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Effect of Aspirin on Biomarkers of Barrett's Esophagus After Successful Eradication of Barrett's Esophagus with Radiofrequency Ablation

Consortium Name: **University of Texas MD Anderson Early Phase Clinical Research Consortium**

Name of Consortium Principal Investigator:
Powel H. Brown, M.D., Ph.D.
Professor and Chair, Department of Clinical Cancer Prevention
University of Texas MD Anderson Cancer Center
1515 Holcombe Boulevard, Unit 1360
Houston, TX 77030-4009
Telephone: (713) 792-4509
Fax: (713) 794-4679
phbrown@mdanderson.org

MD Anderson Consortium Administrative Director:
Lana A. Vornik, M.H.A., M.S.
Associate Director, Research Planning and Development
Department of Clinical Cancer Prevention
University of Texas MD Anderson Cancer Center
1515 Holcombe Boulevard, Unit 1360
Houston, TX 77030-4009
Telephone: (713) 792-9594
Fax: (713) 745-4230
lavornik@mdanderson.org

Organization Name: University of Texas MD Anderson Cancer Center
Protocol Principal Investigator:
Robert S. Bresalier, M.D.
Department of Gastroenterology, Hepatology and Nutrition
The University of Texas MD Anderson Cancer Center
1515 Holcombe Boulevard Unit 1466
Houston, Texas 77030
713-745-4340
713-745-9295
rbresali@mdanderson.org

Organization Name: University of Texas MD Anderson Cancer Center
Investigator:
Marta Davila, M.D.
15151 Holcombe Boulevard Unit 1466
Houston, Texas 77030-4900
Tel: 713-563-8906
mdavila@mdanderson.org

Organization Name: University of Texas MD Anderson Cancer Center
Investigator: PeiyingYang, Ph.D. †
Integrative Medicine Research
1515 Holcombe Boulevard Unit 1414
Houston, Texas 77030-4009
Tel: 713-563-7531
pyang@mdanderson.org

Organization Name: University of North Carolina School of Medicine
Protocol Co-Principal Investigator: Nicholas Shaheen, M.D.*
CB#7080, 130 Mason Farm Rd, Suite 4150
Chapel Hill, NC 27599-7080
(919) 962-9791
nicholas_shaheen@med.unc.edu

Organization Name: Mayo Clinic Rochester
Protocol Co-Principal Investigator: Navtej S. Buttar, M.D.*
Mayo Clinic Department of Gastroenterology and Hepatology
200 First Street SW
Rochester, MN 55905
Tel: 507-284-2511
Fax: 507-255-7612
Email: buttar.navtej@mayo.edu

Organization Name: Mayo Clinic Rochester
Investigator: Prasad G. Iyer, M.D.
Mayo Clinic Department of Gastroenterology and Hepatology
200 First Street SW
Rochester, MN 55905
Tel: 507-284-6930
Fax: 507-255-7612
Email: iyer.Prasad@mayo.edu

Organization Name: Kansas City VA Hospital
Protocol Co-Principal Investigator: Prateek Sharma, M.D.*
Gastroenterology
4801 E. Linwood Blvd. (111)
Kansas City, MO 64128
Tel: 816-861-4700, ext 57456
Fax: 816-922-3362
E-mail: psharma@kumc.edu

Organization Name: Baylor College of Medicine
Investigator: Hashem B. El-Serag, M.D.
Michael E. DeBakey VA Medical Center
2002 Holcombe (152)
Houston, Texas 77030
Tel: 713-794-8640
Email: hasheme@bcm.tmc.edu

Organization Name: Baylor College of Medicine
Investigator: G. Avinash Ketwaroo, M.D. MSc
Michael E. DeBakey VA Medical Center
(Mail code: 111-D)
2002 Holcombe Boulevard
Houston, Texas 77030
Tel: 713-791-1414 (work)
617-955-7517 (cell)
Fax: 713-794-7472
Email: gyanprakash.ketwaroo@bcm.edu

Organization Name: Northwestern University
Investigator: Srinadh Komanduri, M.D.
Feinberg School of Medicine
Chicago, Ill
Tel: 312-933-4873
sri-komanduri@northwestern.edu

Organization Name: St. Michael's Hospital
Investigator: Norman Marçon, M.D.
30 Bond Street – Suite 16-062
Toronto, Ontario, Canada M5B 1W8
Tel: 416- 864-3092
norman.marcon@utoronto.ca

Organization Name: University of California, Los Angeles
Investigator: V. Raman Muthusamy, M.D.
Gastroenterology/Interventional Endoscopy
200 UCLA Medical Plaza, Room 330-37
Los Angeles, CA 90095
Tel: 310-825-1892
Fax: 310-825-5492
raman@mednet.ucla.edu

Organization Name: University of Colorado Anschutz Medical Campus
Investigator: Sachin Wani, MD
Medical Co-Director Esophageal and Gastric Center
Therapeutic and Interventional Endoscopy
1635 Aurora Ct, Rm 2.031
Aurora, CO 80045
Telephone Academic: 720-848-2786
Clinical: 720-848-2746
Fax: 720-848-2749
E-mail sachin.wani@ucdenver.edu

Organization Name: University of Pennsylvania
Investigator: Gary Falk, MD, MS
Gastroenterology
1 Convention Avenue
9th Floor Penn Tower Building

Division of Gastroenterology
Philadelphia, PA 19104
Tel: 215-662-7655
Fax: 215-615-3756
Email: gary.falk@uphs.upenn.edu

Organization Name: Baylor University Medical Center at Dallas

Investigator: Rhonda F. Souza, MD, AGAF, FASGE †
Investigator/Professor
Co-Director, Center for Esophageal Research
Baylor Scott & White Research Institute
Baylor University Medical Center
Center for Esophageal Research
2 Hoblitzelle, Suite 250
3500 Gaston Avenue
Dallas, TX 75246
Office phone: 214 865-2243
Email: Rhonda.Souza@BSWHealth.org

MD Anderson Consortium Statistician: University of Texas MD Anderson Cancer Center
J. Jack Lee, Ph.D.
1515 Holcombe Boulevard, Unit 447
Houston, TX 77230
Telephone: (713) 794-4158
Fax: (713) 563-4242
jjlee@mdanderson.org

*Protocol Co-Principal Investigators
†Biomarker Laboratories

Sponsor: NCI/Division of Cancer Prevention
9609 Medical Center Drive, Rm 5E-536
Rockville, MD 20850
(240) 276-7050

IND# N/A

Agent(s)/Supplier: NCI DCP

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SCHEMA

Effect of Aspirin on Biomarkers of Barrett's Esophagus After Successful Eradication of Barrett's Esophagus with Radiofrequency Ablation

Study Population: Participants who have received radiofrequency ablation for Barrett's Esophagus (BE) and have documentation of complete ablation on at least 2 occasions 3 months apart (second demonstration of ablation may be qualifying exam)

↓

Screening to determine initial eligibility (see inclusion/exclusion criteria) Chart review and telephone interview: No invasive cancer, no contraindication to aspirin use (bleeding diathesis, platelet disorder, history of GI or intracranial bleed on aspirin, previous UGI surgery, renal insufficiency), inability to tolerate aspirin, no regular aspirin/NSAID use for 1 month, no medical contraindications)

↓

Consent / Registration: Followed by qualifying endoscopic exam. This exam will be clinically indicated second clearing or subsequent surveillance exam to document complete eradication of BE (completion of ablation should have occurred no greater than 36 months prior to randomization). Clinically indicated biopsies (Seattle protocol) will be taken and sent to local Pathologist. Baseline study biopsies 1 cm above GE junction, midway through area defined by length and location of previous Barrett's segment, 2 cm above extent of original Barrett's segment: 2 biopsies in each of 4 quadrants at each location (1 processed for immunohistochemistry, remainder snap frozen)

↓

Eligibility Confirmation
Participants have no residual BE (biopsy documented)
Laboratory tests (CBC, platelets, creatinine, pregnancy test in persons of childbearing potential) WNL and normal physical exam

↓

Randomization

↓

Intervention
Duration 12 months (+/-30 days)
Aspirin 325 mg/day or
Placebo

↓

Scheduled Visits
At Month 1: Interview by phone regarding somatic symptoms, illnesses, doctor's visits, medication, over-the-counter medications, compliance with study medications.
Every 3 months during entire course of study: Interview by phone or mail regarding somatic symptoms, illnesses, doctor's visits, medication, over-the-counter medications, compliance with study medications. Dispensation of study agent by mail.
At one year: As above plus SF-36 Health Survey and Interval Questionnaire plus follow-up exam
Toxicity Assessment at Months 1,3,6,9 by follow-up phone calls or mail and in person at 1 year and 18 months

↓

Post-Treatment EGD and Tissue Acquisition at 12 and 18 months after randomization
EGD will be performed and clinically indicated biopsies (Seattle protocol) will be taken at scheduled surveillance exam and sent to local Pathologist. Study biopsies 1 cm above GE junction, midway through area defined by length and location of previous Barrett's segment, 2 cm above extent of original Barrett's segment: 2 biopsies in each of 4 quadrants at each location (1 processed for immunohistochemistry, remainder snap frozen)
Physical exam and laboratories at 12 months

↓

1. **Primary Endpoints:**
a) Difference in the change of tissue CDX2 mRNA levels in esophageal mucosa between participants taking aspirin and placebo at 12 months
2. **Secondary Endpoints**
a) Safety Profile (Toxicity Assessment) and tolerability of aspirin at 12 months.
b) Difference in the change of tissue CDX2 mRNA levels in esophageal mucosa between participants taking aspirin and placebo at 18 months
c) Differences in activation of NF-κB between groups by assessing levels of total and phospho-p65 and cytoplasmic to nuclear translocation of phospho-p65
d) Differences at 12 and 18 months in prostaglandin E2 levels
e) Differences in the expression of proinflamatory cytokines TNFα, Il-1β, Il-6, IL-10, Il-17A, IL-23

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1. OBJECTIVES

1.1 Primary Objectives – We plan to conduct a randomized, double blind, placebo-controlled Phase II chemoprevention trial, investigating **whether supplementation with aspirin 325 mg/day for 12 months is safe and reduces the expression of CDX2 mRNA (a biomarker which has been associated with the risk of developing Barrett's Esophagus [BE]) in comparison to placebo after successful radiofrequency ablation (RFA).**

1.2 Secondary Objectives – Secondary endpoints will assess safety at 12 months. Secondary endpoints will also assess differences in the expression of CDX2 at 18 months, **activation status of NF-kB** by assessing levels of total and phospho-p65 and cytoplasmic to nuclear translocation of phospho-p65 which is likely to be affected by aspirin. Additional secondary endpoint markers will be the prostanoid marker, **prostaglandin E_2**, and prostaglandin synthases, which are known to respond to aspirin and to correlation with clinicopathological factors in the esophageal cancer PGE2 was previously shown to be reduced in Barrett's mucosa in response to a combination of acid inhibition and high-dose (but not low dose) aspirin. Furthermore, differences in the expression of proinflammatory cytokines known to induce activation of NFkB, i.e., **TNFα, IL-1β, IL-6, IL-10, IL-17A, IL-23** will be measured. These inflammatory mediators have been implicated in the pathogenesis of Barrett's Esophagus and esophageal adenocarcinoma. **Recurrent Barrett's Esophagus** (at 12 and 18 months) will be ascertained completely and accurately. **While we will not have sufficient power to assess differences in histological recurrence of BE during the short course of the study, we will examine this as an exploratory secondary endpoint.** ***This study will generate data designed to lead to the submission of an adequately powered multi-center prospective chemoprevention trial with histologic recurrence of BE after RFA as the clinically relevant primary endpoint.***

2. BACKGROUND

2.1 Study Disease: Barrett's Esophagus (BE)

The incidence of adenocarcinoma of the esophagus (EA) has increased dramatically over the past three to four decades in western countries. This increase has occurred in all race and gender groups, most dramatically in white males. According to data from the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program, the incidence of esophageal adenocarcinoma (EAC) in white males rose from 0.8 per 100,000 in 1973 to 5.4 per 100,000 in 2002, a near-annual increase of 8% . Barrett's esophagus (BE) is being increasingly recognized in western countries and is widely considered the major risk factor for EAC (1). Activation of signaling pathways may facilitate the healing of reflux-damaged squamous cells through metaplasia (manifested as BE). Identifying these pathways could provide molecular targets for chemoprevention to prevent Barrett's Esophagus from recurring in patients who have undergone endoscopic ablation of Barrett's epithelium. Molecular, biochemical, and immunohistochemical studies have focused on potential pathways and markers that may result in Barrett's metaplasia and subsequent transformation and progression to adenocarcinoma (1-4). Cancers are believed to arise through the development of genetic instability and clonal expansion driven by selection for mutations in cancer genes. A combination of genetic instability and clonal expansion is associated with progression to adenocarcinoma in BE. Chronic exposure to both acid and bile in the gastroesophageal refluxate promotes damage and inflammation in the esophageal epithelium which may trigger these changes (3). Within the epithelial cells, the combination of reactive oxygen species (ROS) damage, alterations in DNA methylation, activation of the NFKB signaling pathway by cytokines and the acid/bile refluxate contribute to activation of target genes that further amplify inflammation and promote intestinal differentiation (5-7). These changes are associated with biological and morphological changes which are manifested as "biomarkers" which are associated with disease progression. Cdx homeotic genes of the

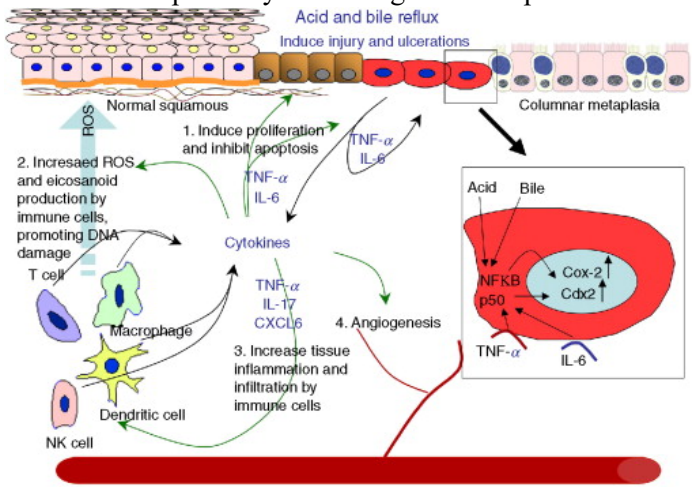
para-homoeobox family appear to direct the formation of simple columnar epithelia, and the metaplastic epithelium of BE express CDX messenger RNA and protein (5, 8). **Existing data from our group (see below) suggest that acid and bile salts induce CDX2 in esophageal squamous cells from patients with BE, but not from GERD patients without BE** (8). These differences may underlie the development of BE and could serve to predict development of metaplasia both de novo and after ablation.

2.2 Study Agent: Aspirin 325 mg/day for 12 months

Endoscopic ablative techniques such as radiofrequency ablation (RFA) have provided an alternative to surgery in treating high-risk lesions including high grade dysplasia associated with BE (9,10). Long-term follow-up has, however demonstrated recurrence of BE after RFA ranging from 8% to 25% (9-12). Long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs), especially **aspirin**, has been associated in observational studies with a decreased risk of EAC (OR 0.54 in one pooled analysis) and a decreased risk of neoplastic progression in BE (13). NSAIDs may be especially associated with decreased EAC risk in individuals with high-risk markers predictive of conversion to EAC (4). Aspirin may play a protective role against the development of EAC by inhibiting chronic inflammation and NFkB-dependent transcriptional induction of CDKN2A and prostaglandin biosynthesis genes which have been shown to contribute to carcinogenesis (4, 5,13). Aspirin-induced suppression of NF-KB target genes is significant, because acid and bile salts activate NF-kB to induce CDX2, which has been recently linked to the development of Barrett's metaplasia (8). Using PGE2 concentrations as a surrogate for NF-KB activation levels, aspirin (325 mg/day) in combination with acid inhibition significantly reduces NF-KB activation in esophageal tissue from individuals with BE (14) Moreover, aspirin has been used extensively in chemoprevention trials of gastrointestinal and other forms of neoplasia with an acceptable safety profile (15).

2.3 Rationale

CDX2 and the Development of Intestinal Metaplasia: Metaplastic conditions like BE are associated with a high rate of cell turnover that may be stimulated by chronic inflammation (16). The continuous cycle of injury and repair that accompanies chronic inflammation predisposes to alterations in the pattern of gene expression by the epithelial cells. Metaplasias occur when such alterations affect homeotic genes, like CDX2, that control tissue phenotypes. In one study of esophageal biopsy specimens taken from patients with BE, CDX2 expression levels were approximately 400 times higher in the Barrett's metaplasia than in the normal esophageal squamous epithelium, in which the levels were almost undetectable (17). In fact, CDX2 has been implicated as the "master switch" in the formation of Barrett's metaplasia. In a rat model of reflux esophagitis and intestinal metaplasia, basal cells in the esophageal squamous epithelium begin to express CDX2 prior to the development of intestinal metaplasia suggesting that CDX2 expression precedes the development of Barrett's metaplasia and that GERD may initiate the development of intestinal metaplasia by increasing CDX2 expression in esophageal stem cells (18,19).



Reflux Esophagitis and the Induction of CDX2*:* There are at least two ways in which GERD might induce CDX expression in the esophagus: 1) Certain components of the refluxed gastric juice (e.g. acid, bile salts) stimulate CDX expression by esophageal stem cells or 2) Esophageal inflammation (reflux esophagitis) stimulates CDX expression by esophageal stem cells**.** Following ablation of BE, re-epithelialization by squamous cells is favored in the setting of acid suppression

whereas recurrent Barrett's epithelium occurs when acid suppression is inadequate (20,21). Thus, it is recommended that patients with BE should take PPIs as a means to prevent recurrence in the setting of ongoing reflux (22). However, PPIs do not correct the underlying reflux diathesis and *Barrett's patients who take PPIs still have frequent reflux of weakly acidic (pH 4-7) material (including bile acids) (23).* Perhaps this is why a substantial proportion of patients with BE have recurrence of intestinal metaplasia following RFA. Differences in gene expression profile within the regenerated squamous epithelium may predict which Barrett's patients recur and subsequently require recurrent endoscopic intervention. We have previously shown that esophageal squamous epithelial cells from GERD patients with BE increase the expression and activation of CDX2 in response to exposure to acid, bile salts, and the combination of both. **In contrast, esophageal squamous cells from patients who have GERD without Barrett's Esophagus fail to induce CDX2 expression in response to any of these exposures** (8)**.** Our preliminary data demonstrate that compared to squamous cells from Barrett's patients, squamous cells from GERD patients exposed to acid and bile salts exhibit less phosphorylation of IKKα/β and IKB, and less nuclear translocation of p50. Unlike the squamous cells from Barrett's patients, furthermore, the GERD squamous cells show no nuclear translocation of p65, and p50 does not bind their CDX2 promoter. In squamous cells from Barrett's patients, knockdown of p65 by siRNA inhibits the induction of the CDX2 promoter suggesting that nuclear translocation of p65 is functionally relevant for the induction of CDX2 in response to acid and bile salts. These data suggest that phenotypic differences among patients in how the NF-KB pathway in their esophageal squamous cells responds to gastric reflux might determine whether they develop Barrett's metaplasia. We propose to investigate the chemopreventive potential of aspirin, which has been shown to affect the expression and activation of NF-KB related pathways in the esophagus. CDX2 expression will serve as a surrogate marker of recurrence of Barrett's metaplasia after RFA.

3. SUMMARY OF STUDY PLAN

3.1 Target Population

- Age ≥ 18 years
- Known diagnosis of histologically-confirmed BE with or without dysplasia (as defined by the presence of specialized columnar epithelium anywhere in the tubular esophagus with ≥ 1 cm of circumferential or non-circumferential involvement of specialized columnar epithelium) requiring radiofrequency ablation.
- Documentation of complete ablation of BE after radiofrequency ablation on two endoscopic examinations at least 3 months apart (including no evidence of BE on surveillance and biopsies). Completion of ablation should have occurred no greater than 36 months prior to randomization.

3.2 Sample Size and Duration of Accrual

This is a randomized, double-blind placebo controlled study with 2 intervention arms. We plan to enroll up to 20 participants per group.

3.3 Intervention Arms

Participants who have successfully completed radiofrequency ablation for BE with or without dysplasia and who have been histologically-confirmed to be free of Barrett's will be randomly assigned to a 12 month intervention with:

Arm A - aspirin 325 mg PO QD
Arm B – aspirin placebo QD

All participants will take proton pump inhibitor in the FDA approved healing dose as clinically

indicated (omeprazole or S-omeprazole 40 mg twice daily, lansoprazole 30 mg twice daily or equivalent).

Participants will continue study agent up to the day of the 12-month endoscopic assessment (see below).

Study Assessments
Endoscopic examination of the esophagus will be performed as part of a clinically prescribed protocol for patients undergoing radiofrequecy ablation for BE. Participants who have documentation of complete ablation on at least 2 separate occasions 3 months apart will be eligible to participate in the study. An endoscopy performed for a second demonstration of ablation may serve as a qualifying exam. Clinically indicated biopsies will be performed using the Seattle protocol and sent for local Pathology assessment. Additional study biopsies will be performed at baseline and at 12 and 18 months in locations 1 cm above the gastroesophageal junction, midway through an area defined by the length and location of the pre-ablation Barrett's segment, and 2 cm above the upper extent of the original Barrett's segment. Standardization of endoscopic techniques, assessment of landmarks and tissue procurement methods across Participating Organizations will be achieved through a series of pre-trial discussions with the Protocol Lead Investigators. Details of the biopsy protocol are provided in Section 7.6.1 and 7.6.2.

All participants will have a telephone interview at Month 1 regarding symptoms possibly related to the agent, illnesses, doctors' visits, hospitalizations, mediations (including over the counter medications), and compliance with study tablets. Adherence with the study medication will be discussed and reinforced; pill diary will be reviewed.

All participants will have a telephone interview and/or be contacted by mail every 3 months regarding symptoms possibly related to the agent, illnesses, doctors' visits, hospitalizations, mediations (including over the counter medications), and compliance with study tablets.

Participants will undergo a clinically indicated surveillance EGD at 12 months following randomization at which time clinically indicated surveillance biopsies and research biopsies will be obtained. End of treatment questionnaires and SF-36 Health Surveys, physical exam and laboratories will also be performed.

Participants will be followed off aspirin or placebo for an additional 6 month period (with an interval phone interview at 13 months post-randomization). Participants will undergo a clinically indicated surveillance EGD at 18 months following randomization at which time clinically indicated surveillance biopsies and research biopsies will be obtained.

Expression of CDX2 mRNA and differences in the activation status of NF-κB will be analyzed in the laboratory of Dr. Rhonda Souza at the Center for Esophageal Research, Baylor Scott & White Research Institute, Dallas, TX. Prostaglandin E2 levels and expression of pro-inflammatory cytokines will be assessed in the laboratory of Dr. Peiying Yang Ph.D. at the University of Texas MD Anderson Cancer Center.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Age ≥ 18 years

4.1.2 Known diagnosis of histologically-confirmed BE with or without dysplasia (as defined by the presence of specialized columnar epithelium anywhere in the tubular esophagus with ≥ 1 cm of circumferential or non-circumferential involvement of specialized columnar epithelium) requiring radiofrequency ablation.

4.1.3 Documentation of complete ablation of BE after radiofrequency ablation on two endoscopic examinations at least 3 months apart (including no evidence of BE on surveillance biopsies) as determined by the pathologist at each site. Completion of ablation should have occurred no greater than 36 months prior to randomization.

4.1.4 The effects of the candidate chemoprevention agents on the developing human fetus remain incompletely defined. For this reason, persons of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a participant become pregnant or suspect she is pregnant while participating in this trial, she should inform the research personnel and her clinical care provider immediately.

4.1.5 Willingness to provide tissue samples for research purposes.

4.1.6 No chronic use of aspirin or NSAIDs or selective COX-2 inhibitors during one month prior to randomization. Chronic use is defined as any aspirin or NSAID use on ≥ 7 days during one month preceding the beginning of randomization.

4.1.7 Normal organ and marrow function, as defined below, based on laboratory studies obtained ≤ 45 days prior to randomization:

- Hemoglobin ..... ≥10 g/dL or Hematocrit ≥ 30 %
- Leukocyte count..... ≥3,000/microliter
- Platelet count ..... ≥100,000/microliter
- Absolute neutrophil count..... ≥1,500/microliter
- Creatinine..... ≤2.5 x institutional ULN
- OR GFR ..... >30ml/min/1.73m2
- Total bilirubin ..... ≤2 x institutional ULN
- AST (SGOT)..... ≤2.5 × institutional ULN
- ALT (SGPT) ..... ≤2.5 × institutional ULN

4.1.8 A negative serum pregnancy test at Baseline, but within 21 days of Randomization, for persons of childbearing potential only.

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

4.1.10 Ability to understand and the willingness to sign a written informed consent document. A Legally Authorized Representative (LAR) may sign Informed Consent for persons who do not have the capacity to legally consent to take part in the study.

4.2 Exclusion Criteria

4.2.1 Inability to abstain from NSAID (including aspirin) and selective COX-2 inhibitor therapy at the time of randomization through the completion of the study (the study period is defined as baseline to exit endoscopy at 18 months after randomization which defines the completion of the study). Participants may take Tylenol and Non-NSAID pain relievers.

4.2.2 Current or planned use of anticoagulant drugs such as: warfarin, heparin, low molecular weight heparin, Plavix, or Aggrenox throughout the course of the study.

4.2.3 Individuals taking the drugs listed below may not be randomized unless they are willing to stop the medications (and possibly change to alternative non-excluded medications to

treat the same conditions) no less than 1 month prior to starting aspirin or placebo on this study. Consultation with the participant's primary care provider will be obtained prior to stopping any agent. The use of the following drugs or drug classes is prohibited during aspirin/placebo treatment:

NSAIDs: such as aspirin, Naprosyn, ketorolac and others NSAIDs

COX-2 inhibitors: such as Celecoxib, Rofecoxib

Valproic acid

Sulfinpyrazone

Probenecid

Corticosteroids (other than short-term use defined as less than 2 weeks or prn use of an inhaler less than twice per month)

Platelet aggregation inhibitors, except in a monitored antithrombotic regimen

Methotrexate (MTX)

Vaccines containing live viruses

Gingko

4.2.4 Individuals with uncontrolled renal insufficiency or renal failure.

4.2.5 Participants with fundoplication within the past year, bariatric surgery or any other major upper GI surgery. Fundoplication more than one year ago will not be grounds for exclusion. Cholecystectomy will not be grounds for exclusion.

4.2.6 History of invasive cancer diagnosis ≤ 12 months prior to randomization, excepting non-melanoma skin cancer. Patients with T1a adenocarcinoma of the esophagus arising in the setting of Barrett's esophagus are eligible for enrollment in the trial.

4.2.7 History of cancer treatment ≤ 12 months prior to randomization, excepting hormonal therapy (except treatment for non-melanoma skin cancer or carcinoma-in-situ of the cervix).

4.2.8 Receipt of any other investigational agents ≤ 3 months prior to randomization, except innocuous agents with no known interaction with the study agents (e.g., standard dose multivitamins or topical agents for limited skin conditions), at the discretion of the Protocol Lead Investigator at each Participating Site.

4.2.9 History of allergic reactions attributed to aspirin or compounds of similar chemical or biologic composition to the study agent.

4.2.10 History of endoscopically or radiographically diagnosed peptic ulcer disease with upper GI bleeding during the past 5 years or history of endoscopically or radiographically diagnosed peptic ulcer disease with upper GI bleeding any time while taking aspirin.

4.2.11 Uncontrolled intercurrent illness including, but not limited to: ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, bleeding disorder, vitamin K deficiency, alcohol abuse (defined as ingestion of 3 or more drinks per day) or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.12 Pregnant women. Note: because the teratogenic or abortifacient effects of the study agents remain incompletely defined.
4.2.13 Breast feeding women. Note: because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with the study agents, women who are breast-feeding will be excluded.
4.2.14 Surveillance biopsies demonstrating residual BE at qualifying exam.
4.2.15 Presence of an esophageal stricture defined as "any recognizable change in esophageal luminal caliber that is accompanied by symptoms of dysphagia, or any asymptomatic narrowing that either will not allow any adult endoscope to pass or allows passage with resistance."
4.2.16 Patients with human immunodeficiency virus (HIV) infection.

4.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial. However, BE is more common among Caucasian males and we expect our recruitment to reflect the known demographics of this condition. There is no information available regarding differential effects of the study agents in population subsets defined by race, gender, or ethnicity, and there will be no reason to expect such differences exist. Therefore, although the planned analysis will look for differences in intervention effect based on racial and gender groupings, the sample size is not increased in order to provide additional power for these subset analyses.

4.4 Recruitment and Retention Plan

See Document A for details.

5. AGENT ADMINISTRATION

Intervention agents will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

Doses of the study agents will be either
(a) Aspirin 325 mg PO QD or
(b) Matching placebo PO QD

Agents will be administered for 12 months after randomization.

5.2 Aspirin Administration

Aspirin or aspirin placebo will all be administered once in the morning with a meal. All study agents will be self-administered by the participants. Participants will be given sufficient agent supplies to last for a 3 month period (between phone interviews or mail contacts) plus a short time period beyond their scheduled interview to account for unavoidable scheduling delays.

First Randomized Pill Bottle: Participants who continue to be eligible based on qualifying endoscopy and biopsies and interested in study participation will receive a supply of study pills. The supply will be the randomly assigned study agent. Participants will be instructed to start this

new bottle immediately upon receipt and to record the date that the bottle is started. The study agent may be dispensed to the participant at the randomization visit or shipped directly to the participant.

Subsequent Randomized Pills: Participants will be instructed to take at least 3 months of pills from each bottle prior to starting their new one and to record the date that the new bottle is started. Bottles containing randomized pills will be mailed in approximately three month intervals adjusting for the previous pill bottle start date. Special shipments will also be made upon request, if, for example, a participant loses their bottle or does not receive their new bottle of pills.

5.3 Run-In Procedures

This trial will not include a placebo or active agent run-in period

5.4. Contraindications

Exclusions as outlined in Section 4.2

5.5 Concomitant Medications

Concurrent use of aspirin with valproic acid has been reported to increase the plasma concentration of valproic acid and induce valproic acid toxicity (see exclusions). Concurrent use of aspirin with high-dose corticosteroids may increase the risk of gastrointestinal side effects (see exclusions). Concurrent use with platelet aggregation inhibitors is prohibited, except in a monitored antithrombotic regimen, because the risk of bleeding may be increased. Sulfinpyrazone and probenecid may decrease renal clearance and increase plasma concentrations and toxicity of salicylates (see exclusions). Aspirin may block excretion of methotrexate (MTX) and increase MTX toxicity (see exclusions). No vaccines containing live viruses can be administered during the study due to an increased risk of Reye's syndrome (see exclusions). Gingko may be associated with increased risk of bleeding; patients will not be allowed to use gingko on this study. All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: start and stop dates (if known; if unknown, will be marked "unknown" in the database), and dose. Medications taken for a procedure (e.g., biopsy) will also be included.

5.6 Dose Modification

There will be no dose modification. Participants with Grade 4 adverse events possibly, probably or definitely related to study agent, or Grade 3 adverse events possibly, probably or definitely related to study agent will be taken off study and followed off study agent for at least 30 days or for the remainder of the planned intervention period (whichever is longer). Participants with Grade 2 adverse events possibly, probably or definitely related to the study agent can be taken off study agent for up to 1 week. If the adverse event resolves, the study agent may be resumed. If the adverse event continues, participants will be taken off study. Participants with any Grade 1 adverse event may continue the study agent at full dose, unless either the participant or the investigator finds the adverse event to be intolerable. In this instance, the study agent can be stopped and restarted as described for participants with Grade 2 adverse events. Rechallenge of participants who experience Grade 2 or intolerable Grade 1 adverse events with the study agent

will be instituted only after the adverse event has been resolved to the satisfaction of the Protocol Principal Investigator. If the Grade 2 or intolerable Grade 1 adverse event recurs after re-challenge, the participant will be withdrawn from the study and followed off study agent for at least 30 days or for the remainder of the planned intervention period (whichever is longer).

5.7 Adherence/Compliance

Although drug concentration will not be measured in the blood, compliance will be monitored in the following ways:

Pill diary completion: Compliance will be monitored by a pill diary that all participants will be asked to complete (see Appendix B). Participants will be asked to mail back their pill diary and their unused agent supplies after each phone call at Month 3, 6, and 9 visits and to bring both their pill diary and their unused agent supplies to the Post-Intervention evaluation at Month 12 visit.

Dose count: Randomized participants will receive their initial 3 month (90 pills plus 10 additional pills) supply of study agent at the randomization visit. At Month 3, 6, and 9 visits the participants will be asked to return all full and empty bottles by mail, and to start taking pills from their new 3 month (90 pills plus 10 additional pills) supply that has been mailed to them. After each visit at Months 3, 6, 9 and 12 compliance will be measured as follows:

number of tablets taken (i.e., number of tablets given - number of tablets returned) / number of tablets that should have been taken during that period of time x100 = % compliance.

Compliance (acceptable adherence to study agent) will be defined as ingestion of ≥ 80% of the planned agent doses for the purposes of secondary data analyses. In cases where the tablets are not returned, the compliance has to be calculated from the calendar. In cases of non-compliance with the study agent, the participant will be reinstructed. The research nurse or study coordinator and the physicians will provide education for participants, indicating when and how should the tablets be administered.

In case of concomitant medications, subjects are asked to contact the study staff in order to check whether any potential interference is expected. Data on drug suspension or interruption will be reported in the CRFs.

6. PHARMACEUTICAL INFORMATION

6.1 Study Agent (IND #, IND Sponsor)

This study will not be conducted under an IND.

Aspirin (325 mg enteric coated tablets) and placebo will be supplied by NCI, DCP. Aspirin is an odorless white, needle-like crystalline or powdery substance. When exposed to moisture, aspirin hydrolyzes into salicylic and acetic acids, and gives off a vinegary-odor. It is highly lipid soluble and slightly soluble in water. The 325 mg aspirin tablet contains 325 mg aspirin and the following excipients: carnauba wax, cellulose, D&C Yellow #10 Aluminum Lake, FD&C Yellow #6 Aluminum Lake, hypromellose, iron oxides, methacrylic acid copolymer, polysorbate 80, propylene glycol, shellac, sodium lauryl sulfate, starch, titanium dioxide, triacetin.

6.1.1 Rescue Medication:
Participants will be allowed to take acetaminophen in clinically indicated doses for headache or pain, but will not be allowed to take aspirin or other NSAIDS during the entire study period (baseline to 18 months after randomization).

6.2 Reported Adverse Events and Potential Risks

Aspirin has been associated with gastrointestinal (GI) side effects. GI side effects include stomach pain, heartburn, nausea, vomiting, and gross GI bleeding. Minor upper GI symptoms, such as dyspepsia, are common and can occur anytime during therapy. Aspirin has also been associated with an increased risk of hemorrhagic stroke. Aspirin has also been associated with elevated hepatic enzymes, blood urea nitrogen and serum creatinine, hyperkalemia, proteinuria, and prolonged bleeding time. High-dose aspirin has been associated with tinnitus.

6.3 Availability

Aspirin and aspirin placebo are investigational agents supplied to investigators by NCI, DCP.

6.4 Agent Distribution

Agents will only be released by NCI, DCP after documentation of IRB approval of the DCP-approved protocol and consent is provided to DCP and the collection of all Essential Documents is complete (see DCP website for description of Essential Documents).

NCI, DCP-supplied agents may be requested by the Investigator (or their authorized designees) at each Organization. DCP guidelines require that the agent be shipped directly to the institution or site where the agent will be prepared and administered. DCP does not permit the transfer of agents between institutions (unless prior approval from DCP is obtained). DCP does not automatically ship agents; the site must make a request. Agents are requested by completing the DCP Clinical Drug Request form (NIH-986) (to include complete shipping contact information) and faxing or mailing the form to the DCP agent repository contractor:

John Cookinham
MRIGlobal
DCP Chemoprevention Agent Repository
425 Volker Blvd.
Kansas City, MO 64110
FAX: (816) 753-5359
Emergency Telephone: (816) 360-3800
Email: NCI.DCP@mriglobal.org

6.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP using the NCI Drug Accountability Record Form (DARF) or an institutionally-approved accountability system. The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. Include on receipt record from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed to and returned by each participant. The protocol site PI(s) or their representative will be responsible for study agent accountability for participants at his/her participating site.

6.6 Packaging and Labeling

Aspirin and aspirin placebo will be packaged by NCI, DCP.

6.7 Storage

Study drug will be stored in a secure location, at controlled room temperature (59°F and 86°F) under low humidity. All agents should be kept in a secured storage area.

6.8 Registration/Randomization

Screening and Registration into the DMI Database:
Once informed consent has been signed, participants will be registered into the DMI database. The DMI database will assign a participant's ID upon completion of the registration process.

Randomization:
Participants will be assigned a randomization number once the following has been accomplished: eligibility has been verified at the site level, eligibility has been confirmed by the site PI, and eligibility CRF has been entered into the DMI web application. The randomization number will be generated by the database and assigned to the participant. Refer to Section 13.2 for details of randomization.

Screening/Registration/Randomization into site-specific databases:
The DMI is the database of record for the study. Registration and randomization should occur per the procedures outlined above. If the site staff need to enter study data into site-specific electronic databases per their institutional requirements, they should do so in accordance with their institutional policies and procedures.

Appropriate CRFs must be completed for any participant who signs an informed consent. If a consented participant is a screen failure and deemed ineligible, the following CRFs must be completed: 1) the Registration CRF; 2) the Randomization CRF with the eligibility box checked "no", 3) the Inclusion and Exclusion CRFs showing why the participant is ineligible, 4) the Off-Study CRF, 5) the Adverse Event CRF, 6) the Concomitant Medication CRF and 7) the Verification CRF. If no Adverse Event and/or Concomitant Medications were assessed by the time the participant is deemed ineligible, the "NONE" box will be checked to complete both CRFs. All participants who sign an informed consent must formally go off study. All participant registration information will be entered into DMI. If a participant experiences a serious adverse event during the screening process, an SAE form must be completed.

6.9 Blinding and Unblinding Methods

6.9.1 Participants will be blinded to aspirin or placebo.

6.9.2 The Statistician and the Site Study Pharmacist will not be blinded to aspirin or placebo.

6.9.3 All other Investigators will be blinded to aspirin or placebo and to the dose level.

6.9.4 All participants will take one 325 mg tablet per day of aspirin or an identical-appearing placebo tablet.

6.9.5 Study assignments will be unblinded to the Study Investigators and Site Coordinators after all of the data are collected and the study database has been locked. Unblinding will also occur if the participant's physician deems that unblinding is necessary, such as in the case of unacceptable toxicity thought to be related to the study agent or progressive disease, or if the participant becomes pregnant.

6.9.6 The Data and Safety Monitoring Board will also be blinded unless unblinding is warranted (if the participant's physician deems that unblinding is necessary, if the

participant becomes pregnant, or after all of the data are collected and the study database has been locked).

6.9.7 Unblinding will only take place after consultation with the NCI, DCP Task Order Monitor (Medical Monitor). Unblinding will be conducted as follows:

1) The Site PI contacts the Protocol Chairman (the Protocol Principal Investigator) and requests the participant's treatment status be unblinded.
2) The Protocol Chairman (the Protocol Principal Investigator) contacts the NCI, DCP Task Order Monitor (Medical Monitor) and requests the participant's treatment status be unblinded. The Protocol Chairman then conveys the Task Order Monitor's decision to the Site PI. The Site PI then proceeds with unblinding as written out below.
3) If the NCI Task Order Monitor cannot be reached and the participant requires emergency care, the Protocol Chairman (the Protocol Principal Investigator) may authorize the site PI to break the blind.
4) If the Site PI is unable to reach the Protocol Chairman and the participant requires emergency care, then the Site PI must proceed with unblinding as written out below.
5) The Site PI requests the participant's treatment status be unblinded by the research pharmacist (or designated individual responsible for dispensing drug).
6) The Site PI officially takes the participant off-study.
7) The date and reason for breaking the blind must be submitted by the Site PI to the Protocol Chairman, Robert Bresalier, MD, as soon as possible.
8) It is the responsibility of the Study Chairman to report the date and reason for breaking the blind to the **NCI Task Order Monitor,** Gary Della'Zanna, DO as soon as possible after receiving this information from the Site PI.

   NCI Task Order Monitor:
   Gary Della'Zanna, DO, MSc
   National Cancer Institute
   Division of Cancer Prevention
   Gastrointestinal and Other Cancers Research Group
   9609 Medical Center Drive, MSC-9782
   Rockville, MD 20850
   Phone: 240-276-7042
   Fax (with cover sheet, Attn: Dr. G. Della'Zanna): 240-276-7848
   Email: gary.dellazanna@nih.gov
9) The date and reason for breaking the blind must be submitted by the Study Chairman to the MD Anderson Consortium Principal Investigator, Powel H. Brown, MD, PhD, or designee as soon as possible via email to phbrown@mdanderson.org.
10) The date and reason for breaking the blind will be reported by the Study Chairman to the MD Anderson DSMB as soon as possible.

6.10 Agent Destruction/Disposal

At the completion of investigation, all unused study agent will be returned to NCI, DCP Repository according to the DCP "Guidelines for AGENT RETURNS" and using the DCP form "Return Drug List".

The guidelines and the form are available on the DCP website.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

SCHEDULE OF EVENTS

Evaluation/ Procedure	Screening	Baseline Visit/ Eligibility Verification	Randomization (within 45 days of qualifying endoscopy)[b]	Month 1 (+/- 7 days) Phone contact	Months 3,6,9 (+7 days) and Month 15 (+/- 14 days) Phone or mail contact	Month 12 (+/- 30 days) EGD with biopsies. End of treatment	Month 18 (+/- 30 days) EGD with biopsies. Study termination
Informed Consent		X					
Assess Eligibility	X	X					
Baseline History Assessment	X	X[a]					
Symptom Assessment Questionnaire	X						
Symptom Assessment		X	X	X	X	X	X
Physical Exam		X				X	X
Vital Signs/ Height and Weight[c]		X				X	X
Laboratory Tests		X				X	
Pregnancy Test[d]		X	X				
Qualifying EGD with Biopsies		X[g]				X[h]	X[h]
Biomarkers		X				X	X
Concomitant Medications	X	X	X	X	X	X	X
Dispense Study Agent			X In person or by mail		X Mail[e]		
Collect Study Agent					X[f]	X	
Review Pill Diary/Record			X	X	X	X	
Adverse Events			X	X	X	X	X
Telephone Contact	X		X[b]	X	X		
Interval questionnaire					X	X	
Your Health and Well-Being (SF-36) survey		X				X	

[a] Do not repeat the Baseline History Assessment Questionnaire at Baseline. If participant is registered to study, enter Baseline History Assessment Questionnaire captured at Screening.
[b] In person or by phone.
[c] Do not repeat height measurement after Baseline Testing.
[d] In persons of childbearing potential a serum pregnancy test must be done at the baseline visit. If positive at that baseline visit that participant is a screen failure. Randomization may occur within 21 days from the baseline visit negative pregnancy test without repeating the test. For randomization to occur after this 21 day window from the baseline visit negative pregnancy test date a repeat pregnancy test must be done and must be known to be negative prior to randomization and medication dispensing.
[e] Study medication is distributed after randomization and at 3, 6, and 9 months only.

[f] Study medication is collected at 3, 6, 9 and 12 months.
[g] Qualifying endoscopy.
[h] A clinically indicated surveillance endoscopy.

7.2 Baseline Testing/Prestudy Evaluation

7.2.1 Screening

Eligible participants will be identified from patients undergoing or who have undergone radiofrequency ablation for BE and are participating in or will participate in an ongoing surveillance program. Potentially eligible participants will be identified through database/charts/clinic lists prior to Baseline Visit. A letter may be mailed to potentially eligible participants introducing the study to the patients. If the Site chooses to utilize a letter, the letter will be signed by the Site PI who is familiar to the patient as the doctor following their medical condition. If the Site PI is not the patient's physician who is treating their BE, the letter will be co-signed by the physician who is familiar to the patient. To facilitate screening, the Study Coordinators are advised to call potential participants and introduce the study over the phone, as well as administer the screening questionnaires. However, the study team may choose to administer the questionnaires in person when the participant is seen at the clinic. Screening to identify potentially eligible participants takes place prior to obtaining Informed Consent. The following questionnaires will be administered during Screening:

- Baseline history assessment questionnaire, to include a review of cancer history and previous medical history, previous surgery, chemotherapy, radiation, and other cancer-directed therapy history, family history, demographic information, including age and race, history of allergies or intolerance to aspirin, aspirin containing products or NSAIDs.
- Symptom Assessment Questionnaire, to include a review of the following symptoms: gastrointestinal bleeding, hematemesis, hematochezia, melena, abdominal pain/cramping, heart burn, irregular heart rate/palpitations, chest pain, shortness of breath, edema, itching, constipation, diarrhea, headache, dizziness, drowsiness, lightheadedness, vertigo, easy bruising/bleeding.
- Use of concomitant medications will be collected.

If the participant is found to be potentially eligible for the study, a Baseline visit will be scheduled.

7.2.2 Baseline Testing/Eligibility Verification: Consent; Registration; Qualifying EGD with biopsies
The following procedures will be conducted at the Baseline Testing/Eligibility Verification Visit

- Informed consent must be obtained prior to starting any further study procedures.
- Registration: Registration will be completed during the screening period once informed consent has been signed. All consented participants will be assigned a PID number pre-generated by the electronic database. All participants will be entered with the Data Management Initiative (DMI) database and Participating Organizations registry databases as applicable. Participants will also be registered into site-specific registry databases as applicable.
- Enter Baseline history assessment into the DMI.
- Symptoms assessment.
- Participants will be given a "Your Health and Well-Being (SF-36)" survey. If the survey cannot be completed during the visit due to time constraints, the participant may be asked to complete the survey at a later date, but prior to randomization.
- Use of concomitant medications will be collected.
- Physical examination will be performed (to include assessment of vital signs, height and

weight)

- Laboratory tests: CBC (must include hemoglobin, leukocyte count, platelet count) with differential (must include absolute neutrophil count), Creatinine, electrolytes (must include: sodium, potassium), AST/SGOT, ALT/SGPT, TBILI. Standard of care labs are acceptable for use to confirm eligibility if done within 30 days of Qualifying Visit.
- In persons of childbearing potential a serum pregnancy test must be done as part of the baseline visit/eligibility verification and must be negative. If positive the participant is a screen failure. A participant with a negative pregnancy test at that baseline visit maybe randomized up to 21 days from that negative pregnancy test without repeating the test. If randomization occurs after that 21 day period a repeat negative pregnancy test must be done and must be known to be negative prior to randomization and medication dispensing. If pregnancy is found at any time after randomization such participants are not considered screen failures and the proper procedure for taking a participant off study should be followed. See Notes below.
- Esophagogastroduodenoscopy and biopsies will be performed as part of a clinically indicated surveillance exam to document complete eradication of BE and absence of Barrett's metaplasia or associated dysplasia.
- At the time of qualifying and subsequent EGDs standard of care mucosal biopsies will be performed according to the Seattle protocol (4 quadrant biopsies every 2 cm in individuals with a history of metaplasia or low grade dysplasia, every 1 cm in those with a history of high grade dysplasia)
- Additional research study biopsies will be performed as follows: 4 quadrant biopsies 1 cm above the gastroesophageal junction, at a site mid-way through an area defined by the length and location of the original Barrett's segment, and normal-appearing squamous mucosa 2 cm above the upper extent of the original Barrett's segment (2 biopsies in each of 4 quadrants to be snap frozen plus 1 biopsy for formalin fixation and paraffin embedding, or 9 biopsies in each segment, at 3 separate segments, for a total of 27 additional research biopsies).
- Eligibility will be determined by absence of residual Barrett's metaplasia or dysplasia at this qualifying exam as determined by the local Pathology reading.

Notes:

- Any screen failed participant who is deemed ineligible based on medical history, physical exam, laboratory values, pregnancy test or persistence or BE at qualifying exam after consent is obtained will need to have a screen failure case report form completed. Information will be recorded on the appropriate CRF(s) up until the point at which the participant was found ineligible and screen failed.
- Participants who are ineligible due to regular aspirin or NSAID use at the time of screening may be reevaluated after refraining from aspirin or NSAID use for one month.
- If a pregnancy is discovered after Randomization, instruct the participant to stop taking drug, take participant off-study, complete the Off-Study CRF, follow the pregnancy to term and complete the Outcome of Pregnancy CRF.

7.2.3 Randomization

Subjects meeting the criteria outlined above and whose standard of care surveillance biopsies document absence of residual Barrett's metaplasia or dysplasia will be eligible for randomization which will take place within 45 days of the qualifying endoscopy. The randomization visit may be in person or by phone.

- Randomization: Once registration is complete, eligibility has been confirmed and eligibility CRF is entered into the web application, participants will be assigned a randomization number pre-generated by the database. Participants will be randomized to receive either active agent (aspirin 325 mg) or placebo. Both the participant and the investigator will be blinded to the agent.
- Symptom assessment.
- Concomitant medications.
- Randomized participants will receive a 3-month supply of study agent (90 pills plus 10 additional pills). The drug supply may be mailed to the participant or given to the participant in person.
- Review pill diary and pill intake instructions with the participant. Participants must be instructed to take at least 3 months of pills from their first bottle prior to starting the next bottle and to record the date that the new bottle is started.
- Assess adverse events.

7.3 Evaluation During Study Intervention

Month 1 (+/- 7 days) Phone contact

- All participants will have a telephone interview at Month 1 (+/- 7 days) regarding symptoms possibly related to the agent, illnesses, doctors' visits, hospitalizations, mediations (including aspirin, NSAIDs and over the counter medications), adverse events, and compliance with study tablets. Adherence with the study medication will be reinforced.
- Adverse Events assessment.
- Concomitant medications assessment.
- Symptom assessment.
- Review pill diary.

Months 3, 6, and 9 (+7 days) Phone or mail contact

- All participants will have a telephone or mail interview every 3 months (+7 days)regarding symptoms possibly related to the agent, illnesses, doctors' visits, hospitalizations, mediations (including aspirin, NSAIDs and over the counter medications), adverse events, and compliance with study tablets.
- After documentation of continued eligibility and compliance, participants will be instructed to return any unused study agent at this time. Prior to Months 3,6, and 9 (+7 days) visit the site coordinator should mail to the participant their next 3-month supply of study agent or placebo (90 pills plus 10 additional pills). The participant is instructed to take at least 3 months of pills from each bottle before switching to the next bottle.
- Interval questionnaire.
- Adverse Events assessment.
- Concomitant medications assessment.
- Symptom assessment.
- Review pill diary.

7.4 Evaluation at Completion of Study Intervention (12 months from randomization +/- 30 days)

A clinically indicated surveillance endoscopy exam will be performed at 12 months (+/- 30 days) from randomization. This will also serve as the end of treatment exam. At this time the following

will be performed:

- Symptom assessment.
- Physical examination will be performed (to include assessment of vital signs and weight).
- Laboratory tests: CBC (must include hemoglobin, leukocyte count, platelet count) with differential (must include absolute neutrophil count), Creatinine, electrolytes (must include: sodium, potassium), AST/SGOT, ALT/SGPT, TBILI.
- Esophagogastroduodenoscopy and biopsies will be performed as part of a clinically indicated surveillance exam to document complete eradication of BE and absence of Barrett's metaplasia or associated dysplasia.
- Standard of care mucosal biopsies will be performed according to the Seattle protocol (4 quadrant biopsies every 2 cm in individuals with a history of metaplasia or low grade dysplasia, every 1 cm in those with a history of high grade dysplasia).
- Additional research study biopsies will be performed as follows: 4 quadrant biopsies 1 cm above the gastroesophageal junction, at a site mid-way through an area defined by the length and location of the original Barrett's segment, and normal-appearing squamous mucosa 2 cm above the upper extent of the original Barrett's segment (2 biopsies in each of 4 quadrant to be snap frozen plus 1 biopsy for formalin fixation and paraffin embedding in each segment, or 9 biopsies in each segment, at 3 separate segments, for a total of 27 additional research biopsies).
- Interval questionnaire.
- SF-36 health survey.
- Any unused study medication will be returned (participants will also have returned unused study medications at 3, 6 and 9 months).

7.5 Post-intervention Follow-up Period

A phone and/or mail interview to track adverse events and medication use (including aspirin and NSAID use) will be performed at Month 15 (+/- 14 days). Refer to the schedule of events for study procedures at Month 15 (+/- 14 days) visit.

A clinically indicated surveillance endoscopy exam will be performed at 18 months (+/- 30 days) from randomization. At this time the following will be performed:

- Symptoms assessment.
- Adverse events assessment.
- Concomitant medications assessment.
- Physical examination will be performed (to include assessment of vital signs and weight).
- Esophagogastroduodenoscopy and biopsies will be performed as part of a clinically indicated surveillance exam to document complete eradication of BE and absence of Barrett's metaplasia or associated dysplasia.
- Standard of care mucosal biopsies will be performed according to the Seattle protocol (4 quadrant biopsies every 2 cm in individuals with a history of metaplasia or low grade dysplasia, every 1 cm in those with a history of high grade dysplasia).
- Additional research study biopsies will be performed as follows: 4 quadrant biopsies 1 cm above the gastroesophageal junction, at a site mid-way through an area defined by the length and location of the original Barrett's segment, and normal-appearing squamous mucosa 2 cm above the upper extent of the original Barrett's segment (2 biopsies in each of 4 quadrant to be snap frozen plus 1 biopsy for formalin fixation and paraffin embedding in each segment, or 9 biopsies in each segment, at 3 separate segments, for a total of 27 additional research biopsies).

Subsequent to study participation, the patients will be followed through routine clinically indicated surveillance endoscopy exams and biopsies performed at 6 to 12 month periods.

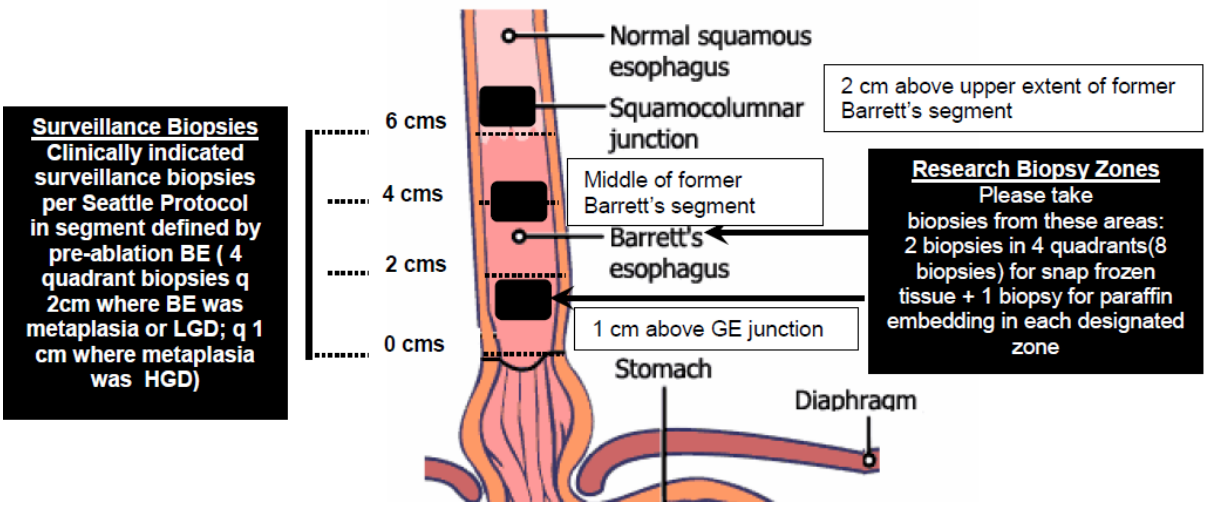
7.6 Methods for Clinical Procedures

Clinically indicated surveillance esophagogastroduodenoscopy (EGD) will be performed at qualifying exam, 12 months (+/- 30 days) from randomization and 18 months (+/- 30 days) from randomization. Standard of care mucosal biopsies will be performed according to the Seattle protocol (4 quadrant biopsies every 2 cm in individuals with a history of metaplasia or low grade dysplasia, every 1 cm in those with a history of high grade dysplasia)

Additional research study biopsies will be performed as follows: 4 quadrant biopsies 1 cm above the gastroesophageal junction, at a site mid-way through an area defined by the length and location of the original Barrett's segment, and normal-appearing squamous mucosa 2 cm above the upper extent of the original Barrett's segment (2 biopsies in each of 4 quadrant to be snap frozen plus 1 biopsy for formalin fixation and paraffin embedding in each segment, or 9 biopsies in each segment, at 3 separate segments, for a total of 27 additional research biopsies)

Surveillance Mucosal Biopsies

Kits are required for research biopsies. The kit contains supplies and instructions for collecting, processing, and shipping research biopsy specimens. Participating Sites may obtain specimen collection kits by faxing the Fax Supply Order Form to the number provided (found in the Forms Packet). At least two weeks should be allowed to receive the kits. Kits will not be sent to the Participating Sites by FedEx ®. Because charges are incurred for all outgoing kits, a small but sufficient supply of the specimen collection kits should be ordered prior to participant entry.
Endoscopic research biopsies will be obtained with the Boston Scientific Radial Jaw
RJ4 large capacity biopsy forceps (provided by coordinating center) from 1 cm above the level of the lower esophageal sphincter, just above the proximal margin of the gastric folds, in a zone corresponding to the middle of the former Barrett's segment, and a zone 2 cm above the upper extent of the former Barrett's segment.



8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

The **primary endpoint** of the study will be **differences in the change of CDX2 mRNA levels from baseline to 12 months post-randomization in esophageal squamous tissue between participants taking aspirin supplementation versus those taking placebo. CDX2 mRNA levels will be compared at baseline versus at 12 months within each participant, and the change in CDX2 mRNA levels compared between participants taking aspirin participants taking placebo (similar comparisons will be made for differences in the change of secondary biomarkers-see below).** Our *rationale* to use this biomarker is based on the direct link between aspirin, chronic inflammation, and the downregulation of NF-kB-induced downstream target genes among which is CDX2 (4,5,8).Total RNAs will be isolated from squamous and neosquamous mucosal biopsy specimens using Trizol (Invitrogen, Carlsbad, CA) and quantitated by spectrophotometry. Reverse transcription will be performed using QuantiTect Reverse Transcription kit (Qiagen, Valencia, CA) per manufacturer's instructions. Real-time PCR for CDX2 mRNA will be carried out using rapid cycling with the StepOnePlus Real-Time PCR System and SYBR mix (Life Technologies, Carlsbad, CA) using previously published CDX2 primer sequences (8).

8.2 Secondary Endpoints

8.2.1 Safety will be assessed by comparison of adverse events using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated in Section 11.1.3.1. We will closely monitor the toxicity profile and tolerability in each group. Safety will be assessed by monitoring routine clinical parameters at baseline and at each visit throughout the trial (including Month 1, every 3 months by phone calls and/or mail interviews and in person at 12 and 18 months), and by monitoring laboratory parameters at baseline and at 12 months. All AEs will be recorded and graded during clinical visits, whether or not they are considered drug-related. All participants will be monitored for toxicity from the time of randomization. Each treatment arm will be monitored separately for severe toxicities (grade 3 and 4 and SAEs). We will be especially vigilant about the incidence of gastrointestinal (gastric and duodenal ulcers, gastritis, gastrointestinal bleeding or perforation, hematemesis) and hematological (anemia, thrombocytopenia) events. Toxicity will be considered drug-related based on clinical assessment. In addition, at the completion of the study, the frequency of toxicities will be compared between the placebo and aspirin groups.

The SF-36 is a multi-purpose, short-form health survey with 36 questions. It yields an 8-scale profile of functional health and well-being scores as well as psychometrically-based physical and mental health summary measures and a preference-based health utility index. It is a generic measure, as opposed to one that targets a specific age, disease, or treatment group. Accordingly, the SF-36 has proven useful in surveys of general and specific populations, comparing the relative burden of diseases, and in differentiating the health benefits produced by a wide range of different treatments. Since a secondary endpoint of our study is to determine safety in a specific clinical setting (use of aspirin following radiofrequecy ablation, we wish to compare measures of health and well-being between those receiving aspirin and those receiving placebo. Bivariate analyses will be performed on study entry and completion of the on-drug period. Multivariate analyses will be performed between groups after controlling for potential confounders.

8.2.2 **Differences in the change of CDX2 mRNA levels from baseline to 18 months post-randomization** in esophageal squamous tissue between participants taking aspirin supplementation versus those taking placebo. Total RNAs will be isolated from squamous and neosquamous mucosal biopsy specimens using Trizol (Invitrogen, Carlsbad, CA) and quantitated by spectrophotometry. Reverse transcription will be performed using QuantiTect Reverse Transcription kit (Qiagen, Valencia, CA) per manufacturer's instructions. Real-time PCR for CDX2 mRNA will be carried out using rapid cycling with the StepOnePlus Real-Time PCR System and SYBR mix (Life Technologies, Carlsbad, CA) using previously published CDX2 primer sequences (8).

8.2.3 **Differences in the activation status of NF-kB by assessing levels of total and phospho-p65 and cytoplasmic to nuclear translocation of phospho-p65 which is likely to be affected by aspirin** (baseline, 12 and 18 months**).** Fractionation and Western blot for the p65 NF-kB family member: Using esophageal squamous and neosquamous mucosal biopsies taken before and after treatment with aspirin, levels of phospho-p65 and total p65 will be determined by Western blot and quantitated by densitometry. We will then evaluate the cytoplasmic to nuclear translocation of phospho-p65 protein as previously described by us. Nuclear extracts will be isolated from tissues using the NE-PER Nuclear and Cytoplasmic Extraction kit (Thermo Fisher Scientific, Rockford, IL) per manufacturer's instructions. Blots will be probed with antibodies to phospho-p65 and total p65. Tubulin and transcription factor IIB (TFIIB) antibodies will be used to assure the purity of the cytoplasmic and nuclear subcellular fractions, respectively.

8.2.4 **Differences in the prostanoid marker, prostaglandin E_2, and prostaglandin synthases** (baseline, 12 and 18 months**)**, which are known to respond to aspirin and to correlation with clinicopathological factors in the esophageal cancer (14, 24). PGE2 was previously shown to be reduced in Barrett's mucosa in response to a combination of acid inhibition and high-dose (<u>but not low dose</u>) aspirin. Endogenous eicosanoids will be extracted from snap frozen biopsies and analyzed as we have previously described (25). Immediately following isolation, biopsies will be placed in individual vials containing 5 µg/mL aqueous indomethacin, instantly snap frozen in liquid nitrogen and maintained in liquid nitrogen. Using this regimen, measured PGE_2 reflects the actual concentration in vivo (26).

8.2.5 **Differences in the expression of proinflammatory cytokines known to induce activation of NFkB, i.e., TNFα, IL-1β, IL-6, IL-10, IL-17A, IL-23** will be measured (baseline, 12 and 18 months). These inflammatory mediators have been implicated in the pathogenesis of BE and esophogeal adenocarcinoma. Snap frozen biopsies will be homogenized in lysis buffer and endogenous IL-1β, IL-6, IL-10, IL-17A, IL-23, and TNFα will be simultaneously measured utilizing a human Bio-Plex customized multiplex kit (Bio-Rad) and the Bio-Plex 200 System as we have previously described (27).

8.2.6 **Recurrent Barrett's Esophagus** (at 12 and 18 months) will be ascertained completely and accurately. While we will not have sufficient power to assess differences in histological recurrence of BE during the short course of the study, we will examine this as an exploratory secondary endpoint. *This study will generate data designed to lead to the submission of an adequately powered multi-center prospective chemoprevention trial with histologic recurrence of BE after RFA as the clinically relevant primary endpoint.* At all endoscopic examinations after randomization, all raised mucosal lesions, and all flat lesions suspicious for neoplasia, will be biopsied and excised by EMR, their location and size noted, and pathology material reviewed by the study pathology center for confirmation of diagnosis. For each participant, the surface area of recurrent BE will be estimated along with its Prague classification, histological characteristics, and location. A designated study pathologist will review all pathology specimens without knowledge of treatment assignment. Each biopsy

will be classified (using standard criteria) as normal, Barrett's metaplasia, BE with low- or high-grade dysplasia, neoplasia, indefinite for dysplasia, intramucosal or invasive carcinoma.

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate agent supply, noncompliance, concomitant medications, medical contraindication. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events. We plan to enroll up to 20 participants per group.

8.4 Off-Study Criteria

Participants may go 'off-study' for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, adverse event/serious adverse event, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, death, determination of ineligibility (including screen failure), pregnancy.

8.5 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

Methodology employed is based on existing literature and previously generated data from the biomarker laboratories and investigators involved in this study.

Preliminary Data to Justify Primary Endpoint CDX2 and Assessment of Differences in the activation status of NF-kB by assessing levels of total and phospho-p65 and cytoplasmic to nuclear translocation of phospho-p65 which is likely to be affected by aspirin:

In Esophageal Squamous Cells Derived from GERD Patients with and without Barrett's Esophagus, There Are Differences in NF-κB Activation after Acid and Bile Salt Exposure. In human esophageal squamous cell lines derived from GERD patients *with* BE, we have shown that acid and bile salts cause nuclear translocation of both the p50 and p65 subunits of NF-KB, but only p50 binds the CDX2 promoter to induce CDX2 expression. In contrast, esophageal squamous cells from patients who have GERD without BE fail to induce CDX2 expression in response to any of these exposures (8). To further explore this mechanism, esophageal squamous cells from GERD patients *with* (NES-B3T & NES-B10T) and *without* (NES-G2T & NES-G4T) BE were exposed to acidic media (pH5.5), neutral bile salt media (pH 7.2), and acidic bile salt media (the same bile salt solution at pH5.5) and activation of NF-κB pathway proteins were determined. Compared to the Barrett's squamous cell lines, the GERD squamous cell lines exhibited less phosphorylation of IKKα/β and IKB after exposure to all three test media; levels of phospho-IKKα did not differ among the cell lines (Figure 1 and data not shown).

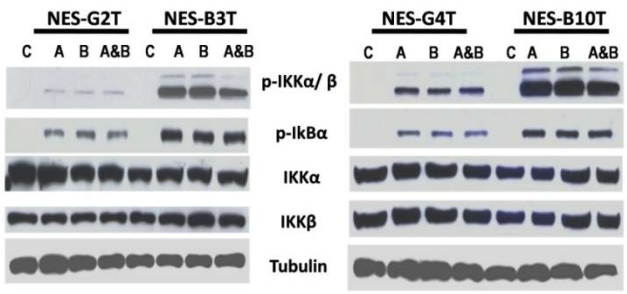
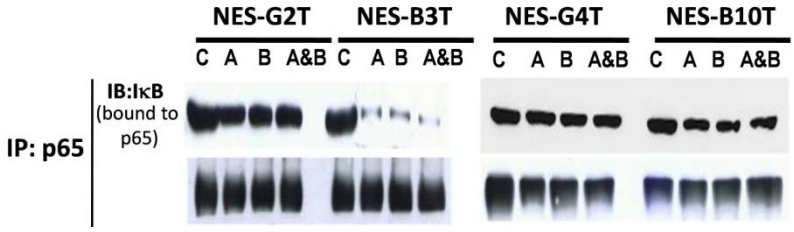


Figure 1. NES-G2T and NES-G4T exhibit less phosphorylation of IKK α/β and IκB than NES-B3T and NES-B10T in response to exposure to acid, bile salts, or the combination of both. C, non-treated control cells; A, acidic media alone; B, bile salt media alone; A&B, acidic bile salt media.

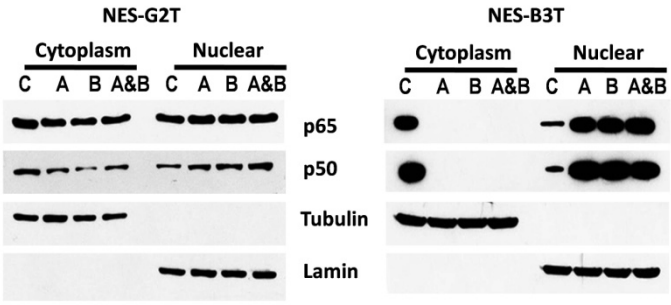
Compared to Barrett's squamous cell lines, the GERD squamous cell lines exhibited more cytoplasmic binding of p65 to IKB after exposure to all three test media (Figure 2).

Figure 2. NES-G2T and NES-G4T exhibit more cytoplasmic binding of p65 to IκB than NES-B3T and NES-B10T in response to exposure to acid, bile salts, or the combination of both. C, non-treated control cells; A, acidic media alone; B, bile salt media alone; A&B, acidic bile salt media.



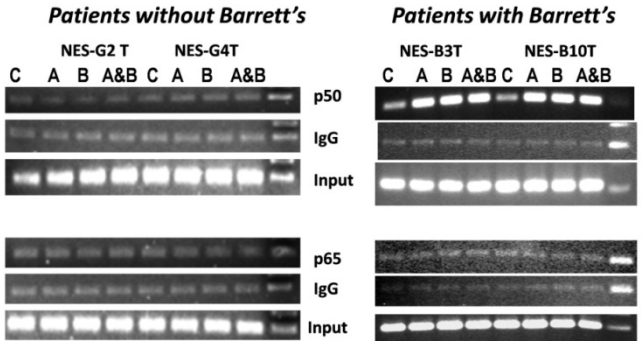
The NES-G lines also exhibited less nuclear translocation of p50, and there was no apparent nuclear translocation of p65 (Figure 3); these results were confirmed with immunofluorescence (data not shown).

Figure 3. NES-G2T exhibits less nuclear translocation of p50 and no apparent translocation of p65 compared to NES-B3T in response to exposure to acid, bile salts, or the combination of both. C, non-treated control cells; A, acidic media alone; B, bile salt media alone; A&B, acidic bile salt media.



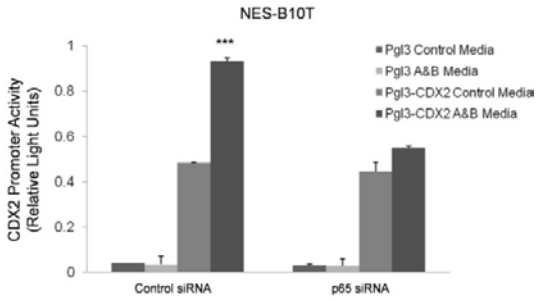
Using ChIP, we determined whether p50 and/or p65 bound to the CDX2 promoter. We observed no increase in binding of the p65 subunit to the CDX2 promoter in response to any of the test media in the squamous cells from GERD patients with and without BE. Following exposure to our test media, squamous cells from GERD patients with BE has a marked increase in DNA binding of p50, whereas squamous cells from patients without BE demonstrated no DNA binding of p50 to the CDX2 promoter (Figure 4). These data suggest that although p65 does not bind directly to the CDX2 promoter that nuclear translocation of p65 may be necessary to allow for p50 to bind the CDX2 promoter.

Figure 4. NES-G2T and NES-G4T have no apparent binding of p50 to the CDX2 promoter whereas as NES-B3T and NES-B4T demonstrate a marked increase in DNA binding of p50 in response to exposure to acid, bile salts, or the combination of both. None of our cells demonstrated p65 binding to the CDX2 promoter. C, non-treated control cells; A, acidic media alone; B, bile salt media alone; A&B, acidic bile salt media.



To determine whether nuclear translocation of p65 was required for p50 to bind to CDX2 promoter, we transfected NES-B10Tcells with p65 siRNA and with a CDX2 promoter-reporter construct and exposed the transfected cells to acidic bile salt media. As expected, acidic bile salt media significantly increase CDX2 promoter activity in NES-B10T cells containing the control siRNA. In contrast, acidic bile salt media did not increase CDX2 promoter activity in NES-B10T cells containing p65 siRNA (Figure 5).

Figure 5. Acidic bile salts cause a significant increase in CDX2 promoter activity in NES-B10T cells containing control siRNA but this same exposure does not increase CDX2 promoter activity in NES-B10T cells containing p65 siRNA. ***, p<0.001



These findings suggest that acidic bile salt media-induced nuclear translocation of p65 is necessary for the induction of CDX2 expression through the binding of p50 to the CDX2 promoter in esophageal squamous cells. **Therefore, reflux-induced induction of CDX2 might be prevented by agents that reduce phosphorylation and nuclear translocation of p65 and may be useful chemopreventive agents to prevent recurrent Barrett's metaplasia after RFA.**

Assessment of Differences in prostaglandin E2 are based on data from investigators involved in this study which demonstrate that a combination of esomeprazole and aspirin reduces tissue concentrations of prostaglandin E2 in patients with BE (14), and the methodology involved in that study.

Assessment of differences in the expression of proinflammatory cytokines known to induce activation of NFkB, i.e., TNFα, IL-1β, IL-6, IL-10, IL-17A, IL-23 will be measured according to standard methodology (27).

9.2 Comparable Methods

The methodology described above are those previously used (see details above), and the resulting data will be able to be compared to existing data.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

The laboratory of Dr. Rhonda Souza MD at the Center for Esophageal Research, Baylor Scott & White Research Institute, Dallas, TX will serve as the biomarker laboratory for assessment of the primary biomarker (differences in the change of CDX2 mRNA levels from baseline to 12 months post-randomization in esophageal squamous tissue between participants taking aspirin supplementation versus those taking placebo) and two important secondary biomarkers (differences in the change of CDX2 mRNA levels from baseline to 18 months post-randomization in esophageal squamous tissue between participants taking aspirin supplementation versus those taking placebo and differences in the activation status of NF-kB by assessing levels of total and phospho-p65 and cytoplasmic to nuclear translocation of phospho-p65. Dr. Souza's research has focused on the pathogenesis of reflux esophagitis, BE and esophageal adenocarcinoma. For the past 19 years, she has worked closely with Dr. Stuart Spechler to establish a translational research program at the Dallas VA that incorporates cell culture models, animal models, and *in vivo* patient studies to answer research questions related to GERD, BE, and esophageal

adenocarcinoma. Dr. Souza and Dr. Huo in her laboratory will provide expertise in molecular and cell biology as well as our technical experience in investigating the role of phospho-p65 and CDX2 as biomarkers in esophageal squamous in predicting the recurrence of Barrett's metaplasia after radiofrequency ablation.

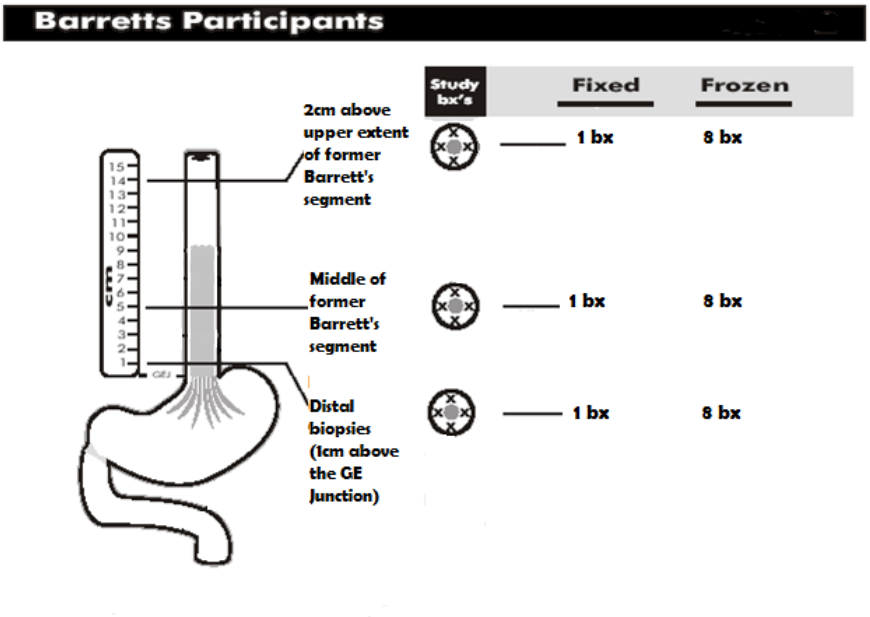
Differences in the the prostanoid marker, prostaglandin E_2, and prostaglandin synthases and Differences in the expression of proinflammatory cytokines known to induce activation of NFkB, i.e., TNFα, IL-1β, IL-6, IL-10, IL-17A, IL-23 will be measured in the laboratory of Dr. PeiyingYang Ph.D. at the University of Texas MD Anderson Cancer Center. Dr. Yang's lab have developed highly sensitive and specific methods for the analysis of both endogenous and exogenous bioactive lipids, such as prostaglandins, leukotrienes and HETEs, derived from cyclooxygenase and lipoxygenase enzymes. These methods have been applied to *in vitro* and *in vivo* studies in support of cancer research already used extensively by numerous investigators nationally. Dr Yang served as a leader of Analytical Core for the P01 project, led by Dr. Raymond Dubois to understand the role of bioactive lipid in inflammation and cancer and her lab helped analyze eicosaniods levels in over 2000 samples in various biological matrices. The methodology for these analyses is based primarily on an LC/MS/MS method using stable isotope dilution techniques employing deuterated internal standards (IS). The eicosanoid analyses are routinely carried out by Dr. Yang's laboratory. Additionally, the availability of the methods to simultaneously quantify COX and LOX metabolites has allowed a number of studies to illustrate the interrelationship of COX and LOX metabolites in certain malignancies.

10.2 Collection and Handling Procedures

Research Mucosal Biopsy Specimens

Esophageal mucosa biopsy techniques have been described in Section 7. Types of assay conducted for each biopsy specimen are described in Section 8. Research biopsies will be obtained at baseline (after consent but prior to randomization), at 12 months and at 18 months as described in Section 7.
Endoscopic research biopsies will be obtained with the Boston Scientific Radial Jaw
RJ4 large capacity biopsy forceps (provided by coordinating center at no additional cost to participating sites) from 1 cm above the level of the lower esophageal sphincter, just above the proximal margin of the gastric folds, in a zone corresponding to the middle of the former Barrett's segment, and a zone 2 cm above the upper extent of the former Barrett's segment.

Tissue kits will be provided by the coordinating Center at the University of Texas MD Anderson Cancer Center. Tissue collection, handling, and shipping instructions will be included with each kit. Participating Sites may obtain tissue kits by faxing the Supply Order Form to the number provided (found in the Forms Packet). At least two weeks should be allowed to receive the shipping kits. Kits will be sent via FedEx ® at no additional cost to the participating institutions. They will not be forwarded by FedEx ® rush delivery service unless the participating institution provides their own FedEx ® account number or alternate billing number for express service. Because charges are incurred for all outgoing kits, a small, but sufficient, supply of the specimen collection kits should be ordered prior to participant entry.



Biopsy #	Planned Use
Level 1 (1 cm above GE junction)	
Biopsy A1 (Formalin fixed)	Histological evaluation and immunohistochemistry
2 Biopsy A2 (RNAlater®)	CDX2 mRNA
2 Biopsies A3 (Flash Frozen)	CDX2 mRNA
Biopsy A4 (Flash Frozen)	total and phospho-p65 and cytoplasmic to nuclear translocation of phospho-p65
Biopsy A5 (Flash Frozen)	PGE2 and proinflammatory cytokines
Biopsy A6 (Flash Frozen)	PGE2 and proinflammatory cytokines
Biopsy A7 (Flash Frozen)	PGE2 and proinflammatory cytokines
Level 2 (middle of former Barrett's segment)	
Biopsy B1 (Formalin fixed)	Histological evaluation and immunohistochemistry
2 Biopsies B2 (RNAlater®))	CDX2 mRNA
2 Biopsies B3 (Flash Frozen)	CDX2 mRNA
Biopsy B4 (Flash frozen)	total and phospho-p65 and cytoplasmic to nuclear translocation of phospho-p65
Biopsy B5 (Flash frozen)	PGE2 and proinflammatory cytokines
Biopsy B6 (Flash Frozen)	PGE2 and proinflammatory cytokines
Biopsy B7 (Flash Frozen)	PGE2 and proinflammatory cytokines
Level 3 (2 cm above former Barrett's segment)	
Biopsy C1 (Formalin fixed)	Histological Evaluation
2 Biopsies C2 (RNAlater®)	CDX2 mRNA
2 Biopsies C3 (Flash Frozen)	CDX2 mRNA
Biopsy C4 (Flash Frozen)	total and phospho-p65 and cytoplasmic to nuclear translocation of phospho-p65
Biopsy C5 (Flash Frozen)	PGE2 and proinflammatory cytokines

Biopsy C6 (Flash Frozen)	PGE2 and proinflammatory cytokines
Biopsy C7 (Flash Frozen)	PGE2 and proinflammatory cytokines

Biopsies A1, B1 and C1 will be placed in neutral buffered formalin at room temperature. Biopsies A2, B2 and C2 will be placed in RNAlater® at 2°-8°C. The remainder of the biopsies will be washed in phosphate buffered saline, each biopsy placed in a cryovial then placed in liquid nitrogen. Vials will be stored at a temperature of -80°C. Specimens must not be thawed before analysis.

Random, blinded, unique ID numbers will be assigned to specimens at each time point (baseline and post-treatment: 12 months and 18 months) and will be distributed with the PID number. All specimens will be labeled with the unique specimen number (XXXX), date (DD-MM-YY) and product code (N) using a permanent, waterproof marker. Laboratories will be blinded from distinguishing participant information and visit point but this information will be kept available in a password protected electronic log at each site to indicate which specimen number belongs to which participant and at what visit and at the Coordinating Center.

10.3 Shipping Instructions

Biopsies taken for research specimens (A1, B1, C1) will be placed in formalin and shipped overnight to the MD Anderson (MDACC) Coordinating Center for further processing. MDACC Research Histology Core will process and paraffin-embed the samples, and then cut one H & E and 20 unstained slides. Dr. Dipen Maru or his designee will provide a diagnosis for these samples (confirmed absence of Barrett's metaplasia or dysplasia, or presence of Barrett's metaplasia or dysplasia).
Biopsies A2, B2 and C2 will be shipped the 3rd week of each month.

Biopsies taken for formalin fixation and paraffin embedding will be placed in prefilled 10% neutral buffered formalin (Azer Scientific 10 ml prefilled in 20 ml containers supplied by the coordinating center). Containers will be wrapped in parafilm, placed in plastic bags, bags sealed and shipped overnight to the coordinating center at the address listed below.

Frozen specimens will be batch shipped on dry ice monthly to the Coordinating Center at MDACC.

- All tissue samples must be kept frozen (-80^{o}C or below) after preparation and until shipment is initiated.
- Wrap the frozen samples in bundles using an elastic band or place samples in appropriate sample storage box and place in a plastic freezer bag.
- Use newspaper or other similar material to insulate the bagged sample bundles from direct contact with the dry ice.
- Shipping containers (e.g., outer foam/cardboard shipping boxes) should be insulated, filled with dry ice and sealed with tape at the time the shipment is made. The shipping labels supplied should be affixed firmly to the outside of each container.
- All samples must be shipped in accordance with local biohazard requirements. Please refer to your institutional policy for biohazard labeling and packaging for shipment of hazardous and infectious human samples. If sealed biohazard containers are used, DO NOT place dry ice inside these containers. Place dry ice outside the containers only. Include a minimum of 10 kg dry ice when shipping by overnight express. Record the estimated weight of the dry ice used per box.
- Shipments should not be made on Friday or the day prior to a public holiday.

- An inventory listing of all the samples must be included in the shipment. The inventory listing should contain the following information from each sample label: protocol number, patient number, patient initials and date and time of collection, along with any other pertinent information. Before placing in the shipping container, put the inventory list in a plastic bag for protection.

Samples will be shipped in containers provided and according to instructions provided by the Coordinating from collaborating Institutions to the following location:

Tamara Tipps
UT MD Anderson Cancer Center
Department of Gastroenterology, Hepatology and Nutrition
1400 Pressler Street, FCT13.6013
Houston, Texas 77030-4900
Telephone 713-794-1439
Back-up Telephone: 713-745-4340
Fax: 713-745-9295
Email: tltipps@mdanderson.org

The Coordinating Center will be notified via e-mail when samples are shipped, so if samples are delayed or lost, tracking may be initiated by the sending site. Sample shipment forms are included with shipments. These data forms describe the date of sample receipt, and availability of sample, along with tracking information. The Coordinating Center will evaluate the sample condition on arrival, enter the sample numbers in the database, verify samples shipped match samples sent, and store at appropriate conditions until shipment to analytical labs.

All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations.

10.4 Tissue Banking

All research specimens are shipped to the study repository at the UTMDACC for storage which will be housed in the laboratory of the Principal Investigator, Robert S. Bresalier, M.D. The laboratory is equipped with five -80 degree freezers which serve as repositories for research samples.

Biologic specimens collected during the conduct of each clinical trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI. At study completion, NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense.

11. REPORTING ADVERSE EVENTS

DEFINITION: AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible.

A list of AEs that have occurred or might occur can be found in §6.2 Reported Adverse Events and Potential Risks, as well as the Investigator Brochure or package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

All AEs that occur after the informed consent is signed and baseline assessments are completed (including run-in) must be recorded on the AE CRF whether or not related to study agent.

11.1.2 AE Data Elements:

The following data elements are required for adverse event reporting.

- AE verbatim term
- CTCAE (MedDRA) System Organ Class (SOC)
- NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) AE term (MedDRA lowest level term
- Event onset date and event ended date
- Treatment assignment code (TAC) at time of AE onset
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a serious adverse event (SAE)
- Whether or not the participant dropped due to the event
- Outcome of the event

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using the CTCAE version 4.0. The CTCAE provides descriptive terminology (MedDRA lowest level term) and a grading scale for each AE listed. A copy of the CTCAE can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AEs will be assessed according to the grade associated with the CTCAE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated below.

CTCAE v4.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.

Grade	Severity	Description
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

ADL

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.1.4 Assessment of relationship of AE to treatment

The possibility that the adverse event is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

11.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

11.2 Serious Adverse Events

11.2.1 Regulations at 21 CFR §312.32 (revised April 1, 2014) defines an SAE as any untoward medical occurrence that at any dose has one or more of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to perform normal life functions
- A congenital anomaly or birth defect
- Important medical events that may not be immediately life-threatening or result in death or hospitalization should also be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require intervention to prevent one of the other outcomes.

11.2.2 Reporting SAEs to DCP

The organization that experiences the serious adverse event (SAE) should report the SAE to the following 3 entities: 1) NCI DCP, 2) DCP's regulatory contractor CCSA, and 3) MDACC, the CLO. Detailed reporting instructions are provided below. In addition, all participating organizations will follow their IRB requirements for SAE reporting.

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE form found at http://prevention.cancer.gov/clinical-trials/clinical-trials-management/protocol-information-office/pio-instructions-and-tools/2012-consortia.

11.2.2.2 Reporting within 24 hours of knowledge of the event.

11.2.2.2(A) **Report to the NCI DCP Medical Monitor within 24 hours:**

Contact the DCP Medical Monitor by phone within 24 hours of knowledge of the event.

Gary Della'Zanna, DO, MSc
National Cancer Institute
Division of Cancer Prevention
Gastrointestinal and Other Cancers Research Group
9609 Medical Center Drive, MSC-9782
Rockville, MD 20850
Phone: 240-276-7042
Fax (with cover sheet, Attn: Dr. G. Della'Zanna): 240-276-7848
Email: gary.dellazanna@nih.gov
DellaZannagj@mail.nih.gov

Include the following information when calling the Medical Monitor:

- Date and time of the SAE
- Date and time of the SAE report
- Name of reporter
- Call back phone number
- Affiliation/Institution conducting the study
- DCP protocol number
- Title of protocol
- Description of the SAE, including attribution to drug and expectedness

11.2.2.2(B) **Report to the Consortium Lead Organization (CLO) PI (Dr. Powel Brown) within 24 hours of knowledge of the event:**

Report all SAEs to the Consortium Lead Organization PI (Dr. Powel Brown) within 24 hours of knowledge of the event. The same information reported to the DCP Medical Monitor should be provided to the CLO Coordinator via email, phone or fax within 24 hours of knowledge of the event.

11.2.2.3 Reporting within 48 hours of knowledge of the event:

11.2.2.3 (A) Email the written SAE reports to the DCP Medical Monitor within 48 hours of learning of the event using the paper SAE form. The SAE forms should be obtained at http://prevention.cancer.gov/files/clinical-trials/SAE_form.doc.

11.2.2.3 (B) The written SAE reports will also be emailed to DCP's Regulatory Contractor, CCS Associates at safety@ccsainc.com

11.2.2.3 (C) The written SAE report will also be emailed to the Consortium Lead Organization PI (Dr. Powel Brown), at PHBrown@mdanderson.org.

It is the responsibility of the CLO to inform the Lead Protocol PI upon receipt of the report from the organization experiencing the event.

11.2.2.4 The DCP Medical Monitor and CCSA regulatory and safety staff will determine which SAEs require FDA submission.

11.2.2.5 The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

11.2.2.6 Follow-up of SAE

Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE form in the appropriate format. Follow-up information should be sent to DCP as soon as available. SAEs related to the study agent will be followed until resolved.

12. STUDY MONITORING

12.1 Data Management

This study will report clinical data using the Data Management Initiative (DMI) web-based application managed by the Consortium Biostatistics and Data Management Core. Data Management Initiative (DMI) infrastructure has been developed in the Division of Quantitative Sciences (DQS), MD Anderson Cancer Center. This infrastructure supplies integrated database and software services for web-based data collection, randomized treatment assignment, reporting, query, data download, and data quality management. The DMI will be the database of record for the protocol and participant to NCI and FDA audit. All DMI users will be trained to use the DMI system and will comply with the instructions in the protocol-specific "DMI User Manual" as well as applicable regulatory requirements such as 21 CFR; Part 11. Data management procedures for this protocol will adhere to the Data Management Plan (DMP) on file at the DCP for contract HHSN261201200034I.

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRF) developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDEs). The approved CRFs will be used to create the electronic CRF (e-CRF) screens in the DMI application. Site staff will enter data into the e-CRF. Amended CRFs will be submitted to the DCP Protocol Information Office for review and approval. Approved changes will be programmed into the DMI database by the Consortium Biostatistics and Data Management Core.

12.3 Source Documents

Source documentation will include only those documents containing original forms of data, including clinic charts, shadow files, hospital charts, and physician notes. Data recorded directly on the CRFs designated as source documents (i.e., no prior written or electronic record of data) will be considered source data. All other data recorded on the CRFs will not be considered source documentation.

12.4 Data and Safety Monitoring Plan

The Data and Safety Monitoring Plan for the MD Anderson Consortium is on file at the DCP. This study will be monitored by the MDACC Data and Safety Monitoring Board, the data and safety monitoring board of record for this study. The Data Safety and Monitoring Board (DSMB) reports to the President, or his designee, as the on-campus representative of The University of Texas Board of Regents. It oversees the data and patient safety issues for randomized clinical trials that originate at MD Anderson; that are coordinated or analyzed by MD Anderson and are not being monitored by any other DSMB; or have been

designated as requiring DSMB monitoring at the request of the IRB, the CRC, or institution. The primary objectives of the DSMB are to ensure that patients' rights pertaining to participation in a research study are protected, and that patients' interests are prioritized over the interests of the scientific investigation. Responsibilities include:

(a) Review interim analyses of outcome data (prepared by the study statistician or other responsible person at the time points defined in the study) approved by the IRB and additional time points as determined by the DSMB, and to recommend, if necessary, whether the study needs to be changed or terminated based on these analyses;
(b) Determine whether, and to whom, outcome results should be released prior to the reporting of study results;
(c) Review interim toxicity data and efficacy of treatment;
(d) Review major research modifications proposed by the investigator or appropriate study committee prior to implementation (e.g., termination, dropping an arm based on toxicity results from the study or results of other studies, increasing target sample size).

Refer to the Data and Safety Monitoring Plan for the MD Anderson Consortium on file at the DCP for further details.

12.5 Sponsor or FDA Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc*.), as well as IRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

12.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

The agent(s) supplied by DCP, NCI, used in this protocol, is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA) between the Pharmaceutical Company(ies) (hereinafter referred to as Collaborator(s)) and the NCI Division of Cancer Prevention. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator@ contained within the terms of award, apply to the use of Agent(s) in this study:

12.7.1 Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational agents contain confidential

information and should not be shared or distributed without the permission of the NCI. If a patient participating on the study or participant's family member requests a copy of this protocol, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from the DCP website.

12.7.2 For a clinical protocol where there is an Investigational Agent used in combination with (an) other investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-party Data"). This section is not applicable to the present protocol.

12.7.3 NCI must provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol. This section is not applicable to the present protocol.

12.7.4 Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval, or commercialize its own investigational agent.

12.7.5 Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational agent.

12.7.6 Clinical Trial Data and Results and Raw Data developed under a collaborative agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate. All data made available will comply with HIPAA regulations.

12.7.7 When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators of Collaborator's wish to contact them.

12.7.8 Any manuscripts reporting the results of this clinical trial must be provided to DCP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days (or as specified in the CTA) from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to DCP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to DCP prior to release. Copies of any manuscript, abstract, and/or press release/ media presentation should be sent to the Protocol Information Office at NCI_DCP_PIO@mail.nih.gov.

The Protocol Information Office will forward manuscripts to the DCP Project Officer for distribution to the Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

This a placebo-controlled phase II study to evaluate the effect of aspirin on the modulation of CDX2 mRNA levels from baseline to 12 months post-randomization in esophageal squamous tissue in participants with BE after successful eradication with radiofrequency ablation.

13.2 Randomization/Stratification

Randomization will be performed using the random permutated block design to ensure the balance between the two arms (aspirin and placebo). The randomization list will be generated by the study statistician and incorporated into the data base in a double blind fashion, i.e. both the study investigators and the study participants are blinded to the designation of the specific treatment arm to which the participant is assigned.

13.3 Accrual and Feasibility

1. **Planned duration of accrual: Two years (including study initiation)**
2. **Expected accrual rate per month: 5-7**
3. **Number of expected participants registered per month per site: approx. 1-2**

The investigators participating in this study have extensive experience in the treatment and study of patients with BE, and specifically follow large cohorts who have undergone RFA for this disease. Active and ongoing programs insure entry of new eligible participants who will undergo RFA for Barrett's (range 50 to 120/site/year). RFA cohorts (completed and currently under active treatment; new RFA pts/year) at each site include MDACC (250;120), UNC (386;70), St Michael's Hospital (370;80), Mayo Rochester (320;110), Northwestern U (310;100), UCLA (250;48), U Colorado (150;70), U Pennsylvania (130;50), U Kansas VA (70;30), BCM (115;35).

13.4 Primary Objective, Endpoint(s), Analysis Plan

The **primary endpoint** of the study will be **differences in the change of CDX2 mRNA levels from baseline to 12 months post-randomization in esophageal squamous tissue between** participants **taking aspirin supplementation versus those taking placebo.**

Proposed Sample Size: The primary endpoint is the change of CDX2 mRNA expression from baseline to 12-months post-treatment. The sample size is determined based on the precision of measuring the CDX2 mRNA expression at baseline as well as the change from baseline to 12-months post-treatment.
Repeated Measures: The effect size of aspirin on CDX2 mRNA expression in tissues is not known. However, we have prior data on phosphorylation of p65 in esophageal tissue after esophageal perfusion with bile acids which revealed an effect size (*d* values) that was > 1.0. It is assumed that CDX2 will correlate with p65 as it is a downstream target gene. Without treatment, it is expected that CDX2 mRNA levels will increase after radiofrequency ablation and that the increase in CDX2 mRNA is associated with the development of metaplasia. As shown in the table below, after normalization, the expected CDX2 mRNA level at baseline is 100 in both groups. In the placebo group, we assume that CDX2 mRNA levels will increase to 120 at 12 months. On the other hand, with conservative estimates, we expect that the level for the aspirin treated group will remain at 100 at 12 months. Hence the difference of CDX2 mRNA change from baseline to 12 months is assumed to be 20 between the two groups. Assuming a standard deviation of 20 for the change in CDX2 mRNA, the corresponding effect size is 1.0. An effect size of 1.0

on the biomarker modulation is a conservative estimation for similar agents in chemopreventive settings. For example, sulindac was assumed to have an effect size of 1.06 in detecting the PGEM level in a Phase I trial. (32) Naproxen was assumed to have an effect size of 1.25 in modulation of the tissue PGE2 level in a Phase Ib biomarker trial. (33)
With a two-sided 5% type I error, we will have 83% power for detecting the difference of CDX2 change between the two groups with 18 participants per group using the two-sample t-test.

In addition, the CDX2 mRNA level will be quantified in the baseline and the follow-up samples for both randomized and non-randomized patients. Specifically, the CDX2 mRNA level in patients with continued presence or recurrence of Barret's mucosa will be analyzed to explore the value of CDX2 as a marker for residual disease. The analysis can be done within the same patient if CDX2 mRNA measures are available in both Barret's mucosa and normal tissue. Otherwise, the analysis will be done between patients. The distribution of CDX2 mRNA level across different tissue types, different treatment groups, and different follow-up time will be exploratory in nature to help us in better quantify the expression level for understanding its value in designing future studies. With a sample size of 18 and 36 (two treatment groups combined), the standard error of the mean can be reduced by 4.2 and 6.0 folds of its standard deviation, respectively. We plan to enroll a minimum of 20 participants per group to account for a potential 10% attrition rate.

	Baseline	12-month
Aspirin Group	100	100
Placebo group	100	120

Statistical Analyses: We will perform both parametric (t-test) and non-parametric (Wilcoxon) tests for detecting the effects of aspirin supplementation on the primary and the secondary endpoints of the study. Benjanimi-Hochberg FDR correction will be applied to account for multiple testing for the bioinformatics analyses when appropriate.

13.5 Secondary Objectives, Endpoints, Analysis Plans

Secondary Endpoints

Safety will be assessed by comparison of adverse events using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (Refer to Section 8.2.1). Safety will be assessed by monitoring routine clinical parameters at baseline and at each visit throughout the trial (including every 3 months by phone calls and/or mail interviews and in person at 12 and 18 months), and by monitoring laboratory parameters at baseline and at 12 months. All AEs will be recorded and graded during clinical visits, whether or not they are considered drug-related. All participants will be monitored for toxicity from the time of randomization. Each treatment arm will be monitored separately for severe toxicities (grade 3 and 4 and SAEs). We will be especially vigilant about the incidence of gastrointestinal (gastric and duodenal ulcers, gastritis, gastrointestinal bleeding or perforation, hematemesis) and hematological (anemia, thrombocytopenia) events. Toxicity will be considered drug-related based on clinical assessment. In addition, at the completion of the study, the frequency of toxicities will be compared between the placebo and aspirin groups.

The SF-36 is a multi-purpose, short-form health survey with 36 questions. It yields an 8-scale profile of functional health and well-being scores as well as psychometrically-based physical and mental health summary measures and a preference-based health utility index. It is a generic

measure, as opposed to one that targets a specific age, disease, or treatment group. Since a secondary endpoint of our study is to determine safety in a specific clinical setting (use of aspirin following radiofrequecy ablation, we will compare measures of health and well-being between those receiving aspirin and those receiving placebo. Bivariate analyses will be performed on study entry and completion of the on-drug period. Multivariate analyses will be performed between groups after controlling for potential confounders.

Differences in the change of CDX2 mRNA levels from baseline to 18 months post-randomization in esophageal squamous tissue between participants taking aspirin supplementation versus those taking placebo.

Differences in the activation status of NF-kB by assessing levels of total and phospho-p65 and cytoplasmic to nuclear translocation of phospho-p65 which is likely to be affected by aspirin (comparison between baseline, 12 and 18 months).

Differences in the prostanoid marker, prostaglandin E_2, and prostaglandin synthases (comparison between baseline, 12 and 18 months)

Differences in the expression of proinflammatory cytokines known to induce activation of NFkB, i.e., TNFα, IL-1β, IL-6, IL-10, IL-17A, IL-23 will be measured (comparison between baseline, 12 and 18 months).

Recurrent Barrett's Esophagus (at 12 and 18 months) will be ascertained completely and accurately. While we will not have sufficient power to assess differences in histological recurrence of BE during the short course of the study, we will examine this as an exploratory secondary endpoint.

Statistical Analyses: In addition to the standard statistical hypotheses testing for the effect of aspirin on each individual biomarker in the proposed panel, we will use select tools from the fields of *Machine Learning* and *Pattern Recognition* to build multivariate classification models, e.g. Linear Discriminant Analyses (LDA) and k-Nearest Neighbours (kNN), based on combinations of the proposed biomarkers. This is an exploratory analysis to search for the joint effect of multiple biomarkers in predicting the outcome of potential clinical interest. The application of classification is facilitated by the presence of different cases with different levels of CDX2 modulation where we will be interested in the strength of separation between the participants who belong to one or the other class of interest. Given that the biomarkers will be measured at baseline, 12 months and 18 months, we will evaluate the difference of the change on these biomarkers at 12 months and 18 months between two treatment arms by employing repeated measures analysis, including generalized mixed effect model and generalized estimating equations. The purpose of the exploratory analysis is for hypothesis generating and the findings will need to be validated in future studies.

13.6 Reporting and Exclusions

Adherence to treatment will be assessed in two ways: 1) Participant-reported compliance: every 3 months, during the telephone and/or mail interview with the study coordinator, the participant will be asked to report how many pills they took per week on average since their last phone interview. A report of 7 pills would represent 100 % compliance, 6 pills would represent 86 % compliance, etc... 2) Computed compliance based on participant-reported pill bottle start dates: participants will be asked to record and report to their study coordinator the date that they start each new bottle of study pills. Given the start date of each pill bottle and the total number of pills in each bottle (which the participant will be instructed to finish prior to starting the next bottle) we will calculate a compliance rate for each bottle. To maintain

blinding, these data will only be used to measure compliance at the level of treatment groups. The Project Coordination Center will regularly summarize the pill-taking status of each center's participants, and list for each coordinator the relevant study ID numbers of those who have reported changed or inadequate adherence.

Compliance will be defined as ingestion of ≥ 80% of the planned agent doses for the purposes of data analyses.

13.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose of aspirin or placebo .

13.8 Evaluation of Response

All participants included in the study will be assessed for response to intervention, even if there are major protocol deviations or if they are ineligible.

All of the participants who met the eligibility criteria (with the possible exception of those who did not receive study agent) will be included in the main analysis. All conclusions regarding efficacy will be based on all eligible participants.

Subanalyses may be performed on the subsets of participants, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of intervention, major protocol violations, etc.). However, subanalyses may not serve as the basis for drawing conclusions concerning efficacy, and the reasons for excluding participants from the analysis should be clearly reported. For all measurements of response, the 95% confidence intervals should also be provided.

13.9 Interim Analysis

There is no formal interim analysis planned for this study.

13.10 Ancillary Studies

There are no plans for ancillary studies.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Lead Investigator at the Lead or Participating Organization(s) will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators, at each site that will participate in the protocol. All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be listed on Form FDA 1572.

14.2 Other Required Documents

14.2.1 Current (within two years) CV or biosketch for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.2 Current medical licenses (where applicable) for all study personnel listed on Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.3 Lab certification (*e.g.*, CLIA, CAP) and lab normal ranges for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

14.2.4 Documentation of training in "Protection of Human Research Subjects" for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.5 Documentation of Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.6 Signed Investigator's Brochure/Package Insert acknowledgement form

14.2.7 Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the Principal Investigator for each site and initialed by all study personnel listed on the form

14.2.8 Signed and dated NCI, DCP Financial Disclosure Form for all study personnel listed on Form FDA 1572 for the Lead Organization and all Participating Organizations

Form FDA 1572 and all of the documents listed in Section 14.2 will be collected by the Consortium Lead Organization on behalf of the Protocol Principal Investigator, Robert S. Bresalier, M.D.

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Individuals who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option may be included within the informed consent document

or may be provided as an addendum to the consent. A Model Consent Form for Use of Tissue for Research is available through a link in the DCP website.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the IRB at each Organization at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by NCI, DCP, the Consortium Lead Organization's IRB, and then submitted to each organization's IRB for approval prior to initiation.

14.5 Submission of Regulatory Documents

All regulatory documents are collected by the Consortia Lead Organization and reviewed for completeness and accuracy. Once the Consortia Lead Organization has received complete and accurate documents from a participating organization, the Consortium Lead Organization will forward the regulatory documents to DCP's Regulatory Contractor:

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates, Inc.
1923 Landings Drive
Mountain View, CA 94043
Phone: 650-691-4400
Fax: 650-691-4410

E-mail Submissions:
regulatory@ccsainc.com

Regulatory documents that do not require an original signature may be sent electronically to the Consortium Lead Organization for review, which will then be electronically forwarded to DCP's Regulatory Contractor.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

Participants will not be responsible for the costs of this study. Study agent will be provided at no cost to the participant. If, as a result of participation in this study, an individual experiences injury from known or unknown risks of the research procedures as described in the informed consent, immediate medical care and treatment, including hospitalization, if necessary, will be available. No monetary compensation is available for the costs of medical treatment for an injury, thus, the participant will be responsible for the costs of such medical treatment, either directly or through their medical insurance and/or other forms of medical coverage.

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APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	**Descriptions**	**Percent**	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Appendix B: Pill diary/calendar

Patient Name: _____PID: _____Visit #: _____

Number of Pills Given:_____
Total Daily Dose:_____
(To be completed by RN/PI Designee)

Pill Bottle(s) returned: Circle **Yes** or **No**
Number of Pills returned:_____

This calendar will be provided to you every three months in order to record the drug daily intake. Please do not forget to take 1 tablet a day, every day, in the morning with a meal.

There are 100 pills in each bottle. Please take at least 3 months of pills from each bottle prior to starting the next bottle.
Please do not take an extra dose to 'make up' for the missed/vomited dose. Take your next dose as scheduled.
Cross out the corresponding day of the calendar if you have taken your tablet (1 cross per day to indicate tablet taken).
Do not cross out the day if you have forgotten to take your medication.
Should you experience any symptom during the study, please write it down in the space provided and should you need any medical assistance, please contact [the local PI or designee name/phone #] during working hours.
Please do not discard any empty bottles and do not forget to mail back or bring with you all the drug bottles (empty or full) together with the present calendar to the next clinic visit.

PLEASE BRING THIS SHEET TO YOUR NEXT VISIT OR SHIP IT BACK WITH YOUR RETURNED MEDICATIONS

Month: _____

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Notes:

Month:_____

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Notes:

Please fill also the back page

Month:_____

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Notes:

Month:_____

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Notes:

Patient Signature: _____ Date: _____
Consenting Professional/Research RN /PI designee Signature: _____ Date: _____
Consenting Professional/Research RN/PI designee Comments: _____
_____
_____
_____