumors.

2.4.3 **Addressing Intra-Patient Heterogeneity Through Space**

In order to address whether the strategy delineated in section 2.5.1.6 is beneficial, we must be confident that patients are classified appropriately at the time of diagnosis/trial enrollment. We and others have demonstrated that patients' tumors frequently experience molecular evolution from primary to metastatic lesion, including HER2 status (**Figure 5**). Given that most diagnostic biopsies are from the primary tumor, most molecular assays are performed on these tumors. However, approximately 10-20% of cases may evolve to acquire the presence of any given biomarker (e.g. HER2 or MET amplification, PI3K mutation etc.) that is only present in the metastatic lesion. In the instance of profiling only the primary lesion, a patient would be classified as negative for the biomarker, and therefore not assigned treatment with the appropriate therapy, thus biasing the results of the trial towards the null. Therefore, with biopsy of a dominant metastatic lesion (liver, lung, peritoneum, lymph node) as determined by the radiologist and treating oncologist, one will be able to assess both the primary and metastatic lesion. In the event of discrepancy, the molecular profile of the metastatic lesion will trump the primary tumor molecular profile, in terms of the final classification; it is important to note that there are few reports (if any) of LOSS of the status of the proposed biomarkers (HER2 amp+, MET amp+, KRAS/PIK3CA, FGFR2 amp+, EGFR amp+) in metastatic lesions when present in the primary tumor. Therefore we are mostly attempting to detect those tumors in the metastatic space that have evolved to acquire the aberration during the passage from primary tumor location to metastatic site, although it is possible that some aberrations will be lost from primary to metastatic lesion. Importantly, assessment of multiple biomarkers will be done, such that although any one biomarker may not change status through space, this does not exclude the possibility that at least one concurrent aberration does. Therefore, estimated tumor evolution rates of 10-15% as determine by evaluating only 3 aberrations may actually be higher when looking at more biomarkers, supporting a biopsy of the distant site to adequately classify a patient’s tumor (**Figure 3,5**).

2.4.4 Rationale of Serial Biopsy and Biologic Beyond Progression (BBP)

2.4.4.1 Addressing Intra-Patient Heterogeneity Through Time with Serial Biopsies

Despite accurate biomarker assessment and classification and optimal response to treatment, ultimately, tumors will evolve to demonstrate resistance to a given therapeutic regimen. We have shown that serial biopsies over time can identify resistance mechanisms that are either inherently present in a proportion of cells at the onset (original tumor biopsy) that are clonally selected by exposure to a particular therapy, or mechanisms that are up-regulated in cells as an acquired ‘response’ in order to maintain viability, despite exposure to the treatment.[98,111] This has been demonstrated across tumor types, including GEC. Importantly, results from repeat biomarker assessment in these serial biopsies will allow for patients to cross over to alternate treatment categories if their disease evolves into that respective category, providing incentive and direct potential benefit to the patient undergoing the biopsy. An example is a patient with EGFR status (B8, **Figure 3**) who develops MET amplification after treatment with anti-EGFR agents as determined by post-treatment biopsy at PD1. This patient would be reclassified as MET+ (B2, **Figure 3**) in the treatment algorithm, and treated accordingly.

2.4.4.2 Rationale of Continuation of Biologic Agents Beyond Progression (BBP)

Significant evidence exists that maintenance of biologic ‘pressure’ on the presumed ‘driver’ with a BBP approach, improves clinical outcomes, including mOS (eg. BCR-ABL for CML, KIT mutation for GIST, HER2+ breast cancer, and anti-VEGF for colorectal cancer). Two strategies of BBP have been examined. The first is to declare the targeted inhibitor failed, but resume therapy with another inhibitor of the same target (eg. BCR/Abl-CML, KIT-GIST, HER2-breast,GEC).[112,119] On the other hand, continued inhibition of the target with the same molecular inhibitor but alteration of the cytotoxic chemotherapy backbone has also been successful (anti VEGF-A – Colorectal).[113,114] Discontinuation of an active biologic agent (eg MET inhibitor for MET amplified tumor) at developed resistance (acquisition of KRAS amplification in addition to MET amplification) allows the tumor to revert back to its original MET amplification only status as the sole driver, suggesting that maintaining MET inhibition is required for best tumor control.[107] Another advantage of BBP in the PANGEA trial is that it will allow for a very accurate mOS since patients will be uniformly treated through three lines of therapy, which clearly does not occur in 'one-line' trials where patients' treatment plans diverge significantly at second/third line therapy, and those enrolled in second or third line trials represent a selection bias not representing all patients with newly diagnosed GEC. Therefore, PANGEA will be able to account for post-first (and second) line treatments, and compare to the historical control (in this pilot trial) or a randomized placebo control (in future planned randomized phase II trials). (**Figure 1,2**)

2.5 Biomarker Assessment and Treatment Algorithm

2.5.1 **Rationale of Molecular Categories and Treatment Assignment**

Molecular profiling of tumors has led to the observation that although 'driver-oncogene' aberrations can be determined with novel medium-high throughput assays, the various genomic aberrations are not necessarily mutually exclusive (**Table 1,2**).[1,2,115-118] One tumor sample may have up to 10 genomic aberrations. On average, we have observed ~3-5 genomic changes (mutations, amplifications, translocations) in GEC patients (**Figure 6**).[1,2] Adding proteomic expression data to the genomic data adds another layer of complexity and 'over-lapping' of potential treatment categories.[119] Thus, in order to assign a patient to a predefined categorical molecular group that will be treated with one matched therapy, we have set a prioritization algorithm based on known clinical and preclinical information regarding the various potential molecular abnormalities (**Figure 3**). For instance, given the known benefit of HER2+ GEC with trastuzumab therapy, HER2+ status trumps all, and patients will be assigned to this group. (However, if other genes have higher amplification, they will take precedence). The next priority goes to MET amplification, which has strong preclinical evidence of therapeutic benefit from anti-MET agents, and also has positive randomized phase II data to support this. However, MET over-expression is treated with less priority than EGFR and FGFR2 gene amplification, given each of these scenarios having strong preclinical evidence of 'driver' status. Therefore, MET overexpression (non-amplified) is lower than these groups. MSI-H/EBV/TMB>15mt/Mb/ PDL1+ CPS>10 patients, typically mutually exclusive to the Amplified patients will be next priority, with strong evidence to date with checkpoint inhibitors for these patients. Next would be the RAS/PI3K-like groups (having activation of either RAS/RAF/MEK and/or PI3K/mTOR/AKT and/or GNAS). Because less overwhelming data exists for a matched agent for these groups, they take lower precedence, should a concurrent aberration be present (eg MET amp+, MET expression, etc.). Finally, for those 'all-negative' tumors, an anti-EGFR agent will be used here for those expressing EGFR by Mass Spectrometry (MS) (B8, **Figure 3**), and with VEGFR inhibition for MS negative (B9, **Figure 3**), with the rationale that other molecular groups that would be considered unlikely to respond to anti-EGFR (or anti-VEGFR) have been removed, thus enhancing the possibility of benefit of the remaining patients to this therapy. Assays used for biomarker assessment via the biomarker assessment and treatment algorithm are described in Section 2.5.2.

*While we await drugs for arms other than those available, if a tumor is classified in a category where the drug is not yet available, they will proceed to the next priority level or treated as standard of care, per physician discretion, in this pilot study.

2.5.1.1 **HER2**

As discussed in 2.1.7, HER2 gene amplification and overexpression (HER2+) was evaluated in the ToGA trial where the addition of Trastuzumab to Fluoropyrimide/platinum resulted in improved OS in these HER2+ patients, becoming a new standard of care in the first line setting.[72] Continuation of anti-HER2 therapy after progression is currently unsupported and not recommended in GEC. However, the TYTAN study recently showed that HER2+ patients do benefit in terms of mPFS and mOS in the second line therapy with lapatinib, after Trastuzumab failure, supporting the notion of continued pressure on HER2 after first progression.[120] Moreover, in HER2+ breast cancer, both lapatinib and TDM-1 have shown survival benefit with continuing HER2 inhibition after first progression with trastuzumab. This trial will test the continued use of trastuzumab while altering backbone chemotherapy in order to improve HER2+ patient outcomes over standard cytotoxic second/third line therapy alone.

2.5.1.2 **MET**

As discussed in section 2.2, another promising molecular subgroup of GEC are MET-driven tumors, either by MET gene amplification and consequent protein-overexpression or by over-expression without gene amplification.[83,86,121,122] A number of MET inhibitors are in development, including monoclonal antibodies to the ligand (eg. Rilotumumab, ficlatuzumab) as well as to the receptor itself (eg emibetuzumab, onartuzumab, ABT-700). Additionally, small molecule inhibitors, both classic ATP-competitive (Type I and II) as well as non-ATP-competitive inhibitor (ie. tivatinib), are in development.

A phase II trial using anti-HGF antibody (rilotumumab) treatment (the growth factor for MET) improved mOS of stage IV GEC patients from 8.9 to 11.1 months for all (unselected) patients. However, in a retrospective analysis using an arbitrary cut-off of ≥50% MET expression (≥ 1+) by IHC in tumor cells as a predictive biomarker (~42% of patients on trial), it was observed that mOS was improved from 5.7 to 11.1 months in this sub-population.[83] This confirmed MET high expression to be a poor prognostic marker and positive predictive marker to MET signaling pathway inhibition, as previously described. Importantly, those patients with low MET expression (~58% of patients) did worse than in the placebo arm, strengthening the argument and importance of patient selection for treatment.

MET has been noted to be a resistance mechanism to a number of other targeted therapies. It is thus very possible that other treatment categories will ‘migrate’ to the MET+ category at PD1 and/or PD2, thus potentially benefiting from anti-MET therapy at this time.

Selection of MET positive tumors (defined as gene amplified or overexpressed by mass spectrometry) for treatment with a MET pathway inhibitor will comprise a molecular treatment category in the PANGEA trial (**Figure 1,3**).

Interim reports indicated that the phase III trial of ECX+/-rilotumumab would be halted for fultility (no safety issues) of reaching the primary endpoint,[123] and we reported this recently at ASCO 2015. It should be noted that the selection of MET+ patients was via IHC testing, and ~80% of screened patients were deemed MET+ (likely to high). We propose in PANGEA that the cut-off of MET positivity should be much more stringent, based on our recent studies using mass spectrometry of approximately 25% positive rate.[124] We will therefore use Mass Spectrometry (Oncoplex Dx) in a CLIA certified commercially available assay to determine treatment with **MET antibody (TBD),** not IHC, and believe that the potential benefit outweighs any potential risks. In fact, only <10% of patients actually had IHC2+ or greater and we believe that any true benefit in this subgroup would be concealed by the overwhelming majority of cases that were only 1+. Also, the recent onartuzumab phase II and III trials were reported both negative ITT. However, there were only 6 vs 8 patients in each arm with IHC 2+/3+ in the phase II, and therefore would be unable to detect even large benefit with such low numbers. Moreover, the phase III onartuzumab trial only had 40% of patients with IHC2+/3+ and in this subset of patients the HR for OS was 0.64 with p=0.06. The argument derived from both the rilotumumab and onartuzumab trials is that there was not sufficient power in the small subset of patients enrolled with truly high met expression in order to detect a HR of 0.5-0.8, and that evaluating these patients specifically is still warranted and excluding those low-level expressors.

2.5.1.3 **EGFR**

EGFR is expressed in most GEC tumors at low/moderate levels. Gene amplification has been described in a much smaller subset of approximately 5%.[117,122] Numerous clinical trials have been conducted in GEC with various anti-EGFR therapies, either alone or in combination, in unselected patient populations, including REAL3 (panitumumab),[77] EXPAND (cetuximab),[79] as well as trials with erlotinib.[125,126] All of these trials reported unimpressive results. In stark contrast to the HER2 (2.5.1.1) and MET (2.5.1.2) trials with their positive results, anti-EGFR therapy was not targeted towards tumors containing driver EGFR status (i.e. amplification, since EGFR mutation has not been described in GEC). It is likely that anti-HER2 therapy would also have failed, or had only marginal benefit, if given to all GEC without selection, and similarly with anti-MET therapy. Moreover, recent analyses of both the REAL3 and EXPAND trials revealed that a subset of highly expressing EGFR tumors and/or those tumors possessing mutations that would predict lack of response (KRAS mutation etc.) derived OS survival benefit.[77,127]

Given this background, PANGEA seeks to exclude tumors with known drivers that would be likely to render anti-EGFR therapy ineffective (HER2 amp+, MET amp+, FGFR2 amp+, KRAS amp+, PI3K mutation, etc.) and additionally select for those tumors that may actually be driven via the EGFR pathway from gene amplification. Preclinical models of cell lines with EGFR amplification are as sensitive to anti-EGFR therapy as HER2 amplified cell lines are to anti-HER2 therapy, and anti-MET therapy for MET amplification.[128]

2.5.1.4 **FGFR2**

FGFR2 is amplified in a subset of GEC of approximately 5-10%.[129] Preclinical models with FGFR2 amplified GEC cell lines have demonstrated extreme sensitivity to FGFR inhibition, similar to the HER2, MET, and EGFR cases.[129-133]

Antibodies and small molecules are in development targeting the FGFR2 pathway in early phase clinical trials, many selecting for FGFR 'activated' status by either mutation or amplification in various tumor types.[131] PANGEA includes FGFR2 as a molecular category within the trial.

2.5.1.5 **MSI-H/EBV+/TMB>15mt/Mb/** PDL1+ CPS>10

Microsatellite instability (MSI) can be assessed with routine clinical testing (using next generation sequencing NGS). MSI-H tumors occur in approximately 5% of metastatic GEC. Reports now have established that these tumors may derive benefit from immune checkpoint inhibitors.

EBV positivity (Ebstein Bar Virus, EBV+) can be assess by routine pathology EBER-ISH assay. EBV+ tumors occur in ~5% of metastatic GEC. Reports now have established that these tumors may derive benefit from immune checkpoint inhibitors.

High Tumor Mutation Burden (TMB >15 mutations/Mb), usually occurs with MSI-High tumors as above, but can sometime occur without MSI-High. These tumors have also had reported benefit from immune checkpoint inhibitors in other tumor types.

PDL1+ CPS>10% can be assessed by routine IHC by the FDA approved companion diagnostic and can co-occur with any of the above (MSI-High, EBV+, TMB+) but can be present in the absent of these as well. These tumors have also had reported benefit from immune checkpoint inhibitors in this is other tumor types.

Therefore, these groups are included and will be matched with anti-PD1 antibodies along with standard chemotherapy in PANGEA.

2.5.1.6 **KRAS/PIK3CA/AKT/GNAS ("KRAS-like": VEGFR2)**

KRAS mutation is the most common oncogenic aberration in human malignancy.[134] However, it is very rare in GEC amounting to 3% of cases.[100] However, we and others have reported that KRAS gene amplification (and less commonly NRAS mutation) can account for up to 10-15% of GEC cases, with consequent overexpression and dependence on the RAS/RAF/MEK pathway, and also co-dependence on the RAS-PI3K/mTOR/AKT pathway.[87,99,135,136] Moreover, PI3K mutation is frequently observed in up to 15% GEC patients, often in combination with HER2 amplification and KRAS amplification,[137] with suggestion that this aberration renders resistance to anti-EGFR therapy similar to KRAS mutation for colon cancer.[138] PTEN loss, BRAF mutation, and other aberrations in these signaling pathways including GNAS mutation, although relatively rare, and KRAS mutation, will also contribute to this group, which is herein referred to as "KRAS-like".

In contrast to HER2, MET, EGFR, and FGFR2 receptor tyrosine kinase, the ability to directly inhibit KRAS has not been accomplished to date and the target has remained un-druggable to date likely due to inherent aspects of KRAS activation and affinity of GTP. Therefore, various indirect inhibition strategies have been or are being tested including farnesyl transferase inhibitors (inhibit cell surface association of KRAS), synthetic lethal partner inhibition, or inhibition of downstream effectors. The first two approaches have resulted in unimpressive results, while the latter is the current approach undergoing active investigation.[134] Another strategy of inhibition for KRAS driven GEC which has support preclinically and clinically is targeting blood vessel formation and angiogenesis.[139-143]

The AVAGAST trial evaluated the anti-VEGF antibody, bevacizumab, with standard chemotherapy versus chemotherapy alone.[78] Although mPFS was improved with statistical significance, mOS was not. Again, this trial was not designed to select any particular subset that may be more likely to derive benefit.

Additionally, despite the negative results from AVAGAST, the REGARD trial was a global, randomized, double-blind, placebo-controlled trial of 355 (unselected) patients with disease progression on first-line platinum- or fluoropyrimidine-containing combination therapy.[144] It showed that the addition of ramucirumab, an anti-VEGFR2 antibody, to best supportive care significantly prolonged median overall survival—the primary endpoint—from 3.8 to 5.2 months ($p = 0.0473$). This translated into a 22% reduction in the risk of death with ramucirumab. Ramucirumab monotherapy also prolonged median progression-free survival from 1.3 months to 2.1 months when added to best supportive care (hazard ratio: 0.483; $p < 0.0001$). Ramucirumab plus best supportive care more than doubled the disease control rate compared with best supportive care alone (48.7% vs 23.1%; $p < 0.0001$).

The randomized phase III clinical trials assessed combinations of ramucirumab with first- and second-line chemotherapy regimens in patients with advanced GEC. The results of the phase III RAINBOW trial, evaluating second-line treatment with ramucirumab in combination with paclitaxel compared with paclitaxel alone, met its endpoint of improving overall survival: The OS hazard ratio (HR) was 0.807 (95% CI 0.678, 0.962; $p=0.0169$). Median OS was 9.63m for RAM+PTX and 7.36m for PTX. The HR for PFS was 0.635 (95% CI 0.536, 0.752; $p < 0.0001$). Median PFS was 4.40m versus 2.86m for the control arm. ORR was 28% RAM+PTX;16% PTX ($p=0.0001$).[145] Moreover, if a subset of patients could be identified that responded with better outcomes, most patients could be spared from unnecessary treatments (cost and toxicity) while those who would be most likely to derive benefit would be treated. Therefore, treatment with anti-angiogenic agents is planned for the “KRAS-like” molecular category on PANGEA.

In addition to the preclinical models discussed above regarding anti-angiogenesis therapy in KRAS/PIK3CA driven cancer,[143] it has been reported that the benefits of anti-VEGF therapy observed in colon cancer trials did not depend on KRAS or PI3K mutation status,[140] suggesting that inhibiting the VEGFR pathway downstream from these genomic events was effective in these patients. Given the preclinical and clinical evidence that KRAS driven tumors and PI3K driven tumors rely heavily on neo-angiogenesis, as well as the positive results from anti-VEGFR2 and/or anti-VEGF (mPFS) to date in GEC, a reasonable strategy would be to target this downstream effector for this molecular category. PANGEA seeks to evaluate anti-angiogenesis in this study in this “KRAS-like” subgroup.

2.5.1.7 Rationale of Prioritized Treatment Algorithm

Above we have discussed a finite number of biomarker categories (HER2, MET, EGFR, KRAS/PI3K, FGFR2). This may be considered ‘futuristic’ in its approach to treating GEC particularly within one trial. Given the current strategy of defining HER2 positive versus negative, but otherwise treating all GEC patients similarly, the PANGEA design may be considered complicated and difficult to implement – this is the reason to commence with this pilot trial first for feasibility.

However, it is becoming well-recognized that there are infinite possible molecular profiles (“n-of-one”), even within HER2 positive tumors (**Table 1**). An example of several GEC patients evaluated by NGS and FISH are demonstrated in **Table 1**. One can clearly observe the profound variability from one patient to the next in terms of molecular profiles.
The 5-category biomarker classification as determined by the algorithm in **Figures 3 and 6** is therefore largely a compromise from the true nature of inter-patient heterogeneity. The 5 categories have been chosen based on their known preclinical and clinical evidence of ‘driver oncogene’ status and available therapeutic agents.[2,87] It is very possible, and not uncommon, to have concurrent activation of one or more of the 5 categories as discussed above in section 2.5.1. Clear examples include *HER2* amplification along with *PI3K* mutation and/or *KRAS* amplification. Therefore, a prioritization of biomarker classification and treatment assignment (**Figure 3**) is required. This prioritization is proposed and follows logical reason, discussed in section 2.5.

The highest priority is *HER2* amplification and immunooncology (IO), given known clinical significance and the demonstrated evidence for anti-HER2 and anti-checkpoint therapy benefit for these patients.

Next is the other receptor tyrosine kinase amplifications including *MET* amplification which has strong preclinical evidence of driver oncogene status and predictive value with respect to anti-MET therapy. Similarly, *FGFR2* and *EGFR* gene amplifications, since these are similar to *HER2* and *MET* as ‘driver’ aberrations. Although the 4 said genes are usually mutually exclusive, if in the event there is concurrent *HER2, MET, FGFR2,* and/or *EGFR* gene amplification in any combination, treatment category will be assigned by metatastatic samples (over primary samples) and prioritized treatment on the gene with the highest copy number. Example, if *HER2* gene copy is 8 and *EGFR* is 70 (or ratio of 2 versus 20+ for instance), treatment assignment will logically be EGFR.

The next (sixth) category encompasses mutations and amplifications in the RAS/RAF/MEK/ERK and PI3K/PTEN/mTOR/AKT pathways (when the higher priority biomarkers are considered normal). This “KRAS-like:VEGFR2” category will be assigned to anti-VEGFR2 therapy, as in Section 2.5.1.5.

Finally relegation cohorts #7 and #8 are if all other biomarkers are negative, where patients' tumors having EGFR expression by Mass Spectrometry with be assigned to anti-EGFR therapy (ABT-806) and 'all negative' will be VEGFR2 (ramucirumab). The rationale for EGFR as this 'otherwise negative' category (if expressing EGFR by MS) can be supported since we are excluding patients with other known driver oncogenes (HER2, MET, FGFR2, KRAS/PIK3CA, etc) that would predict against anti-EGFR therapy, enriching for a cohort that may gain benefit from targeting EGFR. If patients have QNS metastatic sample for full profiling and assignment, ctDNA NGS can be used, and if that is unrevealing, primary tumor can be used. If molecular profiling is overall QNS, then patients are assigned to #8 anti-VEGFR2.

2.5.2 **Background of Biomarker Assessment Techniques**

Bioassays and limited tissue (also see Section 9)

Various techniques are available for tumor molecular characterization, each with its own sensitivity, specificity, advantages and disadvantages.[146] Namely, high and medium 'targeted' throughput assays can quickly interrogate whole exomes/genomes for genomic aberrations or proteomic expression data in a timely manner, while two dimensional (2-D) histological data and tissue architecture is lost. Conversely, low-throughput assays do maintain tissue architecture, which is of extreme relevance when considering intra-PH within the tumor site, but is limited to one/few biomarker(s) and, in the case of FISH, is laborious, costly and time consuming. Clinically for GEC, low-throughput techniques, including IHC and/or FISH have been approved for determination of protein overexpression and/or gene amplification, respectively, for HER2 status.[146,71,84] Similarly, IHC is currently the chosen method to select MET 'positive' patients for clinical trial enrollment.[83] Moreover, there is an increasing trend for clinical trials to specify enrollment criteria based on the availability of tissue for screening and selection for one target of interest (eg. HER2, MET, etc.).

However, with increasing evidence of inter-PH as discussed above, and the <u>scarcity of tissue</u> from endoscopic or core needle diagnostic biopsies (particularly after standard clinical diagnostic tests are completed), there are significant limitations as to the number of low-throughput tests that can be performed on a given sample.[146] With the introduction of several clinical trials operating in the same clinical indication, each requiring tissue for trial enrollment, an economical and pragmatic algorithm utilizing available bioassays and common molecular aberrations within GEC is desperately needed to best ration limited tissue samples and to best molecularly classify tumors for translational correlative research (**Figure 4**).

Over the years, in order to facilitate high throughput testing of a limited tissue sample for gene expression, for both research purposes and clinical use, high/medium throughput gene expression assessments were accomplished by RNA extraction and either CHIP micro-arrays or RNA next-generation sequencing (NGS) assays.[146] This was mostly due to the initial lack of availability of medium/high throughput proteomic assays. However, these RNA methods are highly dependent on tissue fixation, resulting in significant variation in reproducibility of results. In general, clinical tissues are formalin fixed and paraffin embedded (FFPE) and by this process of fixation, time to fixation, along with storage time, the RNA becomes significantly degraded, subjecting these high-throughput assays to multiple deficiencies.[146,147] Moreover, RNA expression, even in the best preserving circumstances, often does not correlate well with protein expression (as low as r^2=0.3).[88,148] However, selected reaction monitoring mass spectrometry (SRM-MS) has recently become available for targeted medium throughput assessment of protein expression in FFPE tissues, and therefore is no longer a limiting step. We have shown using the 'GEC-plex' that FFPE tissues can be used for proteomic profiling that is not dependent on time to fixation or time from fixation or time from cutting the tissue block.[119] This is in stark contrast to IHC and RNA-based tests, making this technology particularly attractive as a diagnostic clinical test.

Given advances in technology available for molecular testing, there is increasing appreciation of tumor complexity and heterogeneity both between and within patients. There is also appreciation of the need to move away from a 'one-size-fits-all' treatment strategy towards a more therapeutic approach premised on oncogenic 'drivers'.[82] There is significant uncertainty and controversy as to how to best accomplish the 'personalized' treatment of GEC. Our preliminary data and the planned correlative studies within PANGEA-IMBBP aim to i) refine our understanding and evaluate the degree of inter-PH and intra-PH, through space and time using four (redundant) bioassays (proteomic: IHC, MS; genomic: FISH, NGS) in a panel of GEC cell lines and FFPE tumor tissues; ii) evaluate the correlation of results between each of these bioassays; and iii) further develop an algorithm to categorize patients into molecular subgroups that may better predict treatment benefit with currently available targeted agents.[1,2]

2.5.2.1 **Fluorescence in Situ Hybridization (FISH)**

FISH testing is considered the gold-standard for gene copy number (GCN) testing within nuclei of interest.[71] The test is low-throughput yet has many advantages over medium-high throughput assays that may also be able to determine GCN. Advantages include direct visualization of the probe within each individual nucleus, and therefore allows for counting of several nuclei (20-100, depending on study referenced) and 'averaging' of GCN/nucleus. Additionally, a control probe (CEP) for each gene is simultaneously hybridized in a different fluorescent wavelength, allowing for differentiation of increased GCN of the gene of interest (GOI) between amplification and mere polysomy (trisomy vs. low vs. high). A third probe for a second GOI on the same chromosome may be multi-plexed (eg. tri-color FISH with MET/EGFR/CEP7 on chromosome 7). The ratio of the GOI/CEP is used to determine amplification status ($\geq$2 usually for GEC, such as for HER2 standard testing). Furthermore, maintenance of 2D tissue architecture allows for evaluation of tumor heterogeneity within the sample being assessed, as well as tumor GCN evolution from adjacent normal→metaplasia→dysplasia→ carcinoma histological progression.

FISH probes for each proposed GOI (HER2, MET, FGR2, KRAS, EGFR) have been characterized and evaluated extensively with reproducibility in the University of Chicago Clinical Cytogenetics Core Facility (CLIA).[2,119] The HER2 testing is performed per FDA guidelines (IHC and FISH).

However, disadvantages to this low-throughput technique are the cost, time, and use of 4uM of tissue per hybridization. Often, multiple 4uM slides are required per gene, in order to get an adequate tissue digestion and hybridization for reliable scoring. Additionally, this is operator dependent and scoring of nuclei can be subjective. This requires pathologist oversight to ensure proper nuclei are being scored. False results can be attained due to cross sectioning of nuclei whereby some signals would be lost.

GCN by FISH has been shown to correlate well with protein expression by IHC for several genes, including HER2, MET, FGFR2, EGFR and KRAS in GEC and other tumor types. However, there is a discordance rate – for example several FISH+ patients in the ToGA trial were scored as IHC 1 or 0 occurring in 131/584 = ~22% of patients enrolled.[72] Importantly, the low expressing FISH+ patients derived no benefit from the addition of trastuzumab.

Prospective evaluation of concordance of FISH status to IHC, MS, and NGS will be done, as well as correlation with clinical outcomes, both as univariate and multivariate (all genes of interest) analyses (see statistical methods section).

Ultimately, for Gene Copy number and amplification should there be discrepancy between FISH and NGS, NGS will take precedence and be defined as >6 copies as in the Foundation One report.

2.5.2.2 **Immunohistochemistry (IHC)**

IHC is a standard method to determine expression levels of a protein of interest.[71,149] It is routinely used for HER2 evaluation in all GEC patients in the University of Chicago Department of Pathology (CLIA) per FDA guidelines. Similarly, MET, EGFR, KRAS, FGFR2, and PTEN have been validated for testing. Advantages of IHC are the relative ease, familiarity and expedient nature of the test. The 2D tissue architecture is maintained and therefore allows for assessing tumor heterogeneity, similar to FISH testing.

On the other hand, each protein of interest tested requires 4uM of tissue, and given limited tissue availability, there is a finite number of tests that can be performed. Moreover, time to fixation, time from fixation, and time from cutting the FFPE tissue block can affect the accuracy of the results. Moreover, the primary antibody chosen to evaluate the expression may also affect results.

Antibodies for the proteins of interest (HER2 – already clinical standard testing; and MET – MET4 antibody) have been validated and will be performed in the CLIA setting at Quintiles Westmont where already being performed in the RILOMET phase III trial.

Prospective evaluation of concordance of IHC status to FISH, MS, and NGS will be done, as well as correlation with clinical outcomes, both as univariate and multivariate (all genes of interest) analyses.

Ultimately, for protein expression of Met and Egfr, Mass Spectrometry will take precedence for assigning to the Met+ arm (#5 Figure 3) and the Egfr+ arm (#7 Figure 3) using the Oncoplex Dx CLIA certified assay as in section 2.5.2.3.

2.5.2.3 **Mass Spectrometry (MS)**

Over the years, in order to facilitate high throughput testing of a limited tissue sample for gene expression, for both research purposes and clinical use, high/medium throughput gene expression analyses were accomplished by RNA extraction and either CHIP micro-arrays or RNA next-generation sequencing (NGS) assays.[146] This was mostly due to the initial lack of availability of medium/high throughput proteomic assays. However, these RNA methods are highly dependent on tissue fixation, resulting in significant variation in reproducibility of results. In general, clinical tissues are formalin fixed and paraffin embedded (FFPE) and by this process of fixation, time to fixation, along with storage time, the RNA becomes significantly degraded, subjecting these high-throughput assays to multiple deficiencies.[146,147] Moreover, RNA expression, even in the best preserving circumstances, often does not correlate well with protein expression (as low as r^2=0.3).[88,148] However, selected reaction monitoring mass spectrometry (SRM-MS) has recently become available for targeted medium throughput assessment of protein expression in FFPE tissues, and therefore is no longer a limiting step. We have shown using the 'GEC-plex' that FFPE tissues can be used for proteomic profiling that is not dependent on time to fixation or time from fixation or time from cutting the tissue block.[103,119,150] This is in stark contrast to IHC and RNA-based tests, making this technology particularly attractive to develop as a diagnostic clinical test (**Figure 4**).

Oncoplex Dx will perform exploratory MS analysis with CLIA/CAP certification, of all samples within PANGEA using the 'GEC-plex' assay, consisting of HER2, EGFR, MET, FGFR2, KRAS, and other peptides of interest including HER3, RON, SRC, IGF1R, FGFR1,PDL1, E-Cadherin, and Vimentin.

Prospective evaluation of concordance of MS status to FISH, IHC, and NGS will be done, as well as correlation with clinical outcomes, both as univariate and multivariate (all genes of interest per laboratory correlatives Section 9) analyses. **As in Figure 3, MS will take precedence when determining therapy in this pilot trial for the Egfr (#7) and Met expression arms (#5).**

2.5.2.4 **Next-Generation Sequencing (NGS)**

Our ability to amass large amounts of genetic information has far surpassed our experience and expertise regarding the clinical application of the derived material. Never has this discrepancy been more magnified - and have our limitations been so apparent - as with the advent of next-generation sequencing (NGS) (massive parallel end sequencing) technology and its role in modern-day oncologic practice.[146,151,152]

Clinical Oncology is in the midst of a major paradigm shift. Fueled by tremendous advances in molecular biology and technology, decisions regarding cancer care are increasingly being driven by data derived from NGS. NGS will likely exponentially increase in the near future and may become universal as we strive for "personalized medicine."

However, clinical trials, such as PANGEA, seek to evaluate the utility of NGS in routine clinical practice (**Figures 4,6**, **Tables 1,2** in section 2.5.1.6). The profound inter-patient heterogeneity of tumor genomes and the large number of possible aberrations make this technology very powerful in order to quickly assess all potential relevant mutations, translocations, and/or gene copy changes with an economical amount of tissue using a 'targeted' oncology platform. This platform consists of known oncogenes and suppressors frequently (relatively) altered in cancers. Foundation One is a platform in routine use and will be performed for exploratory purposes on all samples within the trial. The methods are validated and the test is performed with CLIA certification.

Prospective evaluation of concordance of NGS GCN and MSI status to FISH, IHC, and MS will be done, as well as correlation with clinical outcomes, both as univariate and multivariate (for genes of interest) analyses.

Ultimately, for Gene Copy number and amplification should there be discrepancy between FISH and NGS, NGS will take precedence and be defined as >6 copies as in the Foundation One report.

2.5.2.1 **ctDNA NGS**

We will incorporate clinically available ctDNA NGS results at baseline and at progression to assist with treatment assignment, in particular when tissue biopsy of metastatic/progression site is not available/insufficient.

2.6 Investigational Biologic Therapies

2.6.1 Personalized Approach: Treating Oncogenic Drivers

2.6.2 HER2: (Trastuzumab)

Trastuzumab (lyophilized formulation) is available for commercial use as a freeze-dried preparation. All trastuzumab is supplied for IV administration. Trastuzumab is formulated in histidine, trehalose, and polysorbate 20. Vials of trastuzumab are shipped with cool packs at a temperature ranging from 2°C−8°C (36°F−46°F) and must be placed in a refrigerator (same temperature range) immediately upon receipt to ensure optimal retention of physical and biochemical integrity. Temperature logs must be maintained (in accordance with local pharmacy practice) on the refrigerator to ensure proper storage conditions. Do not use beyond the expiry date stamped on the vial. DO NOT FREEZE.

Trastuzumab may be sensitive to shear-induced stress (e.g., agitation or rapid expulsion from a syringe). DO NOT SHAKE. Vigorous handling of solutions of trastuzumab results in aggregation of the protein and may create cloudy solutions. Trastuzumab should be carefully handled during reconstitution. Causing excessive foaming during reconstitution or shaking the reconstituted trastuzumab may result in problems with the amount of trastuzumab that can be withdrawn from the vial.

<u>Dosage, Administration, and Schedule: See section 5.1.6.</u>

2.6.3 MET: (TBD)

.Dosage, Administration, and Schedule: See section 5.1.7.

2.6.4 EGFR: (ABT806)

The study drug and diluent must be refrigerated at 2°C to 8°C/36°F to 46°F, protected from light. A storage temperature log will be maintained to document proper storage conditions. The refrigerator temperature must be recorded on a daily basis on the temperature log to record proper function. Temperature excursions must be reported to the Sponsor immediately. The investigational products are for investigational use only and are to be used only within the context of this study. The study drug supplied for this study must be maintained under adequate security and stored under the conditions specified on the label until dispensed for subject use or returned to the destruction facility.
Study drug in vial form will be packaged in cartons. Each vial and carton will be labeled per country requirements. Labels must remain affixed to the vial and carton. Refer to the detailed guideline: Study Medication Preparation Guidelines, provided as a separate document outside of this protocol.

Dosage, Administration, and Schedule: See section 5.1.8

2.6.5 FGFR2 (TBD)

Dosage, Administration, and Schedule: See section 5.1.9

2.6.6 MSI-H/EBV+/TMB>15mt/Mb/ PDL1+ CPS>10 (nivolumab)

Nivolumab is approved for various cancers. Although not approved for first line GEC, it is approved for MSI-H colon cancer tumors currently in the second line or higher, and also pembrolizumab another checkpoint inhibitor is approved for any tumor type with MSI-H including GEC. Pembrolizumab is now also approved for PDL1+ tumors in the third line setting or higher of GEC. Nivolumab is approved for GEC in the third line setting or higher in Japan. Therefore treatment with nivolumab will be conducted per package insert for MSI-High patients, as well as EBV+ and TMB>15mt/Mb and PDL1+ CPS>10 patients as these patients may also derive significant benefit from checkpoint inhibitors.

2.6.7 KRAS/PIK3CA (Ramucirumab)

Ramucirumab is now FDA approved for gastric, esophagogastric, and esophageal adenocarcinoma based on the REGARD[153] and RAINBOW[154] trials as monotherapy or combination with cytotoxic therapy, respectively. Therefore treatment with ramucirumab will be conducted per package insert.

3. PATIENT SELECTION

3.1 Eligibility Criteria

1. Histologically confirmed metastatic gastric or esophagogastric junction (type I,II,III Siewert) adenocarcinoma

2. Newly-diagnosed chemo-naïve or recurrent after curative-intent surgery
    - ≥6 months after completion of adjuvant therapy (including chemotherapy and/or radiotherapy)
    - No prior treatment with any targeted agent
    - Patients who have started first line mFOLFOX6 therapy (+/- trastuzumab for HER2 amplified tumors) may be considered for trial participation if they have received no more than 4 doses of therapy at the time of consent and screening.
        1. These patients will be required to meet 'next cycle' parameters for eligibility before commencing treatment on trial (as per Section 6) rather than being required to meet parameters as indicated below in #12 which is for previously untreated metastatic/recurrent patients.

3. Measurable metastatic disease by RECIST criteria,
    - Must be amenable to ultrasound or CT-guided biopsy of one metastatic lesion
    - Peritoneal disease as the sole site of occult metastasis or presenting as malignant ascites is acceptable if a cell block of tumor cells can be obtained showing >20% viable tumor cells.

4. No currently active second malignancy

5. No uncontrolled intercurrent illness or infection

6. No peripheral edema ≥ grade 2 at baseline.

7. No peripheral neuropathy ≥ grade 2 at baseline.

8. No diarrhea ≥ grade 2 at baseline.

9. No autoimmune disease or chronic steroids (dose of >10 mg/day prednisone equivalent) or other immunosuppressive medications within 7 days of randomization (for MSI-H/EBV+/TMB-High≥15mt-Mb/PDL1+ CPS≥10% nivolumab group)

10. ECOG PS 0-2

11. Age ≥ 18 years

12. No CVA within 6 months, no recent MI within 6 months

13. Patients must have normal organ and marrow function as defined below:

    - granulocytes ≥1,500/mcL

- platelets $\geq$100,000/mcL
- total bilirubin $\leq$ 1.5 x ULN, $\leq$1.8 x ULN with liver metastases
- AST(SGOT)/ALT(SGPT)
  $\leq$2.5 X ULN without liver metastases;
  $\leq$5 X ULN with liver metastases
- creatinine within normal institutional limits (<1.5)

OR

- creatinine clearance $\geq$50 mL/min/1.73m^{2},
  (for creatinine level above normal)
- INR: $\leq$ 1.5 (patients on warfarin need to be converted to LMWH during study participation to be eligible)

14. Consent to baseline metastatic and progressive disease biopsy (of metastatic/progressing lesion) for enabling biomarker assessment and treatment assignment (at each time point – baseline, PD1, PD2, PD3) as well as for correlative studies.
    - Consent to baseline and serial blood draws for plasma/serum/whole blood banking for correlative studies

15. Ability to understand and the willingness to sign a written informed consent document and consent to the serial nature of the proposed PANGEA treatment with first, second and third line therapy as tolerated.

16. Ability to comply with requirements of the protocol, as assessed by the investigator by the patient signing the consent form.

17. If history of exposure to anthracyclines during perioperative treatment, the following cumulative doses of anthracyclines must be less than:
    Epirubicin < 720 mg/m2
    Doxorubicin or liposomal doxorubicin < 360 mg/m2
    Mitoxantrone > 120 mg/m2 and idarubicin > 90 mg/m2
    If more than one anthracycline has been used, then the cumulative dose must not exceed the equivalent of 360 mg/m2 of doxorubicin.

18. Cardiac Ejection Fraction >50% (for HER2+ patients) as assessed by echocardiogram, MUGA scan, or cardiac MRI

19. Willingness to use effective and reliable methods of contraception (For appropriate methods of contraception considered acceptable see Appendix B).

20. To commence second line irinotecan, bilirubin should be < 1 mg/dL. If between 1-2 mg/dL initial dose should be reduced by one dose level. If >2 mg/dL, then irinotecan will not be used.

21. To commence third line docetaxel, patients must have grade 2 or less neuropathy from prior oxaliplatin treatment. Also bilirubin > upper limit of normal (ULN), or AST and/or ALT > 1.5 x ULN concomitant with alkaline phosphatase > 2.5 x ULN are not eligible for docetaxel therapy.

22. Patients are allowed to consent to PANGEA as long as they have received 2 months (4 doses) or less of FOLFOX (with or without 5FU/LV bolus) (plus trastuzumab if HER2 amplified) chemotherapy.

3.2 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

All patients should be registered by the responsible Clinical Research Associate and/or Research Nurse in the eVelos Database prior the start of protocol-specific assessments. All selection criteria listed in Section 3.0 should be confirmed prior to registration.

Following registration, patients should begin protocol treatment with FOLFOX chemotherapy and biologic agent (if assigned) within 14-days. Issues that would cause treatment delays should be discussed with the Principal Investigator.

*****For HER2- subjects, they will receive standard chemotherapy alone.*** *If a targeted biologic becomes available for one or more of the other groups, the protocol will be amended to include new agents for the matched molecular group. Patients that started with chemotherapy alone will be candidates for adding the biologic agent when it becomes available along with whichever cytotoxic backbone regimen they are currently receiving, and will be considered having progression if CT shows progression after receiving 4 cycles of cytotoxic and backbone chemotherapy. IF PD on 4 cycles of cytotoxic chemotherapy prior to obtaining treatment assignment, patients can proceed to second line FOLFIRI and when assignment is learned added to FOLFIRI. However, if at C5 treatment assignement is known, patients may continue on FOLFOX first line therapy plus the newly assigned targeted therapy.*

Similarly, upon documented progression of disease by CT at first progression (PD1), ALL patients will undergo repeat biopsy and then change therapy to FOLFIRI cytotoxic and continue the biologic agent as assigned in the first line (**Figure 2**). Upon determination of biomarker classification and treatment assignment of the PD1 biopsy, patients may change to a new biologic therapy (with FOLFIRI) if the molecular category changes from the originally assigned category. Similarly, at PD2, patients will change to FOLTAX chemotherapy and continue the biologic determined after PD1, and will switch biologic if the PD2 biopsy assessment indicates to do so. The Principal Investigator will determine which biologic category will be assigned originally, and at PD1 and PD2, using the 4 diagnostic assays (FISH, IHC, MS, and NGS) as in section 2.5.2 and the biomarker assessment and treatment algorithm (**Figure 3**). Patients initially in one biomarker category that demonstrate tumor evolution into a different category, will change therapy to both the appropriate biologic therapy along with next cytotoxic therapy.

4.2 Registration Process & Data Submission for Consortium Affiliates

Not Applicable

4.3 Data and Safety Monitoring

Data Safety and Monitoring will occur at the weekly University of Chicago GI Research Program meetings, which are led by senior level medical oncologists. At each meeting, the study will be reviewed for safety and progress toward completion. Toxicities and adverse events will be reviewed at each meeting.

5. TREATMENT PLAN

5.1 Agent Administration and use of palliative therapy (ie Radiation)

Treatment will be administered on an outpatient basis and follow the treatment strategy from first to second to third line (as tolerated) as depicted in the treatment schema and treatment strategy in **Figures 1 and 2**, respectively. A treatment cycle is defined as 2 weeks (14 days). Biologic agents will be assigned based on the biomarker category that the patient's tumor is determined to be (**Figure 3**) at each time point (baseline, PD1, PD2). Appropriate dose modifications for each biologic agent and cytotoxic agent are described in Section 6. Reported adverse events and potential risks for each biologic agent and each chemotherapy backbone (mFOLFOX6 → FOLFIRI → FOLTAX) are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy. In determining treatment doses for all agents, actual height and weight will be used for all calculations. **Patient height and weight at screening will be used. This dose will be recalculated only if > 10% change in weight as per standard practice**. There should be no adjustment to "ideal" weight. **In the event that scheduled intravenous treatment and/or clinic visits fall on holidays, the **treatment may be given +/-3 days before or after** these particular days.

Palliative Radiation: Treatment interruption for palliative procedures such as radiation to painful lesions or bone metastases or an EGJ primary tumor causing dysphagia, as would be done standardly, is allowed, as long as systemic treatment is resumed within 6 weeks from last due dose.

5.1.1 **Safety Lead-In**

Because each chemotherapy regimen has not been previously combined, in some cases, with each proposed biologic agent, an initial group of 6 patients will receive open-label biologic agent within each treatment category (HER2+, MET+, etc.) for each cytotoxic backbone (mFOLFOX6, FOLFIRI, FOLTAX), with standard-doses of each cytotoxic, and each 6-patient cohort will be closely monitored.

We conclude that a formal phase I study is not indicated for any of the investigational/cytotoxic combinations, since pre-clinical and clinical experience with these proposed monoclonal antibody agents have shown that they do not substantially alter the toxicity profile of various chemotherapy regimens, including the cytotoxic agents included in PANGEA. This safety lead in will ensure that this is indeed the case prior to continuing with further enrollment.

The initial 6 patients for any given treatment category and cytotoxic backbone will have completed 4 weeks of therapy with the combination and all data has been reviewed by the PI and drug sponsors (if applicable) prior to proceeding with the remaining portion of this study thereafter with respect to that biologic agent (treatment category) and cytotoxic backbone. Using the NCI CTC for adverse events v4.0, DLT will be defined as grade 4 thrombocytopenia, grade 4 neutropenia lasting more than 7 days, grade 3 neutropenia complicated by fever or infection, or grade 3 or greater toxicity in other organ systems possibly, probably or definitely related to the biologic agent. If 1 DLT develops, 6 additional patients will be accrued for a total of 12 patients. If 2 DLTs occur, accrual will be halted, and a dose de-escalation will be considered.

If no investigator-determined DLTs have occurred in the first 6 patients on each combination or no more than 1 DLT in 12 patients on each combination, then the study can proceed to regular treatment within this particular category and cytotoxic regimen, once the Principal Investigator, has reviewed the data and concurs with the safety of this combination.

5.1.2 **Treatment Strategy: PANGEA**

Treatment will be administered on an outpatient basis and follow the treatment strategy from first to second to third line (as tolerated) as depicted in the treatment schema and treatment strategy in **Figures 1 and 2**, respectively. The biologic agents will be assigned based on the biomarker category the patient's tumor is determined to be (**Figure 3**) at each time point (baseline, PD1, PD2).

The pilot IMBBP with 4 of 6 molecular categories and biologic agents secured (IO: nivolumab; HER2++: trastuzumab; EGFR++: ABT-806; VEGFR2++/+: Ramucirumab).

The trial will be amended on a 'rolling basis' as the other drugs are secured for the other molecular arms. For patients' tumors found to be in a molecular category without drug available at the time of enrollment (eg.FGFR2 amplified) they will be based on the prioritized treatment assignment in **Figures 2 and 3**). Accrual will be completed upon enrolling 68 patients within HER2++, MET++, EGFR++/+, VEGFR2++/+, and FGFR2++/MET++ if treated with anti-FGFR2/MET antibodies] tumors, per the primary endpoint intention to treat (ITT) delineated in Section 13.

Treatment assignment will be performed by the Principal Investigator, after reviewing all of the biomarker data for the patients' tumors, at each timepoint (baseline primary/metastatic lesion, PD1, PD2) using the pre-specified treatment algorithm (**Figure 3**).

5.1.3 **mFOLFOX6**

On day one of each treatment session patients will receive mFOLFOX6 with Oxaliplatin 85 mg/m2 given as a two-hour intravenous (IV) infusion. The dose of Leucovorin will remain fixed at 200 mg/m2 as a two-hour IV infusion followed by 5-fluorouracil 400 mg/m2 IV push (bolus) and 5-fluorouracil 2400 mg/m2, given as a forty-six to forty-eight hour infusion (Continuous Infusion).

mFOLFOX6 REGIMEN DESCRIPTION (FIRST LINE REGIMEN)

Agent	***Dose***	***Route***	***Schedule***	***Cycle Length***
Oxaliplatin	85mg/m2 in 500ml D5W	IV over 2 hours **before** 5FU	Day 1, week 1	2 weeks (14 days)
Leucovorin	200mg/m2 in 250ml D5W	IV over 2 hours, can be given at the same time as Oxaliplatin in separate bags using a Y-line	Day 1, week 1	
5-FU Bolus	400mg/m2	IV push	Day 1, week 1	
5-FU	2400mg/m2	IV Via an ambulatory infusion pump of choice over 46-48 hours	Day 1,2 week 1	
Biologic Agent (as assigned)	See respective 5.1.(6-10) section depending on treatment category assignment	IV (see dosing for each specified biologic agent in respective sections)	Day 1, week 1	

- Oxaliplatin may be given concurrently with Leucovorin 200 mg/m2. Oxaliplatin must not be mixed with normal saline; therefore, when Leucovorin and Oxaliplatin are given concurrently via a Y-connector, both drugs should be administered in D5W

- There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore, **all dosing is to be determined solely by the patient's BSA as calculated from actual weight.** This will eliminate the risk of calculation error and the possible introduction of variability in dose administration. **Failure to use actual body weight in the calculation of drug dosages will be considered a protocol deviation.** Physicians who are uncomfortable with administering chemotherapy dose based on actual body weight should not enroll obese patients on this protocol.

- Oxaliplatin is emetogenic. All patients receiving Oxaliplatin should be pre-medicated with an acceptable antiemetic regimen. Patients may receive dexamethasone 10-20 mg IV as pre-treatment antiemetic unless there is a relative or absolute contraindication to corticosteroids. Other antiemetics may be used in addition to the suggested regimen, if clinically indicated.

- Hypersensitivity: Platinum hypersensitivity can cause dyspnea, bronchospasm, itching and hypoxia. Appropriate treatment includes supplemental oxygen, steroids, antihistamines, and epinephrine; bronchodilators and vasopressors may be required. Platinum hypersensitivity is an extremely rare event and should be treated promptly. Oxaliplatin hypersensitivity occurs in approximately 0.5% of patients receiving this agent.

- Pharyngo-laryngo dysesthesias: Oxaliplatin may cause discomfort in the larynx or pharynx associated with dyspnea, anxiety, swallowing difficulty and is exacerbated by cold. Appropriate therapy includes use of anxiolytics, cold avoidance and monitoring.

- Appropriate dose modifications for mFOLFOX6 are described in Section 6.

- Oxaliplatin may be held for up to 2 months for toxicity or other (i.e. break per OPTIMOX strategy), per physician discretion, and resumed by 2 months if: i) toxicity resolved to grade 1 or less, or if PD on maintenance 5FU. **PD on mFOLFOX6 (PD1) is declared when:** i) PD by RECIST 1.1 (section 11) after a two month round (4 cycles) of full cytotoxic backbone (mFOLFOX), ii) PD on 5FU/LV alone and unable to resume Oxaliplatin due to toxicity > G1 (usually neuropathy) despite being off Oxaliplatin for 2 months, and ultimately iii) physician's discretion.

5.1.4 **FOLFIRI (Second Line Chemotherapy Backbone)**

FOLFIRI REGIMEN DESCRIPTION (SECOND LINE REGIMEN)				
Agent	***Dose***	***Route***	***Schedule***	***Cycle Length***
Irinotecan	180mg/m2 in 500ml D5W	IV over 2 hours **before** 5FU	Day 1, week 1	2 weeks (14 days)
Leucovorin	200mg/m2 in 250ml D5W	IV over 2 hours, can be given at the same time as Oxaliplatin in separate bags using a Y-line	Day 1, week 1	
5-FU Bolus	400mg/m2	IV push	Day 1, week 1	
5-FU	2400mg/m2	IV Via an ambulatory infusion pump of choice over 46-48 hours	Day 1,2 week 1	
Biologic Agent	See respective 5.1.(6-10) section	IV	Day 1, week 1	

(as assigned)	depending on treatment category assignment	(see dosing for each specified biologic agent in respective sections)		

- FOLFIRI consists of 5-FU, Leucovorin, and Irinotecan**.** The dosing regimen for each subject will adhere to the protocol specifications. FOLFIRI dosing regimen will consist of *l*-LV 200 mg/m2 or *dl*-LV 400 mg/m2 as a 2-hour infusion, and Irinotecan 180 mg/m2 given as a 90-minute infusion in 500 mL dextrose 5% via a Y-connector, followed by bolus FU 400 mg/m2 and a 46-hour infusion FU 2400 mg/m2.

- Appropriate dose modifications for mFOLFIRI are described in Section 6.

- Per treating physician discretion, testing for UGT1A1 genotype and dose reduction can be performed per clinical standards.

- Doses of 5FU (bolus and continuous) within FOLFIRI should be continued from prior regimen dosing (ie mFOLFOX6), if dose reductions have occurred in the first line.

- Irinotecan may be held for up to 2 months for toxicity or other (i.e. break per OPTIMOX/OPTIMIRI strategy), per physician discretion, and resumed by 2 months if: i) toxicity resolved to grade 1 or less, or if PD on maintenance 5FU**. PD on FOLFIRI (PD2) is declared when:** i) PD by RECIST (section 11) after a two month round (4 cycles) of full cytotoxic backbone (FOLFIRI), ii) PD on 5FU/LV alone and unable to resume Irinotecan due to toxicity > grade 1 despite being off Irinotecan for 2 months, and ultimately iii) physician's discretion.

5.1.5 FOLTAX (Third Line Chemotherapy Backbone)

mFOLTAX REGIMEN DESCRIPTION (THIRD LINE REGIMEN)				
Agent	***Dose***	***Route***	***Schedule***	***Cycle Length***
Docetaxel	50mg/m2 in 500ml D5W	IV over 2 hours **before** 5FU	Day 1, week 1	2 weeks (14 days)
Leucovorin	200mg/m2 in 250ml D5W	IV over 2 hours, can be given at the same time as Oxaliplatin in separate bags using a Y-line	Day 1, week 1	
5-FU Bolus	400mg/m2	IV push	Day 1, week 1	

5-FU	2400mg/m2	IV Via an ambulatory infusion pump of choice over 46-48 hours	Day 1,2 week 1	
Biologic Agent (as assigned)	See respective 5.1.(6-10) section depending on treatment category assignment	IV (see dosing for each specified biologic agent in respective sections)	Day 1, week 1	

- FOLTAX consists of 5-FU, Leucovorin, and Docetaxel**.** The dosing regimen for each subject will adhere to the protocol specifications. FOLTAX dosing regimen will consist of *l*-LV 200 mg/m2 or *dl*-LV 400 mg/m2 as a 2-hour infusion, and docetaxel 180 mg/m2 given as a 90-minute infusion in 500 mL dextrose 5% via a Y-connector, followed by bolus FU 400 mg/m2 and a 46-hour infusion FU 2400 mg/m2.

- Appropriate dose modifications for mFOLTAX are described in Section 6.

- Doses of 5FU (bolus and continuous) within FOLTAX should be continued from prior regimen dosing (i.e. mFOLFOX6, FOLFIRI), if dose reductions have occurred in the first line/second line.

- **Patients being considered for third line mFOLTAX are required to have grade 2 or less neuropathy from prior oxaliplatin treatment to be eligible.**

- Docetaxel may be held for up to 2 months for toxicity or other (i.e. break per OPTIMOX/OPTIMIRI/OPTITAX strategy), per physician discretion, and resumed by 2 months if: i) toxicity resolved to G1 or less, or if PD on maintenance 5FU**. PD on FOLTAX (PD3) is declared when:** i) PD by RECIST (section 11) after a two month round (4 cycles) of full cytotoxic backbone (FOLTAX), ii) PD on 5FU/LV alone and unable to resume Docetaxel due to toxicity > G1 (usually neuropathy) despite being off Docetaxel for 2 months, and ultimately iii) physician's discretion.

5.1.6 **Trastuzumab (Herceptin) – HER2++ Patients**

- Trastuzumab will be administered intravenously on Day 1 of each treatment cycle, using an initial dose of 6 mg/kg for Cycle 1, followed by doses of 4 mg/kg Q2W for subsequent treatment cycles.

- Trastuzumab will be given until disease progression in the first line (PD1) (mFOLFOX6+trastuzumab) for HER2+ patients, or the development of unacceptable toxicity, or the patient is withdrawn from study treatment for another reason.

- The initial dose of Trastuzumab (Cycle 1, Day 1) will be administered over 90 (± 10) minutes, after the patient will be observed for infusion associated reactions (IARs) such as fever, chills, headache, pruritus, nausea or vomiting, changes in vital signs, etc. If such symptoms occur, slowing or interruption of the infusion may be helpful, and the infusion can be resumed when symptoms abate. If the initial infusion is well tolerated, subsequent infusions may be administered over 30 (± 10) minutes, followed by an observation period of 30 minutes.

- Patients who experience infusion-associated symptoms may be premedicated for subsequent infusions using acetaminophen/paracetamol and antihistamines. Dose reduction of Trastuzumab for toxicity is not permitted. Dose delays are permitted for toxicity, including cardiotoxicity documented by a symptomatic or an asymptomatic decrease in LVEF (see Section 6). See section 6 for cardiac imaging specifications (at least every 3 months while on Trastuzumab).

- If the patient misses a dose of Trastuzumab for any cycle (i.e., the two sequential administration times are 4 weeks or more apart), a reloading dose of 6 mg/kg of Trastuzumab should be given. If reloading is required for a given cycle, the study therapies should be given at the same schedule. Subsequent maintenance Trastuzumab doses of 4 mg/kg will then be given Q2W, starting 2 weeks later.

- HER2+ patients will continue with Trastuzumab therapy after PD1 and PD2 with the other cytotoxic backbones, FOLFIRI and FOLTAX, respectively, unless HER2+ status changes to one of the other 4 categories at PD1 or PD2 biopsy and molecular assessment. If molecular classification changes at either of these two time points, biologic treatment will change to the new appropriate drug for that molecular category. Trastuzumab will continue with second/third line cytotoxic regimen until biomarker assessment results are available from the PD1/PD2 biopsy.

5.1.7 **MET++ Patients (TBD)**

Patients may be able to receive anti-MET antibodies on other studies, and if so, they will be followed for survival outcomes and other translational correlatives as per the PANGEA study, but treated according to protocol of the other specific study with the anti-MET antibodies. At disease progression, they could then proceed with PANGEA protocol for subsequent lines of therapy per PANGEA protocol. These patients will be considered towards intention-to-treat population if indeed they receive the appropriate antibodies at each line of therapy along with chemotherapy, otherwise they will be included in a secondary exploratory analysis.

5.1.8 (ABT806): EGFR++/+ patients

ABT-806 24 mg/kg will be given every other week by IV infusion preceded approximately 30 minutes prior with 650 mg acetaminophen and 25 to 50 mg IV diphenhydramine. Administration rate should not exceed 15 mg/min or be completed earlier than 15 minutes.

Subjects will be closely monitored for treatment-related adverse events, especially allergic reactions, during the infusion and the post-infusion observation hour. For the initial ABT-806 infusion, pre-infusion vital signs should be taken. Direct observation is required for the first 15 minutes of the infusion and subjects should be closely monitored during the post-infusion hour. For subsequent infusions, direct observation is not required; however, pre-infusion vital signs should still be taken.

Severe allergic reactions (Grade 3 or 4) require the immediate interruption of ABT-806 treatment and permanent discontinuation from further treatment. Moderate allergic reactions (Grade 1 or 2) will also require the immediate interruption of ABT-806 treatment. Once symptoms have resolved, retreatment is allowed with a 50% reduction of the infusion rate.
Guidelines for dose, dose preparation, volume, rate of infusion and type of infusion pump device will be supplied by Abbott. Acetaminophen and diphenhydramine will be supplied by the site.

5.1.9 (TBD): FGFR2++ patients

Patients may be able to receive anti-FGFR2 antibodies on other studies, and if so, they will be followed for survival outcomes and other translational correlatives as per the PANGEA study, but treated according to protocol of the other specific study with the anti-FGFR2 antibodies. For example, the Phase I FIGHT study is a study assessing the new combination of FPA144 plus FOLFOX chemotherapy. For this phase I open label study, this FOLFOX-FPA144 combination allows for any line of therapy. So should a patient require anti-FGFR2 therapy at any line of therapy during enrollment on PANGEA due to FGFR2 amplification, then the patient could enroll in the FIGHT study to gain access to the FGFR2 inhibitor, as long as FOLFOX therapy could be considered appropriate for them. At disease progression, they could then proceed with PANGEA protocol for subsequent lines of therapy per PANGEA protocol. These patients will be considered towards intention-to-treat population if indeed they receive the appropriate antibodies at each line of therapy along with chemotherapy, otherwise they will be included in a secondary exploratory analysis.

5.1.10 (Nivolumab) MSI-H/EBV+/TMB-High≥15mt-Mb/PDL1+ CPS≥10%

- Nivolumab will be administered intravenously on Day 1 of each treatment cycle, using an initial dose of 3 mg/kg for each Cycle Q2W
- Treatment will be administered per FDA approved package insert

5.1.11 (Ramucirumab): VEGFR2++/+ patients

- Ramucirumab will be administered intravenously on Day 1 of each treatment cycle, using an initial dose of 8 mg/kg for each Cycle Q2W.
- Treatment will be administered per FDA approved package insert

5.2 General Concomitant Medication and Supportive Care Guidelines

5.2.1 **Antiemetics:** Antiemetic medication may be used prior to treatments at the treating physician's discretion.

5.2.2 **Growth Factors:** Colony-stimulating factors (i.e., G-CSF) may be used if required. CSFs should be used according to ASCO guidelines.

5.2.3 **Central Access Device:** is required for 5FU continuous infusion.

5.3 Duration of Therapy on Trial

In the absence of treatment delays due to adverse events, treatment on trial (mFOLFOX6→FOLFIRI→FOLTAX, each with appropriate biologic therapy) may continue until one of the following criteria applies:

- Disease progression as defined by PD3 or after 4 years on trial, whichever occurs first.
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse events(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- Patients who are discontinued from 1 or more agents for reasons of toxicity may continue on the remaining agents until one of the criteria above is reached.
- Death

5.4 Duration of Follow Up

All patients will be followed until disease progression (PD1, PD2, PD3), and for any therapies received (what they are, and their duration) after PD3, and for survival until death or at 5 years from enrollment.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose Modification

Chemotherapy dosing will be based on the patient’s baseline weight measurement. Weight will be measured on Day 1 of each 2-week treatment cycle. If there is a ≥10% change from the patient’s baseline weight measurement, the chemotherapy dose should be recalculated using the new weight.

To the extent possible, the causal relationship(s) for each AE should be considered independently for each component of study treatment, and dose modifications (dose delays or dose reductions) should be applied only for the component(s) of study treatment that are causally related to a given AE.

For **non-hematologic toxicities** that are likely a combination of both cytotoxic agents, **<u>removal of the 5FU bolus/LV will be the first dose modification</u>**, without altering the other cytotoxic (5FU continuous infusion, oxaliplatin, irinotecan, or docetaxel**). Subsequent recurrences** of a similar toxicity will result in dose reduction of **both cytotoxics (5FU continuous infusion and one**

of oxaliplatin, irinotecan, docetaxel) as in T**ables 3-7** below**, or per the discretion of the treating physician.**

6.1 5-FU/Leucovorin – Non-Hematologic

Re-treatment should be delayed until all non-hematologic toxicities have subsided to Grade ≤ 1. Treatment may be delayed up to 4 weeks (i.e., 6 weeks since the start of the previous treatment cycle) to allow for this recovery. If the patient cannot meet the re-treatment criteria at this time point, 5-FU should be permanently discontinued because of unacceptable toxicity.

***First, omit the bolus 5FU and LV for any grade 2 or higher Hematologic or Non-Hematologic toxicity that may be attributable to 5FU either alone or in combination of other cytotoxics. Treatment should be held until resolution to Grade 1 or less, and then resume without the 5FU bolus.**

Then, based on the most severe toxicity experienced since the previous treatment, the following dose modifications should be used for non-hematologic toxicities**. Table 3** provides further 5FU dose reductions for the first appearance (after dropping 5FU/LV bolus) of the specified toxicities.

At each subsequent appearance of the toxicities, despite a prior dose reduction, the 5-FU dose could be further adjusted, if the treating physician considers the reduction to be in the best interest of the patient (otherwise the treatment should be discontinued). (eg. The second dose adjustment is by 25% of the previous dose (consistent with dose changes in **Table 3**)).

Table 3: 5-FU Dose Modifications for Non-Hematologic Toxicities (if recurrent after dropping bolus)

Toxicity	Grade	5-FU
		First drop bolus for any toxicity, then:
Nausea or vomiting, or both	3 or 4	75% of previous dose
Diarrhea	3 or 4	75% of previous dose
Stomatitis	3	75% of previous dose
Stomatitis	4	50% of previous dose
Cardiac toxicity (vasospasm) (attributed to 5-FU)	2	Stop 5-FU permanently
Skin toxicities (HFS)	3 or 4	75% of previous dose
Fatigue	3 or 4	75% of previous dose

Nausea and Vomiting
For Grade 3 or 4 nausea or vomiting, or both, the patient must have recovered to Grade ≤1 before treatment can be re-initiated.

Diarrhea
Doses should be reduced according **to Table 3**. After Grade 3 or 4 diarrhea, the patient must have recovered to Grade ≤ 1 before treatment can be re-initiated.

Stomatitis
After Grade 3 or 4 stomatitis, the patient must have recovered to Grade ≤ 1 before treatment can be re-initiated.

Skin Toxicity (Hand-Foot-Syndrome)
Treatment will be withheld for Grade 3 or 4 toxicity until recovery to Grade ≤1.
Fatigue
Treatment will be withheld for Grade 3 or 4 toxicity until recover to Grade ≤1.

Other Toxicities
Other toxicities not listed above should be managed symptomatically, if possible, if they are Grade ≤2. For Grade 3 AEs, chemotherapy should be withheld for a maximum of 4 weeks beyond the scheduled treatment date (6 weeks since the start of the previous treatment cycle) until toxicity is resolved to Grade ≤1, then continued at a lower dose, if medically appropriate. After recovery from Grade 3 to Grade ≤1, a dose reduction of 25% of the previous dose should be made for subsequent cycles. If there is no resolution to Grade ≤1 after a maximum of 4 weeks beyond the scheduled treatment date of the next cycle (6 weeks since last dose), the patient should be permanently discontinued from 5-FU treatment. In case of Grade 4 severity of toxicities other than those specified above, the patient will have 5-FU treatment held and the AE will be followed up until resolution of toxicity according to the protocol (grade 1 or less) and then resume at the appropriate at 75% of the previous dose, or not resume at all, ultimately per physician discretion.

NOTE: There are no dose reductions for leucovorin. The dose remains fixed at 200 mg/m^2. Leucovorin is discontinued only when 5-FU bolus is discontinued.400 mg/m^2 racemate (d,l-leucovorin) may be a replacement in shortage of l-leucovorin.

Thromboembolism
Subjects who experience any grade ≥ 3 venous thromboembolic event while on study treatment may be treated with full dose anti-coagulation therapy with low molecular weight heparin (LWMH) (preferred) or other standard anti-coagulation if unable to receive LWMH.

6.2 Oxaliplatin

First, omit the bolus 5FU and LV for any Hematologic or Non-Hematologic toxicity that may be attributable to 5FU either alone or in combination of other cytotoxics. If the patient requires further oxaliplatin dose reductions than listed in the protocol (**Tables 4-7**), the patient will be removed from treatment with that agent. If the patient is removed from treatment with 5-FU or 5-FU treatment is held, oxaliplatin should be discontinued or held until 5-FU is resumed. If unable to resume 5FU, proceed to next line of therapy (irinotecan) alone without 5FU.

6.3 Irinotecan

To commence second line irinotecan, bilirubin should be < 1 mg/dL. If between 1-2 mg/dL initial dose should be reduced by one dose level. If >2 mg/dL, then irinotecan will not be used. First, omit the bolus 5FU and LV for any Hematologic or Non-Hematologic toxicity that may be attributable to 5FU either alone or in combination of other cytotoxics. If the patient requires further irinotecan dose reductions than listed in the protocol (**Tables 4-7**), the patient will be removed from treatment with that agent.

6.4 Docetaxel

To commence third line docetaxel, patients must have grade 2 or less neuropathy from prior oxaliplatin treatment. Also bilirubin > upper limit of normal (ULN), or AST and/or ALT > 1.5 x ULN concomitant *with* alkaline phosphatase > 2.5 x ULN are not eligible for docetaxel therapy. First, omit the bolus 5FU and LV for any Hematologic or Non-Hematologic toxicity that may be attributable to 5FU either alone or in combination of other cytotoxics.

If the patient requires further docetaxel dose reductions than listed in the protocol (**Tables 4-7**), the patient will be removed from treatment with that agent.

Table 4: Dose Adjustment Levels for Any Toxicities for Cytotoxics (Oxaliplatin, Irinotecan, and Docetaxel).

	First Line Oxaliplatin	Second Line Irinotecan	Third Line Docetaxel
Starting Dose	85mg/m^2	180mg/m^2	50mg/mg^2
Dose -1	65mg/m^2	135mg/m^2	37.5mg/m^2
Dose -2	50mg/m^2	100mg/m^2	25mg/m^2

If the patient requires further dose reductions than listed in the protocol in the table above, the patient will be removed from treatment with that agent. If the patient is removed from treatment with 5-FU or 5-FU treatment is held, oxaliplatin should be discontinued or held until 5-FU is resumed. Single agent irinotecan or docetaxel is permitted.

Table 5: <u>Hematologic</u> Dose Reductions (to be followed after 5FU bolus and LV dropped for first attributable toxicity occurrence as in Section 6.1).

Toxicity	Action	Dose Modification
Neutropenia[1]		
Grade 1 (ANC>1.5 x 10^9/L)	Continue Treatment	Maintain Dose Level
Grade 2 (ANC 1.0 x 10^9/L – 1.5 x 10^9/L)	Continue Treatment	Maintain Dose Level
Grade 3 (ANC 0.5 x 10^9/L – 1.0 x 10^9/L)	**Hold Treatment** Recheck blood counts weekly until ANC $\geq$ 1 x 10^9/L, then restart treatment.	**Decrease Cytotoxic one dose level.** [1] If the patient is not receiving cytotoxic, decrease continuous 5FU 75% from prior dose.

Grade 4 (ANC<0.5 x 10^9/L)	**Hold Treatment** Recheck blood counts weekly until ANC $\geq$ 1 x 10^9/L, then restart treatment.	**First Occurrence:** Reduce Cytotoxic one dose level.[1] **Second Occurrence:** Decrease 5-FU 75% from prior dose and cytotoxic one dose level.[1]
Neutropenic Fever[1]		
Grade 3 (ANC 0.5 < 1.0 x 10^9/L) AND fever >38.5°C	Treat neutropenic fever according to standard guidelines. Proceed with next cycle when fever is resolved and ANC $\geq$ 1.25 x 10^9/L.	**First Occurrence:** Reduce Cytotoxic one dose level.[1] **Second Occurrence:** Decrease 5-FU 75% from prior dose and cytotoxic one dose level.[1]
Grade 4 (ANC 0.5 < 1.0 x 10^9/L) AND fever >38.5°C **Life-threatening consequences including septic shock, hypotension, acidosis**	Treat neutropenic fever according to standard guidelines. Proceed with next cycle when fever is resolved and ANC $\geq$ 1.25 x 10^9/L.	**First Occurrence:** Reduce Cytotoxic one dose level.[1] **Second Occurrence:** Decrease 5-FU 75% from prior dose and cytotoxic one dose level.[1]
Thrombocytopenia		
Grade 1 (PLT >75 0 x 10^9/L)	Continue Treatment	Maintain Dose Level
Grade 2 (PLT 50 x 10^9/L - 75 x 10^9/L)	Continue Treatment	Maintain Dose Level
Grade 3 (PLT 50 x 10^9/L - 75 x 10^9/L)	**Hold treatment.** Recheck blood counts weekly until PLT $\geq$ 75 x 10^9/L, then restart treatment.	Reduce Cytotoxic one dose level. If the patient is not receiving cytotoxic, decrease continuous 5FU 75% from prior dose.
Grade 4 (PLT < 25 x 10^9/L)	**Hold treatment.**	Reduce Cytotoxic one dose level. If the patient is not receiving cytotoxic, decrease

	Recheck blood counts weekly until PLT ≥ 75 x 10^9/L, then restart treatment.	continuous 5FU 75% from prior dose.

ANC = absolute neutrophil count; PTL = platelet count

[1] At the investigator's discretion growth factors may be used according to standard practice guidelines.

Table 6: Non-Hematologic (except Neurotoxicity) Dose Reductions (to be followed after 5FU bolus and LV dropped for first attributable toxicity occurrence as in Section 6.1).

Toxicity	Action	Dose Modification
Grade 1	Start Medical management. Continue Treatment	Maintain Dose Level
Grade 2	Start Medical management. Continue treatment.	If grade 2 toxicity (eg. diarrhea) persists despite aggressive medical management, may drop continuous 5FU and partner cytotoxic one dose level at physician discretion.
Grade 3	Start Medical management. Hold all treatment until resolved to Grade ≤1	Decrease 5FU (per **Table 3**) and partner cytotoxic one dose level.
Grade 4	Start Medical management Hold all treatment until resolved to Grade ≤1	Decrease 5FU (per **Table 3**) and partner cytotoxic one dose level.

Table 7: Dose Modifications for Oxaliplatin or Docetaxel[3] Induced Neurotoxicity

Toxicity	**Duration of Toxicity**		**Persistent Toxicity (at next due cycle)**
	1-7 Days	**>7 Days & <14 Days**	
Grade 1 **Paresthesias/dysesthesias**[1] of short duration that resolves and do not interfere with function	No Change	No Change	No Change
Grade 2 **Paresthesias/dysesthesias**[1] interfering with function, but not activities of daily living (ADL).	No Change	No Change	Decrease cytotoxic by one dose level.

Grade 3 Paresthesias/dysesthesias[1] with pain or with functional impairment that also interfere with ADL.	**1st event:** Decrease cytotoxic by 1 dose level. **2nd event:** Decrease cytotoxic by a second dose level.	Discontinue cytotoxic	Discontinue cytotoxic
Grade 4 Paresthesias/dysesthesias[1] that are disabling or life-threatening.	Discontinue cytotoxic	Discontinue cytotoxic	Discontinue cytotoxic
Pharyngo-laryngeal dysesthesias (All Grades)	Increase duration of infusion to 6 hours[2].	Increase duration of infusion to 6 hours[2].	Increase duration of infusion to 6 hours[2]

[1]May be cold-induced.

[2]Infusions extended to 6 hours because of toxicity may be administered over 2 days. Treatment continuation or discontinuation ultimately up to treating physician.

[3] To commence third line docetaxel, patients must have grade 2 or less neuropathy from prior oxaliplatin treatment. Treating physician may start docetaxel at one dose level lower for grade 2 oxaliplatin-induced neuropathy, at his/her discretion.

6.5 Trastuzumab

Trastuzumab administration may be delayed to assess or treat AEs, such as cardiac events. **However, no dose reduction is permitted for Trastuzumab**.

If Trastuzumab is held for more than two cycles or needs to be permanently discontinued, the patient will be continued with cytotoxic treatment (and other biologics if appropriate), and the patient will continue to be followed after treatment as described in Section 5.

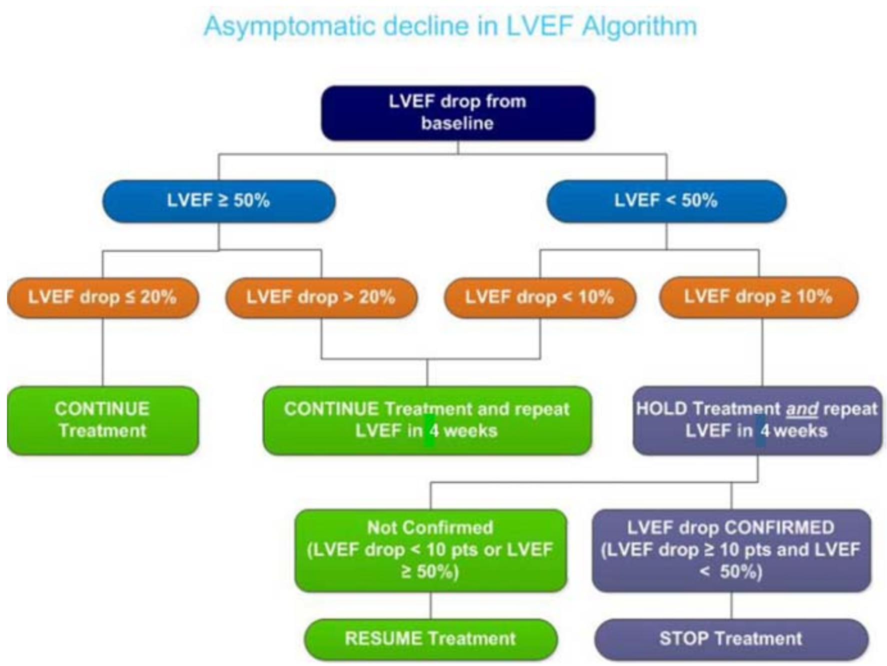
Dose Delays or Discontinuations due to Cardiac Events

In this study, all patients must have a baseline LVEF value ≥ 50%, and LVEF is to be monitored at least every 12 weeks during Trastuzumab antibody treatment. To ensure patient safety, if an investigator assesses that an AE may be related to cardiac dysfunction, an additional LVEF measurement should be performed, as well as other appropriate procedures such as chest X-ray, and the scheduled cardiac toxicity assessments will continue unchanged.

If symptomatic LVSD (CHF) is confirmed by a cardiologist's evaluation in any patient, Trastuzumab should be permanently discontinued, and the patient will continue cytotoxic chemotherapy (and other biologic therapy as appropriate) as tolerated, and followed per protocol. Symptomatic LVSD should be treated and monitored according to standard medical practice.

At the present time, there are inadequate data available to assess the prognostic significance of clinically asymptomatic decreases in LVEF values. However, **if a patient's LVEF value decreases to $<$ 50% and with an LVEF decrease of $\geq$ 10 points from baseline in the absence of symptoms, treatment with Trastuzumab should be withheld temporarily and the LVEF measurement repeated in <u>4 weeks</u>.** If decreased LVEF is confirmed, Trastuzumab should be permanently discontinued and the patient may be treated with cytotoxic therapy as tolerated, per protocol (**see figure below**).

LVEF, the algorithm shown in the Figure below should be followed to assess the LVEF decrease and determine whether to stop or continue study treatment (with both antibodies



and chemotherapy, if the patient is still receiving chemotherapy).

Infusion-Associated Reactions
Administration of Trastuzumab may result in infusion-associated symptoms such as nausea, pyrexia, diarrhea, chills, fatigue, and headache, or allergic reactions. The majority of hypersensitivity reactions was mild or moderate in severity and resolved upon treatment.

Study treatment will be administered in a clinical treatment setting with emergency equipment and staff who are trained to monitor for and respond to medical emergencies. Any patient who experiences a Grade 4 allergic reaction, bronchospasm, or acute respiratory distress syndrome (ARDS) associated with Trastuzumab will not be rechallenged with Trastuzumab.

Patients who experience infusion-associated symptoms may be managed by slowing or interrupting the Trastuzumab infusion and by providing supportive care with oxygen and medications (e.g., beta-agonists, antihistamines, antipyretics, corticosteroids), as determined by the investigator to be clinically appropriate.

In patients who have experienced infusion-associated symptoms, premedication with antipyretics, antihistamines, or corticosteroids may be used before subsequent infusions of Trastuzumab.

Any patient who experiences infusion-associated symptoms should be monitored under clinical observation until complete resolution of all signs and symptoms of the infusion-associated reaction.

On very rare occasions, patients have experienced the onset of infusion symptoms or pulmonary symptoms more than 6 hours after the start of the Trastuzumab infusion. Patients should be warned of the possibility of such a late onset and should be instructed to contact their physician if these symptoms occur.

If a patient cannot tolerate Trastuzumab infusions, Trastuzumab treatment will be permanently discontinued, but chemotherapy backbone may continue.

Incomplete Dose Administration
If a patient receives an incomplete dose of Trastuzumab because of an infusion related reaction or another reason, the following guidelines should apply: The patient should receive at least 50% of the dose during the first week of the treatment cycle.

Therefore, if the patient receives < 50% of the dose, the patient should receive the remainder before Day 15 preferably within the first week. Thereafter, the patient should receive the usual maintenance dose 2 weeks after the first interrupted dose as routinely scheduled.

- If the patient has received 50%–75% of the dose during the first week of the treatment cycle, the remainder should be given during the same treatment cycle (i.e., before Day 15 of that cycle), preferably before the end of the first week (i.e., before Day 8).

- If the patient received > 75% of the dose, the remainder should be given during the same treatment cycle (i.e., before Day 15 of that cycle). Alternatively, the patient may be given a reloading dose on Day 1 of the following treatment cycle.

- Please refer to Section 5.1.6 for guidance on re-loading in the event of missed Trastuzumab dose.

6.6 FGFR2 antibody (TBD)

6.7 ABT-806

If **at the start of a cycle** (ie, day 1 of a cycle) cytotoxic chemotherapy is held due to toxicity, then ABT-806 treatment will be held until the subject has recovered (in accordance with protocol guidelines). If ABT-806 is held due to toxicities, but cytotoxic chemotherapy has not met criteria for withholding per protocol, then cytotoxic chemotherapy may be administered. A missed dose of ABT-806 will not be made up.

If a subject experiences a grade 3 or 4 adverse event (thought secondary to ABT-806 such as edema), ABT-806 administration will be postponed until the toxicity resolves to grade 1 or returns to the subject’s baseline value with the exception that

ABT-806 may resume with grade 2 neutropenia (absolute neutrophil count (ANC) ≥ 1 x109/L).

Grade 1 and 2 infusion reaction: If a ABT-806 infusion is interrupted because of a grade 1 or 2 infusion reaction, the infusion may be restarted but at a reduced rate (50% rate reduction) after resolution of the event and stabilization of the subject (total infusion time increased up to 120 ± 10 minutes). All subsequent infusions should be administered at the reduced rate. If a subject is unable to complete an ABT-806 infusion on the day of the infusion due to an infusion reaction, then the remainder of the infusion will not be made up on subsequent days. For subjects who have had a grade 1 or grade 2 ABT-806 infusion reaction (that did not result in interruption of ABT-806 infusion) decreasing the ABT-806 infusion rate is at the discretion of the investigator.

Grade 3 infusion reaction: Subjects, who experience a grade 3 infusion reaction during ABT-806 administration, will have the ABT-806 infusion stopped and the remainder of that ABT-806 infusion will not be administered. Continuation of subsequent ABT-806 dosing after a grade 3 infusion reaction will be based on the severity and resolution of the event, and the grade 3 infusion reaction must be reviewed by the PI to determine if the subject can resume ABT-806. If a decision is made to continue ABT-806 treatment, all subsequent infusions should decrease the ABT-806 infusion rate by 50% (eg, increase the ABT-806 infusion duration from 60 ± 10 to 120 ± 10 minutes).

Grade 4 infusion reaction: Any subject who experiences a grade 4 infusion reaction must permanently discontinue ABT-806.

If ABT-806 is discontinued, but chemotherapy has not met withdrawal criteria per protocol, then cytotoxic chemotherapy may be continued.

6.8 Ramucirumab – dose adjustments and delays will be conducted per package insert.

If ramucirumab is discontinued, but chemotherapy has not met withdrawal criteria per protocol, then cytotoxic chemotherapy may be continued.

6.9 Nivolumab - – dose adjustments and delays will be conducted per package insert.

If nivolumab is discontinued, but chemotherapy has not met withdrawal criteria per protocol, then cytotoxic chemotherapy may be continued

6.10 MET Ab (TBD)

7. ADVERSE EVENTS: Definitions AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **Attribution** of the AE:
  - Definite (5) – The AE *is clearly related* to the study treatment.
  - Probable (4) – The AE *is likely related* to the study treatment.
  - Possible (3) – The AE *may be related* to the study treatment.
  - Unlikely (2) – The AE *is doubtfully related* to the study treatment.
  - Unrelated (1) – The AE *is clearly NOT related* to the study treatment.

7.2. Adverse Event Definitions

7.2.1 **Adverse Event**

An adverse event is defined as any untoward medical occurrence in a clinical trial subject. The event does not necessarily have a causal relationship with study treatment. The investigator is responsible for ensuring that any adverse events observed by the investigator or reported by the subject are recorded in the subject's medical record. The definition of adverse events includes worsening of a pre-existing medical condition. Worsening indicates the pre-existing medical condition (eg, diabetes, migraine headaches, gout) has increased in severity, frequency, and/or duration, and/or has an association with a significantly worse outcome. A pre-existing condition that has not worsened during the study, and involves an intervention such as elective cosmetic surgery or a medical procedure while on study is not considered an adverse event.

Disease progression itself is not considered an adverse event; however, signs and symptoms of disease progression may be recorded as adverse events or serious adverse events. Deaths due to progressive disease during treatment or 30 days after the protocol specified therapy is stopped, whichever is later, should be recorded as due to the primary tumor. If a new primary malignancy appears, it will be considered an adverse event.

The treating physician is responsible for reviewing laboratory test results and determining whether an abnormal value in an individual study subject represents a change from values before the study. In general, abnormal laboratory findings without clinical significance (based on the treating investigator's judgment) should not be recorded as adverse events; however, laboratory value changes requiring therapy or adjustment in prior therapy are considered adverse events. Where applicable, clinical sequelae (not the laboratory abnormality) should be recorded as the adverse event.
A persistent AE is one that extends continuously, without resolution, between patient evaluation time points. Such events should only be recorded once on the Adverse Event eCRF. The initial severity of the event should be recorded, and the severity should be updated to reflect the most extreme severity any time the event worsens. If the event becomes serious, the Adverse Event eCRF should be updated to reflect this.

A recurrent AE is one that resolves between patient evaluation time points and subsequently recurs.

The treating investigator's clinical judgment will be used to determine whether a subject should be removed from treatment or from the study due to an adverse event. A subject, or subject's parent/legal guardian, may also voluntarily withdraw from treatment due to an adverse event. If the subject withdraws full consent, the subject should be encouraged to undergo, at a minimum, an end-of-study assessment.

7.2.2 **Serious Adverse Events**

An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) Life-threatening (e.g. places subject at immediate risk of death, this does not include events that might have caused death if they occurred a greater severity)
3) Results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.

Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events could include allergic bronchospasm, convulsions, and blood dyscrasias, DILI or events that necessitate an emergency room visit, outpatient surgery, or other events that require other urgent intervention.

Since the criteria for CTCAE v4.0 severity differs from the regulatory criteria for serious adverse events, if adverse events correspond to grade 4 "life threatening" CTCAE v4.0 severity criteria (eg, laboratory abnormality reported as grade 4 without manifestation of life threatening status), it will be left to the investigator's judgment to also report these abnormalities as serious adverse events. For any adverse event that applies to this situation, comprehensive documentation of the event's severity status must be recorded in the subject's medical record.

7.2.3 **Unexpected Events**

Unexpected events are those not listed at the observed specificity or severity in the protocol (section 6), informed consent*, investigator brochure, or FDA-approved package insert.* An event is considered unexpected if it is listed as occurring within the class of drugs or otherwise expected from the drug's pharmacological properties but which has not been previously observed with this specific investigational agent.

7.2.4 **Adverse Reactions**

An adverse event is considered to be an adverse reaction if there evidence to suggest a causal relationship to the study agent. This may include a single occurrence of an event strongly associated with drug exposure (e.g. Stevens-Johnson Syndrome), one or more occurrence of an event otherwise uncommon is the study population, or an aggregate analysis of specific events occurring at greater than expected frequency.

7.3 Reporting Procedures for Serious Adverse Events

7.3.1 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported using the Serious Adverse Event Reporting Form and/or MedWatch Form discussed below must <u>also</u> be reported in routine study data submissions.**

The principal investigator is responsible for ensuring that all adverse events observed by the investigator or reported by the subject that occur on or after the first treatment of study therapy through 30 (+ 3) days after the last administration of protocol specified therapy are reported using the applicable eCRF (eg, Adverse Event Summary eCRF).

The investigator must assign the following adverse event attributes:

- Adverse event diagnosis or syndrome(s), if known (if not known, signs or symptoms),
- Dates of onset and resolution,
- Severity (and/or toxicity per protocol),
- Assessment of relatedness to biologic (ie trastuzumab or rilotumumab) and/or cytotoxic backbone (mFOLFOX6, FOLFIRI, FOLTAX)
- Action taken.

The adverse event severity grading scale used will be the CTCAE v4.0.

The treating investigator (in communication with the PI) must assess whether the adverse event is possibly related to the investigational biologic product in question (eg. Trastuzumab or rilotumumab) and/or cytotoxic chemotherapy (mFOLFOX6, FOLFIRI, FOLTAX). This relationship is indicated by a “yes” or “no” response to the question: “Is there a reasonable possibility that the event may have been caused by IP and/or cytotoxic regimen?”

The treating investigator (in communication with the PI) must assess whether the adverse event is possibly related to any study-mandated procedure or activity. This relationship is indicated by a “yes” or “no” response to the question: “Is there a reasonable possibility that the event may have been caused by a study procedure/ activity?”

7.3.2 **Serious Adverse Event Reporting**

All serious adverse events (as defined in section 7.3) occurring on this study require expedited reporting to the University of Chicago Comprehensive Cancer Center (UC CCC) Clinical Trials Office (CCTO). The responsible Research Nurse or other designated individual should report the SAE to the CCTO by the end of the business day when s/he becomes aware of the event. Events occurring after business hours should be reported to the CCTO by 12pm (noon) the next business day. Reports should be made using the eVelos database 'Serious Event Report' Form.

All serious and unexpected adverse reactions must also be reported to the FDA. The responsible Research Nurse or other designated individual should provide a complete written report using the FDA MedWatch 3500A form. The completed form should be sent to the CCTO at qaccto@bsd.uchicago.edu , within the specified timelines below regardless of whether all information regarding the event is available. If applicable, a follow-up report should be provided to the CCTO if additional information on the event becomes available.

Research staff (e.g. responsible research nurse) should NOT forward any adverse events directly to the FDA. The CCTO will report all events to the FDA as per the current FDA guidelines.

Fatal or Life-threatening Events: within 4 calendar days from treating investigator knowledge of the event

All Other Reportable Events: within 10 calendar days of treating investigator knowledge of the event

Any new information relating to a previously reported serious adverse event must be submitted to Amgen within 24 hours following knowledge of the new information. The investigator may be asked to provide additional follow-up information, which may include a discharge summary or extracts from the medical record. Information provided about the serious adverse event must be consistent with that recorded on the applicable CRF (eg, Adverse Event Summary eCRF).

When required, events requiring anticipated protocol-specified procedures such as hospitalization for administration of chemotherapy, blood product transfusion, central line insertion, or disease progression of a primary tumor are exceptions to the reporting of serious adverse events.

All serious adverse events should also be reported to the local IRB of record according to their policies and procedures.

For patients receiving Rilotumumab: Amgen will be notified of pregnancy and lactation within 10 days of sponsor awareness.

8. PHARMACEUTICAL INFORMATION

8.1 Biologic Agents

8.1.1 **Trastuzumab (Herceptin)**

This study will use commercially available Trastuzumab. For further details, see the current FDA approved Package Insert.

8.1.2 **MET Ab (TBD)**

8.1.3 **ABT806**
Abt-806 will be supplied by ABBVIE. For further details see the current Investigator Brochure for the agent.

8.1.4 FGFR2 ab (TBD)

8.1.5 **Nivolumab (Opdivo)**

This study will use commercially available Nivolumab. For further details, see the current FDA approved Package Insert.

8.1.6 **Ramucirumab (Cyramza)**
This study will use commercially available Ramucirumab. For further details, see the current FDA approved Package Insert.

8.2 Commercial Cytotoxic Agents

8.2.1 **5-Fluorouracil/Leucovorin**

This study will use commercially available 5-Fluorouracil and leucovorin. For further details, see the local prescribing information for each agent. Treating physician has discretion upon use (or not) of the bolus 5FU/LV depending on clinical circumstances.

8.2.2 **Oxaliplatin**

This study will use commercially available oxaliplatin. For further details, see the local prescribing information for each agent.

8.2.3 **Irinotecan**

This study will use commercially available irinotecan. For further details, see the local prescribing information for each agent.

8.2.4 **Docetaxel**

This study will use commercially available docetaxel. For further details, see the local prescribing information for each agent.

9. BIOMARKER ASSESSMENT METHODS

9.1 Background and Algorithm
Molecular profiling of tumors has led to the observation that although 'driver-oncogene' aberrations can be determined with novel medium-high throughput assays,the various genomic aberrations are not necessarily mutually exclusive. One tumor sample may have up to 10 genomic aberrations.[2,155] On average, we have observed ~3-5 genomic changes (mutations, amplifications, translocations) in GEC patients. Adding proteomic expression data to the genomic data adds another layer of complexity and 'over-lapping' of potential treatment categories.

Thus, in order to assign a patient to a predefined categorical molecular group that will be treated with one matched therapy, we have set a prioritization algorithm based on known clinical and preclinical information regarding the various potential molecular abnormalities (**Figure 3, 6**). Patients will be prioritized first by metastatic site if this is discordant from the primary tumor, then by prioritization as in **Figure 3.** If patients are negative for all biomarkers, or a treatment assignment is unable to be obtained, patients are assigned to group #8 (**Figure 3**) with anti-angiogenesis ramucirumab. Assays used for biomarker assessment via the biomarker assessment and treatment algorithm are described in Section 2.5.

9.2 Tissue Biomarker Assessment Methods[156]

Assays used for biomarker assessment via the biomarker assessment and treatment algorithm are described in Section 2.5. These will be performed at diagnosis/enrollment on **both the primary tumor sample and the metastatic lesion sample**. (ie. If diagnostic biopsy is from the primary site, after enrollment patients will get a biopsy of a metastatic site or vice versa). **The biomarker algorithm will be applied to both samples (primary/metastasis), and if discordant in terms of the results, biomarker classification and consequent recommended treatment assignment, the metastatic lesion will be prioritized and takes precedence.**

The **assays will also be performed on serial biopsies taken of a progressing lesion** each time of progression (PD1, PD2, PD3). The biomarker assay and treatment algorithm will be performed again at each time point, as described in Section 2.5. **While awaiting testing results, subjects should continue the current molecularly assigned therapy until results are available. If a biopsy cannot be performed (eg if no safe lesion to biopsy is identified) or biopsy is inadequate and rebiopsy is not possible or also failed, ctDNA results may be applied to guide targeted therapy, and subjects should move to the next line of standard cytotoxic treatment (FOLFIRI or FOLTAX). IF a patient withdraws consent for serial biopsy when possible during the trial, they will come off study and be treated with standard therapy.**

****Estimated # Biopsies: Baseline primary tumor, Baseline Metastatic Lesion, PD1, PD2, PD3 = 5.** Biopsies obtained for other clinical reasons along with ctDNA NGS results may also be used to guide therapy at progression points if these are all that is available.

9.2.1 Fluorescence in situ hybridization (FISH)

9.2.1.1 HER2

FISH for *HER2* will be conducted in the University of Chicago CLIA certified Core Cytogenetics Laboratory in the Department of Pathology and scored per standard clinical *HER2* FISH guidelines.[71] This will be done as a routine diagnostic test (regardless of HER2 IHC results) prior to starting therapy, in order to determine HER2 status and eligibility for Trastuzumab therapy.

9.2.1.2 **MET**

FISH for *MET* will be conducted a CLIA certified Core Cytogenetics Laboratory in the Department of Pathology or Clarient/Neogenomics, and scored per standard clinical *HER2* FISH scoring guidelines for GEC.

9.2.1.3 **FGFR2**

FISH for *FGFR2* will be conducted in the University of Chicago CLIA certified Core Cytogenetics Laboratory in the Department of Pathology or Clarient/Neogenomics, and scored per standard clinical *HER2* FISH guidelines for GEC.

9.2.1.4 **KRAS**

FISH for *KRAS* may be conducted in the University of Chicago CLIA certified Core Cytogenetics Laboratory in the Department of Pathology or Clarient/Neogenomics, and scored per standard clinical *HER2* FISH guidelines for GEC.

9.2.1.5 MSI-H/EBV+/TMB>15mt/Mb/ PDL1+ CPS>10%

NGS testing will include MSI status (MSI-H vs MSS) as well as tumor mutation burden (>15mt/Mb will be considered 'high').

EBV testing will be conducted at the University of Chicago using routine clinical assay called Eber ISH.

PDL1+ CPS≥10% is scored with the routine FDA approved IHC 22C3 PharmDx assay

9.2.1.6 **EGFR**

FISH for *EGFR* will be conducted in the University of Chicago CLIA certified Core Cytogenetics Laboratory in the Department of Pathology or Clarient/Neogenomics, and scored per standard clinical *HER2* FISH guidelines for GEC

.

9.2.2 Next-Generation Sequencing: Foundation One

Next Generation Sequencing (NGS) will be performed using the medium throughput clinically available Foundation One test evaluating 315 oncogenes and tumor suppressors, and includes MSI-High testing and Tumor Mutation Burden Testing (TMB). This is a CLIA certified routinely ordered test. Results will be applied to the biomarker and treatment assignment algorithm, as described in section 2.5.

Circulating Tumor DNA (ctDNA) will be obtained throughout patient care using routine clinically available CLIA certified laboratory, Guardant Health with the Guardant360 assay. These ctDNA NGS results will be largely correlated with biopsy results as a correlative study, but these results will be used to direct targeted therapy if/when biopsy results are not obtainable/insufficient from metastatic/growing disease at baseline/progression.

9.2.3 Immunohistochemistry

9.2.3.1 HER2

Immunohistochemistry for HER2 will be performed as a routine diagnostic test at the time of diagnosis per standard protocol (at the University of Chicago), prior to starting therapy, in order to determine HER2 status and eligibility for Trastuzumab therapy.[71] Results will be applied to the biomarker and treatment assignment algorithm, as described in section 2.5.

PDL1+ CPS>10%. – will be performed as a routine diagnostic test at the time of diagnosis using the 22C3 PharmDx IHC assay.

9.2.4 Mass Spectrometry: GEC-plex

Mass Spectrometry (MS) will be performed (Oncoplex Dx) using the medium throughput clinically available GEC-plex test evaluating 15 peptides.[119] Peptides include HER2, EGFR, HER3, MET, RON (3 peptides), FGFR1, FGFR2, KRAS (2 peptides), IGF1R, SRC, ROS1, PDL1, E-cadherin, Vimentin, and controls. This is a CLIA certified and routinely ordered test. Results will be applied to the biomarker and treatment assignment algorithm, as described in section 2.5.

9.2.5 Collection, Handling, and Shipping of Specimens

<u>Collection of Tissue Specimens (see Appendix C)</u>

- This pilot phase study is being conducted at the University of Chicago. Specimens will be collected in the routine diagnostic manner following standard procedure, including from surgical, core needle, and endoscopic biopsies.

- Patients will sign IRB protocol 16294A, the umbrella GI tissue and blood banking protocol, concurrently with the PANGEA consent.

- **Patients on anticoagulation will be bridged in the routine clinical manner usually followed for invasive procedures (using enoxaparin or other accepted clinical standard). Warfarin is not allowed** on protocol (see eligibility).

- Specimens will be formalin fixed and paraffin embedded following routine pathologic procedures in of the Department of Clinical Pathology. Routine diagnostic testing will be performed if there is yet to be an established diagnosis of cancer. If diagnosis is established, the obtained sample will be evaluated with one H&E slide to confirm adequate viability and percentage of tumor cells (>20%), as indicated in section 2.5.3 and **Figure 3**.

- One Core may be flash frozen and stored in the Catenacci laboratory for future use (eg. PAMGENE kinase activity analyses).

<u>Core Biopsies via Interventional Radiology (IR):</u>

- When obtaining Core biopsies, discussion with the research nurse and/or PI with Dr. Paul Chang or colleague in the Department of IR at UC will be done prior to each specified biopsy time point, based on most recent imaging, in order to determine the method (ultrasound or CT guided) and location (liver, lung, peritoneal etc) of biopsy to be conducted. **Safety of obtaining the biopsy will take precedence over the location. Every attempt will be made, however, to biopsy a progressing lesion (ie. that lesion felt most responsible for, or at least contributing to, progression by RECIST).**

An order for the biopsy will be placed as follows:

"Core needle biopsy with 19 gauge (or less) needle. Please obtain at least 6 core biopsies and process through routine diagnostic methods (formalin fix and send to diagnostic pathology). Please indicate 'For molecular testing, require only H&E to confirm viable tumor. Please place the four cores in parallel on on FFPE block' labeled on the sample. Please obtain an additional (at least 1) frozen core and page Leah Chase Clinical Research Associate for preparation of container and dry ice and pick up."

Leah Chase contact information:
lmchase@medicine.bsd.uchicago.edu
Pager:7918
If not available paging the PI Dr. Catenacci p3044

****The labeling on the biopsy sample, 'For molecular testing, require only H&E to confirm viable tumor. Please place the six cores in parallel on 2 FFPE blocks (3 cores each block)' (see Appendix C), will indicate that only H&E staining should be done ONLY if diagnostic biopsies have ALREADY been obtained for the patient and a confirmed diagnosis is already available.**

The core biopsy that is frozen will be stored in the Catenacci laboratory at -80 degrees Celsius for future studies.

<u>Handling, Allocation/Coordination, and Shipping of Specimens</u>

The specimens that are obtained and sent to pathology will be processed and assessed in the routine diagnostic manner. After diagnostic assessment of the percentage of viable tumor cells in the sample is completed by the pathologists (along with indicated tests including IHC HER2 and MET), the samples will be sent to central holding in Pathology. **There, Venessa Perez (with Emily O'Day) will coordinate ordering, shipping, and retrieval of the FFPE blocks and the results to/from the University of Chicago Core Diagnostics**

Laboratories and Quintiles Westmont (for IHC and FISH) and the other Commercial Diagnostics Laboratories (for Mass Spec and NGS) in the routine clinical manner (see Appendix C). Slides/tissue for testing on the blocks will be done in the following manner of prioritization:

1. **IHC** HER2 (HER2 at U of C Department of Pathology)
    a. HER2 will be performed in the usual clinically indicated manner
2. **FISH** HER2, MET, EGFR, KRAS, FGFR2 (Department of Pathology – Cytogenetics Laboratory)
    a. HER2 FISH will be performed in the usual clinically indicated manner
    b. MET, EGFR, KRAS, FGFR2 will be performed in the Cytogenetics laboratory via the laboratory developed test (LDT) and scoring methods.
3. **GEC-plex** (Oncoplex Dx, Rockville, MD, USA)
4. **Foundation One** (Foundation Medicine, Cambridge, MA, USA) Other commercial vendors for NGS may be considered if appropriate.

Tissue will be saved by cutting fifteen 4uM sections for the planned IHC and FISH initially, at one time, as well as onto three 10uM laser capture microdissection (LCM) slides (Oncoplex Dx slides), to limit waste from facing the block several times, as we have previously demonstrated.[1,2] An H&E will be done on the 1st and 15th 4uM slide cut. The three 10uM LCM slides and H&E (made from blank slide #15) will be sent to Oncoplex Dx for testing. The remaining FFPE tissue block will then be sent to Foundation Medicine. This will provide results from the four diagnostic modalities (IHC, FISH, MS, NGS) as efficiently and expediently as possible. The tissue remaining in the FFPE block will be returned to the University of Chicago and stored as is routinely done in the Department of Pathology. Results from these studies will be applied to the biomarker assessment and treatment algorithm as delineated in Section 2.5 and **Figure 3**.

9.3 Blood Banking

9.3.1 Collection of Specimens

Serum, plasma, and whole blood will be collected at C1D1 and C4D1 of each line of therapy, and at last disease progression. This amounts to a maximum of 7 draws per patient as below.
Patients may sign IRB protocol 16294A, the umbrella GI tissue and blood banking protocol, concurrently with the PANGEA consent, and PANGEA consent allows for samples to be collected under 16294A (ie one or the other or both are equivalent for banking, but will be stored under the same IRB 16294A for parsimony purposes).
All blood specimens MUST BE LABELED as follows:

1. Patient name (Full first and last name)
2. Medical record number
3. Protocol number
4. Date/time of blood draw
5. Indication of the time point of the draw (ie OxC1D1, End of Study-EOS):
    i) OxC1D1
    ii) OxC4D1
    iii) IriC1D1
    iv) IriC4D1

v) TaxC1D1
vi) TaxC4D1
vii) EOS

9.3.2 **Handling of Specimens and Blood Banking**

Blood will be collected as in 9.3.1, with one red top (serum), one green top (plasma) in 1ml aliquots and whole blood (one 10ml purple tops) for future analyses. The purpose of such analysis will be to look at other potential biomarkers and relation to clinical outcomes. Long term storage of samples (at least 5 years) will be at –80ºC in the Phase I/GI Freezer space.

9.4 Shipping of Specimens

Specimens will be shipped in the standard fashion to the outside clinical laboratories as in section 9.2.5 (Oncoplex Dx for Mass Spec, Foundation Medicine for Next-Generation Sequencing) per routine care in coordination with the University of Chicago Department of Pathology. This will be coordinated by Venessa Perez (Pathology) and Leah Chase, pager 7918(email lmchase@medicine.bsd.uchicago.edu) as is routinely done for clinical samples for patients not enrolled in clinical trials. Upon completion of testing, the pathology slides and block will be returned to the University of Chicago Department of Pathology.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 14 days prior to start of study treatment. Radiological scans must be done ≤4 weeks prior to the start of therapy, and it is preferred that the scans be done at the University of Chicago. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	Pre-Study	Wk 1 C1D1	Wk 2	Wk 3 C2D1	Wk 4	Wk 5 C3D1	Wk 6	Wk 7 C4D1	Wk 8	Wk 9 [G] C5D1	Wk 10	Wk 11 C6D1	Wk 12[H]	Off Study Visit[E]
Biologic Agent[A,K]: i)Trastuzumab for HER2+ [G] ii) nivolumab for MSI-H/EBV+/TMB-High>15mt-Mb/PDL1+ CPS>10% [G] *iii)* ABT-806 for EGFR+ [G] *iv)* Ramuciruamb for VEGFR2+ [G]		X--X								*X---------------------X* *X---------------------X* *X---------------------X*				
Cytotoxic[B] Chemotherapy (ie. 1^{st}, 2^{nd}, 3^{rd} line: i) mFOLFOX6 ii) FOLFIRI, iii) FOLTAX[L])		X		X		X		X		X		X		
Informed consent	X													
Medical history	X													
Concurrent meds	X	X--X												
Physical exam	X	X				X				X				X
Vital signs	X	X				X				X				X
Height	X													
Weight	X	X				X				X				X
Performance status	X	X				X				X				X
CBC w/diff, plts	X	X	X	X		X		X		X		X		X
Serum chemistry[C]	X	X	X	X		X		X		X		X		X
Ca 19-9 and CEA[I]	X	X								X				X
EKG	X													
Adverse event evaluation		X---X												X
Tumor measurements/ Radiologic evaluation with CT C/A/P infused	X[J]	**Tumor measurements are repeated every *8* weeks (4 cycles)**. Radiologic documentation must be provided for patients removed from study for progressive disease. ***Patients should be re-evaluated for response every 8 weeks (2 months) irrespective of receiving all intended chemotherapy doses or delays.**												X
MUGA or 2D cardiac ECHO[D]	X												X	
Biopsy/Tumor Blocks/slides[M] (see section 9.2.5)	X													X[M] (at PD1, PD2,PD3)
Blood for correlatives[F]		X						X						X

Opthalmology Exam[N]	X													

A: *Biologic Agent*:

- HER2 Positive: Trastuzumab 6 mg/kg IV C1D1 loading dose, then 4 mg/kg every cycle thereafter (every two weeks).
- MET Positive: Rilotumumab 10 mg/kg IV C1D1, every cycle thereafter (every two weeks).
- EGFR Positive: ABT-806 24 mg/kg IV C1d1, every cycle thereafter (every two weeks). See section 5.1.8 for premeds
- VEGFR2 Positive: Ramucirumab 8mg/kg IV C1D1, every cycle thereafter (every two weeks).

B: Patients are treated with standard cytotoxic chemotherapy, as tolerated, and changed to second, third line therapy, as tolerated, upon i) disease progression, ii) intolerance to prior regimen (see Section 11 and 6, respectively).

- **mFOLFOX6**
  On day one of each treatment session patients will receive mFOLFOX6 with oxaliplatin 85 mg/m2 given as a two-hour intravenous (IV) infusion. The dose of leucovorin will remain fixed at 200 mg/m2 as a two-hour IV infusion followed by 5-fluorouracil 400 mg/m2 IV push (bolus) and 5-fluorouracil 2400 mg/m2, given as a forty-six to forty-eight hour Infusion (Continuous Infusion).

- **FOLFIRI**
  FOLFIRI consists of 5-FU, leucovorin, and irinotecan**.** The dosing regimen for each subject will adhere to the protocol specifications. FOLFIRI dosing regimen will consist of *l*-LV 200 mg/m2 or *dl*-LV 400 mg/m2 as a 2-hour infusion, and irinotecan 180 mg/m2 given as a 90-minute infusion in 500 mL dextrose 5% via a Y-connector, followed by bolus FU 400 mg/m2 and a 46-hour infusion FU 2400 mg/m2.

- **FOLTAX**
  FOLTAX consists of 5-FU, leucovorin, and docetaxel**.** The dosing regimen for each subject will adhere to the protocol specifications. FOLTAX dosing regimen will consist of *l*-LV 200 mg/m2 or *dl*-LV 400 mg/m2 as a 2-hour infusion, and docetaxel 50 mg/m2 given as a 90-minute infusion in 500 mL dextrose 5% via a Y-connector, followed by bolus FU 400 mg/m2 and a 46-hour infusion FU 2400 mg/m2.

***5FU reductions (bolus and/or continous infusion) will carry over to second and third line therapy.**

C: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

D: Echocardiogram or MUGA at screening for HER2 positive patients, to evaluate baseline cardiac ejection fraction. Repeat every 3 months. **If patients are not HER2 positive at baseline, but become HER2 positive at serial biopsy (ie PD1, PD2) and it is determined that the patient be treated with trastuzumab, a baseline study must be done at that time prior to commencing trastuzumab, to ensure EF>50%.

E: Off-study evaluation within 30 days of last dose of therapy.

F: Blood collection and banking as in section 9.3, prior to administration of C1D1 and C1D4 of each line of chemotherapy and after last progression (ie PD3 or prior) for end of study sample, for a maximum of 7 blood draws throughout the trial. The Blood Collection Form (Appendix A) is required for each sample collection to indicate the time point (eg OxC1D1, see Section 9.3.1) that the sample was acquired. *Patients will sign IRB protocol 16294A, the umbrella GI tissue banking protocol, concurrently.

G. Note that HER2 patients begin therapy with trastuzumab from C1D1 per clinical standards, if Her2 testing results available from the metastatic site,

All other patients (HER2- will be treated with chemotherapy only (mFOLFOX6→FOLFIRI→FOLTAX) in the standard manner, until molecular profiling results become available at which time patients will be assigned and treated within the appropriate group.

H. The studies continue as indicated for each consecutive cycle until progression. At PD1 cytotoxic therapy is changed to second line FOLFIRI. At PD2, cytotoxic therapy is changed to FOLTAX. Assigned biologic agent continues beyond progression, unless repeat molecular testing suggests migration to a different molecular category, at which point the biologic therapy is changed to the appropriate new drug that matches the new molecular class – see protocol for further details. (see Section 5.1.2)

I. CEA Ca19-9 will be drawn at baseline and at least every two months with CT scans and at progression/off study evaluation.

J. Baseline CT for staging must be within 4 weeks of starting treatment, preferred at the University of Chicago. Baseline CT prior to starting off protocol treatment will be compared to the next CT and going forward, per RECIST, and included and assess as ORR1 best response evaluation.

K. *Allow for up to 2 months for molecular testing when the molecularprofiling results become available.*

L. Pre-med for the Docetaxel will premedicate with decadron 16mg/day (8mg twice daily) orally for 3 days, starting the day before docetaxel administration.

M. Biopsies through the course of therapy will be at the time of PD1 (first progression on FOLFOX), PD2 (second progression on FOLFIRI), and PD3 (third progression on FOLTAX). (See section 2.4, 2.4.4, and section 9)

N. Ophthalmology exam baseline for patients assigned to FGFR2 arm (drug TBD).

*** ALL lab tests, imaging, and treatment dosing may be done within a +/- 7 day window for holidays, weekends, unforeseen events (eg. inclement weather) and scheduling convenience. Routine labs may be drawn closer to home for**

patient convenience. CTs and prospective tumor biopsies are preferred to be done at the University of Chicago Hyde Park Site.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, **patients should be re-evaluated for response every 8 weeks (2 months) irrespective of receiving all intended chemotherapy doses or delays.**

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1). Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used in the RECIST criteria.

11.1.1 **Definitions**

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment on C1D1.

Evaluable for objective response. All registered patients will be considered evaluable for response by intention to treat. Subset analysis of patients receiving one or more doses will also be done. These patients will have their response classified according to the definitions stated below and in section 13.

11.1.2 **Disease Parameters**

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area will not be considered measurable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be **≥15 mm** in **short axis** when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and **in follow-up**, **only the short axis will be measured** and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Discussion with interventional radiology (Paul Chang and/or colleague) to determine the best baseline metastatic lesion (representative of disease, feasible/safe) to biopsy should be done, and this lesion that is ultimately biopsied included in the measurable lesions.   Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

At disease progression by RECIST, or clinician's discretion of clinical progression despite RECIST, another discussion with interventional radiology with ensue in order to determine best progression lesion to re-biopsy, and similarly the lesion that is ultimately biopsied included in RECIST calculation, if not already, moving forward.

11.1.3 **Methods for Evaluation of Measurable Disease**

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Conventional CT and MRI This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Ultrasound Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Ascites:

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

If previous ascites and/or pleural effusion is stable by RECIST, but the effusion worsens and is amenable to therapeutic decompression, and the patient is otherwise tolerating therapy and otherwise clinically stable, the patient may be considered stable with final decision per discretion of the treating physician and PI.

11.1.4 **Response Criteria**

11.1.4.1 **Evaluation of Target Lesions**

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

11.1.4.2 **Evaluation of Non-Target Lesions**

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis)

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Although a clear progression of “non-target” lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 **Evaluation of Best Overall Response**

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

<table>
<tr><th>Target Lesions</th><th>Non-Target Lesions</th><th>New Lesions</th><th>Overall Response</th><th>Best Response for this Category Also Requires:</th></tr>
<tr><td>CR</td><td>CR</td><td>No</td><td>CR</td><td>≥4 wks. Confirmation**</td></tr>
<tr><td>CR</td><td>Non-CR/Non-PD</td><td>No</td><td>PR</td><td rowspan="2">≥4 wks. Confirmation**</td></tr>
<tr><td>CR</td><td>Not evaluated</td><td>No</td><td>PR</td></tr>
</table>

PR	Non-PD/not evaluated	No	PR	
SD	Non-PD/not evaluated	No	SD	documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	
Any	PD*	Yes or No	PD	no prior SD, PR or CR
Any	Any	Yes	PD	

* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

** Only for non-randomized trials with response as primary endpoint.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

11.1.5 **Duration of Response**

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

11.1.6 **Progression-Free Survival**

PFS is defined as the duration of time from enrollment/registration to time of progression or death from any cause (PFS1+2+3).
On this trial patients we will have up to three phases of treatment, as tolerated and indicated, as follows:

PFS_1: This, a secondary objective, is defined as the duration of time from enrollment/registration to time of progression on mFOLFOX6 chemotherapy and assigned biologicUpon first progression (PD_1) by RECIST, patients change to second line therapy as tolerated.

PFS_2: This is defined as the duration of time from declaration of PD_1 to time of progression on second line FOLFIRI chemotherapy and assigned biologic Upon second progression (PD_2) by RECIST, patients change to third line therapy as tolerated.

PFS_3: This is defined as the duration of time from declaration of PD_2 to time of progression on third line FOLTAX chemotherapy and assigned biologic. Upon third/final progression (PD_3) by RECIST, patients are removed from study.

11.1.7 **Response Review**

Response rate is a secondary objective. As above, RR at each line of therapy (RR_1, RR_2, RR_3) will be recorded. In the event that patients are receiving mFOLFOX6 alone while molecular testing is being conducted and repeat imaging is performed, this will be denoted as RR_0.

11.2 Other Response Parameters and Objectives

11.2.1 **Overall survival,** a primary objective, is defined as the duration of time from enrollment/registration to the time of death, of any cause.

11.2.2 **Toxicity**, a secondary objective, is defined as in sections 6 and 7.

11.2.3 **Safety**, a primary objective, will be assessed with respect to SAE due to baseline and serial biopsies. A rate of $\leq$5% SAE will be deemed acceptable for the a) elective baseline and b) serial biopsies, considered separately. Considering all enrolled patients (n=68), this will produce a 95% confidence width of approximately +/- 4.3% (+/-5.3% for the primary endpoint within the cohort of 68 patients undergoing study treatment) at for the baseline biopsy and the same for serial biopsies (this is a conservative estimate of precision given the possibility of multiple biopsies in each patient - up to 3). Additionally, the safety lead-in of novel combinations of chemotherapy regimen with cytotoxic regimen will be followed, as in section 5.1.1.

11.2.4 **Feasibility,** a primary objective, will be assessed and the trial deemed feasible if i) $\geq$85% of enrolled patients **are successfully assigned, using our biomarker assessment and treatment assignment algorithm, by two months** from biopsy; and ii) if $\geq$85% of enrolled patients have successful serial biopsy at first progression (PD1) and successful reassignment to biomarker group and matched treatment. Considering all enrolled patients (n=68), this produces a 95% confidence width of approximately +/- 7% (+/-8.7% for the primary endpoint within the cohort of 68 patients) for the baseline biopsy and the same for serial biopsies (again, this is conservative given the possibility of multiple biopsies in each patient - up to 3). Patients can initiate cytotoxic therapy (FOLFOX) while awaiting biologic results/assignment, upon which the biologic is added to next cycle.

12. DATA SAFETY REVIEW

The PI will meet by teleconference with relevant pharmaceutical collaborators twice monthly during phase I safety lead-in (section 5.1.1) and at least monthly thereafter. Weekly meetings within the GI Oncology group will occur and review the previous week's events, AEs, SAEs, and other pertinent information. The DSRC will be comprised of the study Principal Investigator, Dr. Catenacci and co-investigators of the study.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

Safety, a primary objective, will be assessed with respect to SAE due to baseline and serial biopsies. A rate of $\leq$5% SAE will be deemed acceptable for the a) elective baseline and b) serial biopsies, considered separately. Considering all enrolled patients (n=104), this will produce a 95% confidence width of approximately +/- 4.3% (+/-5.3% for the primary endpoint within the cohort of 68 patients undergoing study treatment) at for the baseline biopsy and the same for serial biopsies (this is a conservative estimate of precision given the possibility of multiple biopsies in each patient - up to 3). Additionally, the safety lead-in of novel combinations of chemotherapy regimen with cytotoxic regimen will be followed, as in section 5.1.1.

Feasibility, a primary objective, will be assessed and the trial deemed feasible if i) $\geq$85% of enrolled patients are successfully assigned, using our biomarker assessment and treatment assignment algorithm, by two months from biopsy; and ii) if $\geq$85% of enrolled patients have successful serial biopsy at first progression (PD1) and successful reassignment to biomarker group and matched treatment. Considering all enrolled patients (n=68), this produces a 95% confidence width of approximately +/- 7% (+/-8.7% for the primary endpoint within the cohort of 68 patients) for the baseline biopsy and the same for serial biopsies (again, this is conservative given the possibility of multiple biopsies in each patient - up to 3).

In addition to Safety and Feasibility, an efficacy **primary endpoint** (in addition to the safety and feasibility endpoints above and in 11.2.3 and 11.2.4) of this single arm pilot feasibility and exploratory 'phase IIa' pilot trial is median overall survival (mOS) of the all ITT categories combined, as defined in section 11.2.1. The main analysis will be an intent-to-treat (ITT) analysis incorporating all screened patients included/enrolled into the study (N=68).

A mOS time of approximately 12 months in gastric/gastroesophageal adenocarcinoma is observed historically, regardless of which first line and subsequent lines of chemotherapy used, including mFOLFOX6.[14] This will be used as the estimated mOS This will require a total of approximately 68 patients.

Survival time will be measured from the date of initial enrollment. We will estimate overall survival using the Kaplan-Meier (1958) method, and compare the observed 12-month survival rate of the combined ITT targeted therapy categories to 50%, using a binomial test if there is no censoring, or a z-test based on the Greenwood standard error to accommodate censoring if necessary. Assuming mOS of 12 months in the historical control group, and 18 months in the personalized treatment approach (HR=0.67), and exponential survival, a total sample size of 68 subjects provides 80% power to detect a difference of this magnitude (63% 12-month survival rate), using a one-sided test at the 0.10 significance level. This assumes 24 month accrual period, subsequent 12 month follow-up period, with expected (H_0) total events (death) of 34 (0.50*68), compared to (H_1) 25 ((1-0.63)*68), in this single arm exploratory study**.**

***It is recognized that only 4 of 6 drugs are currently available for the proposed arms of personalized therapy.** Notably, as molecular categories (FGFR2, MET) have yet to secure a drug for treatment, the initial iteration of the trial will be assessing the survival primary endpoint for only those treated by intention to treat the combined

HER2++, MSI-H/EBV+/TMB-High$\geq$15mt-Mb/PDL1+ CPS$\geq$10%, EGFR++/+, VEGFR2++/+ categories, along with the safety and feasibility endpoints. Patients who obtain anti-FGFR2 or anti-MET therapies will also be included in ITT analyses. For patients in any category initially *without* intended biologic therapy available, they will be treated with standard of care.

On a rolling basis, as new drugs become available for the remaining vacant arms (ie. FGFR2, MET) ongoing enrollment of patients will be assigned to the appropriate therapy after amendment of the protocol has been approved to include the acquired agent.

13.2 Sample Size/Accrual Rate

The planned sample size will be 68 initial patients, as in section 13.1, for the per protocol ITT population (treated from the onset with appropriate targeted therapy). **The primary endpoint is evaluating the personalized treatment strategy compared to historical control in this Type IIa Expansion Platform Clinical Trial Design.[157]**

13.3 Analysis of Secondary Endpoints

13.3.1 **Secondary endpoints**

Secondary endpoints will include **mPFS** and overall response rates (**ORR**) for the combined HER2++, - MSI-H/EBV+/TMB-High$\geq$15mt-Mb/PDL1+ CPS$\geq$10%, EGFR++/+, VEGFR2++/+ categories and any patient treated with antiFGFR2/MET during the course of the study (N=68), evaluated at each interval of therapy and total ($_{1,2,3}$ and $_{1\text{-}3}$). mPFS will be compared to historical controls of 6 months for $mPFS_1$, 4 months for $mPFS_2$, and 2 months for $mPFS_3$. **ORR** will be compared to a historical control of 30% for ORR1, 20% for ORR2 and 10% for ORR3 using an exact binomial test.

Adverse events will be summarized by type and grade and compared between groups using chisquare or Fisher exact tests, as appropriate.

13.3.2 **Subset Analyses between and within Molecular Categories**

A number of preplanned subset analyses will be performed. This is due to
i) Inter-category variation. The varying prognostic implications of each molecular category (ie MET+ have worse prognosis than HER2+ with mOS of ~11.8 months for HER2+ versus 5.7 months for MET+ without matched biologic agent).
ii) Intra-category variation. We will assess differences (exploratory) between HER2 positive patients that are 2+ IHC/FISH+ and IHC3+/FISH+ as well as by Mass Spectrometry HER2+ expression. Similarly, for the MET+ category, this will consist of patients with *MET* amplification and consequent MET overexpression, as well as MET overexpression without gene amplification. These subgroups will be assessed individually in an exploratory and descriptive manner. The other subgroups will also be evaluated as such including but not limited to EGFR amplified versus EGFR overexpressing, KRAS amplified versus KRAS mutant versus relegation ‘not fitting into any other category’ (B9, **Figure 3**).

13.4 Reporting and Exclusions

13.4.1 **Evaluation of toxicity**

All patients will be evaluable for toxicity from the time of their first treatment with any biologic therapy. Adverse events will be summarized by type and grade and compared between groups using chi-square or Fisher exact tests.

13.4.2 **Evaluation of response**

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) will be included in the main analysis of the response rate. Patients in response categories 4-9 will be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration will not result in exclusion from the analysis of the response rate.

All conclusions will be based on all eligible patients. Subanalyses will then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses will not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis will be reported.

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PANGEA

IRB# 14-0141

Appendix A
The University of Chicago

Blood Sample Collection Form

PANGEA-IMBBP: Personalized ANtibodies for Gastro-Esophageal Adenocarcinoma - A 1st Pilot Metastatic Trial of Biologics Beyond Progression

Blood Banking: i) Banking of serum (1 red top), ii) plasma (1 green top), and iii) Whole Blood for DNA (1 purple top) for future studies

Clinician/Research Nurse: Please Fill Out

Blood Samples

Patient Name: ____________________ Timpoint (eg OxC1D1 __________________

Patient Protocol ID #:_______________ Date Blood Obtained: __________________

Date of Birth: ______________________ Attending Physician: __________________

Visit	Collection Tubes to use:	Date Drawn	Processing	Please check all samples collected
OxC1D1	One 6ml Red (serum) Top One 6ml Green (plasma) Top One 10ml Purple (whole blood) Tops		Two 1 ml aliquots Two 1 ml aliquots No processing purple tops	
OxC4D1	One 6ml Red (serum) Top One 6ml Green (plasma) Top One 10ml Purple (whole blood) Tops		Two 1 ml aliquots Two 1 ml aliquots No processing purple tops	
IriC1D1	One 6ml Red (serum) Top One 6ml Green (plasma) Top One 10ml Purple (whole blood) Tops		Two 1 ml aliquots Two 1 ml aliquots No processing purple tops	
IriC4D1	One 6ml Red (serum) Top One 6ml Green (plasma) Top One 10ml Purple (whole blood) Tops		Two 1 ml aliquots Two 1 ml aliquots No processing purple tops	
TaxC1D1	One 6ml Red (serum) Top One 6ml Green (plasma) Top One 10ml Purple (whole blood) Tops		Two 1 ml aliquots Two 1 ml aliquots No processing purple tops	
TaxC4D1	One 6ml Red (serum) Top One 6ml Green (plasma) Top One 10ml Purple (whole blood) Tops		Two 1 ml aliquots Two 1 ml aliquots No processing purple tops	
EOS	One 6ml Red (serum) Top One 6ml Green (plasma) Top One 10ml Purple (whole blood) Tops		Two 1 ml aliquots Two 1 ml aliquots No processing purple tops	

APPENDIX B

ACCEPTABLE AND UNACCEPTABLE FORMS OF CONTRACEPTION FOR WOMEN OF CHILDBEARING POTENTIAL

Women of childbearing potential are required to use two forms of acceptable contraception, including one barrier method during participation in the study and for the 12 months following the last dose.

Acceptable forms of contraception for women of childbearing potential:

Primary Forms

- Tubal ligation
- Partner's vasectomy
- Intrauterine device
- Hormonal (combination birth control pills, skin patches, injections, implants, or vaginal ring)
- Barrier forms (always used with spermicide)

Secondary Forms

- Diaphragm
- Cervical cap
- Barrier form (used with or without spermicide)
- Male latex condom
- Vaginal sponge (contains spermicide)

Unacceptable forms of contraception for women of childbearing potential:

- Birth control pills without estrogen
- IUD progesterone T
- Female condom
- Natural family planning (i.e., rhythm method) or breastfeeding
- Fertility awareness
- Withdrawal
- Cervical shield

APPENDIX C

COLLECTION OF TISSUE SPECIMENS ALGORITHM