Protocol for:
VICC GI1527: Targeted chemoprevention of gastric carcinogenesis in high risk populations

Co-Principal Investigator, Contact PI:
Keith Wilson, MD

Co-Principal Investigator, Clinical Trial oversight:
Douglas Morgan, MD, MPH

Study Scientific Oversight:
Division of Gastroenterology, Hepatology, and Nutrition
[redacted]

Study Coordination Center:
Vanderbilt Ingram Cancer Center (VICC)
Clinical Trials Shared Resource (CTSR)
[redacted]

Study Centers:
- **Ministry of Health, Hospital de Occidente, Copán, Honduras, Central America**
  Dr. Ricardo Dominguez
- **University of Puerto Rico (UPR) Comprehensive Cáncer Center**
  Dr. Marcia Cruz Correa

[redacted]

Study Agent: Eflornithine (DFMO)

Current Date and Protocol version number:
2022 April 28, Version 6

Vanderbilt IRB designation: 141521 (Study umbrella)
Vanderbilt IRB designation: 150411 (Clinical trial)

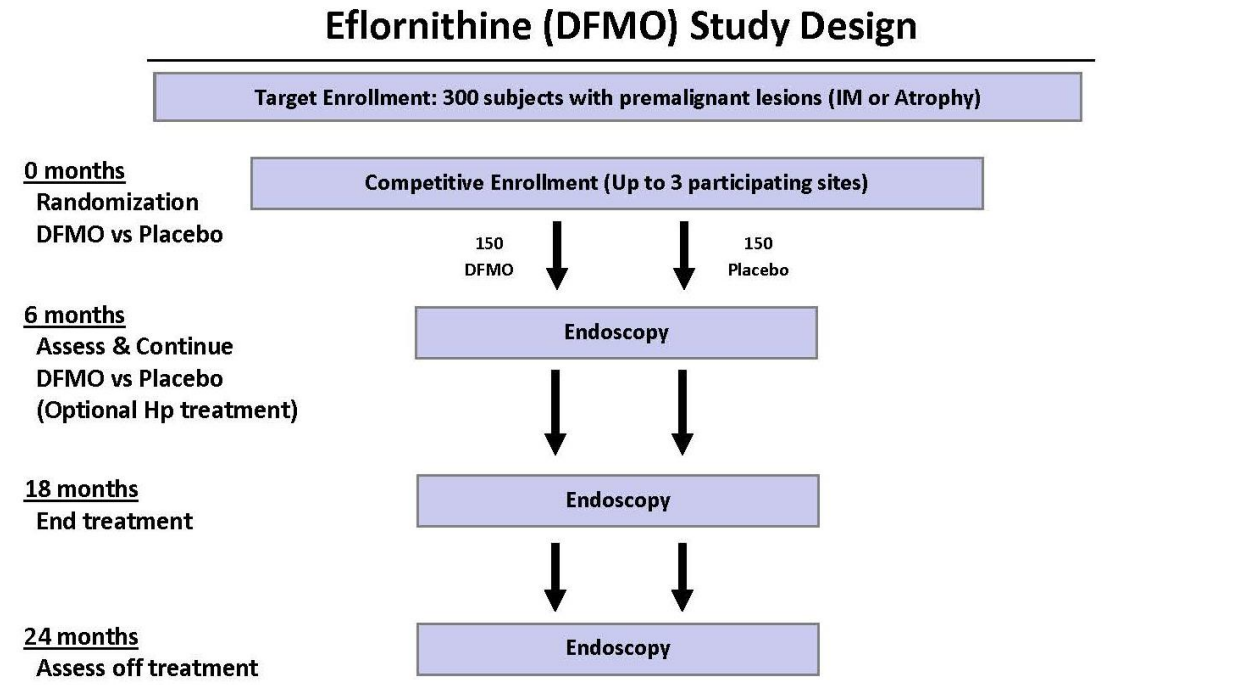
IND number: 140718

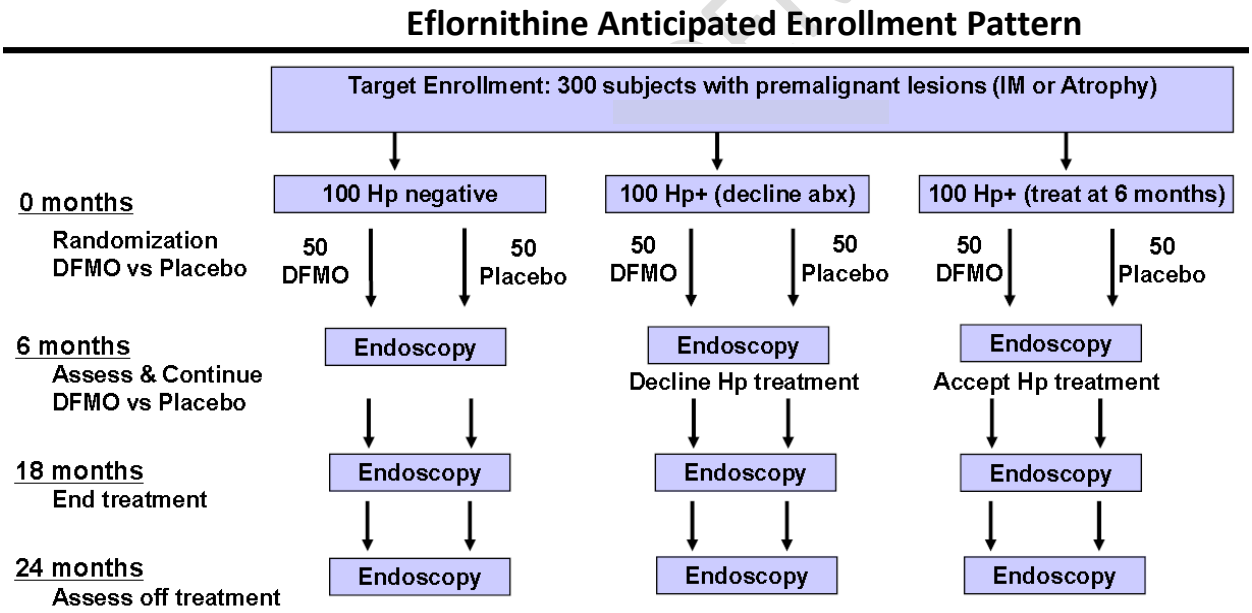
NCT number: NCT02794428

TABLE OF CONTENTS

Contents

STUDY SCHEMA





Notes:
Please refer to Section 6.0 (Stratification Factors) for further details of the Study Schema.

Abbreviations:
DFMO, α-difluoromethylornithine (eflornithine)
IM, gastric intestinal metaplasia
Atrophy, multifocal atrophic gastritis (MAG)
Hp, *H. pylori*, *Helicobacter pylori* (Hp+, *H. pylori* positive)
Abx, antibiotic treatment for *H. pylori* eradication

1.0 OBJECTIVES

Overview of Study Design

This is a clinical study of the efficacy of oral alpha-difluoromethylornithine (eflornithine or DFMO) in male and female subjects ages 30-69 with gastric premalignant lesions in high risk regions of Latin America. The primary intervention is the randomized, double-blind assignment of patients to once daily eflornithine (500 mg) versus placebo for an 18 month treatment period. The primary endpoint is gastric epithelial cell DNA damage, measured at the 6 month time point, assessed by percent positive cells in each patient, measured by gamma H2AX immunohistochemistry (IHC). Gastric precancerous lesions are defined as chronic atrophic gastritis (CAG) and intestinal metaplasia (IM). Patients will be clinically assessed with endoscopy and gastric biopsy at four time points: 0, 6, 18, and 24 months the assessments at 0 and 24 months are considered part of usual clinical care in subjects with precancerous lesions in high risk regions. Overall, the efficacy of eflornithine is assessed by its effect on: 1) DNA damage, 2) histology scoring, and 3) gastric polyamine levels.

Primary Objective

1.1 The difference in cell DNA damage between patients treated with DFMO and patients treated with placebo at 6 months. The cell DNA damage is measured using the percent positive gastric epithelial cells assessed by IHC for gamma H2AX. The mean difference between the two groups at 6 months will be calculated, accounting for their baseline measurements.

Secondary Objectives

1.2 The difference in cell DNA damage between patients treated with DFMO and patients treated with placebo for 18 months, and then followed for an additional 6 months. The cell DNA damage is measured using the percent positive gastric epithelial cells assessed by IHC for gamma H2AX. The mean difference between the two groups at 18 and 24 months will be calculated, accounting for their baseline measurements.

1.3 The differences in the gastritis histopathology score between patients treated with DFMO and patients treated with placebo for a total of 18 months, and followed for an additional 6 months. The gastritis histopathology score is measured with a quantitative scale 0.0-6.0, for atrophy, intestinal metaplasia, and dysplasia. The mean differences between the two groups at 6, 18, and 24 months will be calculated using mixed models, accounting for their baseline measurements.

1.4 Number of patients with quantitative toxicities. Toxicities will be assessed per CTCAE criteria, and each toxicity will be assigned an adverse event (AE) term according to CTCAE definitions (each AE term = unique representation of a specific event used for medical documentation and scientific analyses), and graded as defined by CTCAE (grade 1 = mild; grade 2 = moderate; grade 3 = severe or significant but not immediately life-threatening; grade 4 = life-threatening; grade 5 = death).

1.5 To evaluate whether candidate single nucleotide polymorphisms (SNPs) relevant to eflornithine (DFMO) efficacy.

2.0 BACKGROUND

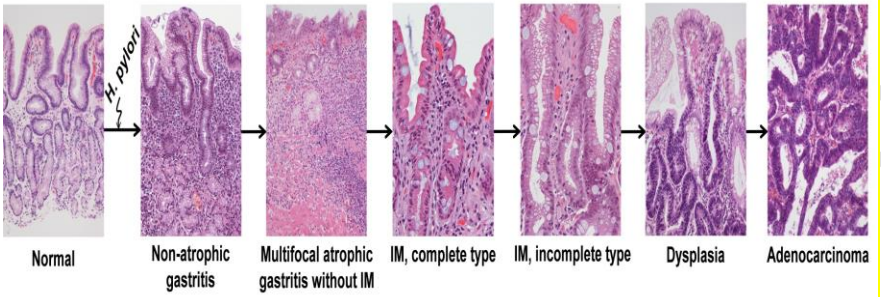
Burden of gastric cancer

Gastric adenocarcinoma is a leading global cause of cancer mortality, and the leading global infection-associated cancer.[1] Latin America has a significant disease burden, with a concentration of disease in the mountainous regions of the Pacific littoral zone.[2-4] The "altitude enigma" of gastric cancer in Latin America affords the unique opportunity for accelerated scientific discovery and focused prevention programs. The stomach cancer mortality-to-incidence ratio of 0.82 in the region underscores the need to improve cancer control.[2] In the USA, gastric cancer represents a marked health disparity: non-whites, including Hispanics, have nearly twice the incidence rates, which is largely un-investigated.[5,6]

General rationale for chemoprevention

H. pylori is the most common bacterial infection in humans, and causes gastritis in all individuals. Gastritis progresses along the "Correa cascade" from gastritis to the precancerous stages of atrophic gastritis (CAG) and intestinal metaplasia (IM), to gastric adenocarcinoma.[7] The validated Correa histopathology score provides quantitative assessment of the gastric mucosa, including changes over time and with interventions.[8-10]

Gastric Cancer Cascade



Important issues related to *H. pylori* infection and chronic gastritis include the following:

1) More than half of humans are infected [11], and over 80% in high incidence regions.
2) Biomarkers are lacking to delineate strain virulence and predict who will develop cancer.
3) *H. pylori* mimics a commensal organism [12] and is inversely correlated with asthma [13] and esophageal cancer. [14]

Arguments against universal *H. pylori* eradication in the gastritis stage in high risk populations include: development of antibiotic resistance, frequency of re-infection, potential role as a beneficial commensal, and lack of definitive RCT evidence. [15-17] The optimal approach for subjects with gastritis await the discovery and validation of both novel biomarkers and new *H. pylori* eradication strategies.

Important issues regarding the premalignant stage are:

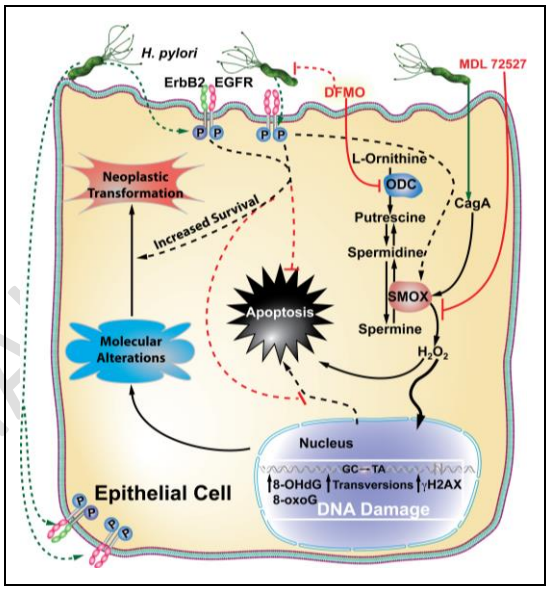
1) Organism loss and a "point of no return" is observed with the progression to atrophy, metaplasia and cancer. [18]
2) While antibiotic eradication may provide some benefit, it cannot serve as the sole intervention, and consistent evidence is lacking that this approach prevents cancer at this stage. [15-17]
3) Endoscopic surveillance for early gastric cancer (e.g., Japan, Korea) is resource intensive and impractical in most global settings with current technology.
4) Chemoprevention agents for gastric precancerous lesions are lacking.

In summary, eflornithine (DFMO), with its body of scientific and safety literature, in conjunction with the detailed preclinical investigations, is an ideal candidate to evaluate for the prevention of gastric cancer. [19-26]

Polyamine pathway

The polyamine synthesis pathway and its regulation have been subject to extensive investigation. [27-30] In excess, polyamines are implicated in epithelial carcinogenesis, including gastric carcinogenesis. [31,32] Difluoromethylornithine (eflornithine, DFMO) is an irreversible inhibitor of the first enzyme (ornithine decarboxylase [ODC]) in the polyamine synthesis pathway and has been useful in identifying the various roles of polyamines in cellular growth, invasion and metastases.[33] A large number of laboratory investigations of polyamine biochemistry have been reported, and a number of prevention and therapeutic clinical trials of eflornithine have been conducted and are ongoing.

Evidence for eflornithine (DFMO) in chemoprevention



This project will directly evaluate, for the first time, the role of polyamines in human gastric inflammation and carcinogenesis. Altered L-arginine/polyamine metabolism have been implicated in immune dysregulation and DNA damage caused by *H. pylori*. L-arginine is the substrate for generation of nitric oxide (NO) via inducible NO synthase (iNOS) and polyamines via ODC. *H. pylori* upregulates iNOS *in* vitro [34-39] and *in vivo* [40, 41]. The mammalian polyamines are putrescine, spermidine, and spermine.[42] ODC forms putrescine from L-ornithine, and spermidine is formed from putrescine, and converted to spermine;[43] the production of spermidine and spermine from putrescine requires the activity of the enzyme S-adenosylmethionine decarboxylase (SAMDC, aka AMD1) along with spermidine and spermine synthase.[44] Polyamines have been implicated in carcinogenesis due to effects on epithelial cell growth[45,46] and apoptosis,[46,47] and levels are increased in *H. pylori* gastritis[48,49]. Spermine is back-converted by spermidine oxidase (SMOX) to spermidine, generating H_2O_2.[69] Cellular injury is mediated by polyamines in macrophages and epithelial cells exposed to *H. pylori*7,[22,23,34-37] due to effects of SMOX [22,23] rather than the alternative pathway where spermidine/spermine N1-acetyltransferase (SSAT) acetylates spermidine or spermine, followed by oxidation by acetyl polyamine oxidase (PAO). While SMOX is the major source of oxidative DNA damage and its inhibition prevents this, data show that inhibition of ODC with DFMO is equally beneficial in *in vitro* and *in vivo* models. While there are inhibitors of polyamine oxidation, they are not completely specific for SMOX, and none are available for use in humans.

Toxicity of eflornithine

The overall eflornithine toxicity profile is modest, and the main considerations are reversible hearing loss and mild gastrointestinal (GI) side effects. In prior studies of DFMO efficacy for colon adenoma prevention, the overall and upper GI symptoms and toxicity were similar to placebo, after three years treatment duration, although small sample sizes may have limited detection. (28, 51, 52) Modest reversible hearing loss was noted in early studies of DFMO plus sulindac. A significant incidence of DFMO-associated ototoxicity is not anticipated, based on recent literature.[52]

Rationale for correlative pharmacogenetic analyses

Precision medicine holds great promise in the immediate future for the rational improvement of prevention and treatment modalities based on the individual's environmental exposure and genetic background. The impact of personalized medicine is already considerable in cancer research and particularly in cancer genetics.[53] Cancer chemoprevention comprises the treatment of ostensibly healthy individuals who are at risk and, more generally, of cohorts with premalignant lesions and early stage cancers or otherwise at high risk for new cancers or cancer recurrence. Cancer chemoprevention, therefore, requires the application of low to non-toxic agents over extended periods to individuals most likely to respond to such treatment (cohort enrichment). Specifically, applying pharmacogenetic testing to the field of cancer chemoprevention can help achieve the goal of improving the probability of a patient response, while minimizing adverse effects.

ODC1 (ornithine decarboxylase-1)
DFMO directly binds to and inhibits the ODC enzyme, thereby decreasing polyamine synthesis. In colon adenoma studies with DFMO and sulindac, genotyping (e.g., ODC+316 SNP) appears to identify patients who are both more likely to respond to eflornithine and also those with fewer adverse effects.[54] This SNP variant in ODC1 intron 1 has been shown to modulate ODC expression, and this gene is thought to be the direct target of eflornithine.[54, 55]

Inclusion of Women and Minorities:
This study was designed to include women and minorities, but is not designed to measure differences of intervention effects. The study sites locations dictate the enrollment of primarily Hispanic subjects. The anticipated accrual in the ethnicity/race and gender categories is shown in the table below.

Accrual Targets			
Ethnic Category	**Female**	**Males**	**Total**
Hispanic or Latino	150	150	300
Not Hispanic or Latino	0	0	0
Ethnic Category: Total	150	150	300
Racial Category	**Female**	**Males**	**Total**
American Indian or Alaskan Native	0	0	0
Asian	0	0	0
Black or African American	0	0	0
Native Hawaiian or Other Pacific	0	0	0
White	150	150	300
Racial Category: Total	150	150	300

3.0 DRUG INFORMATION

Investigator's Brochures.
For this study, eflornithine is investigational. Eflornithine is being provided under an IND held by Cancer Prevention Pharmaceuticals, LLC (CPP). The current version of the Investigator Brochure is provided by the Sponsor per protocol.

3.1 Eflornithine (NSC-337250) (**103678.**)

a. DESCRIPTION
This trial will use low dose oral eflornithine hydrochloride. Eflornithine is a member of the following drug classes: 1) inhibitor of ornithine decarboxylase (ODC), 2) hair growth retardant. Eflornithine is FDA approved as a cream for female hirsutism, and in intravenous form for trypanosomiasis. The oral tablet form is not available outside of the clinical trial setting in the U.S. The formulation used in this trial is similar to that reported previously in a Phase III colon adenoma clinical trial in combination with sulindac.[28] This formulation of eflornithine is under study in U.S. and international trials both for chemoprevention (low dose) and cancer therapy (high dose). (See Appendix).

b. TOXICOLOGY
Contraindications: Prior hypersensitivity to eflornithine. Precaution in patients with bone marrow suppression or hematologic disorders.

Side effects: The side effects reported herein are those observed in all studies to date, and for both high and low dosing. This study will utilize the low dose of eflornithine (500mg per day).

Common Side Effects (most frequently reported AEs in NCI, DCP-sponsored chemoprevention studies): diarrhea (9.0%), headache (7.5%), nausea (6.5%), hearing loss (5.6%), tinnitus (4.3%) and asthenia (4.7%).

Less Common Side Effects (2 – 3%): epigastric pain, flatulence, dyspepsia, anemia, dizziness and skin rash.
Rare Side Effects (1 - 2%): stomatitis, rhinitis, insomnia, infections, vomiting, vasodilation, dry mouth, constipation, dry skin, menstrual disorders, pharyngitis, emotional lability, pruritis, myalgia, and pain (miscellaneous).

There have been rare but serious reports of seizures in some subjects receiving high doses of eflornithine (15 g/day, ~215 mg/kg-bw/day). These instances occurred in subjects who were trypanosomiasis, glioma, or AIDS patients, and were suspected of having an impaired blood-brain barrier due to their disease or prior drug treatments.

Pregnancy and Lactation: Eflornithine is contraindicated in women who are or may become pregnant and in nursing mothers. It is likely that eflornithine will cause fetal harm when administered to pregnant women. Eflornithine has been shown to have a contragestational effect in mice, rats, and rabbits, when given in doses equivalent to approximately fifty-times the dose used in human chemoprevention trials. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. Eflornithine is also contraindicated in patients who are hypersensitive to eflornithine or any of the inactive ingredients.

c. PHARMACOLOGY
Kinetics: Therapeutic drug concentration is not established. Time to peak concentration for oral eflornithine is 4-6 hours. Absorption: for the oral solution is 54-58% and is unaffected by feeding status. Distribution: no protein binding sites, crosses blood-brain barrier. Volume of distribution is 0.3-0.35 liters/kg.
Metabolism: urinary recovery of unchanged drug as eflornithine is 86% and essentially not metabolized. Excretion: renal excretion. Elimination half-life: 3-3.5 hours but once daily dosing is sufficient to maintain efficacy.
Formulation: Eflornithine 250 mg and placebo are supplied as tablets.
Composition of Eflornithine: The active compound is DFMO (as the monohydrate monohydrochloride monohydrate) (Eflornithine HCl) 250 mg; with microcrystalline cellulose, starch 1500, silicon dioxide, magnesium stearate and opadry.
Composition of Eflornithine placebo: Dibasic calcium phosphate dihydrate, microcrystalline cellulose, starch 1500, hypromellose, silicon dioxide, magnesium stearate, opadry (yellow).
Storage and Stability: Tablets are stored at room temperature.
Administration: Two tablets by mouth daily taken with food. The tablets are a light tan color.
Supplier: Eflornithine and placebo will be supplied by Cancer Prevention Pharmaceuticals, Tucson, AZ, USA.

3.2 Agent Supply and Labeling
Eflornithine 250 mg tablets and matching placebo will be provided free of charge by Cancer Prevention Pharmaceuticals (CPP). CPP will provide eflornithine and placebo to Sharp Clinical Services, Inc., who will then label and distribute to the study sites.

Eflornithine and matching placebo will be supplied in white plastic bottles with a child resistant cap and tamper evident seal, each containing 100 tablets of either 250 mg eflornithine or matching placebo.

Each bottle will be labeled with:

- The protocol number
- The patient study number (e.g., "999999")
- The number of tablets (e.g., “100 tablets”)
- The agent identification (e.g., "eflornithine 250 mg or placebo")
- Administration instructions (i.e., " 2 tablets once a day")
- The date (year, month, day as xxxx.yy.zz)
- The bottle labels will have a line for the patient initials
  The convention for patient initials and names is "ABCD" for which A represents the first name, B is the second (middle) name, C is the first surname, and D is the second surname (if used by the patient).

- The lot number (e.g., “9999”)
- The caution information (e.g., “Keep out of reach of children”)
- The storage information (e.g., temperature range)
- The clinical study restriction (e.g., “For clinical study use only”)
- The sale information (e.g., “Not for sale”)
- The study contact information (e.g., Investigator, Sponsor, Distributor)

3.3 Drug Orders, Transfers, Accountability, Emergency Unblinding
Drug Orders. Blinded specific clinical supplies of eflornithine and placebo will be sent to the registering investigator, identified by randomized patient study number. Replacement bottles will be available at each site.
Drug Transfers. Bottles may not be transferred from one patient to another patient.
Drug Accountability. The site investigator must maintain a careful record of the receipt, dispensing, and return of all agents. Undispensed agent supplies should be destroyed on-site. Opened bottles or kits with remaining tablets should be documented (i.e., logged as “returned by patient” and logged as “destroyed on site”) and destroyed on site in accordance with institutional policy.
Emergency Unblinding. See Appendix for emergency unblinding instructions.

4.0 PATHOLOGY CRITERIA

4.1 Pathology criteria. To be eligible to participate, subjects must have the histology-based diagnosis of a gastric premalignant lesion, either chronic atrophic gastritis (CAG) or intestinal metaplasia (IM). The updated Sydney system for gastritis provides standard assessment of the CAG and IM diagnosis, and includes severity and associated inflammation.[56] The histology assessment by the site clinical pathologist(s) will be recorded at study entry, serving as the basis for study eligibility, and at each study endoscopy. While dysplasia is considered a precancerous lesion, patients with dysplasia are not eligible for participation.

4.2 Additional pathology assessment. The Correa histopathology score accurately quantifies the histology for the atrophy and intestinal metaplasia, and dysplasia categories; this scoring system has been shown to be precise and reliable, including for longitudinal cohort studies. [8-10] The subtypes of intestinal metaplasia, incomplete (“colonic-type”) versus complete (“small intestinal type”), and extensive versus limited, may further stratify risk, and are part of the baseline evaluation and each endoscopy. Incomplete IM may reflect a higher risk subset, and is common among the patient populations in this study.[57,58]

4.3 *H. pylori* infection status assessment. Active *H. pylori* is diagnosed by standard histology assessment, and assessed at each endoscopy. Active *H. pylori* infection will also be assessed by stool antigen study. The diagnosis of active *H. pylori* infection for each patient is based upon either a positive histology OR stool antigen study, and is assessed at the time of each endoscopy. Subjects may be positive or negative for active *H. pylori* infection at the time of enrollment and throughout the study. (See Sections 6.2 and 6.3). Lastly, *H. pylori* antibody by ELISA testing will be performed at enrollment for ever-infected *H. pylori* status, for completeness.

5.0 ELIGIBILITY CRITERIA

5.1 Patient population. Subjects will be evaluated at the time of a scheduled visit (clinic or endoscopy) at the study centers. Dyspepsia is the principal indication for endoscopy, and the majority (90%) of patients have *H. pylori*-associated gastric pathology, and 20-30% have precancerous lesions. The primary inclusion criteria are the presence of a precancerous lesion on index endoscopy, age 30-69, willingness to participate, and the absence of exclusion criteria.

5.2 Eligibility criteria. Each of the criteria in the following section must be met in order for a patient to be considered eligible for registration. All potential eligibility issues should be addressed by the study site investigator in consultation with the study PIs.

1. Patients must have a history of a premalignant lesion of the stomach, atrophic gastritis or intestinal metaplasia. Subjects with dysplasia (indeterminate, low grade, high grade) are not eligible for participation.
2. Patients must be ages 30 to 69 years of age.
3. Patients must not have a significant medical or psychiatric condition that would preclude study completion.
4. Patients must not have a significant cardiovascular disease history, including uncontrolled blood pressure (sBP > 150 mmHg), myocardial infarction, cerebrovascular accident, or heart failure (New York Heart Association Class III, or IV).
5. Patients must not have a history of gastric or esophageal cancer, gastric resection or surgery, peptic ulcer disease (within 6 months), *H. pylori* treatment (within 6 months), or inflammatory bowel disease.
6. No prior malignancy is allowed except for adequately treated basal cell or squamous cell skin cancer, in situ cervical cancer, or other cancer for which the patient has been disease-free for >5 years.
7. Patients must not have known hypersensitivity to eflornithine or the excipients.
8. Patients must not be receiving corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), or anticoagulants on a regular or intermittent basis.
9. Patients must have a pure tone audiometry evaluation to document air conduction within 60 days prior to randomization. Patients with hearing loss ≥30 dB in any of the tested frequencies (250 Hz, 500 Hz, 1,000 Hz, 2,000 Hz, 4,000 Hz, 8,000 Hz) are not eligible.
10. Patients must have adequate blood counts as evidenced by the following results (obtained within 60 days):
    a. Blood counts: WBC ≥4.0 /mcL, platelets ≥100,000 /mcL and hemoglobin ≥11.0 g/dL
    b. Kidney function: Creatinine <1.6 x IULN (institutional upper limit of normal)
    c. Liver function tests: Bilirubin ≤2.0 mg/dL and AST (SGOT) or ALT (SGPT) ≤2 x IULN
11. Patients must not be pregnant or nursing (due to eflornithine pregnancy class C). Women and men of reproductive potential must have agreed to use an effective contraceptive method.

6.0 REGISTRATION PROCEDURES

All patients MUST be registered with the Coordinating Center prior to the start of protocol treatment. Registration can only be conducted during the business hours of 8AM – 5PM Central Standard Time Monday through Friday.

1) All sites must email the VICC CTSR Coordinating Center at Coordinating.Center@vumc.org to notify of each patient enrollment within 24 – 48 hours of randomization. The following information should be included in your email:
    - Copy of the patient's signed and dated Informed Consent including documentation of the consent process.
    - VICC Patient Enrollment Form

Issues that would cause treatment delays should be discussed with the Protocol Chair. Any requests for eligibility exceptions and/or deviations must be approved in writing by the Protocol Chair and the VICC DSMC.

As is generally accepted, standard of care procedures performed prior to consent, but within the protocol defined screening window for each assessment, can be used for study purposes. All research-only procedures must be performed after patient consent.

7.0 STRATIFICATION FACTORS

Patients will be randomized to one of two treatment groups: eflornithine versus eflornithine placebo, with 150 subjects in each treatment group. A dynamic balancing algorithm will be used for randomization, with stratification by study site. *H. pylori* status is integrated into the analysis, but stratification is not performed by *H. pylori* status.

7.1 Study sites. Study enrollment will be competitive across up to three participating sites. The enrollment at each individual site will be targeted at 240 (80%), with the exception of extenuating circumstances (e.g., natural disaster, recruitment limitations, etc.).

7.2 *H. pylori* subgroups. Three subgroups of subjects with precancerous lesions will be studied as a secondary analysis, based on *H. pylori* status and acceptance of eradication treatment at enrollment: 1) *H. pylori* negative; 2) *H. pylori* positive, decline antibiotic treatment (50%); and 3) *H. pylori* positive, accept antibiotic treatment (50%). Historically, given the high prevalence of *H. pylori* infection and the natural history of reinfection, about 50% of subjects decline or accept antibiotic eradication, respectively. Thus, these 3 subgroups are common clinically we expect a *balanced distribution* at our study sites (see study schema). In summary, we expect 150 subjects in each treatment group (eflornithine, placebo) and 100 subjects enrolled in each *H. pylori* subgroup.

7.3 *H. pylori* eradication. The secondary intervention is *H. pylori* treatment of subjects of the subgroup who are *H. pylori* positive and desire treatment, per usual standard of care. Subjects are treated with standard triple therapy (clarithromycin 500 mg, amoxicillin 1000 mg, lansoprazole 30 mg) twice daily for 14 days. This is the optimal regimen in Latin America, based on RCT results.[59] Patients are permitted to choose alternative *H. pylori* eradication regimen, per usual standard of care, in consultation with the site investigator. Patients will be treated at the 6 month time point. This *H. pylori* eradication treatment timing permits the maximum number of study subjects on DFMO monotherapy for the 6 month primary endpoint. This timing is ethical given the lifelong infection with *H. pylori* in >80% of our populations, the natural history of reinfection, and the limited short term benefit of *H. pylori* eradication for subjects with premalignant lesions . Subjects who elect to change their decision on *H. pylori* treatment during the initial 6 month period are permitted to do so. In addition, we expect infection recurrence rates of 8% in Honduras at the 18 month point based upon our Latin America RCT. If infected at 24 months, subjects will be offered treatment per local standards of care and outside of this protocol. [59,60]

8.0 TREATMENT PLAN

8.1 Randomization

Three-month supplies will be provided for each patient for the duration of the intervention. Careful instructions regarding medication administration will be provided verbally, and patients will be instructed call or visit the clinic or call the research team at any time if necessary.

8.2 Study Intervention Schedule

Agent	Dose	Route	Interval
Eflornithine placebo	2 tablets	Oral	Daily for 18 months
Or			
Eflornithine*	2 tablets	Oral	Daily for 18 months

*Each eflornithine tablet is 250mg

Initial Visit (pre-randomization): Patient should have history and physical, weight, and blood draw (include CBC: Hgb, hematocrit, WBC, platelets; liver tests: bilirubin, AST, ALT; and creatinine). Additionally, women of child bearing potential will have a pregnancy test (serum or urine) within 14 days prior to start of study drug. Patients will be evaluated via endoscopy at baseline. Audiometry testing to document air conduction will be conducted.

Follow-up during intervention (Months 0-18): During the 18 month intervention, patients will be followed at three month intervals with clinic visits which include a physical exam and blood draw (CBC, chemistries). Endoscopic surveillance is incorporated into the follow up at 6 and 18 months. Study drug dispensation coincides with clinic visits. More frequent patient contact is planned (e.g., phone follow-up: weekly in months 0-6, and monthly thereafter), at the discretion of the site investigator, in consultation with the study PIs, as in prior clinical trials.[59,60]

Final intervention visit (Month 18): At Month 18, all patients will have a complete physical exam, blood draw (CBC, chemistries), and pure tone audiogram.

Post intervention follow-up (Month 24): All patients will be followed for 6 months after completion of the 18 months of intervention off of study medications.

Patient removal from protocol: If the patient terminates the protocol intervention between completion of Month 3 and Month 18, the complete physical exam, blood draw (CBC, chemistries), pure tone audiogram, and endoscopy will be

offered to the patient at that time. If the patient terminates the protocol intervention prior to Month 3, the physical exam, blood draw (CBC, chemistries), and pure tone audiogram are recommended, without endoscopy. Endoscopy will be offered to the patient at Month 24 as part of usual care and clinical surveillance protocol at the sites for subjects with premalignant lesions.

8.3 Endoscopy Procedures (Months 0, 6, 18, 24):

The endoscopy with standard biopsies of the gastric mucosa will be done at four time points: Months 0 (pre-study), 6, 18, and 24. Patients are screened at the time of the index endoscopy, previously scheduled for clinical indications per local standard of care. Samples obtained at the time of the index endoscopy performed per standard of care prior to informed consent for this study may be submitted for research once informed consent is obtained. The Month 0 (pre-study) and Month 24 endoscopies are part of usual care clinical screening and surveillance endoscopies in high risk regions, per local and emerging international standards.[61] The allowable timeframe for each endoscopy is - 30/+60 days. The gastric mapping biopsy protocol for histology follows the international protocols,[61] and with additional research biopsies per longstanding protocol. (Appendix 16.2).

Biopsy mapping of the gastric mucosa for histopathology is part of usual care of patients at study sites, and adherent to emerging international standards for stomach precancerous lesions. Five formalin-fixed biopsies are obtained from the antrum (2), corpus (2), and incisura (1). Two sets of research small pinch biopsies are obtained for the study, preserved for study analyses.

The potential risks of endoscopy include perforation, bleeding, infection, and drug reaction. Biopsies of the stomach are considered a routine component of diagnostic upper endoscopy. The serious adverse event rate for upper endoscopy with biopsies is less than 1/6000 cases (<0.02%).[62] Perforation and bleeding may require a repeat endoscopy, and in some cases, surgery. Infection at the biopsy sites is highly unlikely. Allergic reactions to medications used for procedure sedation are extremely uncommon, and would be treated immediately if they should occur.

8.4 Patient Pill Diary and Pill Dispensing

A pill diary will be provided for patient use to facilitate tracking and adherence of the assigned intervention. Three-month supplies of study drug are issued throughout the intervention.

8.5 Adherence/Compliance

Adherence to the blinded drug in this study will be measured by returned tablet count. All unused study medication is returned to the clinic at each follow-up assessment every 3 months. The remaining tablets are counted and the number of pills dispensed and returned will be documented. An individualized adherence intervention strategy is used when the adherence level is below 75%. The Coordinating Center [redacted]) should be notified within 7 days whenever an intervention strategy needs to be implemented for a patient. The following information should be included in this notification:

- Protocol Number
- Subject ID
- % compliance noted
- Intervention strategy implemented

8.6 Study Blinding Information and Criteria for Removal from Protocol Treatment

a. All patients, study centers, pathology and laboratory personnel will be blinded to study treatment. The study pharmacist at each study site is unblinded to facilitate randomization.
b. Laboratory and pathology specimens will be labeled by patient identification number and initials only.
c. The study drug may be discontinued without unblinding the patient. Patient's treatment will be unblinded only if the treating physician demonstrates a compelling medical need for this information (Appendix).
d. As study arms will be analyzed using an intent-to-treat method, patients are never "dropped" from the study.

e. Patients will be removed from protocol treatment under the following circumstances, and all reasons for discontinuation will be documented:
   - Unacceptable toxicity (Section 8.0).
   - A patient who becomes pregnant or declines contraception.
   - Intercurrent illness which would affect assessments, in the opinion of the treating physician.
   - Cumulative delay of study intervention >90 days for any reason.
   - The patient may withdraw from the study at any time for any reason.

9.0 TOXICITIES TO BE MONITORED AND DOSAGE MODIFICATIONS

9.1 This study will utilize the CTCAE version 4.0 (NCI Common Terminology Criteria for Adverse Events) for toxicity and Serious Adverse Event (SAE) reporting.

9.2 Guidelines for monitoring expected and unexpected symptoms/toxicities are defined below.
All interventions can be dose reduced based on toxicity or participant perceived toxicity. All toxicities are recorded on the Adverse Events Summary Form. All patients who discontinue treatment after randomization will continue to be followed for endoscopy results.

Guidelines for Monitoring of Expected and Unexpected Symptoms/Toxic Events:

- ***Grade 0***
  No monitoring necessary.

- ***Grade 1 and Grade 2***
  Monitor as needed until problem has resolved (i.e., Grade 0), stabilized (i.e., remains as Grade 1 or Grade 2), or is otherwise explained. If Grade 1 or Grade 2 symptoms persist, the physician will assess whether study medication is to be discontinued or whether symptom management should be initiated.

- ***Grade 3 and Grade 4 Toxicities:***
  If any Grade 3 or Grade 4 toxicity occurs, ascertain cause of event and study relatedness. If symptoms are related to the study drug, the dose of the drug should be reduced by 50% as follows:

Study Drug	50% Dose Reduction	Frequency	If toxicity improves to grade 0, 1, or 2
Eflornithine or matched placebo	250 mg (1 tab)	Daily	Continue dosing at 50%

  If Grade 3 or 4 toxicity persists at 50% dose level, the patient can be given a 4 week drug holiday. After the 4 week drug holiday, the patient may be re-challenged at the aforementioned 50% dose reduction level for 4 weeks. Patient will be removed from protocol treatment if there is a cumulative delay of study intervention >90 days.

10.0 STUDY CALENDAR

Required Studies	Month 0 (Pre-Study)	Study Start	Month 3 (± 14 days)	Month 6 (± 30 days)	Month 9 (± 14 days)	Month 12 (± 14 days)	Month 15 (± 14 days)	Month 18 [10] (± 30 days)	Month 21 (± 14 days)	Month 24 (± 30 days)
PATIENT EVALUATIONS										
Consent & Enrollment [14]	X									
Patient information[1]	X									
History & Physical [13]	X		X	X	X	X	X	X	X	X
Pill Diary [2]		X	X	X	X	X	X	X		X
Toxicity Notation		X	X	X	X	X	X	X	X	X
Laboratory tests [3]	X		X	X	X	X	X	X		X
Pregnancy Test [12]	X									
Audiometry [4]	X							X		
DIAGNOSTIC EVALUATIONS										
Endoscopy [5]	X			X				X		X
Histology assessment	X			X				X		X
H. pylori status [11]	X			X				X		X
INTERVENTIONS										
Dispense study drug [6]		X	X	X	X	X	X			
Adherence assess [7]			X	X	X	X	X	X		
H. pylori treatment [8]				X						(X) [9]

Study Calendar notes:

1. The subject assessment at enrollment includes demographic information and risk factor assessment.
2. While participant is on the protocol intervention, the pill diary is reviewed at each visit.
3. The laboratory tests include CBC (Hgb, Hematocrit, WBC, platelets), liver tests (bilirubin, AST, ALT) and creatinine.
4. Audiometry is performed at the pre-study visit (after all eligibility criteria are met), and at 18 months. Patients are screened for hearing impairment at each study visit, and referred for audiometry, if indicated.
5. Endoscopy with gastric biopsy mapping for histology assessment is the standard of care. The index endoscopy is performed for clinical indications. Samples obtained at the time of the index endoscopy per SOC and prior to patient consent for this study may be submitted for research once informed consent is obtained. The Month 24 endoscopy is considered a surveillance endoscopy in patients with precancerous lesions.[61]
   The allowable timeframe for each follow-up endoscopy is – 30/+60 days.
6. A three-month supply is dispensed on Day 1 and every 3 months through Month 15. Patients will dose for a total of 18 months and then be followed for an additional 6 months.
7. The adherence assessment consists of the pill count. Phone contact (weekly in months 0-6; monthly in months 6-18) is planned with the patients for adherence assessment, and is performed at the discretion of the study centers.
8. *H. pylori* treatment consists of the standard antibiotic regimen, per usual standard of care. Patients who are *H. pylori* positive at study enrollment are offered therapy at Month 6. (Section 6.0).
9. If a patient is *H. pylori* positive at 24 months, they will be offered additional treatment through their local providers. This intervention is outside of the scope of the study.
10. If the patient terminates the protocol intervention between completion of Month 3 and Month 18, the complete physical exam, blood draw (CBC, chemistries), pure tone audiogram, and endoscopy will be offered to the patient at that time. If the patient terminates the protocol intervention prior to Month 3, the physical exam, blood draw (CBC, chemistries), and pure tone audiogram are recommended, without endoscopy. Endoscopy will be offered to all patients at Month 24 as part of the clinical surveillance protocol (usual care) at the sites for subjects with premalignant lesions.
11. *H. pylori* infection diagnostic evaluations. Active *H. pylori* infection status (positive or negative) is assessed by gastric histology and stool antigen testing at the time of each endoscopy. In addition, *H. pylori* antibody ELISA testing is performed at the first endoscopy only, for assessment of ever-infected status.
12. Pregnancy testing (serum or urine) is required for all women of childbearing potential (WOCBP) within 14 days prior to the first dose of study drug. WOCBP of childbearing potential are defined as those not surgically sterile or not post-menopausal (i.e. if a female patient has not had a bilateral tubal ligation, a bilateral oophorectomy, or a complete hysterectomy; or has not been amenorrheic for 12 months in the absence of an alternative medical cause, then patient will be considered a female of childbearing potential). Postmenopausal status in females under 55 years of age should be confirmed with a serum follicle-stimulating hormone (FSH) level within laboratory reference range for postmenopausal women.
13. Each physical exam should include assessment of vital signs.
14. Consent should be obtained within 60 days prior to the start of study treatment.

11.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

Primary Objective

11.1 The difference in cell DNA damage between patients treated with DFMO and patients treated with placebo at 6 months. The cell DNA damage is measured using the percent positive gastric epithelial cells assessed by IHC for gamma H2AX. The mean difference between the two groups at 6 months will be calculated, accounting for their baseline measurements.

Secondary Objectives

11.2 The difference in cell DNA damage between patients treated with DFMO and patients treated with placebo for 18 months, and then followed for an additional 6 months. The cell DNA damage is measured using the percent positive gastric epithelial cells assessed by IHC for gamma H2AX. The mean difference between the two groups at 18 and 24 months will be calculated, accounting for their baseline measurements. The additional measure of DNA damage, gamma H2AX by IHC and by flow cytometry will also be assessed at 0, 6, 18, and 24 months.

11.3 The differences in the gastritis histopathology score between patients treated with DFMO and patients treated with placebo for a total of 18 months, and followed for an additional 6 months. The gastritis histopathology score is measured with a quantitative scale 0.0-6.0, for atrophy, intestinal metaplasia, and dysplasia. The mean differences between the two groups at 6, 18, and 24 months will be calculated using mixed models, accounting for their baseline measurements.

11.4 Number of patients with quantitative toxicities. Toxicities will be assessed per CTCAE criteria, and each toxicity will be assigned an adverse event (AE) term according to CTCAE definitions (each AE term = unique representation of a specific event used for medical documentation and scientific analyses), and graded as defined by CTCAE (grade 1 = mild; grade 2 = moderate; grade 3 = severe or significant but not immediately life-threatening; grade 4 = life-threatening; grade 5 = death).

10.5 To evaluate whether candidate single nucleotide polymorphisms (SNPs) relevant to eflornithine (DFMO) efficacy.

12.0 STATISTICAL CONSIDERATIONS

12.1 Data Analyses for the primary and secondary endpoints

We hypothesize that high risk subjects with precancerous gastric lesions will benefit from eflornithine, by reducing DNA damage, inflammation, and bacterial virulence, leading to attenuated histopathology and cancer risk. The primary objective is to assess whether eflornithine affects gastric epithelial cell DNA damage, measured at the 6 month time point, as assessed by percent positive cells in each patient, measured by IHC for gamma H2AX. We expect 150 patients in each group (eflornithine vs. placebo). We expect 100 patients in each *H. pylori* subgroup (*H. pylori* negative, *H. pylori* positive treated, *H. pylori* positive untreated [decline treatment]). We will examine the main effect of treatment and the effect modification from *H. pylori* status and treatment in a multivariate model.

We hypothesize a 50% reduction in DNA damage from baseline at the 6 and 18 months. We expect a modest reduction of eflornithine (10%) effect on DNA damage after treatment cessation (months 18-24), which nonetheless may remain statistically significant. We also expect a reduction in the histopathology score by 0.16-0.18 units at 18 months. We base these estimations on our prior published studies.[8,9] We assume a two-sided alpha of 0.05 and 80% power. For the main comparison, DFMO vs. placebo at 6 months (150 subjects each), a difference in DNA damage of ≥18% (IHC for gamma H2AX) is hypothesized. A difference of ≥0.1 units in histopathology score at 18 months is expected.

Subgroup analysis (50 subjects each) at 6 months, where the comparator is *H. pylori* negative may detect a difference of ≥36% and ≥31% in DNA damage, respectively, and may detect at 18 months a difference of ≥0.15 units in histopathology score for both comparisons. These calculations assume normally distributed endpoints and use standard deviations from our prior work. The size of differences chosen for computations has been reduced to allow a 10% loss to follow up.

Generalized linear models with a Gaussian link are used to analyze difference in means of DNA damage (flow cytometry, immunostaining), and histopathology at 18 months. The primary endpoint will be reported in terms of marginal means and their difference (DFMO/placebo) with 95% confidence intervals (95%CI) adjusted for baseline variables (age, gender, etc.) and baseline dependent variable values. Mixed models will be used to analyze secondary endpoints including the longitudinal changes from baseline to 6, 18, and 24 months. Time between measurements will be included in the model so that random slopes for the dependent variable at each point in time and random intercepts for each subject will be initially assumed. Adjusted marginal means and 95%CI will be reported with significance levels. The subgroup analysis will involve entering the subgroup analysis variable into generalized linear mixed models: Negative *H. pylori* status, *H. pylori* positive and treated, and *H. pylori* positive and refused treatment. Marginal means and 95%CI are reported for each subgroup after adjusting for baseline variables and including the main independent variable (treatment). A main effects model and an interaction model will be assessed for the treatment and subgroup variables. Prior experience with all three measurements has shown them to be normally distributed. Comparisons of AEs and SAEs between each of the treatment groups will also be performed using person-times poisson rates, with rates being reported using exact 95% confidence intervals. Lastly, we will examine the interaction of treatment arm and genotype expression with respect to different outcomes. STATA 13 is used for computations.

12.2 Accrual Monitoring. The historical experience for patient recruitment is approximately 150 patients per year. The estimated 10% dropout rate is based on prior studies, with excellent 1-year follow-up of >93% at these same study sites.[59,60] The general populations are keenly aware of the impact of gastric cancer in their regions, and the need for prevention. We anticipate balanced recruitment between the two study sites, 150 subjects per site, but with a flexible recruitment strategy. The enrollment at each individual site will be capped at 240 (80%).

13.0 SPECIAL INSTRUCTIONS

13.1 Collection of blood, tissue and stool specimens for biobanking is optional for patients.
The study sites will seek additional patient consent to bank blood, tissue and stool for future translational medicine studies (including genotyping and biochemical assays). This is the established research protocol at the study sites.

14.0 ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Components of sound research practice:
Informed Consent.
The principles of informed consent are described by the U.S. Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). They must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations.

Patient Confidentiality.
Confidentiality of patient's personal data will be protected in accordance with the Health Insurance Portability and Accountability Act of 1996 (HIPPA) and national data protection laws, as applicable. HIPPA regulations require that, in order to participate in the trial, a patient must sign an authorization from the trial that he or she has been informed of the following:

- What protected health information (PHI) will be collected from patients in this trial;
- Who will have access to that information and why;
- Who will use or disclose that information;
- That health information may be further disclosed by the recipients of the information, and that if the information is disclosed the information may no longer be protected by federal or state privacy laws;
- The information collected about the research trial will be kept separate from the patient's medical records, but the patient will be able to obtain the research records after the conclusion of the trial;
- Whether the authorization contains an expiration date; and
- The rights of a research patient to revoke his or her authorization.

In the event that a patient revokes authorization to collect or use his or her PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of patient authorization. For patients that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e., that the patient is alive) at the end of their scheduled trial period.

In compliance with ICH GCP guidelines and applicable parts of 21 CFR it is a requirement that the investigator and institution permit authorized representatives of Sponsor, the regulatory authorities and the IRB direct access to review the patient's original medical records at the site for verification of trial-related procedures and data.
The investigator agrees to keep all information provided by this study in strict confidence and to request similar confidentiality from his/her staff and the IRB/IEC/REB. Study documents provided (protocols, investigators' brochures, CRFs and other material) will be stored appropriately to ensure their confidentiality.

Patient medical information obtained by this study is confidential, and disclosure to third parties other than those noted in the informed consent form is prohibited. Upon the patient's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. This medical information must be made available to the IRB and DSMC, upon request, for source verification of study documentation. Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA, local health authorities, the Study Chair and his authorized representative(s), collaborators and licensees, and the IRB for each study site, if appropriate.

All reasonable efforts will be made to keep patient's PHI private and confidential. PHI will only be utilized or relinquished according to U.S. federal privacy guidelines. There are many safeguards in place to prevent the unintentional disclosure of information obtained for or produced by this study. Research data, including the data collected from the medical charts will be entered into a password-protected database. Any publications or public disclosure of data relating to the patient's tumor will be done without any identifying information.¬

Confidentiality and security will be maintained for the biospecimen collection within this study. All research biospecimens obtained for this study will be assigned a code and this code used to identify the sample. The samples will not be labeled with the patient's name, address or other information that would identify them. All information will be coded to maintain privacy. Research data, including the data collected from the medical charts will be entered into a password-protected database.

Institutional Review. This study must be approved by an appropriate institutional review committee or ethics committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46).

Drug Accountability. An investigator is required to maintain adequate records of the disposition of investigational drugs according to procedures and requirements governing the use of investigational new drugs as described in the Code of Federal Regulations 21 CFR 312.

Study Monitoring. This study will be monitored by the VICC Clinical Trials Shared Resource (CTSR). Details are provided in section 15.3.

Study Confidentiality. Please note that the information contained in this protocol is considered confidential and should not be used or shared beyond the purposes of completing protocol requirements unless permission is obtained.

14.2 Record Retention and Documentation of the Trial.

14.2.1 Amendments to the Protocol

Amendments to the protocol shall be planned, documented and signature authorized prior to implementation.
If an amendment to the protocol is required, the amendment will be originated and documented by the Coordinating Center in collaboration with Sponsor. The written amendment must be reviewed and approved by the Sponsor, and submitted to the IRB at the Study Chairs' facility for the board's approval.

Amendments specifically involving change to trial design, risk to patient, increase to dosing or exposure, subject number increase, addition or removal of new tests or procedures, shall be reviewed and approved by the IRB at the Study Chairs' facility. Once approved, the amendment will be distributed to the participating sites for submission to their institutional review boards (IRB) or Ethics Committees (EC). Updates to the participating site ICF must be reviewed and approved by the Coordinating Center prior to the site submission to their IRB.

The amendment will be submitted formally to the FDA or other regulatory authorities [redacted] applicable, after IRB approval and specifically when an increase to dosing or patient exposure and/or subject number has been proposed; or, when the addition or removal of an Investigator is necessitated.

Items requiring a protocol amendment with IRB and/or FDA approval include, but are not limited to, the following:

- Change to trial design
- Risk to patient
- Increase to dose or patient exposure to drug
- Subject number increase
- Addition or removal of tests and / or procedures
- Addition/removal of a new Investigator

It should be further noted that, if an amendment to the protocol substantially alters the trial design or the potential risks to the patients, their consent to continue participation in the trial should be obtained.

14.2.2 Documentation Required to Initiate a Trial

Before the trial may begin, certain documentation required by FDA regulations must be provided by the Investigator to the [redacted].
Documents at a minimum required to begin a trial in the US include, but are not limited to, the following:

- A copy of the Form FDA 1572 signed and dated by the Principal Investigator from the participating site.
- A copy of a current CV and Medical Licenses for all Investigators listed on the Form FDA 1572.
- Lab credentials (i.e. CAP, CLIA, ranges, etc.) as applicable for all labs listed on the Form FDA 1572 being used by the participating site.
- A copy of the Protocol Acceptance Page signed and dated by the Principal Investigator from the participating site.
- A copy of the Financial Disclosure completed and signed by all investigators listed on the Form FDA 1572 for the participating site.
- A copy of the delegation of authority log must be sent to the Coordinating Center
- A copy of documentation of training for study staff members must be sent to the Coordinating Center.
- A copy of the consent form that has been approved by the Coordinating Center and the Institutional Review Board for the participating site.
- The IRB approval letter for the participating site.
- The IRB Committee Roster for the participating site.
- The drug destruction policy for the participating site.
- A copy of a fully executed contract must be on record in the contracts office
- Site qualification reports, where applicable;

- Verification of Principal Investigator acceptability from local and/or national debarment list(s)

14.2.3 Trial Documentation and Storage

The site PI must maintain a list of appropriately qualified persons to whom he/she has delegated trial duties and should ensure that all persons assisting in the conduct of the trial are informed of their obligations. All persons authorized to make entries and/or corrections on the CRFs are to be included on this document. All entries in the patient's CRF are to be supported by source documentation where appropriate.

Source documents are the original documents, data, records and certified copies of original records of clinical findings, observations and activities from which the patient's CRF data are obtained. These can include, but are not limited to, hospital records, clinical and office charts, clinical research unit charts, laboratory, medico-technical department and pharmacy records, diaries, microfiches, ECG traces, copies or transcriptions certified after verification as being accurate and complete, photographic negatives, microfilm or magnetic media, X-rays, and correspondence.

The PI and trial staff for each clinical trial site are responsible for maintaining a comprehensive and centralized filing system (Site Trial File/SSF or ISF) of all trial-related (essential) documentation, suitable for inspection at any time by representatives from the Sponsor and/or applicable regulatory authorities. The ISF/SSF must consist of those documents that individually or collectively permit evaluation of the conduct of the trial and the quality of the data produced. The ISF/SSF should contain as a minimum all relevant documents and correspondence as outlined in ICH GCP Section 8 and 21 CFR Part 312.57, including key documents such as the IB and any amendments, protocol and any amendments, signed ICFs, copies of completed CRFs, IRB approval documents, Financial Disclosure forms, patient identification lists, enrollment logs, delegation of authority log, staff qualification documents, laboratory normal ranges, records relating to the trial drug including accountability records. Drug accountability records should, at a minimum, contain information regarding receipt, shipment, and disposition. Each form of drug accountability record, at a minimum, should contain PI name, date drug shipped/received, date, quantity and batch/code, or lot number for identity of each shipment. In addition, all original source documents supporting entries in the CRF must be maintained and be readily available.

The Sponsor shall maintain adequate investigational product records and financial interest records as per 21CFR Part 54.6 and Part 312.57 for no less than 2 years after the last marketing application has been approved by FDA; or, in the event that the marketing application has not been approved by FDA, for no less than 2 years after the last shipment / delivery of the drug for investigational use is discontinued and FDA has been notified of the discontinuation.
The IRB shall maintain adequate documentation / records of IRB activities as per 21CFR Part 56.115 for at least 3 years after completion of the research.

The Site Investigator shall maintain adequate records of drug disposition, case histories and any other trial-related records as per 21 CFR Part 312.62 for no less than 2 years after the last marketing application has been approved by FDA; or, in the event that the marketing application has not been approved by FDA, for no less than 2 years after the last shipment / delivery of the drug for investigational use is discontinued and FDA has been notified of the discontinuation.

To enable evaluations and/or audits from regulatory authorities or from the Sponsor or its representative, the investigator additionally agrees to keep records, including the identity of all participating patients (sufficient information to link records e.g., CRFs and medical records), all original, signed informed consent forms, and copies of all CRFs, SAE Reporting forms, source documents, detailed records of treatment disposition, and related essential regulatory documents. The documents listed above must be retained by the investigator for as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or after the last marketing approval). The VICC Multi- Institutional Coordinating Office will notify the investigator(s)/institutions(s) when the trial- related records are no longer required.

If the investigator relocates, retires, or for any reason withdraws from the trial, both Coordinating Center and the Sponsor should be prospectively notified. The trial records must be transferred to an acceptable designee, such as another investigator, another institution, or to sponsor. The investigator must obtain the sponsor written permission before disposing of any records, even if retention requirements have been met. All trial files will be maintained by the Coordinating Center throughout the trial, and prepared for long term storage by Coordinating Center at the conclusion of the trial.

14.2.4 Data Collection

Data will be collected and entered by the participating sites using a centralized Electronic Data Capture (EDC) system called REDCap.

The Vanderbilt University Office of Research will be used as a central location for data processing and management. Vanderbilt University, with collaboration from a consortium of institutional partners, has developed a software toolset and workflow methodology for electronic collection and management of research and clinical trial data. REDCap (Research Electronic Data Capture) is a secure, web-based application that is flexible enough to be used for a variety of types of research. REDCap provides an intuitive user interface that streamlines project development and improves data entry through real-time validation rules (with automated data type and range checks). REDCap also provides easy data manipulation (with audit trails for reporting, monitoring and querying patient records) and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). In addition to traditional data capture functionality, REDCap's survey capabilities are a powerful tool for building and managing online surveys. The research team can create and design surveys in a web browser and engage potential respondents using a variety of notification methods. All data collection projects rely on a thorough, study-specific data dictionary, defined by all members of the research team in an iterative, self-documenting process. This iterative development and testing process results in a well-planned and individualized data collection strategy.

REDCap servers are housed in a local data center at Vanderbilt, and all web-based information transmission is encrypted. REDCap was developed specifically around HIPAA-Security guidelines and is recommended to Vanderbilt researchers by both our Privacy Office and Institutional Review Board. REDCap has been disseminated for local use at more than 940 other academic/non-profit consortium partners in 75 countries. Vanderbilt leads the REDCap Consortium, which currently supports more than 99,000 projects and 128,000 users. More information about the consortium and system security can be found at [redacted]

In order to maintain confidentiality, only trial number, patient number, initials and year of birth will identify the patient in the CRF. If the patient's name appears on any other document (e.g. laboratory report), it must be obliterated on the copy of the document to be supplied to [redacted] and replaced instead with the patient number and patient's initials. The investigator will maintain a personal patient identification list (patient numbers with corresponding patient identifiers) to enable records to be identified and verified as authentic. Patient data/information will be kept confidential, and will be managed according to applicable local, state, and federal regulations.

All data requested on the CRF must be supported by and be consistent with the patient's source documentation. All missing data must be explained. When a required laboratory test, assessment, or evaluation has not been done or an "Unknown" box is not an option on the CRF, a note should be created verifying that the field was "Not Done" or "Unknown". For any entry errors made, the error(s) must be corrected, and a note explaining the reason for change should be provided.

15.0 SAFETY MONITORING AND REPORTING ADVERSE EVENTS

15.1 The Data and Safety Monitoring Board (DSMB) will oversee the conduct of the study. The DSMB will receive confidential reports every 6 months. The focus of these reports will be on study progress and safety. The DSMB will be

responsible for decisions regarding possible termination and/or early reporting of the study and they will have access to any available data needed to inform their decisions. The Data and Safety Monitoring Committee (DSMC) of the Vanderbilt Ingram Cancer Center (VICC) will also oversee the study safety.

15.2 The Data and Safety Monitoring Committee (DSMC)

The Vanderbilt-Ingram Cancer Center (VICC) oversees patient safety and data monitoring for its investigator-initiated and NIH-NCI funded clinical trials through its Data and Safety Monitoring Committee (DSMC). The purpose of the DSMC is to ensure the efficient implementation and management of VICC Data and Safety Monitoring Plan (DSMP). The Committee maintains authority to intervene in the conduct of studies as necessary to ensure clinical research performed at VICC achieves the highest quality standards.

The VICC DSMC meets on a quarterly basis and ad hoc to discuss data and safety monitoring of clinical trials and to oversee the VICC DSMP. Internal audits for compliance with adverse event reporting, regulatory and study requirements, and data accuracy and completion are conducted according to the VICC DSMP according to study phase and risk. The committee reviews all serious adverse events (SAE) on Vanderbilt sponsored investigator-initiated studies on a quarterly basis and provides DSMC SAE review reports to the Vanderbilt IRB.

15.3 VICC Multi-Institutional Coordinating Center

The trial additionally will be monitored by the VICC Multi-Institutional Coordinating Center. The actual frequency of monitoring will depend on the enrollment rate and performance of the site. Monitoring will be conducted through onsite and remote monitoring, teleconferences with the Investigator and site staff, and appropriate communications by mail, fax, email, or telephone. The purpose of monitoring is to ensure that the study is conducted in compliance with the protocol, standard operating procedures (SOPs), and other written instructions, and to ensure the quality and integrity of the data.

During scheduled monitoring visits, investigators and the investigational site staff must be available to discuss the progress of the trial, make necessary corrections to case report form entries, respond to data clarification requests, provide required regulatory documents, and respond to any other trial-related inquiries of the monitor.
In addition to the above, the Sponsor, CPP, and the FDA may review the conduct or results of the study at the investigational site.

15.3 Adverse Event Reporting Purpose.

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Certain adverse events must be reported in an expedited manner. Adverse events should be reported from the start of study drug through 30 days after the last dose of study treatment.

Any event that occurs more than 30 days after the last dose of study agent and is attributed (possible, probable, or definite) to the study agent(s) must be reported according to the instructions outlined in section 15.

15.4 Adverse Event Definitions.

15.4.1 Adverse Event (AE)

An adverse event is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

15.4.2 Serious Adverse Event (SAE)

A serious adverse event is an undesirable sign, symptom or medical condition which:

- is fatal or life-threatening;
- requires or prolongs inpatient hospitalization;
- results in persistent or significant disability/incapacity;
- constitutes a congenital anomaly or birth defect; or
- jeopardizes the participant and requires medical or surgical intervention to prevent one of the outcomes listed above.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission respite care

15.4.3 Expectedness

- Expected: Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.
- Unexpected: An adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk

15.4.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment
- Probable – The AE is likely related to the study treatment
- Possible – The AE may be related to the study treatment
- Unlikely - The AE is doubtfully related to the study treatment
- Unrelated - The AE is clearly NOT related to the study treatment

15.5 Adverse Event Reporting methods.

15.5.1 General Considerations

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs, and avoid colloquialisms and abbreviations. If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g. record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is acceptable to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

All deaths that occur during the protocol-specified AE reporting period, regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death." Deaths that occur during the protocol specified adverse event reporting period that are attributed by the investigator solely to progression of disease should be recorded only in the study eCRF and not reported as an SAE.

A pre-existing medical condition is one that is present prior to initiation of protocol specified treatment. Such conditions should be reported as medical and surgical history. A pre-existing medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the pre-existing condition has changed by including applicable descriptors (e.g., "more frequent headaches").

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a patient is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a patient is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for pre-existing conditions; or
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study; or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

15.5.2 Serious Adverse Event Reporting

All serious adverse events, regardless of causality to study drug, will be reported to the Principal Investigator and/or the Study Coordinator at each institution, and also to the Coordinating Center.

All serious adverse events must be reported to the Coordinating Center within 24 hours of the site becoming aware of the event. Events should be reported using the Vanderbilt Coordinating Center SAE form, located in the packet of supplemental forms. This form must be fully completed and emailed (preferred), faxed, or scanned to:

[redacted]

If SAE documents are faxed, the Coordinating Center must be notified via email as well. Follow-up information must also be reported within 24 hours of receipt of the information by the investigator.

The Coordinating Center will disseminate information regarding serious adverse events to the participating sites as described in FDA guidance only in the case that the event(s) is/are unexpected, and is/are believed to be related (i.e., possibly, probably or definitely) to the study device/medication. The Coordinating Center will be responsible for reporting of events to the FDA and supporters, as appropriate (outlined below).

15.5.3 Institutional Review Board/Ethics Committee

All adverse events and serious adverse events will be reported to the IRB/EC per current institutional standards. If an adverse event requires modification of the informed consent, these modifications will be provided to the IRB with the report of the adverse event. If an adverse event requires modification of the study protocol, these modifications will be provided to the IRB as soon as is possible.

15.5.4 Food and Drug Administration (FDA)

In this trial, unexpected serious adverse events believed to be definitely, probably, or possibly related to study treatment (as determined by the sponsor-investigator) will be reported to the FDA via MedWatch 3500A (available at https://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf.

Submissions by the Sponsor can be submitted via fax or email and must be addressed to Regulatory Project Manager in the FDA review division that has responsibility for review of the IND. The Sponsor, Cancer Prevention Pharmaceuticals, will be responsible for reporting to the FDA as appropriate.

15.5.5 Reporting to the Sponsor

The Coordinating Center will submit all SAEs, regardless of causality, to Cancer Prevention Pharmaceuticals (CPP) within 24 hours of the Coordinating Center becoming aware of the event. [redacted]

15.6 Reporting Pregnancy, Fetal Death, and Death Neonatal Pregnancy.

Study participants who become pregnant while on study; that pregnancy should be reported to the Coordinating Center using the Vanderbilt Coordinating Center SAE form within 24 hours of the site becoming aware.

Fetal Death. Fetal Death defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation" should be reported to the Coordinating Center using the Vanderbilt Coordinating Center SAE form within 24 hours of the site becoming aware.

Death Neonatal. Neonatal death is defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent or

intervention should be reported expeditiously. A neonatal death should be reported to the Coordinating Center using the Vanderbilt Coordinating Center SAE form within 24 hours of the site becoming aware

Pregnancy, Fetal Death and Death Neonatal will all be reported by the Coordinating Center to the Sponsor, Cancer Prevention Pharmaceuticals, within 24 hours of the Coordinating Center becoming aware.

16.0 BIBLIOGRAPHY

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer* 127:2893-917, 2010.
2. Goss PE, Lee BL, Badovinac-Crnjevic T, et al. Planning cancer control in Latin America and the Caribbean. *Lancet Oncol.* 14:391-436, 2013.
3. Torres J, Correa P, Ferreccio C, Hernandez-Suarez G, Herrero R, Cavazza-Porro M, Dominguez R, Morgan D. Gastric cancer incidence and mortality is associated with altitude in the mountainous regions of Pacific Latin America. *Cancer Causes Control* 24:249-56, 2013. PMCID:3697934
4. Dominguez RL, Crockett SD, Lund JL, Suazo LP, Heidt P, Martin C, Morgan DR. Gastric cancer incidence estimation in a resource-limited nation: use of endoscopy registry methodology. *Cancer Causes Control* 24:233-9, 2013. PMCID:3815449
5. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA. Cancer J. Clin.* 62:10-29, 2012.
6. Siegel R, Naishadham D, Jemal A. Cancer statistics for Hispanics/Latinos, 2012. *CA. Cancer J. Clin.*62:283-98, 2012.
7. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res.* 52:6735-40, 1992.
8. de Sablet T, Piazuelo MB, Shaffer CL, Schneider BG, Asim M, Chaturvedi R, Bravo LE, Sicinschi LA, Delgado AG, Mera RM, Israel DA, Romero-Gallo J, Peek RM, Jr., Cover TL, Correa P, Wilson KT. Phylogeographic origin of *Helicobacter pylori* is a determinant of gastric cancer risk. *Gut* 60:1189-95, 2011. PMCID:3133872
9. Mera R, Fontham ET, Bravo LE, Bravo JC, Piazuelo MB, Camargo MC, Correa P. Long term follow up of patients treated for *Helicobacter pylori* infection. *Gut* 54:1536-40, 2005.
10. Kodaman N, Pazos A, Schneider BG, Piazuelo MB, Mera R, Sobota RS, Sicinschi LA, Shaffer CL, Romero-Gallo J, de Sablet T, Harder RH, Bravo LE, Peek RM, Jr., Wilson KT, Cover TL, Williams SM, Correa P. Human and *Helicobacter pylori* coevolution shapes the risk of gastric disease. *Proc. Natl. Acad. Sci. U. S. A.* 111:1455-60, 2014.
11. Peek RM, Jr., Blaser MJ. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer* 2:28-37, 2002.
12. Blaser MJ. Ecology of *Helicobacter pylori* in the human stomach. *J. Clin. Invest.* 100:759-62, 1997.
13. Chen Y, Blaser MJ. *Helicobacter pylori* colonization is inversely associated with childhood asthma. *J.Infect. Dis.* 198:553-60, 2008.
14. Varanasi RV, Fantry GT, Wilson KT. Decreased prevalence of *Helicobacter pylori* infection in gastroesophageal reflux disease. *Helicobacter* 3:188-94, 1998.
15. You WC, Brown LM, Zhang L, Li JY, Jin ML, Chang YS, Ma JL, Pan KF, Liu WD, Hu Y, Crystal-Mansour S, Pee D, Blot WJ, Fraumeni JF, Jr., Xu GW, Gail MH. Randomized double-blind factorial trial of three treatments to reduce the prevalence of precancerous gastric lesions. *J. Natl. Cancer Inst.* 98:974-83, 2006.
16. Ma JL, Zhang L, Brown LM, Li JY, Shen L, Pan KF, Liu WD, Hu Y, Han ZX, Crystal-Mansour S, Pee D, Blot WJ, Fraumeni JF, Jr., You WC, Gail MH. Fifteen-year effects of *Helicobacter pylori*, garlic, and vitamin treatments on gastric cancer incidence and mortality. *J. Natl. Cancer Inst.* 104:488-92, 2012. PMCID:3309129
17. Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK, Chen JS, China Gastric Cancer Study G. *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 291:187-94, 2004.
18. Correa P, Piazuelo MB, Wilson KT. Pathology of gastric intestinal metaplasia: clinical implications. *Am.J.Gastroenterol.* 105:493-8, 2010. PMCID: 2895407.
19. Hardbower DM, de Sablet T, Chaturvedi R, Wilson KT. Chronic inflammation and oxidative stress: The smoking gun for *Helicobacter pylori*-induced gastric cancer? *Gut Microbes* 4:2013.
20. Chaturvedi R, de Sablet T, Peek RM, Wilson KT. Spermine oxidase, a polyamine catabolic enzyme that links *Helicobacter pylori* CagA and gastric cancer risk. *Gut Microbes* 3:48-56, 2012. PMCID:3337125
21. Chaturvedi R, Asim M, Romero-Gallo J, Barry DP, Hoge S, de Sablet T, Delgado AG, Wroblewski LE, Piazuelo MB, Yan F, Israel DA, Casero RA, Jr., Correa P, Gobert AP, Polk DB, Peek RM, Jr., Wilson KT. Spermine oxidase mediates the gastric cancer risk associated with *Helicobacter pylori* CagA. *Gastroenterology* 141:1696-708 e1-2, 2011. PMCID:3202654

22. Xu H, Chaturvedi R, Cheng Y, Bussiere FI, Asim M, Yao MD, Potosky D, Meltzer SJ, Rhee JG, Kim SS, Moss SF, Hacker A, Wang Y, Casero RA, Jr., Wilson KT. Spermine oxidation induced by *Helicobacter pylori* results in apoptosis and DNA damage: implications for gastric carcinogenesis. *Cancer Res.* 64:8521-5, 2004.
23. Chaturvedi R, Cheng Y, Asim M, Bussiere FI, Xu H, Gobert AP, Hacker A, Casero RA, Jr., Wilson KT. Induction of polyamine oxidase 1 by *Helicobacter pylori* causes macrophage apoptosis by hydrogen peroxide release and mitochondrial membrane depolarization. *J. Biol. Chem.* 279:40161-73, 2004.
24. Martinez ME, O'Brien TG, Fultz KE, Babbar N, Yerushalmi H, Qu N, Guo Y, Boorman D, Einspahr J, Alberts DS, Gerner EW. Pronounced reduction in adenoma recurrence associated with aspirin use and a polymorphism in the ornithine decarboxylase gene. *Proc. Natl. Acad. Sci. U. S. A.* 100:7859-64, 2003. PMCID:164678
25. Hubner RA, Muir KR, Liu JF, Logan RF, Grainge MJ, Houlston RS, Members of the UC. Ornithine decarboxylase G316A genotype is prognostic for colorectal adenoma recurrence and predicts efficacy of aspirin chemoprevention. *Clin. Cancer Res.* 14:2303-9, 2008.
26. Zell JA, McLaren CE, Chen WP, Thompson PA, Gerner EW, Meyskens FL. Ornithine decarboxylase-1 polymorphism, chemoprevention with eflornithine and sulindac, and outcomes among colorectal adenoma patients. *J. Natl. Cancer Inst.* 102:1513-6, 2010. PMCID:2950167.
27. Meyskens FL Jr., Gerner EW. Development of difluoromethylornithine (DFMO) as a chemoprevention agent. Clin Cancer Res 5:945-51, 1999.
28. Meyskens FL Jr., McLaren CE, Pelot D, Fujikawa-Brooks S, Carpenter PM, Hawk E, Kelloff G, Lawson MJ, Kidao J, McCracken J, Albers CG, Ahnen DJ, Turgeon DK, Goldschmid S, Lance P, Hagedorn CH, Gillen DL, Gerner EW. Difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas: a randomized placebo-controlled, double-blind trial. Cancer Prev Res 1:32-38, 2008.
29. Gerner EW, Meyskens FL Jr, Goldschmid S, Lance P, Pelot D. Rationale for, and design of, a clinical trial targeting polyamine metabolism for colon cancer chemoprevention. Amino Acids 33(2):189-95. Epub, 2007.
30. Zell JA, Ignatenko NA, Yerushalmi HF, Ziogas A, Besselsen DG, Gerner EW, Anton-Culver H. Risk and risk reduction involving arginine intake and meat consumption in colorectal tumorigenesis and survival. Int J cancer 120(3):459-68, 2007.
31. Erdman SH, Ignatenko NA, Powell MB, Blohm-Mangone K, Holubec H, Guillen-Rodriquez J, Gerner EW. APC-dependent changes in expression of genes influencing polyaminic metabolism and consequences for gastrointestinal carcinogenesis in the Min mouse. Carcinogenesis 20:1709-1713, 1999.
32. Thomas T, Thomas TJ. Polyamine metabolism and cancer. J Cell Mol Med 7:113-26, 2003.
33. Auvinen M. Cell transformation, invasion, and angiogenesis: a regulatory role for ornithine decarboxylase and polyamines? J Natl Cancer Inst 89:533-7, 1997.
34. Chaturvedi R, Asim M, Lewis ND, Algood HM, Cover TL, Kim PY, Wilson KT. L-Arginine availability regulates inducible nitric oxide synthase-dependent host defense against *Helicobacter pylori*. *Infect. Immun.* 75:4305-15, 2007.
35. Bussiere FI, Chaturvedi R, Cheng Y, Gobert AP, Asim M, Blumberg DR, Xu H, Kim PY, Hacker A, Casero RA, Jr., Wilson KT. Spermine causes loss of innate immune response to *Helicobacter pylori* by inhibition of inducible nitric-oxide synthase translation. *J. Biol. Chem.* 280:2409-12, 2005.
36. Gobert AP, Cheng Y, Wang JY, Boucher JL, Iyer RK, Cederbaum SD, Casero RA, Jr., Newton JC, Wilson KT. *Helicobacter pylori* induces macrophage apoptosis by activation of arginase II. *J. Immunol.* 168:4692-700, 2002.
37. Gobert AP, McGee DJ, Akhtar M, Mendz GL, Newton JC, Cheng Y, Mobley HL, Wilson KT. *Helicobacter pylori* arginase inhibits nitric oxide production by eukaryotic cells: a strategy for bacterial survival. *Proc.Natl. Acad. Sci. U. S. A.* 98:13844-9, 2001.
38. Wilson KT, Ramanujam KS, Mobley HLT, Musselman RF, James SP, Meltzer SJ. *Helicobacter pylori* stimulates inducible nitric oxide synthase expression and activity in a murine macrophage cell line. *Gastroenterology* 111:1524-33, 1996.
39. Gobert AP, Mersey BD, Cheng Y, Blumberg DR, Newton JC, Wilson KT. Cutting Edge: Urease release by *Helicobacter pylori* stimulates macrophage inducible nitric oxide synthase. *J. Immunol.* 168:6002-6, 2002.
40. Fu S, Ramanujam KS, Wong A, Fantry GT, Drachenberg CB, James SP, Meltzer SJ, Wilson KT. Increased expression and cellular localization of inducible nitric oxide synthase and cyclooxygenase 2 in *Helicobacter pylori* gastritis. *Gastroenterology* 116:1319-29, 1999.

41. Mannick EE, Bravo LE, Zarama G, Realpe JL, Zhang XJ, Ruiz B, Fontham ET, Mera R, Miller MJ, Correa P. Inducible nitric oxide synthase, nitrotyrosine, and apoptosis in *Helicobacter pylori* gastritis: effect of antibiotics and antioxidants. *Cancer Res.* 56:3238-43, 1996.
42. Pegg AE, McCann PP. Polyamine metabolism and function. *Am. J. Physiol.* 243:C212-21., 1982.
43. Marton LJ, Pegg AE. Polyamines as targets for therapeutic intervention.
Annu. Rev. Pharmacol. Toxicol. 35:55-91, 1995.
44. Casero RA, Jr. Say what? The activity of the polyamine biosynthesis inhibitor difluoromethylornithine in chemoprevention is a result of reduced thymidine pools? *Cancer Discov.* 3:975-7, 2013. PMCID:3786367
45. Ray RM, McCormack SA, Johnson LR. Polyamine depletion arrests growth of IEC-6 and Caco-2 cells by different mechanisms. *Am. J. Physiol. Gastrointest. Liver Physiol.* 281:G37-43, 2001.
46. Thomas T, Thomas TJ. Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. *Cell. Mol. Life Sci.* 58:244-58, 2001.
47. Li L, Rao JN, Bass BL, Wang JY. NF-kappaB activation and susceptibility to apoptosis after polyamine depletion in intestinal epithelial cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* 280:G992-G1004, 2001.
48. Messa C, Di Leo A, Greco B, Caradonna L, Amati L, Linsalata M, Giorgio I, Jirillo E. Successful eradicating treatment of *Helicobacter pylori* in patients with chronic gastritis: gastric levels of cytokines, epidermal growth factor and polyamines before and after therapy. *Immunopharmacol. Immunotoxicol.* 18:1-13., 1996.
49. Linsalata M, Russo F, Notarnicola M, Berloco P, Di Leo A. Polyamine profile in human gastric mucosa infected by *Helicobacter pylori*. *Ital. J. Gastroenterol. Hepatol.* 30:484-9., 1998.
50. Ha HC, Woster PM, Yager JD, Casero RA, Jr. The role of polyamine catabolism in polyamine analogue induced programmed cell death. *Proc. Natl. Acad. Sci. U. S. A.* 94:11557-62., 1997.
51.. Zell JA, Pelot D, Chen WP, McLaren CE, Gerner EW, Meyskens FL. Risk of cardiovascular events in a randomized placebo-controlled, double-blind trial of difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas. Cancer Prev Res (Phila Pa) 2:209-12, 2009.
52. McLaren CE, et al. Detailed ototoxicity assessment in the difluoromethylornithine plus sulindac for the Prevention of sporadic colorectal adenomas. Cancer Prev Res 1(7):514-521, 2008. 53. Gutman S. Draft guidance for industry and FDA staff — pharmacogenetic tests and genetic tests for heritable markers.
http://www.fda.gov/cdrh/oivd/guidance/1549.html US Food and Drug Administration, 2006.
54. Zell JA, Ziogas A, Ignatenko N, Honda J, Qu N, Bobbs AS, Neuhausen SL, Gerner EW, Anton-Culver H. Associations of a polymorphism in the ornithine decarboxylase gene with colorectal cancer survival. Clin Cancer Res 1;15(19):6208, 2009.
55. Martinez ME, O'Brien TG, Fultz K.E, Babbar N, Yerushalmi H, Qu N, Guo Y, Boorman D, Einspahr J, Alberts DS, Gerner EW. Pronounced reduction in adenoma recurrence associated with aspirin use and a polymorphism in the ornithine decarboxylase gene. Proc Natl Acad Sci U S A 100:7859-7864, 2003.
56. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am. J. Surg. Pathol.*20:1161-81, 1996.
57. Gonzalez CA, Sanz-Anquela JM, Gisbert JP, Correa P. Utility of subtyping intestinal metaplasia as marker of gastric cancer risk. A review of the evidence. *Int. J. Cancer* 133:1023-32, 2013. PMCID:3732516
58. Gonzalez CA, Pardo ML, Liso JM, Alonso P, Bonet C, Garcia RM, Sala N, Capella G, Sanz-Anquela JM. Gastric cancer occurrence in preneoplastic lesions: a long-term follow-up in a high-risk area in Spain. *Int.J. Cancer* 127:2654-60, 2010.
59. Greenberg ER, Anderson GL, Morgan DR, Torres J, Chey WD, Bravo LE, Dominguez RL, Ferreccio C, Herrero R, Lazcano-Ponce EC, Meza-Montenegro MM, Pena R, Pena EM, Salazar-Martinez E, Correa P, Martinez ME, Valdivieso M, Goodman GE, Crowley JJ, Baker LH. 14-day triple, 5-day concomitant, and 10-day sequential therapies for *Helicobacter pylori* infection in seven Latin American sites: a randomized trial. *Lancet* 378:507-14, 2011. PMCID:3313469
60. Morgan DR, Torres J, Sexton R, Herrero R, Salazar-Martinez E, Greenberg ER, Bravo LE, Dominguez RL, Ferreccio C, Lazcano-Ponce EC, Meza-Montenegro MM, Pena EM, Pena R, Correa P, Martinez ME, Chey WD, Valdivieso M, Anderson GL, Goodman GE, Crowley JJ, Baker LH. Risk of recurrent *Helicobacter pylori* infection 1 year after initial eradication therapy in 7 Latin American communities. *JAMA* 309:578-86, 2013. PMCID:3697935

61. Dinis-Ribeiro M, Areia M, de Vries AC, et.al., European Society of Gastrointestinal E, European Helicobacter Study G, European Society of P, Sociedade Portuguesa de Endoscopia D. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). *Endoscopy* 44:74-94, 2012. PMCID:3367502
62. Wolfsen HC, Hemminger LL, Achem SR, Loeb DS, Stark ME, Bouras EP, DeVault KR. Complications of endoscopy of the upper gastrointestinal tract: a single-center experience. Mayo Clin Proc. 2004;79(10):1264.
63. Mamont PS, Duchesne MC, Grove J, Bey P. Anti-proliferative properties of DL-alpha-difluoromethyl ornithine in cultured cells. A consequence of the irreversible inhibition of ornithine decarboxylase.
Biochem. Biophys. Res. Comm. 1978; 81(1):58-66.
64. Priotto G, Kasparian S, Mutombo W, et. al. Nifurtimox-efl ornithine combination therapy for second-stage African Trypanosoma brucei gambiense trypanosomiasis: a multicenter, randomized, phase III, non-inferiority trial.
Lancet. 2009; 374(9683):56-64.

17.0 APPENDIX

17.1 Eflornithine (DFMO) Hydrochloride Supplementary Information

17.2 Standard endoscopy gastric biopsy mapping

17.3 Emergency Unblinding Guidelines

17.1 Eflornithine (DFMO) Hydrochloride Supplementary Information

Eflornithine was synthesized by Merrell Research Institute in the 1970's.[63]. It was originally synthesized as a potential cancer therapeutic agent. Subsequent clinical trials in both Europe and the U.S. failed to establish efficacy of this drug for therapy of any specific cancer.[29] More recent clinical investigations have focused on the ability of eflornithine to prevent or treat risk factors for cancer.[29] Eflornithine has also been developed for treatment of other human conditions, including human African trypsanosomiasis (HAT) and excess facial hair in women. The tables summarize the status of current eflornithine approvals, as well as ongoing chemoprevention and treatment clinical trials.

Eflornithine (trade name Ornidyl) has been used to treat human African trypanosomiasis (HAT). Aventis, then the manufacturer of Ornidyl halted its production in 1995 for economic reasons. In 2001, Sanofi-Aventis agreed to resume manufacturing for a 5 year period and at a financial commitment of US$5 million per year. No publicly available summary is available of total HAT patients treated to date with Ornidyl. One recent publication reports the results of 287 patients treated with Ornidyl alone or in combination with Nifurtimox. [64]

The status of current approvals of eflornithine

Trade name	Dose Form	Company	U.S. NDA	Approval dates	Indications	Populations	Countries approved
Ornidyl	200 mg/ml injectable	Sanofi Aventis	019878	11-28-1990	HAT	Sub-Saharan Africa	USA*
Vaniqa	Topical Cream-13.9%	Bristol-Myers Squibb	021145	07-27-2000	Facial hair	Women	39 countries**
Eflornithine	Oral	ILEX	302087+	02-19-2002	FAP***	FAP	EU++

Notes:

*marketing withdrawn; marketing rights transferred to World Health Organization (WHO)

**including the United States, Canada, the European Union, and major Latin American countries

***FAP, Familial adenomatous polyposis

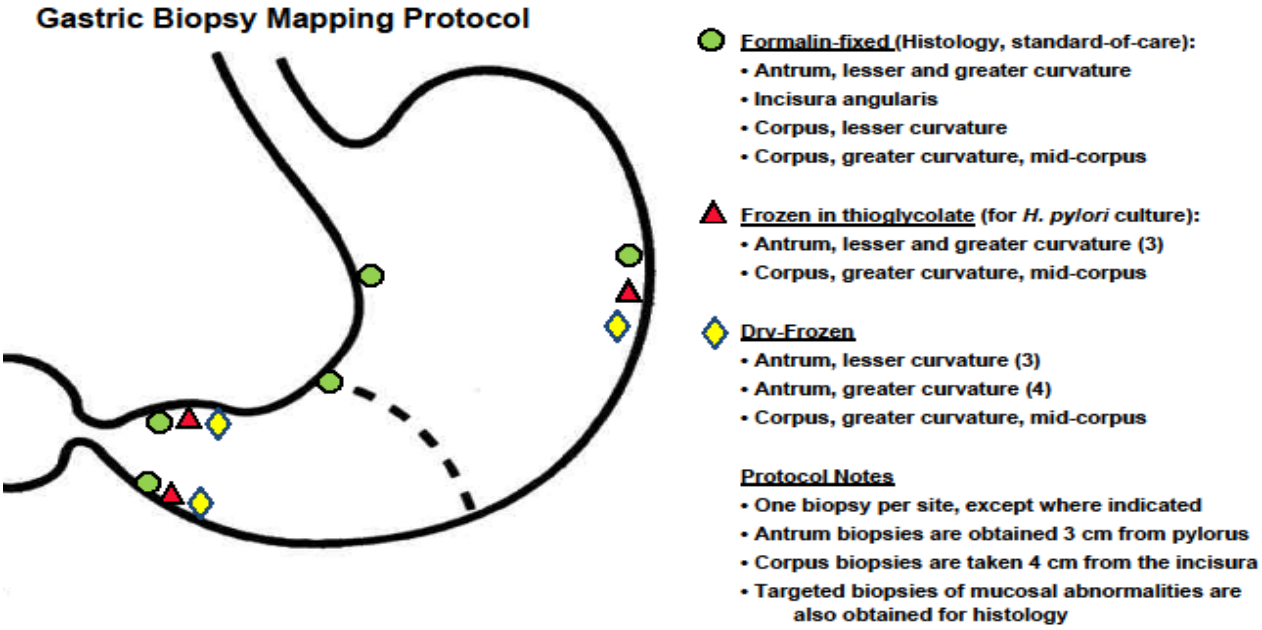
+European Union (EU) number

++ orphan drug; withdrawn from the Community Register of designated Orphan Medicinal Products in April 2003 on request of the sponsor.

Ongoing clinical trials with eflornithine

Trial	Patient Risk Group	Treatment Arms	Treatment Duration, Number of Patients	Start Date (actual/projected)
Colon adenomas SWOG S0820	Moderate	? Placebo ? DFMO ? Sulindac ? DFMO plus Sulindac	3 years, 1340 patients	3rd Quarter 2013
FAP (adult) FAP-310	High	? DFMO plus sulindac ? DFMO plus sulindac placebo ? Sulindac plus DFMO placebo	2 years, 150 patients	4th Quarter 2013
Colorectal Cancer	High	? Placebo ? DFMO plus aspirin	1 year, 104 subjects	3rd Quarter 2009
Neuroblastoma NMTRC003	High	? DFMO	2 years, 74 subjects	2nd Quarter 2012
Neuroblastoma NMTRC006	High	? DFMO	1 year, 24 subjects	2nd Quarter 2012
Neuroblastoma NMTRC010	High	? DFMO plus velcade	3.5 years, 44-62 subjects	2nd Quarter 2014
Neuroblastoma NANT N2012-01	High	? DFMO plus celecoxib, cyclophosphamide, and topotecan	1 year, up to 24 subjects	2nd Quarter 2014

17.2 Standard endoscopy gastric biopsy mapping



Biopsy mapping of the gastric mucosa for histopathology is part of usual care of patients at study sites, and adherent to the local standards and the emerging international standards for stomach precancerous lesions.[61] Five - six formalin-fixed biopsies are obtained from the antrum (2), corpus (2), and incisura (1-2). Two sets of research small pinch biopsies are obtained for the study, preserved for study analyses.

Pathology criteria. To be eligible to participate, subjects must have the histology-based diagnosis of a gastric precancerous lesion, either chronic atrophic gastritis (CAG) and intestinal metaplasia (IM). The updated Sydney system for gastritis provides standard assessment of the CAG and IM diagnosis, and includes severity and associated inflammation.[56] The histology assessment and Sydney system will be recorded at study entry, and at each study endoscopy. While dysplasia is considered a precancerous lesion, patients with dysplasia are not eligible for participation.

Additional pathology assessment. The histopathology score accurately quantifies the histology for the atrophy and intestinal metaplasia, and dysplasia categories; this scoring system has been shown to be precise and reliable, including for longitudinal cohort studies. [8-10] The subtypes of intestinal metaplasia, incomplete ("colonic-type") versus complete ("small intestinal type"), and extensive versus limited, may further stratify risk, and are part of the baseline evaluation and each endoscopy. Incomplete IM may reflect a higher risk subset, and is the most common premalignant condition among the patient populations in this study. [57, 58]

17.3 Guidelines for Emergency Unblinding of Coded Drug

A. The following events MAY require emergency unblinding of Coded Drug:

- A compelling medical need as determined by a physician, e.g., occurrence of a severe or life-threatening reaction, inclusive of an adverse drug reaction, which may have been attributable to Coded Drug, or existence of a condition where the knowledge of the patient's treatment assignment would directly influence his/her immediate care;
- Ingestion of the Coded Drug by persons other than the patient or in excessive quantity;
- Exposure of a pregnant woman to the Coded Drug;
- Exposure of a child to the Coded Drug;

B. Procedure for emergency unblinding

The procedure for unblinding the treatment assignment for a patient is as follows:

- All unblinding must be done by the registering physician or designee.
- Contact the Vanderbilt Ingram Cancer Center (VICC) Coordinating Center for unblinding calls.
  The Coordinating Center contact information: 3322 West End Avenue, Suite 1000, Nashville, TN, 37203, USA.
  Coordinating.Center@vumc.org
- Provide the CTSR with the following information:
  - Study number: **XXXX**
  - Patient number
  - Patient name
  - Name and telephone number of the caller
  - Reason unblinding is required
- Unblinding for ingestion of the Coded Drug by a child will not require the authorization of a resource physician.
- Unblinding for ingestion of the Coded drug by a pregnant woman will not require the authorization of a physician.
- Unblinding for ingestion of the drug either in excessive amounts or by a person other than the patient will be done ONLY when a compelling medical need exists and/or unblinding has been authorized by a resource physician.
- Unblinding for a "compelling medical need" must be authorized by a physician designated as a resource physician for this protocol. The treating physician (or designee) would provide the Coordinating Center with the information needed to determine if unblinding is required for the patient. Based on the decision of the resource physician, the Coordinating Center would call the treating physician with either the unblinded treatment assignment or a treatment recommendation from the resource physician. If a resource physician cannot be reached, treatment of the patient should proceed as if the drug ingested were an active agent.
- Unblinding of Coded Drug for any reason must be documented.