Ramucirumab plus irinotecan in patients with previously treated advanced gastric or gastro-esophageal junction adenocarcinoma

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Study Drug(s): Ramucirumab (Cyramza)
Irinotecan (Camptosar)

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Principal Investigator Signature Page

Principal Investigator:	Kian Huat-Lim, M.D., Ph.D.	
	Signature of Investigator	Date
	Printed Name of Investigator	
	By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.	

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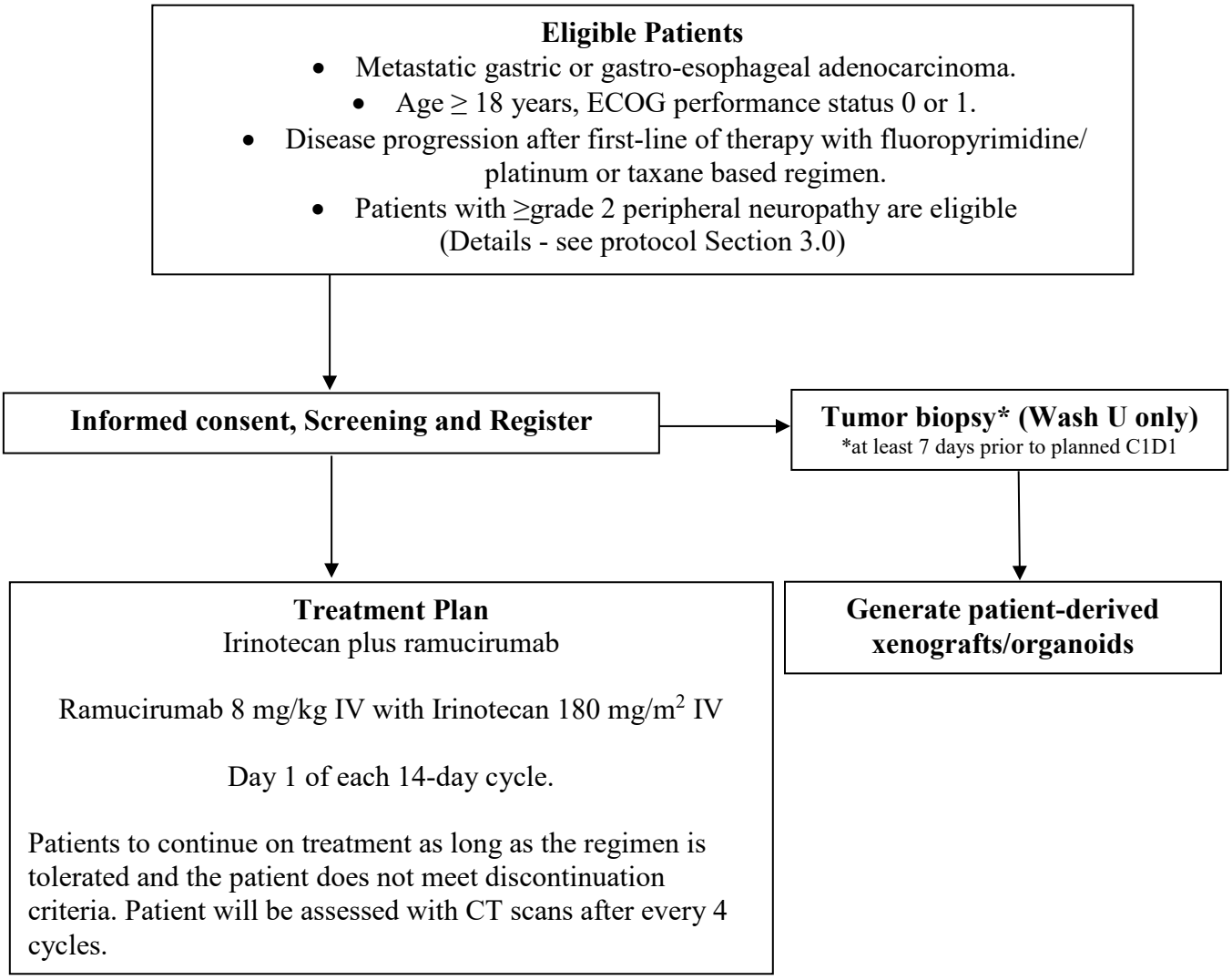
SCHEMA



Glossary of Abbreviations

5-FU	5-fluorouracil
AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
B-HCG	Beta human chorionic gonadotropin
BOR	Best overall response
CBC	Complete blood count
CBR	Clinical benefit rate
CFR	Code of Federal Regulations
CR	Complete response
CRF	Case report form
CST	Central standard time
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DNA	deoxyribonucleic acid
DSM	Data and Safety Monitoring
DSMC	Data Safety Monitoring Committee
DVT	Deep vein thrombosis
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
FWA	Federal wide assurance
GEJ	Gastro-esophageal junction
GI	Gastrointestinal
HGF	Hepatocyte growth factor
HIV	Human Immunodeficiency Virus
HRPO	Human Research Protection Office (IRB)
IND	Investigational New Drug
INR	International normalized ratio
IRB	Institutional Review Board
IULN	Institutional upper limit of normal
IV	Intravenous
LV	Leucovorin
MRI	Magnetic resonance imaging
NCI	National Cancer Institute
NIH	National Institutes of Health
NSCLC	Non-small cell lung cancer
NYHA	New York Heart Association
OHRP	Office of Human Research Protections

ORR	Overall response rate
OS	Overall survival
PD	Progressive disease
PE	Pulmonary embolism
PET	Positron emission tomography
PFS	Progression-free survival
PI	Principal investigator
PK	Pharmacokinetics
PR	Partial response
PTT	Partial thromboplastin time
QASMC	Quality Assurance and Safety Monitoring Committee
RECIST	Response Evaluation Criteria in Solid Tumors (Committee)
RPLS	Reversible posterior leukoencephalopathy syndrome
SAE	Serious adverse event
SCC	Siteman Cancer Center
SD	Stable disease
TTP	Time to progression
UA	Urinalysis
UPN	Unique patient number
VEGF	Vascular endothelial growth factor
WUSM	Washington University School of Medicine

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1.0 BACKGROUND AND RATIONALE

1.1 Gastric Cancer

Gastric cancer is the fifth most common malignancy, and the third leading cause of cancer mortality worldwide.[1] Currently, platinum-based and fluoropyrimidine-based combinations are accepted worldwide as established first-line drug regimens for advanced gastric adenocarcinoma, with a median survival ranging from 8 months to 10 months.[2] Selected second-line chemotherapy including docetaxel and irinotecan improved overall survival compared with best supportive care in randomized trials, however median survival remained dismally poor at less than 6 months.[3-5] Newer, more active second line systemic therapy options are needed.

1.2 VEGF in Gastric Cancer

Vascular endothelial growth factor (VEGF) and VEGF receptor-2 (VEGFR-2)-mediated signaling and angiogenesis contribute to the pathogenesis of gastric cancer. In patients with gastric cancer, circulating VEGF levels are associated with increased tumor aggressiveness and reduced survival.[6,7] In animal models of gastric adenocarcinoma, VEGFR-2 inhibition reduced tumor growth and vascularity.[8] Ramucirumab, a human IgG1 monoclonal antibody VEGFR-2 antagonist, prevents ligand binding and receptor-mediated pathway activation in endothelial cells.[9]

1.3 Chemotherapy for Metastatic Gastric Cancer

Ramucirumab monotherapy has shown overall survival benefit after first-line chemotherapy in advanced gastric cancer.[10] Second-line treatment with ramucirumab plus paclitaxel after progression on frontline chemotherapy (platinum plus fluoropyrimidine with or without an anthracycline) showed an improvement in overall survival to more than 9 months in patients with metastatic gastric or gastro-esophageal junction cancer.[11] This is now being regarded as a new standard in second-line treatment for patients with advanced gastric cancer.

Despite these advances, many patients may be unable to receive this combination due to prior taxane exposure, as taxane containing regimens are a standard option as first line therapy.[12] Additionally, peripheral neuropathy after frontline platinum-based chemotherapy, may preclude patients from receiving paclitaxel in the second line setting, or can significantly worsen with paclitaxel leading to early discontinuation. Most platinum containing gastric cancer regimens have reported very high rates of peripheral neuropathy. In the REAL-2 (Randomized ECF for Advanced and Locally Advanced Esophagogastric Cancer 2) phase III trial, peripheral neuropathy (all grades) was seen in 30-36% patients in cisplatin arms and 80-84% patients in oxaliplatin arms.[13] Similarly, neurosensory toxicity was fairly common (all grades: 62.5%, grade 3 or 4: 14.3%) with oxaliplatin and 5-fluorouracil/leucovorin use in the first line setting.[14] In a large meta-analysis, platinum based therapy compared to non-platinum based therapy containing S-1, taxanes or irinotecan was associated with two-fold risk of neurotoxicity (RR = 2.01, 95%CI, 1.05–3.86, p = 0.03).[15]

Furthermore, in the RAINBOW trial, peripheral neuropathy was more common in the ramucirumab plus paclitaxel arm (all grades: 46%) compared with paclitaxel alone (all grades: 37%) and was associated with a higher cumulative paclitaxel dose.[11] Therefore, there is a need to evaluate newer chemotherapy combinations with ramucirumab for advanced gastric cancer.

1.4 Irinotecan (Camptosar)

Irinotecan is an antineoplastic agent of the topoisomerase I inhibitor class. Irinotecan is an active drug for gastric cancer, and is not neurotoxic. Irinotecan based combinations have shown survival benefit in first-line,[16,17] second-line[4,5,18] and third-line[19] treatment of advanced gastric cancer. Common adverse reactions observed in single agent therapy clinical studies are: nausea, vomiting, abdominal pain, diarrhea, constipation, anorexia, neutropenia, leukopenia (including lymphocytopenia), anemia, asthenia, fever, body weight decreasing, and alopecia.

Irinotecan has been studied in clinical trials in combination with 5-fluorouracil (5-FU) and leucovorin (LV) and as a single agent. When given as a component of combination-agent treatment, irinotecan was either given with a weekly schedule of bolus 5-FU/LV or with an every-2-week schedule of infusional 5-FU/LV. Weekly and once-every-3-week dosage schedules were used for the single-agent irinotecan studies.

1.5 Ramucirumab (Cyramza)

Ramucirumab is a human vascular endothelial growth factor receptor 2 antagonist indicated: as a single agent or in combination with paclitaxel for treatment of advanced gastric or gastro-esophageal junction adenocarcinoma with disease progression on or after prior fluoropyrimidine- or platinum-containing chemotherapy; in combination with docetaxel for treatment of metastatic non-small cell lung cancer with disease progression on or after platinum-based chemotherapy; and in combination with FOLFIRI for the treatment of metastatic colorectal cancer with disease progression on or after prior therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine. Ramucirumab alone and ramucirumab in combination with chemotherapy (paclitaxel) has shown prolonged survival in the REGARD and RAINBOW trial.

The most common adverse reactions observed in single-agent ramucirumab-treated patients were hypertension and diarrhea. Ramucirumab increases the risk of: hemorrhage and gastrointestinal hemorrhage, including severe and sometimes fatal hemorrhagic events; gastrointestinal perforation; and impaired wound healing.

1.6 Study Rationale

Ramucirumab monotherapy as well as combination of paclitaxel plus ramucirumab has shown overall survival benefit after first-line chemotherapy (platinum plus fluoropyrimidine with or without an anthracycline) in advanced gastric or gastro-esophageal junction cancer.[10,11] Irinotecan is active in gastric cancer and has shown

survival benefit, that is similar to taxanes in second-line setting.[4,5,18] Safety of 5-fluorouracil/Irinotecan (FOLFIRI with irinotecan at a dose of 180 mg/m^2 – similar to present study) and ramucirumab combination has been studied in second-line treatment for metastatic colon cancer, where this combination is safe and provides survival benefit.[20] Therefore, studying irinotecan plus ramucirumab in the second-line setting for gastric cancer after fluoropyrimidine and platinum based therapy is prudent. If active, this would offer a non-neurotoxic treatment combination of chemotherapy and ramucirumab for patients with advanced gastric cancer and GEJ cancers.

We hypothesize that this combination regimen of irinotecan plus ramucirumab administered as second line treatment will be tolerated and lead to improved outcomes similar to paclitaxel plus ramucirumab in patients with advanced gastric and GEJ cancers. This study proposes a phase II clinical trial with irinotecan plus ramucirumab for treatment of patients with metastatic gastric and GEJ adenocarcinoma who have progressed after first line chemotherapy. To our knowledge, this regimen has not been previously administered to this patient population, so safety and tolerability will be monitored and reported.

1.7 Correlative Studies

1.7.1 Patient-derived xenograft and organoid in gastroesophageal cancer

The use of biomarkers to predict response to novel targeted therapies have ushered in an ever- expanding era of precision oncology. However, development of targeted treatments in upper gastrointestinal cancers has been met with, at best, modest success. HER2 and VEGF-targeting agents are the only approved targeted therapy for metastatic gastric and GEJ cancers. Several targeted therapy agents have been tested in clinical trials, but did not show significant improvement over traditional chemotherapy, in part due to limitations in patient selection. The advent of patient-derived xenograft and organoid modeling systems has enabled novel approaches to truly personalize and improve cancer treatment.

Patient-derived xenograft (PDX) models are generated by growing cells or tissue from a patient's cancer in an immunodeficient mouse. These PDXs have been shown to recapitulate many key aspects of the original tumor biology, and, more importantly, allow predictions of clinical efficacy for new targeted treatments in that patient whose cell or tissue is derived from. [21,22] Therefore, PDXs derived from gastric and GEJ cancers are used to screen novel agents[23-26] (Figure 1A).
Patient-derived organoid (PDO) models are generated by culturing epithelial tumor cells or tissue in 3D matrigel. Novel "biobanks" have been established in many tumor types for use in genomic analyses, transcriptome studies, large scale drug screens, and the development of personalized medicine allowing predictions of future drug response.[27] Gastric and esophageal cancer have been shown to amenable to these culture techniques with high concordance between primary tissue and organoid histology, genomic landscape, and transcriptomic expression profiles[28,29] with the ability to carry out large scale drug screens as shown in Figure 1B.

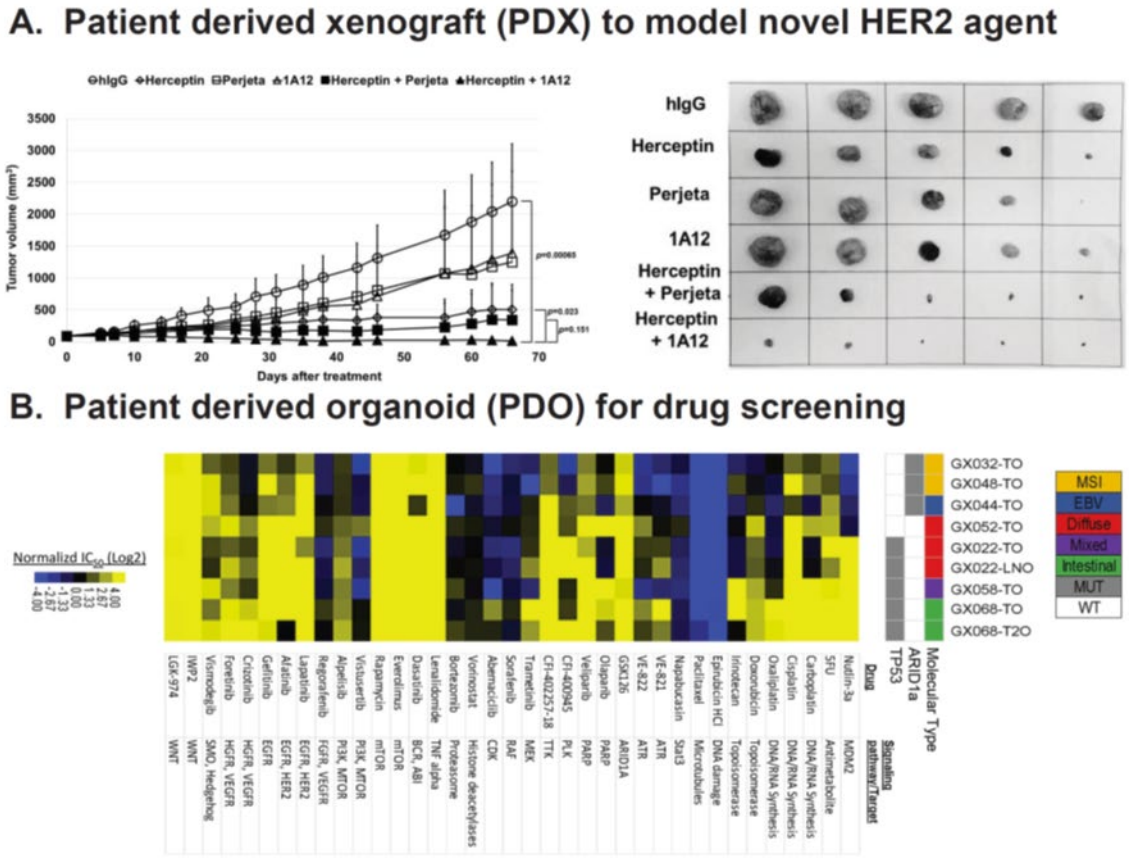

Figure 1. Applications for PDX and PDO models in cancer biology. A) Five GC PDX models were generated and tested for tumor volume response after treatment with trastuzumab, pertuzumab and 1A12 (a novel HER2 antibody). **B)** Nine GC PDO models were used to screen drug sensitivities (IC_{50}) for 37 compounds.

Both techniques have certain advantages and disadvantages as pre-clinical models summarized in Table 1. But despite the advancement in PDX and PDO technology, there have been few studies that have utilized both in direct comparison. PDX models require less amount of patient tissue than PDO, but takes longer time to generate. Therefore, PDO models are likely better suited for rapid drug screen, and potentially can be used for real-time treatment decision making. Furthermore, prospective studies demonstrating the ability of either of these models to predict or recapitulate individual patient treatment response are lacking. This correlative study would be wholly novel in utilizing both techniques in the context of a clinical trial allowing prospective investigation of both models and actual patient response to the same treatments.

We plan to generate PDX and PDO models from enrolled patient derived samples. Using these models, we will specifically assay efficacy of ramucirumab and irinotecan and compare with prospective clinical data regarding actual patient treatment response. This will serve as a pilot study for future clinical trials that may

integrate PDX/PDO models to provide truly personalized cancer therapy options to patients with gastroesophageal cancer.

Features	Patient Derived Organoids (PDO) Model	Patient Derived Xenograft (PDX) Model
Success rate of initiation	+++	++
Ease of maintenance	++	+/-
Resource consumption	Medium	High
Expansion	++	+
Retention of *in vivo* histology	++	+++
Retention of genetic features	++	++
Amenable to genetic modification	+++	-
Tumour microenvironment interation	++	++
Low-thoughput drug screens	+++	+
High-throughput drug screens	++	-
Biobanking	+++	-

Table 1. Comparison of PDO and PDX models. Features were determined as best (+++), suitable (++), possible (+), not very suitable (+/-) or unsuitable (−).

1.7.2 Circulating blood biomarkers including angiome profiles and cell-free DNA

Accumulating preclinical and clinical evidence suggests antiangiogenic therapy affects the level of several factors, and that changes in these factors with therapy may have predictive value. This trial provides an excellent correlative opportunity to assess markers that may predict response to angiogenesis inhibition.

For this trial, blood will be collected to measure alterations in circulating angiogenic factors (plasma angiome profile) during therapy and to assess correlations with outcome, and to identify relevant pharmacogenetic markers, mechanisms of resistance and correlate with response.

Further rationale for correlative studies is described later in the protocol (Section 9.0).

2.0 OBJECTIVES

2.1 Primary Objective

To determine the progression-free survival (PFS) with irinotecan plus ramucirumab in patients with metastatic gastric and gastro-esophageal junction cancer who have progressed after first line chemotherapy.

2.2 Secondary Objectives

1. To determine the overall survival (OS) of patients treated with irinotecan plus ramucirumab.
2. To determine time to progressive disease (TTP) in patients treated with irinotecan plus ramucirumab.

3. To determine the best overall response (BOR) of confirmed Complete Response (CR), confirmed Partial Response (PR), Stable Disease (SD) or Progressive disease (PD) in patients treated with irinotecan plus ramucirumab.
4. To determine the objective response rate (ORR) (defined as confirmed CR + confirmed PR) in patients treated with irinotecan plus ramucirumab.
5. To determine clinical benefit rate (CBR) (percentage of combined patients who have achieved confirmed CR, confirmed PR and SD) in patients treated with irinotecan plus ramucirumab.
6. To evaluate toxicity and tolerability of irinotecan plus ramucirumab as measured by NCI-CTCAE version 4.03.

2.3 Exploratory Objectives

1. To determine the success rate of PDX/PDO generation from patients with metastatic gastroesophageal cancer.
2. To evaluate the responses to treatment with ramucirumab and irinotecan in PDX/PDO models
3. To characterize molecular changes in PDX/PDO models along with corresponding patients' clinical courses to identify potential predictors of response and mechanism of resistance
4. To investigate changes blood based angiome profile and cell free DNA in patients following treatment with ramucirumab and irinotecan to identify potential mechanism of response and resistance

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. Histopathologically or cytologically confirmed diagnosis of gastric or gastroesophageal junction (GEJ) adenocarcinoma that is metastatic or locally advanced and unresectable.

2. Measurable disease defined as lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 10 mm with CT scan (or MRI at the discretion of the PI), as ≥ 20 mm by chest x-ray, or ≥ 10 mm with calipers by clinical exam.

3. Either primary or non-osseous metastatic site amenable for research biopsy for patients enrolled at Washington University, if safe and feasible, as confirmed by scheduling of biopsy procedure. Other methods to obtain appropriate cancer cells such as large-volume paracentesis or thoracentesis can be allowed at PI discretion. Biopsy or other procedures should be performed at least 7 days prior to C1D1.

4. Experienced documented objective radiographic or clinical disease progression during first-line therapy or within 4 months after the last dose of first-line therapy with any platinum/fluoropyrimidine doublet with or without anthracycline (epirubicin or doxorubicin) or taxane (docetaxel) for unresectable or metastatic disease.

   NOTE: This is not intended to be an exclusive list of allowed agents. The targeted therapies such as Herceptin and ADC, or immunotherapies without cytotoxic chemotherapy, are permitted.

5. At least 18 years of age.

6. ECOG performance status ≤ 1 (see Appendix A).

7. Normal bone marrow and organ function as defined below:
   a. Absolute neutrophil count (ANC) ≥ 1,500/μL
   b. Hemoglobin ≥ 9.0 g/dL (5.58 mmol/L)
   c. Platelets ≥ 100,000/μL
   d. Total bilirubin ≤ 1.5 mg/dL (25.65 μmol/L)
   e. AST(SGOT)/ALT(SGPT) ≤ 3.0 x IULN (or ≤ 5.0 x IULN in the setting of liver metastases)
   f. Creatinine ≤ 1.5 x IULN OR creatinine clearance ≥ 40 mL/min/1.73 m^2 for patients with creatinine levels > 1.5 x IULN (that is, if serum creatinine is > 1.5 x IULN, a 24-hour urine collection to calculate creatinine clearance must be performed)
   g. Urinary protein ≤ 1+ on dipstick or routine UA; if dipstick or routine UA is ≥ 2+, a 24-hour urine collection for protein must demonstrate < 1000 mg of protein in 24 hours
   h. Adequate coagulation function as defined by INR ≤ 1.5 and PTT ≤ 5 seconds above the ULN (unless receiving anticoagulation therapy). Patients receiving warfarin must be switched to low molecular weight heparin and have achieved stable coagulation profile prior to first dose of protocol therapy.

8. All clinically significant toxic effects (except peripheral neuropathy) of prior locoregional therapy, surgery, or other anticancer therapy have resolved to ≤ CTCAE grade 1.

9. Women of childbearing potential and men must agree to use two forms of adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately. Women of childbearing potential must have a negative serum pregnancy test within 7 days of study entry.

10. Ability to understand and willingness to sign an IRB approved written informed consent document (or that of legally authorized representative, if applicable).

3.2 Exclusion Criteria

1. Squamous cell or undifferentiated gastric cancer.

2. Received any chemotherapy (including irinotecan) other than platinum and fluoropyrimidine with or without anthracycline or taxane for advanced gastric or GEJ adenocarcinoma.

3. Received previous systemic chemotherapy with a cumulative dose of > 900 mg/m^2 of epirubicin or > 400 mg/m^2 of doxorubicin.

4. Received any previously systemic therapy (including investigational agents) targeting VEGF or the VEGFR signaling pathways. Other previous targeted therapies are permitted if stopped at least 28 days prior to start of treatment.

5. A history of other malignancy ≤ 3 years previous with the exception of basal cell or squamous cell carcinoma of the skin which were treated with local resection only or carcinoma *in situ* of the cervix or other solid tumors treated curatively and without evidence of recurrence.

6. Currently receiving any other investigational agents.

7. History or evidence of known brain metastases or carcinomatous meningitis. Patients with known brain metastases must be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

8. A history of allergic reactions attributed to compounds of similar chemical or biologic composition to monoclonal antibody treatment, any components used in the ramucirumab DP preparation, irinotecan, or other agents used in the study.

9. Any grade 3-4 GI bleeding within 3 months prior to enrollment.

10. History of gastrointestinal perforation and/or fistulae within 6 months prior to enrollment.

11. History of deep vein thrombosis, pulmonary embolism, or any other significant thromboembolism (venous port of catheter thrombosis or superficial venous thrombosis are not considered "significant") during the 3 months prior to enrollment.

12. History of any arterial thromboembolic event, including but not limited to myocardial infarction, transient ischemic attack, cerebrovascular accident, or unstable angina within 6 months prior to enrollment.

13. Diagnosis of symptomatic congestive heart failure (NYHA II-IV) or symptomatic or

poorly controlled cardiac arrhythmia.

14. Uncontrolled or poorly controlled hypertension (> 160 mmHg systolic or > 100 mmHg diastolic for > 4 weeks) despite standard medical management.

15. Presence of serious or nonhealing wound, ulcer, or bone fracture within 28 days prior to enrollment.

16. Major surgery within 28 days prior to first dose of protocol therapy.

17. Minor surgery/subcutaneous venous access device placement within 7 days prior to first dose of protocol therapy.

18. Receiving chronic antiplatelet therapy, including aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs, including ibuprofen, naproxen, and others), dipyridamole or clopidogrel, or similar agents. Once-daily aspirin use (maximum dose 325 mg/day) is permitted.

19. The patient has elective or planned major surgery to be performed during the course of the clinical trial.

20. Bowel obstruction, history or presence of inflammatory enteropathy or extensive intestinal resection (hemicolectomy or extensive small intestine resection with chronic diarrhea), Crohn's disease, ulcerative colitis, or chronic diarrhea.

21. Cirrhosis at a level of Child-Pugh B (or worse) or cirrhosis (any degree) and a history of hepatic encephalopathy or clinically meaningful ascites resulting from cirrhosis (i.e. ascites from cirrhosis requiring diuretics or paracentesis). Patients with ascites not related to cirrhosis, such as malignant ascites, are allowed.

22. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, metabolic disorders or other nonmalignant organ or systemic disease or secondary effects of cancer that induce a high medical risk and make assessment of survival uncertain, or psychiatric illness/social situations that would limit compliance with study requirements.

23. Pregnant and/or breastfeeding.

24. Known HIV-positivity on combination antiretroviral therapy because of the potential for pharmacokinetic interactions with ramucirumab and irinotecan. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

3.3 Inclusion of Women and Minorities

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

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility by Washington University
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

Once the patient has been entered in the Siteman Cancer Center database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below and scanning and emailing it to the research coordinator listed in the *Siteman Cancer Center Clinical Trials Core Protocol Procedures for Secondary Sites* packet at least one business day prior to registering patient:

1. Your name and contact information (telephone number, fax number, and email address)
2. Your site PI's name, the registering MD's name, and your institution name
3. Patient's race, sex, and DOB
4. Three letters (or two letters and a dash) for the patient's initials
5. Currently approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of enrollment
8. Completed eligibility checklist, signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email within one business day. Verification of eligibility and registration should be kept in the patient chart.

All patients at all sites must be registered through the Siteman Cancer Center database at

Washington University.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

5.0 TREATMENT PLAN

5.1 Premedication Administration

Consider prophylactic or therapeutic administration of 0.25 to 1 mg of IV or SC atropine to prevent early diarrhea (occurring during or shortly after infusion of irinotecan).

It is recommended that patients receive premedication with antiemetic agents on the day of treatment, starting at least 30 minutes before administration of irinotecan. Suggested antiemetic agents are 10 mg of dexamethasone given with a 5HT3 blocker (e.g. ondansetron or granisetron).
Prior to each ramucirumab infusion, premedicate all patients with an intravenous histamine H1 antagonist (e.g., diphenhydramine). For patients who have experienced a grade 1 or 2 infusion-related reaction, also premedicate with dexamethasone (or equivalent) and acetaminophen prior to each ramucirumab infusion.

5.2 Agent Administration

Patients will receive ramucirumab intravenously on an outpatient basis at a dose of 8 mg/kg over the course of 60 minutes on Day 1 of each 14-day cycle. They will then receive irinotecan intravenously at a dose of 180 mg/m^2 over the course of 90 minutes on Day 1 of each 14-day cycle. With approval by the Washington University PI, the agents may be given on different days within the protocol specified window.

5.3 Evaluability

All patients who receive any study treatment are evaluable for toxicity. Patients are evaluated from first receiving study treatment until a 30-day follow up after the conclusion of treatment or death.

All patients are evaluable for disease response unless they discontinue treatment prior to completion of Cycle 4 and have not had any disease assessment.

5.4 General Concomitant Medication and Supportive Care Guidelines

Patients receiving warfarin must be switched to low molecular weight heparin and have achieved stable coagulation profile prior to the first dose of protocol therapy.

Do not administer strong CYP3A4 inducers or inhibitors while patients are receiving irinotecan (refer to Appendix B).

UGT1A1 inhibitors (atazanavir, gemfibrozil, indinavir) are prohibited.

Late diarrhea associated with irinotecan can be life-threatening since it may be prolonged and may lead to dehydration, electrolyte imbalance, or sepsis. Patients should have loperamide readily available to begin treatment for late diarrhea. Begin loperamide at the first episode of poorly formed or loose stools or the earliest onset of bowel movements more frequent than normal. One dosage regimen for loperamide is 4 mg at the first onset of later diarrhea and then 2 mg every 2 hours until the patient is diarrhea-free for at least 12 hours. Loperamide is not recommended to be used for more than 48 consecutive hours at these doses because of the risk of paralytic ileus.

Patients with unresected primary tumors (or local recurrence) who develop grade 3 or 4 venous thromboembolism may also receive anticoagulation and continue ramucirumab therapy provided that the tumor does not confer an excessive bleeding risk, in the opinion of the patient's physician.

5.5 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative serum pregnancy test within 7 days prior to the first day of treatment.

Female and male patients (along with their female partners) are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for 3 months following the last dose of either study drug.

If a patient is suspected to be pregnant, ramucirumab and irinotecan should be immediately discontinued. In addition, a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient or female partner of a male patient becomes pregnant during therapy or within 3 months after the last dose of either study drug, the investigator must be notified in order to facilitate outcome follow-up.

5.6 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented

in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue indefinitely or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug, including:
    - Grade 3 or 4 infusion reaction
    - Grade 4 hypertension
    - Grade 3 or 4 arterial thromboembolic event, or any PE/DVT occurring or worsening during anticoagulant therapy
    - One occurrence of urine protein level > 3 g/24 hours, three occurrences of urine protein level > 2 g/24, or urine protein level > 2 g/24 hours for more than 2 weeks
    - Gastrointestinal perforation or fistula formation
    - RPLS
    - Hepatic encephalopathy or other serious signs of liver impairment such as hepatorenal syndrome
- General or specific changes in the patient's condition render the patient unable to receive further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.7 Duration of Follow-up

Patients will be followed every 2 months after discontinuation of treatment for progression and survival for 30 months. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6.0 DOSE DELAYS/DOSE MODIFICATIONS

6.1 Dose Modifications for Ramucirumab

Treatment modifications for toxicities not listed below will be made at the discretion of the PI. Treatment may be held for a maximum of 4 weeks before a patient must be removed from study treatment. Treatment may be held beyond 4 weeks at the discretion of the PI.

6.1.1 Infusion-Related Reactions

Reduce the infusion rate of ramucirumab by 50% for grade 1 or 2 infusion-related reactions. Permanently discontinue ramucirumab for grade 3 or 4 infusion-related reactions.

6.1.2 Hypertension

Interrupt ramucirumab for severe hypertension until controlled with medical management. Permanently discontinue ramucirumab for severe hypertension that cannot be controlled with antihypertensive therapy or any grade 4 hypertension.

6.1.3 Proteinuria

If urinalysis or dipstick shows protein ≥ 2+, a 24-hour urine collection must be collected and ramucirumab cannot be dosed until the below criteria are met. Interrupt ramucirumab for urine protein levels ≥ 2 g/24 hours. Reinitiate treatment at a reduced dose once the urine protein level returns to < 2 g/24 hours. If the protein level ≥ 2 g/24 hours reoccurs, interrupt ramucirumab and reduce the dose again once the urine protein level returns to < 2 g/24 hours. Permanently discontinue ramucirumab for urine protein level > 3 g/24 hours, if there is a third occurrence of > 2 g/24 hours, if the protein level does not return to < 2 g/24 hours within 2 weeks, or in the setting of nephrotic syndrome. If it is anticipated that the urine protein will be elevated, a 24-hour urine collection can be collected and evaluated for dosing criteria independent of the urinalysis or urine dipstick.

Initial dose	8 mg/kg
First dose reduction	6 mg/kg
Second dose reduction	5 mg/kg

6.1.4 Venous and Arterial Thromboembolic Events, Gastrointestinal Perforation, Fistula, or Bleeding

Ramucirumab should be permanently discontinued in the event of a gastrointestinal perforation or fistula formation.

Patients with unresected primary tumors (or local recurrence) who develop grade 3 or 4 venous thromboembolism may receive anticoagulation and continue ramucirumab therapy provided that the tumor does not confer an excessive bleeding risk, in the opinion of the patient's physician.

Grade 3 or 4 arterial thromboembolic events, or any PE/DVT occurring or worsening during anticoagulant therapy, require permanent discontinuation of ramucirumab therapy. Any venous or arterial event leading to discontinuation of ramucirumab therapy will be considered serious and should be reported via the SAE mechanism.

For any clinically significant bleeding events deemed at least possibly related to Ramucirumab, patients must be discontinued from further treatment unless permission is granted from the Washington University PI to continue study therapy.

6.1.5 Hepatic Encephalopathy or Other Serious Signs of Liver Impairment

Permanently discontinue ramucirumab. Signs of serious liver impairment include hepatorenal syndrome or other signs as determined by the treating physician.

6.1.6 Reversible Posterior Leukoencephalopathy Syndrome

If RPLS is diagnosed, ramucirumab must be permanently discontinued. All cases of RPLS must be reported via the SAE mechanism.

6.2 Dose Modifications for Irinotecan

Treatment modifications for toxicities not listed below will be made at the discretion of the PI. Patients requiring a dose reduction beyond that outlined in the study protocol will have the agent discontinued. The other agent may be continued independently in keeping with the applicable dose modifications for that agent. Treatment may be held for a maximum of 4 weeks before a patient must be removed from study treatment. Treatment may be held beyond 4 weeks at the discretion of the PI.

Initial Dose	180mg/m^2
First Dose Reduction	150 mg/m^2
Second Dose Reduction	120 mg/m^2

6.2.1 Late Diarrhea

Treatment should be delayed until the return of pre-treatment bowel function for at least 24 hours without anti-diarrhea medication. Patients must not be treated with

irinotecan until resolution of any bowel obstruction. If grade 2, 3, or 4 late diarrhea recurs, subsequent doses of irinotecan should be decreased to 150 mg/m^2.

6.2.2 Myelosuppression

Hold irinotecan if neutropenic fever occurs or if ANC < 1000/mm^3. After recovery to an ANC ≥ 1000/mm^3, subsequent doses of irinotecan should be reduced by one level.

6.2.3 Hypersensitivity

Discontinue irinotecan if anaphylactic reaction occurs.

6.2.4 Pulmonary Toxicity

If Interstitial Pulmonary Disease is diagnosed, irinotecan and other chemotherapy should be discontinued and appropriate treatment instituted as needed.

7.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outline below.

Please refer to Appendix C for definitions and Appendix D for a grid of reporting timelines.

Adverse events will be tracked from start of treatment through 30 days following the last day of study treatment. All adverse events must be recorded on the toxicity tracking case report form.

Refer to the data submission schedule in Section 11 for instructions on the collection of AEs in the EDC.

Reporting requirements for Washington University study team may be found in Section 7.1. Reporting requirements for secondary site study teams participating in Washington University-coordinated research may be found in Section 7.2.
Eli Lilly requires that all events be reported as outlined in Section 7.6.

7.1 Sponsor-Investigator Reporting Requirements

7.1.1 Reporting to the Human Research Protection Office (HRPO) at Washington University

Reporting will be conducted in accordance with Washington University IRB Policies.

Pre-approval of all protocol exceptions must be obtained prior to implementing the change.

7.1.2 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The Washington University Sponsor-Investigator (or designee) is required to notify the QASMC of any unanticipated problems involving risks to participants or others occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within 10 days of receipt of IRB acknowledgment via email to a qasmc@wustl.edu. Submission to QASMC must include the myIRB form and any supporting documentation sent with the form.

For events that occur at secondary sites, the Washington University Sponsor Investigator (or designee) is required to notify the QASMC within 10 days of Washington University notification via email to qasmc@wustl.edu. Submission to QASMC must include either the myIRB form and supporting documentation or (if not submitted to myIRB) the date of occurrence, description of the event, whether the event is described in the currently IRB approved materials, the event outcome, determination of relatedness, whether currently enrolled participants will be notified, and whether the informed consent document and/or any study procedures will be modified as a result of this event.

7.1.3 Reporting Requirements for Secondary Sites

The research team at each secondary site is required to promptly notify the Washington University Sponsor-Investigator and designee of all serious adverse events (Appendix C, Section D) within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report (using either an FDA 3500a MedWatch form and the Washington University Serious cover sheet (Appendix E). A formal written report must be sent to the Washington University Sponsor-Investigator and designee**4 calendar days** (for fatal of life-threatening suspected adverse reactions) or 11 calendar days (for serious unexpected suspected adverse reactions) of the occurrence of the event or notification of the secondary site's PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines. . The research team at Washington University is responsible for reporting all applicable events to the FDA, IBC, and as needed.

Washington University pre-approval of all protocol exceptions must be obtained prior to implementing the change. Local IRB approval must be obtained as per local guidelines. Washington University IRB approval is not required for protocol exceptions occurring at secondary sites.

7.1.4 Reporting to Secondary Sites

The Washington University Sponsor-Investigator (or designee) will notify the research team at each secondary site of all unanticipated problems involving risks to participants or others that have occurred at other sites within **10 working days** of the occurrence of the event or notification of the Sponsor-Investigator (or designee) of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable. Refer to Section 16.0 (Multicenter Management) for more information.

7.1.5 Reporting to Eli Lilly

Any venous or arterial event leading to discontinuation of ramucirumab therapy will be considered serious and should be reported via the SAE mechanism.

Lilly will be notified within twenty-four (24) hours of Investigator and/or Institution receiving notification of any "serious" adverse event experienced by a patient participating in the Study and receiving Study Drug. For purposes of this requirement, "serious" means: (1) death; (2) in-patient hospitalization or prolonged hospitalization; (3) life-threatening; (4) persistent or significant disability or incapacity; (5) congenital anomaly or birth defect; or (6) other serious events that may jeopardize the patient and may require medical or surgical intervention to prevent one of the other five listed outcomes. Serious adverse events should be reported to Lilly using a CIOMS Form or other form acceptable to Lilly. Institution further agrees to make available promptly to Lilly such records as may be necessary and pertinent for Lilly to further investigate an adverse event in the Study that is possibly associated with the Study Drug.

Lilly will be notified within twenty-four (24) hours of Investigator and/or Institution receiving notification or becoming aware of any product complaint related to the Study Drug. For purposes of this requirement, a product complaint is any written, electronic, or oral communication that alleges deficiencies of a drug or drug delivery systems related to: (1) identity, (2) performance, (3) reliability, (4) safety, (5) quality, (6) durability, (7) purity, or (8) effectiveness.

The Lilly Global Patient Safety Fax Number for all SAEs is 866-644-1697 or 317-453-3402.

7.1.6 Timeframe for Reporting Required Events

Adverse events will be tracked for 30 days following the last day of study treatment. With the exception of progressive disease, all adverse events that occur between the first treatment on study and 30 days after the last day of study treatment will be recorded on the case report forms. This includes all adverse events that are CTCAE version 4.03 gradable laboratory values, regardless of attribution or clinical significance. Progressive disease should not be reported as an AE.

In addition to the planned revision to the protocol detailed above, please find guidance below for tracking of abnormal labs in the study EDC:

- For patients currently on treatment at the time this clarification is released, enter the highest occurrence of each gradable abnormal lab to date with drug attribution and the highest gradable abnormal labs with drug attribution per cycle moving forward (regardless of clinical significance) on the AE case report form.

- For patients off study at the time this clarification is released, enter the highest grade of each abnormal lab with drug attribution for the duration of the patient's time on study treatment on the AE case report form.

- Please note that medical history data is not required to be entered on the AE case reporting form.

8.0 PHARMACEUTICAL INFORMATION

8.1 Ramucirumab (Cyramza)

8.1.1 Ramucirumab Description

Ramucirumab is a recombinant human IgG1 monoclonal antibody that specifically binds to vascular endothelial growth factor receptor 2. Ramucirumab has an approximate molecular weight of 147 kDa. Ramucirumab is produced in genetically engineered mammalian NS0 cells.

8.1.2 Clinical Pharmacology

Ramucirumab is a vascular endothelial growth factor receptor 2 antagonist that specifically binds VEGF Receptor 2 and blocks binding of VEGFR ligands, VEGF-A, VEGF-C, and VEGF-D. As a result, ramucirumab inhibits ligand-stimulated activation of VEGF Receptor 2, thereby inhibiting ligand-induced proliferation, and migration of human endothelial cells. Ramucirumab inhibited angiogenesis in an in vivo animal model.

8.1.3 Pharmacokinetics and Drug Metabolism

The pharmacokinetic (PK) characteristics of ramucirumab are similar for patients with gastric cancer, NSCLC, and mCRC based on a population PK analysis. The mean (% coefficient of variation [CV%]) clearance for ramucirumab was 0.015 L/hour (30%) and the mean terminal half-life was 14 days (20%).

8.1.4 Supplier(s)

Ramucirumab will be provided free of charge by Eli Lilly and Company.

8.1.5 Dosage Form and Preparation

Ramucirumab is available in two dose strengths:

- 100 mg/10 mL (10 mg per mL) solution, single dose vial
- 500 mg/50 mL (10 mg per mL) solution, single dose vial

8.1.6 Storage and Stability

Store vials in a refrigerator at 2°C to 8°C (36°F to 46°F) until time of use. Keep the vial in the outer carton in order to protect from light.

8.1.7 Administration

Calculate the dose and the required volume of ramucirumab needed to prepare the infusion solution. Vials contain either 100 mg/10 mL or 500 mg/50 mL at a concentration of 10 mg/mL solution of ramucirumab.

Withdraw the required volume of ramucirumab and further dilute with only 0.9% Sodium Chloride Injection in an intravenous infusion container to a final volume of 250 mL. Do not use dextrose containing solutions.

Gently invert the container to ensure adequate mixing.

DO NOT FREEZE OR SHAKE the infusion solution. DO NOT dilute with other solutions or co-infuse with other electrolytes or medications.

Store diluted infusion for no more than 24 hours at 2°C to 8°C (36°F to 46°F) or 4 hours at room temperature (below 25°C [77°F]).

Discard vial with any unused portion of ramucirumab.

Visually inspect the diluted solution for particulate matter and discoloration prior to administration. If particulate matter or discolorations are identified, discard the solution.

Administer diluted ramucirumab infusion via infusion pump over 60 minutes through a separate infusion line. Use of a protein sparing 0.22micron filter is recommended. Flush the line with sterile sodium chloride (0.9%) solution for injection at the end of the infusion.

8.2 Irinotecan (Camptosar)

8.2.1 Irinotecan Description

Irinotecan is an antineoplastic agent of the topoisomerase I inhibitor class. It is a semisynthetic derivative of camptothecin, an alkaloid extract from plants such as *Camptotheca acuminata* or is chemically synthesized.

The chemical name is ***(S)***-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1***H***-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate, monohydrochloride, trihydrate.

Its empirical formula is $C_{33}H_{38}N_4O_6 \cdot HCl \cdot 3H_2O$. Its molecular weight is 677.19.

8.2.2 Clinical Pharmacology

Irinotecan is a derivative of camptothecin. Camptothecins interact specifically with the enzyme topoisomerase I, which relieves torsional strain in DNA by inducing reversible single-strand breaks. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex and prevent relegation of these single-strand breaks. Current research suggests that the cytotoxicity of irinotecan is due to double-strand DNA damage produced during DNA synthesis when replication enzymes interact with the ternary complex formed by topoisomerase I, DNA, and either irinotecan or SN-38. Mammalian cells cannot efficiently repair these double-strand breaks.

8.2.3 Pharmacokinetics and Drug Metabolism

After intravenous infusion of irinotecan in humans, irinotecan plasma concentrations decline in a multiexponential manner, with a mean terminal elimination half-life of about 6 to 12 hours. The mean terminal elimination half-life of the active metabolite SN-38 is about 10 to 20 hours. The half-lives of the lactone (active) forms of irinotecan and SN-38 are similar to those of total irinotecan and SN-38, as the lactone and hydroxy acid forms are in equilibrium.

Irinotecan exhibits moderate plasma protein binding (30% to 68% bound). SN-38 is highly bound to human plasma proteins (approximately 95% bound). The plasma protein to which irinotecan and SN-38 predominantly binds is albumin.

Irinotecan is subject to extensive metabolic conversion by various enzyme systems, including esterases to form the active metabolite SN-38, and UGT1A1 mediating glucuronidation of SN-38 to form the inactive glucuronide metabolite SN-38G. Irinotecan can also undergo CYP3A4-mediated oxidative metabolism to several inactive oxidation products, one of which can be hydrolyzed by carboxylesterase to release SN-38. *In vitro* studies indicate that irinotecan, SN-38 and another metabolite aminopentane carboxylic acid (APC), do not inhibit cytochrome P-450 isozymes. UGT1A1 activity is reduced in individuals with genetic polymorphisms that lead to reduced enzyme activity such as the UGT1A1*28 polymorphism. Approximately 10% of the North American population is homozygous for the UGT1A1*28 allele (also referred to as UGT1A1 7/7 genotype).

The disposition of irinotecan has not been fully elucidated in humans. The urinary excretion of irinotecan is 11% to 20%; SN-38, <1%; and SN-38 glucuronide, 3%. The cumulative biliary and urinary excretion of irinotecan and its metabolites (SN-38 and SN-38 glucuronide) over a period of 48 hours following administration of irinotecan in two patients ranged from approximately 25% (100 mg/m2) to 50% (300 mg/m2).

8.2.4 Supplier(s)

Irinotecan is commercially available and will be billed to insurance.

8.2.5 Dosage Form and Preparation

Irinotecan is available in three single-dose sizes:

- 2 mL via containing 40 mg irinotecan hydrochloride injection
- 5 mL via containing 100 mg irinotecan hydrochloride injection
- 15 mL via containing 300 mg irinotecan hydrochloride injection

8.2.6 Storage and Stability

Store at controlled room temperature (15° to 30°C / 59° to 86°F). Protect form light.

8.2.7 Administration

See Section 5.2.

8.2.8 Special Handling Instructions

Care should be exercised in the handling and preparation of infusion solutions prepared from irinotecan injection. The use of gloves is recommended. If a solution of irinotecan contacts the skin, wash the skin immediately and thoroughly with soap and water. If irinotecan contacts the mucous membranes, flush thoroughly with water.

9.0 CORRELATIVE STUDIES

9.1 Rationale for patient-derived xenograft and organoid generation

Patient-derived xenograft (PDX) and patient-derived organoid (PDO) models have been shown to be important emerging preclinical models in oncology. Recent work has focused on developing these models for application in gastric and GEJ cancers to screen novel agents.[23-26,28,29] Despite the advancement in PDX and PDO technology, there have been few studies that have utilized both in direct comparison. Furthermore, studies are also lacking demonstrating the prospective ability of either of these models to predict or recapitulate individual patient treatment response. We propose to generate PDXs and PDOs from patient derived biopsy samples prior to initiation of trial treatment when the biopsy procedure is both safe and feasible. We plan a prospective comparison between both models and actual patient response to the same trial treatments of ramucirumab and irinotecan.

9.1.1 Collection of Specimens

For patients enrolled at Washington University School of Medicine, prior to study treatment, fresh tissue samples will be obtained either by surgical laparoscopic sampling (preferred), endoscopic biopsy, or image-guided core biopsy. If safe and feasible, biopsy will be done at least 7 days prior to planned C1D1. Additional methods to obtain adequate samples for PDX/PDO generation, including large-volume paracentesis or thoracentesis, will be allowed at PI discretion.

9.1.2 Handling of Specimens

Samples will be stored in ice cold PBS prior to downstream processing. Samples will be immediately delivered to the Mills Laboratory (Washington University School of Medicine, Division of Gastroenterology, Bldg: CSRB-NTA, Rm 927, 425 S Euclid Ave, St Louis, MO 62110 United States) and stored until processed. All samples will be processed by Mills Lab and Fields Lab for PDX/PDO related experiments.

9.1.3 PDX generation

To generate gastric cancer PDX models, typical procedures are as follows; however, standard procedures at the Fields Laboratory and PDXNet will be followed (HRPO #201803010).

Primary patient tumor tissue will be implanted subcutaneously into the flank of 6-week-old NODSCID mice (Charles River Laboratories, Wilmington, MA, USA) for the first passage. When the tumor size reaches 500 mm3 (approximately 3months), the mice will be sacrificed, tumors will be surgically removed, and implanted into male athymic nude mice (BALB/c-nude; 6 weeks old; Japan SLC, Hamamatsu, Japan) for amplification. After four consecutive passages, the xenografts will be amenable to drug treatment.

9.1.4 PDO generation

To generate gastric cancer PDO models, typical procedures are as follows; however, standard procedures at the Mills Laboratory will be followed if there is any discrepancy.

Primary patient tumor tissues will be rinsed with advanced DMEM/F12 containing 1 x P/S twice, and then incubated at 37 C with digestion buffer (advanced DMEM/F12 containing 1x P/S, 1mg/ml primocin, 2.5% FBS, 0.6mg/mL collagenase, 20mg/mL hyaluronidase and 10mM Y-27632). Tissue debris will be removed by passing the mixture through a 100 μm cell strainer. Cell clusters will be washed twice using AdDMEM medium followed by centrifugation at 1000 g for 5 min, and resuspension in Matrigel, and plated in a 24-well plate. 500 ml of standard gastric organoid medium (advanced DMEM/F12, 1x GlutaMax, 1x HEPES, 1x P/S, 50% Wnt3a, 10% RSPO-1, 10% Noggin, 1xB27, 50ng/mL EGF, 200ng/mL FGF10, 1mM N-Acetylcystenine, 1nM Gastrin, 2mM A83-01), 10mM Y-27632 and 1mg/ul primocin were added to each well. Fresh medium was changed every 2-3 days and passage of the organoid cultures will be performed every two weeks.

9.1.5 PDX and PDO treatment

For treatment of PDX models, after maturation of xenografts, athymic nude mice will be treated with ramucirumab (27 mg/kg) every 3 days for 3 weeks and irinotecan (40 mg/kg according to previous studies[30,31]). Tumors will be measured twice weekly with calipers, and tumor volumes will be calculated.

For treatment of PDO models, one day after splitting, tumor organoids will be treated with ramucirumab and irinotecan diluted according to a seven-point half-log scale, with a concentration range spanning patients' therapeutic levels[28]. For each biological run, raw data from three technical replicates across three plates will be averaged and compared with the mean of the positive control (10mM MG132) and negative control (DMSO). The effect of the drugs on the tumor organoids, in terms of cell viability, will be quantitated using a Cell titer-Glo 2.0 assay (Promega). Dose response curves, absolute IC50 and AUC will be calculated.

Based on further molecular characterization as noted below, additional therapeutic agents may be used to treat these models.

9.1.6 PDX and PDO characterization

For our PDX and PDO models, we plan for additional characterization experiments to be performed before and after drug treatment. These important additional experiments will assess concordance between PDX and PDO models through histology, transcriptome, and whole genome analyses. In addition, pre and post

treatment data will allow further exploratory analyses to be made between treatment response and molecular characteristics to generate hypotheses regarding resistance/sensitivity mechanisms. First we will process a portion of xenograft tumors and organoids by standard formalin fixation and paraffin embedding for H&E pathohistological analyses and further serial unstained sectioning. Additional samples from xenograft tumors and organoids will be processed for high quality RNA and DNA isolation using Qiagen isolation kits.

Dr. Mills is a human pathologist who will analyze morphological and cytological features of PDO and PDX samples analyzed for histology. Specifically, he will be interested in whether PDOs and PDXs and original tumor resection/biopsy specimens are concordant with level of differentiation (well, moderate, poor), pattern of invasion (single-cell, clusters, glands), morphology of tumor as a whole (single-cells with signet ring features vs. "intestinal"-type), cellular morphology (degree of atypia, N:C ratio, nuclear/nucleolar features).

A portion may be sent to the Washington University Genome Technology Access Core (GTAC) for library preparation and RNA and DNA sequencing (e.g., on an Illumina HiSeq-3000). Downstream analyses will be performed on Partek Genomics Suite. Additional analyses including comparison between PDX and PDO models, and pre- and posttreatment molecular responses using exome sequencing and transcriptomics via RNA seq may be performed. We are cognizant that the tumor organoids are an epithelial-biased system. Methods, such as single-cell RNAseq or epithelial cell enrichment through flow cytometry, may be used to distinguish and analyze the contribution of non-epithelial, non-tumor tissue in our PDX models.

9.2 Rationale for assessment of circulating angiogenic factors

Circulating levels of angiogenic factors have been increasingly evaluated in cancer patients receiving antiangiogenesis therapy. The results of such correlative studies are well documented.[32] Overall, correlations have been inconsistent and have not identified clear predictive markers. Such data in patients with gastroesophageal cancer is sparse, given the lack of Phase 2 experience in delivering antiangiogenic therapy in this disease. However, certain candidate factors warrant further investigation in ways which account for our growing appreciation of the complexity of interwoven pathways. Angiogenesis appears to be important in the progression of this disease and, therefore, correlative studies to improve our understanding of drug mechanisms and improve patient selection are warranted.

The association between circulating angiogenesis factors in gastroesophageal cancer has been explored. Gastric cancer cells have been shown to generate high VEGF levels.[33] Circulating VEGF levels have correlated with microvessel density in primary gastric tumors[34] and with higher stage.[35,36] In esophageal cancer, serum VEGF levels have been found to be raised in patients with cancer, compared with controls.[37,38] Serum VEGF has been associated with tumor size, tumor depth, lymph node metastasis, distant metastasis, response to chemotherapy, and survival.[37,39]

9.3 Blood-based Angiome profiling using multiplex technology

In the past, the gold standard for detection of growth factors and cytokines in blood was the use of ELISAs; however, multiplex technology offers an attractive alternative approach for cytokine and growth factor analysis. This novel technology allows for the measurement of multiple analytes simultaneously from a single sample. The advantages of multiplex technology compared to traditional ELISA assays are conservation of patient sample, increased sensitivity, and significant savings in cost, time and labor. Furthermore, all plate designs have been validated in order to 1) limit cross-reactivity of the antibodies 2) optimize sensitivity and specificity and 3) maximize the linearity of the assay's dynamic range.

Several systems exist, the plate-based platforms being the Meso Scale Discovery (MSD) multiplex system and the SearchLight system, produced by Aushon Biosystems. The assay design in both cases is similar to a sandwich ELISA, except multiple capture antibodies are pre-spotted into individual wells of a 96-well plate. Samples or standards are added which bind to the specific capture antibodies and are detected using various outputs. Over the past 7 years, the design of customized multiplex ELISA plates has been optimized via extensive collaborations with Aushon Biosystems. This has led to the development of an appropriately designed panel for the simultaneous evaluation of 28 regulators of tumor and normal angiogenesis.

The table below lists the panel of markers currently optimized; that would be analyzed for this study. This will be performed in collaboration with Duke Molecular Reference Laboratory at Duke University Medical Center (Dr. Andrew Nixon).

Table: Plasma-based marker identification

Soluble Angiogenic Factors		Matrix-Derived Factors	Markers of Vascular Activation and Inflammation
ANG-2	PDGF-BB	sEndoglin	CRP
bFGF	PlGF	Osteopontin	ICAM-1
HGF	VEGF-A	TGFβ1	IL-6
IGFBP1	VEGF-D	TGFβ2	PAI-1 Active
IGFBP2	sVEGFR1	TGFβRIII	PAI-1 Total
IGFBP3	sVEGFR2	TIMP1	SDF-1
PDGF-AA	sVEGFR3	TSP2	VCAM-1

9.3.1 Collection of Specimens

Ten mL of blood will be collected into one lavender top (K2EDTA) tube at each of the following time points:

- Cycle 1 Day 1 (prior to start of study treatment)
- Cycle 5 Day 1 (at the time of first restaging evaluation, prior to treatment)
- Cycle 9 Day 1 (at the time of second restaging evaluation, prior to treatment)
- End of treatment

9.3.2 Handling of Specimens

For plasma samples:

1. Invert tubes 10 times to mix blood
2. Centrifuge at 4°C at 2500 x g for 15 minutes (or in accordance with centrifuge manufacturer's instructions)
3. Remove plasma from each tube and transfer equally into two separate clean 15ml polypropylene tubes
4. Repeat centrifuge at 4°C at 2500 x g for 15 minutes (or in accordance with centrifuge manufacturer's instructions)
5. Aliquot approximately 1.0ml of plasma from each tube into each 2.0ml cryovial. For the EDTA, aliquot into pink capped cryovial. Total of 4 pink capped cryovials needed for EDTA plasma.
6. Label and freeze at -80°C* (see labeling instructions below)

- If the participating site does not have a -80°C freezer, samples should be shipped on dry ice on the day of collection. If unable to ship samples on the day of collection, please place the samples on dry ice until they can be shipped. Samples can be stored on dry ice for no more than 48 hours prior to shipping. Please replenish dry ice as needed to ensure samples stay frozen and there is enough to last throughout shipment.
- Biomarker assays are time sensitive. Samples must be processed within one hour of collection. Complete the Biomarker Flowsheet, insert a copy of the flowsheet with shipment, and fax to ATTN: BIOMARKER LABORATORY at 919-668-3925.
- Any questions regarding biomarker processing, supplies and shipping, should be directed to 919-681-2239

All samples have appropriate chain-of-custody documentation to ensure compliance with FDA and IRB regulations. Systems are in place detailing the location, transfer, and use of any and all human research subject samples. Any discrepancies or omissions in flow sheets and/or sample labels are resolved upon

receipt of the sample in the lab. All sample and data handling procedures will be fully compliant with the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

9.3.3 Shipping of Specimens

Plasma samples will be stored for future translational studies with blood based angiome-profiling.

Plasma-containing tubes need to be labeled with the following information (using a Sharpie or Cryopen):

- Protocol Name
- Subject Study Number
- Subject Initials
- Sample Date and Time
- Sample Type (ie. whole blood, EDTA plasma, citrate plasma, serum, urine)

Biomarker samples will be shipped within 48 to 72 hours of completed processing with a completed Biomarker Flowsheet with shipment.

All biomarker samples (whole blood, plasma, serum and urine) must be shipped on dry ice by overnight delivery Monday through Thursday (no holidays) to the following address:

Phase I Biomarker Laboratory
ATTN: Andrew Nixon, PhD
Duke University Medical Center
395 MSRB, Research Drive
Durham, NC 27710

9.4 Blood for Cell-Free DNA

The analysis of cell free or circulating tumor DNA is a promising area of investigation that allows for interrogation of tumor-specific molecular alterations in the circulation. One of the key advantages of cfDNA analyses is the high degree of specificity offered, because mutations found in cfDNA are in essence integral agents of an individual's cancer and are defined by their presence in tumor DNA and absence in matched normal DNA. In terms of sensitivity, levels of cfDNA are abundant in most patients with advanced cancer, allowing for the assessment of molecular heterogeneity, monitoring of tumor dynamics, identification of genetic determinants for therapy, tracking of genomic evolution, and development of acquired resistance.[40]

9.4.1 Collection of Specimens

Ten mL of blood will be collected into EDTA tubes prior to treatment at each of the following time points:

- Cycle 1 Day 1
- Cycle 3 Day 1
- Cycle 5 Day 1
- Cycle 7 Day 1
- Cycle 9 Day 1
- End of treatment

9.4.2 Handling of Specimens

See Section 9.3.2

9.4.3 Shipping of Specimens

Plasma samples will be stored for future translational studies using circulating cell-free DNA.

See Section 9.3.3

9.5 HER-2 Status Collection

Because of its involvement in the development of different types of cancer, HER-2 gene/protein status information will be collected for all patients during the study. No formal testing will take place but information will be obtained from prior testing available in medical records or tests that have occurred for other medical reasons during the trial as a part of routine medical care.

10.0 STUDY CALENDAR

Screening/baseline evaluations are to be conducted within 28 days prior to start of protocol therapy. Cycles are 14 days long but may be extended to up to 21 days at physician discretion.

	Screening	**Day 1 of each cycle (+/- 3 days)**	**Every 8 weeks (+/- 3 days**	**EOT (+/- 3 days)**	**F/U[1]**
Informed consent	X				
Demographics and medical history	X				
ECOG PS, Vitals, weight	X	X			
Height	X			X	
Physical exam	X	X		X	X[5]
EKG	X				
CBC	X	X		X	
CMP	X	X		X	
PTT/INR	X				
Urinalysis or Urine Dipstick[6]	X	X		X	
β-hCG[2]	X				
Imaging[3] and RECIST 1.1 time point response	X		X	X[4]	
Ramucirumab		X			
Irinotecan		X			
Tumor Biopsy (Wash U only)	X[7]				
Blood for angiome profiling		Before treatment on C1D1, C5D1, and C9D1		X	
Blood for cfDNA		Before treatment on C1D1, C3D1, C5D1, C7D1, and C9D1		X	
Collection of HER2 status	At some point during the study				
Adverse event assessment	X ---------- X				X
Survival information					X

1. 30 days after EOT visit, then every 2 months. AEs only collected for 30 days after treatment discontinuation.
2. Women of childbearing potential only
3. Imaging (CT or MRI) will be performed every 8 weeks, +/- 7days, regardless of dosing/cycle delays up to 1 year. After 1 year, the imaging frequency is at the discretion of the treating physician. Information from lesion evaluation by imaging done as part of routine care will be collected until progressive disease has been objectively determined if the patient discontinued treatment due to any other reason than progressive disease.
4. EOT imaging not required if most recent scan has been performed within 21 days prior to EOT.
5. Physical exam will only be done if the follow-up visit involves an in-person office visit.
6. A urine dipstick may be performed in lieu of a full urinalysis, however, if the dipstick is abnormal, confirm urine protein with a full urinalysis. If urinalysis or dipstick shows protein ≥ 2+, a 24-hour urine collection must be collected.
7. If safe and feasible, biopsies are to be performed at least 7 days prior to the planned C1D1. See Section 9.1 for additional information.

11.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
Registration Form Eligibility Form On-Study Form	Prior to starting treatment
Treatment Form	Every cycle
Toxicity Form	Continuous
Correlative Form	Per protocol
Treatment Summary Form	Completion of treatment
Follow Up Form	Every 2 months
Tumor Measurement Form	Submit at imaging time points. See section 10.0 for imaging time points.
MedWatch Form	See Section 7.0 for reporting requirements

Any queries generated by Washington University must be responded to within 28 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

11.1 Adverse Event Collection in the Case Report Forms

All adverse events that occur beginning with start of treatment (minus exceptions defined in Section 7.0) must be captured in the Toxicity Form. Baseline AEs should be captured on the Toxicity Form.

Participant death due to disease progression should be reported on the Toxicity Form as grade 5 disease progression. If death is due to an AE (e.g. cardiac disorders: cardiac arrest), report as a grade 5 event under that AE. Participant death must also be recorded on the Death Form.

12.0 MEASUREMENT OF EFFECT

12.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 8 weeks regardless of dosing/cycle delays. In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

Information from lesion evaluation by imaging done as part of routine care will be collected until progressive disease has been objectively determined if the patient discontinued treatment due to any other reason than progressive disease.

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).[41] Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

12.2 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 >20 mm by chest x-ray, 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).

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. 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.

12.3 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.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

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).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

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.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [JNCI 96:487-488, 2004; J Clin Oncol 17, 3461-3467, 1999; J Clin Oncol 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [JNCI 92:1534-1535, 2000].

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

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.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up

corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

- FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

12.4 Response Criteria

12.4.1 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.

12.4.2 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. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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).

12.4.3 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.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	>4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	>4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once >4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
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.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

12.4.4 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 progressive 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, including the baseline measurements.

12.4.5 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first. For those who are alive and do not experience progression, we censor them at the time of loss to follow-up (very few) or at 12 months from the study entry, which come first.

12.4.6 Response Review

It is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images is the best approach.

13.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, an independent Data and Safety Monitoring Board (DSMB) will be specifically convened for this trial to review toxicity data. A DSMB will consist of no fewer than 3 members including 2 clinical investigators and a biostatistician. DSMB members must be employed by Washington University, Barnes-Jewish Hospital, or St. Louis Children's Hospital. Like investigators, DSMB members are subject to the Washington University School of Medicine policies regarding standards of conduct. Individuals invited to serve on the DSMB will disclose any potential conflicts of interest to the trial principal investigator and/or appropriate university officials, in accordance with institution policies. Potential conflicts that develop during a trial or a member's tenure on a DSMB must also be disclosed.

Until such a time as the first secondary site enrolls its first patient, a semi-annual DSM report to be prepared by the study team will be submitted to the Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning 6 months after study activation at Washington University (if at least one patient has been enrolled) or one year after study activation (if no patients have been enrolled at the 6-month mark).

The DSM report for the DSMB will be prepared by the study team with assistance from the study statistician, will be reviewed by the DSMB, and will be submitted to the QASM Committee. The DSMB must meet beginning six months after enrollment of the first patient at a secondary site, no more than one month prior to the due date of the DSM report to QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date, accrual by site, and accrual by arm
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites and separated by arm
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Further DSMB responsibilities are described in the DSMB charter.
The study principal investigator and coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines (Please refer to section 7.0).

Refer to the Washington University Quality Assurance and Data Safety Monitoring Committee Policies and Procedures for full details on the responsibilities of the DSMC at https://siteman.wustl.edu/research/clinical-research-resources/protocol-office-prmcqasmc/.

14.0 AUDITING

Since Washington University is the coordinating center, each site will be audited annually by Siteman Cancer Center personnel (QASMC) unless the outside institution has an auditing mechanism in place and can provide a report. The outside sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.

Additional details regarding the Auditing Policies and procedures can be found at http://www.siteman.wustl.edu/uploadedFiles/Research_Programs/Clinical_Research_Resources/Protocol_Review_and_Monitoring_Committee/QASMC%20Policies%20and%20Procedures%205-3-13.pdf

15.0 STATISTICAL CONSIDERATIONS

15.1 Design and Sample Size

This is a multi-institutional, open-labeled, Phase II study. The study will be conducted at five institutions (including Washington University). Patients with previously treated, metastatic esophagogastric adenocarcinomas will be assigned to treatment with irinotecan plus ramucirumab.

Target sample size for this single arm, phase II study would be **40 patients**. A sample size of 40 has 85% power at a 0.050 significance level to detect a median progression-free survival time of 4 months in the treatment group when the median survival time of the

historic control group is 2.5 months. These estimations are based on historical data from irinotecan studies and a PFS improvement of 1.5 months seen in the RAINBOW study (PFS for paclitaxel alone = 2.9 months, paclitaxel plus ramucirumab = 4.4 months). Calculation is based on one-sided, one-sample log-rank test and assumes that subjects will be accrued for a period of 12 months, and follow-up continues for a period of 12 months after the last subject is enrolled.[42,43] The survival time distributions of both groups are assumed to follow an exponential distribution.

15.2 Primary Efficacy Measure

Progression-Free Survival (PFS):
PFS will be measured from date of study entry to first radiographic progression or death due to any cause. Radiographic progressive disease (PD) will be defined using Response Evaluation Criteria in Solid Tumors v1.1 (RECIST v1.1).[41] For those who are alive and do not experience progression, we censor them at the time of loss to follow-up (very few) or at 12 months from the study entry, which come first.

15.3 Secondary Efficacy Measures

- *Overall Survival Time (OS):*
  OS time will be measured from date of study entry to date of death from any cause. For those who are alive, we censor them at the time of loss to follow-up (very few) or at 30 months from the date of treatment discontinuation, which come first.
- *Time to Progressive Disease (TTP):*
  TTP is defined as the time from study entry until date of radiographic PD using RECIST v1.1 criteria. For those who are alive and do not experience progression, we censor them at the time of loss to follow-up (very few) or at 12 months from the study entry, which come first. For those who are dead before progression, we will consider death as the competing risk. If the number of death are very small, we will censor them at time of death.
- *Best Overall Response (BOR) of Complete Response (CR), Partial Response (PR), Stable Disease (SD) or PD:*
  BOR is defined as the best response across all time points from randomization until radiologically confirmed PD using RECIST, v1.1 criteria. CR is defined as the disappearance of all target and non-target lesions and any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm and normalization of tumor marker level of non-target lesions. PR is defined as having a ≥30% decrease in sum of longest diameter (LD) of target lesions. PD was defined as having a ≥20% increase in sum of LD of target lesions and ≥5 mm increase above nadir. SD was defined as small changes that did not meet above criteria.
- *Objective Response Rate (ORR):*
  ORR is defined as the percentage of participants with confirmed CR or confirmed PR.
- *Clinical Benefit Rate (CBR):*
  CBR is defined as percentage of combined patients who have achieved confirmed CR, PR and SD.

15.4 Anticipated Findings

We anticipate prolongation of PFS with combination of irinotecan and ramucirumab. This may provide an early signal of activity of this combination in second line treatment of esophagogastric adenocarcinomas, and would lay the foundation for future phase III studies.

15.5 Exploratory Analyses

For PDX/PDO models, successful generation would be defined by growth in size/number ability to expand, and ability to passage. Treatment response will be assessed by cell death by counting or using a cell death assay. The correlation coefficient from the PDX and PDO models will be compared with the clinical outcome from the patients (progression vs no progressive disease) using a point biserial model which will give us a measure on the strength of the relationship between the two variables. Paired analyses for correlation will be underpowered (67% power to detect effect size of 0.6 and 10% significance level under null hypothesis of a medium effect size of 0.3 with 10 paired samples). Therefore, all analyses will be descriptive and hypothesis generating in nature, and it will be used to guide future clinical trial development incorporating PDX/PDO models.

For circulating biomarkers, levels of plasma-based biomarkers will be presented in descriptive summary. Spearman correlations among analytes may be calculated and illustrated. The prognostic value of each analyte as a continuous measure may be assessed by a univariate Cox proportional hazards model for PFS with corresponding hazard ratios and 95% confidence intervals. Cox regression analyses with each analyte adjusting for clinical variables, including age, race, gender and performance status may be performed.

16.0 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Regulatory Coordinator (or designee) prior to IRB/IEC submission.

Each participating institution must have the following documents on file at Washington University prior to first subject enrollment:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.
- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality Assurance and Safety Monitoring Committee (QASMC), the DSMC (if applicable), and the Washington University study team.
- Documentation of FWA, signed FDA Form 1572, and signed and dated CVs of all participating investigators.
- Documentation of training in protection of human subjects by all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The Principal Investigator is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. There will be one current version of the protocol document at any given time and each participating institution will utilize that document. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 2 weeks of obtaining Washington University IRB approval with acknowledgement of receipt requested. Secondary sites are to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt, and confirmation of submission must be forwarded to the appropriate contact person on the Washington University study team at the time of submission. Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

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APPENDIX A: ECOG Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B: CYP3A4 Strong Inhibitors and Inducers

Strong CYP3A4 Inhibitors	Strong CYP3A4 Inducers
Indinavir	Efavirenz
Nelfinavir	Nevirapine
Ritonavir	Barbiturates
Clarithromycin	Carbamazepine
Itraconazole	Glucocorticoids
Ketoconazole	Modafinil
Nefazodone	Oxcarbazepine
Saquinavir	Phenobarbital
Suboxone	Phenytoin
Telithromycin	Pioglitazone
Lopinavir	Rifabutin
Telaprevir	Rifampin
Voriconazole	St. John's wort
	Troglitazone

http://medicine.iupui.edu/clinpharm/ddis/main-table

APPENDIX C: Definitions for Adverse Event Reporting

A. Adverse Events (AEs)

As defined in 21 CFR 312.32:

Definition: any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.03 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

B. Suspected Adverse Reaction (SAR)

As defined in 21 CFR 312.32:

Definition: any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. "Suspected adverse reaction" implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

C. Life-Threatening Adverse Event / Life Threatening Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: any adverse drug event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator, its occurrence places the patient at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

D. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: an adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- Death
- A life-threatening adverse event

- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Any other important medical event that does not fit the criteria above but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

E. Protocol Exceptions

Definition: A planned change in the conduct of the research for one participant.

F. Deviation

Definition: Any alteration or modification to the IRB-approved research without prospective IRB approval. The term "research" encompasses all IRB-approved materials and documents including the detailed protocol, IRB application, consent form, recruitment materials, questionnaires/data collection forms, and any other information relating to the research study.

A minor or administrative deviation is one that does not have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

A major deviation is one that does have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

APPENDIX D: Reporting Timelines

Expedited Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Eli Lilly**
Serious AND unexpected suspected adverse reaction			Report no later than 15 calendar days after it is determined that the information qualifies for reporting	Report to Eli Lilly within 24 hours of Sponsor-Investigator notification.
Unexpected fatal or life-threatening suspected adverse reaction			Report no later than 7 calendar days after initial receipt of the information	Report to Eli Lilly within 24 hours of Sponsor-Investigator notification.
Unanticipated problem involving risk to participants or others	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	Report via email after IRB acknowledgment		Report to Eli Lilly within 24 hours of Sponsor-Investigator notification.
Pregnancy				Notify Eli Lilly as they occur.
Overdose				Notify Eli Lilly as they occur.
Major deviation	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.			
A series of minor deviations that are being reported as a continuing noncompliance	Report within 10 working days.			
Protocol exception	Approval must be obtained prior to implementing the change			
Clinically important increase in the rate of a serious suspected adverse reaction of that list in the protocol or IB			Report no later than 15 calendar days after it is determined that the information qualifies for reporting	
Complaints	If the complaint reveals an unanticipated problem involving risks			

Expedited Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Eli Lilly**
	to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.			
Breach of confidentiality	Within 10 working days.			
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.			

Routine Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Eli Lilly**
Adverse event or SAE that does not require expedited reporting	If they do not meet the definition of an unanticipated problem involving risks to participants or others, report summary information at the time of continuing review	Adverse events will be reported in the toxicity table in the DSM report which is typically due every 6 months.	The most current toxicity table from the DSM report is provided to the FDA with the IND's annual report.	Notify monthly via AE notification or cross-reporting reports/documents.
Minor deviation	Report summary information at the time of continuing review.			
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.			

Routine Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Eli Lilly**
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.			

Expedited Reporting Timelines for Secondary Sites				
Event	**WU (Coordinating Center)**	**Local IRB**	**FDA**	**Eli Lilly**
Serious AND unexpected suspected adverse reaction	Report no later than 11 calendar days after it is determined that the information qualifies for reporting.	Report all applicable events to local IRB according to local institutional guidelines.	The research team at Washington University is responsible for reporting all applicable events to the FDA as needed.	The research team at Washington University is responsible for reporting all applicable events to Eli as needed.
Unexpected fatal or life-threatening suspected adverse reaction	Report no later than 4 calendar days after initial receipt of the information.			
Unanticipated problem involving risk to participants or others	Report no later than 4 calendar days after initial receipt of the information.			
Adverse event or SAE that does not require expedited reporting	As per routine data entry expectations			
Protocol exception	Approval must be obtained prior to implementing the change.			

APPENDIX E: Washington University Serious Adverse Event Reporting Sheet

SAE COVER SHEET- Secondary Site Assessment

Washington University HRPO#:	Sponsor-Investigator:
Subject Initials:	Subject ID:
Treating MD:	Treating Site:
EVENT TERM:	Event Start Date:
EVENT GRADE:	Date of site's first notification:

Treating MD Event Assessment:

Is this event **possibly, probably, or definitely** related study treatment?

☐ yes ☐ no

If yes, please list which drug (if more than one)_____

Explain _____

_____

_____ _____ _____

Physician's Name **Physician's Signature** **Date**