Abbreviated Title: RCT of CDH1 Surveillance

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Title: Phase II Randomized Trial of Bethesda Protocol Compared to Cambridge Method for Detection of Early Stage Gastric Cancer in *CDH1* Mutation Carriers

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PRÉCIS

Background:

- Hereditary Diffuse Gastric Cancer (HDGC) is most often attributed to inactivating germline mutations in the E-cadherin (CDH1) tumor suppressor gene. Mutation carriers have a 24-70% lifetime risk of developing gastric adenocarcinoma.
- International consensus guidelines recommend endoscopic screening and surveillance of *CDH1* mutation carriers who decline risk-reducing total gastrectomy (TG). However, this approach lacks sufficient sensitivity for detection of occult, intramucosal foci of signet ring cancer cells (SRCC), which are pathognomonic of HDGC. Our team has established a systematic endoscopic screening protocol (Bethesda protocol) that demonstrates a higher rate of SRCC detection compared to historic controls using the currently recommended Cambridge method.

Objective:

- Determine if Bethesda protocol provides improved sensitivity for detection of early-stage gastric cancer in *CDH1* germline mutation carriers compared to the Cambridge method.

Eligibility:

- Subjects with pathogenic or likely pathogenic *CDH1* germline mutation.
- Age $\geq$18 years.
- Physiologically able to undergo upper endoscopy

Design:

- Phase II randomized study to compare Bethesda protocol and Cambridge method for detection of intramucosal SRCC in asymptomatic *CDH1* mutation carriers undergoing endoscopic screening or surveillance.

TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

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

- 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; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- Determine if Bethesda protocol provides improved sensitivity for detection of early-stage gastric cancer in *CDH1* germline mutation carriers compared to the Cambridge method.

1.1.2 Secondary Objective

- Define the false negative rate of SRCC detection using Bethesda protocol and Cambridge methods in patients who proceed to risk-reducing total gastrectomy.
- To estimate and compare the difference in crude cancer detection rates between endoscopy using the Bethesda protocol and the Cambridge method.

1.1.3 Exploratory Objectives

- Determine if confocal endoscopic microscopy (CEM) will afford greater sensitivity for detection of SRC foci in *CDH1* germline mutation carriers compared to the Cambridge method
- To characterize gastric tract microbiota of *CDH1* germline mutation carriers.
- To assess esophageal motility after total gastrectomy (in patients who pursue total gastrectomy)
- Apply machine learning algorithms to aid early gastric cancer detection using input from confocal endoscopic microscopy (CEM) images and histopathology images/results, and utilize image-to-image translation to enhance early cancer detection with CEM

1.2 BACKGROUND AND RATIONALE

1.2.1 Study Disease

Hereditary diffuse gastric cancer (HDGC) accounts for approximately 1-3% of gastric cancers globally and is the most common familial form of gastric cancer [1]. Forty percent of HDGC cases are linked to inactivating germline mutations in the E-cadherin (*CDH1)* tumor suppressor gene [2]. Estimates of disease penetrance have demonstrated *CDH1* mutation carriers have a substantially elevated lifetime risk of developing gastric cancer, approximating 37-70% in men and 24-56% in women [3, 4].

The *CDH1* gene encodes the tumor-suppressor protein E-cadherin, which is a glycoprotein localized on the surface of epithelial cells at the adherens junctions [5]. E-cadherin functions to regulate cell-cell adhesion and intercellular communication, which is critical for cellular migration, proliferation, apoptosis and cell differentiation [6-10]. When E-cadherin function is compromised there is loss of cell polarity and epithelial architecture, which interferes with the integrity of the adherens junctions. *CDH1*-null epithelial cells may become inappropriately placed in the cellular matrix of the lamina propria resulting in the formation of signet ring cancer cells (SRCC) (**Figure 1** and **Figure 2**) [11]. It is postulated that gastric *CDH1*-null SRCC can exist for extended periods of time allowing for the accumulation of additional molecular alterations that lead to tumorigenesis and/or metastasis [12]. It is speculated that a second hit to this gastric mucosa, via mutations in *Smad4*, *RHOA,* or *TP53*, ultimately enables tumor cell invasion and metastatic potential [11, 13-15].

Because of the increased lifetime risk of developing gastric cancer, the International Gastric Cancer Linkage Consortium (IGCLC) recommends risk-reducing total gastrectomy to remove all gastric epithelium [3]. At the time of total gastrectomy almost all asymptomatic patients with *CDH1* mutations are found to have SRCC within the stomach. Since 2017, our team has evaluated more than 59 total gastrectomy explants and found 86% harbor SRCC (T1a gastric cancers) on final pathology. However, there is a scientific lack of understanding of how and why these SRCC become invasive cancer.

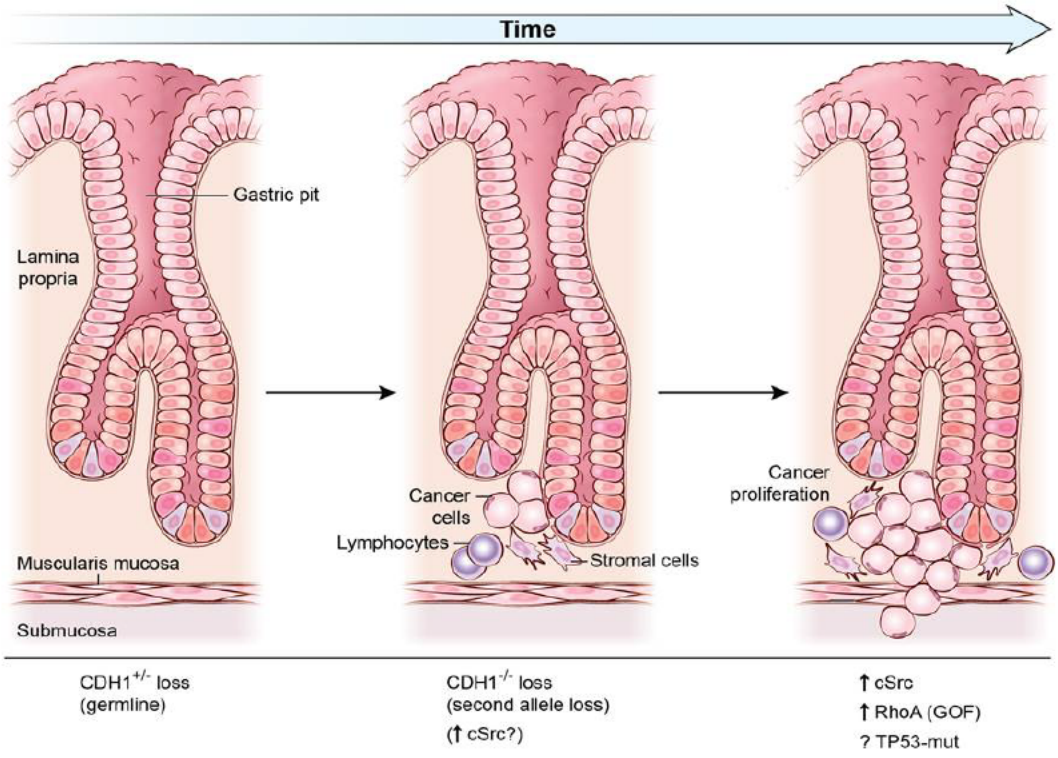

Figure 1. Schematic representation of the formation of signet ring cancer cells (SRCC) from the gastric mucosa in *CDH1* mutation carriers. Left is a diagram of a gastric pit, which comprises normal gastric mucosa. After second allele loss of *CDH1*, SRCC form and accumulate in the lamina propria (middle image). Then, additional loss of cellular regulation mechanism results in tumorigenesis (far right).

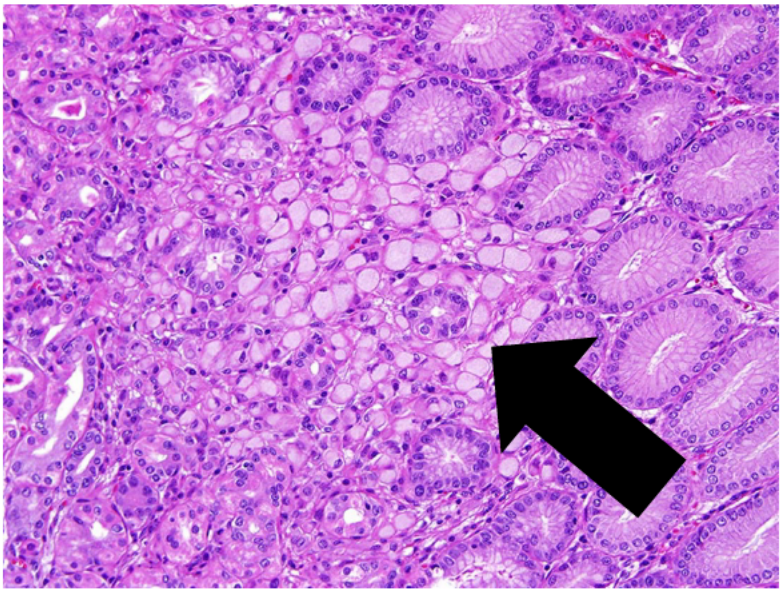

Figure 2. Microscopic image of signet ring cancer cells (black arrow) within the lamina propria of the gastric mucosa

1.2.2 Annual Endoscopic Surveillance

For patients who decline risk-reducing gastrectomy, annual endoscopic surveillance utilizing the Cambridge method is currently recommended by the IGCLC for management patients with pathogenic *CDH1* variants [3]. This endoscopy technique is performed over 30 minutes, using a white light high definition endoscope; it consists of mucosal biopsies of any visible lesions, testing for H. pylori, and 30 random mucosal biopsies from the prepyloric area, gastric antrum, transitional zone, body, gastric fundus, and gastric cardia. However, this technique is not sensitive as it repeatedly has shown to miss early diffuse gastric cancer (DGC) [16-20]. The SRCC detection rate with the standard, Cambridge, method ranges from 15-60% with false negative rates of 37%-84.6% [21-24]. This form of surveillance is unreliable because intramucosal SRCC foci are not visible with standard endoscopy because they make up less than 2% of the gastric mucosa and often measure less than 1 mm in diameter [17].

Our team developed a novel systematic endoscopic screening protocol (Bethesda protocol) that was adapted from a method initially described by Yao [25]. Using white-light upper endoscopy, the Bethesda protocol consists of 88 mucosal biopsies taken from 22 anatomic areas of the stomach. Our group recently compared the sensitivity of SRCC detection using the Bethesda protocol to that of the Cambridge method in a retrospective analysis (**Figure 3**) [21].

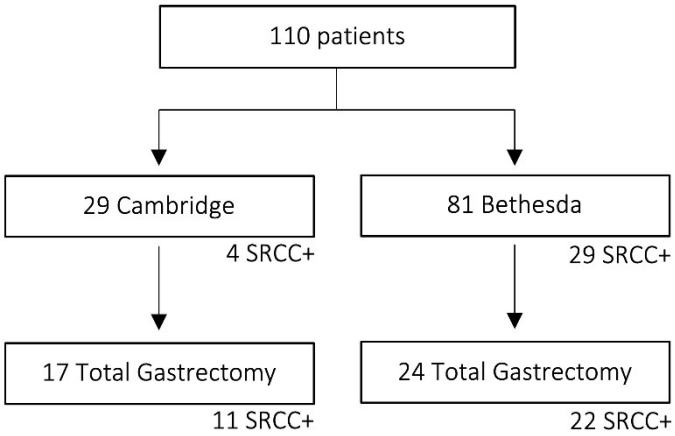

Figure 3. Sensitivity of SRCC detection comparing the Bethesda protocol to that of the Cambridge method in a retrospective analysis.

The analysis consisted of 110 patients with the *CDH1* mutation who were evaluated at NIH Clinical Center. All 110 patients underwent screening gastroscopy; 29 using the Cambridge method and 81 using the Bethesda protocol. Signet ring cancer cells were detected in 4 patients (13.8%) using Cambridge method and 29 (35.8%) with the Bethesda protocol. Over half (17/29) of the Cambridge method patients underwent prophylactic total gastrectomy, of whom 11 had SRCC identified on final pathology. Of those 11, only 2 were positive for SRCC on esophagogastroduodenoscopy (EGD) yielding an 82% (9/11) false negative biopsy rate and an overall sensitivity of 18% (2/11). Comparatively, 24 patients screened with the Bethesda protocol (24/81) later underwent prophylactic total gastrectomy, of whom 22 were found to have SRCC on final pathology. Of those 22, 13 were positive for SRCC on EGD giving the Bethesda protocol a 41% (9/22) false negative biopsy rate and an overall sensitivity of 59% (13/22) for detecting SRCC. Based on these data, we conclude the Bethesda protocol is likely better than the Cambridge method at detecting SRCC in asymptomatic *CDH1* mutation carriers.

Our primary objective with this prospective random-assignment study is to compare the Bethesda protocol (investigational) to the Cambridge method (control) for detection of early stage gastric cancer (e.g. SRCC) in asymptomatic CDH1 mutation carriers.

To fully understand the limitations of endoscopic gastric cancer surveillance, the false-negative cancer detection rate should be defined also, which is a secondary aim of the proposed study. This can only be done through complete pathologic examination of total gastrectomy explants from patients who choose to proceed to prophylactic surgery endoscopic surveillance.

1.2.3 Adjuncts to Endoscopy

Enhanced endoscopic techniques to increase cancer detection rates through improved mucosal visualization have been attempted. Confocal endoscopic microscopy (CEM) is a technique that is used with a standard endoscope to obtain in vivo histology images of gastrointestinal mucosa. Based on data from a Phase II study by our group (18-C-0141), we determined the SRCC detection rate of CEM was slightly improved compared to Cambridge method. Our preliminary results, which included 34 patients accrued over eight months, demonstrated detection rate of CEM to be 28.6% with false negative rate of 71.4%, which appeared only slightly better than 14.3% and 85.7%, respectively, with Cambridge method. It is possible that CEM could improve white light endoscopy detection methods when added to the Cambridge method, as in our Phase II study. Further exploration of CEM added to gastric surveillance is necessary to answer this question. Moreover, interpretation of confocal images obtained during gastric surveillance posed a challenge in our Phase II study. Our group is exploring the possible application of artificial intelligence to endoscopic and histopathologic findings to improve the sensitivity and specificity of gastric cancer diagnosis. Because our use of CEM showed low sensitivity for occult cancer detection, we believe that application of machine learning (i.e., computer models) based on the input provided by CEM imaging will improve our ability to accurately and reliably detect cancer. By incorporating the corresponding histopathology images obtained after gastric biopsy, we will essentially teach the computer how to reliably detect occult gastric cancer through an iterative process.

Accurate detection of early gastric cancer from endoscopic biopsies or total gastrectomy explants requires expertise in gastrointestinal pathology. Briefly, our NCI pathology colleagues have analyzed over 80 gastrectomy specimens and over 1,000 gastric biopsy specimens over the past 3 years. They have a working system for how they approach all of these patients' specimens, such as the use of initial H&E for screening of gastric biopsies followed by selective use of PAS stain. Most often, they are identifying clusters of cells, generally from 100 micrometers up to 1 centimeter in diameter. It is rare for us to identify single cells, but we have identified small areas of carcinoma in situ. The examinations are performed in a systematic manner established by our expert gastrointestinal pathologists. It is most frequently our aim to adhere to international gastric cancer linkage consortium guidelines for pathologic examination based on availability of resources (P. Guilford, *personal communication*). A Level 1 examination is the minimum suggested to obtain sufficient data for appropriate patient care (**Figure 4**). A Level 3 examination would be considered optimal for clinical reporting and support preservation of tissue for future research.

Levels of pathological examination depending on availability of resources

Level	Level 1 Minimum required	Level 2 [level 1 plus…]	Level 3 [Level 2 plus…]
Morphologic	• Pin out and photograph • Sample margins and lymph nodes • Sample tissue from all gastric zones • Map blocks to photo • Examine all slides	• Embed all mucosa, process to paraffin blocks. • Cut a subset of blocks, sampling all gastric zones • Examine sampled slides.	• Cut all blocks. Examine all slides.
Repeat	• Sample tissue from all zones • Map blocks • Examine all slides	• Cut a subset of blocks, sampling all zones • Examine sampled slides.	
Stop	When invasive carcinoma is found or up to arbitrary limit for example 50 blocks	When invasive carcinoma is found, or up to arbitrary limit for example 50 slides.	When all mucosa is examined.
Report	Multiple of foci of pT stage carcinoma in xx% of mucosa examined microscopically	Number of foci of pT stage carcinoma in xx% of mucosa examined microscopically	Number of foci of pT stage carcinoma, all mucosa examined microscopically
Blocks	~ 20-50*	~ 120-270	~ 120-270
Slides	~ 20-50*	~ 20-50*	~ 120-270

Figure 4. Levels of pathological examination depending on availability of resources.

1.2.4 Molecular and Physiologic Sequelae of Hereditary Diffuse Gastric Cancer Syndrome

And, although most patients tolerate life without a stomach, post-gastrectomy syndromes remain poorly understood. This includes everything from malabsorption, urolithiasis, early and late dumping syndrome, and post-gastrectomy dysphagia. For instance, post-gastrectomy dysphagia in the absence of a mechanical cause (i.e., stricture) is present in a subset of these patients for unknown reasons and very little data exist regarding esophageal motility changes following total gastrectomy.

Very few studies have evaluated esophageal motility after total gastrectomy. The limited data available demonstrate that the majority of post-gastrectomy patients (13/20) were symptomatic (with complaints of reflux, dysphagia, or odynophagia) while all demonstrated pathological patterns of contraction on esophageal manometry [26]. Because of this observation of symptomatic dysphagia in the absence of mechanical abnormality (i.e., esophageal stricture), we propose esophageal manometry studies to understand the functional effects of total gastrectomy on the esophagus as part of this endoscopic surveillance protocol.

The advent of highly sensitive analytical methods in recent years has enabled detailed delineation of the gut microbiome in health and disease. Therefore, analysis of the gastric and fecal microbiome may aid our understanding of microbiome diversity and possible dysbiosis. Gastric lavage and gastric biopsy can be used to define and characterize the gastric microbiota. Recent

studies demonstrate a correlation with gastric cancer and opportunistic pathogens within the stomach, however the gastric microbiome of *CDH1* mutation carriers and the microbiome contribution to disease penetrance in this population is unknown [27, 28]. We plan to use pyro-sequencing techniques to survey microbial diversity in patients' gastric and fecal samples. Prior to stool sample collection dietary recordings and detailed medication history will be collected from study participants. The association between specific microbiome maps and the occurrence of bacterial translocation will then be analyzed.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Initial Enrollment

2.1.1.1 Inclusion Criteria

- An individual who harbors a pathogenic, or likely pathogenic, *CDH1* germline variant. **Note:** individuals with *CDH1* variant classified as any of the following are not eligible:
    - variant of uncertain significance
    - benign
    - likely benign.
- Age ≥18 years.
- Physiologically able to undergo upper endoscopy.
- Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.1.2 Exclusion Criteria

- Any clinical contraindication (e.g., known bleeding disorder, thrombocytopenia) to endoscopic biopsy.
- Unstable angina or recent (within 3 months) myocardial infarction.
- Any clinical contraindication to general anesthesia.

2.1.2 Re-Enrollment

2.1.2.1 Inclusion Criteria

- Subject must have previously been enrolled on the study and must have undergone endoscopy. Note: Subject may re-enroll only once after initial endoscopy performed
- Subject must have clinical need for a repeat endoscopy
- Prior on-protocol endoscopy must have occurred at least 6 months (+/- 2 weeks) and no greater than 18 months (+/- 4 weeks)

2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms. Participants may also be recruited through self-referrals, physician referrals, and referrals from the NIH Clinical Center (CC) Office of Patient Recruitment.

2.2 SCREENING EVALUATION

2.2.1 Screening Activities Performed Prior to Obtaining Informed Consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

2.2.2 Screening Activities Performed After a Consent for Screening Has Been Signed

The following activities will be performed only after the subject has signed the consent for this study for screening. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

Within 30 days prior to enrollment:

- Complete medical history and physical examination, including vital signs.
- Review of genetic test results performed in any outside lab if not already completed (Section **2.2.1**).

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2: CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.3.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or 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 (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of non-conclusive genetic test results may be rescreened.

2.3.2 Intervention Assignment Procedures (For Registration Purposes Only)

2.3.2.1 Cohorts

Number	**Name**	**Description**
1	Cohort 1	Subjects with pathogenic, or likely pathogenic, *CDH1* germline mutation

2.3.2.2 Arms

Number	Name	Description
1	Arm 1	Bethesda protocol (*investigational*)
2	Arm 2	Cambridge method (*control*)

2.3.2.3 Arm Assignment and Randomization

Subjects in Cohort 1 will be randomized by CRO to Arm 1 or Arm 2. No stratification is planned.

2.4 BASELINE EVALUATION

Within 30 days prior to study procedure (does not need to be repeated if performed during Screening within designated time period):

- Physical examination, including weight and vital signs.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a Phase II randomized study designed to compare the efficacy of the Bethesda protocol compared to Cambridge method for detecting signet ring cells within gastric mucosa of asymptomatic patients with *CDH1* mutations.

Upon study enrollment, subjects will be randomized to undergo upper endoscopy by either the Bethesda protocol technique (Arm 1) or Cambridge method (Arm 2).

Confocal endomicroscopy (CEM) of the gastric mucosa (Section **3.1.3**) will be performed on enrolled subjects of both study arms unless otherwise indicated* until sufficient data for statistically accurate and reliable application of machine learning (i.e., computer models – refer to Section **5.3.6** for details), currently believed to total the first 50 enrolled participants. *CEM will not be performed in the following circumstances: participant allergy to the contrast (refer to Section **3.1.3**), equipment not available or not functioning properly, or due to operating room scheduling issues/availability.

Note: Biopsies will be obtained as indicated below.

3.1.1 Bethesda Protocol, Arm 1 (Investigational Arm)

Participants will undergo white light endoscopy. The mucosa of the stomach may be thoroughly washed before examination with a combination of mucolytics (N-acetylcysteine) and anti-foaming agents (simethicone) mixed with sterile water, as medically indicated. Inspection will include repeated inflation and deflation to check distensibility and any abnormal appearing areas will additionally be biopsied. Eighty-eight non-targeted biopsies from 22 areas of the stomach, four mucosal biopsies from each, will be obtained using standard cold endoscopic forceps. This technique is estimated to take approximately 45 minutes or less.

- Antrum, antegrade view – anterior (1), lesser curve (2), posterior (3), greater curve (4)
- lower body, antegrade view – anterior (5), lesser curve (6), posterior (7), greater curve (8)

- middle-upper body, antegrade view – anterior (9), lesser curve (10), posterior (11), greater curve (12)
- fundus-cardia, retroflex view – anterior (13), lesser curve (14), posterior (15), greater curve (16)
- middle-upper body, retroflex view – anterior (17), lesser curve (18), posterior (19)
- incisura, retroflex view – anterior (20), lesser curve (21), posterior (22)

3.1.2 Cambridge Method Upper Endoscopy, Arm 2 (Control Arm)

Participants will undergo white light endoscopy. The mucosa of the stomach may be thoroughly washed before examination with a combination of mucolytics (N-acetylcysteine) and anti-foaming agents (simethicone) mixed with sterile water, as medically indicated. Inspection will include repeated inflation and deflation to check distensibility and any abnormal appearing areas will additionally be biopsied. A minimum of 30 non-targeted biopsies will be obtained from 6 anatomic zones of the stomach:

- pre-pyloric area,
- antrum,
- transitional zone,
- body,
- fundus, and
- cardia.

Five mucosal biopsies will be taken from each zone. Biopsies will be taken using standard, cold endoscopic forceps. This technique is estimated to take approximately 30 minutes or less.

3.1.3 Confocal Endomicroscopy of the Gastric Mucosa

Subjects may additionally undergo confocal imaging with fluorescein contrast following white light endoscopy (refer to Section **3.1**). Confocal imaging of the gastric mucosa will be performed using the Cellvizio® Real-Time In Vivo Cellular Imaging Platform with Confocal Miniprobes (refer to Section **3.1**). Focally abnormal areas of gastric mucosa and selected normal-appearing anatomic regions will be selected for digital imaging. The overall procedure time for subjects in which CEM will also be performed is estimated to increase by approximately ≤ 20 minutes.

3.2 SURGICAL GUIDELINES

3.2.1 Preoperative Patient Management

Patients will receive standard preoperative care as appropriate to the planned endoscopic intervention and the patient's underlying health status. This will include sequential compression devices placed on the lower extremities prior to induction of general anesthesia.

3.2.2 White-light, Upper Endoscopy

As described in Sections **3.1.1** and **3.1.2**.

3.2.3 Postoperative Care

- Patients will be monitored in the post anesthesia care unit (PACU) after their procedure prior to being discharged (if outpatient) or transferred to the inpatient ward (if inpatient).

3.2.4 Discharge

- Total hospitalization may be 0-1 day. If patients live in the area they may come in the morning of the procedure and leave on the same day providing they have a companion with them. If the patient is traveling alone (without companion) then they will spend the night in the hospital after the procedure prior to discharge.

3.3 FOLLOW-UP

Patients will return to the NIH Clinical Center approximately two weeks after the EGD to discuss pathology results and any adverse events that they might have experienced. Alternatively, we will discuss pathology results and adverse events (AEs) on the phone.

Gastrectomy is not part of this protocol, but if patient elects to undergo gastrectomy on another protocol, we may perform additional correlative studies before, during and after gastrectomy, including pathology assessment of stomach tissue.

Participants declining total gastrectomy (TG) may be invited to collect stool samples after study intervention as indicated in Section **5.1**.

Participants who elect for TG may be invited to collect stool, blood and oropharyngeal swab/saliva samples pre-gastrectomy and post-gastrectomy as indicated in Section **5.1**.

3.3.1 Repeat Endoscopy

Endoscopy may be repeated once (per patient) for those with clinical need for repeat endoscopy at least 6 months (+/- 2 weeks) and no greater than 18 months (+/- 4 weeks) from the date of initial endoscopy on the current study. Patients who have been taken off study will be re-enrolled (refer to Section **2.1.2**).

Patient will be randomized for second endoscopy by CRO to Arm 1 or Arm 2.

Baseline assessment will be repeated as explained in Section **2.4**.

Endoscopy will be performed as explained in Section **3.2**

Samples for correlative studies will be collected again as explained in Section **5**.

Participants declining TG will be invited to Clinical Center to collect stool samples after second endoscopy (refer to Section **5**).

3.3.2 Long-Term Sequelae of Total Gastrectomy

Esophageal Dysmotility: Very few studies have evaluated esophageal motility after total gastrectomy. The limited data available demonstrate that the majority of post-gastrectomy patients (13/20) were symptomatic (with complaints of reflux, dysphagia, or odynophagia) while all demonstrated pathological patterns of contraction on esophageal manometry [26]. Because of this observation of symptomatic dysphagia in the absence of mechanical abnormality (i.e., esophageal stricture), we propose high resolution esophageal manometry studies to understand the functional effects of total gastrectomy on the esophagus as part of this endoscopic surveillance protocol.

An esophageal manometry is a test to determine how well the muscles of the esophagus work and can help in the evaluation of acid reflux, problems with swallowing, and chest pain that may be coming from the esophagus.

This high resolution esophageal manometry test typically takes 15 to 20 minutes to complete. Patients are asked to stop eating or drinking after midnight on the day of the test. Per standard clinical procedure, a small, flexible plastic tube is passed through the naris (i.e., nostril), down the back of the throat, and into the esophagus. The esophageal manometry probe contains pressure sensors and impedance channels which allow for measurements of the muscle contractions of the esophagus and how liquid or viscous materials are propelled through the esophagus during swallowing. The patient is asked to swallow small sips of water during the test. Afterwards the flexible tube is removed. No activity restriction or dietary restriction is necessary following this test.

Participants planning to undergo total gastrectomy at the NIH will also undergo an optional high resolution esophageal manometry prior to obtaining baseline esophageal function. Following total gastrectomy, additional optional esophageal manometry will be performed at 3 months (+/- 1 month) and 12 months (+/- 2 months) post-gastrectomy. Results of these high resolution esophageal manometry will be shared with participants.

3.4 COST AND COMPENSATION

3.4.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.4.2 Compensation

No compensation is offered on this study.

3.4.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.5 CRITERIA FOR REMOVAL FROM PROTOCOL INTERVENTIONS AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 14 days following study interventions.

3.5.1 Criteria for Removal from Protocol Interventions

- Completion of protocol interventions
- Patient requests to be withdrawn from active intervention
- A serious or intolerable event related to the intervention occurs
- Investigator discretion

3.5.2 Off-Study Criteria

- Completion of 12 months follow up period after last endoscopy procedure (for patients who decline total gastrectomy)

- Completion of 12 months follow up period after total gastrectomy (for patients who opt for total gastrectomy)
- Investigator discretion
- Death
- Participant requests to be withdrawn from the study
- Lost to follow up
- PI decision to close the study
- Screen failure

3.5.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for 4 scheduled visits and is unable to be contacted by the study site staff.

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

- The site will attempt to contact the participant and reschedule the missed visit within 3 weeks 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/video calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). 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.

4 CONCOMITANT MEDICATIONS/MEASURES

During post-operative period, patients will receive all standard of care supportive measures, including pain control and incentive spirometry to prevent atelectasis as indicated.

5 CORRELATIVE STUDIES

5.1 SUMMARY AND BIOSPECIMEN COLLECTION TABLE FOR RESEARCH

Specimen / Assay Method	**Volume (approx.)**	**Type of Collection Tube***	**Collection Point(s)****	**Location of Specimen Processing/ Storage*****	**Location of Specimen Analysis *****
Stool/ Microbiome by DNA + RNA Sequence Analysis	Stool, ≥ 300 mg/ specimen (**or** as available)	Stool Collection Kit (***To BPC within 24 hrs. of collection;*** **refer to Section 5.1.1.3**)	**Participants declining TG:** one-time prior to or day of EGD, AND post- EGD as applicable per PI discretion: 6 months (+/- 1 month) and/or 12-months (+/- 2 months) after study intervention **Participants who elect for TG:** one-time "pre-gastrectomy" (prior to EGD or within 3 months prior to TG), AND at 3 months (+/- 1 month) and 12 months (+/- 2 months) post-gastrectomy	Blood Processing Core (BPC)	CCR Sequencing Facility (CCR-SF)
Serum/ Microbiome by DNA + RNA Sequence Analysis	Blood, 5 mL/ specimen	SST (red/yellow top) tube, or similar (per BPC)	**Participants who elect for TG:** one time "pre-gastrectomy" AND at 3 months (+/- 1 month) and 12 months (+/- 2 months) post-gastrectomy	Blood Processing Core (BPC) **(As indicated in Section 5.1.1.5)**	CCR Sequencing Facility (CCR-SF)

Specimen / Assay Method	**Volume (approx.)**	**Type of Collection Tube***	**Collection Point(s)****	**Location of Specimen Processing/ Storage*****	**Location of Specimen Analysis *****
Gastric Lavage/ Microbiome by DNA + RNA Sequence Analysis	Gastric lavage, 10 mL/ specimen (as available)	Sterile specimen cup or conical tube (or equivalent) **(*To BPC within 2 hrs. of collection;* as indicated in 5.1.1.1)**	One time during upper endoscopy in all study participants (per PI discretion at time of intervention, as applicable)	Blood Processing Core (BPC)	CCR Sequencing Facility (CCR-SF)
Gastric Mucosal Biopsy/ Microbiome by DNA + RNA Sequence Analysis	Non-targeted gastric biopsy; 2 biopsies/ specimen (as available)	Sterile conical tube (or equivalent) with normal saline (without preservative fluid) ***(To BPC within 2 hrs. of collection;* refer to 5.1.1.2)**	One time during upper endoscopy in all study participants (per PI discretion at time of intervention, as applicable)	Blood Processing Core (BPC)	CCR Sequencing Facility (CCR-SF)
Oropharyngeal/ Microbiome by DNA + RNA Sequence Analysis	Oropharyngeal swab, 1 swab **OR** Per salvia collection kit	Cryotube with 500µl RTF buffer (n=1) **OR** Per collection kit (or equivalent) ***(To BPC within 2 hrs. of collection;* refer to 5.1.1.4)**	**Participants who elect for TG:** one time "pre-gastrectomy" AND at 3 months (+/- 1 month) and 12 months (+/- 2 months) post-gastrectomy	Blood Processing Core (BPC)	CCR Sequencing Facility (CCR-SF)

* Tubes/media may be adjusted at the time of collection based upon materials available if approved by the PI/laboratory investigator.

** Note: Specimen defined as "pre-gastrectomy" should be collected anytime within the 3 months **prior** to total gastrectomy.

*** Samples will be sent to Blood Processing Core (BPC) for barcoding and initial processing and storage until they are distributed for sample analysis, if not otherwise specified in the protocol. Samples will be stored and kept frozen in the BPC freezers (e.g., -20/-80°C) according to stability requirements. DNA/RNA extraction in Dr. Heller's lab or per PI discretion at CCR Sequencing Facility (CCR-SF) prior to CCR-SF analysis.

5.1.1 Sample Collection and Processing

Standard pathology evaluation of stomach tissue from biopsies and/or gastrectomy will be performed in the Laboratory of Pathology (Section **5.3.2**).

Note: The handling and processing instructions outlined in the following sections should be followed as closely as possible, however handling and processing may be adjusted at the discretion of the PI and/or supervising laboratory to ensure sample quality.

5.1.1.1 Gastric Lavage/Microbiome by DNA + RNA Sequence Analysis

Gastric lavage will be obtained during upper endoscopy in all study participants (as indicated in Section **5.1**) through the working channel of the upper endoscope. In order to avoid potential contamination, suctioning of oropharyngeal and esophageal secretion will be minimized upon insertion of the endoscope. The gastric lavage will be suctioned into a sterile conical tube. The research study team will be alerted by operating room personnel when the sample is ready for pickup.

The sample will be aliquoted by the study team into approximately 6 smaller containers prior to transfer to the Blood Processing Core (BPC) for storage, or as agreed upon with BPC at the time of transfer (per PI discretion): one 5 mL tube (n=1) and five 1 mL tubes (n=5), for a total of 10 mL (i.e., n=6 total). The samples will be stored at -80°C *within 2 hours of collection* and kept frozen until further analysis. Samples will be initially stored in the Blood Processing Core (BPC) (Section **5.3.1**).

5.1.1.2 Gastric Mucosal Biopsy/Microbiome by DNA + RNA Sequence Analysis

Gastric mucosal biopsy will be obtained during upper endoscopy in all study participants using a sterile standard biopsy forcep (e.g., Standard Radical Jaw™ biopsy forcep, 2.8mm) as indicated in Section **5.1**. Biopsies will be obtained in a standardized fashion, 2 non-targeted biopsies of the gastric mucosa placed in a sterile conical tube in normal saline solution. The research study team will be alerted by operating room personnel when the sample is ready for pickup.

The study team may transfer the specimen to cryotubes prior to delivering samples to the BPC for storage, or as agreed upon with BPC at the time of transfer (per PI discretion). The specimens will be stored at -80°C *within 2 hours of collection* and kept frozen until further analysis. Samples will be initially stored in the Blood Processing Core (BPC).

5.1.1.3 Stool/Microbiome by DNA + RNA Sequence Analysis

Stool specimens will be collected in all study participants.

> **In those who decline total gastrectomy:** the participant will be asked to produce a single stool specimen, to be collected one time prior to or on the day of study intervention (i.e., EGD), followed by potential stool specimen collection twice more post-study intervention (EGD), as applicable per PI discretion (refer to the Section **5.1** specimen collection table).
>
> **In those who elect surgery:** the participant will be asked to produce a single stool specimen, to be collected one-time pre-gastrectomy prior to the endoscopy, then two times post-gastrectomy (refer to the Section **5.1** specimen collection table).

Stool specimens will be collected in a standardized manner using a stool collection kit (**Appendix 3**). Received participant collection kit stool samples will be aliquoted by the research study team into 3 cryotubes containing approximately 300 mg of stool per tube (i.e., n=3 total, if possible

based provided specimen volume only). These samples must be stored at -80°C *within 24 hours after collection* and kept frozen until further analysis. Samples will be initially stored in the Blood Processing Core (BPC).

Note: In circumstances in which a participant is unable to produce a stool sample (i.e., unable to give stool "on demand") at the indicated timepoint, one or more additional scheduled stool sample collection request(s) are permitted to meet study requirements for the collection of the sample per PI discretion.

5.1.1.4 Oropharyngeal/Microbiome by DNA + RNA Sequence Analysis

Oropharyngeal swab or saliva will be collected only from participants who elect for surgery, obtained one time pre-total gastrectomy, and then two times post-gastrectomy (as indicated in Section **5.1**).

Samples will be obtained in a standardized manner and placed in a cryotube with 500µl RTF buffer, or per saliva collection kit. Samples will be stored *at -20°C within 2 hours after collection* and kept frozen until further analysis. Samples will be initially stored in the Blood Processing Core (BPC).

5.1.1.5 Serum/Microbiome by DNA + RNA Sequence Analysis

Serum for bacterial DNA extraction will be collected only from participants who elect for surgery, obtained per the specimen collection table (refer to Section **5.1**). Blood Processing Core (BPC) personnel will be alerted to pick up the specimen from phlebotomy. Serum specimen tubes will be initially processed (spun down) in the BPC and the plasma stored *at -20°C* and kept frozen in the BPC until further analysis.

5.2 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.2.1 Microbiome

The advent of highly sensitive analytical methods in recent years has enabled detailed delineation of the gut microbiome in health and disease. Therefore, analysis of the gastric and fecal microbiome may aid our understanding of microbiome diversity and possible dysbiosis. Gastric lavage and gastric biopsy can be used to define and characterize the gastric microbiota – luminal and mucosal adherent. Recent studies demonstrate a correlation with gastric cancer and opportunistic pathogens within the stomach, however the gastric microbiome of *CDH1* mutation carriers and the microbiome contribution to disease penetrance in this population is unknown [27, 28]. We plan to use pyro-sequencing techniques to survey microbial diversity in patients' serum, oropharyngeal, gastric and fecal samples. The association between specific microbiome maps and the occurrence of bacterial translocation will then be analyzed.

At the time of analysis, we intend to evaluate the relationship between specific microbiome maps and the occurrence of bacterial translocation. In addition, species homology between blood, oropharyngeal, gastric, and fecal samples will be determined by deep sequencing.

Bacterial DNA will be extracted from patient serum, gastric samples, oropharyngeal and stool specimens by the CCR Sequencing Facility (CCR-SF). Metagenomic sequencing techniques will be utilized to characterize the gastrointestinal tract microbiome.

Participant dietary history and prescription, herbal and over-the-counter medication and nutrition supplementation will also be recorded (see Section **6.1**).

5.2.1.1 Dietary History

Participants will be asked to complete two types of validated, online questionnaires for collection of their dietary history: the Automated Self-Administered 24-Hour Dietary Assessment Tool (ASA24) for 24-hour food recall, and the Diet History Questionnaire III (DHQIII) (refer to **Appendix 1** and **Appendix 2**).

For each online questionnaire, the research team will provide each user with a system-generated user ID and password that is unique to each respondent for each survey site. A list of participants' respondent user IDs will be kept in a secure drive that is accessible only to the research team. The data recorded by the survey site will not include any personal identifiable data associated with the study respondents on either questionnaire website. Only this study's investigators and the ASA24 and DHQIII operations teams for NCI (respectively) will have access to the participant response data.

Participants will be contacted by registered mail and/or by phone (or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) as appropriate with instructions on how to complete the questionnaires.

Note: Both the ASA24 and DHQIII questionnaires were developed by NCI for research use.

Automated Self-Administered 24-Hour Dietary Assessment (ASA24) – 24-Hour Food Recall

Participants will be asked to complete the **ASA24 questionnaire** (which once started requests a recall of the last 24 hours from survey start) as follows:

- on the day of each scheduled stool sample collection (Section **5.1.1.3**) (+2 days to align the stool sample collection date with the day of questionnaire completion if needed during the study visit, as the participant may be unable to produce stool at the exact timepoint), and
- on the day prior to scheduled endoscopy (+2 days to align the stool sample collection date with the day of questionnaire completion if needed, as the participant may be unable to produce stool at the exact timepoint).

Note: If the participant is unable to produce stool on the day of the scheduled stool sample collection but has already completed the 24-hour recall questionnaire earlier that day, per PI discretion, the participant may be asked to complete the questionnaire again on the "+1 day" or on the "+2 day" as appliable to when the stool sample is produced (in order to capture approximately the same 24 hours prior to the actual date of stool sample collection as the 24-hour recall questionnaire answers).

Diet History Questionnaire III (DHQIII)

Participants will be asked to complete the **DHQIII questionnaire** as follows:

- prior to each scheduled stool sample collection (Section **5.1.1.3**) (+2 days to align with the stool sample collection date with the day of questionnaire completion if needed during the study visit, as the participant may be unable to produce stool at the exact timepoint), and
- within 4 weeks (i.e., +/- 4 weeks) of scheduled upper endoscopy.

5.2.1.2 Prescription, Herbal, Over-the-Counter Medication and Nutrition Supplementation History

A thorough history of all prescription, herbal and over-the-counter medication and nutrition supplementation use will be collected at each outpatient visit, prior to upper endoscopy and prior to each of the stool collection periods. If the participant is on any laxatives, probiotic supplements, antibiotic medication, stool collection will be deferred until they are off these therapies. (See Section **6.1** for data collection.)

5.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside the National Institutes for Health (NIH) without appropriate approvals and/or agreements, if required.

5.3.1 Samples Managed by Dr. Figg's Blood Processing Core (BPC)

BPC contact information

Please e-mail at [redacted] at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page [redacted]

For immediate help, call [redacted] (main blood processing core number) or, if no answer, [redacted] (main clinical pharmacology lab number).

For questions regarding sample processing, contact [redacted]

The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.

5.3.1.1 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to participants without Labmatrix access. The data recorded for each sample includes the participant ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Participant demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.3.1.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20°C or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a participant withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the participant, if so requested). The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.2**.

Sample barcodes are linked to participant demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to participant information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.3.2 Laboratory of Pathology

Tissues designated for clinical diagnostics are transported to the Laboratory of Pathology (LP) where they are examined grossly, and relevant portions are fixed, embedded in paraffin and sectioned and stained for diagnostic interpretation. Unutilized excess tissues are stored for up to three months, in accordance with College of American Pathologists/Joint Commission on Accreditation of Healthcare Organizations (CAP/JCAHO) guidelines, and then discarded.

Following completion of the diagnostic workup, the slides and tissue blocks are either returned to the originating pathology department if requested or stored indefinitely in the LP's clinical archives. All specimens are catalogued and retrieved utilizing the clinical laboratory information systems, in accordance with CAP/JCAHO regulations. The use of any stored specimens for research purposes is only allowed when the appropriate IRB approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the participant was enrolled.

5.3.3 Center for Cancer Research Sequencing Facility (CCR-SF)

The Center for Cancer Research Sequencing Facility (CCR-SF), frequently referred to as the NCI Frederick Sequencing Facility, is a CCR-dedicated sequencing core facility at the Frederick National Laboratory for Cancer Research (operated by Leidos Biomedical Research, Inc. and sponsored by NCI). The Sequencing Facility processes genomics samples such as DNA or RNA from CCR investigators for genomics analysis including mutation analysis such as whole genome sequencing, transcript profiling and discovery, DNA/RNA protein interaction, exome sequencing or targeted exome sequencing and gene expression analysis.

5.3.3.1 Sequencing Facility Established Procedures of Sample Receiving and Storage

- Samples are received with a service request through the secure online request system (NAS).
- Samples must be coded by the sender to remove any PII. The Sequencing Facility will not receive any samples with PII and does not store such information within their electronic database system (LIMS).
- Samples are inventoried and stored in freezers or refrigerators which are monitored through the alarm system.
- The facility's laboratory is located within a controlled access building.
- Samples are tracked in LIMS in addition to hard copy sample logs.
- Sequencing Facility personnel receive annual updated NIH/CIT training and maintain standards of computer security.
- At the end of the project, leftover samples (if any) will be transferred back to the requestor. The Sequencing Facility does not provide long term storage.

5.3.4 Laboratory of Dr. Heller

Samples collected for the purpose of research under this protocol may undergo DNA/RNA extraction in Dr. Heller's lab after Blood Processing Core (BPC) barcoding, initial processing, and storage. Samples are stored in freezers at -80°C or -20°C according to stability requirements. Samples will be sent on for protocol research analysis as directed by the Principal Investigator.

5.3.5 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described in sections above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reports will be made per the requirements of Section **7.2.1**.

5.3.6 Machine Learning for Early Gastric Cancer Detection

Application of artificial intelligence to radiographic and histopathologic tests is being explored to improve the sensitivity and specificity of cancer diagnosis. Because the use of CEM in our previous Phase II study showed low sensitivity for occult cancer detection, we believe that application of machine learning (i.e., computer models) based on the input provided by CEM imaging, and endoscopic high-resolution video imaging as applicable, will improve our ability to accurately and reliably detect cancer. By incorporating the corresponding histopathology images/results obtained after gastric biopsy, we will essentially teach the computer how to reliably detect occult gastric cancer through an iterative process.

This machine learning for early cancer detection is ONLY being applied post-hoc as a research tool to improve endoscopic diagnosis for protocol endpoints– it will not be used in any way to diagnose or treat patients enrolled on this study.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the start of endoscopy through 14 days following study interventions.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Adverse Events related to study interventions will **only** be recorded if they are serious (all Grades) or unexpected (Grades 3 and above).

We will collect information from participants regarding consuming nutritional or dietary supplementation, antimicrobial medications, pro- or prebiotics, and/or laxative agents; this information may be recorded in the participant's medical record (in addition to the data capture system provided by the NCI CCR) as applicable.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in Section **7.2.1**.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

The PI will share coded linked human data generated in this research for future research:

- in a NIH-funded or approved public repository clinicaltrials.gov, GenBank

- in BTRIS
- in publication and/or public presentations
- with approved outside collaborators under appropriate agreements
- at the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Bacterial gene sequences will be deposited in GenBank.

6.3 Toxicity Criteria

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 Definitions

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#HRPPPolicies-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP Office of Compliance and Training / IRB Reporting

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802: Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#HRPPPolicies-800Series-ComplianceandResearchEventReportingRequirements.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#HRPPPolicies-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI Clinical Director Reporting

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to [redacted] within one business day of learning of the death.

7.4 NIH Required Data and Safety Monitoring Plan

7.4.1 Principal Investigator/Research Team

The clinical research team will meet weekly when patients are being actively enrolled on the trial to discuss each patient.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 STATISTICAL CONSIDERATIONS

8.1 Statistical Hypothesis

Primary Endpoint:

- Determine if Bethesda protocol provides improved sensitivity for detection of early-stage gastric cancer in *CDH1* germline mutation carriers compared to the Cambridge method.
- Secondary Endpoints:
- Define the false negative rate of SRCC detection using Bethesda and Cambridge methods in patients who proceed to risk-reducing total gastrectomy.
- Estimate and compare the difference in crude cancer detection rates between endoscopy using the Bethesda protocol and the Cambridge method.

8.2 Sample Size Determination

For purposes of sample size determination, it will be of interest to determine if the Bethesda protocol provides 60% sensitivity compared to 30% sensitivity for the Cambridge method. For background, results from 110 patients enrolled at the NCI (81 receiving Bethesda protocol and 29 receiving Cambridge evaluations) demonstrated the following. Of the 29 patients who received Cambridge, with 4 having SRCCs identified, 17 underwent total gastrectomy of whom 2 had SRCCs initially identified by Cambridge. Of the 17, 11 had SRCCs on endoscopic biopsy, so the sensitivity of the Cambridge method was 2/11=18%. Similarly, of the 81 patients who received Bethesda protocol, 29 were initially found to have SRCC on endoscopic biopsy. Twenty-four of the 81 underwent total gastrectomy, of whom 13 had SRCCs initially identified by endoscopic biopsy on Bethesda protocol. Of the 24, 22 had SRCCs on endoscopic biopsy, so the sensitivity of Bethesda protocol was 13/22=59%. This also means that in practice, approximately 80% of patients who underwent a gastrectomy would have SRCCs identified.

Based on these values, it is expected that it would be conservative to try to determine if Bethesda protocol would be able to result in 60% sensitivity compared to 30% sensitivity with Cambridge. With 48 patients receiving Cambridge endoscopy with positive biopsy followed by gastrectomy and 48 receiving Bethesda protocol endoscopy with positive biopsy followed by gastrectomy, on a Fisher's exact test with a 0.05 two-sided significance level we would have 80% power to detect

a difference between 30% and 60% sensitivity. In order to yield 48 patients with SRCCs detected on endoscopic biopsy, 60 patients with gastrectomy will be required.

Based on the prior experience with surveillance endoscopy, out of 110 patients, only 17+24=41 (37%) patients underwent a gastrectomy. To provide adequate patients to have a high probability of yielding 60 per arm who have a gastrectomy resulting in 48 with SRCCs, the study will randomize 175 patients to Bethesda protocol and 175 to Cambridge (350 total patients) in order to have 79% probability of obtaining 60 or more patients undergoing each procedure including gastrectomy, if the true probability of undergoing a gastrectomy is 37%. In practice, patients will continue to be randomized up to the limit of 350 total randomized patients, or until 48 patients on each arm have undergone a total gastrectomy and found to have SRCCs. Re-enrolled subjects will be counted separately at each enrollment/randomization.

All randomized patients who receive an endoscopy will have the crude cancer detection rates compared between the two methods. Data from a prior NIH trial indicated that the crude cancer detection rate was 15% using the Cambridge method and 36% using the Bethesda method, based on 26 and 109 evaluable patients respectively. In the present trial, if all 175 patients are required in each arm, then there would be 90.6% power to detect a difference with a two-sided 0.05 significance level between 15% and 30% crude cancer detection rates on the Cambridge method and the Bethesda protocol respectively. If only 135 evaluable patients undergo endoscopy using each method, there would be 81.0% power to detect a difference with a two-sided 0.05 significance level between 15% and 30% crude cancer detection rates.

Since January 2017 through September 2019, we have enrolled over 240 *CDH1* mutation patients on protocol 17-C-0043, performed over 145 surveillance endoscopies (12 performed in September 2019), over 61 risk-reducing gastrostomies (5 performed in September 2019). It is anticipated that more than 100 patients per year will be able to enroll onto this trial. Thus, accrual of up to 350 total subjects is expected to be completed in no more than 3.5 years.

8.3 POPULATIONS FOR ANALYSES

All patients who are randomized on the trial and receive an endoscopy will be used for analysis. Those who undergo gastrectomy will be included in the evaluation of the two methods relative to the biopsy proven results.

8.4 STATISTICAL ANALYSES

8.4.1 General Approach

The patients who undergo gastrectomy will have the sensitivity, and false negative rates of detection of SRCC determined, while all patients undergoing an endoscopy will be used to estimate and compare the crude cancer detection rates for the two methods.

8.4.2 Analysis of the Primary Endpoint

The primary objective of the study is to determine if Bethesda protocol provides improved sensitivity for detection of early stage gastric cancer in *CDH1* germline mutation carriers compared to the Cambridge method. Among patients who undergo gastrectomy, in each of the two arms, the fraction of patients who had SRCCs previously identified by endoscopic biopsy out of those who had SRCCs detected on final pathologic analysis of gastrectomy explants will be used to determine the sensitivity of each arm. These fractions will be reported along with a 95% confidence interval and will be compared between the two arms using a two-tailed Fisher's exact test. This analysis

will also be carried out after 24 evaluable participants on each arm have undergone a total gastrectomy and found to have SRCCs as an interim evaluation, as indicated below in Section **8.4.5**.

8.4.3 Analysis of the Secondary Endpoint

One secondary endpoint is to define the false negative rate of SRCC detection using Bethesda protocol and Cambridge methods in patients who proceed to risk-reducing total gastrectomy. Among patients who undergo the gastrectomy in each of the two arms, the fraction of patients who had SRCC identified on final pathology but were negative for SRCC on EGD will yield the false negative biopsy rate. These fractions will be reported along with a 95% confidence interval and will be compared between the two arms using a two-tailed Fisher's exact test.

The other secondary endpoint is to estimate and compare the crude cancer detection rates by the two methods. The fractions of SRCCs which are found on endoscopy by the two methods will be reported along with a 95% confidence interval, and the difference in the fractions will be compared using a two-tailed Fisher's exact test.

8.4.4 Baseline Descriptive Statistics

Baseline demographic characteristics will be reported overall and by arm.

8.4.5 Interim Analyses

Using the Haybittle-Peto approach, after 24 evaluable participants on each arm have undergone a total gastrectomy and found to have SRCCs, an interim evaluation will be performed. If at this interim point, the difference in the fractions of participants on the two arms with respect to the primary sensitivity endpoint is statistically different with $p < 0.001$, then the trial will stop accrual as soon as this can be determined, and the results will be reported based on these participants. As a result of selecting the very small 0.001 threshold, if the interim analysis does not find a difference at this level, the trial may continue to enroll participants until the intended 48 participants per arm, and the final evaluation will take place using the 0.05 significance level threshold (refer to Section **8.2**).

8.4.6 Analysis of the Exploratory Endpoints

- Determine if confocal endoscopic microscopy (CEM) will afford greater sensitivity for detection of SRC foci in *CDH1* germline mutation carriers compared to the Cambridge method
- To characterize gastric tract microbiota of *CDH1* germline mutation carriers.
- To access esophageal motility after total gastrectomy (in patients who pursue total gastrectomy)
- Apply machine learning for early gastric cancer detection via CEM and histopathology, and utilize image-to-image translation to enhance early cancer detection with CEM

The exploratory objectives are intended to collect data for use in planning future scientific investigations or clinical research. These analyses are expected to be performed primarily using descriptive techniques, reporting descriptive statistics including confidence intervals when appropriate. If any statistical tests are performed for evaluation of exploratory objectives, they will be done without formal adjustment for multiple comparisons, but in the context of the number of tests performed. We will tabulate separately by patient the crude cancer detection results for those

patients who undergo two procedures, and as an exploratory analysis, compare the results within patients and summarize results across patients using descriptive analyses.

9 HUMAN SUBJECTS PROTECTIONS

9.1 RATIONALE FOR SUBJECT SELECTION

Subjects from all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. Efforts will be made to extend accrual to a representative population.

9.2 PARTICIPATION OF CHILDREN

Children are excluded from this study because endoscopic screening for *CDH1* mutation carriers is not recommended in children.

9.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol.

9.4 RISK/BENEFIT ASSESSMENT FOR ALL PARTICIPANTS

9.4.1 Known Potential Risks

9.4.1.1 Risks of Upper Endoscopy

The risks of upper endoscopy include, but are not limited to, temporary throat soreness, gastrointestinal bleeding, and rarely gastrointestinal perforation.

9.4.1.2 Risks of Fluorescein Intravenous Administration

The risks of intravenous administration of the contrast agent fluorescein are: nausea, vomiting, dizziness, headache, and low blood pressure.

9.4.1.3 Risks of General Anesthesia

The risks of general anesthesia include, but are not limited to, temporary confusion and memory loss, dizziness, difficulty passing urine, bruising or soreness from the IV drip, nausea and vomiting, shivering and feeling cold, sore throat, heart attack, pneumonia and stroke.

9.4.1.4 Risk of Biopsies

All care will be taken to minimize risks that may be incurred by tumor sampling. Biopsies will be taken during endoscopy. All procedure-related risks (such as bleeding, infection and visceral injury) will be explained fully during informed consent.

9.4.1.5 Risks of Esophageal Manometry

The risks of esophageal manometry include discomfort from passing a plastic tube through the nostril and possible bleeding.

9.4.1.6 Risks of Oropharyngeal Swabs and Salvia Collection

There are no risks or complications associated with an oropharyngeal swab (cheek cells) or saliva collection. The oropharyngeal swab test may cause momentary gagging because the back of the throat is a sensitive area, but it shouldn’t be painful.

9.4.1.7 Risk of Losing Data

This includes the risk that data obtained during this study that can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release

results to the patients, family members or health care providers, this risk will be included in the informed consent document

9.4.1.8 Risks from Questionnaires

Questionnaires may contain questions that are sensitive in nature. The patients are asked to only answer questions they are comfortable with.

9.4.2 Known Potential Benefits

The benefit is detection of gastric cancer at its earliest stage so that it is potentially curable.

9.4.3 Assessment of Potential Risks and Benefits

A number of clinically appropriate strategies to minimize risks to patients have been built into the protocol through the means of inclusion/exclusion criteria, monitoring strategies, and management guidelines. Overall, the potential benefit of the study interventions in subjects with *CDH1* germline mutations outweigh the risks associated with study interventions.

The potential benefit to a patient that participates in this study is detection of gastric cancer at its earliest stage so that it is potentially curable.

Potential adverse reactions attributable to the study interventions are discussed in Section **9.4.1**. All care will be taken to minimize side effects, but they can be unpredictable in nature and severity.

9.5 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (Non-Electronic) Signature on Electronic Document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,

- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location, but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB) and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about protocol compliance, and data quality are addressed, and satisfy the IRB.

10.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe the site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to source data/documents, and reports for the purpose of monitoring and inspection by local and regulatory authorities.

10.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute (NCI) has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, and their staff. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval.

All research activities will be conducted in as private a setting as possible.

The study monitor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB or Institutional policies.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

11 DEVICE INFORMATION

The NIH IRB has determined that the use of the devices in this study are not investigational and are not the object of the protocol's investigation; therefore, per NIH IRB's guidance, no regulatory device determination language has been included within the protocol.

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13 APPENDICES

13.1 APPENDIX 1: INSTRUCTIONS FOR COMPLETING THE AUTOMATED SELF-ADMINISTERED 24-HOUR DIETARY ASSESSMENT TOOL (ASA24) FOR 24-HOUR FOOD RECALL

See Study Instruments Packet

Citation:

National Cancer Institute, Division of Cancer Control and Population Studies. *Participant Quick Start Guide for 24-Hour Recall using ASA24.* https://epi.grants.cancer.gov/asa24/resources/asa24-quick-start-guide-24hr-recall-06062022.pdf. Published June 6, 2022. Accessed July 24, 2023.

13.2 APPENDIX 2: INSTRUCTIONS FOR COMPLETING THE DIET HISTORY QUESTIONNAIRE III (DHQIII)

See Study Instruments Packet

Citation:

National Cancer Institute, Division of Cancer Control and Population Studies. *Diet History Questionnaire III (DHQ III)*. https://epi.grants.cancer.gov/dhq3/. Publication updated March 15, 2023. Accessed July 24, 2024.

13.3 APPENDIX 3: STOOL SPECIMEN COLLECTION KIT INSTRUCTIONS

See Study Instruments Packet