Phase II study of FOLFIRINOX chemotherapy for treatment of advanced gastric, gastro-esophageal junction, and esophageal tumors

Washington University School of Medicine, Division of Oncology
660 South Euclid Avenue, Campus Box 8056
St. Louis, MO 63110

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Principal Investigator: Haeseong Park, M.D.
Phone: (314) 454-5102
E-mail: haeseongpark@wustl.edu

Sub-Investigators	**Affiliation**
Seth Crosby, M.D.	GPS@WU
Kenneth Krajewski, M.D.	Medical Oncology
Christopher Maher, Ph.D.	Genome Institute at Washington University
Joel Picus, M.D.	Medical Oncology
Robert Pufahl, Ph.D.	Medical Oncology
Caron Rigden, M.D.	Medical Oncology
Rama Suresh, M.D.	Medical Oncology
Benjamin Tan, M.D.	Medical Oncology
Andrea Wang-Gillam, M.D., Ph.D.	Medical Oncology
Kathryn Trinkaus, Ph.D.	Biostatistics

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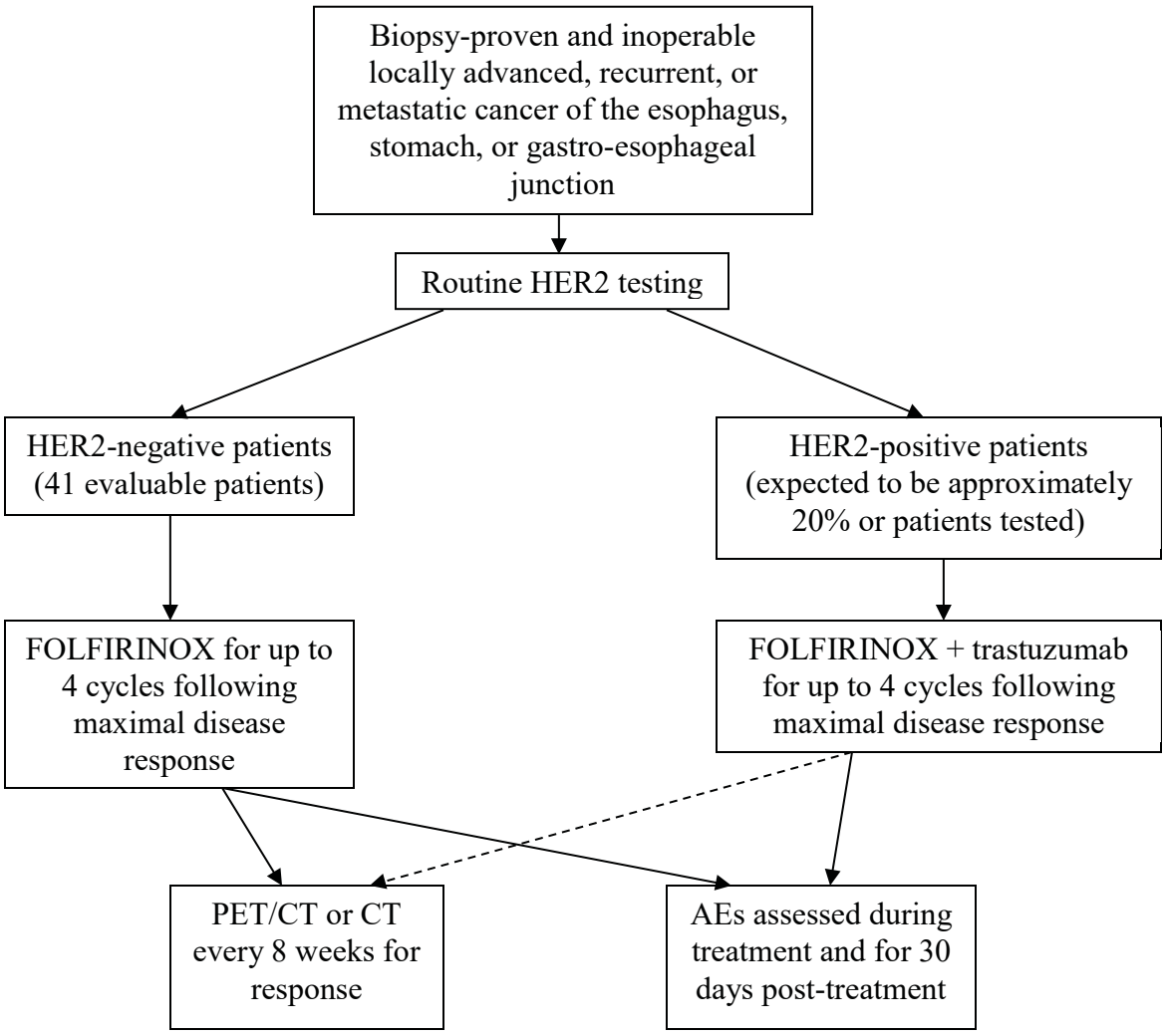
SCHEMA


Biopsy-proven and inoperable locally advanced, recurrent, or metastatic cancer of the esophagus, stomach, or gastro-esophageal junction
Routine HER2 testing
HER2-negative patients (41 evaluable patients)
HER2-positive patients (expected to be approximately 20% or patients tested)
FOLFIRINOX for up to 4 cycles following maximal disease response
FOLFIRINOX + trastuzumab for up to 4 cycles following maximal disease response
PET/CT or CT every 8 weeks for response
AEs assessed during treatment and for 30 days post-treatment

Glossary of Abbreviations

ADCC	Antibody dependent cellular cytotoxicity
ADL	Activities of daily living
AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
AUC	Area under the curve
B-HCG	Beta human chorionic gonadotropin
CBC	Complete blood count
CMP	Comprehensive metabolic panel
CNS	Central nervous system
CR	Complete response
CRF	Case report form
CSF	Cerebrospinal fluid
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DNA	deoxyribonucleic acid
DSM	Data and Safety Monitoring
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
FDA	Food and Drug Administration
G-CSF	Granulocyte colony stimulating factor, filgrastim (Neupogen)
GEJ	Gastroesophageal junction
GI	Gastrointestinal
GPS	Genomic and Pathology Services
HIV	Human Immunodeficiency Virus
HR	Hazard ratio
HRPO	Human Research Protection Office (IRB)
IND	Investigational New Drug
IRB	Institutional Review Board
IULN	Institutional upper limit of normal
IV	Intravenous
LFT	Liver function test
LVEF	Left ventricular ejection fraction
MRI	Magnetic resonance imaging
MS	Median survival
MUGA	Multiple uptake gated acquisition
NCCN	National Cancer Center Network
NCI	National Cancer Institute

NIH	National Institutes of Health
OHRP	Office of Human Research Protections
ORR	Overall response rate
OS	Overall survival
PD	Progressive disease
PET	Positron emission tomography
PFS	Progression-free survival
PI	Principal investigator
PO	Per os (by mouth)
PR	Partial response
QASMC	Quality Assurance and Safety Monitoring Committee
RECIST	Response Evaluation Criteria in Solid Tumors (Committee)
RR	Response rate
SAE	Serious adverse event
SCC	Siteman Cancer Center
SD	Stable disease
SEER	Surveillance Epidemiology and End Results
TTP	Time to progression
UPN	Unique patient number
US	Ultrasound

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1.0 BACKGROUND AND RATIONALE

1.1 Esophageal and Gastric Cancers

Esophageal and gastro-esophageal tumors have been on the rise since 1975 per the SEER database while gastric cancers have remained stable [1]. The American Cancer Society estimates 38,500 new cases of esophageal and gastric cancer are diagnosed annually in the United States [2]. Esophageal cancer has an approximate 86% fatality rate and the gastric cancer fatality rate is approximately 48% [2]. Esophageal cancer presents as incurable locally advanced or metastatic disease in approximately 60% of cases. In the US during 2012, this corresponds to an estimated 17,460 new esophageal cancer cases with a staggering 15,070 expected deaths [3]. Poor long-term survival rates are also seen in advanced gastric cancer; in the United States, approximately 21,320 patients are diagnosed annually, while 10,540 are expected to die from the disease [3]. Most symptomatic gastric cancer patients in the United States already have incurable disease at time of diagnosis. The high mortality rate from these cancers reflects aggressive biology, advanced stage at diagnosis, and lack of effective multimodality therapies. As the goal of therapy is palliation in the majority of cases, the current environment presents many opportunities for improvement.

1.2 Combination Regimens for the Treatment of Esophagogastric Cancers

Standard first-line chemotherapy for metastatic or locally advanced gastroesophageal cancers usually involves a two- or three-drug regimen, typically including a combination of a fluoropyrimidine (5-FU or capecitabine) and a platinum agent (such as cisplatin or oxaliplatin) [4]. Per NCCN, two-drug cytotoxic regimens are preferred because of lower toxicity. Although three-drug cytotoxic regimens are acknowledged for medically fit patients with a good performance status, it also requires access to frequent toxicity evaluations. Preferred regimens with category 1 data are DCF (docetaxel, cisplatin, and fluorouracil), ECF (epirubicin, cisplatin and fluorouracil), and lastly the combination of a fluoropyridine (fluorouracil or capecitabine) and cisplatin [5, 6].

Based on the 2008 study for Cunningham et al, capecitabine and oxaliplatin for advanced esophagogastric cancer is as effective as fluorouracil and cisplatin when compared in this randomized trial [7]. Therefore, combining a fluoropyrimidine with either cisplatin or oxaliplatin are accepted options. A randomized phase III trial of irinotecan/5-fluorouracil showed borderline noninferiority to cisplatin/5-fluorouracil in naïve patients with advanced adenocarcinoma of the stomach or esophagogastric junction and a second-line study evaluating irinotecan demonstrated a survival benefit for patients receiving this medication [8] [9]. Taken together, the efficacy of oxaliplatin and irinotecan has been demonstrated in these cancers and are included in the NCCN guidelines.

The combination of oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX) has become more commonly used in gastrointestinal malignancies in recent years. In 2011, the New England Journal of Medicine published a study of FOLFIRINOX versus

gemcitabine for metastatic pancreatic cancer [10]. The study randomized 342 patients to receive either FOLFIRINOX or single agent gemcitabine (standard of care). The median overall survival was 11.1 months in the FOLFIRINOX group compared to 6.8 months in the gemcitabine group (HR death, 0.57; 95% confidence 0.45-0.73; P<0.001). Also in this study, the objective response rate was 31.6% in the FOLFIRINOX group versus 9.4% in the gemcitabine group (P<0.001). More adverse events were noted in the FOLFIRINOX group, specifically 5.4% percent with febrile neutropenia.

Similarly, a phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) was used as first line treatment for metastatic colorectal cancer [11]. The study randomized 244 patients to receive either FOLFOXIRI or FOLFIRI. The study noted an increase in grade 3 to 4 neutropenia, but not a statistically significant increase in febrile neutropenia with FOLFOXIRI. Both the progression-free survival (PFS) and overall survival (OS) were both significantly improved in the FOLFOXIRI arm (median PFS, 6.9 v 9.8 months, hazard ratio [HR], 0.63; P = 0.0006; median OS, 16.7 v 22.6 months; HR, 0.70; P=0.032). Responses, as assessed by investigators were 41% vs. 66% for FOLFIRI and FOLFOXIRI respectively (P = .0002). Response confirmed by an external panel was 34% versus 60% (P < .0001).

We hypothesize that FOLFIRINOX will result in a similar increase of overall response rate and survival in patients with gastroesophageal cancers.

1.3 HER2 Expression

The HER-2 receptor is a member of the epidermal growth receptor (EGFR) family of receptors which participate in activation of pathways controlling epithelial cell growth and differentiation [12]. Amplification of HER2 or overexpression of its protein product has been widely used as a target for therapy in breast cancer. HER-2 positivity has been identified in 23.7% of gastric cancers, providing the opportunity to target HER2 positivity in advanced disease (4).

1.4 Trastuzumab (Herceptin)

Trastuzumab is a recombinant DNA-derived humanized monoclonal antibody against HER2 and is approved by FDA for treatment of metastatic and early stage breast cancer that overexpresses HER2. It has also been approved by FDA for use with chemotherapy in the treatment of gastric and gastroesophageal tumors that are HER2-positive.

A recent international phase 3 study trastuzumab for Gastric Cancer (ToGA) compared capecitabine plus cisplatin or fluorouracil plus cisplatin given every 3 weeks for six cycles compared to chemotherapy in combination with trastuzumab as first line treatment of HER2-positive advanced gastric or gastro-esophageal junction cancer [13]. The median survival was 13.8 months (95% CI 12-16) in those assigned to trastuzumab plus chemotherapy compared with 11.1 months (10-13) in those assigned to chemotherapy alone (hazard ratio 0.74; 95% CI 0.60-0.91; p=0.0046) [13]. This pivotal study led to the approval of trastuzumab for the treatment of HER-2 expressing gastroesophageal cancers.

1.5 Study Rationale

FOLFIRINOX has been shown to provide marked increases in response rates and survival in patients with cancers of the lower gastrointestinal tract such as colorectal cancer. We hypothesize that this regimen will be tolerated and that similar effects will be seen in patients with cancers of the upper gastrointestinal tract. This study proposes a phase II clinical trial FOLFIRINOX chemotherapy for treatment of advanced gastric, gastro-esophageal junction, and esophageal tumors. Because of the statistically significant improvement in outcomes identified in the ToGA trial, trastuzumab will be added to the FOLFIRINOX regimen for patients who are HER2-positive. To our knowledge, that regimen has not been previously administered to patients, so safety and tolerability will be monitored and reported. Disease response parameters from the HER2-positive patients will be collected for future analysis.

1.6 Correlative Studies Background

It is readily accepted that mutations acquired during cancer initiation and progression can result in altered proteins that contribute to transformation.[14-17] Alternatively, many passenger mutations are acquired as byproducts of the genomic instability accompanying cellular transformation. In either scenario, these genomic alterations may result in the expression of mutant proteins that are perceived as foreign proteins by the immune system and are more exploitable targets for immune-mediated tumor control.[18, 19] Abnormal proteins that arise as a consequence of somatic mutations, termed neoantigens, when recognized by T cells can lead to an immune-mediated anti-tumor response in patients.[20, 21] It has been clinically demonstrated that checkpoint blockade immunotherapy facilitates the expansion of preexisting T cells specific for tumor neoantigens and that mutation-associated neoantigen recognition is an important component of the endogenous antitumor immune response.[22] Therefore the likelihood of generating tumor neoantigens appears to be roughly proportional to the number of somatic mutations present within a given tumor. Our working hypothesis is that the response rates to checkpoint inhibitors may be enhanced by elevating the expression of tumor neoantigens through effective chemotherapy.

1.6.1 Evidence supporting that chemotherapy enhances immune response

Standard cancer chemotherapy can promote tumor immunity by inducing immunogenic cell death as part of its intended therapeutic effect and by disrupting tumoral immune evasion. Chemotherapy drugs at their standard doses and schedule can mediate their antitumor effects by inducing immunogenic cell death involving the concomitant release of tumor antigens and the emission of danger-associated molecular patterns (DAMP) in the tumor microenvironment.[23] However, multiple factors including tumor biology, the particular chemotherapeutic agent and dose/schedule, may influence whether tumor cell death is immunogenic, and which cell death pathway is activated. Other forms of immunogenic chemotherapy-induced cell death include autophagy.[24, 25] It has been suggested that the

generation of endogenous immune mediated anti-tumor responses may be required for durable success of conventional chemotherapies.[26] This hypothesis has led to considerable interest in combining immunotherapy with conventional chemotherapy, even though the optimal methods of combining these treatment strategies are incompletely understood.

Immunotherapy concepts that favor combinations with chemotherapy to augment efficacy include chemotherapy induced enhancement of tumor antigen presentation by upregulating the expression of tumor antigens themselves, or of the MHC class I molecules to which the antigens bind. Alternatively, chemotherapy may upregulate costimulatory molecules (e.g. B7-1) or downregulate coinhibitory molecules (PD-L1/B7-H1 or B7-H4) expressed on the tumor cell surface, enhancing the strength of effector T-cell activity. Chemotherapy may also render tumor cells more sensitive to T cell–mediated lysis through fas-, perforin-, and Granzyme B–dependent mechanisms.[27, 28] Numerous reports confirm the expected synergy between conventional chemotherapy and immune therapy, and as above, the synergy is mediated by diverse mechanisms including preferential depletion of regulatory T reg cells[29-31], liberation of homeostatic or inflammatory cytokines[32, 33] and enhanced immunogenicity of chemotherapy treated tumors.[25, 34] In the context of vaccines targeting self-antigens, chemotherapy given prior to or during[35] vaccination can yield synergy and enhanced survival.[31, 36] In a phase III trial, ipilimumab, a fully human monoclonal antibody against CTLA-4, was used in combination with chemotherapy in patients with metastatic melanoma. Patients receiving ipilimumab in combination with dacarbazine had significantly improved overall survival compared with patients receiving dacarbazine alone.[37] Additionally, a phase II trial in patients with advanced staged lung cancers combined ipilimumab with standard chemotherapy. Treatment timing in this study showed that a 'phased regimen' in which immunotherapy began after chemotherapy resulted in substantially improved progression-free survival (PFS) compared with chemotherapy alone.[38, 39] This study also showed that the clinical effects of administering immunotherapy in combination with chemotherapy may be dependent on the sequencing of treatment.

Conversely, however, other clinical trials have shown that certain standard chemotherapies may also inhibit immunotherapy. One concern has been that the chemotherapy will preferentially kill rapidly proliferating cells - in this case the T cell response required for immune-mediated anti-tumor activity. In clinical trials of pancreatic and prostate cancers, specific vaccine/chemotherapy sequencing strategies may have impaired vaccine-induced immunity.[40, 41] Thus, the optimal integration of immunotherapies with standard cancer therapies to minimize antagonistic interactions and engage potential synergies is therefore of great importance. One strategy is to give immunotherapy in the setting of minimal residual disease, after the tumor mass has been optimally reduced with surgery or effective systemic chemotherapy. This sequencing strategy minimizes the negative impact of tumor bulk (e.g. via tumor-expressed immunosuppressive factors) on the

potency of the antitumor immune response. It also allows chemotherapy to modulate the immune phenotype of any residual tumor cells. Several preclinical and clinical studies have shown improved responses to immunotherapy in the setting of either lower tumor burden or in combination with preparative regimens aimed at cytoreduction.[42, 43] However, the use of distinct chemotherapy drugs which can have differing immune effects, adds to the complexity of determining the relationships between chemotherapy treatments and immune response (reviewed in [27, 44]).

1.6.2 Correlative Study Procedures

Patients undergoing highly active chemotherapy will undergo extensive immune profiling with aim towards characterizing the immune response to this chemotherapy to inform future immunotherapy studies and aid in optimizing these treatments. Assessment of the tumor, tumor microenvironment and immune system will be characterized at baseline, after 2 cycles of chemotherapy and at time of progression.

Biopsies from primary or metastatic lesions: Ten consenting patients with accessible metastatic lesions will undergo optional paired core biopsies from one metastatic lesion prior to the start of FOLFIRINOX chemotherapy and after 2 cycles of therapy. At the time of tumor progression, patients will be approached about having a biopsy of an accessible site of metastatic disease.. Four to 8 cores will be taken at each time point. Biopsy of metastases will be obtained by core needle and will be performed under ultrasound or CT-guidance via interventional radiology. These specimens will be used to characterize changes in biomarkers and immune parameters associated with tumoral response or resistance.

Peripheral blood testing: Participating patients will undergo phlebotomy at the specified time points: Pre-chemotherapy then post-chemotherapy at 2-week intervals at the time of standard-of-care phlebotomy for a maximum of 8 blood draws. Blood will again be drawn at the time of documented tumor progression.

Tumor immune correlates: Analysis will be performed by co-investigators from the Center for Human Immunology and Immunotherapy Programs (CHiiPs) and the McDonnell Genome Institute (MGI). Expression of PD-1, PD-L1, PD-L2, and CTLA-4 (an alternative checkpoint pathway) will be determined by IHC on tumor cells and tumor infiltrating leukocytes (TIL). Tumor samples obtained from primary or metastatic biopsies will be analyzed for these biomarkers. Expression levels of checkpoint molecules (PD-1, PD-L1, PD-L2, and CTLA-4) will be compared in paired primary and metastatic biopsies. Each patient will serve as is its own comparator. The frequency of concordant or discrepant expression of various mechanisms of checkpoint inhibition between primary and metastatic disease sites will be defined.

Single cell suspensions will be made from fresh tissue to characterize the phenotype

of various populations of infiltrating leukocytes (T cells, B cells, NK cells, monocytes/macrophages, dendritic cells) by multi-parametric flow cytometry (FACS) and mass cytometry (CyTOF). Expression of various activation and inhibitory markers on the TIL will also be assessed.

Tumor neoantigen assessments: Mutation burden will be determined by whole exome sequencing. PBMCs will be used to obtain germline sequences for comparison to isolated tumor from tissue biopsies (somatic mutations).

- Tumor cells will be microdissected from tissue biopsies and sent for whole exome sequencing and RNAseq to quantify the mutational landscape as a surrogate of antigenic burden. Whole exome sequencing and RNAseq will be performed on tissue samples to identify potential neoepitopes via MHC class I and II prediction algorithms. Identified neoepitopes will be validated in peptide-specific autologous peripheral blood mononuclear stimulation assays.
- Tumor neoantigen identification and definition by established techniques. MHC tetramers against the specific identified tumor neoantigens will allow the qualitative evaluation of the T cells that are potentially responsible for tumor rejection.

2.0 OBJECTIVES

2.1 Primary Objective

To determine the objective response rate (defined as CR + PR by RECIST 1.1 criteria) of FOLFIRINOX in advanced gastric, GEJ, or esophageal cancer.

2.2 Secondary Objectives

1. To determine the progression-free survival (PFS) of patients treated with FOLFIRINOX.
2. To determine time to progression (TTP) in patients treated with FOLFIRINOX.
3. To determine the overall survival (OS) of patients treated with FOLFIRINOX.
4. To determine the clinical benefit rate of patients treated with FOLFIRINOX. Clinical benefit rate is the percentage of combined patients who have achieved complete response, partial response, and stable disease.
5. To determine the duration of response of patients treated with FOLFIRINOX.
6. To evaluate toxicity and tolerability of FOLFIRINOX +/- trastuzumab as measured by CTCAE version 4.0

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. Biopsy-proven and inoperable locally advanced, recurrent, or metastatic cancer of the esophagus, stomach, or gastro-esophageal junction – adenocarcinoma type.

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, as ≥20 mm by chest x-ray, or ≥10 mm with calipers by clinical exam.

3. Prior single modality radiation therapy is allowed.

4. At least 18 years of age.

5. ECOG performance status ≤ 2 (see Appendix 1)

6. Normal bone marrow and organ function as defined below:
   a. Absolute neutrophil count ≥ 1,500/mcl
   b. Platelets ≥ 100,000/mcl
   c. AST(SGOT)/ALT(SGPT) ≤ 2.5 x IULN unless there is known liver metastases in these instances AST(SGOT)/ALT(SGPT) ≤ 5 x IULN
   d. Creatinine ≤ IULN
      OR
      Creatinine clearance ≥ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal
   e. Total bilirubin ≤ 1.5 mg/dL.

7. HER-2 status may be pending at initiation of FOLFIRINOX, but must be known prior to starting Trastuzumab. If HER-2 is positive, patients must have an LVEF ≥ 50%. HER-2 negative patients are not excluded.

8. Women of childbearing potential and men must agree to use 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.

9. Ability to understand and willingness to sign an IRB approved written informed consent document (legally authorized representative is allowed).

10. Patients already receiving treatment with FOLFIRINOX +/- trastuzumab may participate in the study and have their data collected retrospectively if they met inclusion criteria at the start of therapy and sign consent for study participation moving forward.

3.2 Exclusion Criteria

1. Chemotherapy in the 6 months prior to registration.

2. Any active malignancy within 3 years that may alter the course of esophageal cancer. (Apparently cured localized malignancy or advanced, but indolent malignancy with

significantly more favorable prognosis are allowed).

3. Receiving any other investigational agents at the time of registration.

4. Known untreated brain metastases. These patients 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.

5. A history of allergic reactions attributed to compounds of similar chemical or biologic composition to the agents used in the study.

6. Previous therapy for metastatic gastroesophageal cancer. Previous perioperative chemotherapy is allowed as long as the duration without treatment has been greater than 6 months.

7. A history of congestive heart failure, transmural myocardial infarction, symptomatic valvular disease, or high-risk arrhythmia.

8. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

9. Pregnant and/or breastfeeding. Patient must have a negative urine pregnancy test within 14 days of study entry.

10. Known HIV-positivity and on combination antiretroviral therapy because of the potential for pharmacokinetic interactions with trastuzumab. 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
2. Registration of patient in the Siteman Cancer Center OnCore database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility collecting the information listed below:

1. The registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

All patients must be registered through the Siteman Cancer Center OnCore database.

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 Assessment of HER2 Status

A new biopsy is not required for patient participation. However, if a patient does not have adequate tissue to assess HER2 status, then a fresh biopsy will be necessary as a determination of HER2 status is part of the standard of care therapy.

5.2 Premedication Administration

Given the high incidence of nausea and vomiting associated with the FOLFIRINOX regimen, a combination of aprepitant, 5-HT3 antagonist, and dexamethasone before chemotherapy is strongly recommended.

5.3 Agent Administration

The FOLFIRINOX regimen used for this study is the following drugs given on a 28-day cycle:

- Irinotecan 180 mg/m^2 IV on Days 1 and 15
- Oxaliplatin 85 mg/m^2 IV on Days 1 and 15
- Leucovorin 400 mg/m^2 IV on Days 1 and 15

- 5FU 400 mg/m^2 bolus and 2,400 mg/m^2 continuous infusion over 46 hours beginning on Days 1 and 15

In order for a patient to be treated, his/her total bilirubin must be $\leq$ 1.5 x ULN and his/her AST (SGOT)/ALT (SGPT) must be $\leq$ 3.0 x ULN. Patients with elevated liver function tests may be enrolled (i.e., if they have a biliary obstruction), but their values must have resolved as described above prior to initiation of Cycle 1 treatment with FOLFIRINOX.

For HER2-positive patients, trastuzumab will be administered intravenously with a loading dose of 6 mg/kg on Cycle 1 Day 1, followed by a dose of 4 mg/kg every 2 weeks (Day 15 and Day 1 of all future cycles). In the event a participant's HER2 status is pending at the time treatment is initiated, trastuzumab will be added to the study regimen with the next cycle after determining HER2-positive status.

All study drugs will be administered every 2 weeks on a 28-day cycle as described above. Trastuzumab will be administered first (for patients who are HER2-positive only), followed by irinotecan. Oxaliplatin and leucovorin are then administered concurrently. Lastly, the 5-FU bolus will be given, followed by the 5-FU continuous infusion. In the event of a leucovorin shortage, levoleucovorin may be substituted at a dose of 200 mg/m^2. When supplies of leucovorin become available, change to the protocol-specified dose. If neither leucovorin nor levoleucovorin are available, treatment without leucovorin would be acceptable but should be re-addressed with each treatment.

5.4 General Concomitant Medication and Supportive Care Guidelines

Supportive care: Patients should receive full supportive care, including transfusions of blood and blood products, antibiotics, anti-nausea/diarrhea, etc., when appropriate.

Diarrhea management: Patients should be closely monitored for diarrhea. Dose modification for diarrhea is clearly outlined under Section 6.0. Patients should be instructed to take loperamide at the first sign of poorly formed or loose stools. Patients should take loperamide in the following fashion: 4 mg at the first onset of diarrhea, then 2 mg every 2 hours until diarrhea resolved. The total dose of loperamide should not exceed 16mg/day. Patients are instructed to notify their treating physicians if diarrhea is not resolved within 24 hours. Additional antidiarrheal medications including Lomotil, tincture of opium, or octreotide can be used.

Palliative radiation therapy: Palliative radiation therapy may not be administered concurrently with the trial chemotherapy. The need for palliative radiation therapy will be considered evidence of progressive disease, and patients will be taken off study.

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 urine pregnancy test within 14 days prior to Cycle 1 Day 1.

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 30 days following the last dose of any of the study agents.

If a patient is suspected to be pregnant, all study agents 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 30 days after the last dose of any of the study agents, 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 for up to 4 cycles following disease maximal response (based on CT imaging) 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
- General or specific changes in the patient's condition render the patient unacceptable for 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

If the treating physician determines that a maximal response has been achieved and would like to simplify the study regimen in order to mitigate toxicities for a maintenance strategy, possible options can include but are not limited to modified FOLFOX-6 (with trastuzumab for HER2 positive patients), combination 5-FU/leucovorin (with trastuzumab for HER2 positive patients), FOLFIRI (with trastuzumab for HER2 positive patients), irinotecan (with trastuzumab for HER2 positive patients), or trastuzumab alone (for HER2 positive patients only). Maintenance therapy may also include drugs not specifically part of the

FOLFIRINOX regimen. Patients will continue to have follow up radiological assessments according to treating physician's discretion, until documented progression. Study coordinator should be notified of scans so RECIST can be requested.

A patient who remains on any component of the study regimen or on maintenance therapy will be considered still on study as long as disease progression has not occurred.

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 for toxicities for up to 30 days after the end of study treatment and until death for survival assessment. 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 Reductions

Agent	Level 1 (starting dose)	Dose Reduction -1	Dose Reduction -2
5FU bolus	400 mg/m^2	320 mg/m^2	270 mg/m^2
5FU infusion over 46-48 hours	2400 mg/m^2	1900 mg/m^2	1600 mg/m^2
Oxaliplatin	85 mg/m^2	65 mg/m^2	50 mg/m^2
Irinotecan	180 mg/m^2	150 mg/m^2	120 mg/m^2
Leucovorin*	400 mg/m^2	400 mg/m^2	400 mg/m^2

* If 5FU is skipped, then leucovorin will also not be administered.

6.2 Hematologic Toxicities

The hematologic toxicities associated with FOLFIRINOX are known, based on the large Phase III ACCORD study as well as the institutional experience.

CTCAE Version 4 Terminology	CTCAE Version 4 Grade	FOLFIRINOX
Note: If any patient requires a dose delay > 21 days cumulatively during Cycle 1 or > 14 days cumulatively during Cycle 2, s/he will be removed from the study. Dose modifications will be based on AEs which have occurred either between treatments or on the scheduled treatment day. Patients will not have more than two dose reductions of each agent.		
Neutrophil count decreased (ANC < 999-500/mm^3) *	Grade 3	Delay dose until recovered to 1,200/mm^3. Reduce dose level of 5FU bolus and irinotecan by one dose level for all subsequent cycles.
Neutrophil count decreased (ANC < 500/mm^3) *	Grade 4	Delay dose until recovered to 1,200/mm^3. Reduce dose level of 5FU bolus and irinotecan by one dose level for all subsequent cycles.
Platelet count decreased (49,999-25,000/mm^3)	Grade 3	Delay dose until recovered to 100,000/mm^3. Reduce dose level of 5FU (bolus and infusion), oxaliplatin, and irinotecan by one dose level for all subsequent cycles.
Platelets count decreased (< 25,000/mm^3)	Grade 4	Delay dose until recovered to 100,000/mm^3. Reduce dose level of 5FU (bolus and infusion), oxaliplatin, and irinotecan by one dose level for all subsequent cycles.

*There need not be any dose reduction for grade 3 or 4 neutropenia because patients may receive G-CSF. However, dose reduction for grade 3 or 4 neutropenia in addition to or in place of receiving G-CSF may occur at the PI's discretion.

6.3 Gastrointestinal Toxicities

CTCAE Version 4 Terminology	CTCAE Version 4 Grade	FOLFIRINOX
Note: If any patient requires a dose delay > 21 days cumulatively during Cycle 1 or > 14 days cumulatively during all subsequent cycles, s/he will be removed from the study. All treatment will be delayed until toxicities resolve to ≤ Grade 1.		
Diarrhea (despite administration of antidiarrheal(s))	Grade 3/4	Reduce 5FU (bolus and infusion) and irinotecan by one dose level for all subsequent cycles.
Mucositis	Grade 3	Reduce 5FU (bolus and infusion) by one dose level for all subsequent cycles.
	Grade 4	Reduce 5FU (bolus and infusion) and irinotecan by one dose level for all subsequent cycles.
Vomiting (despite administration of anti-emetic(s))	Grade 3/4	Reduce dose level of 5FU (bolus and infusion), oxaliplatin, and irinotecan by one dose level for all subsequent cycles.
Other clinically significant AE	Grade 3/4	Reduce dose level of 5FU (bolus and infusion), oxaliplatin, and irinotecan by one dose level for all subsequent cycles.
Blood bilirubin increased OR Aspartate aminotransferase increased OR Alkaline phosphatase increased OR Alanine aminotransferase increased	Grade 2**	Reduce dose level of irinotecan by one dose level for all subsequent cycles.
	Grade 3**	Reduce dose level of 5FU (bolus and infusion) and irinotecan by one dose level for all subsequent cycles.
	Grade 4**	Discontinue FOLFIRINOX. *

* Patients who develop elevated LFTs should be carefully evaluated to rule out the possibility of biliary obstruction. If grade 4 toxicities in LFTs are deemed to be caused by treatment, then patient will be removed from the study.
** For patients who have liver metastases with elevated AST, ALT, and/or bilirubin during screening dose modifications should be given based off treating MD discretion.

6.4 Neurotoxicity

Modify dose of oxaliplatin in the event of neurotoxicity as follows:

CTCAE Version 4 Terminology	1-7 days	More than 7 days
Grade 1 paresthesia or dysesthesia (mild sensory alteration)	Maintain dose.	Maintain dose.
Grade 2 paresthesia or dysesthesia (moderate sensory alteration which limits instrumental ADL)	Maintain dose.	Reduce dose by one dose level for all subsequent cycles.
Grade 3 paresthesia or dysesthesia (severe sensory alteration which limits self-care ADL)	First episode: Reduce dose by one dose level for all subsequent cycles. Second episode: Stop oxaliplatin	Stop oxaliplatin.

6.5 Dose Modification for Obese Patients

All chemotherapy dosing will be calculated with actual body weight on the day of treatment. The body weight at time of registration can be used for Cycle 1 treatment if the changes in the weight are less than 10%.

6.6 Dose Modifications for Non-Hematological Toxicities

Treatment may be held and suspect drug(s) may be reduced by one level for all subsequent cycles for any grade 3 or 4 non-hematological toxicity, or any grade 2 non-hematological toxicity of particular concern which the investigator believes is related to the study therapy.

6.7 Management of Trastuzumab Side Effects

For the first (loading) dose of trastuzumab, premedication with acetaminophen 650 mg PO will be given.

Patients should not miss more than one q 2 week dose of trastuzumab consecutively. Patients do not have to make up missed doses.

6.7.1 Dose Modifications for Trastuzumab

Dose modification of trastuzumab is **not** permitted.

6.7.2 Infusion-associated Symptoms with Trastuzumab

Infusion reactions consist of a symptom complex characterized by fever and chills, and on occasion nausea, vomiting, pain (in some cases at tumor sites), headache,

dizziness, hypotension, rash, and asthenia. In postmarketing reports, serious and fatal infusion reactions have been reported. Severe reactions which include bronchospasm, anaphylaxis, angioedema, hypoxia, and severe hypotension were usually reported during or immediately following the initial infusion. However, the onset and clinical course were variable including progressive worsening, initial improvement followed by clinical deterioration, or delayed post-infusion events with rapid clinical deterioration. For fatal events, death occurred within hours to days following a serious infusion reaction.

Interrupt trastuzumab infusion in all patients experiencing dyspnea, clinically significant hypotension, and intervention of medical therapy administered, which may include: epinephrine, corticosteroids, diphenhydramine, bronchodilators, and oxygen. Patients should be evaluated and carefully monitored until complete resolution of signs and symptoms. The rate of infusion should be decreased for mild or moderate (CTCAE grade 1 or 2) infusion reactions. Permanent discontinuation should be strongly considered in all patients with severe (grade 3 or 4) infusion reactions.

7.0 REGULATORY AND REPORTING REQUIREMENTS

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

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 7.2.

The FDA requires that all serious and unexpected adverse events be reported as outlined in Section 7.4. In addition, any fatal or life-threatening adverse experiences where there is a reasonable possibility of relationship to study intervention must be reported.

7.1 Definitions

7.1.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease. Note: For the purposes of this study, abnormal lab values will only be considered adverse events if they are clinically significant.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 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

7.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

All unexpected SAEs must be reported to the FDA.

7.1.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

Events that are both serious AND unexpected must be reported to the FDA.

7.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Life-threatening adverse experiences must be reported to the FDA.

7.1.5 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

- related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.1.6 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

7.1.7 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

7.1.8 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

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

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

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the 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 to the PI of the event.

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

The PI is required to notify the QASMC of any unanticipated problem occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO as reportable. (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 auditor.

7.4 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the investigator to report any unanticipated problem to the FDA as follows:

- Report any unexpected fatal or life-threatening adverse experiences (Section 7.1.4) associated with use of the drug by telephone or fax no later than **7 calendar days** after initial receipt of the information.
- Report any serious, unexpected adverse experiences (Section 7.1.2), as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after initial receipt of this information.

All MedWatch forms will be sent by the investigator or investigator's team to the FDA at the following address or by fax:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Drug Products
5901-B Ammendale Rd.
Beltsville, MD 20705-1266
FAX: 1-800-FDA-0178

7.5 Timeframe for Reporting Required Events

Adverse events will be tracked for 30 days following the last day of study treatment. For the purposes of this study, abnormal lab values will only be collected and documented on CRFs if they are clinically significant. Once a patient begins maintenance therapy, adverse events will no longer be collected and documented on CRFs.

8.0 PHARMACEUTICAL INFORMATION

8.1 Irinotecan (Camptosar®)

8.1.1 Irinotecan Description

Irinotecan is an antineoplastic agent of the topoisomerase I inhibitor class. It is indicated for the treatment of patients with metastatic carcinoma of the colon or rectum whose disease has recurred or progressed following 5FU-based therapy.

Molecular formula: $C_{33}H_{38}N_4O_6 \bullet HCl \bullet 3H_2O$.
Chemical name: (4S)-4, 11-diethyl-4-hydroxy-9-[(4-piperidino-piperidino) carbonyloxy]-1H-pyrano[3',4':6,7] indolizino [1,2-b]quinoline-3,14(4H, 12H)dione hydrochloride trihydrate.
Molecular weight: 677.19.

8.1.2 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 religation 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.

Irinotecan serves as a water-soluble precursor of the lipophilic metabolite SN-38. SN-38 is formed from irinotecan by carboxylesterase-mediated cleavage of the carbamate bond between the camptothecin moiety and the dipiperidino side chain. SN-38 is approximately 1000†times as potent as irinotecan as an inhibitor of topoisomerase I purified from human and rodent tumor cell lines. In vitro cytotoxicity assays show that the potency of SN-38 relative to irinotecan varies from 2- to 2000-fold. However, the plasma area under the concentration versus time curve (AUC) values for SN-38 are 2% to 8% of irinotecan and SN-38 is 95% bound to plasma proteins compared to approximately 50% bound to plasma proteins for irinotecan. The precise contribution of SN-38 to the activity of irinotecan is thus unknown. Both irinotecan and SN-38 exist in an active lactone form and an inactive hydroxy acid anion form. A pH-dependent equilibrium exists between the two forms such that an acid pH promotes the formation of the lactone, while a more basic pH favors the hydroxy acid anion form.

Administration of irinotecan has resulted in antitumor activity in mice bearing cancers of rodent origin and in human carcinoma xenografts of various histological types.

8.1.3 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.

The metabolic conversion of irinotecan to the active metabolite SN-38 is mediated by carboxylesterase enzymes and primarily occurs in the liver. SN-38 subsequently undergoes conjugation to form a glucuronide metabolite. SN-38 glucuronide had 1/50 to 1/100 the activity of SN-38 in cytotoxicity assays using two cell lines in vitro. 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/m^2) to 50% (300 mg/m^2).

8.1.4 Supplier(s)

Irinotecan is considered standard of care for this indication and is commercially available.

8.1.5 Dosage Form and Preparation

Irinotecan is supplied as a sterile, pale yellow, clear, aqueous solution. It is available in two single-dose sizes: 2 mL-fill vials contain 40 mg irinotecan hydrochloride and 5 mL-fill vials contain 100 mg irinotecan hydrochloride. Each milliliter of solution contains 20 mg of irinotecan hydrochloride (on the basis of the trihydrate salt), 45 mg of sorbitol NF powder, and 0.9 mg of lactic acid, USP. The pH of the solution has been adjusted to 3.5 (range, 3.0 to 3.8) with sodium hydroxide or hydrochloric acid. Irinotecan is intended for dilution with 5% Dextrose Injection, USP (D5W), or 0.9% Sodium Chloride Injection, USP, prior to intravenous infusion. The preferred diluent is 5% Dextrose Injection, USP.

The dosage given for this study is 180 mg/m^2.

8.1.6 Storage and Stability

The solution is physically and chemically stable for up to 24 hours at room temperature (approximately 25ºC) and in ambient fluorescent lighting. Solutions diluted in 5% Dextrose Injection, USP, and stored at refrigerated temperatures (approximately 2° to 8°C), and protected from light are physically and chemically stable for 48 hours.

8.1.7 Administration

Irinotecan is administered intravenously over the course of 90 minutes. Irinotecan is emetogenic. It is recommended that patients receive premedication with antiemetic agents. In clinical studies of the weekly dosage schedule, the majority of patients received 10 mg of dexamethasone given in conjunction with another type of antiemetic agent, such as a 5-HT3 blocker (e.g., ondansetron or granisetron). Antiemetic agents should be given on the day of treatment, starting at least 30 minutes before administration of irinotecan. Physicians should also consider providing patients with an antiemetic regimen (e.g., prochlorperazine) for subsequent use as needed.

8.1.8 Special Handling Instructions

As with other potentially toxic anticancer agents, 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.

8.2 Leucovorin

8.2.1 Leucovorin Description

Leucovorin is indicated after high dose methotrexate therapy in osteosarcoma. It is also indicated for use in combination with 5FU to prolong survival in the palliative treatment of patients with advanced colorectal cancer.

Molecular formula: $C_{20}H_{21}CaN_7O_7$.
Chemical name: Calcium N –[*p* –[[[(6RS)-2-amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl]methyl]amino]benzoyl]-L-glutamate(1:1).
Molecular weight: 511.51.

8.2.2 Clinical Pharmacology

Leucovorin is readily converted to another reduced folate, 5,10-methylenetetrahydrofolate, which acts to stabilize the binding of fluorodeoxyuridylic acid to thymidylate synthase and thereby enhances the inhibition of this enzyme.

8.2.3 Pharmacokinetics and Drug Metabolism

The mean peak of 5-methyl-THF was 258 ng/mL and occurred at 1.3 hours. The terminal half-life for total reduced folates was 6.2 hours. The area under the concentration versus time curves (AUCs) for l-leucovorin, d-leucovorin and 5-methyltetrahydrofolate were 28.4 ± 3.5, 956 ± 97 and 129 ± 12 (mg.min/L ± S.E.).

8.2.4 Supplier(s)

Leucovorin is considered standard of care for this indication and is commercially available.

8.2.5 Dosage Form and Preparation

Leucovorin is indicated for intravenous or intramuscular administration and is supplied as a sterile lyophilized powder. The 350 mg vial is preservative free. The inactive ingredient is sodium chloride 140 mg/vial for the 350 mg vial. Sodium hydroxide and/or hydrochloric acid are used to adjust the pH to approximately 8.1 during manufacture. One milligram of leucovorin calcium contains 0.002 mmol of leucovorin and 0.002 mmol of calcium.

Leucovorin Calcium for Injection is supplied in sterile, single-use vials.

The dosage given for this study is 400 mg/m^2.

8.2.6 Storage and Stability

Store at 25°C (77°F); excursions permitted to 15-30°C (59°-86°F). Protect from light.

8.2.7 Administration

Leucovorin will be administered intravenously over the course of 120 minutes.

Because of the calcium content of the leucovorin solution, no more than 160 mg of leucovorin should be injected intravenously per minute (16 mL of a 10 mg/mL, or 8 mL of a 20 mg/mL solution per minute).

8.2.8 Special Handling Instructions

None.

8.3 Oxaliplatin (Eloxatin®)

8.3.1 Oxaliplatin Description

IV Oxaliplatin is FDA approved in combination with infusional 5FU and leucovorin (FOLFOX) for the treatment of patients with metastatic carcinoma of the colon or rectum whose disease has recurred or progressed during or within 6 months of completion of first line therapy with the combination of bolus 5FU/leucovorin and irinotecan.

Molecular formula: $C_8H_{14}N_2)4Pt$.
Chemical name: cis- [(1R,2R)-1,2-cyclohexanediamine-N, N'] [oxaloto (2-)-O, O'] platinum.
Molecular weight: 397.3.

8.3.2 Clinical Pharmacology

Oxaliplatin is a newer platinum derivative with an oxalo ligand group. Although the exact mechanism of oxaliplatin remains unclear, the cytotoxicity of platinum compounds is thought to result from inhibition of DNA synthesis. Intrastrand platinum DNA adducts, the main cytotoxic lesions, are formed by cross-linking activated platinum species and specific base sequences, notably two adjacent guanine residues or two adjacent guanine-adenine bases.

8.3.3 Pharmacokinetics and Drug Metabolism

Time to peak concentration with a single 2-hour infusion of oxaliplatin 85mg/m^2 yielded a peak plasma concentration of 0.814 mcg/mL. The area under the curve for total plasma platinum was 207-290 mg/L per hour. Ultrafiltrable plasma platinum was 11.9-13.6 mg/L per hour. Oxaliplatin has 70-95% platinum-protein binding while 37% represents total platinum taken up by red blood cells (addition of oxaliplatin to whole blood). The volume of distribution is 440 L after a single 2 hour infusion of 85 mg/m^2 oxaliplatin. It undergoes rapid and extensive (30%) nonenzymatic biotransformation. In vitro studies indicate no cytochrome P450-mediated metabolism. Its metabolites include approximately 17 different platinum containing derivatives with some being cytotoxic. It is not known whether oxaliplatin is excreted into breastmilk. Renal clearance is 9.3-17 liters per hour. Elimination half-life: intravenous, total plasma platinum, alpha half-life (0.2-0.43 hours), beta half-life (15-16.8 hours), gamma half-life (252-391 hours). The decline of ultrafiltrable platinum levels is tri-exponential with a relatively short alpha and beta half-life (0.43 and 16.8 hours) and a long terminal gamma half-life (391 hours).

8.3.4 Supplier(s)

Oxaliplatin is considered standard of care for this indication and is commercially available.

8.3.5 Dosage Form and Preparation

Oxaliplatin is formulated as a white freeze-dried powder in amber glass vials and contains 50 mg or 100 mg of oxaliplatin in lactose monohydrate. Each vial is sealed with a stopper with a crimped aluminum cap.

The freeze-dried powder is reconstituted by adding 10-20 mL (for the 50 mg vial) or 20-40 mL (for the 100 mg vials) of sterile water for injection or 5% dextrose solution and then by diluting in an infusion solution of 250 mL or 500 mL of 5% dextrose solution. These manipulations cannot be performed with aluminum needles. The reconstitution or final dilution can't ever be performed with a sodium chloride solution.

The dosage given for this study is 85 mg/m^2.

8.3.6 Storage and Stability

Oxaliplatin is a freeze-dried powder and may be stored at room temperature as long as it is protected from light for up to 3 years. It should not be combined with alkaline medications or media, which cause oxaliplatin to degrade. Do not use needles or IV infusion sets containing aluminum items (risk of degradation of oxaliplatin upon contact with aluminum) for the preparation or administration of oxaliplatin. Oxaliplatin should not be mixed with sodium chloride or other chloride containing solutions. Reconstituted solution must be prepared in a 5% dextrose solution of sterile water for injection in the original vial. This solution may be stored for 24-48 hours at 2-80ºC. Infusion solution: after dilution in 5% dextrose solution, the shelf-life is 24 hours at room temperature.

8.3.7 Administration

Oxaliplatin will be administered intravenously over the course of 2 hours. Antiemetic premedication (5-HT3 blocker with or without dexamethasone) is recommended. Cold temperatures can precipitate/exacerbate neurological symptoms which should be avoided during the infusion of oxaliplatin.

8.3.8 Special Handling Instructions

Oxaliplatin should never be reconstituted/diluted with a chloride-containing solution, and aluminum parts should be avoided when mixing or preparing

oxaliplatin. Oxaliplatin is incompatible with alkaline media (i.e., solutions of 5FU). Oxaliplatin should be prepared in 250-500 mL D5W.

8.4 5FU (5-fluorouracil)

8.4.1 5FU Description

Fluorouracil is effective in the palliative management of carcinoma of the colon, rectum, breast, stomach, and pancreas.

Molecular formula: $C_4H_3FN_2O_2$.
Chemical name: 5-fluoro-2,4 (1H,3H)-pyrimidinedione.
Molecular weight: 130.08.

8.4.2 Clinical Pharmacology

The precise mechanisms of action of fluorouracil have not been fully elucidated. The main mechanism is thought to be the binding of the deoxyribonucleotide of the drug (FdUMP) and the folate cofactor, N5-10-methylenetetrahydrofolate, to thymidylate synthase (TS) to form a covalently bound ternary complex, which inhibits the formation of thymidylate from uracil, thereby interfering with DNA synthesis. In addition FUTP can be incorporated into RNA in place of uridine triphosphate (UTP), producing a fraudulent RNA and interfering with RNA processing and protein synthesis.

8.4.3 Pharmacokinetics and Drug Metabolism

Absorption: Following IV administration of fluorouracil, no intact drug is detected in plasma after 3 hours.

Distribution: Fluorouracil is distributed into tumors, intestinal mucosa, bone marrow, liver, and other tissues. Despite its limited lipid solubility, the drug readily crosses the blood-brain barrier and distributes into CSF and brain tissue. Distribution studies in humans and animals have usually shown a higher concentration of the drug or its metabolites in the tumor than in surrounding tissue or in corresponding normal tissue. It has also been shown that there is a longer persistence of fluorouracil in some tumors than in the normal tissues of the host, perhaps due to impaired uracil catabolism. From these data, it has been suggested that the drug may possibly have some specificity against certain tumors in comparison with normal tissues.

Elimination: Following IV administration, the plasma elimination half-life averages about 16 minutes (range: 8-20 minutes) and is dose dependent. A small portion of fluorouracil is anabolized in the tissues to 5-fluoro-2-deoxyuridine and then to 5-fluoro-2-deoxyuridine-5-monophosphate, the active metabolite of the drug. The major portion of the drug is degraded in the liver. The metabolites are

excreted as respiratory carbon dioxide and as urea, α-fluoro-β-alanine, α -fluoro- β -guanidopropionic acid, and α -fluoro- β -ureidopropionic acid in urine. Following a single IV dose of fluorouracil, approximately 15% of the dose is excreted in urine as intact drug within 6 hours; over 90% of this is excreted in the first hour.

8.4.4 Supplier(s)

5FU is considered standard of care for this indication and is commercially available.

8.4.5 Dosage Form and Preparation

Inspect for precipitate; if found, agitate or gently heat in water bath. Bolus injections are prepared using undiluted drug. Continuous infusions of fluorouracil should be prepared for administration via ambulatory infusion pump according to the individual institution's standards. These solutions may be prepared in D5W or 0.9% NaCl.

The dosage given for this study is 400 mg/m^2 bolus and 2,400 mg/m^2 continuous infusion over 46 hours.

8.4.6 Storage and Stability

Intact vials should be stored at room temperature and protected from light. Slight yellow discoloration does not usually indicate decomposition. 5-FU is stable in syringes for up to 72 hours. Stability in ambulatory pumps varies according to the pump, manufacturer of drug, concentration and dilution. Please refer to appropriate reference sources for additional information.

8.4.7 Administration

5-FU will be administered intravenously, first in as a bolus and then as a continuous 46 hour infusion. Fluorouracil should be administered only intravenously, taking care to avoid extravasation. No dilution is required.

8.4.8 Special Handling Instructions

None.

8.5 Trastuzumab (Herceptin)

8.5.1 Description

Trastuzumab (Herceptin) is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity to the extracellular domain of HER2 (Kd = 5 nM)[45, 46]. The antibody is an IgG_1 kappa that contains human

framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2.

8.5.2 Pharmacokinetics and Drug Metabolism

Trastuzumab administered once weekly demonstrated dose-dependent pharmacokinetics. Mean half-life increased and clearance decreased with increasing dose level. The half-life averaged 1.7 and 12 days at the 10 and 500 mg dose levels, respectively. Trastuzumab's volume of distribution was approximately that of serum volume (44 mL/kg). At the highest weekly dose studied (500 mg), mean peak serum concentrations were 377 mcg/mL.

In studies using a loading dose of 4 mg/kg followed by a weekly maintenance dose of 2 mg/kg, a mean half-life of 5.8 days (range = 1 to 32 days) was observed. Between Weeks 16 and 32, trastuzumab serum concentrations reached a steady state with a mean trough and peak concentrations of approximately 79 microgram/mL and 123 microgram/mL, respectively.

Data suggest that the disposition of Trastuzumab is not altered based on age or serum creatinine (up to 2.0 mg/dL). No formal interaction studies have been performed.

8.5.3 Supplier(s)

Trastuzumab is the standard of care in this patient population and is commercially available.

8.5.4 Dosage Form and Preparation

The diluent provided has been formulated to maintain the stability and sterility of trastuzumab for up to 28 days. Other diluents have not been shown to contain effective preservatives for trastuzumab. Each vial of trastuzumab should be reconstituted with **ONLY 20 mL of BWFI, USP, 1.1% benzyl alcohol preserved, as supplied,** to yield a multi-dose solution containing 21 mg/mL Trastuzumab. Use of all 30 mL of diluent results in a lower-than-intended dose of trastuzumab. THE REMAINDER (approximately 10 mL) OF THE DILUENT SHOULD BE DISCARDED. Immediately upon reconstitution with BWFI, the vial of trastuzumab must be labeled in the area marked "Do not use after:" with the future date that is 28 days from the date of reconstitution.

Trastuzumab is supplied as a lyophilized, sterile powder nominally containing 440 mg Trastuzumab per vial under vacuum.

Each carton contains one vial of 440 mg trastuzumab and one 30 mL vial of Bacteriostatic Water for Injection, USP, 1.1% benzyl alcohol. NDC50242-134-60.

8.5.5 Storage and Stability

Vials of trastuzumab are stable at 2-8°C (36-46°F) prior to reconstitution. Do not use beyond the expiration date stamped on the vial. A vial of trastuzumab reconstituted with BWFI, as supplied, is stable for 28 days after reconstitution when stored refrigerated at 2-8°C (36-46°F), and the solution is preserved for multiple use. Discard any remaining multi-dose reconstituted solution after 28 days. If unpreserved SWFI (not supplied) is used, the reconstituted trastuzumab solution should be used immediately and any unused portion must be discarded. DO NOT FREEZE HERCEPTIN THAT HAS BEEN RECONSTITUTED.

The solution of trastuzumab for infusion diluted in polyvinylchloride or polyethylene bags containing 0.9% Sodium Chloride Injection, USP, may be stored at 2-8°C (36-46°F) for up to 24 hours prior to use. Diluted trastuzumab has been shown to be stable for up to 24 hours at room temperature (2-25°C). However, since diluted trastuzumab contains no effective preservative, the reconstituted and diluted solution should be stored refrigerated (2-8°C).

8.5.6 Administration

Treatment may be administered in an outpatient setting by administration of a 6 mg/kg loading dose by intravenous (IV) infusion over 90 minutes. The maintenance dose is 4 mg/kg every 2 weeks after the loading dose. **DO NOT ADMINISTER AS AN IV PUSH OR BOLUS.** Patients should be observed for fever and chills or other infusion-associated symptoms.

8.5.7 Special Handling Instructions

None.

9.0 CORRELATIVE STUDIES

9.1 Archived Tumor Tissue for Analysis by GPS@WUSTL

Archived tissue will be sent for genetic testing though the Genomics and Pathology Services at Washington University School of Medicine in St. Louis (GPS@WUSTL). The testing evaluates for mutations in 28 genes associated with a variety of cancers involving blood, lymph nodes, and solid tumors. These 28 genes were chosen by GPS@WUSTL as they all have implications in cancer therapy. We hope to identify other possible relationships between treatment outcomes in this study and “actionable” gene mutations noted from the GPS profiling. These results may be useful to select future treatments for the participating patients or help to inform future clinical trials.

Archived specimens from consenting patients will be sent to:
Genomic & Pathology Services

CORTEX Building, 2nd Floor, Room 209
4320 Forest Park Avenue
St. Louis, MO 63108

9.2 Tumor Tissue Correlates – OPTIONAL

Ten consenting patients with accessible metastatic lesions (regardless of whether they are receiving FOLFIRINOX alone or with trastuzumab) will undergo paired core biopsies from the metastatic lesion at the following time points:

- baseline (prior to the start of FOLFIRINOX chemotherapy)
- end of Cycle 2 of FOLFIRINOX
- time of progression (optional even if the patient consented to the paired biopsies)

These specimens will be used to quantify expression of PD-1, PD-L1, PD-L2, and CTLA-4 by IHC on tumor cells and tumor infiltrating leukocytes. Additionally, mutation burden will be determined by whole exome sequencing, with somatic mutations being detected by comparing isolated tumor from tissue biopsies to peripheral blood mononuclear cells (see Section 9.3). Specimens will be evaluated for tumor infiltrating lymphocytes (TIL) to assess possible interplay between these effector and suppressive subsets in determining the response or resistance to chemotherapy.

9.2.1 Specimen Collection

Four to 8 cores will be taken at each time point by ultrasound or CT-guidance.

9.2.2 Processing Instructions

Tissues will be collected and submitted to the TPC. Following processing in the TPC, DNA will be extracted and transported to MGI for sequencing and analysis. Remaining specimens will be stored per standard pathology protocol.

9.3 Peripheral Blood Correlates -- OPTIONAL

Ten consenting patients will have blood drawn at the following time points:

- baseline (prior to start of FOLFIRINOX)
- Day 1 of Cycles 2, 3, and 4
- Day 15 of Cycles 1, 2, 3, and 4
- time of progression

PBMCs will be used to quantify and characterize the immunologic landscape at baseline and various time points during treatment. The T cell repertoire and reactivity to existing and induced neoantigens, pre- and post-treatment will be further characterized by a multi-parametric flow cytometry, spectratyping, and next-generation sequencing. Assessments of various cytokines/chemokines throughout treatment will be performed to assess both temporal and quantitative expression. This will be performed using multiplex-based assays and gene expression profiling. Multiplex analysis and QPCR to assess the following

cytokines: IFN-gamma, IFN-alpha, IFN-beta, IL-2, TNF-alpha, IL-2, IL-4, IL-5, IL-13, IL-10, IL-6, TGF-beta as well as chemokines such as CCL2, CXCL9, CXCL10.

9.3.1 Specimen Collection

Sixty mL of blood will be drawn into 6 10-mL BD Vacutainer® sodium heparin (green top) tubes at each time point.

9.3.2 Processing Instructions

Specimens will be transported to the Tissue Procurement Core within one hour of collection. PBMC single cell suspensions will be obtained and isolated by Ficoll-Hypaque gradient centrifugation and cryopreserved in 10% DMSO according to standard procedures.

10.0 STUDY CALENDAR

Baseline evaluations are to be conducted within 14 days prior to start of protocol therapy. Scans must be done no more than 4 weeks prior to the start of the protocol therapy. There is a window of +/- 2 days for visits and +/- 5 days for scans.

	Screening	Day 1 of each cycle[j]	Day 15 of each cycle[j]	End of every 2nd cycle	End of every 3rd cycle	Progression	F/U[e]
Informed consent	X						
Medical history	X						
Physical exam	X	X[a]	X[a]				
CBC	X	X	X				
CMP	X	X	X				
Pregnancy test[b]	X						
Echocardiogram or MUGA[d, i]	X				X		
PET/CT or CT[l]	X			X			
Archival tissue for GPS@WUSTL	X						
Confirmation of HER2 expression[c]	X						
Trastuzumab[d,h]		X	X				
FOLFIRINOX[h]		X	X				
Research blood collection[p]	X[m]	X[q]	X[r]			X	
Research tissue collection[p]	X[m]			X[n]		X[o]	
Adverse event assessment[f,g]		X ---------- X					

a: Cycle 1 Day 1, Cycle 1 Day 15, and Cycle 2 Day 1 only; thereafter at treating physician's discretion
b: Women of childbearing potential only
c: Can be done either via analysis of archival or freshly collected tissue, but will not be necessary if results are available from prior analysis. HER2 status may be pending at initiation of FOLFIRINOX, but must be known prior to starting trastuzumab. If HER2 is positive, patient must have an LVEF ≥ 50% by echocardiogram or MUGA.
d: HER2-positive patients only
e: Patients should be followed for survival until death. This will occur approximately monthly.
f: Abnormal lab values will be considered AEs and will be captured in the CRFs only if they are clinically significant.
g: Note that once a patient begins maintenance treatment, s/he will no longer be followed for toxicity.
h: Please review Section 5.6 for information regarding maintenance therapy.
i: Echocardiogram/MUGA may have been completed within 60 days prior to the start of protocol therapy, unless the patient has had a cardiac intervention within the last 6 months.
j: Labs may be collected up to 3 days prior to Day 1 and Day 15 of each cycle
l: CT may be used in follow up even if PET/CT was used at baseline
m: May take place at any time between confirmation of eligibility and initiation of FOLFIRINOX
n: End of Cycle 2 only
o: Optional
p: 10 consenting patients only
q: Cycles 2, 3, and 4 only
r: Cycles 1, 2, 3, and 4 only

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 Record	Every cycle
Toxicity Form	Continuous through 30 days post-completion of treatment with FOLFIRINOX +/- trastuzumab (toxicities need not be collected on patients undergoing maintenance therapy)
Treatment Summary Form	Completion of treatment
Tumor Measurement Form	Baseline, end of every even numbered cycles, and end of treatment
Correlatives Form	Baseline Day 15 of Cycles 1, 2, 3, and 4 Day 1 of Cycles 2, 3, and 4 End of Cycle 2 Time of progression
MedWatch Form	See Section 7.0 for reporting requirements
Survival Form	Monthly until death

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.

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) [47]. 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 with the exception of

PET/CT and CT scans. CT scans may be used in follow up if a PET/CT scan was used at baseline. 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.

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.

12.4.6 Overall Survival

OS is defined as the time interval from date of diagnosis to date of death from any cause.

12.4.7 Response Review

Response rate is the primary endpoint. All responses will be reviewed by an expert(s) independent of the study (such as IRAC) at the study's completion.

13.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the Quality Assurance and Safety Monitoring Committee (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
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date

- 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
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

14.0 STATISTICAL CONSIDERATIONS

In prior studies of the FOLFOX regimen in patients with gastroesophageal cancer, an objective response rate (ORR) of approximately 40% and median survival (MS) of 9.6 months has been observed [48-50]. In prior studies in other cancers (i.e. colorectal cancer), the addition of oxaliplatin to FOLFIRI increased the ORR in the treated patients by approximately 20% in comparison to other standard therapies [11]. We therefore propose a favorable response rate of ≥60% for the study regimen (FOLFIRINOX). This Phase II study will use a two-stage Simon MinMax accrual design to ensure that the total number of the patients exposed to this therapy is minimized [51]. A total of 41 evaluable HER2-negative patients will be enrolled in this study. HER2-positive patients will not be considered part of the enrollment goal of 41 evaluable patients. Because approximately 20% of these patients are expected to be HER2-positive, we anticipate enrolling 65 patients total.

If there is evidence that the true underlying overall response rate (CR, PR) is at least 60% in these patients, then the routine administration of the study regimen to patients with this malignancy will be warranted and should be investigated in subsequent trials. However, if the ORR for FOLFIRINOX is lower than 40% in these patients, then the study should be terminated early. Initially, 28 eligible patients will be entered into the study. If there are fewer than 12 responses in these first 28 patients, the trial will be terminated with the conclusion that there is little evidence to suggest that the overall response rate would reach 60%. A sample size of 41 patients will provide a 56% chance to terminate the trial early if the true response rate is 40% or less. If there are 12 or more responses in these first 28 patients, the trial will continue until 41 patients have been treated. If there are 21 or more responses in these 41 patients, then the study will be completed and future studies utilizing this regimen could be considered. This design has a one-sided alpha of 0.10 and a power of 0.90. That is, the probability of declaring a successful trial (RR≥60%) is at least 0.90 if the true ORR rate is ≥ 55%; and the probability of declaring an unsuccessful trial (RR≤40%) is at least 0.90 if the true RR rate is no more than 40%.

The regimen containing FOLFIRINOX plus trastuzumab will only be administered to patients who are HER2-positive. To our knowledge that regimen has not been previously administered to

patients. HER2-positive patient disease response results will not be included in this proposed analysis. Their clinical data will be collected to provide information for possible future studies.

Other Statistical Analysis Plan: Demographic information such as age and race will be tabulated. Descriptive statistics, including means, standard deviations and ranges for continuous parameters, as well as percentages and frequencies for categorical parameters, will be presented. Adverse medical events will be tabulated. NCI toxicity Grade 3 and Grade 4 laboratory abnormalities will be listed. AEs occurring in patients treated with trastuzumab will be included.

Exploratory endpoints: The quantified tumor-associated and systemic biomarkers and immune responses obtained through tumor tissue biopsies and peripheral blood at baseline (pre-chemotherapy), post-chemotherapy, and at disease progression. Paired t-test and/or paired-sample Wilcoxon Signed Rank test will used to compare the antitumor immune responses before and after chemotherapy.

Power Analysis and Sample Size: For the exploratory analysis, approximately 10 patients will be needed. The proposed sample size was chosen to minimize the potential risk to study participants and was not based on either statistical modeling or methods to obtain adequate power for any immune endpoint analysis. However this correlative study design is a pre and post treatment comparison with the same patient being measured twice. The magnitude of TSA changes at baseline and after therapy is unknown. If we assume the pre-treatment and post-treatment TSA magnitude to increase by 50%, with a 10% significance level, we need 10 patients to achieve 80% of power to detect a post-treatment TSA increase compared with the pre-treatment measurements, using a 2-sided one sample paired t test.

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APPENDIX 1: 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.