Abbreviated Title: CEM in subjects with HDGC

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Title: Phase II Study Evaluating Confocal Endoscopic Microscopy for Detection of Early Stage Gastric Cancer in Subjects with Hereditary Diffuse Gastric Cancer Syndrome

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Commercial Devices:

- Cellvizio® 100 series systems with confocal miniprobes™ (Mauna Kea Technologies)
- Olympus GIF 180 endoscope (Global Endoscopy Solutions)

PRÉCIS

Background:

- Hereditary Diffuse Gastric Cancer (HDGC) syndrome is caused by a germline mutation in the CDH1 gene. Carriers of this mutation have a 56-70% lifetime risk of developing gastric adenocarcinoma. Current international guidelines recommend endoscopic screening of CDH1 mutation carriers that consists of systematic biopsies of an otherwise normal appearing stomach. However, this approach lacks sufficient sensitivity for detecting intramucosal foci of signet ring cells (SRC), which are pathognomonic of HDGC syndrome. The goal of the current study is to utilize confocal endoscopic microscopy (CEM) for screening the gastric mucosa in this high-risk population.

Objective:

- Determine if confocal endoscopic microscopy (CEM) will afford greater sensitivity for detection of SRC foci in CDH1 germline mutation carriers.

Eligibility:

- CDH1 germline mutation carriers, or those who meet clinical criteria for HDGC testing but have tested negative for a CDH1 gene mutation or those who have other germline mutations suspected to be, or reported to be, associated with HDGC (e.g. CTNNA1).

Design:

- Phase II, single-institution study of CEM for detection of intramucosal SRC foci compared to current systematic gastric mapping procedure.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- Determine if confocal endoscopic microscopy (CEM) will afford greater sensitivity for detection of SRC foci in CDH1 germline mutation carriers compared to the current method.

1.1.2 Secondary Objective

- Define the false negative rate of CEM detection of SRC foci in those patients who choose to undergo prophylactic total gastrectomy with permanent pathologic analysis.

1.2 BACKGROUND AND RATIONALE

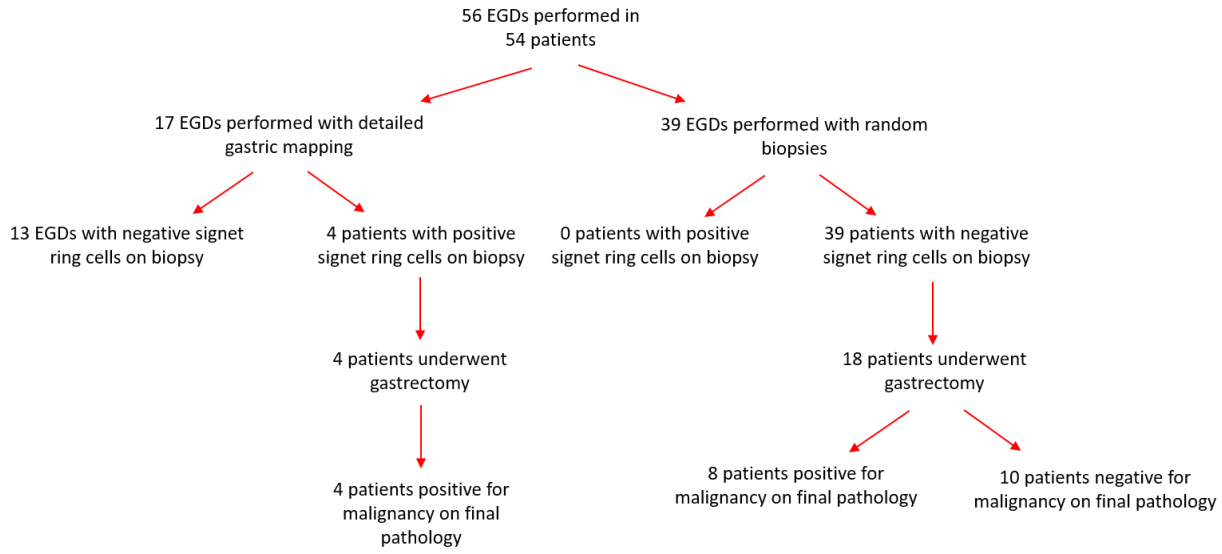
Although gastric adenocarcinoma represents 1.7% of all new cancer cases in the United States it is the fifth most common cancer worldwide.[1, 2] Environmental influences such as H. pylori infection, tobacco smoking, and dietary factors are common risk factors for developing gastric cancer. Hereditary causes account for 1-3% of gastric cancer cases globally. The three main heritable forms of gastric cancer are hereditary diffuse gastric cancer (HDGC) syndrome, gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS), and familial intestinal gastric cancer (FIGC). The commonest example of inherited gastric cancer, HDGC, imparts a 56-70% lifetime risk of developing diffuse gastric cancer, and a 42% risk of developing lobular breast cancer in female carriers.[3, 4] The five-year overall survival rate for patients diagnosed with invasive gastric cancer in the United States is 30%.

HDGC is an autosomal dominant syndrome that is due to a mutation in the CDH1 gene.[4] The CDH1 gene encodes the glycoprotein E-cadherin, which is located on the surface of epithelial cells at the adherens junctions. It plays a crucial role in cell to cell adhesion and communication through homotypic interactions via the extracellular domain. Reduced expression of E-cadherin, often via mutation of the CDH1 gene, interferes with the integrity of the adherens junctions and loss of epithelial architecture, cell polarity, and cell-cell adhesion. Cancer cells with metastatic potential often demonstrate a loss of E-cadherin-mediated cell adhesion. This is how the CDH1 gene came to be known as a tumor suppressor gene.[5] There are more than 100 known pathogenic germline mutations of the CDH1 gene.[6]

For patients diagnosed with a pathogenic germline CDH1 mutation, a risk-reducing total gastrectomy is recommended. This is because mutation carriers are often found to have occult, early-stage (T1a) gastric cancers not visible on routine upper endoscopy. Risk-reducing total gastrectomy is recommended after the age of 20 or 5 years earlier than the age of diagnosis of the youngest affected family member. In addition, initial upper endoscopic gastric screening is recommended in all patients diagnosed with CDH1 mutation. For patients that choose not to proceed with a risk-reducing gastrectomy, annual endoscopic surveillance is currently recommended.[4, 7] Endoscopy should be done under white light with a high-definition endoscope. The International Gastric Cancer Linkage Consortium (IGCLC) recommendation is for six biopsies from each anatomical zone of the stomach (antrum, transitional zone, body, fundus, and cardia) and any visible lesion.[8] If any of the biopsies show signet ring cells on pathology then the patient is advised to undergo a therapeutic total gastrectomy.

Total gastrectomy to reduce gastric cancer risk remains a strong recommendation not only because of the lethality of invasive gastric cancer, but also because cancer screening with endoscopy is ineffective for detecting early, occult gastric cancers. In previously published series of HDGC syndrome patients, signet ring cell foci are reported to make up less than 2% of the gastric mucosa and are often less than 1 mm in diameter.[9] Gastric adenocarcinomas in HDGC syndrome patients are characterized by diffuse-type histology of signet ring cells, often with multiple foci throughout the gastric mucosa. Endoscopy rarely shows gross abnormalities or tumor masses, even though random biopsies have been known to demonstrate intramucosal foci of signet ring cells. Alternative visual enhancement with endoscopy has also been studied, including narrow band imaging, chromoendoscopy, and utilization of Congo red and indigo-carmine dyes.[10, 11] None of these enhancements has led to significantly increased rates of detection. Thus, better screening modalities are needed.

Our group has substantial experience evaluating HDGC syndrome patients with data that support the inadequacy of current endoscopic surveillance methods. As of November 2017, we evaluated 54 patients with predisposition to gastric malignancy at the NIH. Of the 54 patients evaluated, 40 (74%) had a primary genetic abnormality predisposing to gastric malignancy with the remainder having strong family history. All patients were Caucasian, with a majority being female (66%) and a mean age of 44 years. Subjects were screened either through standard upper endoscopy (EGD) with a combination of random and targeted biopsies, or later through a systematic gastric mapping protocol developed by Yao.[12] Of the 56 EGDs performed, 17 (30%) were performed via detailed gastric mapping with resultant 88 individual biopsies in 22 distinct locations in the stomach. Of the remaining 39 (70%) standard EGDs, targeted biopsies of endoscopic abnormalities and random biopsies from the antrum and body were performed. In subjects undergoing systematic gastric mapping, signet ring cells were identified in 4 patients (24%), whereas all 39 standard EGD biopsies were absent of signet ring cells (0%). With regard to long-term follow up, in 22 patients that subsequently underwent prophylactic total gastrectomy, malignancy was identified by histology in 12 (55%) of cases. In the 4 subjects that underwent systematic gastric mapping followed by total gastrectomy, biopsies obtained during gastric mapping matched gastrectomy specimen pathology in all cases (100%). Comparatively, in the 18 subjects that underwent standard endoscopy and gastrectomy, 8 cases of malignancy were identified on gastrectomy specimen pathology (44%). See flowchart below. These preliminary results suggest that while comprehensive gastric mapping biopsies may be superior at detecting early malignancy when compared to standard EGD, the overall detection rate remains too low to recommend surveillance over prophylactic total gastrectomy with confidence.



Similarly, a retrospective study of 23 patients with a CDH1 mutation also demonstrated that EGD is not an adequate screening modality. Of the 23 patients, only 2 were found to have SRC foci on EGD with biopsy. All 23 patients underwent a prophylactic total gastrectomy. On final pathology, 22/23 patients were found to have foci of SRCs. This means that screening with EGD only had a detection rate of 9%.[13]

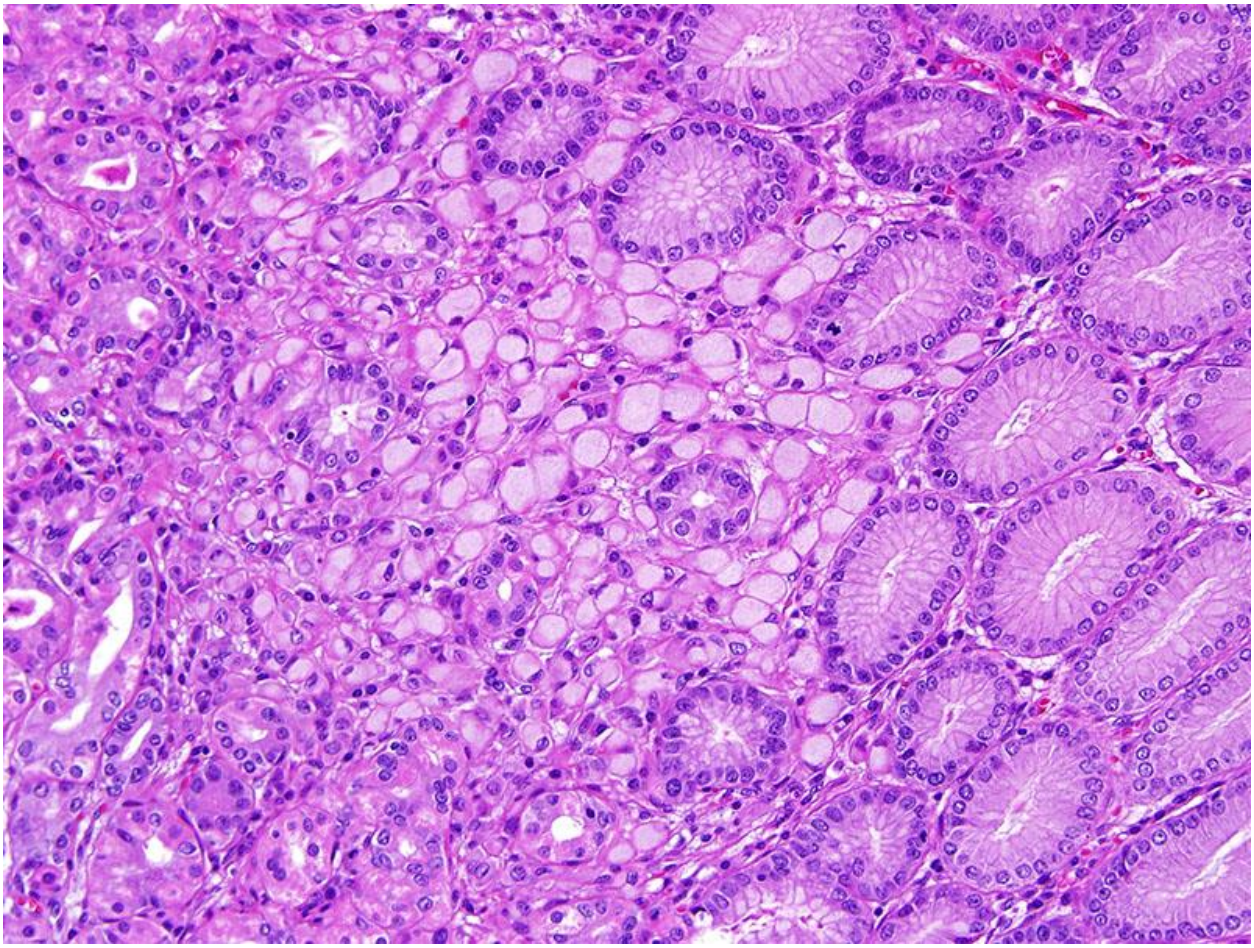
1.2.1 Rationale for Confocal Endoscopic Microscopy

Confocal endoscopic microscopy (CEM) allows physicians to obtain in vivo histology images of the gastrointestinal mucosa. CEM consists of using the tissue's own fluorescent ability or the aid of an IV contrast agent. Contrast will highlight the vasculature, lamina propria, and intracellular spaces allowing for the architecture of each cell to be visualized.[14] A fiber optic bundle with an integrated distal lens is passed through the endoscope channel and connected to a laser scanning unit. This system has a fixed focal length so it scans in only one plane. The GastroFlex probe requires a ≥ 2.8 mm channel. It has a field of view of 250 micrometers, resolution of 1 micrometer, and confocal depth of 55 – 65 micrometers. Each probe can be used on 20 patients. The system is compatible with a standard endoscope. The advantage of a confocal laser microscope is that it is efficient at eliminating out of focus fluorescent light and creating a clear image from a sample. Based on multiple Japanese reports, cancer cells can be distinguished from those of normal mucosa by irregular nuclear size and shape. CEM correlated with H&E stained images.[15] Kitabatake and colleagues used CEM in vivo and found that the confocal images of gastric cancer differed from normal mucosa, which was confirmed with H&E staining. This study also found variability in gastric mucosa affected image capture, but that application of oral pronase and sodium hydrogen carbonate as pretreatment could reduce that variability.[16]

CEM has been used for gastric cancer screening and in a variety of other gastrointestinal neoplastic diseases. Specifically, CEM has been used in patients with intestinal metaplasia of the gastric epithelium and for detection of very early stage gastric cancers in Asian patients. One of the largest studies published to date, the sensitivity and specific of CEM was 89% and 99%, respectively, in detecting superficial cancers and high-grade intraepithelial neoplasia. To the best

of our knowledge, the proposed study is the first time that CEM will be used as a screening modality in patients with pathogenic germline CDH1 mutations.

Most patients with a CDH1 mutation develop multifocal, occult tumors throughout the gastric mucosa with the ability for further invasion and metastatic spread.[17] Since patients already have a CDH1 mutation in one allele, they develop these islands of microscopic signet ring cells due to inactivation of the second allele. Studies have demonstrated that the remaining CDH1 wild type allele may become inactivated through hypermethylation of the CDH1 promoter.[18-20] However, another study found frequently more than one inactivating mechanism of the second allele within the same patient.[21] The H&E photomicrograph below is from a surgical gastrectomy specimen and demonstrates signet ring cells (black arrow) within the lamina propria of the gastric mucosa of a patient with a pathogenic CDH1 mutation.



Aside from loss of CDH1 gene expression in HDGC patients with early-stage, signet ring cell cancers, little is known about subsequent genomic (or epigenomic) events that lead to gastric cancer growth, invasion or metastasis. However, compelling in vitro and pre-clinical data suggest specific additional molecular alterations required for cancer progression beyond the intra-mucosal signet ring cell stage. Humar and colleagues have proposed activation of *c-Src* and its downstream targets correlates well with cancer progression and the differentiated cancer cell phenotype in early HDGC.[22] *c-Src* is known to elevate the expression of β-catenin and activation of the *Wnt* pathway in the epithelial-to-mesenchymal transition cell phenotype.[23]

Another potential molecular driver of diffuse-type gastric cancers includes gain of function RHOA gene mutations that have been described in multiple genomic analyses.[24, 25] Gene expression analysis of gastric signet ring cells from CDH1 patients will likely provide insight into the stepwise progression of gastric cancer. Elucidating the genomic hits necessary for gastric cancer progression and metastasis may demonstrate potential therapeutic targets.

In this study we are going to use Cellvizio® 100 Series systems with Confocal Miniprobes™. These are confocal laser systems with fiber optic probes, approved by FDA, that are intended to allow imaging of the internal microstructure of tissues including, but not limited to, the identification of cells and vessels and their organization or architecture. Prior to patient enrollment in this study, the principal investigator and lead associate investigators have evaluated the CEM probe used in a clinical setting. In addition, *ex vivo* application of the CEM probe to gastric tissue is planned for training of associate investigators. Since many patients undergo total gastrectomy at the NIH Clinical Center, the CEM probe can be used to scan the mucosa of explanted gastrectomy specimens. While this CEM training is not part of the study, it is designed to ensure proper calling of normal and abnormal mucosal images obtained by the CEM probe. There are no inherent risks to the use of the CEM probe per se. Rather, standard training in upper endoscopy should be requisite for use of the CEM probe.

The goal of this study is to determine if CEM will afford a higher level of sensitivity for detection of signet ring cell cancers in CDH1 germline mutation carriers. This technology will be compared to traditional endoscopic surveillance and gastric mapping (consisting of multiple biopsies from each area of the stomach) for patients with a pathogenic CDH1 germline mutation. It is hoped that this technique may lead to fewer gastric biopsies required and a more sensitive way to screen for SRC in patients with CDH1 mutation.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

- Patients with CDH1 germline mutation known to be pathogenic or likely pathogenic, which may also be classified as “significant” or “likely significant” (patients with variants of “uncertain significance” are excluded).

or

- Patients with CTNNA1 and PALB2 germline mutations suspected to be, or reported to be, associated with HDGC.

or

- In the absence of a germline CDH1 mutation, patients must meet clinical criteria for genetic testing due to a history suggestive of Hereditary Diffuse Gastric Cancer (HDGC) syndrome (**Appendix A**).
- Age ≥18 years.
- Physiologically able to undergo upper endoscopy.
- Ability to understand and the willingness to sign a written informed consent document.

- Pregnant women are eligible during second trimester of pregnancy if clinically indicated for evaluation of cancer.

2.1.2 Exclusion Criteria

- Current use of therapeutic anticoagulation medication.
- Known bleeding disorder or thrombocytopenia.
- Unstable angina or recent (within 3 months) myocardial infarction.
- Any clinical contraindication to general anesthesia.

2.1.3 Recruitment Strategies

This study will be posted on NIH websites, NIH social media forums, the CCR website and on clinicaltrials.gov. 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

Within 28 days prior to enrollment unless otherwise noted below:

- Complete medical history and physical examination, including vital signs.
- Review of genetic test results performed in CLIA-approved lab.

2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.3.1 Intervention Assignment Procedures (For Registration Purposes Only):

Cohorts

Number	Name	Description
1	Cohort 1	Subjects with CTNNA1, PalB2 or known to be pathogenic or likely pathogenic CDH1 germline mutations, or those who test negative for these mutations, but meet clinical criteria for HDGC.

Arms

Number	Name	Description
1	Arm 1	Upper white-light endoscopy and confocal endoscopic microscopy

Arm Assignment

Subjects in Cohort 1 will be directly assigned to Arm 1.

2.4 BASELINE EVALUATION

Within 28 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 single arm study designed to evaluate the efficacy of CEM to detect signet ring cell foci within the gastric mucosa of patients with hereditary diffuse gastric cancer syndrome.

At the time of endoscopy, all patients will undergo white-light, upper endoscopy with systematic gastric mapping according to our current practice pattern and as outlined by IGCLC [12] (Section **3.2.2**). In addition, during this endoscopy patients will undergo CEM using the Cellvizio probe (Mauna Kea Technologies) to scan the same anatomic zones as evaluated in the systematic gastric mapping approach (Section **3.2.3**). Any abnormal areas visualized with the CEM probe will be biopsied. Patients are informed of the risks of endoscopy such as gastrointestinal bleeding and perforation. The standard gastric mapping requires approximately 30 minutes of general anesthesia time. The addition of CEM adds another 30 minutes of general anesthesia but no increase in risk of general complications such as bleeding or perforation.

All biopsies are sent to Laboratory of Pathology (see Section **5.1.1**). In patients co-enrolled to protocol 13C0176, leftover biopsy samples will be used for research as described in 13C0176.

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

At the time of endoscopy, all patients will undergo white-light, upper endoscopy with systematic gastric mapping according to IGCLC guidelines.[8] The anatomic zones are outlined below. From each of the five zones, 6 cold forceps biopsies and photos will be obtained. The total number of biopsies will be thirty. Any visibly abnormal areas will also biopsied.

1. Antrum
2. Transitional Zone (antrum-body)
3. Body
4. Fundus

5. Cardia

3.2.3 CEM Analysis

Patients will also undergo CEM analysis of gastric zones using the Cellvizio probe (Mauna Kea Technologies). Abnormal mucosa visualized will have images digitally captured and biopsied.

- Antrum (antegrade view): 4 quadrants (Anterior [A], Lesser curve [L], Posterior [P], Greater curve [G])
- Lower body (antegrade view): 4 quadrants [A, L, P, G]
- Middle-upper body (antegrade view): 4 quadrants [A, L, P, G]
- Fundus-cardia (retroflex view): 4 quadrants [A, L, P, G]
- Middle-upper body (retroflex view): 3 quadrants [A, L, P]
- Incisura (retroflex view): 3 quadrants [A, L, P]

Patients will receive the contrast agent fluorescein prior to endoscopy if no known allergy to fluorescein is documented. Patients who cannot receive fluorescein (e.g. pregnancy or allergy) will have CEM performed in the absence of this contrast agent.

3.2.4 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).

3.2.5 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 from afar then they will spend the night in the hospital after the procedure prior to going discharge.

3.2.6 Post-discharge/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 AEs on the phone.
- If patient elects to undergo gastrectomy, we will request stomach tissue to perform pathology assessment.

3.3 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.

3.3.1 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

3.3.2 Off-Study Criteria

- Investigator discretion
- Death
- Participant requests to be withdrawn from study
- Loss of follow up
- PI decision to close the study

3.3.3 Off Protocol Intervention and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol-intervention and when a subject is taken off-study. A Participant Status Update Form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov.

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 BIOSPECIMEN COLLECTION

Standard pathology evaluation of stomach tissue from biopsies and/or gastrectomy will be performed in the Laboratory of Pathology (see Section **5.1.1**).

Any leftover tissues, and gastric lavage fluid, collected for research, will be processed and stored on protocol 13C0176 if subject is co-enrolled on that study.

5.1 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS Screens 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 NIH without IRB notification and an executed MTA.

5.1.1 Procedures for Storage of Tissue Specimens in the 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 tissue that is not embedded in paraffin is stored in formalin 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 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 and subjects have been co-enrolled on protocol 13C0176. In some cases, this approval has been obtained via the original protocol on which the patient was enrolled.

5.1.2 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**.

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6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (Labmatrix) 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. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. 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 study procedure (see Section **3.2**) 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 endoscopy will **only** be recorded if they are serious (all Grades) or unexpected (Grades 3 and above).

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

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in another public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center).
- Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- An NIH-funded or approved public repository, clinicaltrials.gov.
- BTRIS (automatic for activities in the Clinical Center).
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

No genomic data will be collected.

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 here.

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 here.

- Reports referenced in Policy 801 are made to the IC Specific IRB rather than to the OHSRP Office of Compliance and Training.
- Section 5.2 of the Policy 801 does not apply.
- Policy 802 does not apply.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI Clinical Director Reporting

Problems expeditiously reported to the OHSRP/IRB in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

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

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov 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 on a regular basis (weekly) when patients are being actively treated 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 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 confocal endoscopic microscopy (CEM) affords greater sensitivity for detection of SRC foci in CDH1 germline mutation carriers compared to the current method of standard white light endoscopy.

Secondary Endpoint:

- Define the false negative rate of CEM detection of SRC foci in those patients who choose to undergo prophylactic total gastrectomy with permanent pathologic analysis.

8.2 SAMPLE SIZE DETERMINATION

Subjects who are carriers of a CDH1 mutation and participate in this trial will undergo an EGD and detailed gastric mapping along with confocal laser microscopy. Based on the historical data from NIH, 17 patients who were CDH1 mutation carriers and who underwent an EGD and detailed gastric mapping resulted in 4 with signet ring foci detected. All 4 of these patients eventually were found to have gastric cancer after undergoing a prophylactic gastrectomy. An additional 18 patients who had prophylactic gastrectomy out of 39 who had conventional random biopsies yielded 8 patients with gastric cancer.

Based on the current historical rate of 4/17 (23.5%) of mapped patients who had signet ring foci detected, it would be desirable to see if use of confocal laser microscopy could increase this detection rate substantially. With 36 patients undergoing EGD with detailed gastric mapping and confocal laser microscopy, there would be 89% power to rule out a 24% rate of signet ring detection in favor of a rate of detection consistent with 45%, with a one-sided 0.10 significance level exact binomial test. In practice, if 36 patients would undergo the procedure described and if 13/36 (36.1%) would have signet ring foci identified, then the lower one-sided 90% confidence bound on 13/36 is 25.3%, which would demonstrate statistically greater detection in the subjects tested compared to the historical fraction of 4/17 (23.5%). In addition, the upper one-sided 90% confidence bound on 13/36 is 48.2%, which demonstrates that 13/36 could be shown to be consistent with as great as a 45% detection rate.

It is expected that 18-20 patients may enroll on this study per year. Thus, to enroll 36 evaluable patients, approximately 24 months is the expected accrual period. To allow for a small number of inevaluable patients, the accrual ceiling will be set at 40 patients.

8.3 POPULATIONS FOR ANALYSES

All patients who undergo an EGD and gastric mapping along with confocal endoscopic microscopy.

8.4 STATISTICAL ANALYSES

General Approach

The fraction of patients who undergo the EGD and detailed mapping along with confocal laser microscopy and have signet ring foci identified will be reported.

8.4.1 Analysis of the Primary Endpoints

The fraction of patients who undergo the EGD and detailed mapping along with confocal endoscopic microscopy and have signet ring cell foci identified will be reported along with 80% and 95% two-sided confidence intervals.

8.4.2 Analysis of the Secondary Endpoint(s)

In those patients who choose to undergo prophylactic total gastrectomy with permanent pathologic analysis, the false negative rate, that is, the fraction of patients who have SRC foci

which were not identified by CEM, will be determined and reported along with 80% and 95% two-sided confidence intervals.

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. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **9.5**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

9.4 PARTICIPATION OF PREGNANT WOMEN

Pregnant women with CDH1 germline mutations are at the same risk for developing gastric cancer than non-pregnant women. For this reason and because there is a prospect of direct benefit from research participation (Section **9.5.3**) pregnant women will be included in this study. In addition, some women who are pregnant may require endoscopic screening depending on the timing of diagnosis or the presence of gastrointestinal symptoms. If an endoscopy is indicated, and the patient wishes to be enrolled, there is no reason that they should be excluded from the protocol. Women undergo invasive abdominal and thoracic surgery if needed during pregnancy. Pregnant women will not receive the intravenous contrast agent, fluorescein, if they are enrolled.

9.5 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

These evaluations apply to all participants. All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve risks to patients, which are currently unforeseeable.

9.5.1 Risks for All Participants Including Subjects Unable to Consent

Risks of Upper Endoscopy

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

Risks of Fluorescein Intravenous Administration

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

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.

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.

9.5.2 Benefits for All Participants Including Subjects Unable to Consent

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

9.5.3 Risk/Benefit Analysis for Pregnant Women

The benefit is detection of gastric cancer at its earliest stage so that it is potentially curable. We will be following the anesthesia guidelines for pregnant women, i.e., waiting until the second trimester. All described risks described in Section **9.5.1** apply for pregnant women and there are also additional risks, including fetal loss, premature labor, and delivery.

9.6 CONSENT PROCESS AND DOCUMENTATION

The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and benefits, and potential alternative therapies will be carefully explained to the patient, and a signed informed consent document will be obtained by a study investigator prior to entry onto the study.

The PI or associate investigator will meet with the patient to discuss the protocol treatment and alternative options in detail. It will be stated clearly that participation in the research study is voluntary and that participants can withdraw from the study without losing benefits they would otherwise be entitled to. The patient will be encouraged to ask questions, and additional meetings to discuss the treatment options will be arranged as necessary.

9.6.1 Telephone Consent

The informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. The original informed consent document

will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records.

The informed consent process will be documented in the medical record.

10 DEVICE INFORMATION

There will be no IDE obtained for the use of Cellvizio® 100 Series systems with Confocal Miniprobes™ and Olympus GIF 180 endoscope in this study.

The devices used on this study, the Cellvizio® 100 Series systems with Confocal Miniprobes™ and Olympus GIF 180 endoscope, meet the requirements for exemption under 21 CFR 812.2.C.2, "A device, other than a transitional device, introduced into commercial distribution on or after May 28, 1976, that FDA has determined to be substantially equivalent to a device in commercial distribution immediately before May 28, 1976, and that is used or investigated in accordance with the indications in the labeling FDA reviewed under subpart E of part 807 in determining substantial equivalence."

10.1 SOURCE

Cellvizio® 100 Series systems with Confocal Miniprobes™ will be purchased from Mauna Kea Technologies and Olympus GIF 180 endoscope will be purchased from Global Endoscopy Solutions.

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12 APPENDICES

12.1 APPENDIX A: CLINICAL CRITERIA FOR GENETIC TESTING DUE TO A HISTORY SUGGESTIVE OF HEREDITARY DIFFUSE GASTRIC CANCER (HDGC) SYNDROME

Criteria	Definition
1	Family with 2 or more cases of GC regardless of age, with at least one confirmed DGC
2	One case of DGC at age < 40 years
3	Personal or family history of both DGC and LBC, with one diagnosed at age < 50 years
Expanded criteria	
	Bilateral LBC or family history of 2 or more cases of LBC at age < 50 years
	A personal or family history of cleft lip/palate in a patient with DGC
	In situ signet ring cells and/or pagetoid spread of signet ring cells on stomach biopsy
GC, gastric cancer; DGC, diffuse gastric cancer; LBC, lobular breast cancer	