Cover Page

Study Protocol and Statistical Analysis Plan

Official Title: A RANDOMIZED, OPEN LABELED PHASE II PILOT STUDY OF TOTAL NEOADJUVANT CHEMOTHERAPY WITH FLOT (FLOT-TNT) VS STANDARD PERIOPERATIVE FLOT (FLOTPOP) IN PATIENTS WITH GASTRIC OR GEJ CANCER, AND ASSESSMENT OF ASSOCIATION OF MOLECULAR CORRELATES AND RESPONSE (TOGAR)

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TMH: The Methodist Hospital

Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
Title Page, footer	The co-investigator list was updated, protocol version and version date were updated	Version control/version tracking
Study Schema	Removed duplicate study schema	The duplicate study schema eas removed
Section 2.0 Study Synopsis	The negative diagnostic laparoscopic (DL) assisted cytology of peritoneal fluid cytology was updated from not more than 28 days before registration to not more than 42 days before registration.	A DL at 42 days is an acceptable timeframe in standard of care approach.
Section 12.1 Inclusion Criteria	The negative diagnostic laparoscopic (DL) assisted cytology of peritoneal fluid cytology was updated from not more than 28 days before registration to not more than 42 days before registration.	A DL at 42 days is an acceptable timeframe in standard of care approach.

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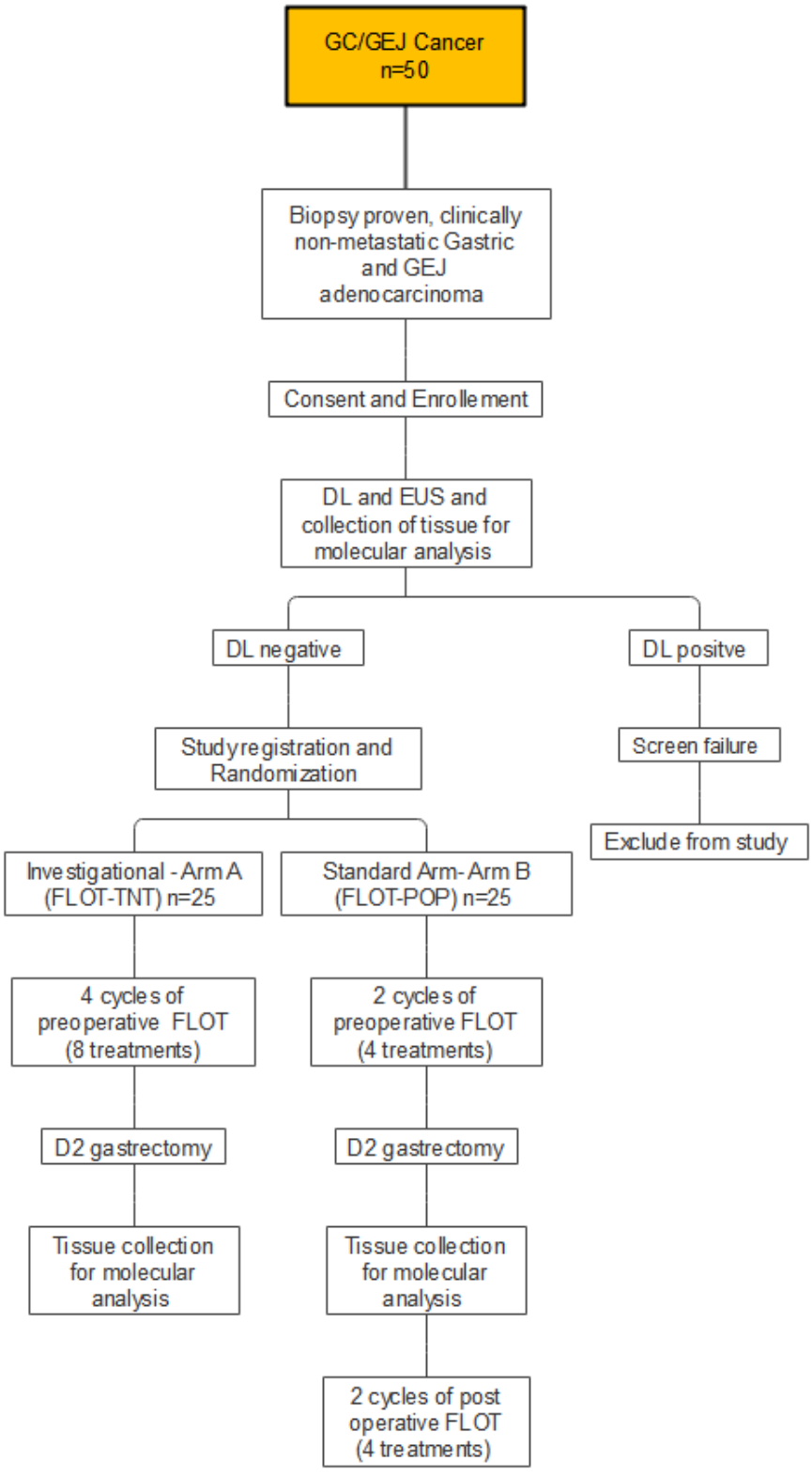
STATEMENT OF COMPLIANCE

This trial will be conducted in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP), and the applicable United States (US) Code of Federal Regulations (CFR). All personnel involved in the conduct of this study have completed Human Subjects Protection and Good Clinical Practice trainings. The protocol, informed consent form, recruitment materials, and all participant materials will be submitted to the IRB for review and approval prior to implementation. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made whether participants will need to be re-consented.

LIST OF ABBREVIATIONS AND DEFINITIONS

Abbreviation	Definition
5FU	5-fluorouracil
AC	Adenocarcinoma
ACRT	Adjuvant Chemoradiation
AE	Adverse Event
BSA	Body Surface Area
CAP	College of American Pathologists
CI	Continuous infusion
CRC	Colorectal Cancer
CT	Computed Tomography
DFS	Disease Free Survival
DL	Diagnostic laparoscopy
ECOG	Eastern Cooperative Oncology Group
EGD	Endoscopic Gastroduodenoscopy
EUS	Endoscopic Ultrasound
FDG	Fluorodeoxyglucose
FLOT	5fu+ LV + Oxaliplatin + Docetaxel
GC	Gastric Cancer
GCSF	Granulocyte Colony-Stimulating Factor
GEA	Gastroesophageal Adenocarcinoma
GEC(s)	Gastroesophageal Cancer(S)
GEJ	Gastroesophageal Junction Cancer
HTAP	Human Tissue Acquisition and Pathology
LNRR	Lymph Node Negativity Rate
LV	Leucovorin
MRD	Molecular Residual Disease
PCR	Pathologic Complete Response
POP	Perioperative Therapy
PRBC	Packed Red Blood Cell
SCC	Squamous Cell Carcinoma
SOX	S-1 And Oxaliplatin
TNT	Total Neoadjuvant Therapy
TRG	Tumor Regression Grade
XELOX	Xeloda And Oxaliplatin

1.0 STUDY SCHEMA



2.0 SYNOPSIS

Title	A Randomized Open Labeled Phase II Pilot Study Of Total Neoadjuvant Chemotherapy With FLOT (FLOT-TNT) vs Standard Perioperative FLOT (FLOT-POP) in Patients with Gastric or GEJ Cancer, and Assessment of Association of Molecular Correlates and Response (TOGAR)
Phase	II Pilot
Number of Subjects	25 evaluable subjects on each arm for a total of 50 evaluable subjects.
Study Design	Total Neoadjuvant Chemotherapy with FLOT (FLOT-TNT) vs Standard Perioperative Chemotherapy with FLOT. Participants will be randomized 1:1 to either Total Neoadjuvant chemotherapy with FLOT-TNT (all 4 cycles of chemotherapy before surgery) or standard of care FLOT-POP (2 cycles of chemotherapy before surgery and 2 cycles of chemotherapy after surgery). Each cycle of chemo is 28 days long with chemo administered on Days 1 and Day 15 of the cycle.
Arm A (Investigational)	FLOT-TNT: Total Neoadjuvant chemotherapy with FLOT
Arm B (Standard)	FLOT-POP: Perioperative chemotherapy with FLOT
Hypothesis	Roughly 40-50% of subjects with GC/GEJ cancer on peri-operative chemotherapy do not complete their planned chemotherapy after surgery. Elimination of micrometastasis and increase in pathological nodal response correlates with survival. Chemotherapy in the post-operative setting is more challenging for the subjects and is frequently incomplete. In this study, we aim to evaluate the feasibility of giving all cycles of chemotherapy in the neoadjuvant setting (FLOT-TNT) to maximize pathological response that is known to impact overall survival. Currently, there are no known predictive or prognostic biomarkers to predict response or survival rates for treatment of locally advanced GC. There is a clear deficiency of knowledge regarding the pathogenesis of gastric and GEJ cancer. We aim to study molecular markers through an integrative multi-“omic” approach in our exploratory analysis to better understand the biological behavior of these cancers. Further elucidation of the molecular proteogenomics of locally advanced GC and GEJ cancer will potentially lead to improvements in patient survival
Primary Objective	The primary objective is to determine the proportion of participants who complete all allocated cycles of chemotherapy by comparing Arms A and B. The completion rate in Arm A: FLOT-TNT is expected to be higher than in Arm B: FLOT-POP.
Secondary Objectives	To estimate the nodal pathological response rate by comparing the two study arms on surgical nodal status. The frequency of positive nodes is expected to be lower in Arm A: FLOT-TNT compared to Arm B: FLOT-POP.
Primary Endpoint	The proportion of participants who complete their allocated cycles of chemotherapy in both Arms A and B.
Secondary Endpoints	In both Arm A and Arm B: • Pathologic response and nodal status based on TRG

Exploratory Objectives	In both arms A and B 1) Elucidate the molecular signature (integrated genomic, transcriptomic, and proteomic changes) associated with pathological complete response rates (as determined by TRG) 2) Assess for molecular (integrated genomic, transcriptomic, and proteomic) changes associated with arm A and arm B 3) Determine the feasibility of establishing gastric and gastroesophageal cancer organoids pre- and post- neoadjuvant FLOT chemotherapy as a mechanism to individualize treatment. 4) Disease free survival
Inclusion criteria	a. Must provide written informed consent. b. Must be ≥18 years of age. c. Must have life expectancy of greater than 3 months. d. Must have pathologically proven Siewert type II and III GEJ or gastric adenocarcinoma from the main tumor or local lymph nodes (pre-neoadjuvant chemo). e. Stage cT2 or higher, any N and M0, are eligible for the study. f. M0 disease must be established by both negative distant metastatic disease on imaging AND negative cytology of peritoneal fluid collected from the diagnostic laparoscopy not more than 42 days before registration. g. Must be a candidate for Neoadjuvant chemotherapy. h. Must be a candidate for curative surgical approach. i. Must have an ECOG performance status 0-2. j. Male or female subjects of childbearing potential must be willing to use contraceptive precautions throughout the trial and for 3 months after discontinuation of study treatment. Female subjects of childbearing potential must have a negative pregnancy test within 28 days of registration. Post-menopausal women must be amenorrhoeic for at least 12 months to be considered of non-childbearing potential. k. Must have adequate kidney, liver, and bone marrow function, within 28 days prior to registration, as follows: i. Hemoglobin ≥ 8.0 gm/dL (PRBC transfusion is allowed to meet this criteria) ii. Absolute neutrophil count (ANC) ≥ 1000 cells/mm3 iii. Platelet count ≥ 100,000 /mm3 iv. Total bilirubin ≤ 1.5 times upper limit of normal (ULN) v. AST (SGOT) and ALT (SGPT) ≤ 3.0 times the ULN vi. Patient must have adequate renal function as evidenced by one of the following: Serum creatinine ≤ IULN OR calculated creatinine clearance ≥ 60 mL/min. This serum creatinine result must be obtained within 28 days prior to registration. Calculated creatinine clearance = $\frac{(140 - \text{age}) \times \text{wt}^* \text{ (kg)} \times 0.85 \text{ (if female)}}{72 \text{ Creatinine}^{**} \text{ (mg/dl)}}$ *The kilogram weight is the subject's actual body weight with an upper limit of 140% of the IBW. ** Actual lab serum creatinine value with a minimum of 0.8 mg/dL

	l. Subjects who have required a short course urgent single modality non curative radiation treatment or gastric artery embolization for the purpose of tumor bleeding control are eligible.
Exclusion Criteria	a. Positive cytology for metastatic disease on diagnostic laparoscopy peritoneal fluid. Reports such as: "cannot rule out malignancy" or "suspicious for malignancy, but not definitive" will exclude the subject from enrolling. b. Seiwert type I GEJ cancer c. Subjects with clinical evidence of metastatic disease. d. Biopsy proven metastatic disease (excluding regional lymph nodes) e. Prior chemotherapy for gastric or GEJ cancer. f. No other prior malignancy is allowed except for the following: adequately treated basal cell or squamous cell skin cancer, current non-advanced prostate cancer per the discretion of the investigator, and any other cancers from which the patient has been disease free for two years. g. Female subjects who are pregnant, breast feeding, or of childbearing potential with a positive pregnancy test prior to baseline. Women of childbearing potential must have a negative serum pregnancy test as a part of eligibility within 28 days of registration. A persistent positive or elevated urine or blood Beta HCG test may be contributed to the primary diagnosis of GC or GEJ cancer after ruling out ectopic and intrauterine pregnancy and germ cell tumors. h. Subjects unwilling or unable to comply with the protocol or provide written informed consent. i. Any medical condition that, in the opinion of the investigator, would exclude the subject from participating in this study and treatment plan. j. ECOG > 2
Criteria For Evaluation	Efficacy: Baseline scans and labs and CEA, RECIST criteria for response, surgical pathology evaluation post operation. Efficacy is analyzed by imaging, pathology review, and tumor marker. Investigational Biomarker: Integrated genomics, transcriptomics, and proteomics based on changes in pre and post treatment tissues.
Statistical Methods	<u>Power Analysis of primary objective:</u> Based on published data, we expect that 45% of subjects in the FLOT-POP arm will complete all allocated chemotherapy. We are expecting 90% of subjects in the FLOT-TNT arm will complete the first 2 cycles [22] and about 90% of those will complete the second 2 cycles resulting in a completion rate of 80%. With 25 evaluable subjects in each group, we will have 85% power (alpha=10%, one-tailed) to detect an absolute improvement of 35% (FLOT-TNT completion rate=80%) using a Fisher Exact test. <u>Analysis Plan and Power of secondary objectives:</u> <u>Response rate:</u> Based on published data, at time of surgery in the FLOT-POP group, we expect to see 49% N0, 16% N1, 13% N2, and 22% N3 (includes not operable). We expect that FLOT-TNT will increase the frequency of N0 and reduce the frequencies of the other categories, especially N2 and N3. The pathological N stage of ypN is an ordinal variable and in this setting, the effect size is measured by the probability that

	a randomly selected subject in the FLOT-TNT population has a lower ypN value than a randomly selected subject from the FLOT-TNT group, expressed as Pr(ypN.FLOT-TNT < ypN.FLOT-POP). When the distribution of ypN is the same in both groups, the null hypothesis probability (p0) is 0.5, like flipping a coin. If, in the FLOT-TNT group, we observe a change to 75% N0, 16% N1, 3% N2 and 6% N3, the corresponding effect size would be p1=0.65. In this pilot study, relatively large effect sizes of this magnitude will be detectable with 80% power (alpha=10%, one-tailed) using a Wilcoxon Rank Sum test. Analysis Plan of exploratory objective: Estimate the association between changes in the integrated genomic, transcriptomic, and proteomic changes , pathological response and DFS. Analysis Plan: Baseline molecular findings should be the same in both groups of this randomized trial, but changes in the molecular findings could portend different likelihood of N0 overall or within groups. Logistic regression with N0 status at surgery as the outcome of interest and treatment group,molecular genomic, transcriptomic, and proteomic findings with their interactions as explanatory variables will be used to look for associations and molecular signatures. We can use the same method to evaluate the predictive value of pre-surgical molecular signatures and/or presurgical changes in molecular signatures. We can also use repeated measures ANOVA or general linear mixed models to examine the pattern of changes in integrated genomic, transcriptomic, and proteomic changes over time, overall and as a function of treatment group.

3.0 BACKGROUND

Gastroesophageal cancers (GECs) are a major global health problem with wide geographic variation. The highest gastric cancer incidence rates occur in East Asia, south and Central America and Eastern Europe. It is the most commonly diagnosed cancer in men in Korea and Japan, and is the leading cause of cancer related mortality in China.[1-3]

In the US, gastroesophageal cancers (GECs) together, represent the fourth most common GI cancer (after colorectal, pancreatic, and hepatobiliary cancers), with the third highest mortality. [4] An estimated 276,600 people were diagnosed, and 11,010 people died of this cancer in 2020.[5]
Five-year survival rates for locally advanced GC remains at 50% [6] despite improvements in surgery technique [7]and chemotherapy [8]

The recent classification of GECs generally comprises three main gastroesophageal subtypes reflecting current understanding of anatomy, histology, etiology, and molecular biology. These include: (i) esophageal SCC; (ii)GEJ AC, which includes ACs in the distal esophagus AC (type I Siewert), at the GEJ AC (type II Siewert), and in the gastric cardia AC (type III Siewert); and (iii) distal gastric AC.[9]
Siewert Type I: adenocarcinoma of the lower esophagus with the epicenter located within 1 cm to 5 cm above the anatomic EGJ. ◊ Siewert Type II: true carcinoma of the cardia with the tumor epicenter within 1 cm above and 2 cm below the EGJ. ◊ Siewert Type III: subcardial carcinoma with the tumor epicenter between 2 cm and 5 cm below the EGJ, which infiltrates the EGJ and lower esophagus from below.

Numerous histologic subtypes have been described pertinent to gastric AC but also for GEJ AC, including histologic differentiation (i.e., well, moderate, poor), Lauren classification (i.e., intestinal, diffuse, mixed type), presence of signet-ring cells or not, and a whole host of other subtypes in the WHO criteria. Despite the noted differences between GEJ and gastric AC, these two subtypes are often grouped together as gastroesophageal adenocarcinoma (GEA) in both the locally advanced and metastatic settings when considering therapy (see Appendix I).

Overall, 95% of gastric cancers are adenocarcinomas and divided to cardia (proximal) and non-cardia (distal) and the most common histological types are intestinal and diffuse types. The intestinal type is usually a well differentiated tumor seen more frequently in high-risk areas and accounts for most of the geographic variation. The diffuse type is defined by being undifferentiated and more prevalent in low-risk areas. The intestinal type is often related to *H. pylori* infection, smoking and high salt intake and alcohol is considered a risk factor for non-cardia gastric cancer. [3, 10-12]
Gastric and GEJ adenocarcinoma patients have a poor prognosis and compared with surgery alone, multimodality intervention including neoadjuvant chemotherapy, adjuvant chemotherapy or chemoradiation improves survival; however, the survival for locally advanced and node positive gastric cancer remains poor. At initial presentation, 50% of the cases are locally advanced and 80% are node positive by clinical staging and upfront surgery is not advised. Surgical resection of adenocarcinoma of the stomach is curative in less than 40% of cases. Surgery alone provides a 5 year OS is 20-30% in locally node positive patients.
The current best perioperative chemotherapy regimens result in pathologic complete response rates as determined by TRG of only 16 percent [13]. In addition, no predictive or prognostic biomarkers exist to predict response or survival rates for treatment of locally advanced GC. There is a clear deficiency of knowledge regarding the pathogenesis of gastric cancer. Specifically, TCGA (The Cancer Genome Atlas program) characterization of GC and GEJ cancer subtypes has not resulted in meaningful clinical improvements [14] Integrated proteogenomics studies are means to further our molecular understanding of cancer, and in particular in how tumors respond to treatment. [15 16, 17 18] Specific applications have been applied towards other GI cancers including colon cancer [19] and pancreatic cancer [20]. Further elucidation into the molecular proteogenomic signatures of locally advanced GC and GEJ cancer will lead to improvements in patient survival.

3.1 Treatment effect and Tumor Regression Grade

Tumor regression grading (TRG) systems are used to categorize the amount of regressive changes after cytotoxic treatment in order to demonstrate potential prognostic information based on objectively determinable histopathologic findings. Multiple TRGs have been developed. The TRG systems according to Mandard, [21] Becker, Dworak, or Rödel are examples for commonly used TRGs, which represent different approaches for estimating the degree of tumor regression. In our study we will be utilizing College of American Pathologists 2020 Grading system for the evaluation of response to neoadjuvant chemotherapy.

Most of TRG systems evaluate the amount of therapy-induced fibrosis in relation to residual tumor only at the primary lesion after neoadjuvant therapy. Patients with complete response (pCR) or near complete regression of the tumor show significant survival benefit and reduced risk of recurrence. [21-23] The value of lymph node regression in gastric cancer was evaluated from a prospective database in patients who had neoadjuvant chemotherapy followed by surgery. Patients with complete response (pCR) or near complete regression of the tumor show significant survival benefit and reduced risk of recurrence. [24-25] Pathological response and histological tumor regression after neoadjuvant therapy have been shown to be predictive of survival in GC patients. The (MAGIC) trial established perioperative Epirubicin, Cisplatin, and Fluorouracil chemotherapy as a standard of care for patients with resectable esophagogastric cancer. Investigators assessed the correlation between node status post treatment and overall survival of patients to identify high risk patients for relapse. Two independent pathologists using the Mandard TRG assessed pathologic regression in resection specimens. Three hundred thirty resection specimens were analyzed. In chemotherapy-treated patients with a TRG of 1 or 2, median OS was not reached, whereas for patients with a TRG of 3, 4, or 5, median OS was 20.47 months.[26] In another study at Cancer Hospital of China Medical University, Liaoning Cancer Hospital, 264 patients with locally advanced gastric carcinoma (including esophagogastric junction carcinoma) treated by perioperative chemotherapy (SOX or XELOX) from May 2012 to December 2017 were prospectively in this study. Tumor regression grade (TRG, Mandard system) and Response Evaluation Criteria in Solid Tumor (RECIST v1.1) was used to evaluate tumor response. The clinical characteristics and the effect on survival were analyzed. On multivariate analysis, the chemotherapy cycle, Lauren classification, vascular invasion or lymphatic invasion, ypN and postsurgical pathologic stage were independent factors for OS and DFS, while TRG were negatively correlated to survival.[27] Lowy et al reported that response to neoadjuvant chemotherapy was the only independent predictor of OS in patients who undergo curative GC surgery.[28] Recently CAP has provided a guideline for reporting treatment effect on gastric cancer. [37]

3.2 Diagnosis and Staging

a. EGD/EUS:

This procedure is used for evaluation of both depth of tumor invasion through the gastric tissue = (T staging) and nodal (N staging).

b. Diagnostic Laparoscopy and peritoneal cytology:

This procedure is used for evaluation of the peritoneal cavity under direct visualization with laparoscopy and to collect peritoneal fluid for cytology evaluation to accurately stage the subject.

c. Imaging:

Radiographic staging is performed with either CT chest with contrast, CT abdomen and pelvis with and without contrast. or MRI of the abdomen and or pelvis with/without contrast and FDG. PET CT scan is performed in special circumstances to clarify radiographic staging.

3.3 Pathology Evaluation:

Initial pathological diagnosis is achieved per EGD/EUS biopsy of the main mass. A full pathological evaluation will be done after surgery has been completed.

Recently, the college of American Pathologist updated their specimen exanimation and reporting protocol on July 2021, and recommended the use of the 4-tier tumor regression score for reporting gastric cancer specimen pathological evaluation after neoadjuvant therapy. Although grading systems for tumor response have not been established in general, 4-category systems provide good inter observer reproducibility [37].

4.0 GENERAL STANDARD TREATMENT APPROACH

A universal treatment of choice for patients with locally advanced GEC has been elusive due to lack of consensus in different parts of the world. The outcomes of these patients are proven to improve with including chemotherapy, surgery and radiation. (Tri-modality).

For any lesions ≥ T3 (tumor invading the muscularis propria [T3]) and/or N+ disease amenable to surgical resection, multimodal management with chemotherapy with or without radiation therapy (RT), before and/or after surgery has become the standard, given high-risk of micrometastastic dissemination. Neoadjuvant chemoradiation followed by surgery (tri-modality therapy) versus perioperative triplet chemotherapy are both standard options to treat locally advanced GEJ AC tumors to decrease risk of recurrence. The optimal approach between these two strategies is unresolved and head-to-head studies are ongoing that are evaluating this question.

There is a great deal of controversy among different regions from Asia to Europe to North America. The different incidences of each of the subtypes of GEC (i.e., esophageal SCC, GEJ AC, and gastric AC) geographically has led to different makeup of these tumor types within various studies, making cross-trial comparisons challenging and potentially misleading. For instance, GEA studies from Asia are almost exclusively of distal gastric AC, without any patients with proximal GEJ AC enrolled, whereas in western countries, GEJ AC accounts for at least half of patients enrolled in GEA studies. Similarly, different makeup of esophageal SCC and AC within different esophageal studies also makes comparison across studies challenging because these subtypes have a different biology, natural history, and sensitivities to the perioperative therapies. As such, various combinations of variables (e.g., the tumor site inclusion, the modalities included, nuanced details of type of chemotherapy or RT, doses and the timing of treatment as related to surgery) were studied in large GEC trials across centers and countries in parallel, each

compared with surgery alone. This ultimately has led to a fragmented treatment approach, with each approach deemed to be more effective than surgery alone, but it remains unclear which, if any, is superior.

4.1 Chemoradiotherapy:

Chemoradiotherapy after Surgery was compared with Surgery Alone for Gastroesophageal Junction Adenocarcinoma in McDonald Intergroup (INT) trial 0116, (SWOG 9008) study.[29] In this trial postop chemo with 5FU+ LV with concurrent radiation followed by 2 months 5FU/LV was compared to surgery alone. Overall survival was 36 vs 27 months and superior in the chemoradiation arm. Local failure was less seen in the chemoXRT arm, however, distant failure was similar. Neoadjuvant chemoradiation followed by surgery vs surgery alone was tested in CROSS trial [30]. In this study, majority 75% of patients had adenocarcinoma (esophageal and GEJ) and 23% had squamous cell carcinoma and their clinical stage consisted of T1N1M0 or T2-3N0-1M0. After a median follow-up for surviving adenocarcinoma patients of 84.1 months overall survival of 43.2 months was seen in the neoadjuvant chemoradiotherapy plus surgery group and 27.1 months in the surgery alone group. The outcomes were significantly better for esophageal and squamous cell GEJ patients (81.6 vs 21.1 months).[30] Distant recurrence remains substantial despite incorporation of local therapies such as radiation and D2 lymphadenectomy. Intergroup 0116 study showed no survival benefit with adjuvant chemoXRT after D2 lymphadenectomy. Similarly, CRTITICS trial showed no benefit with adding radiation to chemo if patients had received neoadjuvant chemotherapy and D2 lymphadenectomy with gastrectomy. [31, 32]

4.2 Perioperative chemotherapy:

Perioperative chemotherapy has been studied in multiple trials. Survival benefit was reported by Perioperative chemotherapy seen in the MAGIC trial.[33] In this trial, 503 patients with resectable adenocarcinoma of the stomach, esophagogastric junction, or lower esophagus were randomly assigned to either perioperative chemotherapy and surgery (250 patients) or surgery alone (253 patients). Chemotherapy consisted of three preoperative and three postoperative cycles of intravenous epirubicin and cisplatin and continuous intravenous infusion of fluorouracil for 21 days. As compared with surgery alone group, peri-operative chemotherapy showed a statistically significant five-year survival rate, 36% vs. 23% and an improved Progression free survival rate. In this trial, 70% of patients had gastric, 12% had GEJ and 15% had lower esophageal adenocarcinoma. In the Magic trial, 43% of subjects did not receive post chemotherapy.

In FNCLCC FFCD/ACCORD Multicenter Phase III Trial French 2011 (n 224) patients consisted of Lower esophagus adeno (15%), GC (20%) and GEJ (70%). Patients had 6 months perioperative chemo (cis + 5FU) vs surgery alone. 5-year OS rate was 38% v 24% in favor of perioperative chemotherapy; however, only 50% got post op chemotherapy completed. Lymph node negativity was seen more commonly in the perioperative group. Patients who had perioperative chemotherapy had less distant metastatic disease (30% vs 38%).[34] Al-Bartan et al studied 700 patients with GC/GEJC and randomized them to a trial evaluating Perioperative chemotherapy with fluorouracil plus leucovorin, oxaliplatin, and docetaxel (FLOT) and compared that with fluorouracil or Capecitabine plus cisplatin

and Epirubicin (ECX or ECF) for locally advanced, resectable gastric or gastro-esophageal junction adenocarcinoma in a randomized, phase 2/3 trial. Four preoperative and four post-operative biweekly cycles of 50 mg/m 2 docetaxel, 85 mg/m 2 oxaliplatin, 200 mg/m 2 leucovorin, and 2600 mg/m 2 fluorouracil as 24-h infusion on day 1 consisted of the FLOT regimen. The results showed the best mOS of 50 vs 35 months in favor of FLOT, where 65 % were GEJ, 44% were gastric adenocarcinoma and 50% of the patients had D2 lymphadenectomy. Three hundred and twenty six (91%) patients in the ECF/ECX group and 320 (90%) patients in the FLOT group completed all cycles of allocated preoperative chemotherapy; however, of all the patients randomized, only 132 (37%) patients in the ECF/ECX group and only 162 (46%) patients in the FLOT group completed all allocated cycles due to lower postoperative chemotherapy completion.[8]

FLOT significantly improved other clinically relevant endpoints such as resectability and disease-free survival. Regarding surgical morbidity and mortality, similar results were observed in both arms in terms of 30-day postoperative death rates (2% in the FLOT group and 3% in the ECF/ECX group) and surgical complications (51% in the FLOT group and 50% in the ECF/ECX group). Node negativity was seen more in FLOT group and it was associated with better OS in this group. This is the first trial to show significant improvement over the available standard of care ECF in the treatment of patients with locally advanced, potentially resectable gastric and gastro-esophageal junction adenocarcinoma. The study showed that perioperative FLOT significantly improved overall survival as compared with perioperative ECF or ECX (Epirubicin and cisplatin plus either fluorouracil or capecitabine. FLOT induced pathological complete regression of up to 17% in phase 2 and retrospective studies.

4.3 Surgery and D2 Lymphadenectomy

Surgery is the main stay of management of GECs; however, in node positive and bulky tumors this modality carries a very poor prognosis.
Gastric cancer is treated with partial or total gastrectomy. Gastroesophageal cancer surgery is divided into three groups based on the Siewert subtype (see appendix I). Siewert Type I is treated with esophagectomy. Siewert Type II is treated with the combination of lower esophagectomy, and total gastrectomy and Siewert Type III is treated with gastrectomy only. In this study only Siewert type II and III are included.
Historically, survival has been poor for patients with GEC treated with surgery alone, with 5-year survival rates ranging from 5% to 34% leading to several studies evaluating the benefit of perioperative therapies. Total or partial gastrectomy with D2 Lymphadenectomy is now adopted as standard of in North America. A D2 lymphadenectomy is defined as the contiguous resection of the second tier of lymph nodes along the branches of the celiac trunk (e.g., common hepatic artery, left gastric artery, and splenic artery). After 15-year follow-up of a randomized Dutch trial that included 1,078 patients with distal gastric AC, D2 lymphadenectomy was associated with lower locoregional recurrence (12% v 22%) and gastric cancer-related death rates (37% v 48%) than D1 (fewer lymph nodes removed) surgery.[35] Although D2 dissection was associated with higher operative morbidity, these data suggest D2 lymphadenectomy should be considered the surgical standard of care. Larger meta-analyses have confirmed this, particularly among patients who have undergone D2 resection who did not undergo resection of the spleen or distal pancreas and nodal stations around these (modified D2), as well as for patients with higher risk T3/T4 or node-positive cancers.[36-37]

5.0 DRUG INFORMATION

For this study, all drugs are commercially available; therefore, Investigator Brochures are not applicable to these drugs. Information about commercial drugs is publicly available in the prescribing information and other resources.

6.0 STUDY RATIONALE

Locally advanced gastric and gastroesophageal adenocarcinoma carries a poor prognosis despite multimodality treatment including surgery, perioperative chemotherapy and chemoradiation. Currently perioperative chemotherapy and D2 lymphadenectomy is the standard of care for GC and GEJ cancers; however, a significant number of subjects are unable to complete all or part of the post op section of their chemotherapy due to surgical recovery. Total neoadjuvant therapy in rectal cancer has proven to achieve higher pathological response rate (PCR) and disease-free survival rates in rectal cancer subjects. Improved outcome in CRC subjects is partially associated with total delivery of uninterrupted systemic therapy and eradication of micrometastasis.[38] Furthermore, higher PCR has proven to be associated with better outcomes in CRC. Here we aim to approach gastric and GEJ subjects with the same approach to investigate whether total neoadjuvant therapy (TNT) improves subject outcomes or not.

To date there are no predictive or prognostic biomarkers to predict treatment response or survival rates for locally advanced GC and GEJ cancer. Here we aim to evaluate the molecular signature (integrated genomic, transcriptomic, and proteomic changes) associated with pathological complete response rates for locally advanced GC and GEJ cancers. We will assess for molecular (integrated genomic, transcriptomic, and proteomic) changes associated with 2 vs 4 cycles of neoadjuvant FLOT chemotherapy and assess the association of DFS with the molecular finding changes. Each cycle consists of two treatments (D1 and D15). We will also determine the feasibility of establishing GC organoids pre- and post- neoadjuvant FLOT chemotherapy as a mechanism to individualize GC treatment.

7.0 HYPOTHESIS

A large percent of subjects with GC/GEJ cancer on peri-operative chemotherapy do not complete their planned chemotherapy after surgery. About 63% of subjects in the MAGIC trial and 54% in FLOT4 trial did not complete the post-operative chemotherapy due to prolonged recovery and/or post-operative complications.[20,22] Elimination of micrometastasis and increase in pathological nodal response correlates with survival.[23-25] Chemotherapy in the post-op setting is more challenging for the subjects and is frequently incomplete. Here we aim to evaluate the feasibility of giving all 4 cycles of chemotherapy in the neoadjuvant setting (FLOT-TNT) and hypothesize that total neoadjuvant therapy (FLOT-TNT) provides an improved completion of intended chemotherapy, and improves nodal clearance that may ultimately translate into better OS in subsequent trials. We also aim to evaluate the molecular (integrated genomic, transcriptomic, and proteomic) findings and their changes associated with cancer response to 2 or 4 cycles of FLOT neoadjuvant chemotherapy.

8.0 OBJECTIVES

8.1 Primary Objective

1. The primary objective is to determine the proportion of participants who complete all allocated cycles of chemotherapy by comparing Arms A and B. The completion rate in Arm A: FLOT-TNT is expected to be higher than in Arm B: FLOT-POP.

8.2 Secondary Objectives

1. To estimate the nodal pathological response rate by comparing the two study arms on surgical nodal status. The frequency of positive nodes is expected to be lower in Arm A: FLOT-TNT compared to Arm B: FLOT-POP.

8.3 Exploratory Objectives

In both arms A and B

1) Elucidate the molecular signature (integrated genomic, transcriptomic, and proteomic changes) associated with pathological complete response rates (as determined by TRG)
2) Assess for molecular (integrated genomic, transcriptomic, and proteomic) changes associated with arm A and arm B.
3) Determine the feasibility of establishing gastric cancer and gastroesophageal junction cancer organoids pre- and post- neoadjuvant FLOT chemotherapy as a mechanism to individualize treatment.

9.0 STUDY DESIGN

9.1 Enrollment

The cancer diagnosis will be established at the time of the initial EGD prior to study enrollment. Subjects must have staging scans and considered non metastatic based on scan reports before they are considered for this study. After enrollment all subjects will get a diagnostic laparoscopy (DL) and an EUS assisted staging of the cancer. Both DL and EUS staging is considered standard of care in the management of gastric and GEJ adenocarcinoma. Subjects with a positive DL tissue diagnosis for metastatic cancer will be excluded from the study as screen failures. Subjects with a negative DL on pathology review will proceed with registration and randomization.

This study is a two-arm phase II pilot design, with eligible subjects randomized 1:1 to either Arm A (Investigational arm) or Arm B (Standard arm). The chemotherapy dose modification will follow routine clinical care. Both arms will receive chemotherapy and curative surgery.

9.2 Arm A: FLOT-TNT (Investigational Arm)

Arm A is the investigational arm with all 4 cycles of FLOT given as total neoadjuvant chemotherapy prior to surgery. Each cycle is 28 days and consists of 2 chemotherapy sessions given every 14 days. The total number of chemotherapy sessions in Arm A is 8. Every effort will be made to have Surgery in week 20 (-1 to +2 weeks), 4 weeks post C4 on arm A.

9.3 Arm B: FLOT-POP (Standard Arm)

Arm B is the standard perioperative arm with 2 cycles of pre-operative FLOT (4 treatment sessions) and 2 cycles (4 treatment sessions) of post-operative FLOT. Post-surgery FLOT will start 4-6 weeks post-surgery. Each cycle of chemotherapy consists of 28 days and consists of 2 chemotherapy sessions given every 14 days. The total number of chemotherapy sessions in arm B is 8. Every effort should be done to have surgery done in week 12 (-1 to +2 weeks) post completion of cycle 2 on Arm B.

9.4 Curative Surgery

Curative gastric and GEJ surgery with D2 lymphadenectomy should be performed per given time frame instruction in table 10.1 and table 10.2. In this study only Siewert type II and III are included. Seiwert type I is considered pure esophageal cancer and not considered in this study (See appendix I).

The subject would not be removed from the study if D2 lymphadenectomy was not carried out per the surgeon's discretion in the operation room for technical or safety reasons. The boundaries of GEJ and GC would be defined by the surgeon performing the surgery. GEJ vs GC should be documented. Siewert level documentation is optional and not required. (See Appendix I).

9.5 Exploratory analysis:

Tissue acquisition (See schema below):

Samples for the exploratory analysis will be collected two times:
1) At the time of EUS staging and 2) After gastrectomy.

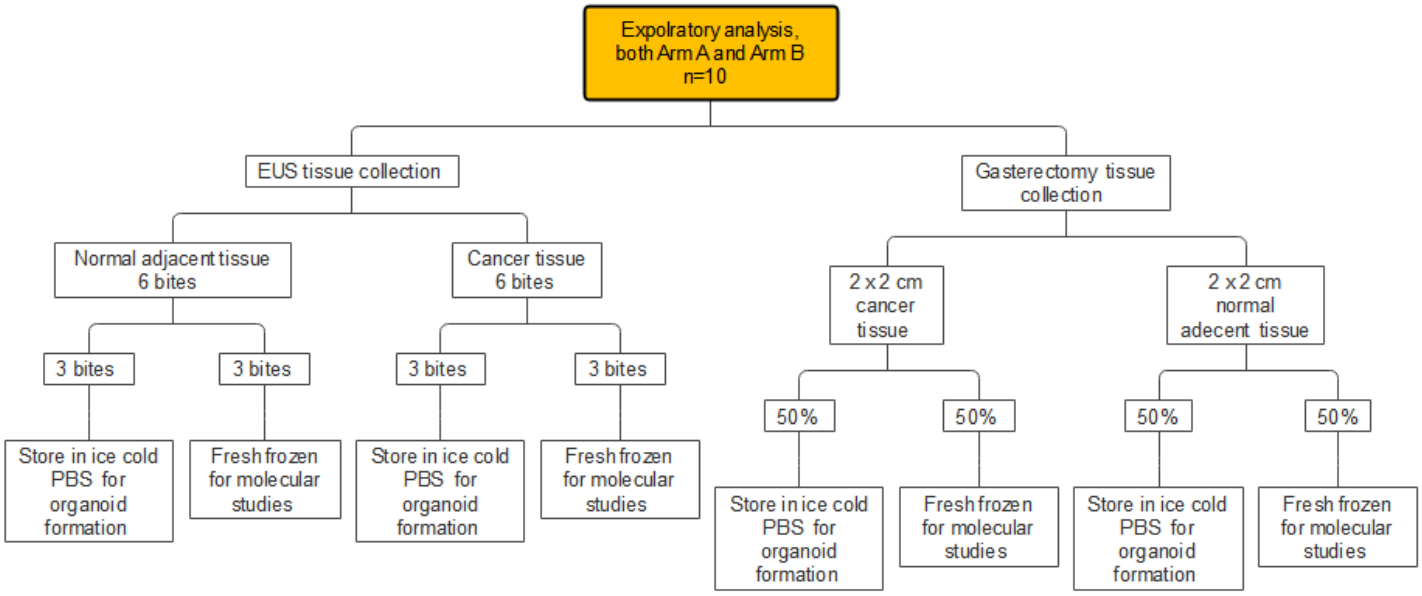
Tissue from a total of first five accrued subjects from each arm (A and B) will be collected for molecular analysis. (total 10 subjects). After the subject is enrolled in the study a standard EUS is performed for staging. During the EUS, six sample biopsy bites will be collected from the cancer tissue and another six sample biopsy bites will be collected from the adjacent normal tissue. Three sample bites from the cancer tissue and three sample bites from the adjacent normal tissue will be immediately flash frozen and stored as fresh frozen samples for molecular studies. The remaining three sample bites from the cancer tissue and the remaining three sample bites from the adjacent normal tissue will be stored in ice cold sterile PBS for subsequent organoid formation.
After gastrectomy is performed, we will obtain a 2 x 2 cm tumor tissue sample and a 2 x 2 cm adjacent normal tissue sample immediately. We will store half of the tumor sample and half of the adjacent normal tissue sample as fresh frozen samples. The other half of cancer

sample and normal tissue sample will be put in ice cold sterile PBS for organoid formation. All frozen samples will be securely stored in a -80C freezer in the lab of Dr. Jason Mills at Baylor College of Medicine until sample processing for molecular studies. All fresh tissues in sterile PBS will be immediately processed for organoid generation. Given the novelty of this approach applied to locally advanced gastric and GEJ cancer, it will initially be difficult to predict the exact number of patients needed. Based on previous studies [18] we believe that only a few samples will yield meaningful results. As a result, we plan to obtain tissue samples from 5 patients in each arm of the study for these exploratory analyses.

Molecular studies:

Fresh frozen specimens will be processed for DNA, RNA, and protein extraction using the Qiagen All Prep DNA/RNA/Protein Mini Kit. Extracted DNA and RNA will be sent for whole-exome and RNA sequencing, respectively (Novogene), and extracted protein will be sent for proteomics and phosphoproteomics using the BCM Lester and Sue Smith Breast Center Proteomics Laboratory [18]. Integrative bioinformatics will be used to extract altered and meaningful molecular pathways in collaboration with Dr. Bing Zhang at Baylor College of Medicine.
For organoid formation studies, further tissue processing will include mechanical stripping of the underlying muscle layers, enzymatic epithelial tissue digestion, and final embedding in Matrigel with previously well-defined culture media conditions.[39] Successful organoid formation is defined as the ability to passage, characterize, and expand each organoid line derived from tumor and normal-adjacent stomach.

Molecular analysis schema: [Q]



Q: A total of ten subjects will provide tissue for molecular analysis. Five subjects from arm A and five subjects from arm B. For each subject, tissue will be collected twice. First at the time of EUS and second at the time of gastrectomy.

10.0 STUDY CALENDAR

10.1 Arm A: FLOT-TNT (Investigational Arm)

Required Studies	**Pre-registration [J]**	EUS [G]	Cycle 1 Wks 1-4		Cycle 2 Wks 5-8		Cycle 3 Wks 9-12		Cycle 4 Wks 13-16		Wk 20 Surgery [K]	Wk 24 Post-surgery [L]	Surveillance [M]
			D 1	D 15	D 1	D 15	D 1	D 15	D 1	D 15			
ASSESSMENTS													
History and Physical Exam [A]	x		x	x	x	x	x	x	x	x		x	x
Weight and Performance status [A]	x		x	x	x	x	x	x	x	x		x	x
Toxicity Assessment [A]				x	x	x	x	x	x	x		x	x
Surgical specimen evaluation												x	
LABORATORY [B]													
CBC [C]	x		x	x	x	x	x	x	x	x		x	x
CMP [D]	x		x	x	x	x	x	x	x	x		x	x
CEA [E]	x				x				x			x	x
Pregnancy test [F]	x												
Research tissue collection		x									x		
SCANS													
CT/MRI/PET for disease Assessment [I]	x						x				x[I]		x
RECIST Radiographic Response							x				x		x
TREATMENT													
FLOT-Chemo			x	x	x	x	x	x	x	x			
Surgery											x		
Survival/Progression													x

10.2 Arm B: FLOT-POP (Standard Arm)

Required Studies	Pre-registration [J]	EUS [G]	Cycle 1 Wks 1-4		Cycle 2 Wks 5-8		Surgery Wk 12 [K]	Cycle 3 Wks 16-20		Cycle 4 Wks 21-24		Week 28 Post chemo [L]	Surveillance [M]
			D 1	D 15	D 1	D 15		D 1	D 15	D 1	D 15		
ASSESSMENTS													
History and Physical Exam [A]	x		x	x	x	x		x	x	x	x	x	x
Weight and Performance status [A]	x		x	x	x	x		x	x	x	x	x	x
Toxicity Assessment [A]				x	x	x		x	x	x	x	x	x
Surgical specimen evaluation								x					
LABORATORY [B]													
CBC [C]	x		x	x	x	x		x	x	x	x	x	x
CMP [D]	x		x	x	x	x		x	x	x	x	x	x
CEA [E]	x				x			x		x			x
Pregnancy test [F]	x												
Research tissue collection [H]		x					x						
SCANS													
CT/MRI/PET scan for disease assessment [I]	x						x[I]					x	x
RECIST Radiographic Response							x					x	x
TREATMENT													
FLOT-Chemo			x	x	x	x		x	x	x	x		
Surgery							x						
Survival/progression													x

Footnotes for Arm A and Arm B Calendars. Note: one cycle = 28 days

A. Assessment must be performed prior to treatment on Days 1 and 15 (+/- 4 days) of each cycle. Assessment of Toxicity during surveillance includes monitoring of long-term side effects of interventions such as chemotherapy related peripheral neuropathy.
B. Labs do not need to be repeated at Cycle 1 Day1 if they were drawn within 14 days of registration. Labs can be drawn at +/- 5 days within the required time frame.
C. CBC: Hemoglobin, WBC, platelet count, and ANC.
D. CMP: Sodium, potassium, bicarbonate, chloride, BUN, calcium, total protein, magnesium, total bilirubin, alkaline phosphatase, albumin, creatinine and GFR
E. CEA must be performed every 3 months during surveillance only in subjects who had an elevated CEA level at baseline.
F. Pregnancy test (urine or plasma) to be performed on all females of childbearing age who have a uterus.
G. EUS will be performed on all subjects after informed consent has been signed for staging and research tissue acquisition
H. Tissue will be collected at the time of EUS and surgery for exploratory analysis, See further instructions under " Exploratory analysis."
I. CT/MRI/ PET scan for disease assessment with imaging to be performed as below:
   Arm A: 28 days prior to subject-registration, on Day 1 of cycle 3, prior to surgery at week 16 to 20 [+ 2 weeks], and every 3 months during surveillance (+/- 2 weeks for all time points except baseline)
   Arm B: 28 days prior to subject registration, before surgery at week 8 to 12 [+2 weeks], at week 28 [+2 weeks post chemo], and every 3 months during surveillance (+/- 2 weeks for all time points except baseline)
   - Every effort should be made to use the same modality of imaging used at screening. CT or MRI must include chest, abdomen, and pelvis. CT scans must be CT chest with contrast, CT abdomen and pelvis with and without contrast. If an MRI is used, it must be an MRI of the chest with contrast, MRI of the abdomen and pelvis with and without contrast and FDG. PET scan is not necessary for initial or subsequent imaging unless needed to clarify imaging status per the discretion of the treating physician.
J. Informed consent should be signed on day -42 to -1 prior to registration.
K. Arm A: surgery should be performed on week 20 (-1 /+2 weeks) 4 weeks post Cycle 4.
   Arm B: surgery should be performed on week 12 (-1 /+2 weeks) post cycle 2.
L. Arm A: post-surgery visit should be done on week 24, 4 weeks after surgery (-1 /+ 2 weeks).
   Arm B: post-surgery visits should be done on week 28, 4 weeks after completion of post-op chemotherapy (-1/+2 weeks).
M. Arm A: Subjects will be followed every 3 months (+/- 3 weeks) for a total of 12 months starting from their post-surgery visit.
   Arm B: Subjects will be followed every 3 months (+/- 3 weeks) for a total of 12 months starting from Cycle 4, Day 15.

11.0 STAGING CRITERIA

AJCC 8th edition will be used for staging before and after surgery and on each restaging scan.

12.0 SUBJECT CRITERIA

12.1 Inclusion Criteria:

a. Must provide written informed consent.
b. Must be ≥18 years of age.
c. Must have life expectancy of greater than 3 months.
d. Must have pathologically proven Siewert type II or III GEJ or gastric adenocarcinoma from the main tumor or local lymph nodes (pre-neoadjuvant chemo).
e. Stage cT2 or higher, any N and M0, are eligible for the study.
f. M0 disease must be established by both negative distant metastatic disease on imaging AND negative diagnostic laparoscopic assisted cytology of peritoneal fluid cytology not more than 42 days before registration.
g. Must be a candidate for neoadjuvant chemotherapy.
h. Must be a candidate for curative surgical approach.
i. Must have an ECOG performance status 0-2.
j. Male or female subjects of childbearing potential must be willing to use contraceptive precautions throughout the trial and for 3 months after discontinuation of study treatment. Female subjects of childbearing potential must have a negative pregnancy test within 28 days of registration. Post-menopausal women must be amenorrhoeic for at least 12 months to be considered of non-childbearing potential.
k. Must have adequate kidney, liver, and bone marrow function, within 28 days prior to registration, as follows:
   i. Hemoglobin ≥ 8.0 gm/dL (PRBC transfusion is allowed to meet this criteria)
   ii. Absolute neutrophil count (ANC) ≥ 1000 cells/mm3
   iii. Platelet count ≥ 100,000 /mm3
   iv. Total bilirubin ≤ 1.5 times upper limit of normal (ULN)
   v. AST (SGOT) and ALT (SGPT) ≤ 3.0 times the ULN
   vi. Patient must have adequate renal function as evidenced by one of the following: Serum creatinine ≤ IULN OR calculated creatinine clearance ≥ 60 mL/min. This serum creatinine result must be obtained within 28 days prior to registration.

Calculated creatinine clearance = $\frac{(140 - \text{age}) \times \text{wt}^* \text{ (kg)} \times 0.85 \text{ (if female)}}{72 \text{ Creatinine}^{**} \text{ (mg/dl)}}$

*The kilogram weight is the subject's actual body weight with an upper limit of 140% of the IBW.

** Actual lab serum creatinine value with a minimum of 0.8 mg/dL

l. Subjects who have required a short course urgent single modality non curative radiation treatment or gastric artery embolization for the purpose of tumor bleeding control are eligible.

12.2 Exclusion Criteria:

a. Positive cytology or histology for metastatic disease on diagnostic laparoscopy peritoneal fluid. Reports such as: "cannot rule out malignancy" or "suspicious for malignancy, but not definitive" will exclude the subject from enrolling.
b. Seiwert type I GEJ cancer
c. Subjects with clinical evidence of metastatic disease.
d. Biopsy proven metastatic disease (excluding regional lymph nodes)
e. Prior chemotherapy for gastric cancer or GEJ cancer
f. No other prior malignancy is allowed except for the following: adequately treated basal cell or squamous cell skin cancer, current non-advanced prostate cancer per the discretion of the investigator, and any other cancers from which the patient has been disease free for two years.
g. Female subjects who are pregnant, breast feeding, or of childbearing potential with a positive pregnancy test prior to baseline. Women of childbearing potential must have a negative serum pregnancy test as a part of eligibility within 28 days of registration. A persistent positive or elevated urine or blood Beta HCG test may be contributed to the primary diagnosis of GC or GEJ cancer after ruling out ectopic and intrauterine pregnancy and germ cell tumors.
h. Subjects unwilling or unable to comply with the protocol or provide written informed consent.
i. Any medical condition that, in the opinion of the investigator, would exclude the subject from participating in this study and treatment plan.
j. ECOG > 2

13.0 TREATMENT PLAN

13.1 Pre-Medication and Supportive Care

Pre-medications and concomitant medicines should be administered to counter the expected common side effects of chemotherapy. These will be ordered by the treating physician per institutional standards and tailored to the subject's condition.

The following is recommended prior to treatment:

a. Supportive care, including but not limited to anti-emetic medications, may be administered at the discretion of the Investigator. Erythropoietin and G-CSF may be administered at the discretion of the investigator, consistent with ASCO guidelines.

b. In the unlikely event of a mild hypersensitivity reaction, premedication may be administered using the premedication regimen the institution typically uses for Oxaliplatin and Docetaxel. In the rare event of a severe hypersensitivity reaction, discontinue the suspected drug.

13.2 Treatment of subjects

Subjects assigned to Arm A and Arm B will both receive the following combination of 5-FU, Leucovorin, Docetaxel and Oxaliplatin also known as FLOT. The FLOT regimen is standard chemotherapy. Arm A will receive FLOT-TNT and Arm B will receive FLOT-POP.

Subjects in both arms will undergo a D2 lymphadenectomy with gastrectomy.

Treatment plan Arm A and B: 5-FU, Oxaliplatin, LV, Docetaxel

Agent	Dose	Route	Day
5-FU	2600 mg/m^2	IV over 26 hours (+/- 1 hour) CI with home pump	1,15
LV	200 mg/m^2	IV over 2 hours	1,15
Oxaliplatin	85 mg/m$_2$	IV over 2 hours	1,15
Docetaxel	60 mg/m^2	IV over 60 minutes	1,15
GCSF©	see below	SC	2,16

13.3 Growth factors

GCSF:

13.3.1 Long acting GCSF (Neulasta of Fulphila- or Biosimilar) is recommended on day 2 and 16 of C1 of the protocol while getting chemotherapy.

13.3.2 GSCF can be discontinued at the discretion of the physician during subsequent cycles if dose modifications to lower-level doses for non–neutropenic toxicities have occurred and in the

investigator's opinion GCSF is no longer necessary to keep the ANC above 1000.

13.3.3 Subject's insurance plan is to be considered for best selection GCSF agent.

GCSF agents include:

a. Neulasta® Onpro- 6 mg subcutaneous administer on day 2 and 16

b. Pegfilgrastim (Neulasta® or a pegfilgrastim biosimilar): 6 mg subcutaneous administer on day 2 and 16

c. Filgrastim (Neupogen® or a filgrastim biosimilar): 5 mcg/kg/day (rounded to nearest syringe size of 300 mcg or 480 mcg)

d. Per physician discretion during neutropenic fever episodes.

14.0 FOLLOW-UP PERIOD

All subjects will be followed up until the end of 12 months of surveillance. After completion of the surveillance period, they will have completed the study. For subjects in Arm A, the surveillance period will start from their post-surgery visit. For subjects in Arm B, the surveillance period will start from cycle 4, day 15 of chemo.

15.0 CRITERIA AT DISEASE PROGRESSION:

15.1 Progression of primary disease confirmed by imaging or EGD and or metastatic disease confirmed by pathological confirmation.

1. Progression is evaluated by evidence of disease progression on scans, preferably using the same scans performed at baseline per RECIST v1.1 criteria.
2. A tissue biopsy is required from the suspected metastatic site including tissue biopsy of peritoneal metastasis deposit or peritoneal cytology if it can be carried out safely and the metastatic deposit is accessible. Biopsy is not required if in the opinion of the investigator progression to metastatic status is unequivocal. The rationale and reason for such conclusion should be clearly documented.

3. Increase of CEA without evidence of measurable disease progression on scans is not considered progression of disease by itself unless accompanied by other evidence such as presence of ascites not explained by any other etiology and strongly believed to be related to progression of the disease in the peritoneum if cytology of the peritoneum is negative.

15.2 Subjects who progress to distant metastatic disease between registration and infusion of chemotherapy on cycle 1, day 1 will enter the surveillance follow-up period and complete the protocol-dictated 12 months of surveillance.

15.3 Subjects who progress during study treatment will enter the surveillance follow-up period and complete the protocol-dictated 12 months of surveillance.

15.4 Subjects who progress during surgery will enter the surveillance follow-up period and complete the protocol-dictated 12 months of surveillance.

16.0 CRITERIA FOR REMOVAL FROM STUDY TREATMENT

16.1 Toxicity that requires all chemotherapy agents to be stopped.

16.2 The subject request to stop chemotherapy at any time for any reason.

16.3 Subjects who are removed from study treatment will enter the 12 month surveillance follow-up period.

17.0 CRITERIA FOR REMOVAL FROM STUDY PROTOCOL

17.1 The subject requests to withdraw from the study at any time for any reason.

17.2 If surgery is deemed not to be the best approach in the opinion of the treating surgeon or subject declines surgery.

17.3 Chemotherapy delay for any reason more than 4 weeks other than the allowed timeframe between chemotherapy and planned gastrectomy per protocol.

17.4 If a subject is a woman of child bearing potential and becomes pregnant during the study.

Subjects who require a short course of urgent non-curative single modality radiation course to stop the bleeding of the tumor when chemotherapy or embolization of the gastric artery is not feasible or effective to stop the acute bleeding are allowed to continue on the protocol and enroll.

18.0 DOSE MODIFICATIONS

18.1 General Considerations:

Dose modifications and treatment delays based on observed drug-related toxicity will be performed as described below:

1. Any toxicity associated or possibly associated with 5FU, Oxaliplatin, LV, Docetaxel treatment should be managed according to standard medical practice.
2. The maximum allowed dose delay of any of the chemotherapy agents for any reason is 4 weeks. If per the investigator's opinion the subject cannot be started on the chemotherapy drug that was held in 4 weeks, that chemotherapy agent will be permanently discontinued form the rest of the treatment.
3. No dose re-escalations are permitted. If the subject experiences toxicity requiring a dose reduction, the dose will remain lowered for subsequent cycles.
4. Subjects on either arm may continue chemotherapy as long as not all drugs need to be discontinued for toxicities.
5. Dose modifications of drug toxicities not specified in the protocol, e.g, abnormal liver function tests, creatinine insufficiency, etc., should follow standard of care approaches and need to be documented.

18.2 Dose Level changes:

18.2.1 For both Arm A and Arm B

Drug	Initial Dose	Dose reduction level -1	Dose reduction level -2**
5FU	2600 mg/m^2	2000mg/m^2	1600 mg/m^2
Oxaliplatin	85 mg/m^2	65 mg/m^2	50 mg/m$^{2\,**}$
Docetaxel	60 mg/m^2	45 mg/m^2	35 mg/m^{2**}
LV	200mg/m^2	none	none

1. Any dose reduction other than instructions mentioned below should be discussed with the study investigator, justified and clearly explained and documented.
2. Reduction of the doses starts with changing the doses of Docetaxel or Oxaliplatin. It is at the discretion of the investigator which drug dose to reduce first. If a subject experiences the first hematologic toxicity, the investigator can choose between Oxaliplatin or Docetaxel to dose reduce one of these 2 drugs to dose level -1. Dose modification occurs with only one drug at a time and one dose level at a time.
3. If a subject experiences a second hematologic toxicity despite reduction of -1 reduction of docetaxel or Oxaliplatin, -1 level dose reduction should be done on the drug that has not been reduced. (Oxaliplatin or Docetaxel)
4. If a subject experiences a hematologic toxicity while Docetaxel is at a dose -2 level, Oxaliplatin will be dose reduced if it is not already at dose -2 level.
5. If a subject experiences a hematologic toxicity while Oxaliplatin is at dose -2 level, Docetaxel will be dose if it is not already at dose -2 level.
6. If subject experiences a hematologic toxicity and Oxaliplatin and Docetaxel are both at dose level -2, 5FU will be reduced to dose level -1.

7. If subject experiences a hematologic toxicity with Oxaliplatin and Docetaxel both at dose level -2 and 5FU at dose level to -1, the investigator is allowed to make a clinical decision to remove Oxaliplatin or Docetaxel in subsequent cycles to preserve the treatment with 5FU at minimum does level -1. Every effort should be made to continue the treatment with 5FU in this scenario even if the toxicity requires it to remain on a single agent 5FU.
8. If a subject experiences hematologic toxicities on single agent 5FU at dose level -1, 5FU should be reduced to dose level -2.
9. If hematologic toxicity continues despite -2 dose level reduction of 5FU, chemotherapy should be stopped.
10. If for a particular drug, a dose reduction is required below Dose Reduction Level 2, that drug must be discontinued.

18.3 Hematological Toxicities:

In the event dose modifications are required at the beginning of a cycle or within a cycle due to hematologic toxicities, doses of 5FU, Oxaliplatin, LV, Docetaxel may be adjusted as detailed below.

18.3.1 Dose Modification For Day 1 And Day 15 Of Each Cycle For Hematologic Toxicity

Day 1, 15 Laboratory results	Day 1, 15 Gemcitabine	Day 1, 15 Docetaxel	Day 1, 15 5FU
ANC > 1000 and Platelets ≥ 75,000	No modification	No modification	No modification
ANC <1000 or Platelets <75,000	Hold until ANC ≥ 1000 And Platelets > 75,000. Delay treatments by 1 week intervals until recovery. Once recovered, treat based on dose reduction per table 18.2.1	Hold until ANC ≥ 1000 And Platelets > 75,000. Delay treatments by 1 week intervals until recovery. Once recovered, treat based on dose reduction per table 18.2.1	Hold until ANC ≥ 1000 And Platelets > 75,000. Delay treatments by 1 week intervals until recovery. Once recovered, treat based on dose reduction per table 18.2.1
Febrile Neutropenia (Grade 3 or 4)*	Hold until ANC > 1500 and fever-free for 48 hours, then treat based on dose reduction per table 18.2.1	Hold until ANC > 1500 and fever-free for 48 hours, then Treat based on dose reduction per table 18.2.1	Hold until ANC > 1500 and fever-free for 48 hours, then Treat based on dose reduction per table 18.2.1

18.3.2 Neutropenic Fever considerations: *

1. If a subject does not experience resolution of neutropenia within 4 weeks despite uninterrupted daily G-CSF treatment, study treatment will be discontinued.

2. Subjects with febrile neutropenia should have their chemotherapy treatment held. Sepsis workup should follow standard approach.

3. Subjects may also receive short acting daily G-CSF, in addition to antibiotic treatment, to hasten the resolution of their febrile neutropenia.

4. In all cases, blood counts must have returned to non-neutropenic levels before resuming chemotherapy treatment.

5. All Subjects are highly recommended to be on a long acting G-CSF agent administered on day 2 and 15 during chemotherapy per the protocol.

6. The additional shot acting G-CSF preparation is optional per the discretion of the investigator with febrile neutropenia. The exact dosage amount and schedule for G-CSF support will be left to the treating physician's discretion.

7. ANC ≥ 1500 AND resolution of fever for at least 48 hours is required to resume chemotherapy with dose reduction per section 18.2.1 after febrile neutropenia has resolved.

18.3.3 Considerations on Anemia and PRBC transfusion:

Hemoglobin should be maintained at ≥7 g/dl. If Hb is between 6 and 7, subject can receive chemotherapy but must receive PRBC in no longer than 48 hours after the end of the chemotherapy infusion. If Hb is <6, chemotherapy should be withheld until PRBC transfusion has been given and Hb is ≥ 7 or the subject has recovered from serious symptoms. Serious anemia related symptoms include but not limited to chest pain, shortness of breath, confusion, etc. Mild to moderate fatigue without other serious symptoms is acceptable and chemotherapy can be continued if Hb is ≥ 7. Treatment can continue with PRBC support despite known GI bleeding if in the opinion of the investigator chemotherapy is believed to contribute to the control of the malignancy bleeding.

18.3.4 Non-hematologic toxicities:

All treatment related non-hematological toxicities (with the exception of hair loss and nausea and vomiting that can be controlled with

antiemetics) should resolve to ≤ Grade 2 prior to starting next cycle of therapy.
Dose modification or delay may occur in the setting of lower Grade toxicity if the treating physician believes that it is in the interest of a subject's safety.
Alopecia and nausea and/or vomiting that can be controlled by antiemetics do not require dose modification. No dose modification will be required for anemia as it can be satisfactorily managed by transfusions.

18.3.5 Dose Modification For Day 1 And Day 15 Of Each Cycle For Non-Hematologic Toxicity *

CTCAE Grade	Treatment Modification
Grade 0-2 Toxicity	Same as Day 1 previous cycle (except for Grade 2 cutaneous toxicity where docetaxel needs to be reduced to a lower dose and Grade 2 diarrhea where 5-FU needs to be reduced to a lower dose)
Grade 3 Toxicity [a, c]	Hold all drugs until resolution to < Grade 1. Then resume treatment at the next lower dose level for all drugs
Grade 4 Toxicity [a, b]	Hold all drugs until resolution to < Grade 1. Then resume treatment at the next lower dose level for all drugs

* Except peripheral neuropathy CTCAE v5.0 table below for sensory neuropathy)

a. If the toxicity only affects neuropathy, then only Oxaliplatin or Docetaxel should be reduced. If the specific toxicity is attributed to only one drug (i.e. Docetaxel skin rash, 5Fu mucositis), only the offending drug should be withheld, and resume treatment at the next lower dose level of only that specific drug when toxicity resolves to < grade 1.

b. Pulmonary embolism (a Grade 4 toxicity in the CTCAE tables) if mild or asymptomatic, will be exempt from this requirement.

c. Excluding electrolyte abnormalities per judgment of the physician/investigator.

Sensory Neuropathy

Modify Oxaliplatin or Docetaxel treatment per below. 5FU administration can continue during this period.

	Duration of Toxicity		Persistent between cycles
Toxicity Grade	1-7 days	> 7 days	
2	No dose modification	No dose modification	Next lowest dose level for Oxaliplatin*
3	Next dose level for Oxaliplatin	Next dose level for Docetaxel	Discontinue
4	Discontinue	Discontinue	Discontinue

Common Terminology Criteria for Adverse Events (CTCAE) v5.0 for Sensory neuropathy
((http://ctep.cancer.gov).

Peripheral sensory neuropathy			
Grade 1	Grade 2	Grade 3	Grade 4
Asymptomatic	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self care ADL	Life-threatening consequences; urgent intervention indicated

Modify Oxaliplatin and Docetaxel treatment for sensory neuropathy per below:

a. 5FU administration can continue during this period.
b. Subjects experiencing peripheral neuropathy that requires a delay in scheduled Oxaliplatin and Docetaxel dosing for > 4 weeks will discontinue study treatment. In those subjects who experience Grade 4 sensory neuropathy, Docetaxel and Oxaliplatin should be stopped.
c. Grade 3 If persistent between cycles: Decrease Oxaliplatin and Docetaxel at next lowest dose level.
d. If peripheral sensory neuropathy stays at grade 2 after dose reduction, dosing can continue at the same dose level if toxicity remains grade 2

e. Grade 3: hold Oxaliplatin and Docetaxel until grade <2 then restart Oxaliplatin and Docetaxel at next lowest dose level

f. Grade 4: Discontinue Oxaliplatin and Docetaxel reduction

Cutaneous Toxicity

Subjects who develop Grade 2 or 3 cutaneous toxicity should have the dose reduced of the drug the local investigator deems most contributory to the next lower dose level. If the subject continues to experience these reactions, despite dose reduction, treatment should be discontinued. Subjects who develop Grade 4 cutaneous toxicity should have treatment discontinued.

Gastrointestinal Toxicity

Subjects who develop Grade 2 or 3 gastrointestinal toxicity (Except nausea and vomiting that can be satisfactorily treated and prevented with antinausea medications) should have their dose reduced to the next lower dose level. If the subject continues to experience these reactions, despite dose reduction, treatment should be discontinued. Subjects who develop Grade 4 gastrointestinal toxicity should have treatment discontinued. e.g. Oral mucositis, diarrhea.

Pulmonary Embolism

Asymptomatic or clinically mild pulmonary embolism can be treated with Anticoagulant of the investigator's choice per standard practice without interruption of therapy. Moderate to severe pulmonary embolism at the discretion of the local investigator will require permanent discontinuation of treatment.

Sepsis

If a subject becomes febrile while not being neutropenic initiate sepsis work up and treatment with broad spectrum antibiotics. Dose modification of any of the chemotherapy agents is required. For febrile neutropenia, withhold all chemotherapy agents until fever resolves for at least 48 hours and ANC ≥ 1500, then resume treatment at reduced dose levels.

Prophylaxis Against Sepsis

Due to the incidences of non-neutropenic sepsis, at the first occurrence of fever ≥ 38.5°C (regardless of neutrophil count), institution of ciprofloxacin (500 mg orally, twice daily) or amoxicillin/clavulanate (500 mg orally, 2-3 times daily) in subjects with allergy to fluoroquinolones should be initiated in the outpatient setting if the subject is clinically stable. If the subject is not clinical stable (e.g. hypotension, tachycardia, altered mental status, etc.) per the discretion of the investigator, the subject should be referred to the emergency room for IV antibiotics and sepsis work up.

Hypersensitivity

Hypersensitivity reactions rarely occur. If they do occur, minor symptoms such as flushing, skin reactions, dyspnea lower back pain, hypotension, or tachycardia may require temporary interruption of the infusion. However, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema or generalized urticarial rash requiring immediate discontinuation of study drug administration and aggressive symptomatic therapy. Subjects who experience severe hypersensitivity reactions to any of the chemotherapy agents should not be re-challenged.

19.0 PERFORMANCE STATUS

Subjects will be graded according to the ECOG Performance Status Scale

Point	Description
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of light and sedentary nature, e.g., light house work
2	Ambulatory and capable of self-care but unable to carry out any work activities. Up and about more than 50% of the waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of the waking hours
4	Completely disabled. Cannot carry on self-care. Totally confined to bed or chair.

20.0 CRITERIA FOR DISEASE EVALUATION DURING STUDY:

20.1. Restaging scans should be compared to the baseline scans during pre-operative chemotherapy per RECIST criteria v1.1 for evaluation of disease status to measure stable disease, partial response, complete response and progression of disease.

20.2. CEA should be measured at specific timeframes per table 5.1 and 5.2 for the respected treatment arms if this marker has been elevated at baseline.

20.3 Surgical pathology after surgery should be reviewed for neoadjuvant chemotherapy effect per standard guidelines and response should be documented per College of American Pathologists version 4.2.1.0 updated on July 2021.

20.4 Investigational Biomarkers will be evaluated for integrated genomics, transcriptomics, and proteomics based on changes in pre and post treatment tissues.

College of American Pathologists definition of Tumor Regression Grade[37]:

Description	Tumor Regression Score
No viable cancer cells (complete response)	0
Single cells or rare small groups of cancer cells (near complete response)	1
Residual cancer with evident tumor regression, but more than single cells or rare small groups of cancer cells (partial response)	2
Extensive residual cancer with no evident tumor regression (poor or no response)	3

21.0 ADVERSE EVENT REPORTING

21.1 Adverse Event:

Any unfavorable medical occurrence in a human subject, including any abnormal sign, symptom, or disease, whether or not it is considered related to the subject's participation in the research.

21.2 Descriptions and Grading:

The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website.

21.3 Attribution / Relatedness:

Each event should be evaluated by the treating investigator for its relatedness to participation in the research:

Definite: The AE is clearly related to the study treatment.
Probable: The AE is likely related to the study treatment.
Possible: The AE may be related to the study treatment.
Unlikely: The AE is doubtfully related to the study treatment.
Unrelated: The AE is clearly NOT related to the study treatment.

21.4 Seriousness:

A Serious Adverse Event (SAE) is any adverse event that:
- Results in death;
- Is life-threatening;
- Results in inpatient hospitalization or prolongation of existing hospitalization;
- Results in a persistent or significant disability/incapacity;
- Results in a congenital anomaly/birth defect; or
- Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

21.5 Expectedness: Definition of expected and unexpected adverse event.

21.5.1 Expected adverse events are those that have been previously identified as resulting from administration of the agent. For purposes of this study, an adverse event is considered expected when it appears in the current adverse event list in the Investigator's Brochure, the package insert, or is included in the informed consent document as a potential risk.

21.5.2 An adverse event is considered unexpected when it varies in nature, intensity, or frequency from information provided in the current adverse event list in the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

21.6 Reporting:

21.6.1 All adverse events that are Grade 3 or above, regardless of relatedness, will be reported and recorded on the appropriate CRFs.

21.6.2 The AE description will include the nature of the experience, the date of onset, the resolution date, the severity of each sign or symptom reported using the NCI-CTCAE, the seriousness of the event, the relatedness to study treatment, the course of action taken (if any), and the outcome of the experience.

21.6.3 Serious adverse events are to be reported to the BCM Institutional Review Board (IRB) according to the board's reporting requirements and required time frame.

21.6.4 Any adverse event that is unexpected, serious, and possible/probably/definitely related to the study should be reported to the review committee (DRC or DSMB) within 15 calendar days of knowledge of the event.

21.6.5 Any event that is reportable to the BCM IRB must also be reported to the DLDCCC Data Review Committee (DRC) via the Patient Safety Officer at dldcc-pso@bcm.edu.

22.0 STATISTICAL CONSIDERATIONS:

22.1. Study Design/Description:

This is a randomized pilot study to evaluate and to compare the completion rates of Total Neoadjuvant chemotherapy with FLOT (FLOT-TNT) and perioperative chemotherapy with FLOT (FLOT-POP).

22.2. Sample Size Justification:

The trial will require 50 evaluable subjects (25 per arm). A subject is evaluable if any chemo treatment has been received. We anticipate at most 10% of subjects will withdraw before beginning study treatment; therefore, we expect to consent accrue at most 56 subjects.
We expect about 5% of evaluable subjects with disease progression. In routine care, they would go to surgery and should be approximately equal between groups. They will go off-treatment and be included for the primary and secondary objectives.

We expect to see the completion rate in FLOT-TNT will be higher than FLOT-POP. Based on published data, we expect that 45% of subjects in the FLOT-POP will complete all allocated chemotherapy. Considering that we expect 90% of subjects complete the first 2 cycles (Lancet ref 24), and if about 90% of those complete the second 2 cycles in the FLOT-TNT arm, then the expected completion rate will be about 80%. With 25 evaluable subjects in each group, we will have 85% power (alpha=10%, one-tailed) to detect an absolute improvement of 35% (FLOT-TNT completion rate=80%) using a Fisher Exact test.

22.3. Randomization/Stratification/Treatment assignment Procedures:

Subjects will be randomized in a 1:1 ratio to either FLOT-TNT group or control FLOT-POP group. Randomization will be open-label and performed by entering data into OnCore. No stratification factors will be used.

22.4. Accrual and Feasibility:

Roughly 10-15 subjects per year are estimated to be enrolled. The accrual period will be 60 months.

22.5. Stopping Rules:

See section 17.0

22.6. Analysis Populations:

22.6.1 Efficacy Intent to Treat (ITT):

The ITT population will consist of all eligible and randomized subjects in the study. Primary objective will be analyzed.

22.6.2. Efficacy Modified Intent to Treat (MITT)

NA

22.6.2 Safety population:

NA

22.7 Statistical Analyses

22.7.1 Primary objective, endpoints, analysis plan:

The primary objective analysis will be based on the efficacy intent to treat (ITT) population. The primary endpoint is the number of subjects who have completed their all-allocated cycles. The proportion of subjects that complete all allocated cycles will be calculated and summarized. Fisher Exact test will be used to compare the proportion between FLOT-TNT group and FLOT-POP group.

22.7.2 Planned Interim Analysis:

N/A

22.7.3 Secondary objective, Exploratory objective, Endpoints, Analysis Plan:

The secondary objectives endpoint analysis will be based on the efficacy intent to treat (ITT) population. The secondary endpoints include pathologic response and nodal status (N stage) based on TRG [CAP criteria (20.1.c)]

The exploratory objective endpoints are organoid growth, and molecular signatures (integrated genomic, transcriptomic, and proteomic) and changes, and disease free survival (DFS) after surgery.

22.8 Response rate:

Based on published data, at time of surgery in the FLOT-POP group, we expect to see 49% N0, 16% N1, 13% N2, and 22%N3 (includes not operable). We expect that FLOT-TNT will increase the frequency of N0 and reduce the frequencies of the other categories, especially N2 and N3. The pathological N stage of ypN is an ordinal variable and in this setting, the effect size is measured by the probability that a randomly selected subject in the FLOT-TNT population has a lower ypN value than a randomly selected subject from the FLOT-TNT group, expressed as Pr(ypN.FLOT-TNT< ypN.FLOT-POP). When the distribution of ypN is the same in both groups, the null hypothesis probability (p0) is 0.5, like flipping a coin. If, in the FLOT-TNT group, we observe a change to 75% N0, 16% N1, 3% N2 and 6% N3, the corresponding effect size would be p1=0.65. In this pilot study, relatively large effect sizes of this magnitude will be detectable with 80% power (alpha=10%, one-tailed) using a Wilcoxon Rank Sum test.
The pathological response rate with frequency of positive nodes and nodal status (ypN) obtained from post gastrectomy surgical specimen will be compared between FLOT-TNT group and FLOT-POP group using Fisher's exact test and Wilcoxon Rank Sum test.

22.9 As a pilot exploration, tissues from a total of five subjects from each arm (A and B) will be collected for molecular analysis at two time points: 1) EUS staging and 2) gastrectomy. Tumor, if any and adjacent normal will be collected from the resection specimen after required pathology samples are taken. If possible, samples will be divided to yield fresh and frozen material. Fresh tissue specimens will be processed in the lab of Dr. Ramon Jin at Baylor College of Medicine for organoid formation and treatment studies. Frozen

specimens will be processed for DNA, RNA, and protein extraction. Extracted DNA and RNA will be sent for whole-exome and RNA sequencing, respectively (Novogene), and extracted protein will be sent for proteomics and phosphoproteomics using the BCM Lester and Sue Smith Breast Center Proteomics Laboratory. Integrative bioinformatics will be performed in collaboration with Dr. Bing Zhang at Baylor College of Medicine

Disease free survival (DFS) is defined as the time between the first day of the chemo treatment and disease progression or death from any cause. A progression is defined by PI's decision after surgery based on standard approaches. Time to first event of DFS will be estimate using the Kaplan-Meier survival method.

23.0 DATA AND SAFETY MONITORING AND QUALITY ASSURANCE:

This study will be monitored regularly by the Data Review Committee (DRC) of the Dan L Duncan Comprehensive Cancer Center, at a frequency of at least once per year, in accordance with the DLDCCC Data and Safety Monitoring Plan. The DRC will monitor the study for progress and enrollment, toxicities, adverse events, and soundness of data.

This study will be monitored by the DLDCCC Quality Assurance program for study conduct and quality of data.

APPENDIX I: ANATOMICAL BOUNDARIES OF GECS

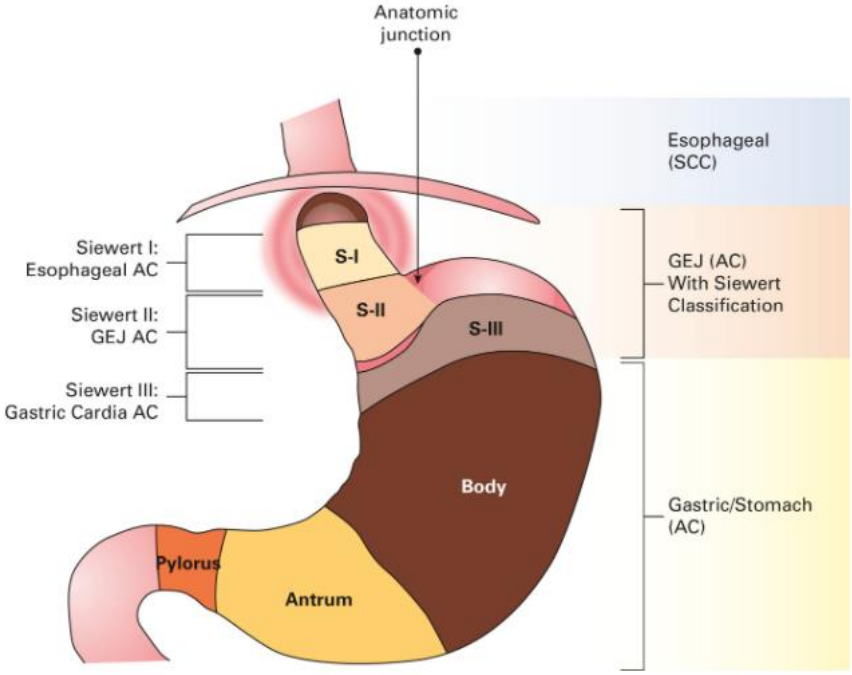


Fig. 13-1:
Anatomical distribution of gastroesophageal cancers.

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