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Phase I Double-Blind, Placebo-Controlled Trial of 27 mg Dolcanatide (SP-333) to Demonstrate Colorectal Bioactivity in Healthy Volunteers

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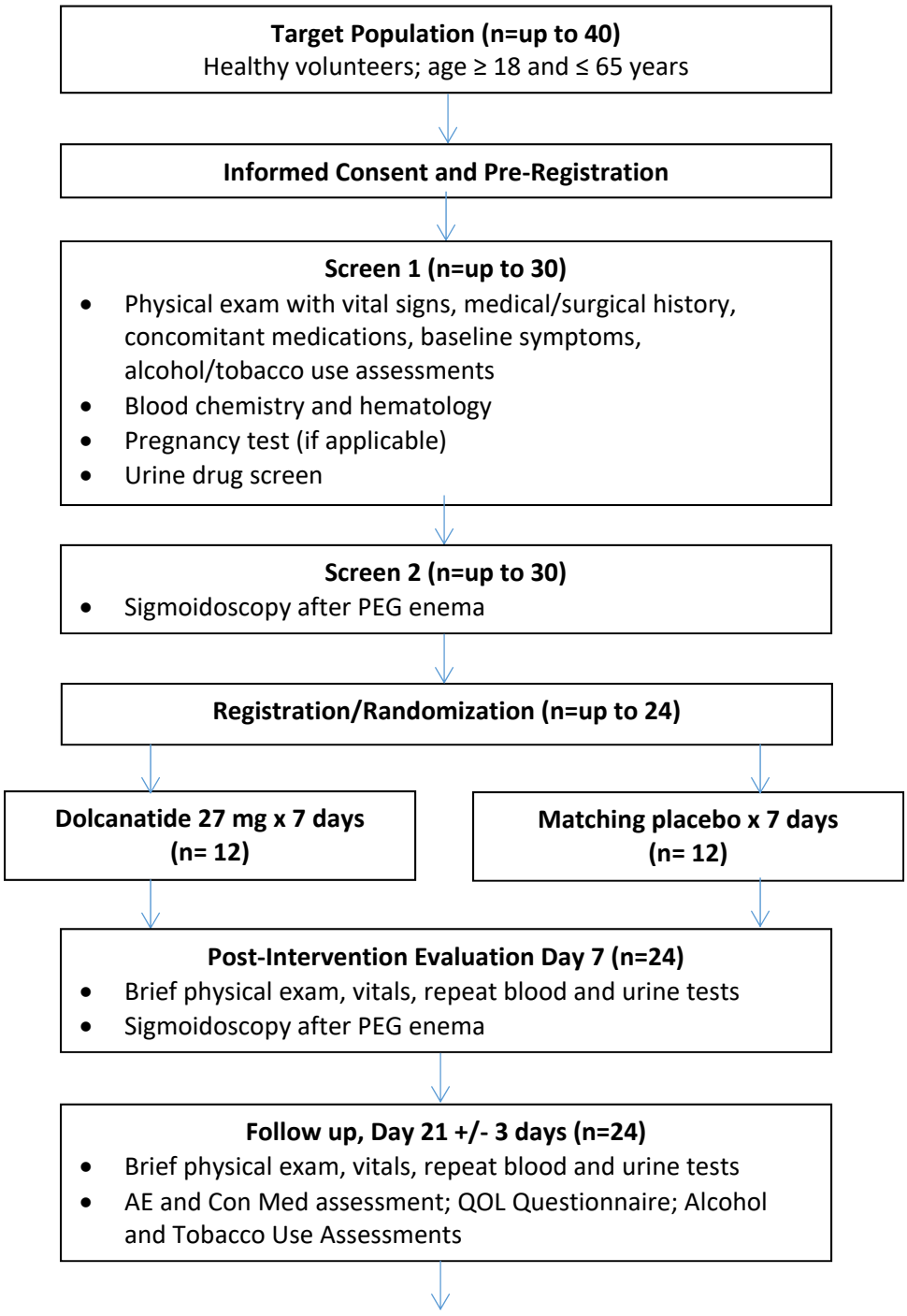
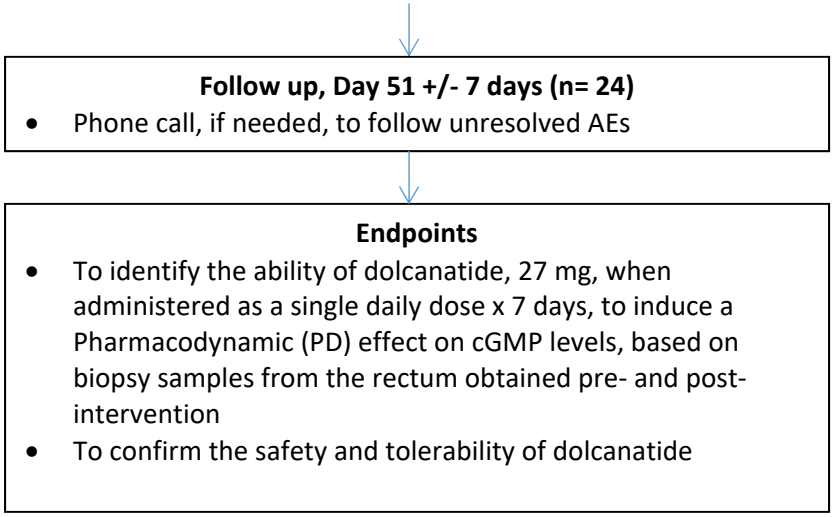
SCHEMA

Phase I Double-Blind, Placebo-Controlled Trial of 27 mg Dolcanatide (SP-333) to Demonstrate Colorectal Bioactivity in Healthy Volunteers





Note: Twenty four (24) participants total will be randomized (12 per arm) to fully evaluate dolcanatide and compare directly to the placebo arm.

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1. OBJECTIVES

Guanylate cyclase C (GCC) is a tumor suppressing receptor whose silencing, due to the loss of expression of the endogenous ligands, the hormones guanylin and uroguanylin, universally contributes to early development of colorectal cancer (CRC). These properties underscore the potential value of oral replacement with GCC agonists as targeted prevention for CRC. Oral GCC agonists have demonstrated impressive safety profiles in pre-clinical through late-stage clinical trials. Given the paucity of agents proven safe and effective for CRC chemoprevention, this class of agents warrants further investigation. Dolcanatide (SP-333) is a GCC agonist currently being evaluated for treatment of opioid-induced constipation and ulcerative colitis. The proposed trial evaluates the effect of 27 mg dolcanatide on the rectal mucosa of humans. Pharmacodynamic (PD) effects of dolcanatide will be evaluated in human rectal biopsy specimens obtained before and after seven days of orally-administered dolcanatide. The goal of this trial is to determine whether or not dolcanatide induces a PD effect on cGMP levels in rectal mucosa.

1.1 Primary Objective: To identify the ability of dolcanatide (SP333), when administered as a single daily dose of 27 mg x 7 days, to induce a direct pharmacological effect on cGMP levels, based on biopsy samples from the rectum obtained pre- and post-intervention, as compared to placebo.

1.2 Secondary Objectives:

- To assess the PD response rate between arms (dolcanatide versus placebo).
- To confirm the safety and tolerability of dolcanatide, as compared to placebo.

2. BACKGROUND

2.1 Colorectal Cancer (CRC)

Unmet Needs for Prevention. CRC is the 4th most commonly diagnosed cancer in the United States, with approximately 150,000 new cases recorded each year.[1] Over the course of a lifetime, about 1 in 18 U.S. residents will be diagnosed with CRC. Despite advances in early detection and treatment, the mortality rate for CRC remains nearly 50%. Although screening and surveillance continues to be the cornerstone of CRC prevention, chemoprevention has emerged as a complementary approach among higher risk participants. To date, aspirin (ASA) and other nonsteroidal anti-inflammatory drugs (NSAIDs) represent the most thoroughly investigated class of CRC chemoprevention agents. However, given the overall risk/ benefit ratio of these agents, the feasibility of widespread ASA or other NSAID use strictly for CRC chemoprevention seems unlikely in average-risk patients. In the current phase I trial, we propose to assess the bioactivity of dolcanatide, a novel GCC agonist.[2] Emerging data suggest that this agent class might favorably affect colorectal mucosal tumorigenesis. Here, the biological effect of dolcanatide once daily x 7 days on colorectal tissue-based molecular markers will be investigated.

GCC Signaling in Human CRC. GCC is the intestinal epithelial cell receptor[3] for the endogenous hormones guanylin and uroguanylin. Hormone-receptor interaction activates the intracellular catalytic domain, which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). This cyclic nucleotide activates downstream effectors, including cGMP-dependent protein kinase (PKG), which phosphorylates the cystic fibrosis transmembrane conductance regulator, producing secretion and, in the case of *E. coli* enterotoxin (ST), a structural and functional homolog of guanylin and uroguanylin, diarrhea.[4-6] Guanylin and uroguanylin are the most commonly lost gene products in CRC in animals and humans.[7-9] Of significance, GCC continues to be produced after epithelial cells undergo

malignant transformation. Interestingly, GCC expression can be identified in a high percentage of primary and metastatic colorectal tumors obtained from CRC patients.[10, 11] Moreover, GCC is over-expressed in colon cancers compared to normal adjacent tissues, and the dormant receptor may serve as a target for colon cancer prevention and therapy.

GCC Signaling Opposes Carcinogenesis. GCC and its ligands regulate intestinal regenerative homeostasis by restricting proliferative dynamics and coordinating cell cycle and metabolic circuits.[12-14] Eliminating GCC expression (GCC-/-)[13, 14] in mice increases the number of crypts, which exhibit higher proliferative indices, associated with an accelerated cell cycle.[2, 15] Hyperproliferation is associated with over-expression of cell cycle drivers, including β-catenin, cyclin D1 and pRb, and decreased cell cycle suppressors, including p27.[14] Proliferative hyperplasia is associated with bioenergetic remodeling including the glycolytic reprogramming that universally characterizes tumorigenesis.[14] GCC-/- mice are more susceptible to ApcMin/+ and azoxymethane (AOM)-induced tumorigenesis, exhibiting increased tumor incidence, multiplicity and burden.[13, 14] Hyperproliferation and glycolytic reprogramming in GCC-/- mice reinforce DNA damage and chromosomal instability producing neoplastic transformation.[13, 14] In contrast, oral supplementation of mice with the GCC paracrine hormone ligand uroguanylin decreases tumor burden in the small intestine and colon of ApcMin/+ mice.[16] Thus, GCC is a tumor suppressor that potentially regulates proliferative and metabolic homeostasis in the human intestine.

Targeted Prevention for Colorectal Cancer. In the healthy state, GCC plays a key regulatory role in proliferative and metabolic processes that oppose tumorigenesis. However, the near universal over-expression of GCC in human CRCs, coupled with the loss of endogenous ligands (guanylin and uroguanylin), highlight a potential targeted, prevention strategy for CRC involving oral replacement therapy. This presumes that during colorectal carcinogenesis, GCC is a dormant tumor-suppressing receptor whose re-engagement by exogenous ligand rescues cell cycle regulation and reprograms glycolytic metabolism to restrict dysregulated growth. GCC signaling inhibits the cell cycle of normal human intestinal cells and human colon carcinoma cells in vitro and ex vivo.[13-15] Activation of GCC induces a G1-S delay that restricts progression through the cell cycle, without apoptosis. Cytostasis induced by GCC downstream signaling was associated with reduced expression of cell cycle drivers like β-catenin, cyclin D1, pRb, and increased expression of cell cycle inhibitors including p27, restricting the transition through the G1/S interface.[13-15] In another two different preclinical models (constitutive gene expression, and E. coli colonization),[17] the murine colons were treated with GCC ligands to examine the efficacy of chronic GUCY2C ligand supplementation to oppose intestinal tumorigenesis. In the gene expression model, endogenous GCC ligand, guanylin, is constitutively expressed under the control of intestine-specific promoter (villin) after tamoxifen induction. Guanylin over-expressing mice exhibited a 90% decrease in tumor burden in the carcinogen-induced tumorigenesis (AOM model) and a 50% decrease in the genetic mutation (APC¬min) model. In the E. coli colonization model, murine colons were colonized with *E. coli* engineered to express bacterial heat-stable enterotoxin (ST), an exogenous GCC ligand. Mice colonized with ST-producing E. coli and similar controls were subsequently exposed to azoxymethane. Control animals formed 60% more colonic macroadenomas than colonized animals, supporting the biologic plausibility of a protective effect derived from chronic GCC stimulation.[18] Further, guanylin over-expressing transgenic mice (chronic exposure to excess GCC ligand) were healthy, had no adverse effects in or outside the GI tract, and were more resistant to DSS-induced colitis.[17] Additionally, in both colonization and genetic over-expression models, the anti-tumor effects of GCC signaling persist over the lifetime of the animal, without obvious attenuation or desensitization. Moreover, no adverse effects of lifetime GCC ligand exposure in colonization and genetic over-expression models have been observed.

2.2 Dolcanatide (SP-333)

Dolcanatide (SP-333), a chemically synthesized hexadecapeptide composed of naturally occurring L-amino acids, shares over 60% amino acid identity with the endogenous peptide hormones guanylin and uroguanylin. The molecular target for these peptides is GCC, a key regulator of intestinal homeostasis in mammals. GCC receptors are activated by guanylin and uroguanylin (and dolcanatide), resulting in the elevation of intracellular cGMP and an increase in chloride and bicarbonate secretion into the intestinal lumen.[19] This anion secretion results in an increase in intestinal fluid secretion and a reflex acceleration of gastrointestinal (GI) transit. Dolcanatide is being evaluated for the treatment of opioid-induced constipation and ulcerative colitis.[19]

2.2.1 Preclinical Pharmacology

Pharmacology Profile: In vitro studies using T84 human colon carcinoma cells demonstrated that dolcanatide stimulated cGMP production in a consistent and concentration-dependent manner, with an average EC50 of 0.58 µM.[20] When evaluated in a panel of 73 other mammalian receptors and ion channels, dolcanatide (10 µM) showed no significant binding or activity.[21] In vivo studies were conducted to evaluate the efficacy of dolcanatide in an animal model of OIC produced by morphine or methadone.[22]

Opioid-Induced Constipation: In the study with morphine, female CD rats (n =6-10/group, median =10) received an intraperitoneal dose of 0 (vehicle, water) or 2.5 mg/kg morphine then 10 minutes later were given an oral dose of 0 (vehicle, water), 0.5, 5 or 50 mg/kg of dolcanatide. A charcoal meal was administered 10 min later, and the rats were euthanized after an additional 10 min. GI transit was then measured (distance traveled by leading edge of charcoal expressed as a percentage of the total length of small intestine). Morphine significantly reduced GI transit (23% vs 53% in vehicle treated; $p \leq 0.001$). Dolcanatide produced a dose-dependent improvement in gut transit in morphine-treated rats: 27%, 37%, 46%, and 48% at 0.5, 2.5, 5, and 50 mg/kg, respectively ($p \leq 0.025$ at all doses except 0.5 mg/kg).

In the study with methadone, female CD rats (n =5-11/group, median =10) received an intraperitoneal dose of 0 (vehicle, pH 6.0 phosphate buffer) or 2.5 mg/kg methadone then 10 minutes later were given an oral dose of 0 (vehicle, pH 6.0 phosphate buffer), 0.1, 0.5, 2.5, 5, or 50 mg/kg of dolcanatide followed immediately by a charcoal meal. The rats were euthanized after an additional 10 min, and GI transit was then measured. Methadone significantly reduced GI transit (28% vs 55% in vehicle treated; $p \leq 0.001$). Dolcanatide generally produced a dose-dependent improvement in gut transit in methadone-treated rats: 31%, 40%, 39%, 44%, and 52% at 0.1, 0.5, 2.5, 5, and 50 mg/kg, respectively ($p \leq 0.025$ at all doses except 0.1 mg/kg).

Ulcerative colitis: An in vivo study was conducted, also, to evaluate the efficacy of dolcanatide in DSS-induced colitis (5% DSS in drinking water) in BDF1 male mice.[23] Animals were randomized to 8 groups (n=10/group): a healthy control group (did not receive DSS or any other test article) and 7 groups given DSS in their drinking water (DSS-groups). One DSS-group did not receive any treatment (untreated DSS-induced colitis), 5 DSS-groups received daily treatment by oral gavage with either dolcanatide vehicle (placebo), dolcanatide at 0.005, 0.05, 0.5, or 5 mg/kg, for 8 days, starting one day before DSS dosing was initiated; 1 DSS-group received 100 mg/kg 5-aminosalicylic acid (5-ASA) daily by oral gavage for 7 days beginning on the day DSS dosing (active control).

A disease activity index (DAI) was derived from the loss of body weight and assessments of diarrhea and bleeding. The DAI score for the 0.05 mg/kg dolcanatide group was significantly lower ($p < 0.05$) than the DAI for the placebo group. The 5-ASA and the other dolcanatide treatment groups demonstrated reduced mean DAI scores relative to the placebo group, but these responses did not achieve statistical significance.

Histopathologic changes in DSS-induced colitis were characterized by changes in crypt architecture, loss of crypts, mucosal erosion, and localized infiltration of inflammatory cells (neutrophils and macrophages). The placebo groups had the worst colitis severity scores. The 5-ASA and dolcanatide treatment groups of 0.005, 0.05, and 5 mg/kg had mean colitis severity scores that were significantly lower than those of the placebo group ($p < 0.05$). The dolcanatide, 0.5 mg/kg group did not demonstrate an effect statistically different from that of placebo because of its larger standard deviation.

Safety Pharmacology Studies: A panel of nonclinical safety pharmacology studies was performed to evaluate the safety and potential effects of dolcanatide in mice and cynomolgus monkeys. The in vitro effect of dolcanatide on ionic currents in voltage-clamped human embryonic kidney cells (HEK293) that stably express the human ether-à-go-go-related gene (hERG) was determined. [24]

Dolcanatide was administered orally to CD-1 mice that were evaluated for effects on neuropharmacological and respiratory function.[25, 26] Administration of dolcanatide at doses up to 1000 mg/kg produced no notable or adverse pharmacological effects in male and female mice and no effects on respiratory function in male mice. All doses were well tolerated, and no safety concerns were identified in these studies, despite administration at dose levels producing clear systemic exposure based on single dose mouse TK data. Dolcanatide also had no effect on cardiovascular function in male cynomolgus monkeys following oral dosing at 25, 175, and 1000 mg/kg.[24] Although minimal dose-dependent increases in heart rate were observed between 4-13 hrs post-dose, these changes were mild and transient in nature and were considered non-adverse. Hence, dolcanatide at doses up to 1000 mg/kg did not produce any treatment-related effects on blood pressure, ECG intervals or duration, or any evidence of electrocardiographic waveform abnormalities in male cynomolgus monkeys.

In the hERG assay in vitro, dolcanatide at 50 and 1682 µg/mL concentrations inhibited hERG current by (Mean ±SEM; n =4) -0.7% and 12.0%, respectively, as compared with -0.8% in the control (hERG). hERG inhibition at 1682 µg/mL was statistically significant ($p < 0.05$) when compared to vehicle control values whereas 50 µg/mL demonstrated no statistically significant effect. Dolcanatide inhibition of the hERG current was <20%. Therefore the 50% maximal inhibitory concentration (IC50) was estimated to be greater than 1000 µM (1682 µg/mL). Under similar conditions, the positive control (0.28 µg/mL terfenadine) inhibited hERG potassium current by 76.5%. Based on the negligible systemic exposure (0.005 µg/mL observed in clinical studies to date, the safety margin (IC50 for inhibitory effect/Cmax) is substantial, so dolcanatide is not to be expected to cause a clinically relevant inhibition of the hERG current.

Summary: The potential side-effects of dolcanatide in humans, based on preclinical studies, are a decrease in stool consistency and potentially diarrhea with doses of 20 mg/day in humans. The death in one monkey in the middle dose group (35 mg/kg) was not considered to be related to dolcanatide.

2.2.2 Clinical Experiences

Dolcanatide has been administered to healthy volunteers in two phase 1 studies: a single ascending dose (SAD) study SP333101 and a multiple ascending dose (MAD) study SP333101-01.[27, 28] Additionally, dolcanatide has been administered to patients in a phase 1b study SP333101-04.[29] These studies were designed to assess the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of dolcanatide in these populations. In these studies, dolcanatide was safe and well tolerated.

Study SP333101 (SAD): The primary objective of this phase 1, first-in-human, single-ascending dose (SAD) study was to assess the safety and tolerability of single oral doses (0.1, 0.3, 1, 3, 10, 20, 30, and 60 mg) of dolcanatide tablets in healthy adult subjects. The secondary objectives were to determine the PK properties and assess the PD effects of dolcanatide tablets following single oral doses in 48 healthy adult subjects. Another 16 subjects received matching placebo tablets.

Dolcanatide was generally safe and well-tolerated during this study. The incidence of diarrhea increased with increasing doses of dolcanatide at doses ≥1 mg; diarrhea did not occur with dolcanatide doses <1 mg. Administration of dolcanatide doses of 10 mg and higher resulted in an AE of mild diarrhea in ≥50% of subjects dosed. Time to onset of diarrhea decreased and the number of watery bowel movements increased with increasing doses of dolcanatide. Overall, there were no clinically important safety issues in this study.

PK parameters showed that there was minimal systemic exposure to subjects. Plasma concentrations of dolcanatide were below the level of quantification (BLOQ) of 1.0 ng/mL for the majority of subjects in this study. The 60 mg dolcanatide group was the only group with quantifiable concentrations at multiple time points. The elimination half-life of dolcanatide was expected to be rapid based on preclinical data; but t½ could not be calculated in this study as there were not enough measureable concentration time points after Cmax for accurate calculation of t½ and the elimination rate (kel). Subjects with quantifiable dolcanatide concentrations in the 60 mg dolcanatide group did not experience any differences in safety measures at the time point of tmax (0.50 hours) compared with all subjects who had dolcanatide concentrations that were BLOQ.[27]

Study SP333101-01 (MAD): The primary objective of this phase 1, multiple-ascending dose (MAD) study was to assess the safety and tolerability of single daily oral doses of dolcanatide tablets in healthy adult subjects. Doses of 0.3, 1, 3, 10, and 30 mg were administered for 14 days to 60 subjects and 60 mg for 7 days to 7 subjects. Matching placebos were administered to 22 subjects. The secondary objectives were to determine the PK properties and assess the PD effects of dolcanatide tablets following repeated, daily, oral doses in healthy adult subjects.

Dolcanatide was generally safe and well-tolerated during this study. The most commonly reported treatment-emergent adverse events (TEAEs) were gastrointestinal (GI) disorders (primarily diarrhea and defecation urgency) of mild or moderate intensity. The GI events occurred at a much greater frequency in dolcanatide treatment groups compared with placebo, however, they did not increase in a dose-dependent manner. Administration of dolcanatide at all doses resulted in an AE of diarrhea in >65% of subjects dosed. The maximum tolerated dose was 30 mg with 60 mg deemed an intolerable dose due to 2 instances of fecal incontinence or soiling in the 6 patients dosed with 60 mg dolcanatide. Time to first bowel movement decreased with increasing doses of dolcanatide. No subject experienced a serious adverse event (SAE) and no subject withdrew from the study due to a TEAE. Overall, there were no

clinically-important safety issues in this study. Systemic exposure following repeated doses was minimal. Pharmacokinetic sampling indicated no detectable (limit of quantitation =1 ng/mL) systemic absorption of dolcanatide following oral doses <10 mg. Quantifiable plasma dolcanatide concentrations were detected in 8 out of 31 subjects who received a 10 mg or higher dose of dolcanatide; 4 of the 8 subjects were in the 60 mg group. Dolcanatide was not detected after 2.0 hours in any of the 8 subjects. Mean PK parameters were highly variable due to the low number of subjects with dolcanatide plasma concentrations greater than the lower limit of quantification. Kel and t½ were not calculated because there were not enough time points.

The few subjects who received 10 mg or greater doses and had quantifiable dolcanatide concentrations did not experience any differences in safety measures compared with subjects who had dolcanatide concentrations that were BLOQ.[28]

The safety and efficacy to induce a PD effect on cGMP concentrations in colorectal mucosa of a different GCC agonist was evaluated in a double-blind, placebo-controlled study (MAY2012-00-01).[30] The trial involved 6 normal healthy volunteers. Three received a single dose of 0.870 mg of a GCC agonist, and 3 received placebo, daily for seven consecutive days. The last dose was administered while the subjects were undergoing bowel prep for colonoscopy. Eight biopsies were collected from each of the cecum, transverse colon and rectum, by colonoscopy following standard colonoscopic bowel preparation, before the first dose and after the last dose of experimental agent. A PD effect on cGMP concentrations was observed in mucosae collected from the cecum, transverse colon, and rectum in two of three patients receiving the active GCC agonist, compared to subjects receiving placebo. Effects of this GCC agonist on cGMP concentrations were associated with increases in phosphorylation of VASP, a downstream protein target of cGMP signaling, and a decrease in epithelial cell proliferation quantified by ki67 immunohistochemistry. **There were no safety concerns observed during the trial.** The most common adverse event in subjects receiving the previous GCC agonist was diarrhea.

This study was extended to a second phase to determine if lower doses of the previous GCC agonist could be used to induce rectal cGMP responses. The first stage involved 6 normal healthy volunteers. Three received a single dose of 0.870 mg of the GCC agonist, and 3 received placebo, daily for seven consecutive days. The last dose was administered while the subjects were undergoing oral bowel prep. Eight biopsies were collected from the rectum by sigmoidoscopy following standard colonoscopic bowel preparation, before the first dose and after the last dose of experimental agent. A PD effect on cGMP concentrations was observed in mucosae collected from the rectum in two of three participants receiving active agent, compared to participants receiving placebo. The second stage of this study reproduced the first stage, except that participants were prepared for sigmoidoscopy using either tap water or PEG enemas. Using either of these approaches, a PD effect on cGMP concentrations was **not** observed in mucosae collected from the rectum in any participants receiving active agent.

As in the first study, **there were no safety concerns observed during the second phase.** It was concluded that under normal conditions of bowel function, including normal levels of stool, the current formulation of the previous GCC agonist, which is targeted to the proximal small bowel, does not produce adequate levels of ligand to induce cGMP production in the distal rectum.[30]

2.3 Rationale

Synergy Pharmaceuticals has made available dolcanatide (SP-333), which is being evaluated for treatment of opioid-induced constipation and ulcerative colitis.

This new agent is presently undergoing phase II testing in patients. In the proposed study, we will test whether or not dolcanatide induces a cGMP response in the distal rectum of healthy volunteers. A PEG enema will be used as a bowel preparation. The PEG enema has been chosen because has been demonstrated to be optimum for preserving epithelial cell integrity for biopsy and subsequent analyses.

In this study, we will test the ability of dolcanatide at a dose of 27 mg/day, administered as a single daily dose for 7 days, to produce a cGMP PD response in rectal mucosa.[17]

Increasing evidence suggests that tobacco and alcohol use are risk factors in the development of intraepithelial neoplasia and cancer. In addition, tobacco and alcohol use may adversely affect agent intervention, for example by altering the safety profile or metabolism of a drug. Standardized assessments of tobacco and alcohol use during clinical trials will aid in understanding the potential relationship between the use of these products and clinical endpoints or cancer prevention biomarkers. Therefore, NCI, DCP is including assessment of tobacco and alcohol use at baseline and Day 21, to determine the potential impact of tobacco and alcohol use on 1) treatment toxicity and symptom burden, and 2) the efficacy of treatment intervention.

2.4 Dose Selection

The initial starting dose of dolcanatide for SP333101 (SAD) was determined using the guidance provided by the Food and Drug Administration, based on dolcanatide's nonclinical safety profile and pharmacological data in animals, as well as information from development of another uroguanylin analog by Synergy, plecanatide.

In the single dose escalation study in the monkey, the no-observed-adverse effect level (NOAEL) 1 mg/kg whereas mild diarrhea was caused by 10 mg/kg.[31] By allometric scaling, the human equivalent dose (HED) for diarrhea would be 192 mg and the HED for the NOAEL would be 19.2 mg. Because the first-in-human study should include a no-effect dose and since the relative sensitivity of humans and monkeys is unknown, the Sponsor decided to initiate the clinical program with a single dose of 0.1 mg.

For the SP333101-01 (MAD) study, the doses were 0.3, 1, 3, 10 mg dolcanatide and were selected based on results from Study SP333101 (SAD). An intolerable dose and, therefore, a maximum tolerated dose were not identified in the SAD study; the highest dose given was 60 mg. The 60 mg dose level (approximately 1 mg/kg), had an adequate safety margin because the NOAEL in the monkey is 175 mg/kg. Based on observations in the SAD study, the acute effects on bowel function (diarrhea, loose stools, abdominal pain), occurred within the first few days of the start of treatment so a 7 day course of treatment with 60 mg dolcanatide was expected to provide data predictive of 14 days of treatment.

For the treatment of OIC in patients with non-malignant chronic pain receiving opioid therapy, the proposed dose range of dolcanatide is 1, 3, or 6 mg daily for 4 weeks. Doses for this study were selected based on the results of phase 1 studies (Study SP333101 [SAD] and SP333101-01 [MAD]) in healthy

subjects. [27, 28] In the SAD study, diarrhea was shown to be generally dose-dependent; in the MAD study TEAEs in the gastrointestinal system/Organ Classification increased with increasing doses.

Oral doses of dolcanatide up to 60 mg daily for 7 days and 30 mg daily for 14 days have been shown to be safe in a phase 1 healthy volunteer study. It is estimated that a maximum of 10-20% of an orally delivered dose of dolcanatide remains pharmacologically active in the distal colon; it is therefore reasonable to conclude that 3 to 6 mg is a safe dose range when the drug is delivered directly to the colon via enema. Data from rodents show that GC-C is expressed at a higher level as samples are taken from the jejunum to the ileum to the proximal colon. Although the exact distribution of GC-C receptors in the human colon relative to the upper GI tract is not completely clear, it is generally thought to be similar to the rodent rat and mouse. Based on clinical observation of mild diarrhea (a clinical pharmacodynamic "marker" of GC-C activation) in healthy volunteers exposed to repeated daily oral doses, it was concluded that 6 mg dolcanatide was pharmacologically active in the upper GI tract. This dose level is predicted to be pharmacologically active in the lower GI tract as well.

The objective of the present study is to define the ability of 27 mg of dolcanatide to produce a PD response in cGMP concentrations in rectal mucosa. **This dose is below the identified maximum tolerated dose**. Up to 24 participants will be examined (12 with dolcanatide versus 12 with placebo). Side effects related to this dose of active agent are not expected to be different from the experiences with MAY2012-00-01 due to a similar lack of bioavailability.[32-34]

2.5 Endpoints

The overall objective of this study is to evaluate the effect of dolcanatide on GCC-related signaling in the colorectum. Tissue samples from the rectum will be collected by flexible sigmoidoscopy before and after administration of dolcanatide or placebo. The primary PD endpoint for this trial is a statistically significant elevation of cGMP after 7 days of dolcanatide administration. A secondary endpoint is to assess the safety and tolerability of dolcanatide at the assigned dose among healthy adult volunteers. Another secondary endpoint is to determine whether or not dolcanatide can induce a PD effect on cGMP in rectal mucosa biopsies when administered as a single daily dose for 7 days without a concurrent colonoscopy bowel preparation.

In the previous study, translational endpoints were focused on examining the impact of dolcanatide on cGMP downstream targets (VASP phosphorylation) and function (regulation of proliferation by Ki67) in intestinal mucosa. Here, rectal mucosa biopsy specimens will be collected and stored to enable future assessment of these translational endpoints to confirm the cGMP PD endpoint, if required.

2.6 Summary

Overall, GCC agonists formulated for oral therapy demonstrate favorable safety profiles in pre-clinical and clinical trials. This class of agent is considered to be high priority for further investigation with respect to CRC chemoprevention. The current Phase I protocol has been developed to maximize the efficiency of further early phase development for these promising compounds. If this study indicates that dolcanatide produces pharmacological coverage of the colorectum for extended administration to patients, we ultimately anticipate initiating a trial focusing on the efficacy of daily doses of dolcanatide to prevent colorectal adenomas in patients at risk for polyp formation. It is anticipated that, should this

development program be successful, patients at elevated risk for colorectal cancer (e.g., polyp-formers, inflammatory bowel disease) may be candidates for extended dolcanatide therapy.

3. SUMMARY OF STUDY PLAN

Healthy volunteers 18-65 years old, without personal or family (first degree relative) history of colorectal neoplasia, inflammatory bowel disease or other recent (≤3 months prior to day 0) or ongoing disease states producing acute or chronic diarrhea will be pre-registered in the study. Pre-registered participants will receive a flexible sigmoidoscopy procedure before receiving the test drug (See Section 7.6 for details concerning type of pre-procedure bowel preparation).

Those who have satisfactory rectal preparation, effective forward progress of the scope to at least 25 cm, and no significant intestinal pathology visualized will be formally registered (accrued). During the pre- and post-intervention sigmoidoscopies, 8 biopsies from the rectum will be taken at each time point. Registered participants will receive dolcanatide at a dose of 27 mg daily or placebo daily for 7 days. Post intervention physical exams, laboratory safety examination, AE monitoring, and questionnaire data will be performed or collected 14 days (+/- 3 days) after the last dose of the assigned study agent.

4. PARTICIPANT SELECTION

4.1 Pre-Registration Inclusion Criteria

4.1.1 Age ≥ 18 and ≤ 65 years

4.1.2 Able to understand and willingness to sign a written informed consent document and follow study procedures

4.1.3 Willing to abstain from grapefruit juice during study

4.1.4 Willing to employ adequate contraception for men and women of childbearing potential. Note: Acceptable methods include double barrier methods, intrauterine device (IUD), postmenopausal status documented by serum FSH, and/or documentation of surgical sterilization

4.1.5 Willing to provide blood and tissue specimens for research purposes

4.2 Pre-Registration Exclusion Criteria

4.2.1 Documented history of advanced adenomas (≥1 cm in maximal diameter, ≥ 3 in total number, villous morphology, or high-grade dysplasia) or colorectal cancer

4.2.2 Family history of polyposis syndrome (e.g., FAP, HNPCC) or colorectal cancer (first degree relatives younger than 60 years old)

4.2.3 History of gastroparesis

4.2.4 History of surgery involving the luminal GI tract, including bariatric surgery
Exception: Prior appendectomy ≥ 60 days prior to pre-registration is not an exclusion criterion

4.2.5 History of celiac disease

4.2.6 Inflammatory bowel disease (Crohn's disease, ulcerative colitis)

4.2.7 Previous diagnosis of irritable bowel syndrome, chronic constipation, functional bowel disorders, colonic motility disorder, or opioid-induced constipation

4.2.8 Any malignancy within 3 years of baseline. Exceptions: Participants with a history of basal cell or squamous cell skin cancer may be enrolled at the discretion of the investigator

4.2.9 Currently receiving any other investigational agents

4.2.10 History of allergic reactions attributed to compounds of similar chemical or biologic composition to dolcanatide or to any of the excipients

4.2.11 History of difficulty with sigmoidoscopy or abnormal colorectal anatomy

4.2.12 Uncontrolled current illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements

4.2.13 Pregnant or lactating women

4.2.14 Current use of laxatives more than 3 times per week

4.2.15 Current use of ≥ 5 cigarettes/day

4.2.16 Current use of ≥ 3 alcoholic drinks/day

4.2.17 Use of anti-coagulants or anti-platelet agents within 5 days prior to anticipated sigmoidoscopy. Exception: Individuals taking aspirin will not be excluded and will not be subject to a wash-out period.

4.2.18 History of bleeding/coagulation problems

4.2.19 Any medical condition reported by the participant or documented in the medical record that is judged by the investigator to constitute a risk to safe participation

4.2.20 Known or suspected mechanical gastrointestinal obstruction

4.3 Registration Inclusion Criteria

4.3.1 Normal organ function and have normal laboratory findings without clinically significant findings defined as follows:

Leukocytes	≥3x10^3/microliter (B/L)
Absolute neutrophil count	≥1.5x10^3/microliter (B/L)
Platelets	≥100x10^3/microliter (B/L)
Total bilirubin	Within normal institutional limits
AST (SGOT)/ALT (SGPT)	≤1.5 × institutional upper limit of normal (ULN)
Creatinine	≤ institutional upper limit of normal

4.3.2 Body Mass Index <35 kg/m^2

4.3.3 No findings in the rectum of advanced adenoma, chronic inflammation, or cancer

4.4 Registration Exclusion Criteria

4.4.1 Sigmoidoscopy findings requiring clinical intervention

4.4.2 Use of any illicit or illegal substances detected by urinary drug screen

4.5 Inclusion of Women and Minorities

There is no information currently available regarding differential effects of dolcanatide in subsets defined by gender, race, or ethnicity, and there is no reason to expect such differences exist. Therefore, although the planned analyses will, as always, look for differences in bioactivity based on gender and racial groupings, the samples sizes are not increased in order to provide additional power for such subset analyses.

To predict the characteristics of volunteers likely to enroll in this trial, we have reviewed the Thomas Jefferson University registration database of healthy volunteers classified by race and gender. This revealed that during the past five years >50% of participants registered in phase I trials were minorities, while about 10% of participants were women. This suggests that, at least one volunteer from a minority population will be enrolled in the study. It is noteworthy that this study is not powered for a separate subset analysis for minority participants, beyond simple inspection of the study results.

4.6 Recruitment and Retention Plan (RR&A)

A study- and site-specific RR&A plan has been developed and will be revised as needed for the purposes of insuring equal access to the clinical trial by individuals of all genders, races, and ethnic groups and for attaining the organization's accrual target. The CPN Operations team will monitor progress toward that target and request a revised/corrective RR&A plan, if necessary. The RR&A plan will be reviewed quarterly, at a minimum, in accordance with a master accrual target plan developed cooperatively between the CPN and DCP teams.

In general, site study team will be identified by the site PI and lead study coordinator. With assistance from the CPN Operations Office, training in study implementation and roles in the RR&A plan will be provided by the site PI and lead study coordinator. Potential participants will be identified by site study

teams. Only individuals who have consented to being contacted for future research will be contacted regarding this clinical trial. The trial will be posted on clinicaltrials.gov and, as needed, the institution's clinical trial web site. Participant consent, screening, and study visits will be implemented per protocol and per institutional policies and procedures. Study teams will maintain contact participants per protocol to improve compliance, retention, and adherence.

5. AGENT ADMINISTRATION

Intervention will be administered on an inpatient and outpatient basis (See Section 7 for details). Reported AEs and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

We will test 27 mg dolcanatide daily for 1 week (7 days), enrolling 24 participants total (12 on active agent and 12 on placebo), to test the hypothesis that a pharmacological effect on cGMP levels can be detected by flexible sigmoidoscopy following PEG enema for dolcanatide vs. placebo. Ultimately, the goal is to determine whether or not 27 mg dolcanatide produces a pharmacological effect on cGMP levels in rectal mucosa sampled by sigmoidoscopy. All participants will undergo sigmoidoscopies at baseline and after seven days of daily dosing with dolcanatide or placebo. For each participant, 8 biopsies will be obtained from the rectum on Day -1 and Day 7. Biopsies will be used to evaluate cGMP levels, and stored for future analyses.

5.2 Dolcanatide (SP-333) Administration

Dolcanatide will be administered at least 30 minutes before food under direct supervision of the Clinical Research Unit (CRU) staff at Thomas Jefferson University. The route of administration is oral.

5.3 Run-in Procedures

There will be no run in procedures.

5.4 Contraindications

Children younger than 6 years of age. Anyone who is allergic to dolcanatide or at risk for mechanical gastrointestinal obstruction.

5.5 Concomitant Medications

Trial participants will not be allowed to take other investigational agents or illicit/illegal substances. Regular use of laxatives (more than 3 times per week) is exclusionary. The use of anti-platelet or anti-coagulation medications is prohibited 5 days prior to a scheduled flexible sigmoidoscopy.

For all participants, on day of screening flexible sigmoidoscopy and on Day 7, PEG enemas (up to 3 times, until clear) will be administered for intestinal preparation, following the TJU flexible sigmoidoscopy standard instructions. The PEG enema will be formulated as follows: One capful of glycolax (17 g of PEG) in 167 cc of deionized water. Flexible sigmoidoscopy will take place at approximately 15:00 on the day of the screening and Day 7.

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: start and stop date, dose and route of administration, and indication. Medications taken for a procedure (e.g., biopsy) will also be included.

5.6 Dose Modification

There will be no dose modifications for this study. It is acceptable to have a single missed dose, with the exception of the final dose (Day 7). Study drug will be permanently discontinued in any patient experiencing a Grade 2+ toxicity, at least possibly related to study drug, except for Grade 2 diarrhea/vomiting that is self-limited (i.e., does not require medical intervention).

5.7 Adherence/Compliance

Dolcanatide will be administered under direct supervision of the Clinical Research Unit (CRU) staff at Thomas Jefferson University every morning between 07:00 and 09:00.

6. PHARMACEUTICAL INFORMATION

6.1 Dolcanatide (SP-333) (IND #139462, NCI, Division of Cancer Prevention)

Dolcanatide, a synthetic hexadecapeptide, is designed to mimic the actions of the GI hormone uroguanylin, an agonist of the GC-C receptor that is expressed on the surface of epithelial cells lining the GI mucosa. Binding of the agonist to GC-C stimulates the intracellular production of cGMP, resulting in decreased Na+ reabsorption through Na+/H+ exchange and in activation of the CFTR, which in turn enhances transepithelial efflux of chloride and bicarbonate ions. Consequently, fluid secretion into the intestinal lumen is stimulated, which facilitates bowel movements.

cGMP signaling mediates anti-inflammatory effects of cellular molecules such as nitric oxide and hemeoxygenase-1. Therapies that induce cGMP and cyclic AMP levels by inhibiting their degradation (phosphodiesterase inhibitors) demonstrate efficacy in murine models of ulcerative colitis.[29] Therefore, a GC-C agonist may ameliorate GI inflammation through enhanced production of cGMP in intestinal epithelial cells. The preclinical profile of dolcanatide has shown that it produces an enhanced cyclic GMP production similar to that produced by the native peptide uroguanylin. The anti-inflammatory activity of dolcanatide has been observed in a murine model of experimental colitis. Treatment with dolcanatide at 0.05 mg/kg was associated with a consistent amelioration of the disease activity index (body weight, diarrhea, and bleeding) and a reduction of myeloperoxidase activity, which were comparable to the effects of oral treatment with 5-ASA at 100 mg/kg.

The systemic absorption of the immediate release formulation of dolcanatide is limited and in monkeys, plasma levels are only observed for up to 4 hours post dose at 35 mg/kg and for up to 8 hours in 3 of 10 monkeys at 175 mg/kg. After single or repeated oral doses of dolcanatide at or below the NOAEL in the monkey, the principal effects were on stool consistency and the incidence of diarrhea. These effects are consistent with the pharmacological effects of dolcanatide when delivered to the proximal intestine, did not lead to weight loss or altered food consumption, and thus are not considered adverse.

6.2 Reported Adverse Events and Potential Risks

The reported adverse events and potential risks described in this section are for dolcanatide.

Potential adverse events associated with the immediate release formulation of dolcanatide treatment may include: headache, diarrhea, bloating, abdominal cramping, nausea and vomiting (See Dolcanatide [SP-333] Investigator Brochure, Version 3.0, April 27, 2017).

Symptoms of overexposure to the immediate release formulation of dolcanatide may include: loose stools, diarrhea, abdominal cramping, bloating, nausea, and vomiting. No reversal agent or specific treatment is available in case of dolcanatide overdose. Symptoms of overdose should be managed by supportive care as appropriate per the Investigator's judgment.

No formal drug-interaction studies have been performed.

It is unknown if dolcanatide has adverse effects on embryo/fetal development and therefore its use should be avoided in pregnant women. Appropriate methods for contraception should be used to ensure women of childbearing potential do not become pregnant during clinical studies of dolcanatide.

6.3 Availability

Dolcanatide will be provided to the NCI under a Clinical Trials Agreement (CTA) between Synergy Pharmaceuticals, Inc., New York, New York, and the NCI, DCP (see Section 12.7).

6.4 Agent Distribution

Dolcanatide (9 mg per capsule) plus matching placebo capsules will be provided by Synergy Pharmaceuticals, Inc. The agent will be released after documentation of CIRB 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). Agent will be shipped directly to the Thomas Jefferson University Hospital Investigational Drug Service Pharmacy (TJUH IDS). The randomization scheme will be generated by Mayo Clinic and sent to the designated Thomas Jefferson University Hospital IDS pharmacy personnel.

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 each sites' licensed pharmacy staff as listed on the Delegation of Tasks documents. 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. All dispensed drug will be stored in the TJU Clinical Research Unit pharmacy storage facility. Participants will be dosed in the CRU under the direct supervision of CRU personnel.

6.6 Packaging and Labeling

Investigational product will be supplied in the form of gelatin capsules. For the intervention period, participants will be assigned dolcanatide (3 capsules each containing 9 mg dolcanatide, daily x 7 days) or identically-appearing placebo (3 capsules daily x 7 days), according to the intervention arm assignment. The placebo capsule will be identical in appearance and will contain methyl cellulose.

All investigational products will be supplied in bottles containing 24 capsules each, and will be labeled with the following content: protocol number, storage information, investigational use language ("Caution: New Drug--Limited by Federal Law to Investigational Use. Keep Out of Reach of Children"), and instructions to take per protocol. The label will also include a tear-off component, to be removed by the unblinded site pharmacist prior to dispensing. The tear-off label component will identify the contents of the bottle as being active agent or placebo. This tear-off label will be maintained in the secure pharmacy records until requested by the study statistical team or if unblinding is required in an emergency situation. The site investigators, study coordinators, and study participants should remain blinded to the intervention assignment.

All study agent will be received and stored at the Thomas Jefferson University Hospital (TJUH) Investigational Drug Service Pharmacy (IDS). Initial dispensing for individual properly randomized participants will be done by TJUH IDS pharmacists. Study agent will be dispensed to designated Research Nurses. Immediately before dispensing investigational product to a participant, the designated Research Nurses will write the participant's initials, participant identification number, and dispensing date on the label. After initial Day 1 dosing, blinded bottles will be stored in the CRU storage pharmacy until end of study and all monitoring on drug has been completed. All dosing of participants will occur in the CRU. Accurate chain of custody logs and dosing records will be maintained.

Dolcanatide 9 mg or Placebo 24 Capsules Protocol MAY2017-09-01 Bottle #_____ Participant ID # _____ Dispensing Date _____ Take per protocol Store refrigerated 36°F - 46°F (2 °C – 8 °C). Keep product in the original container. Do not subdivide or repackage. Protect from moisture. Do not remove desiccant from the container. Keep bottles tightly closed in a dry place. Keep Out of Reach of Children. CAUTION: NEW DRUG LIMITED BY FEDERAL LAW TO INVESTIGATIONAL USE ONLY

Investigational product will be shipped at 2-8^{o}C. Distribution will be done by direct delivery of the appropriately packaged and labeled containers to the Thomas Jefferson University CRU by TJU pharmacy personnel.

The Investigator must ensure that the receipt and use of all study drugs supplied is recorded and must supervise the storage and allocation of these supplies. Whenever study drug is returned, unit counts

must be performed by trial center staff and verified by a site monitor to ensure reliable drug accountability. Before the end of the trial, detailed instructions for the return of all unused capsules and study drug bottles will be provided. Unused capsules and study drug bottles should be returned to Synergy Pharmaceuticals or their designee.

6.7 Storage

Investigational product must be stored in the pharmacy as follows: Store at 2-8°C; excursions permitted between 1°C and 15°C. Protect from moisture. Keep bottles tightly closed in a dry place. Do not subdivide and repackage. Do not remove desiccant from the container. Excursions in temperature during storage should be reported by the site. These will be reviewed to determine if the excursion is allowable and the investigational product can be used.

Once the induction seal has been broken, do not return product to the refrigerator but continue to protect agent from moisture. Once the induction seal has been broken, maintain study agent at room temperature (64-79°F or 18-26°C). Keep bottles tightly closed in a dry place.

6.8 Pre-Registration and Registration/Randomization

6.8.1 Pre-Registration

6.8.1.1To pre-register a participant, send the completed Pre-Registration Eligibility Checklist, to the CPN Registration Office (Email: random01@mayo.edu; Fax: 507-284-0885) between 8:00 a.m. and 4:30 p.m. Central time, Monday through Friday.

The CPN Registration Office will enter the information into the CPN-hosted database. A unique participant identification number (PID) will be assigned.

6.8.1.2 At the time of pre-registration, the following will be verified:

- CIRB protocol approval and CIRB approval for the registering institution
- Participant eligibility (including existence of a signed informed consent document)
- Existence of a signed authorization for use and disclosure of protected health information (USA Institutions only).
- Study agent is available, and Drug Shipment Authorization has been granted to the registering site.

6.8.1.3 The following also will be recorded:

- Participant has/has not given permission to collect specimens and has/has not agreed that specimen samples and related information may be used for the laboratory studies described in this protocol.
- Participant has/has not given permission for the study doctor, or their representative, to contact him/her or his/her physician to learn about the results of these studies.
- Participant has/has not given permission for their alcohol and tobacco use information to be used for future health research.
- Participant has/has not given permission for specimens and related information to be kept in a Biobank for use in future health research.

- Participant has/has not given permission for specimens and related information to be sent to researchers at outside institutions.
- Participant has/has not given permission to his/her doctor, or their representative, to contact them to see if they wish to participate in other research in the future.

6.8.1.6 Registration Office personnel will automatically register participants separately to the translational components of the study (See Section 13).

6.8.2 Registration/Randomization

6.8.2.1 To register a participant, send the completed Registration Eligibility Checklist, to the CPN Registration Office (Email: random01@mayo.edu; Fax: 507-284-0885) between 8:00 a.m. and 4:30 p.m. Central time, Monday through Friday.

The CPN Registration Office will enter the information into the CPN-hosted database. For randomization details, see Section 13.2.

6.8.2.2 Baseline (screening) and on-study evaluations must be completed within the guidelines specified on the Schedule of Events (See Section 7.1).

6.8.2.3 All baseline symptoms must be documented and graded.

6.8.2.4 Intervention cannot begin prior to registration and must begin ≤ 14 days from registration.

6.8.2.5 Intervention on this protocol must commence at a CPN institution under the supervision of a CPN clinician.

6.8.2.6 Stratification Factors: None

6.9 Blinding and Unblinding Methods

Pre-registered participants will be assigned a unique subject number, e.g., an 8-character identification number, e.g. CPN00001. Participants will be randomized at registration on Day 1 to receive active intervention or placebo after they have satisfied the inclusion/exclusion criteria. For each participant, the sequence assignment will be determined according to a prepared randomization schedule at the time of registration/randomization. The assigned study agent bottle number generated for each randomized participant will be sent to the Thomas Jefferson University personnel. Pharmacy personnel will not be blinded to intervention arm assignment.

The study will be double-blinded until after specimen analysis to ensure that the participants, investigators, and clinic staff are unaware of the dosing assignment and to minimize potential for bias in study assessments or in reporting of AEs.

There are two distinct situations in which it will be deemed appropriate to break the randomization assignment for participants enrolled onto this current trial:

- In the event of an *emergency* for an individual participant.
- Serious Adverse Event (SAE) that fulfills the criteria for expedited reporting to the FDA.

In these situations, Participating Organization personnel may assume the participant is on active agent and call the CPN Registration Office within one business day to receive unblinding information. The study name/number, participant identifier, and participant initials will be required to break the randomization code. Thomas Jefferson University personnel are responsible for notifying the medical monitor at DCP, NCI of the unblinding event.

6.10 Agent Destruction/Disposal

At the completion of investigation and after the close-out visit by the CPN Compliance Coordinator, all unused study agent will be returned to Synergy Pharmaceuticals, or their designee.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Study Day	Pre-Registration and Screening 1 ≤ 30days prior to Registration	Baseline		On-Study Procedures				Post-Intervention	
		Screening 2	Registration/ Randomization	Day 1	Days 2-5	Day 6	Day 7	Day 21 (+/- 3 days)	Day 51 (+/- 7 days)
Informed consent	X								
Assess Eligibility	X		X						
Medical History	X								
Tobacco and Alcohol Use Assessments	X							X	
Physical Exam	X						X	X	
Baseline Symptoms	X								
Vital Signs	X	X					X[1]	X[1]	
Safety Blood tests	X						X	X	
Pregnancy Test (urine or serum)[2]	X		X[3]						
ECG	X								
Urinalysis and Urine Drug Screen[4]	X						X	X	
Discharge from CRU		X[5]					X[5]		
Sigmoidoscopy and Biopsy		X					X		
Light Breakfast[6]		X		X	X	X			
Light meal		X					X		
Dose Administration				X	X	X	X		
Adverse Event Recording		X[7]	X[8]	X[9]	X[9]	X[9]	X[7,9]	X	X[10]
Concomitant Medications	X	X	X	X	X	X	X	X[10]	X[10]
WIWI Questionnaire								X[11]	
Follow-up Phone Call									X

1. Heart rate, height, weight, and blood pressure. Height will not be measured at Day 7 or Day 21.
2. For women of childbearing potential only. Negative pregnancy test (serum or urine) must be documented ≤7 days prior to registration.

3. Repeat pregnancy test, only if applicable and not performed ≤7 days prior to registration.
4. May be repeated at any time at the discretion of the investigator.
5. Discharge after full recovery from flexible sigmoidoscopy.
6. The breakfast will be provided by staff from the CRU.
7. Adverse events will be assessed prior to flexible sigmoidoscopy.
8. Adverse events will be assessed at this time point only for individuals found to be ineligible for randomization.
9. Adverse events will be assessed prior to administration of study agent.
10. If any reported AEs are not resolved at the time of the Day 21 follow up, another contact will be made at Day 51 (+/- 3 days) to review AEs. SAEs at least possibly related to the use of study agent will be followed until resolved. Concomitant medications need only be reported if AEs are reported.
11. Was It Worth It (WIWI) Questionnaire and repeat Alcohol/Tobacco Assessments at Day 21 or early termination.

7.2 Baseline Testing/Prestudy Evaluation

Screening 1
Within 30 days prior to registration, participants will undergo a screening visit. Informed consent will be obtained. Potential participants will be pre-registered (See Section 6.8.1). They will undergo medical history, tobacco and alcohol use assessments, physical exam, vital signs (height, weight, heart rate, blood pressure, and ECOG performance status), electrocardiogram, and laboratory safety evaluations. Laboratory safety evaluation will include albumin, total bilirubin, calcium, bicarbonate, chloride, creatinine, glucose, alkaline phosphatase, potassium, total protein, sodium, AST/SGOT, ALT/SGPT, and BUN; CBC with differential; PTT and PT/INR; urinalysis; urine drug screen; and FSH (for post-menopausal women only). They will be asked about baseline symptoms and concomitant medications. Women of childbearing potential must document a negative pregnancy test (urine) ≤ 7 days prior to Registration.

Screening 2
Participants will be limited to clear liquids until noon and then fast, with free access to water, until the flexible sigmoidoscopy at about 3:00 p.m. Participants will be given PEG enemas about 1.5 hours prior to sigmoidoscopy. Up to 3 enemas may be administered until evacuation is clear. PEG enemas are being used because they have been demonstrated to be optimum for preserving epithelial cell integrity for biopsy and subsequent analyses.

Eight mucosal biopsies will be obtained from 8 discrete areas in the rectum (each separated by no less than 0.5 cm in any direction). In addition, all biopsies will be obtained from areas no less than 0.5 cm above the dentate line. All samples will be flash-frozen in liquid nitrogen and stored at -80°C for analysis of cGMP.

Following the sigmoidoscopy, a meal will be provided. Medically stable participants can be discharged at the discretion of the investigator, or participants may be kept overnight for prolonged observation for safety. Specifically, participants who are experiencing pain, bleeding, respiratory distress, fever, or other clinical manifestations that might indicate that a complication occurred during the procedures will be observed overnight or until the symptoms resolve.

Incidentally noted endoscopy findings that are felt to be potentially clinically relevant, in the opinion of the endoscopist, will be biopsied for histologic review. Specimens from any incidentally noted findings obtained by sigmoidoscopy will be fixed in formalin (10%) and sent to the Pathology Department at Thomas Jefferson University for diagnostic assessment. The investigator will review findings with study participants and discuss their continuation in the study. Participants will be referred to primary care physicians or gastroenterologists for further diagnosis and therapy, as appropriate, according to institutional standards of good clinical practice.

7.3 Evaluation During Study Intervention

If participants meet all eligibility requirements, they will be assigned to dolcanatide or placebo. Study agent will be administered daily in the Clinical Research Unit (CRU), Thomas Jefferson University Hospital. No food will be allowed for at least 30 min after dose administration.

Days 2-6
Participants will arrive to the CRU on an outpatient basis at approximately 7:00 a.m., following an

overnight fast, for daily dosing. An oral dose of dolcanatide or placebo will be administered under direct supervision of the CRU staff at the dose time established on Day 1 for each subject.

Participants will be instructed not to eat for 30 minutes following administration of their dose. It is acceptable to have a single missed dose, with the exception of the final dose (Day 7) since the sigmoidoscopy needs to be performed approximately 8 hours after the final dose.

7.4 Evaluation at Completion of Study Intervention

Day 7
Participants will arrive at the CRU in a fasted state in the morning. Adverse events (AE), concomitant medications, and vital signs will be assessed. Blood will be drawn for safety testing (albumin, total bilirubin, calcium, bicarbonate, chloride, creatinine, glucose, alkaline phosphatase, potassium, total protein, sodium, AST/SGOT, ALT/SGPT, BUN, CBC with differential, PTT and PT/INR), urinalysis, urine drug screen, and a brief physical exam will be performed. An oral dose of dolcanatide or placebo will be administered under direct supervision of the CRU staff at approximately 07:00 a.m. Participants will be limited to clear liquids until 12:00 p.m. and remain fasting thereafter until the scheduled sigmoidoscopy, which will take place approximately 8 hours after the dolcanatide /placebo dose time. Participants will be given the first of a maximum of 3 enemas beginning at approximately 1:30 p.m.

Eight mucosal biopsies will be obtained from 8 discrete areas in the rectum (each separated by no less than 0.5 cm in any direction). In addition, all biopsies will be obtained from areas no less than 0.5 cm above the dentate line. All samples will be flash-frozen in liquid nitrogen and stored at -80°C for analysis of cGMP. Any abnormalities identified during the procedure will be handled and followed according to standards of usual clinical practice. Following the sigmoidoscopy the participants will be discharged to the CRU after recovery. A light meal will be provided. Medically stable participants can be discharged at the discretion of the investigator, or participants may be kept overnight or prolonged observation for safety. Specifically, participants who are experiencing pain, bleeding, respiratory distress, fever, or other clinical manifestations that might indicate that a complication occurred during the procedures will be observed overnight or until the symptoms resolve.

7.5 Post-intervention Follow-up Period

On Day 21 (+/- 3 days), approximately 14 days after the last dolcanatide or placebo dosing, participants will come to the CRU at TJU for a post-intervention visit. Participants will undergo physical exam, vital signs (weight, heart rate, blood pressure and temperature), laboratory safety (blood and urine) as previously described, AE and concomitant medication monitoring, complete the alcohol and tobacco use follow up assessments (Appendix D), and complete the Was It Worth It (WIWI) questionnaire (Appendix C). The AE monitoring will include querying whether participants experienced any changes in bowel habits while on study agent and, if so, the duration of these changes and how long it required to return to normal. Similarly, the interview will include querying whether participants experienced any side effects that would prevent them from taking the study agent for long periods.

Participants will receive a follow-up telephone call 30 days (+/- 7 days) after the end of post-intervention to determine the status of any ongoing AEs reported at the Day 21 evaluation. Follow up will be performed via telephone or in person as deemed appropriate by the lead investigator at the participating site.

7.6 Methods for Clinical Procedures

Summary:

- Participants will be administered dolcanatide or placebo in the CRU.
- Participants will receive up to 3 PEG enemas, until evacuations are clear, before the flexible sigmoidoscopy.
- Participants will receive flexible sigmoidoscopy examinations on day 1 and day 7. Eight biopsies will be taken from the rectum at each time point.
- Dolcanatide/placebo will be given at least 30 minutes before any food intake with an 8 ounce glass of water to participants by the staff in the Clinical Research Unit, Thomas Jefferson University. Participants will be instructed to swallow the dosage whole, without biting or chewing the dosage.
- Participants will be instructed to abstain from alcohol and all other medications from the time of first admission to the CRU until after completion of Day 7.
- Participants will be instructed to abstain from alcohol for 24 hours prior to Screening and Post-Intervention visits.
- Participants will be instructed to not consume grapefruit juice during the study.

Sigmoidoscopy will be performed by experienced, board certified Thomas Jefferson Hospital gastroenterologists involved in the study. Endoscopic procedures will be recorded in the appropriate databases, including high quality digital photographs if any lesions are detected. Adequate cleansing prior to sigmoidoscopy will be obtained using PEG enemas. Quality of the preparation will be graded using the Boston Bowel Preparation Scale.

It is important to note that, according to current scientific recommendations, it is not necessary to discontinue aspirin or other NSAID class anti-platelet agents to perform a polypectomy or biopsies. However, participants who are anti-coagulated with warfarin, clopidrogel, or other similar agents will be excluded from the study. Finally, there is significant medical literature demonstrating the safety and tolerability of tandem sigmoidoscopies (one procedure followed immediately by a second) in one participant.[34, 36] Accrual for such studies historically has been without incident.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

The novel role of GCC in suppressing intestinal tumorigenesis, together with the universal disruption of GCC signaling through silencing of hormones early in colon carcinogenesis, underscore the potential value of oral replacement with GCC agonists as targeted prevention for CRC. Available GCC agonists have demonstrated impressive safety profiles in pre-clinical and clinical trials. Given the paucity of safe and effective agents for CRC chemoprevention, we consider this class of agents to be high priority for further investigation with respect to CRC chemoprevention. The biological effects of dolcanatide will be evaluated in human intestinal biopsies obtained before and after seven days of agent administration.

8.1 Primary Endpoint

See Section 13.4

8.2 Secondary Endpoint

See Section 13.5

8.3 Off-Agent Criteria

Participants may stop taking the study agent due to: completion of the planned intervention period, development of an adverse event or serious adverse event, inadequate agent supply, noncompliance, use of concomitant medications, medical contraindication, refusal, ineligibility (see Section 8.4), major treatment violation (see Section 8.4) or alternative treatment. Participants will continue to be followed, if possible, for safety according to the intended schedule of events (see Section 7).

Participants discontinuing the planned intervention prematurely will be encouraged to complete the Post-Intervention Evaluation tests and procedures as appropriate (if participant does not refuse, is not lost to follow-up, or unless it is clinically contraindicated). See Section 8.4 for further details as to data submission for participants deemed Ineligible after starting treatment or classified as a Major Treatment Violation (i.e., protocol requirements regarding intervention during the first week post-registration were severely violated).

8.4 Off-Study Criteria

Participants may go "Off-Study" for the following reasons: development of an adverse event or serious adverse event, death, lost to follow-up, participant withdrawal, physician decision, protocol violation, complete study, or other (with detailed comments provided). Reason(s) will be noted in the participant's research records, with the primary reason clearly identified. The participant will be classified as (Off Study/Off Agent). Data submission and follow-up after participants are determined to be "Off-Study/Off-Agent" for specific situations is noted below:

A registered participant is deemed ineligible if the participant did not satisfy each and every eligibility criterion at the time of study entry, for example, identified based on an audit or through the case evaluation process.

- If participants received study intervention, on-study materials and all data up until the point of confirmation of ineligibility will be submitted.
- If participants did not receive study intervention, on-study materials must be submitted. No further data submission is necessary. No follow-up is required.

Major Treatment Violation: A registered participant is deemed as being in major treatment violation by the coordinating center, if the participant's very first treatment/intervention administration is so grossly administered in error, that the participant's data can no longer be used for the primary endpoint. These cases are typically rare.

- On-study material and all data up until the point of confirmation of a major violation must be submitted.

Cancel/Participant Withdrawal: A registered participant is deemed a cancel if he/she refuses the study or withdraws consent before any study intervention is given. On-study material must be submitted. The Off Study case report form must be submitted. No follow-up is required.

8.5 Study Termination

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

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

It is hypothesized that after oral dolcanatide exposure, biopsies will demonstrate increased cGMP levels in mucosa from the rectum. These analyses will quantify the effects of dolcanatide treatment directly on one central mechanism by which GCC prevents tumorigenesis. These correlative analyses provide a method to assess sensitivity and resistance in participants receiving dolcanatide.

There are several robust analytical techniques for quantifying cGMP in tissue specimens. The most common are commercially-available immunological assays in the form of radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (EIA), both with femtomolar sensitivities. The technique for cGMP determination by RIA and EIA is well defined.[37]

cGMP production: GCC, is a transmembrane receptor-enzyme. Ligand-receptor interaction activates a catalytic domain that converts GTP into cGMP. It is this cyclic nucleotide that produces the downstream signals mediating intracellular responses to GCC, including fluid and electrolyte secretion and regulation of epithelial cell homeostasis mediating tumor suppression. There are several robust analytical techniques for quantifying cGMP in tissue specimens. The most common are commercially-available radioimmunoassays and enzyme-linked immunosorbent assays, both with femtomolar sensitivities for quantifying cGMP in tissue specimens. The technique for cGMP determination is well defined[37] and will be the methodology used in this protocol. Upon collection, rectal mucosal biopsies will be placed in cryogenic tubes, frozen in liquid nitrogen and archived in a -80°C freezer for long-term storage. For analysis, samples will undergo cryopulverization before thawing in 200 µL of pre-cooled 5% trichloroacetic Acid (Sigma-Aldrich). Samples are homogenized on ice using a BeadBeeter (BiospecProducts), followed by centrifugation (15,000 rpm, 15 min, 0-4^{o}C). Four hundred (400) µL of the supernatant will be transferred to a clean test tube. The trichloroacetic acid is extracted from the sample using ether (Sigma-Aldrich). The bottom aqueous layer is for assay analysis. The residual tissue after initial centrifugation is dissolved in 0.2 N sodium hydroxide at 4°C overnight; and protein concentration is determined by BCA protein assay kit (ThermoScientific, Rockford, IL). Cyclic GMP levels will be normalized to the protein content from individual samples.

9.2 Comparable Methods

This study will employ commercially-available kits, reagents and procedures that have been validated previously for clinical use.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

Clinical laboratory analyses: Thomas Jefferson University Clinical Laboratories, 125 South 11th Street, Philadelphia, PA 19107

Colonic mucosal sample analyses: All colonic mucosa samples will be transferred to Dr. Waldman's research laboratory at Thomas Jefferson University (See cover page for contact information) and all analyses for endpoints will be performed there.

10.2 Collection and Handling Procedures

- Eight mucosa biopsies will be collected from the rectum (at or just above the inferior valve of Houston) pre-intervention and post-intervention (Day 7, after administration of dolcanatide or placebo for 7 days).
- Five biopsies will be flash-frozen in liquid nitrogen individually in a screw cap vial and provided a sample-specific ID. Among the 5 biopsies, 2 of them will be evaluated for cGMP concentration. Frozen tissues will be transferred and stored at -80°C in Dr. Waldman's laboratory. In addition, 3 biopsies will be flattened immediately and fixed in 10% formalin within 2 min of collection in pre-labeled cassettes with tracking information provided, for future Ki67 immunohistochemistry analysis, if necessary.
- After formalin fixation overnight, samples will be transferred to 70% ethanol and sent to the Pathology Department for routine tissue processing. Processed tissue will be transferred to Dr. Waldman's laboratory for biomarker studies.
- All specimens from the each biopsy will be put into pre-labeled containers separated for analysis.
- Containers will be labeled with study number, registration number, date/time of collection, anatomical location and biopsy number. For example, 000-D7-Rec-1.
- Specimen manifest will be sent by email to the Biospecimens Resource Manager at the time of shipment.

10.3 Shipping Instructions

All frozen mucosa biopsies will be shipped in a portable liquid nitrogen tank from the department of Gastroenterology (main building 4th floor) to Dr. Waldman's laboratory (JAH 364). Formalin-fixed tissues will be sent to the Pathology Department (BLSB 3rd floor) in plastic containers filled with 70% ethanol.

10.4 Tissue Banking

Biologic specimens collected during the conduct of the 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, remaining frozen biologic specimens will be labeled (study number, participant ID number, specimen type, specimen number, date of collection) batched, and shipped (overnight, M-F) for storage (until request is received to transfer to DCP Biospecimens Repository) to:

Biospecimens Accessioning and Processing (BAP) Freezer
ST SL-16
150 Third Street Southwest
Rochester, MN 55902

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 (per the physician's assessment) should not be reported as AEs.

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

11.1.2 AE Data Elements:

The following data elements are required for 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, or 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 a Serious Adverse Event (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. *(Note: Hospitalization is defined as admission or stay [including Emergency Room] equal to or greater than 24 hours with the exceptions of treatment of a pre-existing condition, outpatient surgery, planned/elective procedure, and procedures described in the protocol [*e.g., *pharmacokinetic sampling, surgery] even if the hospital stay is of the described length; however, it does include events resulting from a protocol procedure that fulfill other serious outcome criteria,* e.g.*, prolonged hospitalization or life-threatening*).
- 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

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

Guidance for the process of coding the SAE can be obtained by contacting the NCI MD Help Desk: adeersmd@tech-res.com

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

Gary Della'Zanna, D.O., M.Sc.
National Institutes of Health, National Cancer Institute
Division of Cancer Prevention
9609 Medical Center Drive
Bethesda, MD 20892
Phone: 240-276-7042
Email: dellazannagj@mail.nih.gov

Include the following information when contacting the Medical Monitor:

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

11.2.2.3 The Participating Organization will submit SAE reports to the following within 48 hours of learning of the event using the DCP SAE Reporting procedure:

1. **DCP Medical Monitor: dellazannagj@mail.nih.gov**
2. **DCP's Regulatory Contractor CCS Associates, Inc.: safety@ccsainc.com**
3. **CPN Operations Office: cancerpreventionnetwork@mayo.edu**
4. **Synergy Pharmaceuticals: pgriffin@synergypharma.com**

Follow up information should also be sent to all of the above.

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

11.2.2.5 The Participating Organization will comply with applicable regulatory requirements related to reporting SAEs to the CIRB.

11.2.3 Follow-up of SAE

Site staff should submit follow-up reports as requested when additional information is available. Follow-up information should be sent to DCP, CCS Associates, and the CPN Operations Office as soon as available. SAEs will be treated according to institutional standards of good clinical practice and followed to resolution.

12. STUDY MONITORING

12.1 Data Management

The Mayo Clinic Cancer Center database will be the database of record for the protocol and subject to NCI and FDA audit. Minimum Data Sets will be submitted to DCP per contract requirements. Please see CPN Master Data Management Plan.

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRF) utilizing NCI-approved Common Data Elements (CDEs). The approved CRFs will be used to create the electronic CRF (e-CRF) screens for data entry into the Mayo Clinic Cancer Center database. Amended CRFs will be submitted to the DCP Protocol Information Office for review and approval.

12.3 Source Documents

A source document is any document, form, or record where *specific participants'* data are first recorded. FDA [21 CFR 312.62 (b)] requires that the investigator "...prepare and maintain accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated with the investigational agent or employed as a control in the investigation."

Among many other items, source documents include:

- Inpatient and outpatient medical records

- Progress notes
- Consults
- Nursing notes
- Pathology reports
- Endoscopy reports
- Medicine/radiation administration records
- Surgical reports
- Laboratory reports
- Admission forms
- Flow sheets and worksheets that are signed and dated
- Protocol or study road maps
- Appointment books
- Participant diaries/calendars
- Blood and tissue collection/submission requisition forms (signed and dated).

12.4 Data and Safety Monitoring Plan (DSMP)

The CPN Master DSMP is applicable to all studies within the CPN Consortium and provides detailed information regarding data and safety monitoring for this study. The trial will be monitored closely for occurrence of adverse events by the study team and by the Mayo Clinic Cancer Center Data Safety Monitoring Board (DSMB). The DSMB (along with the study Medical Monitor) will be consulted regarding whether or not accrual should be suspended to allow for investigation in the occurrence of severe adverse events, particularly for those that are possibly, probably, or definitely related to the study agent.

Additionally, the DSMB (along with the study Medical Monitor) will be consulted regarding whether or not accrual should be suspended to allow for investigation if 25% or more of participants who begin intervention fail to undergo the post-intervention flexible sigmoidoscopy.

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 Clinical Trials Agreement (CTA)

Agent(s) used in this protocol is/are provided to the NCI under a Clinical Trials Agreement (CTA) between Synergy Pharmaceuticals, Inc. and the NCI Division of Cancer Prevention. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborators" contained within the terms of award, apply to the use of Agent(s) in this study:

12.7.1 Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a participant on the study or participant's family member requests a copy of this protocol, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from the DCP website.

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

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

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

DCP Protocol Information Office
E-mail: NCI_DCP_PIO@mail.nih.gov

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

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

Twenty-four (24) participants (12 per arm) will be randomized to dolcanatide 27 mg versus matching placebo (once daily for a period of 7 days). The primary endpoint will be a direct comparison of the pharmacological effect on cGMP levels for dolcanatide arm vs. placebo. Secondary endpoints will compare the Pharmacodynamic (PD) Response rate and adverse events between arms.

13.2 Randomization/Stratification

Participants will be registered on Day 1 after they have satisfied the inclusion/exclusion criteria. All participants will have been assigned a unique subject number [e.g., 7-character subject identification]. Twelve patients will be randomized to each arm (24 total; dolcanatide or placebo, where participants will be treated once daily for a period of 7 days).

13.3 Accrual and Feasibility

We estimate that we will need to screen 40 and pre-register up to 30 participants for eligibility in order to obtain the expected number of registered participants. Participants will be enrolled from a single site (Thomas Jefferson University). This study will enroll 24 participants (12 per arm). With an expected average randomization rate of 4 participants per month, this study is anticipated to require a maximum of 7 months to complete the determination of the primary endpoint. This includes periods during which enrollment traditionally lags, such as holidays.

It is noteworthy that the CRU at Thomas Jefferson University Hospital has achieved 100% enrollment for healthy volunteers in all previous phase I and phase II studies. This has included healthy volunteer studies involving bone marrow biopsy, adipose biopsy, right heart catheterization, and skin biopsy. The CRU has also conducted a number of upper endoscopy studies, including a recent study that employed three endoscopies per subject with direct bile collection for pharmacokinetic evaluation. Based on our previous studies performed in the CRU at Thomas Jefferson University Hospital, an expected screen fall rate is 1:1. This estimation includes, but not limited to, the exclusion of the underlying diseases, positive drug response during screening, or height or weight limitations. Such studies are typically limited to patients younger than 50 years of age. Given the higher age range in the current study and a slightly higher screen fail rate noted in our prior endoscopy study, we expect to have to screen 40 to end up with 24 evaluable for the primary endpoint in this study. Time to study completion, including data entry, clean-up, and analysis, is approximately 1.0 year from study activation.

Planned Accrual Estimates

Racial Categories	DOMESTIC PLANNED ENROLLMENT REPORT				
	Ethnic Categories				
	Not Hispanic or Latino		Hispanic or Latino		Total
	Female	Male	Female	Male	
American Indian/Alaska Native	**0**	**0**	**0**	**0**	**0**
Asian	**0**	**0**	**0**	**0**	**0**
Native Hawaiian or Other Pacific Islander	**0**	**0**	**0**	**0**	**0**
Black or African American	**1**	**5**	**0**	**0**	**6**
White	**7**	**9**	**1**	**1**	**18**
More Than One Race	**0**	**0**	**0**	**0**	**0**
Total	**8**	**14**	**1**	**1**	**24**

Racial Categories	INTERNATIONAL (including Canadian participants) PLANNED ENROLLMENT REPORT				
	Ethnic Categories				
	Not Hispanic or Latino		Hispanic or Latino		Total
	Female	Male	Female	Male	
American Indian/Alaska Native	0	0	0	0	0
Asian	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	0	0	0	0
White	0	0	0	0	0
More Than One Race	0	0	0	0	0
Total	0	0	0	0	0

13.4 Primary Objective, Endpoint(s), Analysis Plan

Primary Endpoint: To a direct comparison of the pharmacological effect on cGMP levels for dolcanatide arm vs. placebo, where this effect is defined below:

Pharmacological Effect on cGMP levels: Arithmetic difference in mean cGMP levels before and after 7 days of dolcanatide from subject biopsies. This represents the increase in cGMP stimulated by 7 days dolcanatide in an individual subject. The mean cGMP value will be calculated based on 8 biopsies from the rectum assessed at each time point.

With a sample size of 12 per arm, we'd have 80% power to detect a significantly increased Pharmacological Effect of the continuous cGMP results for dolcanatide vs. placebo, assuming a 1-sided significance level of 5%. The study would be powered to detect an effect size of 1 (i.e. [difference in means]/SD = 1). Prior data from one of the active doses for the MAY2012-00-01 study found a difference in means of around 4 for the PD effect (5 vs. 1), with an SD of around 4 as well. This would be an example of an effect size of 1 (4/4). Given this prior data, we believe an effect size of 1 is achievable. We'll also report the 2-sided 95% confidence intervals as well for final results. If the cGMP values are not normally distributed, a transformation to a variable with a normal distribution will be performed. See the detailed calculations below:

Let x_{zij} = baseline cGMP level for person i in randomization group z with replicate j.

$x_{zimean} = \Sigma_j x_{zij} / 8$ = mean baseline level

Let y_{zij} = day 7 cGMP level for person i in randomization group z and replicate j.

$y_{zimean} = \Sigma_j y_{zij} / 8$ = mean baseline level

Let i index person and z =0, 1 index group.

Let $d_{zi} = (y_{zimean} - x_{zimean})$ = effect in person I in group z.

$d_{zmean} = \Sigma_i d_{zi} / 12$ = mean effect over all persons in group z

$vard_z = \Sigma_j (d_{zi} - d_{zmean})^2 / 11$

$vard_{zmean} = vard_z / 12$ = variance of mean effect in group z

$dif = d_{1mean} - d_{0mean}$

$vardif = vard_{1mean} + vard_{0mean}$

sedif=Sqrt[vardif].

The final result is dif with two-sided 95% confidence interval, dif-1.96 sedf, dif+1.96 sedif.

The sample size per randomization group is:
N = (za + zpower)^2 vardif /dif^2 , where za and zpower are z-statistics for type I error and power.

The anticipated values are
$vard_{1mean}$ = 4^2 and $vard_{0mean}$ =4^2, and dif= 4;
Substituting into the formula, gives sample size for one arm as
N= (za + zpower)^2 *(32 /16) = ((za + zpower)^2)*2

It is important to keep type error at .05 for the analysis. Let us say it is one-sided. Suppose the power is .8 Then N= ((1.645 + .84)^2)*2 = 12.35, which rounds to 12 per arm.

13.5 Secondary Objectives, Endpoints, Analysis Plans

The following secondary endpoints will be assessed between treatment arms. The analysis of secondary endpoints will include a Bonferroni correction for multiple comparisons.

Estimate the Pharmacodynamic (PD) Response rate across all patients and compare between arms:
Each participant will be assessed for PD response. The calculation is based on the standardized difference in means for the pharmacological effect on cGMP levels at the participant level, as shown in the following mathematical formulas, where a subject with a $z \geq 1.645$ will be considered a PD responder. A participant with a $z < 1.645$ will be considered a non-responder. If the cGMP values are not normally distributed, a transformation to a variable with a normal distribution will be performed. The PD response rate will be compared between the 2 arms using a standard Chi-square or Fisher's exact test. See the detailed calculations below for how the analysis will be done for calculating whether or not a subject is a PD responder:

Let i index persons and z index randomization group. For each time (baseline and final) there are 8 biopsies or replicates.

Let x_{zij} = baseline cGMP level for person i in randomization group z with replicate j.

$x_{zimean} = \Sigma_j x_{zij} / 8$ = mean baseline level

$v_{zi} = \Sigma_j (x_{zij} - x_{zimean})^2 / 7$ = variance of baseline level for one replicate for person i in group z

$v_{zimean} = v_{zi} / 8$ = variance of mean of baseline replicates for person i in group z

Let y_{zij} = day 7 cGMP level for person i in randomization group z and replicate j.

$y_{zimean} = \Sigma_j y_{zij} / 8$ = mean baseline level

$w_{zi} = \Sigma_j (y_{zij} - y_{zimean})^2 / 7$ = variance of final level for one replicate for person i in group z

$w_{zimean} = w_{zi} / 8$ = variance of mean of final replicates for person i in group z

Let $zstat_{zi} = (y_{zimean} - x_{zimean}) / Sqrt[v_{zimean} + w_{zimean}]$

Define PD_{zi} an indicator of a statistically significant increase (at one-sided alpha of .05) in mean cGMP from baseline to final result for person i in group z.

Let $PD_{zi} = 1$ if $zstat_{zi} \geq 1.645$, and 0 otherwise.

To confirm the safety and tolerability of dolcanatide: Summary statistics and frequency tables will be used to describe the data in an exploratory fashion. All adverse events will be summarized by treatment arm and compared between arms using the Fisher's Exact test. All eligible participants initiating study intervention will be considered evaluable for this endpoint.

13.6 Reporting and Exclusions

Given the fact that participants will receive one 7-day course of treatment, we do not anticipate having any drop-outs. There will be no run-in phase for this study. It is acceptable to have a single missed dose, with the exception of Day 7 dose.

13.7 Evaluation of Toxicity

All registered and treated participants will be evaluable for adverse events (AEs) from the time of their first dose of dolcanatide. To evaluate the AE profile for this treatment, the maximum grade for each type of adverse event will be recorded for each participant and frequency tables will be reviewed to determine the overall patterns. The number and severity of adverse events will be tabulated and summarized across all grades. Grade 2+ adverse events will be similarly described and summarized separately. As per NCI CTC Version 4.0, toxicities are defined as adverse events that are classified as either possibly, probably, or definitely related to the interventional agent. Overall toxicity incidence, as well as toxicity profiles will be explored and summarized. Frequency distributions, graphical techniques, and other descriptive measures will form the basis of these analyses. In addition, we will review all adverse event data that are graded as 3, 4, or 5 and classified as either "unrelated or unlikely to be related" to the study intervention in the event of an actual relationship developing.

13.7.1 Adverse Event Stopping Rule

The trial will be monitored closely for occurrence of adverse events by the study team and by the Mayo Clinic Cancer Center Data Safety and Monitoring Board (DSMB) using the adverse event (AE) stopping rule specified below.

Adverse Event Stopping Rule: The stopping rule specified below is based on the knowledge available at study development. We note that the Adverse Event Stopping Rule may be adjusted in the event of either (1) the study re-opening to accrual after any temporary suspension or (2) at any time during the conduct of the trial and in consideration of newly acquired information regarding the adverse event profile of the treatment(s) under investigation. The study team in consultation with the Mayo DSMB may also choose to suspend accrual because of unexpected adverse event profiles that have not crossed the specified rule below.

Accrual will be temporarily suspended to this study if at any time we observe events considered at least possibly related to study treatment (i.e., an adverse event with attribute specified as "possible", "probable", or "definite") that satisfy any of the following criteria:

- If at any time, 1 or more subjects has experienced a Grade 4 or 5 adverse event at least possibly related to study drug.
- The study will be permanently stopped for any Grade 5 adverse event at least possibly related to study drug.

13.8 Evaluation of Response

The determination of response is described in the Section 13.4. All eligible participants having initiated study intervention and having both the pre- and post-intervention biopsies will be considered evaluable for the primary endpoint.

13.9 Interim Analysis

There is no plan for interim analysis. We will randomize 24 subjects total (12 per arm).

13.10 Ancillary Studies

Not applicable

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 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 the Participating Organization.

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 the Participating Organization.

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 the Participating Organization.

14.2.4 Documentation of training in "Good Clinical Practice Training" for all study personnel listed on the FDA Form 1572 and documentation of "Protection of Human Subjects" training for all study personnel listed on the Delegation of Tasks form for the Lead Organization and the Participating Organization.

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 Central Institutional Review Board (CIRB) 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 CIRB. 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 CIRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the CIRB-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.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the CIRB. Any subsequent changes to the informed consent must be approved by NCI, DCP, the CIRB, and then submitted to each organization's appropriate internal approval entities 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 West
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.

14.7 Anticipated Results and Future Directions

Oral GCC ligand replacement therapy holds great promise for prevention of colorectal cancer. These agents have favorable pharmacological characteristics, targeting a receptor primarily expressed by intestinal epithelial cells, and active in the intestinal lumen, without appreciable bioavailability and systemic exposure. The pharmacological effects of these agents following chronic exposure endure, without desensitization, in preclinical and clinical studies. Moreover, chronic administration is without obvious untoward effects in mice and humans. Preliminary studies demonstrated that, following oral colonoscopy preparation 0.870 mg of the previous GCC agonist administered for 7 days increased cGMP concentrations in mucosae from the cecum, transverse colon, and rectum. These changes in cGMP were associated with increased phosphorylation of VASP quantified by immunoblot analysis and a reduction in epithelial cell proliferation quantified by Ki67 immunohistochemistry. However, the previous GCC

agonist was without effect on cGMP levels in the rectum in the absence of oral colonoscopy preparation.

The study proposed here advances the utility of this class of agent to prevent colorectal cancer by determining whether or not dolcanatide at 27 mg daily produces the requisite PD changes in cGMP signaling in rectal mucosa (full organ coverage). Once it is determined that dolcanatide is safe and produces cGMP responses in rectal mucosa, we anticipate advancing to a multi-center chemoprevention trial exploring the ability of dolcanatide to reduce the formation of adenoma in patients following chronic (one year) oral daily dosing. If this novel approach is effective in preventing colorectal cancer in patients, it is anticipated that patients at high risk for colorectal cancer (e.g., polyp formation, inflammatory bowel disease) could be placed on lifetime oral GCC ligand replacement therapy. This is consistent with the anticipated use of oral GCC ligand therapy in constipation, in which patients with irritable bowel syndrome-constipation type or chronic idiopathic constipation will receive chronic oral GCC ligand therapy to normalize bowel function.

15. FINANCING, EXPENSES, AND/OR INSURANCE

No clinical trial-related expenses will be incurred by the study participants and/or their insurance carriers. This does not include costs of tests and procedures that are a part of the participant's normal clinical care. This also does not include any injuries or illnesses the participant may have related to their participation on the study. In the event of an injury or illness, the study participant and/or their insurance carrier will be responsible for all expenses related to the injury or illness. There may be expenses incurred by the trial participants related to their study participation, i.e. child care, transportation, etc. Participants who comply with study requirements will be paid $1320 which is compensation for their time and reimbursement for expenses associated with trial participation.

REFERENCES

1. Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. CA: a cancer journal for clinicians 2010;60:277-300.
2. Jarmuz A, Zielinska M, Storr M, et al. Emerging treatments in Neurogastroenterology: Perspectives of guanylyl cyclase C agonists use in functional gastrointestinal disorders and inflammatory bowel diseases. Neurogastroenterol Motil 2015;27:1057-68.
3. Lucas KA, Pitari GM, Kazerounian S, et al. Guanylyl cyclases and signaling by cyclic GMP. Pharmacological reviews 2000;52:375-414.
4. Field M. Mechanisms of action of cholera and Escherichia coli enterotoxins. The American journal of clinical nutrition 1979;32:189-96.
5. Guerrant RL, Hughes JM, Chang B, et al. Activation of intestinal guanylate cyclase by heat-stable enterotoxin of Escherichia coli: studies of tissue specificity, potential receptors, and intermediates. The Journal of infectious diseases 1980;142:220-8.
6. Guarino A, Cohen M, Thompson M, et al. T84 cell receptor binding and guanyl cyclase activation by Escherichia coli heat-stable toxin. The American journal of physiology 1987;253:G775-80.
7. Steinbrecher KA, Tuohy TM, Heppner Goss K, et al. Expression of guanylin is downregulated in mouse and human intestinal adenomas. Biochemical and biophysical research communications 2000;273:225-30.
8. Notterman DA, Alon U, Sierk AJ, et al. Transcriptional gene expression profiles of colorectal adenoma, adenocarcinoma, and normal tissue examined by oligonucleotide arrays. Cancer

research 2001;61:3124-30.
9. Birkenkamp-Demtroder K, Christensen LL, Olesen SH, et al. Gene expression in colorectal cancer. Cancer research 2002;62:4352-63.
10. Waldman SA, Cagir B, Rakinic J, et al. Use of guanylyl cyclase C for detecting micrometastases in lymph nodes of patients with colon cancer. Diseases of the colon and rectum 1998;41:310-5.
11. Birbe R, Palazzo JP, Walters R, et al. Guanylyl cyclase C is a marker of intestinal metaplasia, dysplasia, and adenocarcinoma of the gastrointestinal tract. Human pathology 2005;36:170-9.
12. Li P, Lin JE, Snook AE, et al. Colorectal cancer is a paracrine deficiency syndrome amenable to oral hormone replacement therapy. Clinical and translational science 2008;1:163-7.
13. Andresen V, Camilleri M, Busciglio IA, et al. Effect of 5 days linaclotide on transit and bowel function in females with constipation-predominant irritable bowel syndrome. Gastroenterology 2007;133:761-8.
14. Bharucha AE, Waldman SA. Taking a lesson from microbial diarrheagenesis in the management of chronic constipation. Gastroenterology 2010;138:813-7.
15. Pitari GM, Di Guglielmo MD, Park J, et al. Guanylyl cyclase C agonists regulate progression through the cell cycle of human colon carcinoma cells. Proceedings of the National Academy of Sciences of the United States of America 2001;98:7846-51.
16. Shailubhai K, Yu HH, Karunanandaa K, et al. Uroguanylin treatment suppresses polyp formation in the Apc(Min/+) mouse and induces apoptosis in human colon adenocarcinoma cells via cyclic GMP. Cancer research 2000;60:5151-7.
17. Busby RW, Kessler MM, Bartolini WP, et al. Pharmacologic properties, metabolism, and disposition of linaclotide, a novel therapeutic peptide approved for the treatment of irritable bowel syndrome with constipation and chronic idiopathic constipation. The Journal of pharmacology and experimental therapeutics 2013;344:196-206.
18. Mann EA, Steinbrecher KA, Stroup C, et al. Lack of guanylyl cyclase C, the receptor for Escherichia coli heat-stable enterotoxin, results in reduced polyp formation and increased apoptosis in the multiple intestinal neoplasia (Min) mouse model. International journal of cancer 2005;116:500-5.
19. Forte LR, Freeman RH, Krause WJ, et al. Guanylin peptides: cyclic GMP signaling mechanisms. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas 1999;32:1329-36.
20. Synergy Pharmaceuticals I. SP-333: stimulation of intracellular cGMP synthesis in T84 cell (Study No. SP PH 007). 2012.
21. Synergy Pharmaceuticals I. SP-333: Data report for pharmacology studies. Activity of SP-333 in a panel of 73 enzyme and radioligand binding assays for various mammalian receptors and ion channels (Study No. AB20754). 2013.
22. Synergy Pharmaceuticals I. Evaluation of the efficacy of SP-333 in a rat model of opioid-induced constipation.
23. Synergy Pharmaceuticals I. A study to determine test item efficacy in an acute DSS-induced colitis model of IBD (Study No. 10/013c, 10/190) 2011.
24. Synergy Pharmaceuticals I. SP-333: Cardiovascular assessment when administered orally in conscious cynomolgus monkeys (Study No. 11-6779) 2012.
25. Synergy Pharmaceuticals I. Neuropharmacologic profile (NPP) of SP-333 following oral gavage administration in mice (Study No. 0200MS58.001). 2012.
26. Synergy Pharmaceuticals I. Evaluation of respiratory function following oral gavage administration of SP-333 in mice (Study No. 1275MS58.002). 2012.
27. Synergy Pharmaceuticals I. A first-in-human, single-blind, placebo-controlled, randomized single

ascending dose study to evaluate the safety, tolerability, and pharmacokinetics of SP-333 tablets in healthy adult subjects (Study No. SP333101). 2013.
28. Synergy Pharmaceuticals I. A single-blind, placebo-controlled, randomized multiple ascending dose study to evaluate the safety, tolerability, and pharmacokinetics of SP-333 tablets in healthy adult subjects (Study No. SP333101-01).
29. Synergy Pharmaceuticals I. A Phase Ib, Exploratory, Double-Blind, Placebo-Controlled, Four week Study of Rectally Administered SP-333 for the Treatment of Patients with Mildly to Moderately Active Left-Sided Ulcerative Colitis (SP333101-04). 2017.
30. Weinberg DS, Lin JE, Foster NR, et al. Bioactivity of Oral Linaclotide in Human Colorectum for Cancer Chemoprevention. Cancer Prev Res (Phila) 2017;10:345-354.
31. Synergy Pharmaceuticals I. SP-333, SP-339 and SP-304: An oral range-finding toxicity study in cynomolgus monkeys (Study No. 30169). 2008.
32. Chey WD, Lembo AJ, Lavins BJ, et al. Linaclotide for irritable bowel syndrome with constipation: a 26-week, randomized, double-blind, placebo-controlled trial to evaluate efficacy and safety. The American journal of gastroenterology 2012;107:1702-12.
33. Lembo AJ, Schneier HA, Shiff SJ, et al. Two randomized trials of linaclotide for chronic constipation. The New England journal of medicine 2011;365:527-36.
34. Ikematsu H, Saito Y, Tanaka S, et al. The impact of narrow band imaging for colon polyp detection: a multicenter randomized controlled trial by tandem colonoscopy. Journal of gastroenterology 2012;47:1099-107.
35. Johnston JM, Kurtz CB, Drossman DA, et al. Pilot study on the effect of linaclotide in patients with chronic constipation. The American journal of gastroenterology 2009;104:125-32.
36. Munroe CA, Lee P, Copland A, et al. A tandem colonoscopy study of adenoma miss rates during endoscopic training: a venture into uncharted territory. Gastrointestinal endoscopy 2012;75:561-7.
37. Steiner AL, Pagliara AS, Chase LR, et al. Radioimmunoassay for cyclic nucleotides. II. Adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in mammalian tissues and body fluids. The Journal of biological chemistry 1972;247:1114-20.

Summary of Changes – Informed Consent Form

NCI Protocol #: MAY2017-09-01
Local Protocol #: MAY2017-09-01
Protocol Version Date: September 6, 2018

Protocol Title: Phase I Double-Blind, Placebo-Controlled Trial of 27 mg Dolcanatide (SP-333) to Demonstrate Colorectal Bioactivity in Healthy Volunteers

Informed Consent Version Date: September 6, 2018

Please note that the page numbers in the table below refer to the Word version of the Informed Consent Form that will be submitted to the CIRB.

#	Section	Page(s)	Change
1.	**Study Calendar**	6-7	"Breathing rate" was removed from the list of evaluations in the study calendar for consistency with Section 7 of the protocol.

Thomas Jefferson University
Informed Consent Document for Human Subjects Research
OHR-8K (v.6/29/2017)

Department: Pharmacology and Experimental Therapeutics- Clinical Research Unit

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Informed Consent

Study Title for Study Participants: A study to see effects of the experimental drug dolcanatide on the colon

Official Study Title for Internet Search on http://www.ClinicalTrials.gov: Phase I, Double-Blind, Placebo-Controlled Trial of 27 mg Dolcanatide (SP-333) to Demonstrate Colorectal Bioactivity in Healthy Volunteers

Introduction

This is a clinical trial, a type of research study. We do research studies to try to answer questions about how to prevent, diagnose, or treat diseases like cancer. 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 informed consent?

You are being asked to take part in a medical research study. As required by federal regulations, this research study has been reviewed and approved by an Institutional Review Board (IRB), which is a committee that reviews, approves, and monitors research involving humans. Before a knowledgeable decision about whether to participate in a research study can be made, the possible risks and benefits related to the study should be understood. This process of learning

and thinking about a study before deciding to participate is known as informed consent and includes:

- Receiving detailed information about this research study;
- Being asked to read, sign, and date this consent form once the nature of the study is understood and a decision is made to participate. If there is anything about the study you don't understand or if there are questions, you should ask for explanations before signing this form;
- Being given a copy of the signed and dated consent form to keep.

An individual who joins a research study has a relationship with the study doctor that is different than the relationship with a treating or personal doctor. A treating doctor treats a specific health condition with the goal of improving that condition. A study doctor treats all participants according to a research plan to obtain information about the experimental drug, device, or procedure being studied and with the understanding that there may or may not be benefit from being in the study. The study doctor and study staff can provide more information about research as opposed to treatment.

Taking part in this study is your choice

You can choose to take part or you can choose not to take part in this study. You also can change your mind at any time. Whatever choice you make, you will not lose your access to medical care or any legal rights.

This informed consent document has key information to help you make your choice. Take time to read it. Talk to your doctor, family, or friends about the pros and cons of taking part in the study. It's important that you have as much information as you need and that all of your questions are answered. See the section "Where can I get more information?" for resources for more clinical trials and general cancer information.

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 take part in a different study, if one is available,
- or you may choose to do nothing.

Why is this study being done?

The purpose of this study is to find out how a drug called dolcanatide affects the colon because researchers think that it may prevent colon cancer. Dolcanatide is also being studied for the treatment of prescription drug induced constipation and irritable bowel syndrome. Dolcanatide has not been approved by FDA. It is considered investigational.

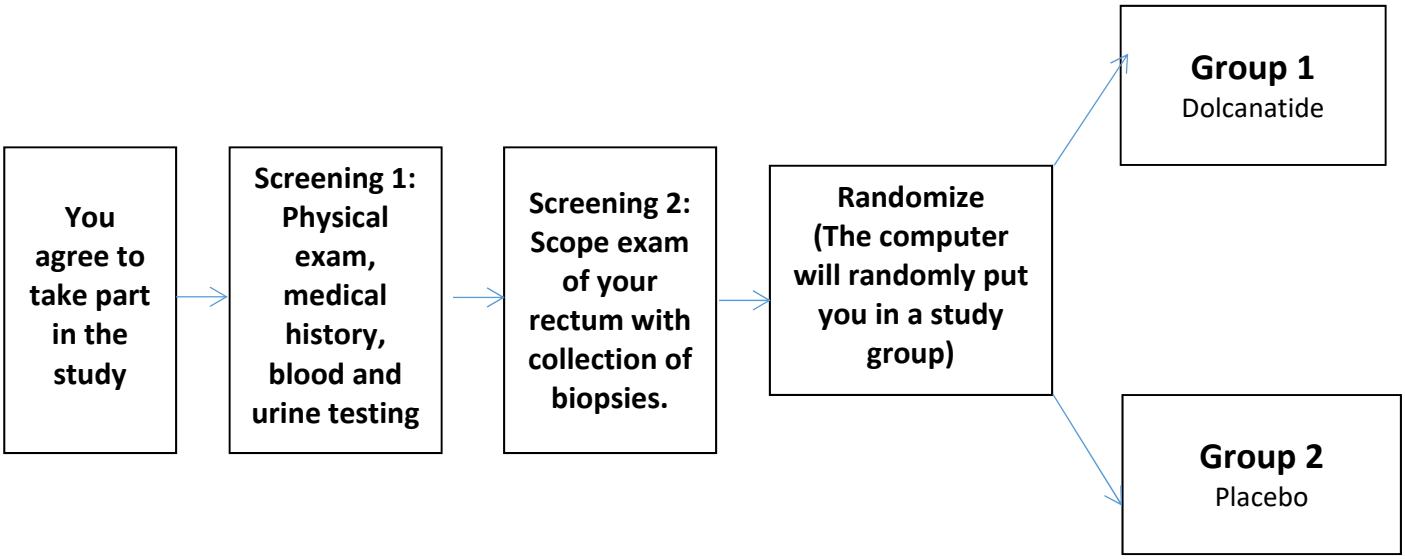
Dolcanatide is similar to a natural hormone released into the intestine. It is thought that people who have low levels of this hormone are more likely to get colon cancer. It may be possible to prevent colon cancer by giving people a drug that is similar to the hormone.

Up to about 40 healthy male and females will be screened for participation in the study at Thomas Jefferson University.

What are the study groups?

This study has two study groups. Group 1 will receive the study drug, dolcanatide, and Group 2 will receive a placebo, a capsule that looks like the study drug but contains no medication.

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?

Your involvement in the study will last up to 8 weeks.

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

Screening 1
Before you begin the study, you will need to have the following exams, tests and procedures to find out if you can be in the study:

- Physical exam
- Review of your medical and surgical history
- Review of any symptoms you are experiencing
- Review of any medications (prescription, over-the-counter, and supplements that you are taking)
- Completion of questionnaires about your alcohol and tobacco use
- Blood tests for safety (you will be asked not to eat or drink anything beginning at Midnight the night before the tests)
- Urine test for safety and for illegal drugs
- Pregnancy or FSH tests, if appropriate
- Electrocardiogram (ECG)

The blood tests for safety include tests of the electrolytes in your blood, tests to check how well your liver and kidneys are working, tests to make sure your blood can clot correctly, and tests to measure the number of red and white blood cells in your blood.

A urine sample will be obtained prior to the start of the study. Some of the urine will be analyzed to check how well your kidneys are working. Some will be used to screen for specific illicit drugs. The drugs tested for include amphetamines (“speed”), benzodiazepines (Xanax), opiates (heroin, codeine), cocaine (“crack”), barbiturates (phenobarbital, Seconal) and cannabinoids (“marijuana”). A positive test for any of these drugs on pre-study examination will cause you to be excluded from participation in this research study and will be maintained as part of your study file. The investigator reserves the right to test for these same drugs during the study period. If at this time your urine drug screen is found to be positive for any of these drugs, your participation may be terminated, a reduction in study payment may be imposed, this result will be maintained as part of your study record, and you will be prohibited from participating in any future studies in the Clinical Research Unit (CRU) at Thomas Jefferson University.

As part of this study you will also be asked to answer questions about your tobacco and alcohol use, both before you begin the study and again at about Day 21. Researchers want to see if tobacco and alcohol use affects the side effects people might get while on this study, or if tobacco and alcohol use modifies the effects of the study agents.

Screening 2
If the exams, tests, and procedures show that you can continue take part in the study, and you choose to, then you will have a flexible sigmoidoscopy. Flexible Sigmoidoscopy is a procedure in which a scope is placed into the anus to look at the rectum and sigmoid colon and obtain biopsies. There are instructions specific to the flexible sigmoidoscopy procedures that you will have to follow. A separate document that includes those special instructions will be provided to you during the study.

Before the flexible sigmoidoscopy, up to three enemas will be administered to clean the lower part of your rectum and colon. An enema is a simple procedure that introduces liquid into the colon to help clean out stool. A bag filled with mildly warm liquid is attached to a lubricated flexible tube. The end of the tube is placed into the rectum and the water is slowly dripped into your colon. The enema will contain a compound called glycolax, which can clean the rectum and colon without damaging or irritating the tissues lining the rectum and colon.

A biopsy is a procedure in which the doctor will remove a small piece of surface tissue from your rectum. Each piece will be smaller than a grain of rice. Doctors will collect a total of 8 pieces of tissue from your rectum. There may be some mild discomfort from the sigmoidoscopy, and you may experience a slight pinch sensation from the biopsies.

If the sigmoidoscopy results are normal, and you still agree to take part in this study, you will be asked to agree to stop drinking grapefruit juice until completion of Day 7.

You will be informed of the results of all of your screening tests and procedures. If any results or worrisome or significant, you will be offered a referral for follow-up care.

During the study (Days 1 to 6):
You will start the study drug on Day 1. You will report to the CRU in the morning without having had anything to eat or drink since Midnight (fasting). You will be given the study drug or placebo and told not to eat or drink anything for 30 minutes after receiving the drug. You will be discharged from the CRU.

During the study (Day 7):
You will arrive in the CRU in the morning without having had anything to eat or drink since Midnight. You will have a brief physical exam and vital signs will be measured. Females will have a pregnancy test. Blood will be drawn for safety testing. You will have urine tests and a repeat urine drug test. You will be given a clear liquid breakfast and be allowed to drink clear liquids until about 11:00 a.m.

At approximately 1:30 p.m. you will have an enema to clear your bowels. No more than 3 enemas will be given. Around 3:00 p.m. you will undergo the flexible sigmoidoscopy in the Jefferson GI (Gastroenterology) offices within the hospital. Prior to the sigmoidoscopy, you will be evaluated by a GI physician to make sure it is safe for you to undergo the procedure. During

the procedure, 8 biopsies will be taken from the rectum. Each biopsy is about the size of a grain of rice. There may be some mild discomfort from the sigmoidoscopy, and you may experience a slight pinch sensation from the biopsies. After the sigmoidoscopy you will return to the CRU and you will be given a light meal. You won't be able to leave the hospital until the doctors and nurses make sure that you are safe to go home.

After the study (Approximately Day 21):
Approximately 14 days after your last dose of dolcanatide or placebo, you will come to CRU after fasting for 8 hours and have a physical exam and have urine collected and blood drawn for laboratory safety evaluations.

You will be asked to complete an optional questionnaire about your experience in this study, Was It Worth It?, which will require about 5 minutes to complete. You will also be asked to complete the follow up Alcohol and Tobacco Use Assessments, which will take about 10 minutes.

For all visits, at the discretion of the study doctor, you may require longer observation in the clinic or additional laboratory testing based on the effects of the study drug or the results of the laboratory tests to make sure there are no problems with your health. The results of all of the safety laboratory tests and your final sigmoidoscopy will be provided to you. If any results are worrisome, you will be offered a referral for follow-up clinical care.

Over the course of this study, a total of about 3 Tablespoons of blood (1½ ounces) will be drawn. This is less than what would be taken if you were donating blood (about 16 ounces).

Follow Up (Approximately Day 51):
You may receive a follow-up telephone call to ask about side effects or symptoms, if any, that you may have reported at the previous visit. This visit is the end of your participation in this study.

This study calendar shows the schedule of events.

Day	Events and Procedures
Screening 1	Physical exam Vital signs (heart rate, blood pressure, temperature, height, and weight) Review of medical and surgical history Questionnaire regarding your alcohol and tobacco use Review of any medications you are taking (prescription, over-the-counter, and supplements) Blood collection for safety tests Urine testing and drug screening ECG (to test your heart function) Pregnancy test, if you are a woman capable of becoming pregnant

Screening 2	Vital signs (heart rate, blood pressure, temperature, and weight) Clear liquid breakfast and clear liquids Sigmoidoscopy procedure with biopsies
Randomization	Assignment to dolcanatide or placebo
Day 1-6	Report to the CRU fasting to receive study drug (do not eat or drink for 30 minutes after receiving the drug) Remember not to take any medications (prescription, over-the-counter, and supplements) while you are on the study Remember not to drink any alcohol while you are on the study Review of side effects and symptoms
Day 7	Report to the CRU fasting Vital signs (heart rate, blood pressure, temperature, and weight) Brief physical exam Review of any side effects, symptoms, and medications you are taking Blood and urine tests for a safety check Sigmoidoscopy procedure with biopsies
Approximately Day 21	Report to the CRU fasting Vital signs (heart rate, blood pressure, temperature, and weight) Review of any side effects and symptoms Blood and urine tests for a safety check Questionnaire: Was It Worth It? Follow up questionnaire about your alcohol and tobacco use
Approximately Day 51	You may have a follow-up telephone call to see how you are doing and ask about any side effects.

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, for example about your alcohol and tobacco use.

The study drug, dolcanatide, 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 study doctor may adjust the study drugs to try to reduce side effects.

Possible Side Effects of Dolcanatide (SP-333):

OCCASIONAL, SOME MAY BE SERIOUS **In 100 people receiving dolcanatide, from 4 to 20 may have:**
• Headache • Loose stools (diarrhea) • Passing gas • Feeling bloated (abdominal distension) • Pain in the belly (abdominal cramping or pain) • Feeling sick to your stomach (nausea) • Throwing up (vomiting)

There have been no deaths related to dolcanatide.

You could potentially lose weight or appetite if the hormone gets absorbed into your bloodstream. However, the chance of the hormone getting into your bloodstream is very low. There may be other side effects or risks that are not known at this time. These may include side effects that are severe or life-threatening. It is important that you tell the study staff about any side effects that you may have had even if you do not think it is related to the study drug.

The study doctor will discuss the possible side effects of dolcanatide with you. You will be told in a timely manner about any important new information that might affect your decision to stay in the study.

Flexible sigmoidoscopy

There are few risks associated with the sigmoidoscopy. You may feel some discomfort when the scope is inserted. There is a slight risk of bleeding and mild pain due to the tissue removal. Any pain and bleeding should stop without any treatment.

The scope may puncture (make a hole) or perforate (tear) your colon. This may cause bowel movement to leak out of the colon into your abdomen. Perforation or puncture occurs in about one person out of every 15,000 people who have had a sigmoidoscopy. You will be monitored throughout the procedure and after the procedure for any of these symptoms.

In this study, you will have two flexible sigmoidoscopies 7 days apart. Biopsies will be taken from slightly different locations during the two procedures. Studies like this one have not shown any additional risks when the procedures are close together. However, it is possible that you will experience pain, bleeding, trouble breathing, or fever.

You should call the study doctor as soon as possible at 215-955-6084 if, during the course of this study, you develop any of these side effects or symptoms. The study doctor has told you that if your condition worsens, if side effects become very severe, or if it turns out that being in this study is not in your best interest, you will be taken out of the study.

Risks related to blood draws

Blood tests can cause mild discomfort, bruising and/or bleeding at the blood draw site. Less likely is the possibility of significant bleeding or infection.

Risks associated with the Electrocardiogram (ECG)

An electrocardiogram is a safe procedure. There may be minor discomfort, similar to removing a bandage, when the electrodes taped to the chest to measure the heart's electrical signals are removed. Rarely, a reaction to the electrodes may cause redness or swelling of the skin. The electrodes only detect electrical impulses produced by the heart. No electricity passes through the body from the machine, and there is no danger of electrical shock.

What are the risks to fetuses, infants and pregnant women?

Pregnant women or women who are breast-feeding should not be in this study because exposure to investigational drugs may be hazardous to an embryo, fetus or nursing infant. Even medications that are well known and prescribed may have adverse effects on an embryo or fetus. As with any medication, there are unknown risks. To be in this study you and your partner must practice adequate birth control measures. The study doctor will discuss acceptable methods of birth control with you. If you are a woman of childbearing potential, you will have a pregnancy test before making a decision about being in this study. This requires either a urine test or that blood be drawn from a vein in your arm (1-2 tsp.) one or two days prior to the start of the study. The results of this pregnancy test will be made available to you prior to the start of the study.

If you become pregnant during the course of this study, you should notify the study doctor as soon as possible. You will be contacted or your medical records will be reviewed to receive information on the pregnancy outcome.

If you are a man participating in this study, you also should practice adequate birth control because investigational drugs may have adverse effects on sperm that could also adversely affect a fetus. If your partner becomes pregnant during the course of the study, the sponsor may want to follow her to receive information on the pregnancy outcome. She will be asked to sign a separate consent form or a form for release of medical information for that.

If you are a person in a same sex relationship, it is not necessary for you to practice birth control. However, if you are female, you will still have to have pregnancy tests according to the study protocol. If you would become pregnant, you will be followed through the pregnancy and receive information on the pregnancy outcome.

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

You will not benefit from taking part in this study. It is for the benefit of research.

If I decide to take part in the study, can I stop later?

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.

For the tobacco and alcohol use questions, you can decide to not answer some or all of the questions. Your decision will not affect whether you can participate in the study, and it will not affect your relationship with your doctor or the study staff.

The study doctor will tell you about any new information or changes in the study that could 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.
- If 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 early for any reason by the sponsor (National Cancer institute, Division of Cancer Prevention [DCP]), the Central Institutional Review Board (CIRB) or the United States Food and Drug Administration (FDA).

If you withdraw from this study, you may continue treatment with your Jefferson doctor, or you may seek treatment from another doctor of your choice.

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 Central Institutional Review Board (CIRB) at 888-657-3711.**

What are the costs of taking part in this study?

The dolcanatide or placebo will be supplied at no charge while you take part in this study. The costs of all study-specific biopsies and exams, tests, and 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.

If you receive a bill that you think is wrong, please discuss it with the study doctor or research coordinator.

Will I be paid for participating in this study?

You will not be paid for participating in this study. However, you may receive payments to help compensate you for your time and to help cover expenses related to study participation, such as travel.

If you abide by all study requirements, the payments you will receive are as follows:

Completion of screening and initial endoscopy: $400.00
Daily visits (Days 1-6) to Clinical Research Unit: $70.00 per visit
Completion of Day 7 visit and endoscopy: $400.00
Completion of Day 21 visit: $100.00

The payments will be authorized at the end of each visit, but it will take some time to process the payment and provide the payment to you.

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 offer to 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. The study doctors 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. However, some of your medical information may be given out if required by law. If this should happen, the study doctors will do their best to make sure that any information that goes out to others will not identify who you are.

Some of your health information, such as your response to cancer treatment, results of study tests, and medicines you took, will be kept by the study sponsor in a central research database. However, your name and contact information will not be put in the database. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

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

- The study sponsor, National Cancer Institute, Division of Cancer Prevention
- The drug company supporting the study, Synergy Pharmaceuticals, Inc.
- The Central Institutional Review Board, CIRB, 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.
- **The National Cancer Institute will obtain information for this clinical trial under data collection authority Title 42 U.S.C. 285.**

The following individuals or entities will have access to your Protected Health Information (PHI) and by law must protect it. These include investigators listed on this consent form and other personnel of Thomas Jefferson University, Jefferson University Physicians, and Thomas Jefferson University Hospitals, Inc. involved in this specific study, the University's Division of Human Subjects Protection and the Institutional Review Board (IRB), and your health insurance company (if necessary for billing for standard medical care).

The following information will be provided to the study sponsor and other entities noted in this consent form:

- **Study data for analysis:** lab results, imaging studies, medical history, medication history, questionnaire results, tissue biopsy, electrocardiogram (ECG)
- **Demographic data:** age, sex, race, ethnicity

If you develop an illness or injury during the course of participation in this study, other PHI about treating and following the condition may be generated and disclosed as it relates to this study.

PHI collected as part of this research may be used/disclosed indefinitely.

You may quit the study and revoke permission to use and share PHI at any time by contacting the principal investigator, in writing, at:

Scott Waldman, MD, PHD
132S. 10th Street, 1170 Main Building
Philadelphia, PA 19107

Further collection of PHI will be stopped on those who quit the study, but PHI that has already been collected may still be used.

Disclosure of Financial Interest

The sponsor of this clinical study, Mayo Clinic Rochester, is paying Thomas Jefferson University to conduct this study.

Dr. Scott Waldman, co-Principal Investigator for this project, has received within the past 12 months consulting income and funding for laboratory research from Synergy Pharmaceuticals, the company providing study agent for this project.

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 US 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 doctors: Scott Waldman, M.D. Ph.D. and Walter Kraft, M.D., 215-955-0058.

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 the studies for any reason, you can still take part in the main study.

Optional Sample Collections for Laboratory Studies and Biobanking for Possible Future Studies

Researchers are trying to learn more about cancer and other health problems using blood and tissue samples from people who take part in clinical trials. By studying these samples, researchers hope to find new ways to prevent, detect, treat, or cure diseases.

Some of these studies may be about how genes affect health and disease, and these are called genomic studies. Other studies may look at how genes affect a person's response to treatment. Genes carry information about traits that are found in you and your family. Examples of traits are the color of your eyes, having curly or straight hair, and certain health conditions that are passed down in families. Some of the studies may lead to new products, such as drugs or tests for diseases. Researchers are interested in the way that genes affect how your body responds to treatment.

If you choose to take part in this study, the study doctor would also like to keep any biopsy samples that are left over after the research described in this consent form is complete. The researchers ask your permission to store and use your samples and health information for medical research. The research that may be done is unknown at this time. Storing samples for future studies is called "biobanking." The Biobank is being run by the Cancer Prevention Network (CPN) and supported by the National Cancer Institute.

WHAT IS INVOLVED?

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

1) Your remaining biopsy samples and some related information may be stored in the Biobank, along with samples and information from other people who take part. The samples will be stored at the Cancer Prevention Network Biobank at the Mayo Clinic in Rochester, MN, 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 Biobank. A research committee 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 if/when research is conducted 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) There is a risk that someone could get access to the personal information in your medical records or other information we have stored about you.
2) 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.
3) 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.

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 or social security number) will be sent. Samples will be identified by a unique study 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 Cancer Prevention Network staff with access to the list must sign an agreement to keep your identity confidential.
3) Researchers to whom the Cancer Prevention Network or NCI, Division of Cancer Prevention 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. 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 doctors: Scott Waldman, M.D. Ph.D. and Walter Kraft, M.D. at 215-955-0058, who will let the researchers know. Then, any sample that remains in the bank will no longer be used. 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 doctors: Scott Waldman, M.D. Ph.D. and Walter Kraft, M.D. at 215-955-0058.

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 specimens collected, and I agree that my specimen samples and related information may be used for the laboratory studies 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 these studies.

YES NO

SAMPLES AND INFORMATION FOR FUTURE RESEARCH STUDIES:

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

YES NO

The information from my tobacco and alcohol use questionnaires may be used in future health research.

YES NO

My specimens and related information may be sent to researchers at outside institutions.

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.

Contact Information

For questions about your rights as a research participant, call:	The Jefferson Institutional Review Board	215-503-8966
For questions, concerns or complaints about the research, or if you suspect a research-related injury, call:	The Principal Investigator, Dr. Scott Waldman or any co-investigator listed at the beginning of this form	215-955-5682
If you have difficulty contacting the study staff, call:	The Jefferson Office of Human Research	215-503-0203

If you want more information about the Jefferson Institutional Review Board or Jefferson's Human Research Protection Program, please visit our website at http://www.jefferson.edu/university/human_research/irb/html

My Signature Agreeing to Take Part in the Main Study

Non-Waiver of Legal Rights Statement

- ✓ **By your agreement to participate in this study, and by signing this consent form, you are not waiving any of your legal rights.**
- ✓ **In order to be in this research study, you must sign this consent form.**

You affirm that you have read this consent form. You have been told that you will receive a copy.

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 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 conducting the informed consent discussion _____

Date of signature_____

Signature of Principal Investigator or Co-Investigator _____

Date of signature_____

APPENDIX A
Performance Status Criteria

ECOG Performance Status Scale

Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g*., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B
Abbreviations

AdEERS	adverse event expedited reporting system
AE	adverse event
AOM	azoxymethane
APC	adenomatous polyposis coli
ASA	aspirin
cGMP	cyclic guanosine monophosphate
CIC	Chronic idiopathic constipation
CIRB	Central Institutional Review Board (NCI)
CPN	Cancer Prevention Network
CRC	colorectal cancer
CRU	clinical research unit
CTA	clinical trials agreement
DARF	Drug Accountability Record Form
DCP	Division of Cancer Prevention
EC	Effective concentration
ECG	electrocardiogram
E. coli	*Escherichia coli*
EIA	enzyme-linked immunosorbent assays
FAP	familial adenomatous polyposis
GCC	guanylate cyclase C
GMP	guanosine monophosphate
GTP	guanosine triphosphate
HNPCC	hereditary nonpolyposis colorectal cancer
HED	Human equivalent dose
IBS-C	Irritable bowel syndrome with constipation
IND	Investigational New Drug
LOI	letter of intent
MRSD	Maximum recommended starting dose
MIN	multiple intestinal neoplasm
NCI	National Cancer Institute
NOAEL	no observed adverse effect level
NSAID	nonsteroidal anti-inflammatory drugs
PEG	polyethylene glycol
PD	pharmacodynamic
PK	pharmacokinetic
PKG	cGMP-dependent protein kinase
pRb	phosphorylated retinoblastoma
RIA	radioimmunoassay
ST	bacterial heat-stable enterotoxin
TEAE	Treatment-emergent adverse event
TJU	Thomas Jefferson University
VASP	vasodilator-stimulated phosphoprotein
WIWI	Was It Worth It

APPENDIX C
Was It Worth It (WIWI) Questionnaire

Visit type (Time point):* ☐ _____

Participating in a clinical trial / research study is a personal choice and an individual experience. We would like to get your feedback on your experience in this research study. Please respond to the following questions as indicated.

☐ Check here if you prefer not to complete this questionnaire
☐ Check here if you prefer not to have your responses added to responses from participants in other clinical trials

Directions: Please answer each question by circling Y (for yes), N (for no), or U (for uncertain).

Was it worthwhile for you to participate in this research study?	Y	N	U
If you had to do it over, would you participate in this research study again?	Y	N	U
Would you recommend participating in this research study to others?	Y	N	U

Directions: Circle one response

Overall, did your quality of life change by participating in this research study?

It improved It stayed the same It got worse

Overall, how was your experience of participating in this research study?

Better than I expected The same as I expected Worse than I expected

If there was one thing **that could have been done to improve your experience in this research study, what would it be?**

_____

_____

_____

Would you like to talk to someone about your concerns (circle one response)? Yes No

_____ _____
Signature Date

Appendix D. Alcohol Assessment: Baseline

Instructions: For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

1. In your entire life, have you had at least 12 drinks of any kind of alcoholic beverage? *(check one)*
   - ☐ Yes
   - ☐ No **(End)**
   - ☐ Choose not to answer **(End)**
   - ☐ Don't know/Not sure

   (If No or Choose not to answer), stop assessment

2. In the past 12 months, on average, how often did you drink any type of alcoholic beverage? *(Enter the number of days you drank based on the timeframe checked below. Enter 0 if you never drank and skip to Question 6.)* _____
   (If more than 0, check one)
   - ☐ Week
   - ☐ Month
   - ☐ Year
   - ☐ Choose not to answer
   - ☐ Don't know/Not sure

3. In the past 12 months, on those days that you drank alcoholic beverages, on average, how many drinks did you have per day?
   *(Enter the average number of drinks per day)*_____
   (If no answer, check one)
   - ☐ Choose not to answer
   - ☐ Don't know/Not sure

4. In the past 12 months, on how many days did you have 5 or more drinks of any alcoholic beverage?
   *(Enter the number of days you had 5 or more drinks, or enter 0 if none.)*_____
   (If no answer, check one)
   - ☐ Choose not to answer
   - ☐ Don't know/Not sure

5. Was there ever a time or times in your life when you drank 5 or more drinks of any kind of alcoholic beverage almost every day?
   (check one)
   - ☐ Yes
   - ☐ No
   - ☐ Choose not to answer
   - ☐ Don't know/Not sure

6. If you do not currently drink alcoholic beverages, but did in the past, how long has it been since you last drank regularly?
   (check one)
   - ☐ Within the past month (0 to 1 month ago)
   - ☐ Between 1 and 3 months (1 to 3 months ago)
   - ☐ Between 3 and 6 months (3 to 6 months ago)
   - ☐ Between 6 and 12 months (6 to 12 months ago)
   - ☐ Between 1 and 5 years (1 to 5 years ago)
   - ☐ Between 5 and 15 years (5 to 15 years ago)
   - ☐ More than 15 years ago
   - ☐ Don't know/Not sure
   - ☐ Never drank regularly
   - ☐ Choose not to answer

Alcohol Assessment: Baseline (continued)

7. At the heaviest point, either now or in the past, on the days when you drank, about how many drinks did you drink a day on the average? *(Enter the number of drinks a day)*_____
(If no answer, check one)
☐ Choose not to answer
☐ Don't know/Not sure

8. How many years have you been drinking (or did drink) regularly? _____ years
(If no answer, check one)
☐ Choose not to answer
☐ Don't know/Not sure

9. At what age did you begin drinking regularly? _____ years of age
(If no answer, check one)
☐ Choose not to answer
☐ Don't know/Not sure

10. What type(s) of alcohol do you drink?

Wine	*(check one)* ☐ Yes	☐ No	☐ Choose not to answer
Liquor	*(check one)* ☐ Yes	☐ No	☐ Choose not to answer
Beer	*(check one)* ☐ Yes	☐ No	☐ Choose not to answer
Wine cooler	*(check one)* ☐ Yes	☐ No	☐ Choose not to answer

_____ _____
Investigator Signature Date

Appendix D. Alcohol Assessment: Follow-Up

Instructions: For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

1. During the past 30 days, did you drink any alcoholic beverages? *(check one)*
   - ☐ Yes
   - ☐ No **(End)**
   - ☐ Choose not to answer **(End)**
   - ☐ Don't know/Not sure

   (If No or Choose not to answer, stop assessment)

2. During the past 30 days, how many days per week or per month did you drink any alcoholic beverages, on the average?
   (Enter number of days you drank based on the timeframe checked below. Enter 0 if you did not drink.) _____
   (If more than 0), specify *(check one)*
   - ☐ Week
   - ☐ Month
   - ☐ Choose not to answer
   - ☐ Don't know/Not sure

3. On the days when you drank, on average, about how many drinks did you have?
   (Enter the average number of drinks you had per day.) _____
   (If no answer, check one)
   - ☐ Choose not to answer
   - ☐ Don't know/Not sure

4. In the past 30 days, on how many days did you have 5 or more drinks per day? _____
   (Enter the number of days when you had 5 or more drinks, or enter 0 if none)
   (If no answer, check one)
   - ☐ None
   - ☐ Choose not to answer
   - ☐ Do not know/Not sure

_____ _____
Investigator Signature Date

Appendix D. Tobacco Assessment: Baseline

Section A. Basic Cigarette Use Information

1. Have you smoked at least 100 cigarettes (5 packs = 100 cigarettes) in your entire life?
   - ☐ Yes
   - ☐ Choose not to answer → **Skip to Section B**
   - ☐ No → **Skip to Section B**
   - ☐ Don't know/Not sure → **Skip to Section B**

2. How old were you when you first smoked a cigarette *(even one or two puffs)*? _____ Years old
   (If no answer, check one)
   ☐ Choose not to answer ☐ Don't know/Not sure

3. How old were you when you first began smoking cigarettes regularly? _____ Years old
   (If no answer, check one)
   ☐ Refused ☐ Don't know/Not sure ☐ Check here if you have never smoked cigarettes regularly.

4. How many total years have you smoked (or did you smoke) cigarettes? Do not count any time you may have stayed off cigarettes.
   (If you smoked less than one year, write "1.") _____ Years
   ☐ Choose not to answer ☐ Don't know/Not sure

5. On average when you have smoked, about how many cigarettes do you (or did you) smoke a day? *(A pack usually has 20 cigarettes in it).* _____ Number of cigarettes per day
   (If no answer, check one)
   ☐ Choose not to answer ☐ Don't know/Not sure

6. Do you NOW smoke cigarettes? *(check one)*
   - ☐ Everyday
   - ☐ Some days
   - ☐ Choose not to answer → **Skip to question 8**
   - ☐ Not at all → **Skip to question 8**

7. How soon after you wake up do you smoke your first cigarette? *(check one)*
   ☐ Within 30 minutes ☐ After 30 minutes ☐ Choose not to answer

8. How long has it been since you last smoked a cigarette (even one or two puffs)? *First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.*

   I smoked a cigarette today (at least one puff) *(check one)* ☐ Yes ☐ No
   1-7 days *(check one)* ☐ Yes ☐ No
   (If yes), Number of days since last cigarette _____
   Less than 1 month *(check one)* ☐ Yes ☐ No
   (If yes), Number of weeks since last cigarette _____
   Less than 1 year *(check one)* ☐ Yes ☐ No
   (If yes), Number of months since last cigarette _____
   More than 1 year *(check one)* ☐ Yes ☐ No
   (If yes), Number of years since last cigarette _____
   Don't know/Don't remember *(check one)* ☐ Yes ☐ No
   Choose not to answer ☐

_____ _____
Investigator Signature Date

Tobacco Assessment: Baseline (continued)

Section B. Use of Other Forms of Tobacco

9. Have you ever used other forms of tobacco, not including cigarettes? *(check one)*
   - ☐ Yes ☐ Choose not to answer
   - ☐ No → **Skip to Section C**

10. How often do you/did you use other forms of tobacco?
   Every day *(check one)* ☐Yes ☐No *(If yes)*, Number of times per day _____
   Some days *(check one)* ☐Yes ☐No *(If yes)*, Number of days _____
   (If yes), Per *(select one)* ☐ Week ☐ Month ☐Year

11. Which of the following products have you ever used regularly? *(Mark yes, no., or choose not to answer for all choices)*

Cigarettes	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Traditional cigars, cigarillos or filtered cigars	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Hookah	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Bidis	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Snus	*(check one)* ☐Yes ☐No ☐ Choose not to answer
E-cigarettes or other electronic nicotine delivery system	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Waterpipe	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Pipes	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Clove cigarettes or kreteks	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Smokeless tobacco *(like dip, chew, or snuff)*	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Paan with tobacco, gutka, zarda, khaini	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Other, please specify: _____	

12. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly? *(check one)*
   - ☐ Within the past month (0 to 1 month ago)
   - ☐ Between 1 and 3 months (1 to 3 months ago)
   - ☐ Between 3 and 6 months (3 to 6 months ago)
   - ☐ Between 6 and 12 months (6 to 12 months ago)
   - ☐ Never used other forms of tobacco regularly
   - ☐ Between 1 and 5 years (1 to 5 years ago)
   - ☐ Between 5 and 15 years (5 to 15 years ago)
   - ☐ More than 15 years ago
   - ☐ Don't know/Not sure
   - ☐ Choose not to answer

Section C. Second-Hand Smoke Exposure

13. Are you currently living with a smoker? *(check one)*
   ☐Yes ☐No ☐ Choose not to answer

14. In the past 30 days, have you lived in a place where other people smoked cigarettes indoors? *(check one)*
   ☐Yes ☐No ☐ Choose not to answer

15. In the past 30 days, have you worked in a place where other people smoked cigarettes indoors? *(check one)*
   ☐Yes ☐No ☐ Choose not to answer

16. Thinking of all your childhood and adult years, have you ever lived in a place where other people smoked cigarettes indoors? *(check one)*
   - ☐Yes → In total, for about how many years? _____ If less than 1, write "1."
   - ☐No
   - ☐ Choose not to answer

17. Thinking of all the years you have worked, have you ever worked in a place where other people smoked cigarettes indoors? *(check one)*
   - ☐Yes → In total, for about how many years? _____ If less than 1, write "1."
   - ☐No
   - ☐ Choose not to answer

_____ _____
Investigator Signature Date

Appendix D. Tobacco Assessment: Follow-Up

1. Do you NOW smoke cigarettes? *(check one)*
   - ☐ Everyday
   - ☐ Some days
   - ☐ Choose not to answer→ **Skip to Question 3.**
   - ☐ Not at all → **Skip to Question 3.**

2. On average, when you smoked, about how many cigarettes do you (or did you) smoke a day? *(A pack usually has 20 cigarettes in it)*.
   _____ Number of cigarettes per day
   (If no answer, check one)
   ☐Choose not to answer ☐Don't know/Not sure

3. How long has it been since you last smoked a cigarette (even one or two puffs)? *First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.*
   I smoked a cigarette today (at least one puff) *(check one)* ☐Yes ☐No
   1-7 days *(check one)* ☐Yes ☐No
   (If yes), Number of days since last cigarette _____
   Less than 1 month *(check one)* ☐Yes ☐No
   (If yes), Number of weeks since last cigarette _____
   Less than 1 year *(check one)* ☐Yes ☐No
   (If yes), Number of months since last cigarette _____
   More than 1 year *(check one)* ☐Yes ☐No
   (If yes), Number of years since last cigarette _____
   Don't know/Don't remember *(check one)* ☐Yes ☐No
   Choose not to answer ☐

4. Since your last visit, have you used other forms of tobacco, not including cigarettes? *(check one)*
   - ☐ Yes
   - ☐ Choose not to answer **(End)**
   - ☐ No (**End**) *(If no)*, stop assessment

5. How often do you/did you use other forms of tobacco?
   Every day *(check one)* ☐Yes ☐No *(If yes),* Number of times per day _____
   Some days *(check one)* ☐Yes ☐No *(If yes),* Number of days _____
   (If yes), per *(select one)* ☐Week ☐Month ☐Year
   ☐Choose not to answer

6. Since your last visit, which of the following products have you used? *(Mark yes or no for all choices)*

Cigarettes	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Traditional cigars, cigarillos or filtered cigars	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Hookah	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Bidis	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Snus	*(check one)* ☐Yes ☐No ☐ Choose not to answer
E-cigarettes or other electronic nicotine delivery system	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Waterpipe	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Pipes	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Clove cigarettes or kreteks	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Smokeless tobacco *(like dip, chew, or snuff)*	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Paan with tobacco, gutka, zarda, khaini	*(check one)* ☐Yes ☐No ☐ Choose not to answer
Other, please specify: _____	

Tobacco Assessment: Follow-Up (continued)

7. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly? *(check one)*
   - ☐ Within the past month (0 to 1 month ago)
   - ☐ Between 1 and 3 months (1 to 3 months ago)
   - ☐ Between 3 and 6 months (3 to 6 months ago)
   - ☐ Between 6 and 12 months (6 to 12 months ago)
   - ☐ Between 1 and 5 years (1 to 5 years ago)
   - ☐ Between 5 and 15 years (5 to 15 years ago)
   - ☐ More than 15 years ago
   - ☐ Don't know/Not sure
   - ☐ Choose not to answer
   - ☐ Never used other forms of tobacco regularly

The following instructions pertain to questions 8 -10. During each of the following time frames, please indicate whether you smoked cigarettes every day, some days, or not at all.

8. During study treatment *(check one)*
   - ☐ Smoked every day
   - ☐ Smoked some days
   - ☐ Did not smoke at all
   - ☐ Don't know/not sure
   - ☐ Choose not to answer
   - ☐ Not applicable

9. After the end of study treatment *(check one)*
   - ☐ Smoked every day
   - ☐ Smoked some days
   - ☐ Did not smoke at all
   - ☐ Don't know/not sure
   - ☐ Choose not to answer
   - ☐ Not applicable (I have not completed the study treatment)

10. Since your last visit to this clinic *(check one)*
    - ☐ Smoked every day
    - ☐ Smoked some days
    - ☐ Did not smoke at all
    - ☐ Don't know/not sure
    - ☐ Choose not to answer
    - ☐ Not applicable (This is my first visit to this clinic)

_____ _____

Investigator Signature Date

Appendix E. Alcohol and Tobacco Cessation Resources

National and local resources to help with alcohol abuse and alcoholism

NIAAA's online guide ***Treatment for Alcohol Problems: Finding and Getting Help*** is written for individuals, and their family and friends, who are looking for options to address alcohol problems. It is intended as a resource to understand what treatment choices are available and what to consider when selecting among them.
https://pubs.niaaa.nih.gov/publications/treatment/treatment.htm

Other resources:

National Institute on Alcohol Abuse and Alcoholism www.niaaa.nih.gov
301–443–3860

National Institute on Drug Abuse www.nida.nih.gov
301–443–1124

National Clearinghouse for Alcohol and Drug Information www.samhsa.gov
1–800–729–6686

Substance Abuse Treatment Facility Locator www.findtreatment.samhsa.gov
1–800–662–HELP

Alcoholics Anonymous (AA) www.aa.org
212–870–3400 or check your local phone directory under "Alcoholism"

Moderation Management www.moderation.org
212–871–0974

Secular Organizations for Sobriety www.sossobriety.org
323–666–4295

SMART Recovery www.smartrecovery.org
440–951–5357

Women for Sobriety www.womenforsobriety.org
215–536–8026

Al-Anon Family Groups www.al-anon.alateen.org
1–888–425–2666 for meetings

Adult Children of Alcoholics www.adultchildren.org
310–534–1815

National and local resources to help with quitting smoking

NCI's Smokefree.gov offers science-driven tools, information, and support that has helped smokers quit. You will find state and national resources, free materials, and quitting advice from NCI.

Smokefree.gov was established by the Tobacco Control Research Branch of NCI, a component of the National Institutes of Health, in collaboration with the Centers for Disease Control and Prevention and other organizations.

Publications available from the Smokefree.gov Web site include the following:

- Clearing the Air: Quit Smoking Today for smokers interested in quitting.
- Clear Horizons for smokers over age 50.
- Forever Free™ for smokers who have recently quit.
- Forever Free for Baby and Me™, in English and Spanish, for pregnant smokers who have recently quit.
- Pathways to Freedom: Winning the Fight Against Tobacco for African American smokers.

NCI's Smoking Quitline at **1–877–44U–QUIT (1–877–448–7848)** offers a wide range of services, including individualized counseling, printed information, referrals to other resources, and recorded messages. Smoking cessation counselors are available to answer smoking-related questions in English or Spanish, Monday through Friday, 8:00 a.m. to 8:00 p.m., Eastern Time. Smoking cessation counselors are also available through LiveHelp, an online instant messaging service. LiveHelp is available Monday through Friday, 8:00 a.m. to 11:00 p.m., Eastern Time.

Your state has a toll-free telephone quitline. Call **1–800–QUIT–NOW (1–800–784–8669)** to get one-on-one help with quitting, support and coping strategies, and referrals to resources and local cessation programs. The toll-free number routes callers to state-run quitlines, which provide free cessation assistance and resource information to all tobacco users in the United States. This initiative was created by the Department of Health and Human Services. For more information about quitlines, speak to an expert on the Smokefree.gov Web site.