COVER PAGE

DCP Protocol #: NWU2015-06-01

Local Protocol #: NCI 2015-06-01

Evaluating Intermittent Dosing of Aspirin for Colorectal Cancer Chemoprevention

Consortium Name: Northwestern Cancer Prevention Consortium

Organization Name*: Northwestern University
Name of Consortium Principal Investigator: Seema A. Khan, MD
303 E. Superior, Suite 4-111
Chicago, IL 60611
Phone: (312)503-4236
Fax: (312)503-2555
E-mail: skhan@nm.org

Organization Name: Vanderbilt University
Protocol Principal Investigator: Qi Dai, MD, Ph.D.
2525 West End Avenue
Suite 800, 8th Floor
Nashville, TN37203
Phone: (615)936-0707
Fax: (615)343-5938
E-mail: Qi.dai@vumc.org

Organization Name: Northwestern University
Protocol Co-PI: Lifang Hou, MD, Ph.D.
680 N Lake Shore Drive, Suite 1400
Chicago, IL 60611
Phone: (312) 503-4798
Fax: (312) 908-9588
E-mail: l-hou@northwestern.edu

Organization: Vanderbilt University
Investigator (Study Chair): Reid Ness, MD, MPH
Gastroenterologist
6th Floor Medical Center East
Nashville, TN 37232
Phone: (615)322-0128
Fax: (615)343-8174
E-mail: reid.ness@vumc.org

Organization: Vanderbilt University
Investigator (Study Physician): Rishi Naik, MD, MSCI

Gastroenterologist
1301 Medical Center Drive
1660 TVC
Nashville, TN 37232
Phone: (615) 343-0109
Fax: (615) 343-8174
E-mail: rishi.d.naik@vumc.org

Organization: Northwestern University
Statistician: Masha Kocherginsky, PhD
680 N. Lake Shore Dr., Suite 1400
Chicago, IL 60611
Tel: (312) 503-4224
Fax: (312) 908- 9588
Email: mkocherg@northwestern.edu

IND Sponsor: NCI/Division of Cancer Prevention
IND# [redacted] (Exempt)
Agent(s)/Supplier: Aspirin / NCI DCP
NCI Contract # HHSN2612201200035I
Protocol Version Date: August 11, 2022

Protocol Version Version 2.19

*No participant accrual occurs at this site

SCHEMA

Colorectal Adenoma Patients

Participants will be recruited from Vanderbilt using electronic medical records and the Personalized Prevention of Colorectal Cancer Trial (PPCCT) through the Survey Research Shared Resource (SRSR).

↓

Pre-Screen Chart Review by Gastroenterologist (n=222)

Confirm diagnosis of colorectal adenoma, review safety profile, review eligibility criteria

↓

Clinic Visit 1: Screening (n=180)

Obtain consent; review medical history, physical including vital signs, and performance status, con meds, symptoms; obtain urine, stool, and blood; pregnancy test; register and randomize

↙ ↓ ↘

Randomization (n=90)

Daily aspirin (n=40) 325mg	Placebo (n=10)	Intermittent aspirin (n=40) • Weeks 1-3: aspirin 325mg • Weeks 4-6: placebo • Weeks 7-9: aspirin 325mg • Weeks 10-12: placebo

↓

Clinic Visit 2: Baseline
Within 6 weeks of Clinic Visit 1

Review questionnaires; review vital signs, con meds, and symptoms; obtain urine, stool, blood, rectal swabs, and rectal biopsies; dispense 3 bottles of study agent

↓

Clinic Visit 3: Intervention[1]
9 weeks after start of intervention (range: 8 weeks to 9 weeks and 2 days)

Review vital signs, con meds, and symptoms; obtain urine, stool, blood, rectal swabs, and rectal biopsies; collect and count unused study agent; dispense fourth bottle of study agent

↓

Clinic Visit 4: Intervention[1] or Off-Agent Visit[2]
12 weeks after start of intervention (range: 11 weeks to 12 weeks and 2 days)

Review vital signs, con meds, and symptoms; obtain urine, stool, blood, rectal swabs, and rectal biopsies; collect and count unused study agent

↓

Endpoints

Primary Endpoint: Ratio of cell proliferation/ apoptosis (rectal biopsies)
Secondary Endpoints: To evaluate the effects of aspirin treatments on:
1) the ratio of cell proliferation (Ki-67)/apoptosis (TUNEL) in rectal biopsies
2) the ratio of cell proliferation (Ki-67)/necroptosis (pMLKL) in rectal biopsies
3) fecal occult blood test (measures of adverse events) as measured by stool samples

Exploratory Endpoints: To evaluate the effects of aspirin treatments on:
1) COX-2 in rectal biopsies
2) BAX in rectal biopsies
3) TRPM7 in rectal biopsies
4) joint index of COX-2 with TRPM7 expression in rectal biopsies;
5) joint index of TRPM7 with BAX in rectal biopsies;
6) methylation assays using the MethylationEPIC BeadChip to identify methylation biomarkers in genes (i.e. CDKN2A (cell cycle regulation), MGMT (DNA repair), DAPK1(apoptosis), CDH1(cell invasion), WNT16 (Wnt pathway) and RASSF1 (RAS signaling)) involved in colorectal carcinogenesis, and other epigenome-wide methylation biomarkers as measured in rectal biopsies;
7) metagenomics analysis to measure abundance of E. coli and Fusobacterium and other microbiota in rectal swabs

[1]Total duration of intervention: 12 weeks (range: 11 weeks to 12 weeks and 2 days). Expected drop outs: n=15.
[2]If a participant comes off-agent (e.g. due to AE or concomitant medication), the Off-Agent Visit will be scheduled to occur as soon as possible after the participant stops the study agent.

Table of Contents

1. OBJECTIVES

1.1 Primary Objectives

The primary objective of the study is to test for the equivalency of the two aspirin schedules. We are going to evaluate the effects of a 3-month intervention of continuous versus intermittent aspirin dosing (i.e. 325 mg daily and 325 mg 3 weeks on/3 weeks off) on the ratio of cell proliferation (Ki-67) /apoptosis (BAX) in rectal biopsies.

1.2 Secondary Objectives

To evaluate the effects of aspirin treatments on:

1) the ratio of cell proliferation (Ki-67)/apoptosis (TUNEL) in rectal biopsies
2) the ratio of cell proliferation (Ki-67)/necroptosis (pMLKL) in rectal biopsies
3) fecal occult blood test (measures of adverse events) as measured by stool samples

1.3 Exploratory Objectives

To evaluate the effects of aspirin treatments on:

1) COX-2 in rectal biopsies
2) BAX in rectal biopsies
3) TRPM7 in rectal biospies
4) joint index of COX-2 with TRPM7 expression in rectal biopsies;
5) joint index of TRPM7 with BAX in rectal biopsies;
6) methylation assays using the MethylationEPIC BeadChip to identify methylation biomarkers in genes (i.e. CDKN2A (cell cycle regulation), MGMT (DNA repair), DAPK1(apoptosis), CDH1(cell invasion), WNT16 (Wnt pathway) and RASSF1 (RAS signaling)) involved in colorectal carcinogenesis, and other epigenome-wide methylation biomarkers as measured in rectal biopsies;
7) metagenomics analysis to measure abundance of E. coli and Fusobacterium and other microbiota in rectal swabs

2. BACKGROUND

2.1 Study Disease: Colorectal Cancer

Approximately 1 in 18 individuals will develop CRC over their lifetime and 40% will die within 5 years of diagnosis, mainly due to diagnosis at a late stage[1;2]. Removal of colorectal adenoma via colonoscopy has been considered the most effective strategy to reduce both incidence and mortality of CRC. However, recent reports found that such an approach is more significantly linked to reduced deaths from **tumors in** *the distal side of the colon than from the proximal side* [3;4]. In as many as 24% of cases, snare polypectomy of exophytic lesions did not inhibit progression to carcinoma[5]. Furthermore, annual recurrence rates in patients with small and advanced adenomas were as high as 19.3% and 22.9%, respectively[6]. Most importantly, CRC still remains the 4th most common incident cancer and the 2nd most common cause of cancer death in the United States[7;8]. *Thus, in addition to colonoscopy, it is critical to develop novel primary and/or secondary preventive strategies for CRC, particularly proximal colon cancer.*
A number of large initiatives over the past decade have generated disappointing results for the prevention of colorectal cancer using vegetables, fiber[9], carotenoids[9], vitamins E and C[10], folic acid[11-13], and calcium and/or vitamin D[14;15].

2.2 Study Agent: Aspirin

Epidemiologic studies and randomized trials have relatively consistently shown that long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin and non-aspirin NSAIDs, and COX-2 inhibitors, is associated with a reduced risk of colorectal cancer and adenoma recurrence [16-21]. Selective COX-2 inhibitors were developed to avoid the potential adverse events of COX-1 inhibition [22]. However, long-term use of COX-2 inhibitors was shown to have severe side effects of cardiovascular disease (**CVD**)[22]. In addition to COX-2 inhibitors, long-term use of non-aspirin NSAIDS causes severe side effects. On July 9th 2015, the U.S. Food and Drug administration (FDA) has strengthened an existing label warning that non-aspirin NSAIDs increase the risk of CVD events http://www.fda.gov/Drugs/DrugSafety/ucm451800.htm.

2.3 Rationale

Efficacy: Unlike COX-2 inhibitors and non-aspirin NSAIDs which may lead to adverse CVD events, aspirin has been commonly used for the prevention of chronic CV diseases[23]. In 1988, use of aspirin was first found to be linked to a reduced risk of CRC [24]. Subsequently, many epidemiologic studies have linked aspirin use to a lower risk of CRC and colorectal adenoma[25;26]. In a pooled analysis of 5 randomized trials for the primary prevention of CV diseases, daily use of aspirin at doses of **75 to 1200** mg for at least 5 years significantly reduced mortality due to CRC by 59%, particularly for proximal colon cancer[27]. Furthermore, clinical trials found use of aspirin at doses of **81 to 325** mg per day reduced adenoma recurrence by 17%[28;29]. In addition, use of aspirin at **600** mg per day reduced risk of CRC among patients with hereditary colorectal neoplasia [23]. However, two randomized trials (i.e. the Physician Health Study (PHS) [30], using 325 mg aspirin every other day, and Women's Health Study (WHS)[31], using **100 mg aspirin every other day**) did not find a beneficial effect. Insufficient dose and short follow-up in the two trials may be the primary reasons[23;26]. Interestingly, although the WHS did not show a reduction in risk of CRC over the first 10-year follow-up[31], the difference emerged after 10 years[32]. After the extended 8-year follow-up, the risk of CRC was reduced by 20% in the aspirin arm, particularly for proximal colon cancer[32] (See more details in the Risk-benefit consideration section). In a review by Chan *et al*, it is concluded after combining extensive data from both observational studies and randomized trials, "there is very strong evidence that aspirin in doses as low as 325 mg/day reduces CRC risk" [23].

Risk-benefit consideration: Thus, aspirin is a very promising chemopreventive agent for the prevention of CRC, particularly proximal colon cancer, which is less effectively prevented by colonoscopy. Further, compared to COX-2 inhibitors and other NSAIDs, aspirin does not have severe CVD side effects. Instead, aspirin was commonly used for the prevention of CV disease[23]. However, as stated by a recent commentary, before aspirin is ready for prime time, "the optimal dosing, frequency and duration and risk-benefit issue have not been yet fully addressed"[33]. The typical daily doses of aspirin used for the prevention of CVD are from 75 to 325 mg which have been shown in previous randomized trials to have similar effects for CRC prevention as high dose aspirin (i.e. 1200 mg/day)[23]. However, long-term use of either enteric-coated or buffered aspirin at low doses of from 75 to 325 mg has been linked to an increased risk of upper and lower gastrointestinal complications (i.e. duodenal and gastric ulcers and gastrointestinal bleeding)[34]. Due to the clear benefit of reducing CVD, it is obvious that among those at risk of CVD, the benefit of aspirin surpassed the bleeding risk[23]. Use of aspirin is also justifiable among patients with hereditary colorectal neoplasia[23]. However, the U.S. Preventive Services task force recommended against the routine use of aspirin to prevent CRC in individuals at average risk of CRC[35]. A meta-analysis of 22 clinical trials found that low doses of aspirin increase the risk of major bleeding by about 70% with no difference between 75-162.5 mg daily versus >162.5-325 mg daily[36]. However, some studies indicated gastrointestinal bleeding was dose-related with doses between 300 and 325 mg/day having higher risk than those between 75 and 162.5 mg/day[23;27;37-39]. In addition to daily use, as we mentioned previously, two randomized trials (WHS and PHS) which used aspirin every other day did not

find any effects in initial reports. After 8-year extended follow-up beyond the initial 10-year follow-up, promising benefits were found in the WHS arm using 100 mg aspirin every other day[32]. In the WHS, the relative risk was 1.14 (95%CI: 1.06 to 1.22) for gastrointestinal bleeding and 1.17 (95% CI:1.09 to 1.27) for peptic ulcers in the aspirin arm[32], which were lower than the RRs observed for daily use of aspirin[36]. On the other hand, it is likely that it takes longer to see a beneficial effect compared to 5-year window for daily use of aspirin[27].

In addition to using aspirin every other day, as mentioned in the NCI TORFP BB-2015, previous animal studies have examined other intermittent dosing strategies in carcinogenesis models[40]. Very recently, a DCP-sponsored rodent study found aspirin delivered on an intermittent schedule (i.e. 3 weeks on followed by 3 weeks off) had an equally high efficacy in preventing colon cancer with aspirin delivered continuously. This is a novel strategy. If confirmed in human studies, it may be able to minimize the adverse event and simultaneously maximize the high efficacy.

Apoptosis Mounting evidence from *in vitro*, *in vivo* and human studies indicates acquired resistance to apoptosis is one hallmark for almost all cancer types [41]. The maintenance of normal colorectal crypt architecture is based on the balance between the apoptosis at the base of crypts and the proliferation at the top [42]. *The transformation from normal colorectal epithelium to carcinoma is accompanied by a progressive resistance to apoptosis* [43]. Dr. Sandler's group found in a prospective study that subjects with the highest tertile level of apoptosis in normal rectal mucosa had a 70% reduction in risk of future colorectal adenoma, compared to the lowest tertile level of apoptosis [44]. Terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) and BAX expression [45] was used to measure apoptosis.

Biomarker for proliferation (Ki-67) Cell proliferation is another critical factor in tumorigenesis [46]. As we mentioned earlier, the balance between apoptosis and proliferation maintains the normal colorectal crypt architecture [42]. Cell proliferation index has been used in many previous intervention trials to assess the effects of chemoprevention agents [47] while Ki-67 is one of the most frequently used natural proteins associated with proliferation [46]. We have identified one published study which found aspirin treatment significantly reduced cell proliferation in young people with familial adenomatous polyposis compared to placebo arm[48].

Therefore, **we hypothesize** that 325 mg aspirin delivered 3 weeks on/3 weeks off has the same efficacy in increasing colorectal apoptosis, reducing colorectal cell proliferation and, in turn, decreasing cell proliferation/apoptosis ratio as aspirin delivered 325 mg daily, but confers reduced side-effect, i.e. gastrointestinal bleeding.

BAX expression reflects earlier signaling of apoptosis and, thus, it is a more sensitive biomarker of apoptosis than TUNEL. Necroptosis is a new pathway for programming cell death. Cell proliferation/ BAX ratio will be used as a primary endpoint and cell proliferation/TUNEL ratio and cell proliferation/necroptosis ratio will be secondary endpoints.

In contrast to apoptosis, which does not induce inflammation[49], necroptosis, a programmed necrosis pathway discovered over the past decade[49,50], elicits robust adaptive immune responses by inducing the inflammatory response cascade and releasing damage-associated molecular patterns against bacterial infection[51,52]. In a recent longitudinal study, we found necroptosis and inflammation plays a key role in colorectal carcinogenesis. Composite index scores of necroptosis (e.g. Transient receptor potential melastatin 7 (TRPM7)) with inflammation (i.e. COX-2) or apoptosis (i.e. BAX) can improve the prediction of metachronous polyp/adenoma risk. Increased COX-2 intensity in the epithelium was seen during carcinogenesis (Ptrend=0.02) and was significantly associated with an increased risk of metachronous polyp (OR=5.12, 95% CI:1.50-17.45, Ptrend=0.008), and for both metachronous adenoma/serrated polyp. Higher expression of TRPM7 was marginally associated with risk of

metachronous adenoma/serrated polyp in a dose-response manner. Combined composite index scores of necroptosis with inflammation or apoptosis using principal component analysis were strongly associated with a 4- to 17-fold increased risk of metachronous adenoma/serrated polyp. We have presented the results in 2022 AACR Annual Meeting. The manuscript will be submitted for publication shortly. Therefore, we propose to add 1) COX-2 expression in rectal biopsy; 2) BAX in rectal biopsies; 3) TRPM7 in rectal biopsies; 4) joint index of COX-2 with TRPM7 expression in rectal biopsies; and 5) joint index of TRPM7 with BAX in rectal biopsies as exploratory endpoints.

3. SUMMARY OF STUDY PLAN

Design
Eligible participants for this intervention study will be colorectal adenoma patients diagnosed at Vanderbilt University Medical Center, with a three-arm Phase IIa randomized placebo-controlled trial design. The three arms include two treatment arms and one placebo arm. Treatment 1 will be 325 mg aspirin orally per day for 12 weeks. Treatment 2 will be 325 mg aspirin orally on an intermittent, "3-weeks active/3-weeks placebo" schedule for 3 months. We will randomize a total of 90 participants into two treatment arms with 40 participants per arm and placebo arm with 10 participants. We anticipate at least 72 participants will complete the study (with a 20% drop-out rate due to both non-compliance and adverse events); we are building in a higher attrition rate than our PPCCT trial of magnesium supplementation because we expect there will be more adverse events related to aspirin use than with magnesium supplementation.

Recruitment and Screening
Participants will be recruited from the Vanderbilt electronic medical records, and from the PPCCT recruitment pool. Study gastroenterologists will perform a medical chart review, and participants who are potentially eligible will be invited for Clinic Visit 1 (screening).

Clinic Visits

Clinic Visit 1: After the consent is signed, screening procedures are performed to determine eligibility, including blood, stool, and pregnancy tests. A medical history, concomitant medications review, and physical including vital signs and ECOG performance status will also be performed. An optional urine sample is collected for future research. Upon final determination of eligibility, the study team will submit the registration information through the study eCRF database. Participant will be randomized according to Section 6.8 procedures.

Clinic Visit 2 (within 6 weeks of Clinic Visit 1): Baseline tests are performed, including the Food Frequency Questionnaire and Baseline Clinic Visit Questionnaire, concomitant medications review, and vital signs. A blood and stool sample are collected for clinical monitoring. Baseline specimens collected include rectal swabs, and biopsies. Another optional urine sample is collected. Three bottles of study medication are dispensed.

Clinic Visit 3 (9 weeks after starting study agent): Concomitant medications are reviewed and vital signs are performed. A blood and stool sample are collected for clinical monitoring. Mid-study specimens collected include rectal swabs, and biopsies. An optional urine sample is collected. Unused pills are collected and counted. The fourth bottle of study mediation is dispensed.

Clinic Visit 4 (12 weeks after starting study agent): Concomitant medications are reviewed and vital signs are performed. A blood and stool sample are collected for clinical monitoring. Post-study specimens collected include rectal swabs, and biopsies. An optional urine sample is collected. Unused pills are

collected and counted.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Diagnosis of colorectal adenoma of any grade.

4.1.2 Age ⩾18 years. Because no dosing or adverse event (AE) data are currently available on the use of aspirin in participants <18 years of age, children are excluded from this study but will be eligible for future pediatric trials, if applicable.

4.1.3 ECOG performance status ⩽1 (Karnofsky ⩾70%)

4.1.4 Participants must have normal organ and marrow function as defined below:

Leukocytes	⩾3,000/microliter
Absolute neutrophil count	⩾1,500/microliter
Platelets	⩾150,000/microliter
Total bilirubin	⩽1.5 x institutional ULN
AST (SGOT)/ALT (SGPT)	⩽1.5 x institutional ULN
Creatinine	⩽1.5 x institutional ULN
Blood hemoglobin	⩾12.0 g/dL
Alkaline phosphatase	⩽1.5 x institutional ULN
Blood urea nitrogen (BUN)	⩽40 mg/dL
eGFR	⩾45 mL/min

4.1.5 Negative fecal occult blood test.

4.1.6 The effects of aspirin on the developing human fetus at the recommended therapeutic dose are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

4.1.7 Ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1 Current (within three weeks of randomization) or planned use during the study intervention of the following:

1. Aspirin, other NSAIDs, or COX-2 inhibitors;
2. Anticoagulants, antiplatelet agents, or corticosteroids;
3. Gingko
4. Ethanol consumption >1 standard drinks/day for women, or >2 standard drinks/day for men.
5. Methotrexate (MTX);

Study participants will be instructed to use Tylenol or some other non-excluded agent to treat common ailments (i.e. headache/minor aches and pains).

4.2.2 History of

1. Any invasive malignancy within the past 2 years, with the exception of non-melanoma skin cancer.
2. Chronic renal diseases or liver cirrhosis;
3. Diseases such as anemia, peptic ulcer, gastrointestinal bleeding, active colitis and inflammatory bowel disease;
4. Hemorrhagic stroke or uncontrolled hypertension.

4.2.3 Participants may not be receiving any other investigational agents.

4.2.4 History of allergic reactions or intolerance attributed to aspirin or compounds of similar chemical or biologic composition.

4.2.5 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness that would limit compliance with study requirements.

4.2.6 Women who are pregnant or breastfeeding. Pregnant women are excluded from this study because aspirin has the potential for abortifacient effects. Because there is an unknown but potential risk for AEs in nursing infants secondary to treatment of the mother with aspirin, breastfeeding should be discontinued if the mother is treated with aspirin.

4.3 Inclusion of Women and Minorities

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

4.4 Recruitment and Retention Plan

Recruitment Pool of non-African Americans

PPCCT (Personalized Prevention of Colorectal Cancer Trial) is an ongoing R01 randomized trial funded by NCI (R01CA149633; Dai & Yu, PI). The trial was conducted among 240 colorectal adenoma polyp patients with calcium/magnesium (Ca/Mg) intake ratio≥**2.60**. We tested **1)** if reducing the Ca/Mg ratio to about 2.2 through Mg supplementation affects carcinogenesis biomarkers in rectal biopsies; and **2)** if the effects differ by *TRPM7* genotype. The PPCCT has enrolled 250 participants, with the original enrollment goal of 240. 239 of them completed the study (dropout rate 4.4%).

Over the past five years, we have successfully established a candidate pool of patients diagnosed with colorectal adenoma during the recruitment process for the PPCCT. An electronic medical record (EMR)-based approach was used for initial screening. Based on over 10,000 screened colorectal polyp patients diagnosed at Vanderbilt University Medical Center by using the Vanderbilt Clinical and Translational Science Awards (CTSA) research database, we have identified approximately 1,033 colorectal polyp patients who donated a DNA sample and are willing to participate in future studies; of these, 739 have a documented colorectal adenoma. The ongoing NCI-funded R01 randomized trial "Fatty acid desaturase activity, fish oil and colorectal cancer chemoprevention" (R01:CA160938; PI: Murff) has used the PPCCT recruitment tool to successfully complete the enrollment of 150 colorectal adenoma patients.

Thus the recruitment pool established based on the PPCCT has laid a solid infrastructure for the proposed study. Dr. Shrubsole has directed the Survey Research Shared Resource (**SRSR**), which has conducted recruitment and data collection and managed the data for the PPCCT. Thus, Dr. Shrubsole and SRSR are very familiar with the study population and relevant data. In the proposed study, Dr Dai, the study PI, and the study coordinator will closely work with Dr. Shrubsole and the **SRSR** for participant recruitment, all communications with potential participants, questionnaire administration, and database maintenance.

Recruitment Pool of African Americans
There are no African Americans in our SRSR database. The PPCCT recruitment pool was funded to exclude African Americans because African Americans are not polymorphic at the genetic locus that is a major aim of the parent study. Therefore, to add AA subjects to the pool, the SRSR staff will screen at least 600 African American patients diagnosed with a colorectal adenoma at Vanderbilt University, as they did previously for the PPCCT.

Prescreening
Vanderbilt hospital's electronic medical record (EMR) may not have the information for the over-the-counter use of aspirin or other NSAID use or may not have the most recently updated information. Also, we predict a fair number of individuals may refuse to participate or have moved. In order to exclude those who are regularly taking NSAIDs (see the exclusion criteria), the **SRSR** will conduct telephone screenings. We estimate over 50% of the study population is regularly taking NSAIDs. Potential participants will be mailed an introductory letter followed by a telephone call about 1 week later. The SRSR staff will place calls during daytime, and on evenings and weekends. For individuals who remain eligible after the telephone prescreening, in **screening 1**, our research nurse will review their EMR to further confirm eligibility. We estimate that about 222, including 24 African Americans, will be willing and eligible for the study. Finally, our gastroenterologists will conduct further review, looking through previous colonoscopies to confirm a diagnosis of colorectal adenomas, reviewing inclusion/exclusion criteria, and reviewing safety profiles. We estimate that about 180 will participate in next phase, i.e. **screening visit 1.**

5. AGENT ADMINISTRATION

Intervention will be administered on an *outpatient* basis. Reported AEs and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

The whole intervention period lasts for 12 weeks (range: 11 weeks – 12 weeks and 2 days):

Control Arm: Placebo (n=10)
Experimental Arm1: Aspirin 325 mg daily (n=40)
Experimental Arm2: Aspirin 325 mg 3 week ON/OFF (n=40). Individuals assigned to 325 mg aspirin 3-week on/3-week off will take placebo during 3-week off period.

5.2 Aspirin Administration

The aspirin and matching placebo capsules will be provided by the National Cancer Institute (NCI) and packaged and labeled by NCI Division of Cancer Prevention's repository contractor, MRIGlobal. The Vanderbilt Investigational Drug Service (VIDS) will be responsible for the storage (controlled room temperature 59° F - 86°F and under low humidity), preparation of all investigational agents, and for maintaining accurate drug storage and dispensing logs.

Each participant will receive four bottles:
- Bottle 1 labeled "Weeks 1-3"
- Bottle 2 labeled "Weeks 4-6"
- Bottle 3 labeled "Weeks 7-9"
- Bottle 4 labeled "Weeks 10-12"

Bottles 1-3 will be dispensed at Clinic Visit 2. Bottle 4 will be dispensed at Clinic Visit 3. The prescription (and, for the first dispense, the signed consent form) will be faxed to VIDS. The research staff will transport the capsules from VIDS to the participant at clinic. Participants will be instructed to take study medication with food to decrease the risk of GI upset.

If the participant is unable to stay at the clinic to receive the capsules, the pharmacist at VIDS will mail the capsules via Fedex within one business day of the Clinic Visit. Research staff will follow-up with a phone call to the participant to ensure receipt of study agent.

5.3 Run-in Procedures

Not applicable.

5.4 Contraindications

Participants should avoid excessive ethanol use (>1 standard drinks/day for women, or >2 standard drinks/day for men).

5.5 Concomitant Medications

While on the study agent, participants should avoid NSAIDs and COX-2 inhibitors. While on the study agent, they should avoid taking anticoagulants, antiplatelet agents, and corticosteroids. Participants should discontinue gingko use while taking the study agent. Participants should avoid taking methotrexate (MTX) while taking the study agent.

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: 1) start and stop date, dose and route of administration, and indication. Medications taken for a procedure (*e.g.*, biopsy) should also be included.

5.6 Dose Modification

Individuals who report any GI symptom $\geqslant$ grade 3, such as dyspepsia or heartburn, will be instructed to discontinue the study medication immediately.

Participants reporting minor upper GI symptoms (dyspepsia or heartburn $\leqslant$ grade 2) who wish to remain on trial will be instructed to treat symptoms with over-the-counter calcium carbonate and/or magnesium hydroxide based antacids (i.e. Tums®, Rolaids®, Mylanta®, etc.) at the manufacturers recommended dose. If symptoms persist or worsen over the next 3 days while taking antacids ($\leq$ grade 2) and the subject still wishes to remain on trial, they will be instructed to treat symptoms with an over-the-counter proton pump inhibitor (PPI). If symptoms persist or worsen while taking a PPI for more than 3 days then the study agent will be discontinued and the participant will be monitored until symptoms resolve. These symptomatic rescue medications will not be provided to the participants by the study.

Individuals who report the following serious side effects that are possibly, probably, or definitely related to the study agent will be withdrawn from the study:

- Bruising grade 2 or above
- Hearing impairment of any grade
- Tinnitus of any grade
- Nausea grade 2 or worse, or nausea grade 1 that lasts for >5 days

- Vomiting grade 2 or worse, or vomiting grade 1 that lasts for >5 days
- Urine output decrease grade 1 or above
- Urine discoloration grade 1,
- Black/tarry stool,
- Blurred vision grade 1 or above
- Headache grade 3 or above
- Serious allergic reaction to the study agent

Individuals who have one of the following results during the intervention will have the result evaluated immediately (preferably within 1 week) and repeated if the treating physician deems necessary. If the treating physician believes the result is possibly, probably, or definitely related to the study agent, or if the repeated test result remains the same, the individual will be withdrawn from the study.

- Blood hemoglobin<12.0 g/dL (anemia, grade 1)
- Platelet count<150,000/microliter (platelet count decreased, grade 1)
- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >1.5 times upper limit of normal (ULN) (AST/ALT increased, grade 1)
- Alkaline phosphatase >1.5 times ULN (alkaline phosphatase increased, grade 1)
- Bilirubin > 1.5 times ULN (blood bilirubin increased, grade 2)
- BUN >40 mg/dL
- eGFR<45 mL/min (chronic kidney failure, grade 2)
- Positive fecal occult blood test

5.7 Adherence/Compliance

5.7.1 Participants will be considered evaluable if they have taken 80% of the prescribed doses.

5.7.2 Participant compliance will be monitored in a variety of ways. Patients will be required to keep a diary documenting drug dosing. Returned pills will be counted.

6. PHARMACEUTICAL INFORMATION

6.1 Aspirin (IND: 130627, Exempt, IND Sponsor: NCI/Division of Cancer Prevention)

Aspirin (325 mg capsules) 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 capsule 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.

6.2 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 elevated hepatic enzymes, blood urea nitrogen and serum creatinine, hyperkalemia, proteinuria, and prolonged bleeding time.

6.3 Availability

Aspirin and aspirin placebo are investigational agents supplied to investigators by the Division of Cancer Prevention (DCP), NCI.

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 Repository
1222 Ozark Street
North Kansas City, MO 64116
Phone: (816) 360-3805
FAX: (816) 753-5359
Emergency Telephone: (816) 360-3800

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). The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility has been delegated to Vanderbilt Investigational Drug Service pharmacists. 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.

6.6 Packaging and Labeling

Aspirin and aspirin placebo will be packaged and labeled by NCI, DCP's Repository Contractor MRIGlobal. Three bottles will be dispensed at Clinic Visit 2. One bottle will be dispensed at Clinic Visit 3. The bottles will include 23 capsules. Bottles will be labeled "Weeks 1-3," "Weeks 4-6," "Weeks 7-9," and Weeks "10-12."

6.7 Storage

Study drug will be stored in a secure location, at controlled room temperature (59° F - 86°F) and under low humidity. The Vanderbilt Investigational Drug Service (VIDS) will be responsible for the storage, preparation all investigational agents, and for maintaining accurate drug storage and dispensing logs.

6.8 Registration/Randomization

In current study, there are three groups, two treatment groups (325 mg aspirin daily and 325 mg aspirin 3-week on/3-week off) and one control group. Individuals assigned to 325 mg aspirin 3-week on/3-week off will take placebo during 3-week off period.

This study will recruit 90 participants with colorectal adenoma. A permuted-block randomization design will be used to allocate the subjects into the treatment/placebo arm, and will be created by the study statistician. Both the subjects and the intervention administering staff will be blinded to assignments.

Procedures

1. A study coordinator must upload into the Northwestern Clinical Trials Management System, a signed and complete informed consent along with HIPAA authorization and a completed eligibility form for each participant identified as eligible to be entered into the study.
2. All participants must be registered in the Northwestern University Robert H. Lurie Comprehensive Cancer Center Clinical Trials Management System (CTMS). Participants must not start protocol treatment prior to registration in the Lurie Cancer Center CTMS.
3. After registration in the CTMS, participants will be assigned a participant identification number.
4. An NCPC Quality Assurance Monitor will submit the following into the REDCap Randomization Information Form: (1) Study Number, (2) Site, (3) Pharmacist Email(s), (4) Participant ID [PID], (5) Participant Initials, and (6) Randomization Date.
5. An Automatic Treatment Assignment Notification email will be sent to the research pharmacist(s) containing: (1) Study Number, (2) Site, (3) Participant ID [PID], (4) Participant Initials, (5) Randomization Date, and (6) Treatment Assignment.
6. The clinical research coordinator(s) will receive a Confirmation of Registration containing the PID via email.
   The research pharmacist will dispense the study agent.

The following people will have a copy of the un-blinded randomization log: the study statistician at NU, the Quality Assurance Team at NU, and the Investigational Pharmacist at Vanderbilt. The following people will have access to the REDCap study project containing randomization information: the study statistician at NU and the Quality Assurance Team at NU. The study statistician will set up randomization blocks.

When possible, the study coordinator will notify an NCPC Quality Assurance Monitor and/or send an email to ncpc@northwestern.edu prior to registering a participant. Prior notification is required for participant randomizations outside the normal business hours of Monday-Friday 9:00am-5:00pm CT.

6.9 Blinding and Unblinding Methods

The research pharmacist will manage the investigational agent. The blind will be maintained through the effort of the research pharmacist and the pharmacy. Unblinding will only occur when it is deemed medically necessary, and will only take place after consultation with the NCI, DCP Task Order Monitor. If the NCI Task Order Monitor cannot be reached and the participant requires emergency care, the Study Chairman may authorize the site PI to break the blind. The date and reason for breaking the blind must be submitted by the site PI to the Study Chairman and the NCI Task Order Monitor as soon as possible.

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

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".

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

	Clinic Visit 1: Screening	Clinic Visit 2: Baseline, within 6 weeks of Clinic Visit 1	Between-Visit Telephone or Email Contacts	Clinic Visit 3: 9 weeks after start of study agent [6]	Clinic Visit 4: 12 weeks after start of study agent [7]; or Off-Agent Visit [8]
Informed Consent	X				
Eligibility	X				
Registration/Randomization [1]	X				
Collect/Review Questionnaires [2]		X			
Medical History	X				
Physical [13]	X				
Vital Signs [12], Body Measurements [3]	X	X		X	X
Con Med Review	X	X		X	X
Assess Adverse Events	X	X		X	X
Pregnancy Test	X				
Blood Sample for Clinical Labs [10]	X	X		X	X
Blood Sample for Research [11]	X	X		X	X
Urine Sample [9]	X	X		X	X
Stool Sample	X	X		X	X
Rectal Swab		X		X	X
Rectal Biopsies [4]		X		X	X
Dispense Study Agent		X		X	
Collect/Count Unused Pills and Review Participant Diary				X	X
Telephone or Email Contact [5]			X		X [14]
Compensation	X				X

[1] Registration and randomization will occur between Clinic Visit 1 and Clinic Visit 2
[2] Clinic Visit 2: Food Frequency Questionnaire and Baseline Clinic Visit Questionnaire
[3] Height; weight; hip and waist circumferences
[4] Verify empty rectal vault prior to rectal biopsy procedure
[5] Phone or email contacts will include the following topics: compliance, adverse events, reminder to switch bottles, reminder to bring unused pills back to clinic. Participants will be contacted within 3 days after each biopsy visit (after Clinic Visits 2, 3, and 4), and at Weeks 1, 3, and 6 (±3 days).
[6] Range: 8 weeks – 9 weeks and 2 days (56 – 65 days) after starting the study agent
[7] Range: 11 weeks – 12 weeks and 2 days (77 – 86 days) after starting the study agent
[8] If a participant comes off-agent (e.g. due to AE or concomitant medication), the Off-Agent Visit will be scheduled for as soon as possible after the participant stops the study agent.
[9] Urine samples will be obtained only from participants who agree to future unspecified research in the consent form.

[10] Clinic Visit 1-4: 1 green top tube for CMP and 1 purple top tube for CBC with differential. CMP and CBC will be processed on the day of the visit.
[11] 1 EDTA purple top tube for plasma, white blood cell (WBC), red blood cell (RBC); 1 red top tube for serum.
[12] ECOG performance status obtained at Screening Visit only. Vital signs include pulse, respirator rate, temperature, and blood pressure.
[13] Physical exam may be performed at any follow-up visit when deemed appropriate by study team due to significant changes or medical events.
[14] Follow-up phone call to assess adverse events 1 month ± 7 days from Clinic Visit 4.

7.2 Pre-study Evaluation

Clinic Visit 1: Screening

- Prior to the visit
  - The research staff will mail the participant a stool collection kit. The participant will collect stool and bring it to the clinic. Participants who fail to bring in the stool sample to the office visit will be asked to try to produce a sample during the clinic visit. If unable, they will be asked to bring or mail the stool sample after Clinic Visit 1 for the fecal occult test.
- During the visit
  - Obtain written informed consent (the study participant will keep a copy).
  - Review medical history, physical including vital signs, ECOG performance status, concomitant medications, and symptoms.
  - Obtain the following specimens:
    - One tube of urine (for optional future research)
    - One tube of stool (brought from home for fecal occult blood test)
    - Two tubes of blood (for clinical laboratory measurements): 1 green top for CMP and 1 purple top for CBC with differential.
    - Two tubes of blood (for research): 1 EDTA purple top for plasma, white blood cell (WBC), red blood cell (RBC); 1 red top for serum.
  - Perform a pregnancy test
- After the visit
  - Research staff will review final inclusion/exclusion criteria based on laboratory results.
  - Participant will be registered in the Northwestern Oncology Trial Information System (NOTIS).
  - Results of the inclusion/exclusion criteria review will be communicated to the participant.

Clinic Visit 2: Baseline
Within 6 weeks of Clinic Visit 1

- Prior to the visit
  - The research staff will mail or give the participant a stool collection kit. The participant will collect stool and bring it to the clinic.
  - The research staff will mail or give the participant the Food Frequency Questionnaire (FFQ) and Baseline Clinic Visit Questionnaire. The participant will complete these questionnaires prior to arriving.
- During the visit
  - Review vital signs, concomitant medications, and symptoms.
  - Physical exam may be performed when deemed appropriate by study team due to significant changes or medical events.
  - Review the FFQ and Baseline Clinic Visit Questionnaire.
  - Obtain the following specimens:
    - One tube of urine (for optional future research)

        - One tube of stool (brought from home for fecal occult blood test)
        - Two tubes of blood (for clinical laboratory measurements): 1 green top for CMP and 1 purple top for CBC with differential.
        - Two tubes of blood (for research): 1 EDTA tube for plasma, white blood cell (WBC), red blood cell (RBC); 1 red top for serum.
        - Rectal swabs
        - Rectal biopsies
    - Dispense first three bottles of study agent and participant diary.
    - Give the participant a stool sample collection kit to use prior to Clinic Visit 3.
- After the visit
    - Within 3 days after the visit, the research staff or nurse will telephone or email each participant to monitor for any adverse events due to biospecimen collection as well as compliance monitoring. Suggested statements for participant communications are provided in Appendix E.

7.3 Evaluation During Study Intervention

Between-Visit Telephone or Email Contacts
Weeks 1, 3, and 6 (±3 days)
The research staff will telephone or email each participant to discuss the following:

- Any adverse events
- Changes in concomitant medications
- Compliance monitoring
- A reminder that, at the beginning of weeks 3 and 6, the participant needs to switch bottles.

The Week 6 Telephone or Email Contact will include the following reminders:

- To bring unused study agent with them to the next Clinic Visit.
- To have a bowel movement prior to their visit, to prepare for the rectal biopsy procedure.

Suggested statements for participant communications are provided in Appendix E.

Clinic Visit 3: 9 Weeks
8 weeks - 9 weeks and 2 days (56 – 65 days) after start of study agent

- Prior to the visit
    - The participant will collect stool using the collection kit given to them during Clinic Visit 2.
- During the visit
    - Review vital signs, concomitant medications, and symptoms.
    - Physical exam may be performed when deemed appropriate by study team due to significant changes or medical events.
    - Obtain the following specimens:
        - One tube of urine (for optional future research)
        - One tube of stool (brought from home for fecal occult blood test)
        - Two tubes of blood (for clinical laboratory measurements): 1 green top for CMP and 1 purple top for CBC with differential.
        - Two tubes of blood (for research): 1 EDTA tube for plasma, white blood cell (WBC), red blood cell (RBC); 1 red top for serum.
        - Rectal swabs
        - Rectal biopsies
    - Collect unused study agent and participant diary and conduct a pill count.
    - Dispense fourth bottle of study agent.
- After the visit

- Within 3 days after the visit, the research staff or nurse will telephone or email each participant to monitor for any adverse events due to biospecimen collection as well as compliance monitoring. Suggested statements for participant communications are provided in Appendix E.

7.4 Evaluation at Completion of Study Intervention

Clinic Visit 4: 12 Weeks
11 weeks – 12 weeks and 2 days (77 – 86 days) after start of study agent
OR
Off-Agent Visit

- Prior to the visit
  - The participant will collect stool using the collection kit given to them during Clinic Visit 3.
- During the visit
  - Review vital signs, concomitant medications, and symptoms.
  - Physical exam may be performed when deemed appropriate by study team due to significant changes or medical events.
  - Obtain the following specimens:
    - One tube of urine (for optional future research)
    - One tube of stool (brought from home for fecal occult blood test)
    - Two tubes of blood (for clinical laboratory measurements): 1 green top for CMP and 1 purple top for CBC with differential.
    - Two tubes of blood (for research): 1 EDTA tube for plasma, white blood cell (WBC), red blood cell (RBC); 1 red top for serum.
    - Rectal swabs
    - Rectal biopsies
  - Collect unused study agent and participant diary and conduct a pill count.
- After the visit
  - Within 3 days after the visit, the research staff or nurse will telephone or email each participant to monitor for any adverse events due to biospecimen collection as well as compliance monitoring. Suggested statements for participant communications are provided in Appendix E.

7.5 Post-intervention Follow-up Period

Follow-Up Telephone or Email Contact
1 month after Clinic Visit 4 or Off-Agent Visit (±7 days)
The research staff will telephone or email each participant to discuss the following:

- Any adverse events

7.6 Methods for Clinical Procedures

7.6.1 Rectal Swab and Biopsy Procedure

The procedure for collecting rectal swabs and biopsies will occur at Clinic Visits 2, 3 and 4. No fasting or bowel cleansing preparations is required. Participants are asked to try to have a bowel movement prior to arriving at clinic. If the participant was unable to clear his/her bowels, the procedure will still proceed. Participant is positioned in the Sims position. See section 10.2 for specimen collection information.

Patient monitoring

After the biopsy, the patient is monitored for thirty minutes. The study staff will check blood pressure and have the patient go to the restroom to check for bleeding prior to leaving the clinic. Patient is told that they can expect some blood on the tissue on the day of the biopsy. Bleeding heavier or more persistent than this should be reported. They should also report anal or lower abdominal pain, fever, a foul anal discharge, or anything else they think could be related to the biopsy. The gastroenterologists and the supporting medical team will handle emergencies. Any other problems discovered by the study team will be discussed at an appropriate level with the patient sufficient to affect referral to the patient's gastroenterologists or primary care physician who will also be informed if the participants agree in the informed consent form (i.e. the following questions will be added in the informed consent form: "If you develop a medical problem while on study, may we inform your physician? If yes, whom would you like us to inform?").

Any possible complications to the biopsies will be recorded on an Adverse Events Form; information will include a description of the complication, a rating of the severity, other essential information needed to evaluate the complication, an assessment of the likelihood that the event was due to a study procedure, and a statement of action taken. Minor problems after the procedure are handled in consultation with the gastroenterologist, and recorded in the subject's study file. If undue complications of biopsies or other procedures arise, the study will be stopped ahead of schedule. In addition, in the event of a severe or life-threatening complication, the Vanderbilt Cancer Center Clinical protocol Review Committee will be immediately notified, and an SAE submitted according to section 11.2. Patients will continue their usual care with the physician. Telephone numbers for the PI and study coordinator will be given to the participant. Emergencies will be handled by the gastroenterologist and supporting medical staff.

7.6.2 Stool Collection Procedure

Before the clinic visit, participants will receive a stool collection kit:

- Sterile stool container with wide mouth
- Zip-lock bags/ biohazard bags
- Insulated bag with ice packs
- Written and verbal instructions for collection of stool samples

Patients will be instructed to collect stool specimen. Collect the stool within 7 days prior to the clinic visit day.

- Place specimen in zip-lock bag / biohazard bag.
- Place zip-locked bag in insulated bag with ice pack.
- Store specimen in the refrigerator until ready to leave for clinic.
- Transport the sample to the clinic and give to study staff.

7.6.3 Research Blood Sample Collection Procedure

Research blood samples will be collected at all Clinic Visits. Blood (25 ml for each person) will be drawn into one EDTA tube for plasma, WBC, RBC and one red top tube for serum.

7.6.4 Urine Sample Collection

The urine samples will be collected Clinic Visits 1-4. Each urine sample will be collected in a sterile urine specimen container containing 100 mg ascorbic acid as a preservative. This sample will be transferred into 1.8ml cryovials.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

BAX expression reflects earlier signaling of apoptosis and, thus, it is a more sensitive biomarker of apoptosis than TUNEL. Necroptosis is a newly described pathway for programmed cell death. To evaluate the effects of a 3-month intervention of continuous versus intermittent aspirin dosing (i.e. 325 mg daily and 325 mg 3 weeks on/3 weeks off), we chose the cell proliferation (i.e. Ki-67)/BAX ratio as a primary endpoint.

8.2 Secondary and Exploratory Endpoints

Immunohistochemistry (IHC)
Cell proliferation/TUNEL ratio, and cell proliferation/Necroptosis ratio will be secondary endpoints, whereas the expression of COX-2, BAX, TRPM7, joint index of COX-2 with TRPM7 and joint index of TRPM7 with BAX in rectal biopsy will be exploratory endpoints.

All biomarkers (TUNEL, pMLKL, COX-2, BAX, TRPM7 and Ki-67) will be detected by an established 6-plex mIHC/IF (multiplex immunofluorescent IHC) technique and analyzed quantitatively using a system composed of an Olympus BX-61 motorized microscope, X-Cite XYLIS Fluorescence Illuminator and imaging software (Q-Capture, ImageJ, CellProfiler). The imaging quantification will be conducted by a research pathologist and the detailed procedures for sequential mIHC/IF staining protocol, quality controls and automated image measurement pipelines are presented in the supplementary file. The final measures are generated automatically as ‘‘total positive cell count / total epithelial nuclei’’ for nuclear staining marker (Ki-67), or ‘‘total positive area / total epithelial cell area’’ for cytoplasmic/membranous staining markers (i.e. pMLKL, TRPM7, COX-2 and BAX). The COX-2 expression in both epithelium and stroma will be separately quantified using pan-CK for tissue segmentation. Quantitative results will be used for endpoints and semi-quantitative results will be used to validate quantitative results.

Fecal occult blood test
Fecal occult blood test will be used to monitor the safety of the treatment at each clinic visit.

Quantification of DNA methylation
Epigenetics, particularly aberrant DNA methylation in gene promoters (i.e. CpG methylation sites), play a critical role during CRC carcinogenesis[55;56]. However, a very recent human study found that use of aspirin reduced the CpG methylation rates at colorectal carcinogenic genes (i.e. CDKN2A (cell cycle regulation), MGMT (DNA repair), DAPK1(apoptosis), CDH1(cell invasion), WNT16 (Wnt pathway) and RASSF1 (RAS signaling))[57].

We will extract high molecular weight genomic DNA from DBS samples with commercially available PureGene Kits (Gentra Systems, Minneapolis, MN). We will use the MethylationEPIC BeadChip to identify methylation biomarkers in candidate genes (i.e. CDKN2A (cell cycle regulation), MGMT (DNA repair), DAPK1(apoptosis), CDH1(cell invasion), WNT16 (Wnt pathway) and RASSF1 (RAS signaling)) involved in colorectal carcinogenesis, and other epigenome-wide methylation biomarkers in rectal biopsies..

Gut microbiota
Gut microbiota dysbiosis[61;62], particularly abundance of ***E. coli*** or E. coli-like bacteria[63;64] and ***Fusobacterium***[65-68], was linked to CRC risk. We will evaluate the effects of aspirin on these bacteria and other microbiota in rectal swabs using whole-genome shotgun metagenomics (WGS) DNA sequencing.

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons:

(1) Individuals who report minor upper GI symptoms such as dyspepsia, heartburn, dyspepsia, or heartburn who do not wish to remain on trial;
(2) Inadequate agent supply;
(3) Noncompliance (e.g. forget taking study agent);
(4) Medical contraindication;
(5) Concomitant medications.

Participants who go off-agent early will have an Off-Agent Visit (clinic visit 4 as listed in "7.1 schedule of events") scheduled as soon as possible.

8.4 Off-Study Criteria

Accrual will be interrupted if at any time toxicity among participants requires withdrawal of 25% of participants based on the toxicity criteria outlined in Section 5.6. Toxicity will be reviewed by the NCI DCP Medical Monitor to determine whether accrual to the study should be permanently stopped.

8.5 Study Termination

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

9. CORRELATIVE/SPECIAL STUDIES

None.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

Dr. Timothy Su at Vanderbilt Epidemiology Biospecimen Core (MCN B-2103, Nashville, TN 37232) will perform immunohistochemical analysis on rectal biopsies.
Dr. M. Andrea Azcarate-Peril at University of North Carolina Microbiome Core Facility (312 Isaac Taylor Hall, Chapel Hill, NC 27599) will conduct microbiome analyses on rectal swabs.
Dr. Lifang Hou's laboratory will perform DNA methylation analysis on rectal biopsies. The assays will be done at University of Chicago Genomics Facility (900 E. 57th Street, Chicago, IL 60637).

10.2 Collection and Handling Procedures

10.2.1 Rectal Biopsies

Rectal biopsies will be collected at Clinic Visits 2, 3, and 4. Biomarkers from rectal biopsies include Ki-67 (cellular proliferation), BAX (pro-apoptosis), TUNEL (apoptosis), pMLKL (necroptosis), COX-2 (inflammation) and TRPM7 (necroptosis) assays. These biomarkers will be analyzed using immunohistochemical (IHC) techniques. Cell proliferation/Apoptosis ratio will be used as our primary endpoint.

Rectal Biopsy Collection

No fasting or bowel cleansing preparations is required. Participants are asked to have a bowel movement prior to arriving at clinic.

- Patient is positioned in the Sims position.
- Biopsies are taken at 10 cm above the anal verge through an anoscope using a large cup Cook flexible 230 cm biopsy forceps (2.4mm cup diameter). Use a standardized circumferential

approach to take samples at 12 o'clock, 1.5 o'clock, 3 o'clock, 4.5 o'clock, 6 o'clock, 7.5 o'clock, 9 o'clock, 10.5 o'clock.

- Care is taken to place the fully open biopsy forceps at right angles to and against the mucosa in order to get a sample of sufficient area and depth.
- At least **eight** adequate biopsies must be obtained, and should be 1-2mm wide and 1mm thick.
- Remove the biopsy from the forceps with a pair of pincers, plastic (single use) or metal (sterilizable). Do not use a needle or other instrument. If the biopsy cannot be teased off using the toothpick, shake it off in the normal saline, remove using a small plastic spoon and put in formalin or in prepared green capped cryovial.
- Study staff will examine biopsies with a magnifying glass to determine adequacy of biopsy. If a biopsy pinch is inadequate, another is obtained.
- **Place 2** rectal biopsy specimens in labeled specimen bottle with 10% normal buffered formalin immediately.
- The remaining rectal biopsy specimens will be snap frozen for future analyses requiring fresh (non-paraffin imbedded) tissue.
- Record the following:
    - Site of biopsy
    - Number of biopsies collected
    - Note any bleeding
    - Enter the time the biopsies were dropped into formalin and/or snap frozen

Rectal Biopsy Processing

During processing, the biopsies are divided into four parts: 1. fresh frozen, 2. embedded, 3. tissue blocks, and 4. slides

1. **Fresh Frozen Specimen**
    - Rectal biopsy in the vials should be frozen almost immediately when placed in the liquid nitrogen or dry ice.
    - Frozen specimen should remain in the liquid nitrogen or dry ice until they can be placed in the -80° C freezer
    - Study staff will then transport specimens to Epidemiology Biospecimen Core MCN B-2103, ext #6-0467
    - Date, time and location of sample in the freezer will be recorded on the specimen log
    - Samples will be stored at –80° C.
    - Specimens will be stored until ready for analysis.
    - All samples will be logged into a log sheet for that day, and signed off by respective personnel along their movement from the participant (retrieval, placing into freezing medium, transportation to the lab)
2. **Embedded Biopsy Specimens**
    - Deliver specimen to Dr. Timothy Su, Epidemiology Biospecimen Core, MCN, B-2103, within one hour of collection
    - Biopsies are approximately 1 mm thick
    - Core laboratory staff will orient specimen tissue samples are placed on a biopsy sponge and visualized under the dissecting microscope.
    - Turn and orient the biopsy mucosal side up so that the specimen is stretched out as flat as possible.
    - Mucosal side can be identified by its honeycomb-like appearance. Handle the specimen as little as possible.
    - Do not touch the mucosal side of the biopsy except at the very edge of the biopsy.
    - Do not worry if the biopsy is not completely flat as long as it is not twisted

- The laboratory/study staff must reorient biopsies within 30 minutes of being obtained.

3. **Tissue Blocks**
   - Embedding Biopsies (embed biopsy specimens as soon as possible after biopsy)
     - Biopsies are fixed and processed for twenty-four hours in a tissue processor.
     - After 24h fixation in neutral buffered formalin, the biopsies are placed in a Shandon Hypercenter Processing Center (ThermoShandon, Pittsburgh, PA), a programmable, automated reagent-moving system used to dehydrate and infiltrate fixed tissues with paraffin.
     - Biopsies will be embedded in one paraffin block. Pay attention to embedding orientation of the tissues so that well-oriented longitudinal crypts can be sectioned.
     - The cassettes are labeled with a # 3 pencil on the front side of each block with the participants ID # and visit date.
     - Initial water residues are removed from tissues by flushing graded alcohols (70, 80, 95, and 100%) sequentially into the reaction chamber.
     - Each alcohol remains in the tissue-containing chamber for approximately 45 minutes, and a 5-minute draining period is allowed between alcohol changes. Two xylene exchanges are followed by two 60C heated paraffin exchanges, each one hour in duration.
     - The paraffin-infiltrated tissues are removed from the Hypercenter, embedded into paraffin blocks, and prepared for shipment.
     - Blocks are then stored in the refrigerator in the core lab at 4 degrees C until ready for analysis.
   - Storage: Blocks are stored in the core lab at 4 degrees C.

4. **Slide preparation**
   - Biopsies will be sectioned with a microtome so that crypts will be longitudinally sectioned from base to lumen.
   - Sections are then placed sequentially on positive charged slides and numbered from 1 to 26.
   - Section levels are Five microns thick, and are taken approximately 50 microns apart. (Taking sections 50 microns apart to avoid double counting of crypts in biopsy specimens)
   - The sections numbered 1, 11 and 21 will be used for H&E staining.
   - Slides are prepared for processing each of the antibodies for apoptosis, cell proliferation, and necroptosis biomarkers (e.g. Ki-67, BAX, TUNEL, pMLKL, TRPM7, COX-2) assay.
   - Multiple biopsies are collected and multiple sections will be prepared. In case of staining run failures, one backup serial section per slide for staining is prepared.
   - All blocks and slides will be stored in a vacuum chamber at 4 degree C in a cold room until analysis.

10.2.2 Rectal Swabs

Rectal Swabs are collected at Clinic Visits 2-4. Biomarkers from rectal swabs include the abundance of E. coli, Fusobacterium, and other microbiota.

Rectal Swab Collection

Patient is positioned in Sims position.
At least 2 rectal swabs will be taken.

- Insert culturette swab about 2 inches into rectum, rotate swab to collect fecal material, and carefully remove swab from rectum

- Insert swab into tube (one swab to one tube). Freeze the tubes and store the sample at -80˚C until future analysis.
- Record the time of collection.
- Send the sample to lab for immediate storage.
- All samples will be logged into a log sheet for that day, and signed off by respective personnel along their movement from the participant (retrieval, placing into freezing medium, transportation to the lab).

10.2.3 Blood

Blood is collected at all Clinic Visits. Blood is used for clinical and research labs. This section describes the blood collected for research labs.

Blood Collection

- Two tubes of research blood are collected: one EDTA for plasma, WBC, RBC, and one red top tube for serum.
- Blood specimens will be stored in CRC or other clinical room refrigerator until processing.

Blood Processing

- Study staff will then transport specimens to Epidemiology Biospecimen Core MCN B-2103, ext #6-0467.
- Specimens will be processed within 4-6 hours after collection.
- All specimen's will be labeled with date, participant ID #
- Serum will be collected after coagulation.
- Whole blood will be processed within 4-6 hours and separated into plasma, buffy coats (white cells), RBC.
- 1-2 ml aliquots of the serum/plasma sample will be pipetted into 3-labeled cryovials.
- Vials will be placed in specimen box for storage
- Date, time and location of sample in the freezer will be recorded on the specimen log
- Samples will be stored at –80° C.
- Blood specimens will be stored until use in relevant assays.

10.2.4 Urine

Urine is collected at Clinic Visits 2-4. Urine collection will be optional for participants. Prior to collection, the participant will need to agree to optional future research on the informed consent form.

Urine Collection

- Each urine sample will be collected in a sterile urine specimen container containing 100 mg ascorbic acid as a preservative.
- Urine specimens will be stored in CRC or other clinical room freezer until processed.

Urine Processing

- Study staff will then transport specimens to Epidemiology Biospecimen Core MCN B-2103, ext #6-0467
- Specimens will be processed within 4-6 hours after collection.
- All specimen's will be labeled with date, participant ID #.
- 1-2 ml aliquots of the urine sample will be pipetted into each of 3-labeled, 1.8ml cryovials.
- Vials will be placed in specimen box for storage.
- Remaining specimen will be discarded.

- Date, time and location of sample in the freezer will be recorded on the specimen log.
- Samples will be stored at –80° C'
- Urine specimens will be stored until ready for analysis.

10.2.5 Stool

Stool samples will be collected for all Clinic Visits. Participants will collect the stool sample at home within 7 days prior to each clinic visit day, and bring it to the clinic. Stool collection kits will be mailed to the participant prior to Clinic Visit 1. Participants will be given stool collection kits at Clinic Visits 1-3 for collection prior to the subsequent Clinic Visit, according to section 7.6. Biomarkers from stool samples include the fecal occult blood test.

Stool Collection

- Each stool sample will be collected in a sterile stool specimen container with wide mouth, labeled "Stool Sample."
- About 2 grams / 2 ml stool will be transferred into 2 labeled plastic tubes with spoon.
- All specimen containers will be labeled with collection date and time, sample ID #, and participant ID #.
- Stool specimens will be stored in CRC or other clinical room freezer.

Stool Processing

- Stool sample tubes will be placed in specimen box for storage.
- Date, time, sample ID #, participant ID #, and location of sample in the freezer will be recorded on the specimen log.
- Samples will be stored at –80° C immediately.
- Stool specimens will be stored until ready for analysis.

10.3 Shipping Instructions

All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations. All specimen shipments should be initiated on Mondays, Tuesdays, or Wednesdays, after communication with the receiving site and notifying ncpc@northwestern.edu of the tracking number.

Tissue samples for DNA methylation will be shipped to Dr. Lifang Hou's laboratory on dry ice via overnight FedEx. Below is the shipping address:

Dr. Lifang Hou/Jun Wang
Department of Preventive Medicine
Feinberg School of Medicine
Northwestern University
680 N Lake Shore Drive, Suite 1400
Chicago, IL 60611
Phone: (312) 503-2965

Rectal Swabs are shipped on dry ice via overnight FedEx to M. Andrea Azcarte-Peril, in one batched shipment at the end of the study.
M. Andrea Azcarate-Peril
Director, Microbiome Core Facility
University of North Carolina at Chapel Hill
Department of Medicine G.I. Division

312 Isaac Taylor Hall
109 Mason Farm Rd
Chapel Hill, NC 27599
Phone: (919) 966-9838

10.4 Tissue Banking

After getting permission from the participants that their samples can be stored and used for future study, the 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 (paper and/or electronic) whether or not related to study agent.

11.1.2 AE Data Elements:

The following data elements are required for AE reporting.

- AE verbatim term
- NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) AE term (MedDRA lowest level term)
- CTCAE (MedDRA) System Organ Class (SOC)
- 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 SAE
- Whether or not the subject 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)*.
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 AE 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 DEFINITION: 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

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

11.2.2.2Contact the DCP Medical Monitor by phone within 24 hours of knowledge of the event.

Gary Della'Zanna, DO, MSc
NCI/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

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

11.2.2.3The Lead Organization and all Participating Organizations will email written SAE reports to the DCP Medical Monitor at gary.dellazanna@nih.gov, to DCP's Regulatory Contractor CCS Associates, Inc. (CCSA; phone: 650-691-4400) at safety@ccsainc.com, and to Northwestern Cancer Prevention Consortium (NCPC, phone 312-503-7821) at ncpc@northwestern.edu within 48 hours of learning of the event using the fillable PDF SAE Report Form.

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

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

11.2.3 Follow-up of SAE

We will follow up after 30 days of the initial event. Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE Report Form in the appropriate format. Follow-up information should be sent to DCP as soon as available.

12. STUDY MONITORING

12.1 Data Management

Data will be managed according to standard operating procedures, which meet the guidelines of DCP Requirements for Data Management and which follow the Data Management Plan that Northwestern University has on file with the Division of Cancer Prevention, NCI. Source data verification will be performed by the Department of Clinical Research Services. The Consortia 2012 Data Management Plan, submitted as part of a contract agreement with the NCI (HHSN261201200035I), was approved.

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRFs) developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDEs). The approved CRFs will be used by Northwestern University to create the electronic CRFs (e-CRFs) screens in the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (Lurie Cancer Center) Clinical Trials Management System (CTMS). Site staff will enter data into the e-CRFs for transmission to DCP according to DCP standards and procedures.

12.3 Source Documents

All source documents will be collected and stored in the Clinical Research Office of the site where the participant was accrued. Any data recorded directly on CRFs that constitute no prior written or electronic record of data, will be specifically identified as source data. Questionnaires completed in person may be completed directly on the paper CRF and need not be transcribed from separate source documentation.

12.4 Data and Safety Monitoring Plan

A comprehensive Data Safety and Monitoring Plan has been submitted by Northwestern University, approved by the DCP, and is on file there. Any future changes will be forwarded for review.

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. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). 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)

Not applicable, since a CTA will not be required for this study.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

This is a Phase IIa, 3 arm, randomized placebo-controlled trial (40 subjects in each of two treatment arms and 10 subjects in the placebo arm) to evaluate whether, use of aspirin at 325 mg in a three weeks on/three weeks off regimen has similar/equivalent efficacy. Our hypothesis is that intermittent use group will show equivalent reduction in the primary endpoint (proliferation: apoptosis ratio) and reduced risk of adverse events, as that using aspirin daily.

13.2 Randomization/Stratification

In current study, there are three arms: two treatment arms (325 mg aspirin daily continuously for 12 weeks, and 325 mg aspirin daily 3-week on/3-week off) and one placebo control arm. Individuals assigned to 325 mg aspirin 3-week on/3-week off will take placebo during 3-week off period.

This study will recruit 90 participants with colorectal adenoma. A permuted-block randomization design will be used to allocate the subjects into the treatment/placebo arm.

13.3 Accrual and Feasibility

Based on our experience from previous R01 trials, we expect to enroll 30-45 participants in year 1 and enroll the remaining in Years 2.

13.4 Primary Objective, Endpoint(s), Analysis Plan

The primary endpoint will be the ratio of proliferation to apoptosis biomarkers (Ki67 index: BAX index, measured continuously) in colorectal mucosa. We anticipate that the two treatment arms are "equivalent" with regard to this main outcome, in the manner described below, for which power analysis is provided. The statistical analysis will consist of examining whether two means are within prescribed limits from each other. Alternatively, two one sided tests can be performed. We have n1=n2=32, which is 40 less 20% dropout; 40-8=32.

Power analysis for the main outcome

The main outcome is defined as the ratio **R= P/A,** where P for measure of Proliferation and A stands for measure of Apoptosis. In principle, intervention increases the Apoptosis and decreases Proliferation,

resulting in R decreasing.

We will measure R for each subject, in each treatment group at baseline (Clinic Visit 2) and at three months (Clinic Visit 4; 12 weeks), obtaining R(i, j), where i= 0, 3 months is the time point, and j=1,2 represents the treatment arm. We will then obtain within-subject changes D(j) = R(3,j) - R(0,j)= for each subject. We expect R to decrease in both groups.

We will then compare mean changes in R between arms to show their equivalence, which we expect, is supported by statistical evidence. To this end, we compute power of a test of equivalence, as defined by following considerations.

The existing information on **R=P/A** is scant. From Dr. Dai's ongoing R01 randomization trial (R01 CA149633; PI: Dai) we have that CV=SD/mean of distribution of R is in the range of 1.5-2 (150-200%).

Variable	Mean	Std Dev
A_P_overall	0.1825	2.5884
A_P_surface	3.2685	4.6094
A_P_bottom	0.0679	3.7236
P_A_overall	2.5444	4.0427
P_A_surface	0.306	4.6094
P_A_bottom	14.733	3.7236

This will limit our ability to argue in favor of equivalence using the usual cutoff points of 10% and 20%. Thus we will have to settle on a broader interval, namely that of 3/4 of standard deviation previously observed in the similar data.

We will assume the correlation between time = 0 and time = 3mo to be Corr[R(3,j), R(0,j)] = 0.5. Simple calculation shows that if pre and post variances are equal to each other σ^2, then Var[D(j)]= σ^2, and thus theoretical standard deviation SD[D(j)]= σ.

From Dr. Dai's data we use mean[R(0,j)]=2.54; sigma[R(0,j)]=4.04. We expect a decrease in R in both groups, on average about one standard deviation. Then the difference post-pre = 4.04 in each group, save for statistical variability. For equivalence interval for comparison of treatments 1 and 2, we take 3/4 of the standard deviation, namely 3.03. For 20% expected drop-out, we have n1=n2=32, power analysis is provided below. We use PASS (www.NCSS.com) for computation. The procedure presented below can be described as follows (PASS Manual, Chapter 260). "This procedure allows you to study the power and sample size of equivalence tests of the means of two independent groups using the two-sample t-test. Schuirmann's (1987) two one-sided tests (TOST) approach is used to test equivalence. For a comprehensive discussion, refer to Chow and Liu (1999)." Essentially, two one sided tests with alpha and beta provide an equivalence test.

Power Analysis of Two-Sample T-Test for Testing Equivalence Using Differences
Numeric Results for Testing Equivalence Using a Parallel-Group Design

Reference Group Sample	Treatment Group Sample	Lower	Upper

Power	Size (N1)	Size (N2)	Equiv. Limit	Equiv. Limit	True Difference	Standard Deviation	Alpha	Beta
0.52	32	32	-2.02	2.02	0.00	4.04	0.10	0.48
0.81	32	32	-3.03	3.03	0.00	4.04	0.05	0.19
0.91	32	32	-3.03	3.03	0.00	4.04	0.10	0.09

The normality assumption for the ratio R will be checked, and data will be logarithmically transformed if the normality assumption does not hold. Assuming log-normal distribution, mean of 2.54 and SD=4.04 on the original scale correspond to mean m=0.3015 and SD_{log} = 1.12 on the log-transformed scale. Further assuming equal variances across time points in both treatment arms, and within-subject correlation 0.5 between pre- and post-treatment values (as above), the standard deviation of changes will also be $SD_{D,log}$ =1.12. The equivalence margin on the log-scale will then be $\delta = 3/4*SD_{D,log} = 0.8423$.

Based on the TOST procedure, equivalence will be established at α=0.05 significance level if the (1-2α)×100% CI for the difference is contained within the interval (-δ, δ). The overall p-value for the equivalence test will be the larger of the p-values of the two one-sided tests.

13.5 Secondary and Exploratory Objectives, Endpoints, Analysis Plans

The secondary objectives are to evaluate the effects of aspirin treatments on:

1) the ratio of cell proliferation (Ki-67)/apoptosis (TUNEL) in rectal biopsies
2) the ratio of cell proliferation (Ki-67)/necroptosis (pMLKL) in rectal biopsies
3) fecal occult blood test (measures of adverse events) as measured by stool samples

The exploratory objectives are to evaluate the effects of aspirin treatments on:

1) COX-2 in rectal biopsies
2) BAX in rectal biopsies
3) TRPM7 in rectal biospies
4) joint index of COX-2 with TRPM7 expression in rectal biopsies;
5) joint index of TRPM7 with BAX in rectal biopsies;
6) methylation assays using the MethylationEPIC BeadChip to identify methylation biomarkers in genes (i.e. CDKN2A (cell cycle regulation), MGMT (DNA repair), DAPK1(apoptosis), CDH1(cell invasion), WNT16 (Wnt pathway) and RASSF1 (RAS signaling)) involved in colorectal carcinogenesis, and other epigenome-wide methylation biomarkers as measured in rectal biopsies;
7) metagenomics analysis to measure abundance of E. coli and Fusobacterium and other microbiota in rectal swabs

The majority of secondary and exploratory endpoints are continuous. Biomarker levels at baseline, 9 and 12 weeks, as well as the corresponding changes from baseline, will be summarized using descriptive statistics including means and standard deviations or medians and interquartile range, as well as graphical methods such as "spaghetti plots" that depict change patterns over time. Distributions of each marker will be examined, and values may be transformed as needed to stabilize the variance or satisfy the normality assumption. Correlation between biomarkers, and between pre- and post-treatment values will be examined using scatterplots with overlayed smoother plots for ease of visual interpretation.

If the normality assumptions are satisfied, paired t-tests will be used to examine whether the biomarkers change from baseline to 12 weeks post-treatment in each treatment arm. Signed rank test will be used if no suitable transformation can be found. Because we expect that the continuous and intermittent dosing regimens will result in similar biomarker changes, we will use the TOST procedure, similar to the primary

endpoint, to test for equivalence. The equivalence margin will be set as 3/4 of the observed standard deviation of the marker at baseline, similar to the primary endpoint.

Additional analyses will be conducted using linear mixed effects models with repeated measures to further explore biomarker change patterns over time across 3 time points, and to compare them between arms. These models use all available observations accommodating data that are missing at random, rather than performing a complete case analysis.

A placebo arm is included in the secondary analyses because the variations on repeated measurement of the new biomarkers of methylation and microbiota are not known from the previous trials, and therefore changes in the treatment arms are difficult to interpret. The placebo arm is used as a reference for background, as a cohort for analysis.

Fecal occult blood tests will be categorized as binary (present vs. absent) at each time visit. Proportion of patients with at least one positive test will be compared between arms using Fishers exact test because incidence of this adverse event is expected to be low.

Exploratory endpoints of joint index of COX-2 with TRPM7 and joint index of TRPM7 with BAX in rectal biopsies will be analyzed similarly. Indices will be calculated using a scoring approach based on factor analysis.

13.6 Reporting and Exclusions

To impute missing data we will use Multiple Imputation via a Semi-Parametric Probability Integral Transformation.This is an approach for imputing continuous, binary, and mixed data by first mapping these data to normally distributed values and then applying multiple imputation so that distributional assumptions for the original data can be relaxed. This method was developed by Irene B. Helenowski, of Northwestern University, in 2012.

http://indigo.uic.edu/bitstream/handle/10027/8652/Helenowski_Irene.pdf?sequence=1

13.7 Evaluation of Toxicity

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

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.

All of the participants who met the eligibility criteria, receive the study agent will be included in the intention-to-treat analysis. Since we will collect rectal biopsies at baseline, week 9 and 12, we will include the participants in the analysis if he/she provides biopsies at week 0 and week 9, but not week 12. Participants will be excluded if he/she only provides one biopsy. All conclusions regarding efficacy will be based on these 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.

We will conduct analysis by the grade of adenomas.

Biomarkers from Week-9 biopsies will also be used in the subanalysis in the intermittent arm.

13.9 Interim Analysis

There will be no interim analysis.

13.10 Ancillary Studies

NA

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. CVs or biosketches do not need to be updated for participating study staff after drug shipment authorization (DSA).

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 Good Clinical Practice training 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

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. Subjects 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.
2001 Gateway Place Suite 350 W
San Jose, CA 95110
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 be compensated $600 for participation (i.e. Clinic Visit 1 $100, Clinic Visit 2 $100, Clinic Visit 3 $100, Clinic Visit 4 $300) if all the procedures are completed. This is provided for the inconvenience of the biopsy protocol, and to compensate for any financial costs due to travel. The participant compensation plan will be pro-rated based on the number of study visits completed. Participant who completes the entire study will receive a total of $600.

REFERENCES

(1) Jemal A, Thomas , Murray T, Thun M. Cancer statistics, 2002. *CA Cancer J Clin* 2002;52:23-47.

(2) Hardy RG, Meltzer SJ, Jankowski JA. ABC of colorectal cancer. Molecular basis for risk factors. *BMJ* 2000;321:886-889.

(3) Nishihara R, Wu K, Lochhead P et al. Long-term colorectal-cancer incidence and mortality after lower endoscopy. *N Engl J Med* 2013;369:1095-1105.

(4) Baxter NN, Goldwasser MA, Paszat LF, Saskin R, Urbach DR, Rabeneck L. Association of colonoscopy and death from colorectal cancer. *Ann Intern Med* 2009;150:1-8.

(5) Hurlstone DP. The detection of flat and depressed colorectal lesions: which endoscopic imaging approach? *Gastroenterology* 2008;135:338-343.

(6) Yamaji Y, Mitsushima T, Ikuma H et al. Incidence and recurrence rates of colorectal adenomas estimated by annually repeated colonoscopies on asymptomatic Japanese. *Gut* 2004;53:568-572.

(7) Rim SH, Seeff L, Ahmed F, King JB, Coughlin SS. Colorectal cancer incidence in the United States, 1999-2004 : an updated analysis of data from the National Program of Cancer Registries and the Surveillance, Epidemiology, and End Results Program. *Cancer* 2009;115:1967-1976.

(8) Ries LAG, Eisner MP, Kosary CL et al. SEER Cancer Statistics Review, 1975-2004. *Natl Cancer Inst Monogr* [serial online] 2004.

(9) Giovannucci E. Diet, body weight, and colorectal cancer: a summary of the epidemiologic evidence. *J Womens Health (Larchmt)* 2003;12:173-182.

(10) Gaziano JM, Glynn RJ, Christen WG et al. Vitamins E and C in the prevention of prostate and total cancer in men: the Physicians' Health Study II randomized controlled trial. *JAMA* 2009;301:52-62.

(11) Cole BF, Baron JA, Sandler RS et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA* 2007;297:2351-2359.

(12) Ulrich CM, Potter JD. Folate and cancer--timing is everything. *JAMA* 2007;297:2408-2409.

(13) Vollset SE, Clarke R, Lewington S et al. Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: meta-analyses of data on 50 000 individuals. *Lancet* 2013.

(14) Wactawski-Wende J, Kotchen JM, Anderson GL et al. Calcium plus vitamin D supplementation and the risk of colorectal cancer. *N Engl J Med* 2006;354:684-696.

(15) Baron JA, Barry EL, Ahnen D.J. et al. A clinical trial of supplementation with vitamin D and/or calcium for the prevention of colorectal adenomas [abstract]Baron JA, Barry EL, Ahnen D.J. et al. *2014 AACR Annual Meeting* 2014;

(16) Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. *J Natl Cancer Inst* 2002;94:252-266.

(17) Chan TA. Nonsteroidal anti-inflammatory drugs, apoptosis, and colon-cancer chemoprevention. *Lancet Oncol* 2002;3:166-174.

(18) Baron JA. Epidemiology of non-steroidal anti-inflammatory drugs and cancer. *Prog Exp Tumor Res* 2003;37:1-24.

(19) Baron JA, Cole BF, Sandler RS et al. A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med* 2003;348:891-899.

(20) Giardiello FM, Hamilton SR, Krush AJ et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. *N Engl J Med* 1993;328:1313-1316.

(21) Steinbach G, Lynch PM, Phillips RK et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 2000;342:1946-1952.

(22) Couzin J. Clinical trials. Halt of Celebrex study threatens drug's future, other trials. *Science* 2004;306:2170.

(23) Chan AT, Arber N, Burn J et al. Aspirin in the chemoprevention of colorectal neoplasia: an overview. *Cancer Prev Res (Phila)* 2012;5:164-178.

(24) Kune GA, Kune S, Watson LF. Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. *Cancer Res* 1988;48:4399-4404.

(25) Dube C, Rostom A, Lewin G et al. The use of aspirin for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. *Ann Intern Med* 2007;146:365-375.

(26) Sehdev A, O'Neil BH. The Role of Aspirin, Vitamin D, Exercise, Diet, Statins, and Metformin in the Prevention and Treatment of Colorectal Cancer. *Curr Treat Options Oncol* 2015;16:359.

(27) Rothwell PM, Wilson M, Elwin CE et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet* 2010;376:1741-1750.

(28) Cole BF, Logan RF, Halabi S et al. Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. *J Natl Cancer Inst* 2009;101:256-266.

(29) Ishikawa H, Mutoh M, Suzuki S et al. The preventive effects of low-dose enteric-coated aspirin tablets on the development of colorectal tumours in Asian patients: a randomised trial. *Gut* 2014;63:1755-1759.

(30) Final report on the aspirin component of the ongoing Physicians' Health Study. Steering Committee of the Physicians' Health Study Research Group. *N Engl J Med* 1989;321:129-135.

(31) Cook NR, Lee IM, Gaziano JM et al. Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. *JAMA* 2005;294:47-55.

(32) Cook NR, Lee IM, Zhang SM, Moorthy MV, Buring JE. Alternate-day, low-dose aspirin and cancer risk: long-term observational follow-up of a randomized trial. *Ann Intern Med*

2013;159:77-85.

(33) Sung JJ. Is aspirin for colorectal cancer prevention on the prime time yet? *Gut* 2014;63:1691-1692.

(34) Sostres C, Lanas A. Gastrointestinal effects of aspirin. *Nat Rev Gastroenterol Hepatol* 2011;8:385-394.

(35) Routine aspirin or nonsteroidal anti-inflammatory drugs for the primary prevention of colorectal cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2007;146:361-364.

(36) McQuaid KR, Laine L. Systematic review and meta-analysis of adverse events of low-dose aspirin and clopidogrel in randomized controlled trials. *Am J Med* 2006;119:624-638.

(37) Seshasai SR, Wijesuriya S, Sivakumaran R et al. Effect of Aspirin on Vascular and Nonvascular Outcomes: Meta-analysis of Randomized Controlled Trials. *Arch Intern Med* 2012.

(38) Mora S. Aspirin Therapy in Primary Prevention: To Use or Not to Use?: Comment on "Effect of Aspirin on Vascular and Nonvascular Outcomes". *Arch Intern Med* 2012.

(39) Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet* 2012;379:1591-1601.

(40) Lubet RA, Szabo E, Christov K et al. Effects of gefitinib (Iressa) on mammary cancers: preventive studies with varied dosages, combinations with vorozole or targretin, and biomarker changes. *Mol Cancer Ther* 2008;7:972-979.

(41) Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57-70.

(42) Yang SY, Sales KM, Fuller B, Seifalian AM, Winslet MC. Apoptosis and colorectal cancer: implications for therapy. *Trends Mol Med* 2009;15:225-233.

(43) Bedi A, Pasricha PJ, Akhtar AJ et al. Inhibition of apoptosis during development of colorectal cancer. *Cancer Res* 1995;55:1811-1816.

(44) Keku TO, Amin A, Galanko J, Martin C, Schliebe B, Sandler RS. Apoptosis in normal rectal mucosa, baseline adenoma characteristics, and risk of future adenomas. *Cancer Epidemiol Biomarkers Prev* 2008;17:306-310.

(45) Fedirko V, Bostick RM, Flanders WD et al. Effects of vitamin D and calcium supplementation on markers of apoptosis in normal colon mucosa: a randomized, double-blind, placebo-controlled clinical trial. *Cancer Prev Res (Phila Pa)* 2009;2:213-223.

(46) Baron JA, Wargovich MJ, Tosteson TD et al. Epidemiological use of rectal proliferation measures. *Cancer Epidemiol Biomarkers Prev* 1995;4:57-61.

(47) Baron JA. Intermediate effect markers for colorectal cancer. *IARC Sci Publ* 2001;154:113-129.

(48) Burn J, Bishop DT, Chapman PD et al. A randomized placebo-controlled prevention trial of aspirin and/or resistant starch in young people with familial adenomatous polyposis. *Cancer Prev*

Res (Phila) 2011;4:655-665.

(49) Pasparakis M, Vandenabeele P. Necroptosis and its role in inflammation. *Nature* 2015;517:311-20

(50) Wallach D, Kang TB, Kovalenko A. Concepts of tissue injury and cell death in inflammation: a historical perspective. *Nat Rev Immunol* 2014;14:51-9.

(51) Parker D, Prince A. Immunoregulatory effects of necroptosis in bacterial infections. *Cytokine* 2016;88:274-5.

(52) Orozco S, Oberst A. RIPK3 in cell death and inflammation: the good, the bad, and the ugly. *Immunol Rev* 2017;277:102-12.

CONSENT FORM

NCI Protocol #: NWU2015-06-01
Local Protocol #: NCI 2015-06-01
Protocol Version Date: v2.19 08/11/2022
Informed Consent Version Date: 08/11/2022

Study Title for Study Participants: Testing aspirin to prevent colorectal cancer after adenoma diagnosis
Protocol Title: Evaluating Intermittent Dosing of Aspirin for Colorectal Cancer Chemoprevention

Introduction

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part in the research. Please take your time to make your decision about volunteering. You may discuss your decision with your friends and family. You can also discuss this study with your health care team. If you have any questions, you can ask your study doctor for more of an explanation. You should only agree to participate in this study when you are comfortable enough with the information so that you can make an informed decision about joining.

What is the usual approach to my polyps?

You are being asked to be a part of this research study because you have had one or more of a certain type of polyp (an adenoma) removed from your colon. People who are diagnosed with adenomas have a higher risk of growing more of these polyps that may become colon or rectal cancer. The usual approach is to be checked every 3 to 5 years by colonoscopy with removal of any new polyps.

What are my other choices if I do not take part in this study?

If you decide not to take part in this study, you have other choices. For example:

- you may choose to have the usual approach described above
- you may choose to take part in a different study, if one is available
- or you could decide not to be treated for polyp formation

Why is this study being done?

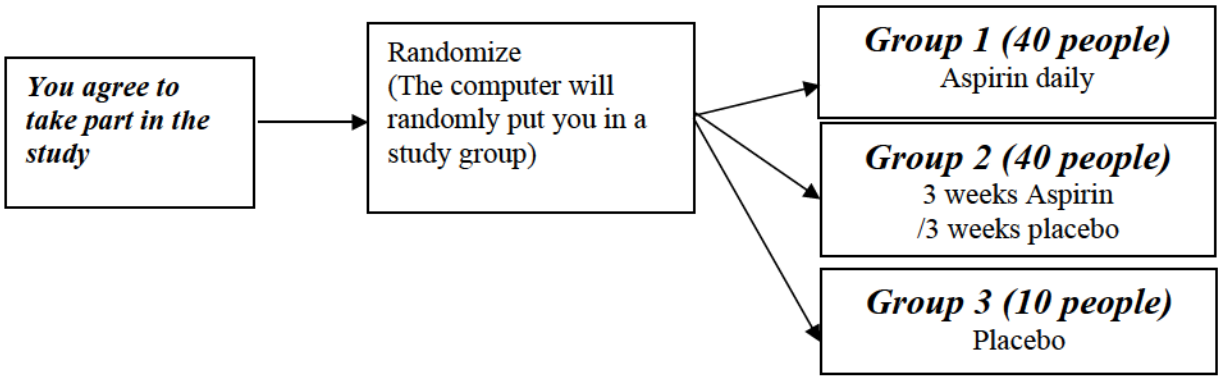
Previous studies have shown that aspirin use may prevent people who have had adenomas from developing colon and rectal cancer. The purpose of this study is to compare the safety and effects of two different dose schedules of aspirin on people and their risk of colon and rectal cancer. In this study you would get either aspirin pills to take daily, or aspirin pills to take daily on an intermittent schedule (3 weeks of daily aspirin followed by 3 weeks of placebo pills), or placebo pills to take daily. The placebo pills look like the study drug but contain no medication. Results from this study will lay a foundation for future studies trying to prevent colorectal cancer. We would like to enroll about 90 participants in this study at Vanderbilt University.

What are the study groups?

This study has three study groups.

- Group 1 will receive the study drug, a daily pill that contains 325 mg aspirin, for 12 weeks.
- Group 2 will receive the study drug, a daily pill that contains 325 mg aspirin for 3 weeks, and then a placebo for 3 weeks; these 3 week on/3-week off cycles will be repeated for a total of 12 weeks. The placebo pill will look like the study drug so that it will not be possible to know which group you are in.
- Group 3 will receive a placebo, a pill that looks like the study drug but contains no medication, for 12 weeks.

A computer will randomly put you in a study group—like a coin toss—to decide what group you get placed into. This is done because no one knows if one study group is better, the same, or worse than the other group. Once you are put in a group, you cannot switch to the other group. Neither you nor your doctor will know if you are receiving the study drug or placebo. Your doctor cannot choose which group you will be in.



How long will I be in this study?

You will receive the study drug for 3 months (12 weeks). After you finish intervention, the research staff or nurse will telephone or email you 1 month after your last visit to ask you about any effects or conditions. The TOTAL duration of the study participation is 4 months.

What extra tests and procedures will I have if I take part in this study?

The whole study will take about 4 months from the time that we first call you until the time that you are finished with the study. Most of the tests and procedures you will need for this study are similar to those you have had before as part of the usual approach for your condition. However, most of the tests and procedures that you have for the study will be in addition to or more often than your usual care.

Before you begin the study (Clinic Visit 1):

- Before you participate in any procedures that are specifically for this research, you will need to come to the Vanderbilt University Medical Center to read and discuss any questions you may have and to sign this consent form.
- To see if you can be in the study, you will have the following extra tests:
    - Physical and vital signs (includes pulse, respiratory rate, temperature, blood pressure, ECOG performance status) and body measurements (includes height, weight, hip and waist circumferences)
    - Blood test – about 1 ⅓ tablespoons of blood taken with a needle from a vein in your arm

- Optional urine test - you will be instructed on how to collect a urine sample
- Stool test - you will be instructed on how to collect a stool sample
- Questionnaires to complete at home that should take 30 minutes. The questions ask about your past medical history, drug allergies, current medications, current symptoms or illnesses, family history and dietary information and other areas in your life that may be related to colorectal cancer risk
- If you are woman able to become pregnant, you will have a urine pregnancy test. Pregnant women, women who are currently breast feeding, and women considering a pregnancy will not be allowed to participate in this study.

<u>During the study</u>
During the study, you will be asked to make four clinic visits:

- Clinic Visit 2 (within 6 weeks of Clinic Visit 1)
- Clinic Visit 3 (9 weeks after starting study agent)
- Clinic Visit 4 (12 weeks after starting study agent) and

*Study agents are dispensed at Clinic Visits 2 and 3.

During these visits:

- You will be asked to come to the Vanderbilt University Medical Center. At each visit, you will bring a stool sample with you. At the clinic, study staff will take a blood sample (about 1 and 2/3 tablespoons) and an optional urine sample (2 tablespoons).
- At visits 2, 3, and 4 the research staff or nurse will take your vital signs (includes pulse, respiratory rate, temperature, blood pressure) and body measurements (includes height, weight, hip and waist circumferences) and will ask you about any changes in your medications or how you are feeling. If there are any major changes or if you are feeling unwell you may also have a physical exam by the doctor.
- At visits 2, 3, and 4, the doctor will insert a swab gently into your rectum to collect fecal material.
- At visits 2, 3, and 4, the doctor will look at your rectum using a plastic tube with a light and will take around eight small pieces (about one-fifth of an inch each) of the normal tissue from your rectum, right after collecting the fecal material and the cells.
- At your 2nd visit, you will be given the pills that have been assigned to you – enough for nine weeks. You will start taking the pills on that day and will continue taking them for 9 weeks. At Visit 3, you will be given more pills for the study and will continue taking them for 3 weeks. You will also be given a diary and asked to keep track of the time of day that you take your pills and any missed doses.
- At visits 3 and 4 you will bring back your pill bottles and any unused pills. The research staff or nurse will count you pills and review your study diary.
- You will be called or emailed (to see how you are feeling) a total of 7 times: After your biopsy visits (Clinic Visits 2, 3, and 4), in Weeks 1, 3, and 6 while you are taking the pills, and 1 month after your last visit (1 month after Clinic Visit 4). You will also be given several ways to contact us in case there is any information you need us to know.

Your rectal biopsies and swabs will be shipped to labs in Northwestern University in Chicago and University of North Carolina at Chapel Hill for lab analyses. Other samples will be tested at the Vanderbilt lab.

All of the information that you provide will be kept strictly confidential. You will be assigned an identification number that will be used in the study to store your information. Only the study staff will have access to the locked key that links your identifying information (for example, name) with your

identification number.

What possible risks can I expect from taking part in this study?

If you choose to take part in this study, there is a risk that you may:

Lose time at work or home and spend more time in the hospital or doctor's office than usual.

Be asked sensitive or private questions which you normally do not discuss. There is a risk someone could get access to the personal information in your medical records or other information researchers have kept about you. Someone might be able to trace this information back to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information. In some cases, this information could be used to make it harder for you to get or keep a job. There are laws against misuse of genetic information, but they may not give full protection. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

There can also be a risk in finding out new genetic information about you. New health information about inherited traits that might affect you or your blood relatives could be found during a study.

You should not take certain painkillers while taking the study drug. These include Motrin, Advil, Celebrex, Naproxen and others. You should talk to your doctor before taking any new pain medicine. You should also not take corticosteroids, warfarin (Coumadin), heparin or other blood thinners. These drugs increase the likelihood of developing serious risks (**e.g. bleeding and gastrointestinal distress**) related to aspirin.

The aspirin used in this study may affect how different parts of your body work, such as your liver, kidneys, heart, and blood. The study doctor will be testing your blood and will let you know if changes occur that may affect your health.

There is also a risk that you could have side effects. Here are important points about side effects:
- The study doctors do not know who will or will not have side effects.
- Some side effects may go away soon, some may last a long time, or some may never go away.
- Some side effects may interfere with your ability to have children.
- Some side effects may be serious and may even result in death.

Here are important points about how you and the study doctor can make side effects less of a problem:
- Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect.
- The study doctor may be able to treat some side effects.

The tables below show the most common side effects that we know about *Aspirin*, some of which may be serious. There might be other side effects that we do not yet know about. If important new side effects are found, the study doctor will discuss these with you.

Possible Side Effects of Aspirin:

COMMON, SOME MAY BE SERIOUS Many side effects of aspirin depend on the dose/ number of capsules taken. The frequency of some side effects is not known.
• Heartburn

COMMON, SOME MAY BE SERIOUS Many side effects of aspirin depend on the dose/ number of capsules taken. The frequency of some side effects is not known.
• Stomach Cramps • Feel Like Throwing Up • Nausea/vomiting, dyspepsia (indigestion) • Drowsiness

RARE, SOME MAY BE SERIOUS Many side effects of aspirin depend on the dose/ number of capsules taken. The frequency of some side effects is not known.
• Bleeding of the Stomach or Intestines (coughing up blood or vomit that looks like coffee grounds, black, bloody, or tarry stools) • Stomach or Intestinal Ulcer • Decreased Blood Platelets • Decreased White Blood Cells • Anemia • Ringing in the Ears • Hepatitis caused by Drugs • Life Threatening Allergic Reaction

Risks related to rectal biopsy

COMMON, SOME MAY BE SERIOUS In 100 people receiving rectal biopsy, more than 20 may have:
• Observe some blood on the tissue on the day of the biopsy. • Pain and/or discomfort from the rectal biopsy.

RARE, SOME MAY BE SERIOUS In 100 people receiving rectal biopsy, 3 or fewer may have:
• Bleeding from the rectum. Bleeding heavier or more persistent than this should be reported. • The biopsy may cause a hole in the rectum which may lead to infection

Reproductive risks:

You should not get pregnant, breastfeed, or father a baby while in this study. The aspirin used in this study could be very damaging to an unborn baby. If a female become pregnant in the study, she should inform the study doctor immediately.

Both females and males must use contraception prior to study entry until the duration of their study participation. Check with the study doctor about what types of birth control, or pregnancy prevention to use.

Risks related to blood tests: Bruising, soreness, or rarely, infection may occur, or you might feel faint as a result of the needle sticks to obtain blood from your vein.

Taking part in this study may be an inconvenience to your time with the telephone or email contacts, the collection of all the samples at the clinic, and the collection of the stool samples at home.

What possible benefits can I expect from taking part in this study?

a) The benefits to science and humankind that <u>might</u> result from this study are: Your taking part may provide information that leads to a safer way of preventing colorectal cancer. Society may benefit from a

low-cost, well-tolerated strategy to reduce death from colorectal cancer.

b) There will be no direct benefits to you for taking part in this study. You will be informed of your renal function during the study. If it is insufficient, you can consult with your healthcare provider.

Can I stop taking part in this study?

Yes. You can decide to stop at any time. If you decide to stop for any reason, it is important to let the study doctor know as soon as possible so you can stop safely. If you stop, you can decide whether or not to let the study doctor continue to provide your medical information to the organization running the study.

The study doctor will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

The study doctor may take you out of the study:

- If your health changes and the study is no longer in your best interest
- If new information becomes available
- If you do not follow the study rules
- If the study is stopped by the sponsor, IRB or FDA.
- If you are not able to provide the blood, urine, or rectal samples.

What are my rights in this study?

Taking part in this study is your choice. No matter what decision you make, and even if your decision changes, there will be no penalty to you. You will not lose medical care or any legal rights.

For questions about your rights while in this study, call the Vanderbilt University Medical Center Institutional Review Board at *(615) 322-2918.*

What are the costs of taking part in this study?

The aspirin will be supplied at no charge while you take part in this study.
The cost of study-specific exams (e.g. pulse, respiratory rate, temperature, blood pressure, height, and weight), tests (e.g. blood, urine, stool test), rectal swab, biopsies and any other procedures will be paid for by the study. Some costs associated with your care may be considered standard of care, and will be billed to you or your insurance company. You will have to pay for any costs (including deductibles and co-payments) not covered by your health insurer. Before you decide to be in the study, you should check with your health plan or insurance company to find out exactly what they will pay for.

You will be compensated for participation: Clinic Visit 1 ($100), Clinic Visit 2 ($100), Clinic Visit 3 ($100), Clinic Visit 4 ($300) for undergoing biopsy procedures, blood draw, stool and urine collections during the study. This is provided for the inconvenience of the biopsy protocol, and to compensate for any financial costs due to travel. Payment will be provided after each visit.

What happens if I am injured or hurt because I took part in this study?

If you feel you have been injured or hurt as a result of taking part in the study, it is important that you tell the study doctor immediately. You will get medical treatment if you are injured or hurt as a result of taking part in this study.

The study sponsors will not pay for medical treatment for injury. Your insurance company may not be willing to pay for study-related injury. If you have no insurance coverage, you would be responsible for any costs. Even though you are in a study, you keep all of your legal rights to receive payment for injury caused by medical errors.

Who will see my medical information?

Your privacy is very important to us and the researchers will make every effort to protect it. The study doctors have a privacy permit to help protect your records if there is a court case. Your information may be given out if required by law. For example, certain states require doctors to report to health boards if they find a disease like tuberculosis. However, the researchers will do their best to make sure that any information that is released will not identify you. Some of your health information, and/or information about your specimen, from this study will be kept in a central database for research. Your name or contact information will not be put in the database.

This study is sponsored by National Cancer Institute, Division of Cancer Prevention. The National Cancer Institute will obtain information for this clinical trial under data collection authority Title 42 U.S.C. 285.

There are organizations that may inspect your records. These organizations are required to make sure your information is kept private, unless required by law to provide information. Some of these organizations are:

- The study sponsor supporting the study.
- The Institutional Review Board, IRB, is a group of people who review the research with the goal of protecting the people who take part in the study.
- The Food and Drug Administration and the National Cancer Institute in the U.S.

Where can I get more information?

You may visit the NCI Web site at http://cancer.gov/ for more information about studies or general information about cancer. You may also call the NCI Cancer Information Service to get the same information at: 1-800-4-CANCER (1-800-422-6237).

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Who can answer my questions about this study?

You can talk to the study doctor about any questions or concerns you have about this study or to report side effects or injuries. Contact the study doctor Dr. Reid Ness at (615) 322-0128, Dr. Rishi Naik at (615) 343-0109.

Sample Collections for Laboratory Studies and/or Biobanking for Possible Future Studies

This section is about optional studies you can choose to take part in.

This part of the consent form is about optional studies that you can choose to take part in. You will not get health benefits from any of these studies. The researchers leading this optional study hope the results will help other people with cancer in the future.

The results will not be added to your medical records, and you or your study doctor may not know the results. You will not be billed for these optional studies.

You can still take part in the main study even if you say 'no' to any or all of these studies. If you sign up for but cannot complete any of these studies for any reason, you can still take part in the main study.

If you choose to take part, the rectal biopsies, rectal swabs, urine, stool and blood samples collected in the main study that are leftover after the main study will be stored for future studies. Storing samples for future studies is called "biobanking" which is being run by the National Cancer Institute.

WHAT IS INVOLVED?

If you agree to take part, here is what will happen next:

1) Your sample and some related health information may be stored in the Biobank, along with samples and information from other people who take part. The samples will be stored at Vanderbilt Epidemiology Biospecimen Core during study until the end of the study, when they may be transferred to the National Institutes of Health.
2) Qualified researchers can submit a request to use the materials stored in the Biobanks. A science committee at the clinical trials organization, and/or the National Cancer Institute, will review each request. There will also be an ethics review to ensure that the request is necessary and proper. Researchers will not be given your name or any other information that could directly identify you.
3) Neither you nor your study doctor will be notified when research will be conducted or given reports or other information about any research that is done using your samples.
4) Some of your genetic and health information may be placed in central databases that may be public, along with information from many other people. Information that could directly identify you will not be included.

WHAT ARE THE POSSIBLE RISKS?

1) The most common risks related to drawing blood from your arm are brief pain and possibly a bruise.
2) There is a risk that someone could get access to the personal information in your medical records or other information researchers have stored about you.
3) There is a risk that someone could trace the information in a central database back to you. Even without your name or other identifiers, your genetic information is unique to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.
4) There are laws against the misuse of genetic information, but they may not give full protection. New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

   A new Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

   - Health insurance companies and group health plans may not request your genetic information that we get from this research.
   - Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.

Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment. All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

HOW WILL INFORMATION ABOUT ME BE KEPT PRIVATE?

Your privacy is very important to the researchers and they will make every effort to protect it. Here are just a few of the steps they will take:

1) When your sample(s) is sent to the researchers, no information identifying you (such as your name) will be sent. Samples will be identified by a unique code only.
2) The list that links the unique code to your name will be kept separate from your sample and health information. Any Biobank and Vanderbilt University Medical Center staff with access to the list must sign an agreement to keep your identity confidential.
3) Researchers to whom Vanderbilt University Medical Center sends your sample and information will not know who you are. They must also sign an agreement that they will not try to find out who you are.
4) Information that identifies you will not be given to anyone, unless required by law.
5) If research results are published, your name and other personal information will not be used.

WHAT ARE THE POSSIBLE BENEFITS?

You will not benefit from taking part. The researchers, using the samples from you and others, might make discoveries that could help people in the future.

ARE THERE ANY COSTS OR PAYMENTS?

There are no costs to you or your insurance. You will not be paid for taking part. If any of the research leads to new tests, drugs, or other commercial products, you will not share in any profits.

WHAT IF I CHANGE MY MIND?

If you decide you no longer want your samples to be used, you can call the Study Coordinator (_____) at (_____) who will let the researchers know. Then, any sample that remains in the bank will no longer be used and related health information will no longer be collected. Samples or related information that have already been given to or used by researchers will not be returned.

WHAT IF I HAVE MORE QUESTIONS?

If you have questions about the use of your samples for research, contact the Study Coordinator (_____) at (_____).

Please circle your answer to show whether or not you would like to take part in each option

SAMPLES FOR THE LABORATORY STUDIES:

I agree to have my specimen collected and I agree that my specimen sample(s) (rectal biopsy/swab, urine, stool and blood) and related information may be used for the laboratory study(ies) described above.

YES NO

I agree that my study doctor, or their representative, may contact me or my physician to see if I wish to learn about results from this(ese) study(ies).

YES NO

SAMPLES FOR FUTURE RESEARCH STUDIES:

My samples and related information may be kept in a Biobank for use in future health research.

YES NO

I agree that my study doctor, or their representative, may contact me or my physician to see if I wish to participate in other research in the future.

YES NO

This is the end of the section about optional studies.

If you develop a medical problem while on study, may we inform your physician?

YES NO

If yes, whom would you like us to inform?

Name: _____ Phone Number: _____

My Signature Agreeing to Take Part in the Main Study

I have read this consent form or had it read to me. I have discussed it with the study doctor and my questions have been answered. I will be given a signed copy of this form. I agree to take part in the main study and any additional studies where I circled 'yes'.

Participant's signature_____

Date of signature_____

Signature of person(s) conducting the informed consent discussion _____

Date of signature_____

Appendix A – Clinic Visit Instructions

Evaluating Intermittent Dosing of Aspirin for Colorectal Cancer Chemoprevention Study

CLINIC APPOINTMENT INSTRUCTIONS

1. You are scheduled for your clinic appointment on

   _____ at _____ AM/PM.

2. Instructions for parking and how to get to the clinic are included in this packet.

3. If you need to change your appointment, please call **XXXXXXXX at XXXXXXXX** to reschedule.

4. Please read the detail "Stool Sample Instructions" and collect your stool sample before your clinic visit. We prefer that you collect the sample up to 3 days before the visit.

5. Remember to place your freezer ice pack into your freezer **at least two days before** your clinic appointment.

6. Please remember to bring to your visit your stool sample along with the ice pack in the foam cooler.

7. If you have any questions, please contact us at XXXXXXXXXX.

Thank you for your participation!

Appendix B – At-Home Stool Collection Instructions

Before you collect your stool sample:

1. We would like you to collect a stool sample **up to 3 days before your clinic appointment**.
2. You may request help reading these instructions from a member of your household, but please **do not let anyone handle** the containers, screw tops, or plastic bags.
3. **At least 2 days before your clinic appointment,** place the freezer pack into your freezer.

When you are ready to collect your stool sample:

1. Wash your hands with soap and warm water.
2. Urinate into the toilet and flush.
3. Lift the toilet seat.
4. Place the support frame on the toilet bowl rim. Place the white plastic bowl into the circle-shaped section of the frame.
5. Lower the toilet seat and pass the stool directly into the white plastic bowl. **Do not mix the stool sample with any urine or water from the toilet.**
6. Put on the gloves and open one of the stool collection containers (brown cap) with the spoon attached to the brown inner lid.
7. Using the spoon attached to the screw top, transfer pieces of stool into the container until the container is filled halfway. If the stool is liquid – pour. If the stool is loose – gently pour.
8. Screw the lid on the container tightly and wipe off any spills from the outside of the container with a clean paper towel. Discard the paper towel.
9. Repeat steps 6, 7, and 8 for the other brown cap stool collection containers.
10. Open the thin collection container with the light green lid.
11. Dip the sample wand attached to the lid into the stool sample which was collected in the white bowl. Cover the grooved portion of the sample wand completely with stool sample.
12. Re-insert the sample wand into the container and push the cap down until a *snap* is heard.
13. Place all 3 samples collection tubes (2 brown caps, 1 light green cap) into the plastic specimen bag and **seal the bag in zip-lock fashion**.
14. Write the **date and time of collection** on the bag with a pen or marker.
15. Place the sealed bag (containing the collection containers) into the second plastic bag. Remove any excess air from the bag and seal the second bag.
16. Wash your hands with soap and warm water. You may discard the gloves and the white stool collection bowl. The frame may be reused if future collections are expected.
17. **Place the sealed bag in the REFRIGERATOR until you bring it to your clinic appointment**

The day of your clinic appointment:

1. Put the sealed bag with the stool samples into the insulated transport bag along with the freezer gel pack.
2. Bring the package with you to your clinic appointment.

A.

B.

C.

This lid with spoon is for illustration only

D.

E.

F.

G.

Contents of Kit:

a. (1) Collection bowl with lid and support frame for toilet rim
b. (2) Tubes with brown caps with spoons attached to inner lids
c. (1) Tube with light green cap with dipstick wand attached to inner lid
d. (1) Pair of non-allergenic nitrile gloves
e. (2) Plastic specimen bags
f. (1) Reusable transport bag

Appendix C – Baseline Clinic Visit Questionnaire

Date: |__|__|/|__|__|/|__|__|__|__| **(mm/dd/yyyy)**

We would like to learn more about your health conditions. Check the appropriate response choice for each question listed below. Please do not skip any of the questions and be careful to mark the correct column for each response choice.

PART 1: Health History

1. **Were any of your first-degree blood relatives, for example your mother, father, siblings, or children ever diagnosed as having cancer?** (not including non-melanoma skin cancer)

☐ Yes
☐ No (Continue with Question 2)
☐ Don't Know (Continue with Question 2)

For each cancer diagnosis, please specify the relative, cancer site, and approximate age at diagnosis of cancer

	RELATIONSHIP TO YOU (for example: sister)	CANCER TYPE (for example: breast)	AGE AT DIAGNOSIS
1.			years
2.			years
3.			years
4.			years
5.			years
6.			years

2. **Were any of your first-degree blood relatives, for example your mother, father, siblings, or children ever diagnosed as having colon or rectal polyps?**

☐ Yes
☐ No (continue with Question 3)
☐ Don't Know (continue with Question 3)

For each polyp diagnosis, please specify the relative and approximate age at diagnosis of polyps.

	RELATIONSHIP TO YOU (for example: sister)	AGE AT DIAGNOSIS
1.	☐	☐☐ years
2.	☐	☐☐ years
3.	☐	☐☐ years
4.	☐	☐☐ years
5.	☐	☐☐ years
6.	☐	☐☐ years

3. **Has a doctor ever told you that you had any of the following conditions? Would you say yes, no, don't know? Please check the correct box. (Do not include borderline high cholesterol, fat; Do not include diabetes you only had when pregnant.)**

Question	Answer		
Has a doctor ever told you that you had…	**Yes**	**No**	**Don't Know**
…high cholesterol?	☐	☐	☐
…high levels of fat (other than cholesterol)?	☐	☐	☐
… diabetes?	☐	☐	☐
…hypertension?	☐	☐	☐
…cardiovascular disease?	☐	☐	☐

PART 2: Medicines

1. **Please list all medicines you have taken in the past 7 days. Please include the amount you took.** (you may use the back of this page if needed):

	Name of each medicine	Dose/unit time
Example		
	Metformin	500 mg once daily
	Verapamil ER 240 mg	1 capsule daily
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.		
32.		

2. Have you ever <u>regularly</u> taken metformin? By regular, it means at least 3 days per week for at least twelve months in a row.

☐ Yes
☐ No [*Skip to Part 3*]
☐ Don't Know [*Skip to Part 3*]

3. When you were regularly taking metformin, how many pills per day or how many per week did you take this medicine?

|___|___|___| pills per day
|___|___|___| pills per week

4. When you were regularly taking metformin, how many months or years did you take this medicine?

|___|___| Months
|___| Years

5. Are you still taking metformin regularly?

☐ Yes
☐ No
☐ Don't Know

6. How many months or years ago did you stop using metformin regularly?

|___|___| Months
|___| Years

PART 3: Supplements

1. **Please list all supplements you have taken in the past 12 months. Please include the amount you took. Please do not list fish oil supplement here.** (you may use the back of this page if needed):

	Name of each supplement	Dose/unit time
Example		
	Centrum calcium	1 tablet daily
	Multivitamin	1 capsule daily
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.		
32.		

2. **Please list fish oil supplements you have taken in the past 12 months. Please include the amount you took.** (you may use the back of this page if needed):

	Name of each supplement	Dose/unit time
Example		
	Omega-3 Fatty Acids	1 capsule daily
	Omega-6 Fatty Acids	1 capsule daily
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.		
32.		

(For women only)

Part 4: Gynecological and Reproductive History

1. **Have you ever had a menstrual cycle or period?**

☐ Yes → 2. **How old were you when you menstruated for the first time (had your first period)?** ☐☐ Years

☐ No → CONTINUE WITH QUESTION 6.

3. **Are you still having menstrual periods?**

☐ Yes → CONTINUE WITH QUESTION 6.
☐ No

4. **How old were you when your periods stopped completely?**

☐☐ Years

5. **What was the reason your periods stopped?** *(Select only one answer)*

☐ Natural menopause (change of life)
☐ Because of hysterectomy (either uterus and/or ovaries were surgically removed).
☐ Took medication that stopped periods
☐ Other (please specify): _____

6. Have you ever taken female hormone pills, skin patches, shots or crèmes other than birth control pills (for example estrogen, progesterone, estrogen and progesterone, conjugated estrogens, Premarin, Provera, Estraderm skin patches, or other not including Tamoxifen, Raloxifen, or Evista)?

☐ Yes → 7. **How old were you when you began taking them?** ☐☐ Years

☐ No → CONTINUE WITH QUESTION 11.

8. **How old were you when you last took them?** ☐☐ Years

9. **Before you started taking female hormones, had your period stopped?**
☐ Yes
☐ No

10. **For many women, taking estrogens involves starting and stopping several times. During the time from starting to finally stopping your use of estrogens, how long, altogether were you actually taking them?**

☐☐ months **OR** ☐☐ years *(Fill in one only)*

11. **Have one or both of your ovaries ever been surgically removed?**
- ☐ Yes-one ovary
- ☐ Yes-both ovaries
- ☐ No
- ☐ Don't Know

12. **Have you ever given birth to a child?**
- ☐ No (Continue with Question 16)
- ☐ Yes

13. **How old were you when you gave birth to your first child?**

☐☐ Years

14. **How many pregnancies have you had that ended with a live birth?**

☐☐ Number of live births

15. **Of these pregnancies, how many were multiples births?** ☐☐ Number of Multiple Births

16a. [***Excluding the above pregnancies***], **did you have any other pregnancies; that is, pregnancies that ended in stillbirths, miscarriages, ectopic pregnancies, or induced abortions?**
- ☐ Yes
- ☐ No

17. **Have you ever taken oral contraceptives (birth control pills)?**

- ☐ Yes →
- ☐ No
- ☐ Don't Know

18. **How old were you when you begin taking them ?** ☐☐ Years

19. **How old were you when you last took them?**
- ☐ Still taking them

☐☐ Years

20. **For many women, taking oral contraceptives involves starting and stopping several times. During the time from starting to finally stopping your use of oral contraceptives, how long, altogether were you actually taking the pills?**

☐☐
- ☐ months
- ☐ years

Reviewer: |__|__|__|__|__|__|__|__| page 8 of 8

Appendix D – Participant Diary

Protocol NWU2015-06-01
Participant ID: ____________
Instructions: Take once per day with food.
Switch to Bottle 2 (labeled "Weeks 4-6") starting on Day 22.
Switch to Bottle 3 (labeled "Weeks 7-9") starting on Day 43.
Switch to Bottle 4 (labeled "Weeks 10-12") after Clinic Visit 3.

DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________	DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________	DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________	DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________	DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________	DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________	DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________
DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________	DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________	DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________	DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________	DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________	DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________	DAY: ___ BOTTLE: ________ DATE: ___/___/___ TIME: ________ Please list new symptoms, new medications, or reasons for missed doses: ______________ ______________ ______________ ______________ ______________

For use by study staff Coordinator's Signature: ______________________________________ Date: ____________

Appendix E – Suggested Statements for Participant Communications

Please use the following suggested statements for telephone or email contacts with participants.

Hello (participant name),

Thank you for participating in the Evaluating Intermittent Dosing of Aspirin for Colorectal Cancer Chemoprevention Trial.

*If you will, **please reply at your earliest convenience** and let me know how your experience has been in since we last spoke. Any and all events are important to record.*

1. *Have you experienced any symptoms or possible side effects?*
2. *Have you taken your study medication daily and marked each dose in your diary? Have you missed any doses?*
   Keep in mind to only take your study medication for 21 consecutive days and then to switch to your next bottle for 21 consecutive days.
3. *Have there been any changes in your other medications/supplements since we last spoke?*

As a reminder, your expected bottle switch date are:
Stop bottle 1/ start bottle 2: (insert date)
Stop bottle 2/ start bottle 3: (insert date)

Our next planned clinic appointment is listed below. Please plan your home specimen collection and bring all study medication bottles with you to this visit.

Clinic visit 3: (insert date)

Please call or email me with any questions.

Thank you,
(insert coordinator name and contact information)