Pre-operative Adaptive Short Course Radiation Therapy in Gastric Cancer

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Protocol Revision History

Initial Approval Version 4	**19 Nov 2019**
Amendment #1 Version 5	**17 Aug 2020**
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Amendment #4 Version 8	**31 Oct 2023**
Amendment #5 Version 9	**16 April 2024**

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Principal Investigator Signature Page

Principal Investigator (printed):

Name of Institution:

PI Signature *Date*

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following ethical guidelines and regulations:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

Glossary of Abbreviations

AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
B-HCG	Beta human chorionic gonadotropin
BMT	Bone marrow transplant
CBC	Complete blood count
CFR	Code of Federal Regulations
CNS	Central nervous system
CR	Complete response
CRc	Cytogenetic complete remission
CRi	Complete remission incomplete
CRm	Morphologic complete remission
CRF	Case report form
CST	Central standard time
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DLT	Dose limiting toxicity
DNA	deoxyribonucleic acid
DSM	Data and Safety Monitoring
DSMC	Data Safety Monitoring Committee
ECG (or EKG)	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDTA	ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
FISH	fluorescent in situ hybridization
FWA	Federal wide assurance
GCP	Good Clinical Practice
HHS	Department of Health and Human Services
HIV	Human Immunodeficiency Virus
HRPO	Human Research Protection Office (IRB)
IND	Investigational New Drug
IRB	Institutional Review Board
MDS	Myelodysplastic syndrome
MM	Multiple myeloma
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCCN	National Cancer Center Network

NCI	National Cancer Institute
NIH	National Institutes of Health
NSCLC	Non-small cell lung cancer
OHRP	Office of Human Research Protections
ORR	Overall response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease
PI	Principal investigator
PR	Partial response
PSA	Prostate-specific antigen
QASMC	Quality Assurance and Safety Monitoring Committee
RECIST	Response Evaluation Criteria in Solid Tumors (Committee)
RFS	Relapse free survival
RR	Response rate
SAE	Serious adverse event
SCC	Siteman Cancer Center
SCT	Stem cell transplant
SD	Stable disease
TSH	Thyroid stimulating hormone
TTP	Time to progression
UPN	Unique patient number
US	Ultrasound
VEGF	Vascular endothelial growth factor
WBC	White blood cell (count)

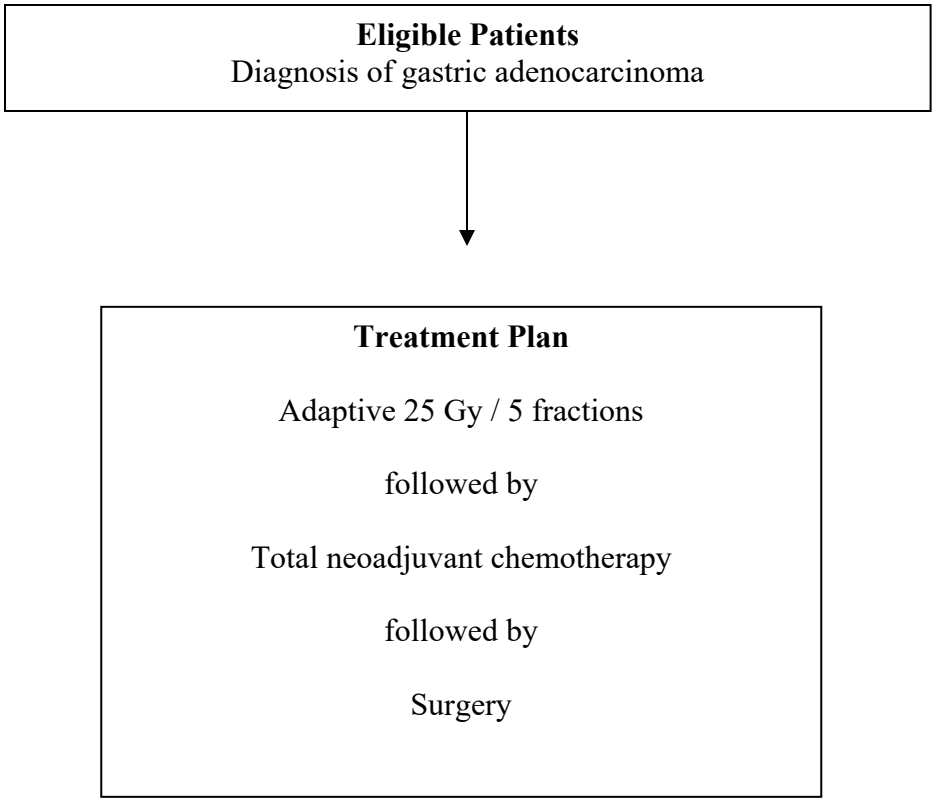
Table of Contents

PROTOCOL SUMMARY

Title:	Pre-operative Adaptive Short Course Radiation Therapy in Gastric Cancer
Study Description:	Hypothesis: Adaptive radiation therapy followed by sequential multi-agent chemotherapy will result in a pathologic complete response (pCR) of at least 20%. In this study, patients with gastric adenocarcinoma will receive hypofractionated adaptive radiation therapy followed by total neoadjuvant chemotherapy followed by surgery.
Objectives:	**Primary Objective:** To quantify the pCR rate in gastric cancer patients treated with preoperative radiation followed by total neoadjuvant chemotherapy. **Secondary Objectives:** 1. To quantify the proportion of patients able to complete a full course of total neoadjuvant chemotherapy 2. To quantify the local control rate of gastric cancer patients treated with preoperative radiation followed by chemotherapy. 3. To quantify the toxicity of gastric cancer patients treated with preoperative radiation followed by chemotherapy. 4. To quantify the overall survival in gastric cancer patients treated with preoperative radiation followed by chemotherapy. 5. To quantify the disease-free survival in gastric cancer patients treated with preoperative radiation followed by chemotherapy. **Exploratory Objectives:** 1. To quantify the deviation in dose to the tumor due to variation in stomach position. 2. To quantify the deviation in dose to the nearby OARs due to variation in OAR position. 3. To determine the distribution of TCGA molecular subtypes in gastric adenocarcinoma after radiation and chemotherapy. 4. To identify differentially expressed post-treatment biomarkers in gastric tumors. 5. To correlate circulating tumor DNA (ctDNA) levels with clinical outcomes.
Endpoints:	**Primary Endpoint:** The rate of pCR (both ypT and ypN) in gastric cancer patients treated with preoperative radiation followed by total neoadjuvant chemotherapy. **Secondary Endpoints:** 1. Proportion of patients able to complete a full course of total neoadjuvant chemotherapy

	2. The local control rate at 1 year post RT. 3. The rate of grade 3 or greater toxicity as defined by CTCAE v 5.0 4. The overall survival at 1 year post RT. 5. The disease-free survival at 1 year post RT. **Exploratory Endpoints:** 1. Average percent difference in coverage of PTV by 95% isodose line. 2. Average percentage difference in dose to nearby OARs (i.e. duodenum, small bowel, large bowel) due to variation in OAR position. 3. The percentage of patients in each TCGA molecular subtype after radiation and chemotherapy. 4. The specific biomarkers 5. ctDNA levels
Study Population:	Thirty-six adults of any gender who have a diagnosis of gastric cancer and are receiving preoperative radiation followed by total neoadjuvant chemotherapy will be enrolled.
Phase:	Not applicable.
Description of Sites / Facilities Enrolling:	This is a multicenter trial coordinated by and enrolling at Washington University in St. Louis. The study will also open to enrollment at other sites in the U.S.
Description of Study Intervention:	Patients undergoing preoperative radiation and chemotherapy for gastric cancer will undergo simulation for radiation treatment planning on a conventional referenced CT scanner and ViewRay or Ethos treatment machines. High resolution volumetric MR or CBCT images will be acquired daily in the treatment position and OARs contoured by the radiation oncologist. The new electronic density map will be used to recalculate dose from the initial radiation plan (based on CT/MR simulation) and treatment delivered to maximize coverage while not exceeding dosimetric constraints.
Study Duration:	36 months.
Participant Duration:	12 months.

SCHEMA


Eligible Patients
Diagnosis of gastric adenocarcinoma
Treatment Plan
Adaptive 25 Gy / 5 fractions
followed by
Total neoadjuvant chemotherapy
followed by
Surgery

1.0 SCHEDULE OF ACTIVITIES

Screening procedures must take place within 60 days of registration.

	Screening	**Baseline**	**Fx1**	**Fx2**	**Fx3**	**Fx4**	**Fx5**	**Chemo[4]**	**Surgery[5]**	**3-mo F/U[6]**	**6-mo F/U[6]**	**12-mo F/U[6]**
Informed consent	X											
H&P, ECOG PS	X									X		
CBC w/diff[1]	X											
CMP[1]	X											
Pregnancy test[2]	X											
EUS	X											
Institutional MRI screening questionnaire	X											
Simulation		X										
CT C/A/P		X							X[8]		X	X
PET/CT		X[11]										
Adaptive SCRT[3]			X	X	X	X	X					
SOC chemotherapy[10]								X	X			
Gastrectomy									X[9]			
Research blood for ctDNA		X						X[7]	X[7]		X[7]	
AE assessment		AEs will be tracked from baseline through 12 months after surgery/definitive end of treatment. Refer to Section 8.0 for more details.										

1. labs will be drawn as per SOC
2. women of childbearing potential only
3. patients will receive 5 fractions of radiotherapy delivered once daily; radiotherapy may take up to 10 business days (due to logistical delays)
4. SOC chemotherapy will begin 2-4 weeks after completion of radiotherapy
5. within 2-4 weeks after completion of SOC chemo
6. follow-up visits will take place at 3 months (+/-2 weeks), 6 months (+/- 4 weeks), and 12 months (+/- 4 weeks) post-surgery; patients who don't undergo surgery will have follow-up visits at 3 months (+/-2 weeks), 6 months (+/- 4 weeks), and 12 months (+/- 4 weeks) from the date of definitive end of treatment.
7. after completion of RT / prior to starting chemo, at completion of chemo / prior to surgery, and 6 months after surgery. See Section 9.2 for further details.
8. prior to surgery
9. tissue for research will be collected from patients who experience pCR
10. Any SOC total neoadjuvant chemotherapy regimen may be given at the discretion of and under the supervision of the treating medical oncologist
11. PET/CT is strongly encouraged at baseline but optional

2.0 INTRODUCTION

2.1 Study Rationale

Gastric cancer is a global health issue as the world's fifth most common malignancy and third leading cause of cancer mortality, respectively[1]. Preoperative radiation therapy may improve overall survival (OS) but is seldom used[2]. There is precedent for preoperative chemoradiation, as it is the standard of care for esophageal and gastroesophageal junction tumors[3]. However, reluctance of physicians to prescribe preoperative radiation therapy in gastric cancer may be due to the large treatment fields necessary to account for stomach motion. Adaptive radiation therapy may permit decreased field sizes and more accurate dose delivery. In traditional CT based radiation delivery the same radiation plan is delivered each day without assessment of inter-fraction or intra-fraction motion. Adaptive radiation therapy permits the physician to contour the unique anatomy daily to generate a new plan to account for day to day organ motion. Real-time imaging is also used during the treatment so that radiation is only delivered when the tumor is within the pre-specified target area. Thus, adaptive radiation therapy may overcome traditional barriers of radiation delivery in gastric cancer and improve oncologic outcomes.

Short course radiation therapy (SCRT) is an increasingly used treatment modality. In rectal adenocarcinoma, SCRT followed by chemotherapy results in double the pathologic complete response (pCR) as long course chemoradiation[4]. SCRT also allows the patient to proceed to multiagent chemotherapy sooner, as the concurrent chemotherapy delivered with long course chemoradiation is usually single agent or decreased systemic dosing. Thus preoperative SCRT followed by chemotherapy may permit more downstaging, pCR and decrease metastatic spread in gastric adenocarcinoma.

2.2 Background

2.2.1 Gastric Cancer

Gastric cancer is a global health issue with 952,000 new cases and 723,000 deaths in 2012, ranking as the world's fifth most common malignancy and third leading cause of cancer mortality, respectively[1]. Perioperative chemotherapy followed by gastrectomy is the category 1 treatment recommendation in the National Comprehensive Cancer Network (NCCN) guidelines[5] for locoregional disease, based on the results of the MAGIC and FLOT trials[6]. The role of radiation therapy in gastric cancer is supported in the adjuvant setting and improves OS, relapse free survival and local failure[7,8]. However, postoperative radiation fields are large and cause significant side effects, with reports of up to 32% acute grade 4 toxicity[6].

Gastric cancer patients with a pCR to preoperative chemoradiation therapy have an improved OS[2,9]. Despite large randomized studies indicating that pCR is rarely achievable with neoadjuvant chemotherapy alone[10], preoperative chemoradiation remains a category 2B recommendation in NCCN guidelines. There is precedent for preoperative chemoradiation, as it is the standard of care for esophageal and

gastroesophageal junction tumors[3]. Single arm studies have shown pCR rates of 16-30%[2,11,12]. Reluctance of physicians to prescribe preoperative radiation therapy in gastric cancer may be due to the large treatment fields necessary to account for stomach motion. Nearby OARs, including the heart, lungs, kidneys, liver, small and large bowel, limit the treatment dose and field size. Fluoroscopic and CT based studies in gastric lymphoma patients have demonstrated inter- and intra-fraction stomach movement requiring 3 cm margins[13,14]. Although preoperative chemoradiation therapy in gastric cancer is currently being evaluated in the international cooperative group TOPGEAR trial, traditional large fields are used without adaptation of radiation plans according to stomach movement[15].

2.2.2 Total Neoadjuvant Therapy

Total neoadjuvant therapy is a treatment paradigm frequently used in rectal adenocarcinoma treatment. By delivering all the planned chemotherapy prior to surgical resection, compliance with chemotherapy increases from 50% to 80%[16]. Data indicate that this treatment paradigm increases pathologic complete response[17] and even permits patients to undergo nonoperative management (omitting expirative surgery) if there is a clinical complete response[18–22]. Data for total neoadjuvant therapy in gastric adenocarcinoma are limited. Delivering total neoadjuvant therapy in gastric cancer may increase the amount of chemotherapy tolerated by the patient, allow increased downstaging, and increase the pathologic complete response rate. Further, if this treatment paradigm results in a high clinical and pathologic complete response, it may be an alternative treatment option for those patients not eligible for surgery.

2.2.3 Adaptive Radiation Therapy

The ViewRay system is a MRgRT coupled LINAC machine (ViewRay Inc., Oakwood Village, OH). The 0.35 Tesla real-time MR imaging allows radiation to be delivered to the tumor only when the target is in the designated treatment area. Traditional 3D CT planned radiation contours include a margin to account for respiratory motion, internal organ motion and setup error. MRgRT may permit smaller margins resulting in lower dose to normal structures[23]. Daily plan adaptation is possible with physicians contouring OARs, and subsequent changes in beam arrangements or dose to meet dosimetric constraints. Not only can this imaging guided treatment ensure prescribed dose delivery, it may permit dose escalation or boost volumes for gross tumor[23]. To date there is no clinical trial that has prospectively evaluated the feasibility or oncologic outcomes of preoperative MRgRT plus concurrent chemotherapy in gastric cancer.

At Washington University, we treat pancreas, liver, breast and other intra-abdominal and thoracic tumors with MRgRT. Our preliminary data for stomach motion during definitive (non-operative) radiation treatment of gastric lymphoma (Figure 1) indicate that even with patient instructions for NPO 3 hours prior to treatment, the daily stomach position can vary up to 2.95 cm from the planning

position. Motion assessment of 5 patients with gastric lymphoma show a mean translational distance (calculated from x, y and z displacement) of 1.3 ± 0.5 cm. The patients' average stomach volume was 763 cc, with 29.5 ± 19.0 cc outside the 5 mm PTV margin due to daily changes in stomach position over 20 fractions.

To date we have treated four patients with gastric adenocarcinoma with MRgRT 25 Gy in 5 fractions. The patients were treated with this fractionation to palliate symptoms or due to medical inoperability. Patients tolerated the treatment without treatment related acute grade 3+ toxicity. One patient that subsequently underwent sequential chemotherapy and surgical resection was not noted to have increased fibrosis or issues with resectability.

The Ethos system is a cone beam CT (CBCT) based radiation treatment device. It obtains high quality CBCT on-table images that allow the physician to contour the patients anatomy, just as in MRgRT. The Ethos system can be equipped with Identify, that permits surface monitoring of the patient's breathing, allowing CBCT imaging and treatment to be delivered in the same anatomical position. Our data indicate high contour reliability and dosimetric feasibility of adaptive radiation therapy using the Ethos platform[24,25]. This is a highly reliable adaptive platform alternative to MRgRT.

2.2.4 Correlative Studies Background

With more effective chemotherapy and radiation treatment delivery it is increasingly important to identify molecular subtypes that may require dose escalated or targeted therapy. The traditional Lauren classification[26] and World Health Organization[27] systems categorize gastric tumors by histology and have had limited utility in guiding oncologic therapies or targeted drug discovery. Currently, the only clinically meaningful molecular biomarker is Her2/neu overexpression, with the TOGA trial demonstrating an OS advantage in Her2/neu positive gastric cancer patients treated with trastuzumab plus chemotherapy versus chemotherapy alone[28]. Unfortunately, only 6-20% of gastric adenocarcinomas demonstrate Her2 overexpression and/or amplification[29]. Other attempts to classify gastric tumors via microarray and whole transcriptome sequencing were limited by small sample sizes, no associated clinical outcomes, and the use of immortalized cell lines instead of original patient pathology[30–33]. In contrast to the parallel technologies that guide the management of breast[34,35] or prostate cancer[36,37], there are no clinically implemented biomarker panels or strata that predict or prognosticate outcomes for gastric cancer.

TCGA performed a multiple platform analysis of 295 treatment naïve gastric cancer samples using array-based somatic copy number analysis, whole-exome sequencing, array-based DNA methylation profiling, messenger RNA sequencing, microRNA sequencing and reverse-phase protein array. They proposed a four molecular subgroup classification system (Figure 1)[38]. However, the study was performed on treatment naïve tissue, with the potential for high molecular heterogeneity inherent to gastric cancer[39]. Due to the sample size that was likely restricted by the high cost of multiple platform genomic analysis, it is possible that the heterogeneous cellular makeup of the gastric tumors deterred the detection of consistently differentially expressed biomarkers in the study. Data in esophageal adenocarcinoma indicate that chemotherapy reduces tumor heterogeneity (Figure 2)[40] and post-chemotherapy cell populations may preferentially express genes that promote survival[41]. Evaluating biomarkers in a more molecularly uniform cell population may increase the ability to detect clinically significant molecular subgroups.

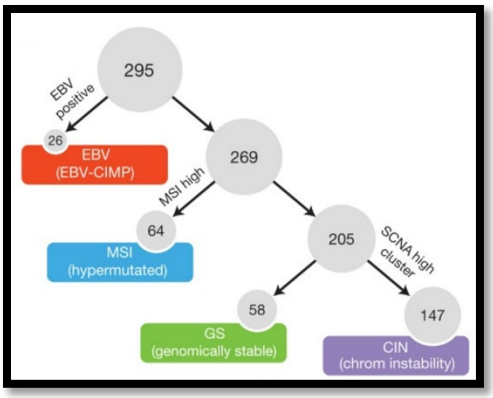


Figure 1. TCGA molecular subtypes of gastric adenocarcinoma. EBV, Epstein-Barr virus; MSI, microsatellite instability; SCNA, somatic copy-number aberrations.

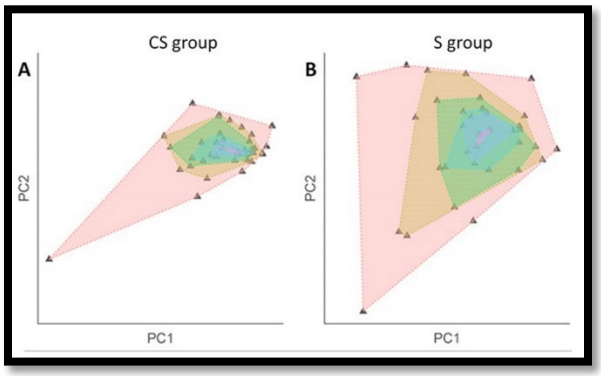


Figure 2.DNA copy number as surrogates for tumor variation; decreased heterogeneity in the chemotherapy plus surgery (CS) vs surgery alone (S).

There are growing data implicating the role of microRNAs (miRs), small non-coding RNAs that regulate mRNA expression, in regulating response to chemotherapy and ionizing radiation[49]. Researchers have demonstrated that increased miR-21 and miR-106a may target phosphatase and tensin homolog (PTEN), resulting in gastric cancer resistance to cisplatin chemotherapy[50,51]. While miR data regarding radiation therapy are limited in gastric cancer due to only sporadic use of preoperative radiation, there are data indicating increased miR-31 levels confer radiation sensitization in esophageal adenocarcinoma[53]. The expression levels of these miRs after preoperative chemoradiation therapy in addition to their prognostic and therapeutic implications in gastric adenocarcinoma are not known.

Cell-free DNA is a powerful non-invasive assay for quantitating tumor disease burden[54,55]. Circulating tumor DNA (ctDNA), the tumor component of cell-free DNA, is indicative of molecular residual disease in colorectal cancer patients and

has prognostic value[56,57]. Other investigators have shown similar findings in breast [58], lung[59], cervical[60], and pancreatic cancer[61]. There are limited data on ctDNA in gastric cancer[62].

2.3 Study Design

2.3.1 Overall Design

The hypothesis of this study is that adaptive SCRT followed by multiagent chemotherapy will result in a pCR of at least 20%.

Thirty-six patients with gastric adenocarcinoma will be enrolled in this study. There will be a lead-in cohort of 5-10 patients (see Safety Cohort and Early Stopping Rules) to ensure there are no grade 5 toxicities related to adaptive SCRT (or SOC chemotherapy after adaptive SCRT). All patients will receive radiation delivered with MR or CBCT guidance followed by sequential chemotherapy. Gastrectomy will occur 2-4 weeks after the completion of chemotherapy. Pathologic response, plan dosimetry, and oncologic outcomes will be evaluated. FDG PET/CTs are strongly encouraged at baseline but optional. Patients will be followed on study for 12 months after surgery.

2.3.2 Scientific Rationale for Study Design

This study will be a non-randomized, single arm, phase II study with all patients receiving preoperative radiation therapy then SOC chemotherapy followed by surgical resection. The reason for performing a non-randomized trial is to evaluate the clinical efficacy of this treatment regimen in a timely manner. Due to the infrequent incidence of this disease in the United States (Washington University in St. Louis performs approximately 30-50 gastrectomies per year for gastric cancer), a randomized control trial would take many years to complete. We will compare the dosimetric and clinical outcomes to historical controls.

2.3.3 Justification for Dose

Twenty-five Gy in 5 fractions is a dose fractionation used for aggressive palliation of intraabdominal malignancies. It is well tolerated and usually prescribed without dose constraints (if delivered with at least four fields). This dose and fractionation to the whole pelvis is a standard of care treatment regimen in patients with rectal adenocarcinoma. This is also the dose and fractionation that is prescribed to the whole pelvis for non-operative management of rectal adenocarcinoma at our institution. at the time of this protocol submission, this short course radiation therapy followed by 4 months of chemotherapy leads to greater than 70% cCR of rectal adenocarcinoma in our institutional experience and prospective phase I trial (NORMAL-R, HRPO# 201512140). We anticipate it will be well tolerated in gastric adenocarcinoma patients with high clinical response when coupled with multi-agent chemotherapy.

3.0 OBJECTIVES AND ENDPOINTS

Objectives	Endpoints	Justification for Endpoints
Primary		
To quantify the pCR rate in gastric cancer patients treated with preoperative radiation followed by total neoadjuvant chemotherapy.	The rate of pCR (both ypT and ypN) in gastric cancer patients treated with preoperative radiation followed by total neoadjuvant chemotherapy.	A complete pathologic response may result in improved oncologic outcomes. High rates of pCR may lead the way for future studies of non-operative management of gastric cancer.
Secondary		
To quantify the proportion of patients able to complete a full course of total neoadjuvant chemotherapy	Proportion of patients able to complete a full course of total neoadjuvant chemotherapy.	Evaluating the feasibility of total neoadjuvant therapy will help guide future trials seeking to maximize response prior to surgery.
To quantify the local control rate of gastric cancer patients treated with preoperative radiation followed by chemotherapy.	The local control rate at 1 year post RT.	Providing radiation therapy prior to chemotherapy and surgery may increase the local control rate.
To quantify the toxicity of gastric patients treated with preoperative radiation followed by chemotherapy.	The rate of grade 3 or greater toxicity as defined by CTCAE v 5.0.	Adding radiation therapy to chemotherapy and surgery may increase the toxicity associated with treatment.
To quantify the overall survival in gastric cancer patients treated with preoperative radiation followed by chemotherapy.	The overall survival at 1 year post RT.	By improving local control and pCR, overall survival may be improved.
To quantify the disease free survival in gastric cancer patients treated with preoperative radiation followed by chemotherapy.	The disease-free survival at 1 year post RT.	By improving the local control, disease free survival (locoregional and distant recurrence) may be improved as well.
Tertiary/Exploratory		
To quantify the deviation in dose to the tumor due to variation in stomach position.	Average percent difference in coverage of PTV by 95% isodose line.	The average percent difference of the five treatments represents approximately what percent of the total planned prescription was delivered to the target. Under-dosing of the tumor due to organ

		motion may result in decreased tumor control.
To quantify the deviation in dose to OARs due to variation in OAR position.	Average percentage difference in dose to nearby OARs (i.e. duodenum, small bowel, large bowel) due to variation in OAR position.	The average percent difference of the five treatments represents approximately what percent of the total planned prescription was delivered to the OAR. Over-dosing of the OARs due to organ motion may result in increased toxicity.
To determine the distribution of TCGA molecular subtypes in gastric adenocarcinoma after radiation and chemotherapy.	The percentage of patients in each TCGA molecular subtype after radiation and chemotherapy.	Treatment with radiation and chemotherapy may select for a different distribution of TCGA molecular subtypes.
To identify differentially expressed post-treatment biomarkers	The specific biomarkers	Treatment with radiation and chemotherapy may select for a less heterogeneous, clinically significant biomarker enriched tumor subtype.
To correlate circulating tumor DNA (ctDNA) levels with clinical outcomes.	ctDNA levels	ctDNA may detect treatment refractory disease and residual tumor earlier than traditional modalities.

4.0 STUDY POPULATION

4.1 Inclusion Criteria

In order to participate in this study, a patient must meet all of the criteria listed in this section:

1. Newly diagnosed histologically or cytologically gastric adenocarcinoma. (Siewert III acceptable: the bulk of tumor should be in stomach; gastric tumors with extension to the gastroesophageal junction are permitted.) Patients with T1-3N0-2 are eligible. Patients with N3, or T4 disease are not eligible.

2. Known T-stage defined by EUS. Must have had CT of the chest/abdomen/pelvis with IV contrast.

3. Medically eligible to receive SOC chemotherapy.

4. At least 19 years of age.

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

6. Normal bone marrow and organ function as defined below:
   a. Absolute neutrophil count ≥ 1,500 cells/mm^3
   b. Platelets ≥ 100,000 cells/mm^3
   c. Creatinine clearance > 50 mL/min

7. The effects of the various chemotherapy agents used in this study on the developing human fetus are unknown. For this reason, 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. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of the study, and one month after completion of the study

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

4.2 Exclusion Criteria

In order to participate in this study, a patient must not meet any of the criteria listed in this section:

1. Prior surgery, radiation, or chemotherapy for gastric or esophageal cancer.

2. Prior surgery to the esophagus or stomach that would alter the radiation treatment field or stomach motion.

3. Siewert I-II GE junction tumor.

4. Any active malignancy within 2 years of enrollment that may alter the course of gastric cancer. (Apparently cured localized malignancy or advanced, but indolent malignancy with significantly more favorable prognosis are allowed).

5. Currently receiving any other investigational agents.

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

7. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, diabetes, symptomatic congestive heart failure, unstable angina pectoris, or cardiac arrhythmia that are considered clinically significant as determined by the treating physician.

8. Pregnant and/or breastfeeding. Women of childbearing potential must have a negative pregnancy test within 14 days of study entry.

9. Patients with HIV are eligible unless their CD4+ T-cell counts are < 350 cells/mcL or they have a history of AIDS-defining opportunistic infection within the 12 months prior to registration. Concurrent treatment with effective ART according to DHHS treatment guidelines is recommended. Recommend exclusion of specific ART agents based on predicted drug-drug interactions (i.e. for sensitive CYP3A4 substrates, concurrent strong CYP3A4 inhibitors (ritonavir and cobicistat) or inducers (efavirenz) should be contraindicated).

4.3 Inclusion of Women and Minorities

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

5.0 REGISTRATION AND ENROLLMENT PROCEDURES

The following steps must be taken before enrolling patients on this study:

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

Patients must not start any protocol intervention or procedures prior to signing of informed consent. All consented patients at all sites must be registered in the Siteman Cancer Center OnCore database at WUSM.

5.1 Registration in OnCore Database

Patient registration to the Siteman Cancer Center OnCore database must occur within one business day of the patient signing consent.

The following patient registration information must be scanned and emailed to the WUSM research coordinator **on the day the patient signs consent**:

1. Complete and submit manual registration form:
   a. Your name and contact information (telephone number, fax number, and email address)
   b. Your site PI's name, the registering MD's name, and your institution name
   c. Patient's race, ethnicity, sex, ZIP code, country, and DOB
   d. Three letters (or two letters and a dash) for the patient's initials
2. Currently approved protocol version date
3. Copy of signed consent form (patient name may be blacked out)
4. Planned date of enrollment

5.2 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study that will include a 3-digit site number (contact the study team for the site number) and a 2-digit sequential number beginning with 01 for the first patient. The UPN must not include patient initials or other identifying information. All data will be recorded with this identification number on the appropriate CRFs.

5.3 Confirmation of Patient Eligibility and Enrollment

Patient eligibility will be confirmed using the information listed below and scanning and emailing it to the WUSM research coordinator **at least two business days prior** to enrolling the patient:

1. Completed eligibility checklist, signed and dated by a member of the study team
2. Copy of appropriate source documentation confirming patient eligibility

Once the patient has been confirmed to be eligible for enrollment, the WUSM coordinator will forward verification of enrollment and the UPN via email.

5.4 Patient Enrollment Submissions

Patient enrollment packets may be submitted Monday through Friday between 8am and 5pm CT. Exceptions will be evaluated and approved on a case-by-case basis. Patient eligibility and subsequent enrollment will be confirmed by the WUSM research coordinator or designee by email within one business day of submission. Verification of eligibility and registration must be kept in the patient chart.

5.5 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (if applicable).

5.6 Strategies for Recruitment and Retention

This trial will be open to all patients irrespective of gender, race, or socioeconomic status. Patients will be identified in both the inpatient (consults) and outpatient setting.

6.0 TREATMENT PLAN

Consenting and eligible patients will receive adaptive SCRT (25 Gy in 5 fractions), followed by standard of care total neoadjuvant chemotherapy followed by standard of care gastrectomy or esophagogastrectomy. The recommended SOC chemotherapy options are CAPOX, FOLFOX, or FLOT, but any SOC total neoadjuvant chemotherapy may be given at the discretion of the treating medical oncologist after consultation with the study chair. Patients who are not able to complete their full total neoadjuvant therapy regimen prior to surgery may complete chemotherapy postoperatively at the discretion of the treating physician.

6.1 Safety Cohort and Early Stopping Rules

There will be a lead-in safety cohort of 5 patients, monitored for 30 days after completion of RT. If there is one treatment-related patient death, then the safety cohort will be expanded to 10 patients. If there are two or more treatment-related deaths, the radiation dose will be decreased to 20 Gy in 5 fractions. The trial will be terminated if there are more than three treatment-related deaths (equaling approximately 10% of the entire projected accrual).

Treatment-related grade 5 toxicities should be reported to the institutional PI within 24 hours and to the WUSM PI according to the timelines in Section 8.2.

For the purposes of the early stopping rules, "treatment-related" refers to both adaptive SCRT and SOC total neoadjuvant chemotherapy.

6.2 Radiation Therapy Guidelines

6.2.1 Dose, Fractionation, and Constraints

When feasible it is strongly recommended that radiotherapy begin on a Monday. It is accepted that occasional logistical delays may occur during radiotherapy treatment due to machine downtime or other issues. Radiotherapy as administered during this study may take up to 10 business days without being considered a protocol deviation.

Radiotherapy will consist of five fractions, delivered once daily, to a total dose of 25 Gy at 5 Gy per fraction. Radiation must be delivered with daily adaptive planning and MR gating or CBCT breath hold treatment. The stomach and OARs must be redrawn each day for the adaptive plan. Plans should be adapted to meet OAR constraints or improve coverage as needed for each day's unique anatomy.

Coverage:
PTV V95% = 100%

Luminal GI structures:
30 Gy < 0.5 cc (hard constraint).
25 Gy < 0.5 cc (soft constraint).

Kidneys V18 < 200cc.
Spinal cord V28 < 0.03cc.

6.2.2 Treatment Planning Procedures

Image-based treatment planning and intensity modulated radiotherapy (IMRT) is permitted. Proton therapy is not permitted. Dose volume histogram (DVH) information for the target volumes, small bowel, and uninvolved colon (defined to be large bowel outside the clinical target volumes) is mandatory. This is to assist in interpreting outcome, including morbidity.

6.2.3 Simulation Procedures/Patient Positioning

Patients should be simulated in supine position. It is recommended to have the left arm raised and right arm down. Both arms down is acceptable as long as dose constraints can be met (avoiding beams through arms). Simulation scan should be obtained on expiratory breath hold.

6.2.4 Clinical Target Volume (CTV) and Planning Target Volume (PTV) Definitions

The CTV_primary will include the stomach GTV (as per PET/CT and endoscopy report) plus a 1-3 cm proximal-distal expansion along the length of the bowel structure (i.e. stomach, esophagus, or duodenum as applicable) This range is provided to allow more coverage near the GE junction (akin to esophageal expansions) but to prevent irradiating the entire stomach. Expansion may extend into esophagus and/or duodenum per tumor location. A 1-1.5 cm radial expansion is generated for CTV primary, cropped to GI luminal structure. Clinically suspicious locoregional lymph nodes as identified by EUS or imaging that are visible on 0.35 T MR may be included with a 1.0 cm expansion to CTV_node (or included in CTV_elective)). Both the CTV_primary and CTV_node must be evaluated for change and re-contoured daily at the machine if significantly changed per reviewing MD assessment. If the LN is not visible then the gross node CTV volume should not be changed.

CTV is generated with a 1-1.5 cm brush, creating a semicircle volume encompassing the proximal 1-1.5 cm of celiac SMA vessel, with the aorta at its posterior border and the anterior border demarcated by bowel or pancreas. Ideally, CTV_elective should not change if treatment imaging is acquired in the same position and phase of breathing as the simulation scan.

PTV is generated by a uniform 1 cm expansion about the CTV. Reducing PTV margins to modify OAR dose(s) is not permitted.

For daily adaptive treatments, patient should be aligned to CTV_elective. GTV is rigidly deformed and redrawn to match daily anatomy of stomach using diagnostic

imaging and prior fraction treatment plan. CTV_primary is created as above.

6.2.5 Normal Tissue Contours

Small bowel, large bowel, duodenum, kidneys, and spinal cord should be contoured. Since absolute rather than relative bowel volumes are to be tracked, it is not necessary to contour the entire large and small bowel. Only those loops within 1.5 cm superior and inferior or within 3 cm axial distance of the PTV must be contoured for daily adaptation. All bowel within 1.5 cm superior and inferior to the PTV should be contoured for initial planning. Gating boundary should be set at 5 mm.

6.2.6 Retrospective Plan Review

Deidentified DICOM datasets will be sent through encrypted transfer to Washington University for central review of contours and treatment plans. Initial radiation treatment plans will be overlaid on each daily anatomy and the change in dose to PTV and OARs will be determined.

6.3 Chemotherapy Guidelines

Chemotherapy will be given as per standard of care and should begin 2 to 4 weeks after completion of radiotherapy. Recommended total neoadjuvant chemotherapy regimens are:

- CAPOX (capecitabine/oxaliplatin)
- FOLFOX (oxaliplatin, leucovorin, and 5-FU)
- FLOT (docetaxel, oxaliplatin, leucovorin, and 5-FU)

However, the treating medical oncologist may prescribe other SOC total neoadjuvant chemotherapy regimen upon discussion with the WUSM PI. Additionally, if one regimen is not tolerated, subjects can move between regimens for a goal of 4 months of chemotherapy treatment total prior to surgery.

Patients who are unable to complete their total neoadjuvant therapy regimen may complete the chemotherapy postoperatively, at the discretion of the treating physician. Postoperative chemotherapy should start within 3 months of surgery. Patients who are medically inoperable will continue to receive chemotherapy until the entire regimen is complete.

A maximum (cumulative) delay of treatment of 4 weeks (or single delay of 3 weeks) is allowed per protocol due to factors/AEs listed in Section 7.0 (i.e., chemotherapy should be completed within a 5-month period). Cumulative delays in excess of 4 weeks, or single delays in excess of 3 weeks, will need to be discussed with the Principal Investigator in regard to proceeding to surgical resection. Such cases will need to be individualized depending on the clinical scenario.

Please note that chemotherapy administration and AE assessment should be performed under the supervision of a medical oncologist. Dosing is to follow standard of care recommendations per institutional guidelines.

Interval imaging during chemotherapy, if obtained, is at the discretion of the treating physician.

6.4 Surgery

Exclusion of metastatic disease will be performed per institutional standard (exploratory laparoscopy or CT imaging).

Surgical resection is dependent on the location of the tumor. Prior to initiation of radiation and chemotherapy, tumor may be tattooed with ink (e.g. carbon black) so that the original tumor extent is identifiable post-radiation and chemotherapy. Tumors should be resected to negative margins as per frozen sections. Subtotal gastrectomy is appropriate for distal tumors, with total gastrectomy at the surgeon's discretion. Total gastrectomy or esophagogastrectomy are appropriate for proximal tumors. The spleen should be preserved when feasible. D2 nodal dissection is mandatory. Clips may be placed to identify areas of tumor adhesion or high risk for residual disease with documentation in the operative report.

Peritoneal washings should be obtained intraoperatively as part of surgical staging.

In the event of negative frozen but positive permanent margin, further treatment will be at the discretion of the treating physician. .

6.5 Patient Clinical Outcomes Data

Clinical outcomes data (including but not limited to patient demographics (age, date of birth, weight, sex, height), tumor staging, treatment delivered, pathologic response, local failure and distant failure) will be recorded in OnCore. Each patient will be deidentified with a unique patient identification number. Only those individuals at the patient's respective institution will have access to the patient's personal health identifiers (e.g. name).

6.6 Definitions of Evaluability

Endpoint	**In order to be evaluable for this endpoint, a patient must…**
Primary	
Rate of pCR	Have received at least one fraction of radiation therapy and undergone surgical resection
Secondary	
Proportion of patients able to complete a full course of total neoadjuvant chemotherapy	Have received at least one fraction of radiation therapy

Local control rate at 1 year post RT	Have received at least one fraction of radiation therapy and undergone at least one disease assessment
Rate of grade 3 or greater toxicity	Have received at least one fraction of radiation therapy
Overall survival at 1 year post RT	Have received at least one fraction of radiation therapy
Disease-free survival at 1 year post RT	Have received at least one fraction of radiation therapy and undergone at least one disease assessment
Exploratory	
Average percent difference in coverage of PTV by 95% isodose line	Have received at least one fraction of radiation therapy
Average percentage difference in dose to nearby OARs due to variation in OAR position	Have received at least one fraction of radiation therapy
Percentage of patients in each TCGA molecular subtype after radiation and chemotherapy	Have completed radiation therapy and neoadjuvant chemotherapy
Specific biomarkers	Have had specimens collected for biomarkers
ctDNA	Have had specimens collected for biomarkers

Note that inevaluable patients will not be replaced.

6.7 Concomitant Therapy and Supportive Care Guidelines

Although addition of trastuzumab is not considered standard of care in the management of locally advanced gastric cancer, if the treating physician feels that adding trastuzumab would be in the patient's best interests, it will be allowed.

6.8 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 pregnancy test within 21 days prior to the first dose of study treatment.

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

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

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

6.9 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, radiation therapy will continue for 5 fractions, neoadjuvant chemotherapy will continue for up to 4 months, and surgery will take place after that. Treatment will be discontinued if 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 unable to receive further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious noncompliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

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

6.10 Duration of Follow-up

Patients will be followed for local recurrence, distant recurrence, and toxicity for 12 months after surgery (or date of definitive end of treatment for patients who do not have surgery). Follow-up visits will be include the following:

- 3-month follow-up: H&P, ECOG PS.
- 6-month follow-up: CT C/A/P, blood for ctDNA.
- 12-month follow-up: CT C/A/P

6.11 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled

visits and is unable to be contacted by the study team.

The following actions must be taken if the participant fails to return to clinic for a required study visit:

- The study team will attempt to contact the participant and reschedule the missed visit within 1 month and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

7.0 DOSE DELAYS/DOSE MODIFICATIONS

7.1 Dosing Adjustments for Radiation Therapy

Radiotherapy for this trial may take place over a maximum of 10 business days before it is considered a protocol deviation. There are no dose adjustments (reductions, omissions) associated with radiotherapy.

7.2 Dosing Adjustments for Chemotherapy

Dose reductions and holds for chemotherapy may be made at the discretion of the treating physician. All chemotherapy is being given as per standard of care, and dosing is not dictated by this protocol. Please refer to the package insert of each drug for recommended dosing adjustments.

8.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below. Please refer to Appendix B for definitions and Appendix C for a grid of reporting timelines. All investigators treating patients on this study are responsible for ensuring that serious adverse events (as defined in Appendix B) are reported to the WUSM PI within an adequate timeframe for the event to be assessed by the WUSM PI for reporting to HRPO and QASMC.

Adverse events will be tracked from baseline through 12 months after surgery (or, for patients who do not undergo surgery, 12 months after definitive end of treatment). All adverse events must be recorded on the toxicity tracking case report form (CRF) with the exception of:

- Baseline adverse events, which shall be recorded on the medical history CRF

- Adverse events that are thought to be at least possibly related to a non-protocol treatment received after the patient comes off SCRT or neoadjuvant chemotherapy

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

Reporting requirements for Washington University School of Medicine study team may be found in Section 8.1. Reporting requirements for secondary site study teams participating in WUSM-coordinated research may be found in Section 8.2.

8.1 WU PI Reporting Requirements

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

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

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

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

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

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

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

8.1.3 Reporting to Secondary Sites

The WUSM PI (or designee) will notify the research team at each secondary site of

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

8.2 Secondary Site Reporting Requirements

The research team at each secondary site is required to promptly notify the WUSM PI and designee of all serious adverse events (refer to Appendix B, Section D) within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report (using FDA Form 3500A (MedWatch) and Washington University's cover sheet (Appendix D)) form if required or an institutional SAE reporting form if not). A formal written report (using FDA Form 3500A) must be sent to the WUSM PI and designee within **4 calendar days** (for fatal or life-threatening suspected adverse reactions) or **11 calendar days** (for serious unexpected adverse reactions) of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines.

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

8.3 Sub-Investigator Reporting Requirements

All investigators treating patients on this study are responsible for ensuring that serious adverse events (as defined in Appendix B) are reported to the WUSM PI within 24 hours of learning of the event in order for the event to be assessed by the WUSM PI for reporting to HRPO and QASMC.

8.4 Exceptions to Expedited Reporting

Events that do not require expedited reporting as described in Section 8.1 include:

- planned hospitalizations
- hospitalizations < 24 hours
- respite care
- events related to disease progression

Events that do not require expedited reporting must still be captured in the EDC.

9.0 CORRELATIVE STUDIES

9.1 Tissue for Research

Patients with pCR will have normal gastric tissue collected at the time of surgery. Fresh tissue will be formalin-fixed and paraffin-embedded (FFPE) per institutional standards.

RNA and whole exome sequencing will be used for formalin-fixed, paraffin-embedded, post-treatment gastric adenocarcinoma tissue samples to determine the post-treatment patient distribution amongst the TCGA molecular subgroups. Blocks will be reviewed by institutional pathologists, H&E slide prepared to identify the block with highest tumor volume and grade (ideally tumor samples with ≥60% tumor nuclei and ≤20% necrosis will be processed for nucleic acid extraction[38]) and then the corresponding block sent for biomarker analysis. Normal tissue adjacent to tumor will also be obtained for differential expression analysis. FFPE tissue will be packaged in insulated Styrofoam packaging and shipped to and sequencing performed at the Washington University Genome Technology Access Center. Cold packs and dry ice are not necessary for FFPE tissue samples. Shipping address is:

Genome Technology Access Center
Cortex, Suite 209
4444 Forest Park
St. Louis, MO 63108

9.1.1 TCGA Molecular Subtype Analysis

Whole transcriptome sequencing (RNA-seq) data will be aligned to Human Genome Reference Consortium Human Build 38 (GRCh38) using HISAT2[63] with Ensembl genes for Homo sapiens version 90 and TCONs. Novel transcripts will be assembled and all gene expression levels (Fragments Per Kilobase per Million fragments, or FPKM) will be calculated using Cufflinks[64]. Read counts will be calculated using featureCounts[65]. Whole exome sequencing data will be aligned to GRCh38 using BWA[66]. Single nucleotide variants will be called using GATK 6 and VarScan2[67]. Small indels and structural variations will be discovered using Pindel[68]. Copy number variations will be analyzed using GISTIC2[69]. RNA-seq data will also be aligned to reference Epstein-Barr Virus (EBV) genes using BLAT[70]. Samples will be classified into subtypes using the methods as described previously[38]. Pearson's chi-squared test ($\chi 2$) will be applied to TCGA molecular subtypes to evaluate the difference in post-treatment data compared to TCGA data.

9.1.2 Differential Biomarker Expression Analysis

Whole transcriptome sequencing data will be pre-processed excluding genes with consistent low expression levels (e.g. <1 FPKM or <200 reads) across all samples[71]. MicroRNA and non-coding small RNA expression will be calculated[72]. The Bioconductor packages including DEseq[74] and edgeR[75] will be applied to the above raw read counts in the unit of genes or pathways. Raw read counts are modeled on the negative binomial (NB) distribution in both packages. This is recommended for proper RNA-seq DE analysis since it will handle the well-known over-dispersion

problem by adjusting relevant variables and solving the multiple testing issue. The ratio of means (e.g. log fold-change > 1.5) will be used as the effect size measure as indicated previously[76], and adjusted false discovery rate (FDR) will be applied to reduce type I errors from a list of differentially expressed genes. All biomarkers discovered will be examined for their predictive and prognostic utility using Kaplan–Meier estimator. Further experiments will include larger discovery and validation patient cohorts using these biomarker panels to verify their association with clinical outcomes in Korean and Western gastric cancer patients[77].

9.2 Blood for ctDNA

Twenty mL of blood will be collected into EDTA tubes at the following time points:

- Prior to the start of radiation
- After the completion of radiation therapy and prior to starting chemotherapy (2-4 weeks after radiation completion)
- At the completion of chemotherapy and prior to surgery (2-4 weeks after chemotherapy completion)
- 6 months after surgery

Use BD Vacutainer purple top 10mL K_2-EDTA tubes for blood collection (Becton Dickinson catalog # 366643). Under no circumstances should blood collected in heparin tubes be used, as heparin greatly inhibits the downstream reactions. Process the blood as soon as possible after drawing (within 2 hours, upper limit 6 hours per *Parpart-Li et al, CCR, 2016*). If there is a delay between phlebotomy and processing, keep tubes in refrigerator at ~4°C. If not processed immediately, note the time delay prior to processing.

Plasma and Plasma Depleted Whole Blood (PWDB) Banking

1. ~20 ml of whole blood will be collected in K_2-EDTA tubes (purple) and placed on ice.
2. Pool whole blood to measure collected volume.
3. Split whole blood into two tubes and spin at 1800 x g for 10 minutes at 15-20^0 C.
4. Remove tubes from centrifuge carefully and place on ice.
5. Remove supernatant (plasma) to fresh tube(s).
6. Spin supernatant a second time, 1800 x g for 5 minutes at 15-20^0 C.
7. Remove cleared plasma supernatant and aliquot into 2 ml vials. (If there is a cell pellet after 2nd spin, suspend these cells in remaining plasma and add this mixture to PDWB, see step 9).
8. Move frozen Plasma vials to a storage box @ -80^o C.
9. During second plasma spin, gently mix remaining PDWB cells from step 5 above.
10. Remove 2 ~1.8 ml PDWB aliquots, and store @ -80^o C.
11. Ship plasma and PDWB on dry ice.

CAPP-SEq analysis will be as previously described, with barcoded adapters for library preparation, targeted DNA hybrid capture, and next-generation sequencing followed by bioinformatic correction of stereotypic errors.

All biospecimens will be marked according to individual country regulations prior to shipment to Washington University. For local specimens, take directly to the Fields lab for processing. For specimens from participating sites, ship processed specimens to the Fields lab at the address below:

Washington University School of Medicine
Department of Surgery
425 South Euclid Avenue
Fields Lab
Clinical Science Research Building, Room 3306
St. Louis, MO 63110

10.0 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
On-Study Form Medical History Form	Prior to starting treatment
MRgRT Treatment Summary Form	End of radiation
SOC Chemo Treatment Form	End of each cycle of SOC chemotherapy
Surgery Form Research Tissue Form	Time of surgery
Research Blood Form	Prior to starting treatment, end of radiation, end of neoadjuvant chemotherapy, 6-month follow-up
Toxicity Form	Continuous until 12 months after surgery/date of definitive end of treatment
Follow Up Form	3 months, 6 months, and 12 months after surgery
Local Control Form	12 months after start of radiation
Progression Form	Time of progression
Death Form	Time of death
MedWatch Form	See Section 8.0 for reporting requirements

Secondary sites are expected to enter data within 10 business days. Any queries generated by WUSM must be responded to within 10 days of receipt by the participating site. The WUSM research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

De-identified patient clinical data will be submitted by all non-Washington University sites on the OnCore website. No patient identifiers will be included for non-Washington University patients with the exception of dates. Unique patient identification numbers will be assigned to each patient and the corresponding patient identifiers (protected health information) will be known only to the investigators within each respective institution.

10.1 Adverse Event Collection in the Case Report Forms

All adverse events that occur beginning with start of treatment (minus exceptions defined in Section 8.0) must be captured in the Toxicity Form. Baseline AEs should be captured on the Medical History Form.

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

11.0 MEASUREMENT OF EFFECT

Effect will be measured by the rate of pCR (primary and nodal) found at the time of surgical resection. Partial pathologic response will be defined as < 10% viable tumor remaining. Residual disease will be documented in the form of % treatment response and % viable tumor. No radiographic response to treatment will be recorded or assessed.

12.0 DATA AND SAFETY MONITORING

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

Until such a time as the first secondary site enrolls its first patient, a semi-annual DSM report to be prepared by the study team will be submitted directly to the Quality Assurance and Safety Monitoring Committee (QASMC) (bypassing the independent DSMB). The first report is required either 30 days after the enrollment of the 5th participant (if sooner than 6 months after study activation) or 6 months after study activation (provided at least one patient has been enrolled; if zero patients have been enrolled at the 6-month mark, the first report will be required one year after accrual opens provided at least one patient has been enrolled).

The DSM report for the DSMB will be prepared by the study team with assistance from the study statistician. Following review by the DSMB, the report and the DSMB's recommendations will be submitted to the QASM Committee. The DSMB must review this study at least every six months

beginning six months after enrollment of the first patient at a secondary site, no more than one month prior to the due date of the DSM report to QASMC. This report will include:

- Study demographic information (local protocol number, protocol title, list of primary study team members, study sites, primary and secondary sponsors, date of most recent QA audit, and study status and history (including activation and suspension dates)
- Accrual information, including study-wide target accrual and actual accrual, anticipated and/or actual accrual end date, and accrual by year by site
- Subject status information presented in both cumulative format (total number of subjects who consented, enrolled, screen failed, started intervention, discontinued intervention, went off study, expired) and current format (number of subjects in screening, on intervention, in follow-up, or off study at time of report)
- Protocol objectives and the number of participants who are evaluable for each objective
- History of study (including summaries of substantive amendments, accrual suspensions and reasons, protocol exceptions, errors, and breaches of confidentiality)
- Summary of exceptions, noncompliance reports, and unanticipated problems reported to the IRB
- Early stopping rules and data describing whether the stopping rules have been met
- Separate SAE and worst grade toxicity tables, each separated by site
- Participant-level response and survival data
- Summary of specimen collection (percentage of participants who have had specimens collected at each required time point)
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety of participants or the ethics of the study

Further DSMB responsibilities are described in the DSMC charter.

The study principal investigator and coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines (please refer to Section 8.0).

Refer to the Quality Assurance and Safety Monitoring Committee Policies and Procedures and the Siteman Cancer Center Independent Data Safety Monitoring Board (DSMB) Policies and Procedures documents for full details on the responsibilities of the DSMB. This is located on the QASMC website at https://siteman.wustl.edu/research/resources-for-researchers/quality-assurance-and-safety-monitoring/.

13.0 AUDITING

As coordinating center of this trial, Washington University (via the Quality Assurance and Safety Monitoring Committee (QASMC)) will monitor each participating site to ensure that all protocol

requirements are being met; that applicable federal regulations are being followed; and that best practices for patient safety and data collection are being followed per protocol. Participating sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the WUSM study team designee, who will obtain the audit materials from the participating institution.

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

Items to be evaluated include:

- Subject screening and enrollment
- Reporting of adverse events
- Maintenance of HIPAA compliance
- Completeness of regulatory documentation
- Completeness of participant documentation
- Acquisition of informed consent
- IRB documentation
- Issues of protocol adherence

Additional details regarding the auditing policies and procedures can be found at https://siteman.wustl.edu/research/resources-for-researchers/quality-assurance-and-safety-monitoring/.

14.0 STATISTICAL CONSIDERATIONS

14.1 Statistical Hypotheses and Sample Size Determination

Chemotherapy alone has a pCR rate of 6-16%[10]. PCR is defined as a pathologic complete response (no tumor on gastrectomy specimen). We hypothesized that preoperative radiation followed by chemotherapy would result in a pCR rate of 20%, and with 36 patients, our confidence interval for this estimate will fall between 9% and 37%.

14.2 Analysis of the Primary Endpoint

The pCR rate will be described as percent with 95% confidence interval. Because only patients who undergo surgery are considered evaluable for the primary endpoint, the denominator used to describe the pCR rate will be the number evaluable. This will be described clearly when study results are reported.

14.3 Analysis of Secondary Endpoints

Secondary endpoints include the following:

- The proportion of patients that can complete total neoadjuvant chemotherapy.
- The local control rate at 1 year will be described as percent with 95% confidence interval. Local control is defined as no local recurrence, calculated from RT start.
- The rate of grade 3 or higher toxicity will also be described as percent with 95% confidence interval.
- Kaplan-Meier estimates will be used to describe disease-free survival (no cancer evident or death) and overall survival (survival from start of RT) at 1 year.

14.4 Baseline Descriptive Statistics

Baseline demographic characteristics will be summarized: Continuous factors will be presented as median with range; categorical factors will be presented as counts and percentages.

15.0 MULTICENTER REGULATORY REQUIREMENTS

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

Site activation is defined as when the secondary site has received official written documentation from the coordinating center that the site has been approved to begin enrollment. At a minimum, each participating institution must have the following documents on file at Washington University prior to study activation:

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

The coordinating center Principal Investigator (or designee) is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 4 weeks of obtaining Washington University IRB approval. Activated secondary sites are expected to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt unless otherwise noted. Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

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

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

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APPENDIX A: ECOG Performance Status Scale

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

APPENDIX B: Definitions for Adverse Event Reporting

A. Adverse Events (AEs)

As defined in 21 CFR 312.32:

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

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 5.0 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

B. Suspected Adverse Reaction (SAR)

As defined in 21 CFR 312.32:

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

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

As defined in 21 CFR 312.32:

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

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

As defined in 21 CFR 312.32:

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

- Death
- A life-threatening adverse event

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

E. Protocol Exceptions

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

F. Deviation

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

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

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

APPENDIX C: Reporting Timelines

Expedited Reporting Timelines		
Event	**HRPO**	**QASMC**
Unanticipated problem involving risk to participants or others	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	Report via email after IRB acknowledgment
Major deviation	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	
A series of minor deviations that are being reported as a continuing noncompliance	Report within 10 working days.	
Protocol exception	Approval must be obtained prior to implementing the change	
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.	
Breach of confidentiality	Within 10 working days.	
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.	

Routine Reporting Timelines		
Event	**HRPO**	**QASMC**
Adverse event or SAE that does not require expedited reporting	If they do not meet the definition of an unanticipated problem involving risks to participants or others, report summary information at the time of continuing review	Adverse events will be reported in the toxicity table in the DSM report which is typically due every 6 months.
Minor deviation	Report summary information at the time of continuing review.	
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1	

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

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

APPENDIX D: Washington University Unanticipated Problem Reporting Cover Sheet

SAE COVER SHEET- Secondary Site Assessment

Washington University HRPO#:	WUSM PI:
Subject Initials:	Subject ID:
Treating MD:	Treating Site:
EVENT TERM:	Admission Date:
EVENT GRADE:	Date of site's first notification:

Treating MD Event Assessment:

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

   ☐ yes ☐ no

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

2. Is this event **unexpected**?

   ☐ yes ☐ no

Explain _____

_____

_____ _____ _____

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

APPENDIX E: Study-Specific DSM Tables

Protocol Objectives and Subject Evaluability	
Objective	**# of patients evaluable for this endpoint to date**
Primary	
To quantify the pCR rate in gastric cancer patients treated with preoperative radiation followed by total neoadjuvant chemotherapy.	
Secondary	
To quantify the proportion of patients able to complete a full course of total neoadjuvant chemotherapy	
To quantify the local control rate of gastric cancer patients treated with preoperative radiation followed by chemotherapy	
To quantify the toxicity of gastric cancer patients treated with preoperative radiation followed by chemotherapy	
To quantify the overall survival in gastric cancer patients treated with preoperative radiation followed by chemotherapy	
To quantify the disease-free survival in gastric cancer patients treated with preoperative radiation followed by chemotherapy	
Exploratory	
To quantify the deviation in dose to the tumor due to variation in stomach position	
To quantify the deviation in dose to the nearby OARs due to variation in OAR position	
To determine the distribution of TCGA molecular subtypes in gastric adenocarcinoma after radiation and chemotherapy	
To identify differentially expressed post-treatment biomarkers	
To correlate circulating tumor DNA (ctDNA) levels with clinical outcomes	

Interim Analysis and Early Stopping Rules
Does the study design include an interim toxicity analysis? No
Does the study design include an interim futility analysis? No
Are there early stopping rules that outline circumstances under which the study must be suspended or closed? Yes
If yes, please insert text describing early stopping rules from the protocol:

There will be a lead-in safety cohort of 5 patients, monitored for 30 days after completion of RT. If there is one treatment-related patient death, then the safety cohort will be expanded to 10 patients. If there are two or more treatment-related deaths, the radiation dose will be decreased to 20 Gy in 5 fractions. The trial will be terminated if there are more than three treatment-related deaths (equaling approximately 10% of the entire projected accrual).
If yes, please provide data describing whether the stopping rules have been met (by indicating whether there have been any treatment-related grade 5 toxicities in the safety lead-in cohort):

Response					
UPN	**On tx date**	**# fx SCRT complete**	**# cycles chemo complete**	**Pathologic response**	**Date of progression**

Treatment Discontinuation and Survival				
UPN	**Off tx date**	**Reason off tx**	**Vital status**	**If dead, cause**

Summary of Specimen Collections			
Type of specimen	**Time point**	**# of patients eligible for collection at this time point**	**% of patients who have reached this time point and had the specimen collected**
Tissue	Surgery		
Blood for ctDNA	Prior to start of RT		
	After completion of RT but before starting chemo		
	After completion of chemo but before surgery		
	6 months after surgery		