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Personalized Prevention of Colorectal Cancer Trial

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Qi Dai, M.D., Ph.D., Professor
Division of Epidemiology, Department of Medicine
Vanderbilt University

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1.0 Background

Colorectal cancer is the fourth most common incident cancer and the second most common cause of cancer death in the United States, with approximately 150,000 new cases and 57,000 deaths per year. About 1 in 18 individuals will develop colorectal cancer over their lifetime and 40% will die within 5 years of diagnosis, mainly due to diagnosis at a late stage. Therefore, development of primary preventive strategies for colorectal cancer is very critical. Colorectal cancer is believed to arise in the overwhelming majority of cases from adenomatous polyps via the well-established adenoma-carcinoma sequence. Furthermore, 41% of patients presenting an initial hyperplastic polyp subsequently developed adenomatous polyps. Growing evidence has highlighted a potentially important role in colorectal carcinogenesis of the serrated pathway comprising hyperplastic polyps and serrated adenomas.*Thus, investigation of preventive strategies for colorectal adenoma will provide primary preventive strategies for colorectal cancer.*

Thus far, endoscopy, particularly colonoscopy, has been regarded as the most effective preventive strategy to reduce both incidence of colorectal cancer and mortality due to colorectal cancer. In recent years, both incidence and mortality of colorectal cancer in the U.S. has decreased, possibly because of the rapidly increased use of colonoscopy or improved treatments. However, colorectal cancer still has one of the highest rates of incidence and mortality in the U.S. One possible explanation is that the routine screening rate is still not sufficiently high. On the other hand, a very recent population-based report found that colonoscopy is associated with reduced deaths from the left side of the colon, but not the right. It was also found, in as many as 24% of cases, snare polypectomy of exophytic lesions was unable to inhibit progression to carcinoma. Consistent with several large studies conducted in other Western countries, very recently, using an elective colonoscopy technique not used in regular clinical practice, a large US prevalence study with 1,819 patients found an overall prevalence rate of 9.35% for flat or depressed adenoma which are frequently missed in standard of care colonoscopy. The flat or depressed lesions were about 10 times more likely to contain carcinoma than polypoid lesions, with more than half of the carcinomas diagnosed among flat or depressed lesions [21]. *These findings suggest that new preventive strategies, including chemoprevention, are needed to prevent both polypoid and flat or depressed adenomas as well as adenomas in the proximal colon.*

A number of initiatives and studies have been proposed to develop preventive strategies for colorectal adenoma and colorectal cancer. In contrast to earlier studies, recent epidemiologic studies and clinical trials, however, have generated disappointing results on the association between fiber or vegetables and colorectal cancer, and recent intervention trials also did not support an association of dietary intake of vitamin C and E and carotenoids with colorectal adenoma. Very recently, the Physicians' Health Study II randomized controlled trial found individual supplements of 400 IU of vitamin E every other day and/or 500 mg of vitamin C daily provided no overall benefit for incidence or mortality of cancer, including colorectal cancer after a mean follow-up of 8 years. Furthermore, a recent large randomized trial of healthy women taking two other promising chemopreventive agents, anti-inflammatory aspirin and antioxidant vitamin E, for 10 years did not reduce the risk of cancer. Also, long-term use of non-steroidal anti-inflammatory drugs, particularly COX-2 inhibitors, was recently found to have a severe side effect of an increased risk of cardiovascular disease. Calcium, vitamin D and folate are the three other remaining promising protective agents with convincing evidence in colorectal cancer. Unfortunately, a large randomized clinical trial (Women's Health Initiative, WHI) found that calcium plus vitamin D supplementation for seven years had no effect on the incidence of colorectal cancer. Very recently, the first clinical trial of folic acid found the intervention does not reduce recurrent colorectal adenoma risk. Moreover, folic acid supplementation might increase

the risk of colorectal neoplasia for subjects with adenoma.

Consistent with the disappointing findings for colorectal cancer, a number of large-scale clinical trials over the past half year reported no protective effects and, possibly even adverse effects in several cases, for supplementation with vitamins C, D, and E, selenium, calcium, and folate on not only colorectal cancer, but also prostate, breast or total cancer. The only beneficial effect was reported in a clinical trial conducted in China with supplementation in the Dietary Reference Intake (DRI) range. In a very recent commentary in *JNCI*, internationally renowned investigators in the field commented that growing evidence suggests that some nutrients or metals, including calcium, showed protective effects at normal range, but showed opposite effects at a high dose. Before the initiation of new large-scale intervention trials, it is essential to "rethink" the mechanisms, study design and dose-range of supplementation by conducting smaller-scale studies to understand nutrient-nutrient and gene-nutrient interactions, particularly in early tumorigenesis. Our proposed study is designed in such a manner to address many of the issues raised in the commentary, such as using biomarkers related to earlier tumorigenesis, supplementation of magnesium will not exceed the DRI for supplemental magnesium intake by more than 40 mg, supplementation dosage according to baseline dietary intake, nutrient-nutrient interaction (calcium/magnesium balance), and gene-nutrient interaction. Our findings may eventually lead to personalized strategies in the prevention of colorectal cancer and, perhaps, many other cancers or chronic diseases which are both linked to magnesium deficiency and common in Western societies.

2.0 Rationale and Specific Aims

High intake of calcium, particularly when intake of vitamin D is also high, may protect against both colorectal cancer and adenoma, however, results have been inconsistent. Three recent studies have linked a high intake of magnesium to a significantly reduced risk of colorectal cancer. Nevertheless, one recent cohort study has not found such an association. We reported recently in the Tennessee Colorectal Polyp Study (TCPS; P50CA95103) that intake of magnesium is related to a significantly reduced risk of adenoma and hyperplastic polyps. This inverse association primarily appeared among those with a low ratio of calcium to magnesium intake (Ca/Mg intake ratio). We have found similar results for intake of calcium. Very recently, we have confirmed these novel findings in a large clinical trial study (unpublished results). Furthermore, in the TCPS, we found that the Ca/Mg intake ratio significantly interacted with the common Thr1482Ile polymorphism (rs8042919, G→A) of the *TRPM7* gene, in relation to both adenomas and hyperplastic polyps**.** The *TRPM7* gene is involved in calcium and magnesium re(absorption) and homeostasis. We also found the associations between intakes of calcium or magnesium and risk of colorectal adenoma and hyperplastic polyps may differ by the Thr1482Ile polymorphism. Therefore, our findings may partially explain the inconsistency in previous studies of the association of calcium and magnesium with risk of colorectal cancer or adenoma.

To follow-up on these novel and promising findings, we propose to conduct an intervention trial using biomarkers linked to colorectal carcinogenesis. In the proposed study, we will enroll 288 colorectal adenoma or/and hyperplastic polyp patients, polyps free participants but being at high risk of colorectal polpys or cancer from the TCPS study, the TIARS, Vanderbilt University Hospital, or from other resources, who consume a high dietary Ca/Mg intake ratio and whose daily calcium consumption is equal to or greater than 700 mg. Furthermore, these participants will be chosen from among those for whom Thr1482Ile polymorphism (G→A) genotyping was conducted in our previous and ongoing studies. We will enroll 152 who carry the GG genotype and 136 who possess at least one variant A allele (i.e. either GA or AA genotype), 10 of whom carry the AA genotype.

The **primary specific aims** of this study are to (*hypotheses are described in Italics)*:

Aim 1: Conduct a randomized intervention trial (120 patients in each treatment or placebo arm) to evaluate the main effect of reducing the dietary Ca/Mg intake ratio to under 2.6 by magnesium supplementation on:

1) Primary endpoints: Increasing the expression of apoptosis biomarkers (e.g. TUNEL and Bax) and reducing expression of TRPM7, COX-2 (inflammation) and Ki-67 (proliferation index), pMLKL in colorectal mucosa.
2) Secondary endpoints: Increasing serum levels of magnesium and vitamin D and reducing plasma concentration of C-reactive protein. Reducing urinary excretion of prostaglandin E_2 metabolite (PGE-M). Increasing body magnesium store (magnesium tolerance test).

We hypothesize the reduction in the dietary Ca/Mg intake ratio among those with a high ratio may improve magnesium status, reduce magnesium transporter expression, reduce level of colorectal and systemic inflammation, increase colorectal apoptosis, reduce colorectal cell proliferation and, in turn, lead to a decreased risk of colorectal cancer.

Aim 2: Evaluate the interaction between magnesium treatment and the Thr1482Ile polymorphism (G→A). The intervention trial will be a 2-factor factorial design. The first factor is magnesium supplementation (modulation of the dietary Ca/Mg intake ratio). The second factor is the Thr1482Ile polymorphism genotype. Therefore, in either treatment or placebo arms (120 patients in each arm), we will randomly include 60 participants who carry the GG genotype and 60 participants who possess at least one A allele through genotype screening, then we will randomly assign them to the treatment or placebo arms with a 1:1 ratio. We expect to have about 10 subjects with AA genotype. For the AA carriers, the randomization will be stratified on genotype to ensure an equal number of AA genotypes will be assigned to the two arms.
We hypothesize that reducing the dietary Ca/Mg intake ratio will more significantly improve the body magnesium status and lead to more pronounced changes on primary endpoints among those who carry at least one variant A allele, particularly those with the AA genotype, compared to those who possess the GG genotype.
The focus of this proposed study is on the evaluation of gene-nutrient (Ca/Mg intake ratio) interaction in the development of colorectal cancer. The hypotheses proposed in the application are novel and based on strong biological plausibility and our recent findings from the TCPS and several other studies.

3.0 Animal Studies and Previous Human Studies

Early epidemiologic studies found that there was no or very little inverse association between calcium intake and colorectal cancer and adenoma. One recent pooled-analysis of 10 cohort studies suggested that the highest quintile of total calcium consumption from both dietary and supplemental sources may confer a 14% reduction in colorectal cancer risk versus the lowest quintile, with the association only being significant among people with high intake of vitamin D. Consistent with the results from these epidemiologic studies, two large intervention trials observed that calcium supplementation moderately reduced the risk of recurrent colorectal adenoma, while one trial found that this protective effect may only be present in those with a high blood level of vitamin D. Baron et al found in the observational phase of a clinical trial that the protective effect of calcium supplementation on colorectal adenoma recurrence rate extends up to 5 years after cessation of active treatment. However, results from a very recent large-scale randomized clinical trial (Women's Health Initiative, WHI) do not support an effect of calcium plus vitamin D supplementation on the incidence of colorectal cancer after 7 years of follow-up.

Four prospective studies conducted in Western societies have investigated the association between intake of magnesium and risk of colorectal cancer and found an inverse association in two studies, a reduced risk with colon cancer among overweight subjects in the third study, and no association in the fourth study.

Previous studies including both epidemiologic studies and clinical trials have generated inconsistent findings on the effects of calcium and magnesium intake for the prevention of colorectal cancer and adenoma. We found that the mean magnesium intake in the US population is not different from East Asian populations traditionally at low risk for colorectal cancer and other chronic diseases, while the Ca/Mg intake ratio is much higher. We also found in the TCPS that the inverse association between intakes of calcium and magnesium only appear among those with a low Ca/Mg intake ratio. Very recently, we confirmed this finding in a large clinical trial of colorectal adenoma recurrence. Furthermore, we found in the TCPS that the Ca/Mg intake ratio significantly interacts with the Thr1482Ile polymorphism (rs8042919, G→A) in the *TRPM7* gene, critical to calcium and magnesium (re)absorption and balance; and we also found that the protective effect of calcium and magnesium on the risk of colorectal adenoma may differ by the polymorphism. Based on these findings, the inconsistency on the associations between calcium and magnesium and risk of colorectal cancer could be due to: 1) Inter-person variation in the ability to absorb or reabsorb calcium or magnesium was not considered in any previous study; 2) Ca/Mg intake ratio has not been considered in any previous studies.

To further evaluate these novel findings, we propose *a novel personalized intervention trial* in which treatment (reducing Ca/Mg intake ratio) is based on the individual's baseline dietary Ca/Mg intake ratio. Furthermore, we will be able to examine the potential gene-treatment interaction. Tumorigenesis-related biomarkers, including apoptosis and proliferation index, COX-2 expression in rectal mucosa as well as magnesium status and urinary PGE-M, a non-invasive biomarker newly developed at Vanderbilt, will be used as primary endpoints in the proposed study. The progressive resistance to apoptosis is one hallmark for almost all cancer types. The apoptosis index is a strong predictor of future adenoma occurrence. The resistance to apoptosis is accompanied by an elevation in COX-2 expression during tumorigenesis. Macrophages are the primary cells generating COX-2 in adenoma. These cells are elevated as one of the earliest events during magnesium deficiency. We found in a cohort study that an increased level of urinary PGE-M was associated with a substantially increased risk of colorectal cancer. In addition, *no cohort or clinical trial study has examined the effect of supplementation of magnesium (modulating Ca/Mg intake ratio) on secondary endpoints, such as serum CRP and plasma vitamin D.*

4.0 Inclusion/Exclusion Criteria

We will recruit participants who are in general good health and able to participate in a low to moderate intensity supplement intervention. Additional exclusion criteria are intended to avoid potential side effects and control for potentially confounding variables that might limit our data analysis. This research will not involve fetuses, neonates, pregnant women, children, prisoners, institutionalized individuals, or others who may be considered a vulnerable population. Participants are anticipated to be between the ages of 40 to 85 years of age. Participants will be primarily recruited from two existing large adenoma studies, TCPS and TIARS, who agreed to be contacted for future studies. We will also recruit participants with colorectal adenoma or/and hyperplastic polyp diagnosed at Vanderbilt University Hospital. In addition, Participants with adenoma or/and hyperplastic polyp will be recruited from other resources, such as from those who contact us after they see the study advertisements including the information posted on the ClinicalTrials.gov website.

Inclusion Criteria:

- Hyperplastic polyp or/and Adenoma cases
- Polyps free participants with any of the following high risk of colorectal polyps or cancer: (1) family history of colorectal cancer or polyps; (2) current cigarette smoker; (3) obesity (BMI≥30 kg/m2); (4) low intake of fiber (lowest fiber intake quartile: daily intake <16.6g); (5) high intake of red meat and well-done or processed meat (mutageneity index ≥5852).
- Aged 40-85 years
- Consent to be contacted for future studies
- Participants with a calcium intake ≥ 700 mg/day measuring with 24 hour dietary recalls
- Participants with a calcium intake < 2000 mg/day measuring with 24 hour dietary recalls
- Participants with a calcium/magnesium intake ratio > 2.6
- Participants with known genotype for Thr1482Ile polymorphism in TRPM7
- Will live in Nashville or surrounding area for the next 6 months
- No history of Inflammatory bowel disease

Exclusion Criteria:

- Intolerance to magnesium glycinate or microcrystalline cellulose (placebo)
- Current breastfeeding
- Current or planned pregnancy
- Chronic renal diseases and hepatic cirrhosis
- Chronic ischemic heart disease with unstable angina, chronic heart failure at class III or IV, and myocardial infarction in the last 6 months
- Chronic diarrhea
- Type I diabetes mellitus
- Pituitary dwarfism
- Individuals with a history of colon resection or colectomy due to any reason
- Individuals with any history of cancer other than non-melanoma skin cancer
- Individual with history of any organ transplantation
- Individual with history of gastric bypass due to any reason
- Use of digoxin and licorice
- Current use of blood anticoagulant drugs such as Dicumarol (Warfarin), Clopidogrel (Plavix), Prasugrel HCl (Efficent), Ticlopidine (Ticlid), Lovenox (Enoxaparin), Fragmin (Dalteparin), Innohep (Tinzaparin), Eptifibatide (Integrilin), Tyrofiban (Aggrastat), and Abciximab (Reopro)
- Current use of lithium carbonate therapy (Eskalith, Lithobid, Lithonate, Lithotabs, Apo-Lithium carbonate, Apo-Lithium carbonate SR, Carbolth, Duralith, PMS-Lithium carbonate, PMS-Lithium citrate)
- Individuals with Inflammatory bowel disease
- Individuals if creatinine clearance is < 50
- Currently institutionalized
- Homeless individuals (address, telephone etc.)
- Unable to provide informed consent
- Any condition that in the opinion of the investigator raises concerns about protocol compliance

5.0 Enrollment/Randomization

Enrollment will require a process involving identification of eligible candidates for the magnesium intervention study, recruitment letter, telephone contact, and clinic eligibility and consent visit. Participants may be excluded at any of these four steps.

Eligibility and inclusion criteria must be met before a participant can be considered eligible for the study. Eligible participants will be identified based on the screening criteria by reviewing the data collected in the Tennessee Colorectal Polyp Study (TCPS) and Tennessee-Indiana Adenoma Recurrence Study (TIARS) and will be limited to participants who have previously provide written informed consent to be contacted about future research studies. Patients with colorectal adenoma or/and hyperplastic polyp diagnosed at Vanderbilt Hospital will also be identified for further screening. In addition, participants will be recruited from other resources, such as from those who contact us after they see the study advertisements including the information posted in the ClinicalTrials.gov website (ELIGIBILITY FORM 1, Appendix B). This generated list of potential participants will be delivered to the research staff for this new study. Participants still eligible after a review of the medical record for eligibility (ELIGIBILITY FORM 2, Appendix B) for an initial screening will be mailed an introductory letter signed by the parent study PI and another introductory letter signed by the PI and the gastroenterologist describing the study and inviting them to participate. A few days after the letter is mailed, the trained research staff of the Survey Research Shared Resource will call the potential participant to provide more detailed information about the study, answer questions about the study, and to see if they may be interested in participating. During the telephone conversation, a series of YES/NO screening questions are administered to determine eligibility for the intervention based on the exclusion criteria (Eligibility Form 3, Appendix B). If the person appears eligible, implied verbal consent will be obtained to conduct the baseline interview survey and 24 - hour dietary recalls prior to the first in-person visit. For potential participants from Vanderbilt University Hospital, an implied verbal consent will also be obtained to provide a mouthwash sample for genetic analysis before conducting 24 hour dietary recalls and baseline survey. If the person meets the study inclusion criteria, an appointment will also be made with the participant for the baseline in-person visit. The purpose of this visit is to orient the participant to the study protocol, further evaluate interest in participation, and to obtain written informed consent. The research staff member describes the purpose of the study, the study design, and the biospecimen and data collection protocols. The risks and benefits of the study are discussed prior to obtaining signatures on the consent form. Participants will be told that participation is voluntary. The candidate has the opportunity to read the consent form, ask questions, and discuss any aspect of the study. The candidate is asked to sign the consent form, and the participant will be provided a copy of their signed form. We will recruit and randomly assign participants to magnesium supplementation (treatment) and placebo arms until these participants have completed the entire study process. (See details in section 6.0)

This study will enroll 288 participants, 152 of whom have the GG genotype and 136 of whom have GA/AA genotype. Within the two genotype groups, a permuted-block randomization algorithm will be used to allocate the subjects into the treatment/placebo arm. These participants will be randomized to either treatment or placebo using a double-blind two-factorial (Ca/Mg intake ratio and genotype) design so that each treatment/placebo arm will contain 60 participants with the GG genotype and 60 participants with at least one A allele. (See details in Section 6.0)

Once our study is approved by Vanderbilt IRB, we will register our trial with ClinicalTrials.gov via a web based data entry system called the Protocol Registration System (PRS). Our trial will be registered in full not later than 21 days after the first patient is enrolled.

6.0 Study Procedures

To clearly describe each procedure of the intervention trial, we provide all of the study protocols as follows.

A. Protocol Summary

1. Protocol Summary
2. Study Procedure Calendar (Appendix A)

B. Eligibility and Recruitment

1. Eligibility and Recruitment Protocol
2. Eligibility forms (Appendix B)
    1) Eligibility Form 1 --- TCPS Data or Electronic Medical Record Data Abstraction
    2) Eligibility Form 2 --- Medical Record Abstraction
    3) Eligibility Form 3 --- Recruitment Eligibility (*for TCPS participants, for TIARS participants, and non-TCPS participants respectively*)
3. Introductory Letters for TCPS participants, TIARS participants, and *non-TCPS participants from Vanderbilt University Hospital* (Appendix B)
4. Letter of collecting mouthwash sample for *non-TCPS participants* (Appendix B)
5. Instruction for collecting mouthwash sample for *non-TCPS participants* (Appendix B)
6. Script for collecting mouthwash sample for *non-TCPS participants*(Appendix B)
7. Script for Post Clinic Visit #1 Ineligibility Call (Appendix B)
8. IRB Application Form --- Form # 1100
9. Standard Informed Consent Form

C. Dietary Intake Assessment

1. Dietary Assessment Protocol
2. Script for 24 hour Dietary Recall (Appendix C)
3. Food Amount Booklet and letter (Appendix C)
4. Letter for Record Assisted Dietary Recall (Appendix C)
5. Food Record Instructions (Appendix C)
6. Food Record Form (Appendix C)
7. Script for Record Assisted Dietary Recall (Appendix C)
8. Telephone script and letter for the eligible participant post the 2nd 24h dietary recall (Appendix C)

D. Baseline Interview

1. Baseline Telephone Questionnaire in combination with scheduling Clinic Visit Appointment (Appendix D)

2. Clinic Visit Schedule Form (Appendix D)

E. Randomization and Intervention

1. Randomization Protocol
2. Intervention Protocol
3. Formulas (Appendix E)
   1) Magnesium Glycinate 515mg
   2) Magnesium Glycinate 515mg Placebo
   3) Magnesium Glycinate 694mg
   4) Magnesium Glycinate 694mg Placebo
   5) Magnesium Glycinate 489mg
   6) Magnesium Glycinate 489mg Placebo
4. Instruction Sheet for Taking Pills (Appendix E)

F. Clinical Visits

1. Clinic Visits
   1. Study Procedures for each Clinic Visit
   2. Clinic Visit Reminder Call Scripts (Appendix F)
      - Reminder Call Script for Clinic Visit #1
      - Reminder Call Script for Clinic Visit #2
      - Reminder Call Script for Clinic Visit #3
      - Reminder Call Script for Clinic Visit #4
   3. Study Activity Calendar (Appendix F)
   4. Clinic Progress Notes (Appendix F)
   5. Montreal Cognitive Assessment Forms
      - Montreal Cognitive Assessment Form for Clinical Visit #1
      - Montreal Cognitive Assessment Form for Clinical Visit #3
      - Instructions for Cognitive Assessment
   6. Health Assessment Forms
      - Health Assessment Form for Clinical Visit #1
      - Health Assessment Form for Clinical Visit #2
      - Health Assessment Form for Clinical Visit #3
      - Health Assessment Form for Chinese Participants
   7. Email Script for Chinese Participants
2. Biological Specimen Collection Protocols

   --- Rectal biopsy

   1. Biopsy Procedures and Initial Handling Protocol
   2. Study Notes
      - Study Note for Dr. Ness

- Study Note for Dr. Seidner

3. Participant Instruction --- Post Biopsy Safety Handout (Appendix G)

--- Blood

1. Blood Draw Protocol

--- Urine

1. Urine Collection and Handling Protocol

--- Stool

1. Stool Collection and Handling Protocol

--- Saliva

1. Saliva Collection and Handling Protocol

--- Hair

1. Hair Collection and Handling Protocol

--- Skin Swab

1. Skin Swab Collection and Handling Protocol

--- Nail

1. Nail Collection and Handling Protocol

3. Instructions for Urine, Stool, and Saliva Samples Collection, Clinic Visit Questionnaire Form and other Questionnaire Forms (Appendix G)
    1. Instruction Booklet Cover sheet
    2. Instruction for Urine Sample Collection
    3. Instruction for Stool Sample Collection
    4. Instruction for Saliva Sample Collection
    5. Instruction for 24 Hour Urine Collection
    6. Clinic Visit Questionnaire Form
    7. Participant Folder Contents and Instructions
    8. Diet Questionnaire
        - Diet Questionnaire for Clinic Visit #1
        - Diet Questionnaire for Clinic Visit #3
    9. Health Questionnaire
        - Health Questionnaire for Clinic Visit #1
        - Health Questionnaire for Clinic Visit #2
        - Health Questionnaire for Clinic Visit #3
        - Health Questionnaire for Chinese Participants
        - Health Questionnaire for Chinese Participants (Chinese version)
    10. Probiotics Questionnaire
        - Probiotics Questionnaire for Clinical Visit #1

- Probiotics Questionnaire for Clinical Visit #2
- Probiotics Questionnaire for Clinical Visit #3

4. Biological Specimen Collection Forms (Appendix H)
    1. Biological Specimen Collection Form H_1
    2. Biological Specimen Collection Form H_2
    3. Biological Specimen Collection Form H_3
    4. Biological Specimen Collection Form H_4

G. Data and Safety Monitoring Plan

1. Safety and Compliance Monitoring Protocol
2. Phone Script for Magnesium Supplementation Safety and Compliance Monitoring (Appendix I)
3. Data and Safety Monitoring Plan

H. Payment

1. Compensation Protocol
2. Request for Payment Form and W8 Form ((Appendix J)

I. Data Analysis

1. Data Analysis Protocol

A. Protocol Summary

The goal of this study is to evaluate the main effect of reducing the dietary Ca/Mg intake ratio to under 2.6 by magnesium supplementation on inflammatory biomarkers and magnesium status and to estimate the potential interaction between magnesium treatment and the Thr1482Ile polymorphism (G→A) of the *TRPM7* gene. The study will enroll 288 participants with colorectal hyperplastic polyp or/and adenoma, polyps free but being at high risk of colorectal polyps or cancer. 152 of whom have the GG genotype and 136 of whom have at least one A allele including10 of whom have the AA genotype. Participants will be randomized to receive either magnesium supplementation (at least 120 participants) or placebo (at least 120 participants) using a double-blind two-factorial (Ca/Mg intake ratio and genotype) design such that each treatment arm will contain 60 participants with the GG genotype and 60 participants with at least one A allele.

Participants will complete 24-hour dietary recalls and collection of rectal biopsy, blood and urine samples, and possibly collection of stool, rectal swab, hair, nail and saliva samples at baseline, and at the end of treatment, and will complete study questionnaires at baseline, throughout the treatment, and at the conclusion of treatment. Questionnaire data to be collected include diet, medication use, health history, and other cancer risk factors. Biospecimens collected at baseline and at the end of intervention include a rectal biopsy sample, a first morning urine specimen, a blood sample (possibly fasting), stool, rectal swab, hair, nail and saliva. An additional collection of a blood sample, a urine sample, stool, rectal swab, and saliva will be done at the midpoint of the intervention phase. Medical records will be reviewed for hyperplastic polyp or/and adenoma pathology information at baseline. In addition to two baseline 24-hour dietary recall assessments, two 24-hour dietary recalls will be conducted during weeks 1 - 6, and two at weeks 7 - 12 of the trial. The research staff in the SRSR will also contact the participants to monitor for any adverse events and the study compliance, 12 times in total (4 times together with 24- hour dietary recalls). The duration of the study from recruitment through the end of the intervention is approximately 14 weeks including a 12-week intervention.

Throughout the course of the introductory and intervention phases, the participant will have multiple contacts with the study staff including both in-person and telephone contacts. Provided below are details related to each of these contacts and time frame of this study. Participants will be compensated $250 -300 for participation at the end of the study depending upon the participant's provision of fasting blood collection.

Table A. Participant Study Protocol

	Baseline		Week of Intervention											
	-0	0	1	2	3	4	5	6	7	8	9	10	11	12
Introduction Letter	X													
Telephone Contact														
Questionnaire	X													
24 hr Dietary Recall(s)*	XX				XX						XX			
*Monitoring/Compliance**					XXX						XXX			
In-person visit														
Questionnaires	X	X						X						X
*Monitoring/Compliance**								X						X
Rectal Biopsy Sample		X												X
Rectal Swab		X												X
Blood Sample		X						X						X
Urine Sample		X						X						X
Stool Sample		X						X						X
Saliva Sample		X						X						X
Skin Swab Sample		X						X						X
Hair Sample		X												X
Nail Sample		X												X
Health Assessment		X						X						X
Magnesium Tolerance Test														X
Supplementation		X	X	X	X	X	X	X	X	X	X	X	X	X
Compensation														X

*Phone safety and compliance monitoring × 6 (3 times at wks 1-6 and 3 times at wks 7-12), dietary recall × 4 (2 times at wks 1-6 and 2 times at wks 7-12), and In-person visit × 3 (wk 1, 6, 12).

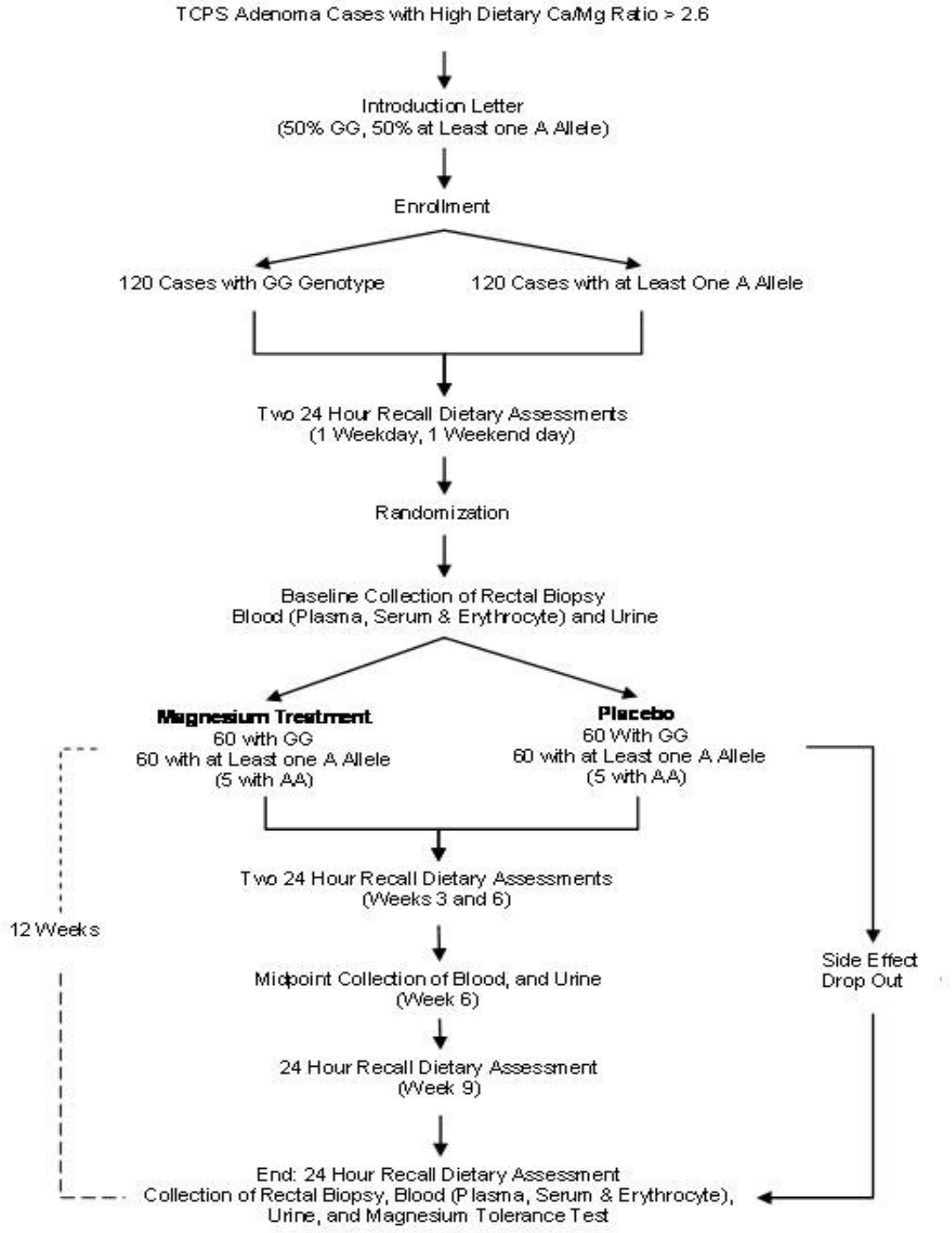


Figure A. The panned study design

B. Eligibility and Recruitment

Eligibility and Recruitment Protocol

Eligibility ascertainment will require a process involving identification of eligible candidates for the magnesium intervention study, recruitment letter, telephone contact, and clinic eligibility and consent visit. Participants may be excluded at any of these four steps.

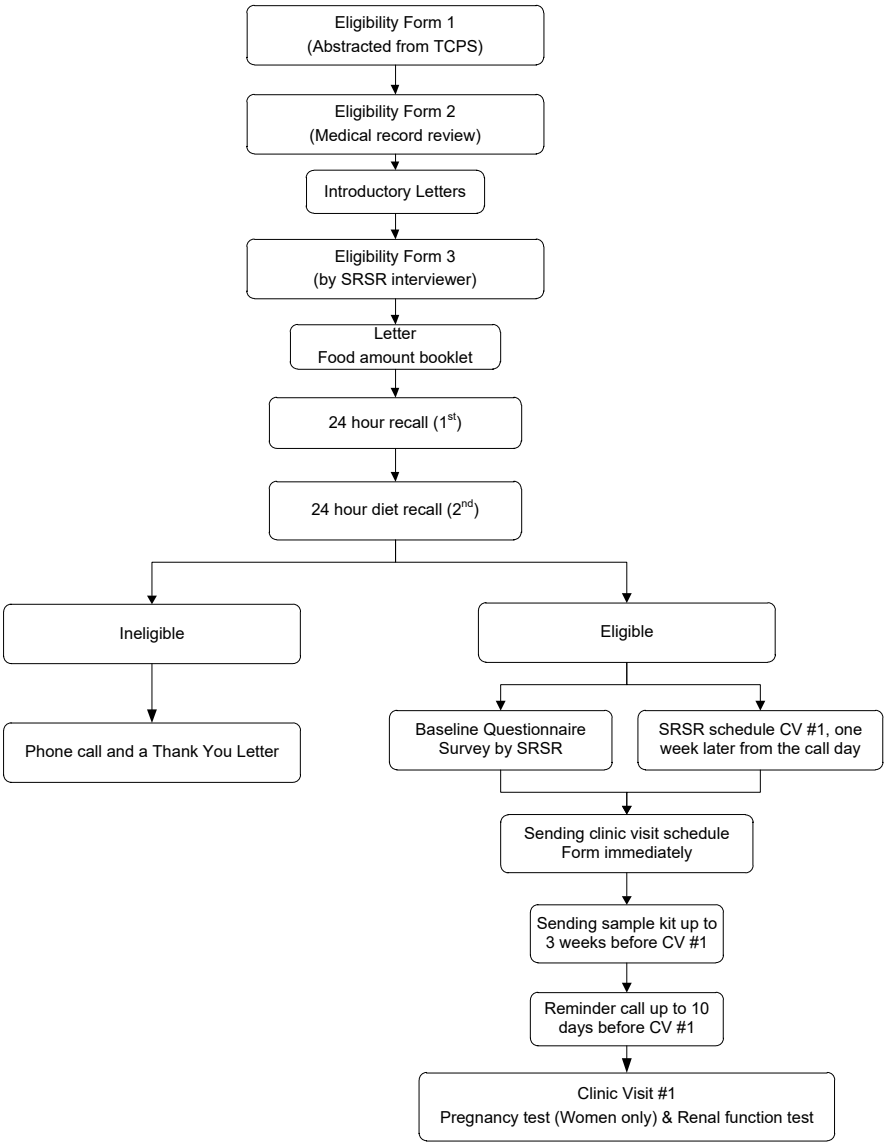
We will recruit participants who are in general good health and able to participate in a low to moderate intensity supplement intervention. Additional exclusion criteria are intended to avoid potential side effects and control for potentially confounding variables that might limit our data analysis. . This research will not involve fetuses, neonates, pregnant women, children, prisoners, institutionalized individuals, or others who may be considered a vulnerable population. Participants are anticipated to be between the ages of 40 to 85 years of age. Participants will be primarily recruited from two existing large adenoma studies, TCPS and TIARS, who agreed to be contacted for future studies. We will also recruit participants from patients with colorectal adenoma or/and hyperplastic polyp diagnosed at Vanderbilt University Hospital if needed.

Eligibility and inclusion criteria must be met before a participant can be considered eligible for the study. Eligible participants will be identified based on the screening criteria by reviewing the data collected in the Tennessee Colorectal Polyp Study (TCPS, IRB # 090235) or in the Tennessee Indiana Adenoma Recurrence Study (TIARS, IRB # 020462), and will be limited to participants who have previously provide written informed consent to be contacted about future research studies. Eligible participants will also be identified based on the screening criteria by reviewing the Electronic Medical Record data from the Vanderbilt University Hospital. In addition, participants from other resources will be recruited to screen for the trial, such as from those who contact us after they see the study advertisements including the information posted on the ClinicalTrials.gov website. This generated list of potential participants will be delivered to the research staff for this new study. Participants still eligible after a review of the medical record for eligibility (ELIGIBILITY FORM 2, Appendix B) for an initial screening will be mailed an introductory letter signed by the parent study PI and another introductory letter signed by the PI and the gastroenterologist describing the study and inviting them to participate. A few days after the letter is mailed, the trained research staff of the Survey Research Shared Resource will call the potential participant to provide more detailed information about the study, answer questions about the study, and to see if they may be interested in participating. During the telephone conversation, a series of YES/NO screening questions are administered to determine eligibility for the intervention based on the exclusion criteria (RECRUITMENT ELIGIBILITY AND BASELINE INTERVIEW FORM 3, Appendix B). If the person appears eligible, implied verbal consent will be obtained to conduct the baseline interview survey and 24 - hour dietary recalls prior to the first in-person visit. For the eligible participants from Vanderbilt University Hospital, implied verbal consent will be obtained to collect mouthwash sample for genetic analysis and then to conduct 24 hour dietary recalls and a baseline interview survey. An appointment will also be made with the participant for the baseline in-person visit. The purpose of this visit is to orient the participant to the study protocol, further evaluate interest in participation, and to obtain written informed consent. The research staff member describes the purpose of the study, the study design, and the biospecimen and data collection protocols. The risks and benefits of the study are discussed prior to obtaining signatures on the consent form. Participants will be told that participation is voluntary. The candidate has the opportunity to read the consent form, ask questions, and discuss any aspect of the study. The candidate is asked to sign the consent form, and the participant will be provided a copy of their signed form. We will recruit and randomly assign participants to magnesium supplementation (treatment) and placebo arms until these participants have completed the entire study process.

Procedures

A. Recruitment procedures for participants from the existing adenoma studies, TCPS and TIARS.

PPCCT Recruitment Procedures
(For TCPS participants)



1. Identification of eligible candidates from those who had participated in TCPS or in TIARS. TCPS or TIARS research staff will provide the PPCCT research staff with a list of eligible candidates based on the screening inclusion criteria (ELIGIBILITY FORM 1, Appendix B).
   Screening Criteria:
   - Hyperplastic polyp or/and Adenoma cases
   - Polyps free participants with any of the following high risk of colorectal polyps or cancer: (1) family history of colorectal cancer or polyps; (2) current cigarette smoker; (3) obesity (BMI≥30 kg/m2); (4) low intake of fiber (lowest fiber intake quartile: daily intake <16.6g); (5) high intake of red meat and well-done or processed meat (mutageneity index ≥5852).
   - Aged 40-85 years
   - Consent to be contacted for future studies

- Prioritize participants with a calcium intake ≥ 600 mg/day. Participants with a calcium intake <600 mg/day may be considered
- Prioritize participants with a calcium intake < 2000 mg/day. Participants with a calcium intake ≥2000 mg/day may be considered
- Prioritize participants with a historical calcium/magnesium intake ratio > 2.6. Participants with a historical intake ratio <= 2.6 may be considered to measure their current dietary intake. If the participant's intake levels of calcium and magnesium are unknown, two 24-hour dietary recalls will be conducted to evaluate his/her current calcium and magnesium intakes, to determine eligibility.
- Participants with known genotype for Thr1482Ile polymorphism in TRPM7

2. PPCCT research staff will initiate a participant eligibility form.
    - The eligibility form will document:
    - Name
    - Date of birth
    - Age
    - Sex
    - Identification Number in TCPS or TIARS
    - Date of enrollment in the TCPS or TIARS
    - Daily calcium intake
    - Daily magnesium intake
    - Dietary calcium/magnesium intake ratio
    - Race
    - Mailing address
    - Telephone number
3. PPCCT research staff will review medical record and record the potential participant's current address and contact information (ELIGIBILITY FORM 2, Appendix B).

    Exclusion Criteria:
    - Intolerance to magnesium glycinate or microcrystalline cellulose (placebo)
    - Current breastfeeding
    - Current or planned pregnancy
    - Chronic renal diseases and hepatic cirrhosis
    - Chronic ischemic heart disease with unstable angina, chronic heart failure class III or IV, and myocardial infarction in the last 6 months
    - Chronic diarrhea
    - Type I diabetes mellitus
    - Pituitary dwarfism
    - Medication use
        - Current use of digoxin and licorice
        - Current use of blood anticoagulant drugs such as Dicumarol (Warfarin), Clopidogrel (Plavix), Prasugrel HCl (Efficent), Ticlopidine (Ticlid), Lovenox (Enoxaparin), Fragmin (Dalteparin), Innohep (Tinzaparin), Eptifibatide (Integrilin), Tyrofiban (Aggrastat), and Abciximab (Reopro)
        - Current use of lithium carbonate therapy (Eskalith, Lithobid, Lithonate, Lithotabs, Apo-Lithium carbonate, Apo-Lithium carbonate SR, Carbolth, Duralith, PMS-Lithium carbonate, PMS-Lithium citrate)
    - Individuals with a history of colon resection or colectomy due to any reason
    - Individuals with any history of cancer other than non- melanoma skin cancer
    - Individual with history of any organ transplantation

- Individual with history of gastric bypass due to any reason
- Individuals with any history of cancer other than non- melanoma skin cancer
- Individuals with Inflammatory bowel disease

4. PPCCT research staff will mail a recruitment letter describing study and invitation to potential participants.
    - Mail eligible candidates an introductory letter from the parent study PI (Appendix B) and another introductory letter from the current study PI and the gastroenterologist (Appendix B) describing the study and inviting them to participate.
    - A study project telephone number and email address are included with the recruitment letter.
    - Letters will be mailed in waves such that 50% of candidates carry the GG genotype and the other 50% possess the GA or AA genotype. We will repeat this procedure with subsequent waves until 120 with the GG genotype and 120 with either the GA or AA genotype complete the study process.
    - In the meanwhile the introductory letters will be sent to the potential participants by email.
5. PPCCT research staff will place a follow-up phone call (RECRUITMENT ELIGIBILITYFORM 3, Appendix B) beginning seven days after the letter is sent to answer questions, evaluate interest and determine the participants' potential eligibility.
    - Attempts will be made to telephone each participant for the purposes of recruitment
        - Attempt includes leaving a message on an answering machine or with a household member to call regarding study.
    - Answer questions about the study
        - Verify preliminary contact information
        - Response of participant
    - Preliminary screening questions. The screening questionnaire (RECRUITMENT ELIGIBILITY FORM 3, Appendix B) will be administrated to determine the eligibility for the intervention.
        - Evaluate interest in participating
        - Possibility to come to Vanderbilt Clinic Research Center
        - Availability for the duration of the study
        - Ask preliminary eligibility questions to determine eligibility to the study
        - Review screening Inclusion / Exclusion Criteria

          Exclusion Criteria:
            - Intolerance to magnesium glycinate or microcrystalline cellulose (placebo)
            - Current breastfeeding
            - Current or planned pregnancy
            - Chronic renal diseases and hepatic cirrhosis
            - Chronic ischemic heart disease with unstable angina, chronic heart failure class III or IV, and myocardial infarction in the last 6 months
            - Chronic diarrhea
            - Type I diabetes mellitus
            - Pituitary dwarfism
            - Current use of digoxin, and licorice
            - Current use of blood anticoagulant drugs such as Dicumarol (Warfarin), Clopidogrel (Plavix), Prasugrel HCl (Efficent), Ticlopidine (Ticlid), Lovenox (Enoxaparin), Fragmin (Dalteparin), Innohep (Tinzaparin), Eptifibatide (Integrilin), Tyrofiban (Aggrastat), and Abciximab (Reopro)

- Current use of lithium carbonate therapy (Eskalith, Lithobid, Lithonate, Lithotabs, Apo-Lithium carbonate, Apo-Lithium carbonate SR, Carbolth, Duralith, PMS-Lithium carbonate, PMS-Lithium citrate)
- Individuals with a history of colon resection or colectomy due to any reason
- Individuals with any history of cancer other than non-melanoma skin cancer
- Individual with history of any organ transplantation
- Individual with history of gastric bypass due to any reason
- Individuals with Inflammatory bowel disease
- Currently institutionalized
- Homeless individual (address, telephone etc.)
- Unable to provide informed consent
- Any condition that in the opinion of the investigator raises concerns about protocol compliance

  - If candidate is not eligible, no further contact is required. Eligibility status is recorded.
  - If the candidate does not consent to further contact, no further contact is required. Refusal status will be recorded.
  - If candidates eligible, but he/she is on another clinical trial, we will call back after he/she completes the current clinic trial.
  - If candidate is eligible after the Recruitment Eligibility Form_3 interview, continue with the following procedures.

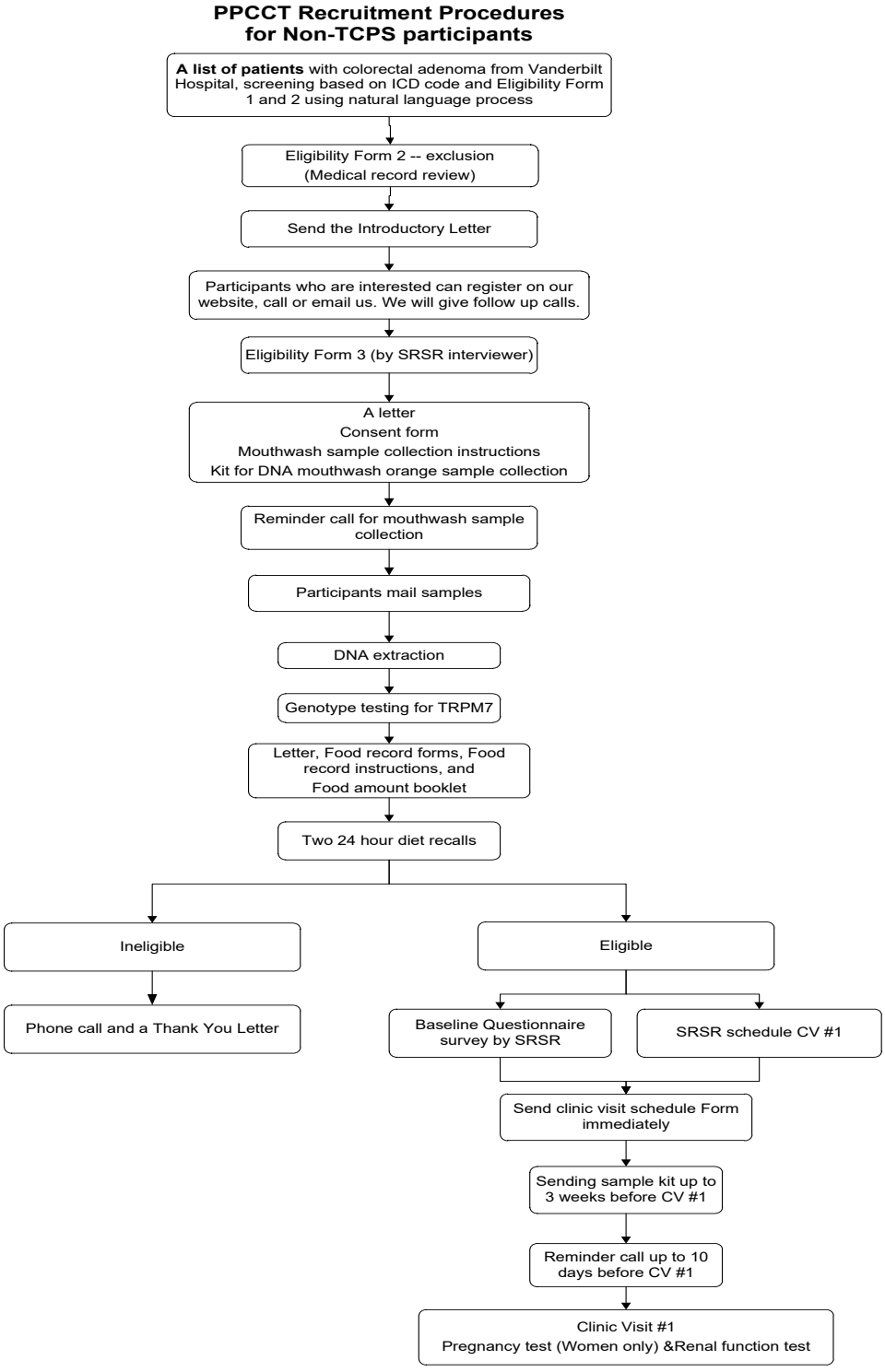
6. PPCCT research staff will mail a Foods Amount booklet and place phone calls (Script for 24 Hour dietary Recall, Appendix C) beginning seven days after the letter is sent to perform two 24 hour recalls and further determine the participants' eligibility.
   - If candidate is eligible after the Recruitment Eligibility Form_3 interview:
     - Verify and collect all contact information
     - Explain the study protocol in lay terminology
     - Send a food amounts booklet mail to the participant candidate.
     - Conduct or schedule appointment for two 24 hour dietary recalls (one on weekday and the other during weekend).
     - Candidates with a calcium intake ≥ 700 mg/day and < 2000 mg/day and also with a calcium/magnesium ratio>2.6 will be considered eligible and recruited into the study.
     - If candidate is not eligible after the 2nd recalls, SRSR interviewer will notify of his/her eligibility. A thank you letter will be mailed. Eligibility status is recorded.
     - If candidate is eligible after the 2nd diet recall, SRSR interviewer will schedule or conduct a Baseline Questionnaire interview.
     - SRSR interviewer will schedule in-person clinic visit #1 and #3. The date for clinic visit #1 should be more than **one week** later from the call day. The date for clinic visit #3 is the end of week of 12 or the beginning of week 13.
     - Send a mail containing the Clinic Visit Schedule Form to Participants **immediately** after the eligibility confirmed after the 2nd diet recall.
     - Sample collection kit package will be mailed **up to 3 weeks** before clinic visit #1. It includes
       a) kits for urine, stool, and saliva sample collection
       b) an instruction booklet
       c) clinic visit questionnaire form
       d) clinic visit schedule form, and
       e) diet questionnaire form for clinical visit #1

f) health questionnaire form for clinical visit #1
g) Probiotics questionnaire for clinical visit #1
h) Informed consent form
i) Participant's folder #1 contents

- Reminder call for scheduled clinic appointment will be made **up to 10 days** before the first clinic visit (REMINDER CALL SCRIPT for CLINIC VISIT #1, Appendix F)
  a) Remind not to eat anything approximately 8 hours before clinic visit except a glass of water.
  b) Remind to collect urine, saliva, and stool samples at home the day before clinic visit.
  c) Remind to bring all medications and supplements to this appointment. Research staff will record what medications and supplements are being taken

7. Clinic eligibility and consent visit
   - Visit will be at Vanderbilt Clinical Research Center, Medical Center North 3rd Floor.
   - The PPCCT research staff member will meet with the participant and describe the study protocol in lay terminology, including the purpose of the study, the study design and the biospecimen and data collection protocols in the study.
   - The risks and benefits of the study are discussed prior to obtaining signatures on the consent form. The participant will be told that participation is voluntary
   - Further evaluate the interest in participation.
   - Review Inclusion / Exclusion Criteria.
   - The research staff will provide ample time for the participant to have questions answered prior to enrollment.
   - Obtain written informed consent.
   - The research staff will also provide the study participant with a copy of the consent form.
   - During the clinic visit, pregnancy test will be done on the female candidates. The participants with a positive pregnancy test will be informed of their ineligibility to participate this study.
   - 5 ml of blood from the participant will be drawn in a green top tube and will be delivered to the Vanderbilt hospital laboratory to measure serum creatinine level for renal function assessment.
8. Renal Function Assessment
   - The PPCCT research staff will abstract the lab result of serum creatinine level from the StarPanel approximately 2 hours after the blood sample is delivered to the hospital lab.
   - The estimated Glomerular Filtration Rate (eGFR) for each participant will be calculated based on the serum creatinine level, the gender, age, and the race of the participant.
   - The participants with an eGFR < 50 will be ineligible for this study.
9. Exclusion of participants with decreased renal function and positive pregnancy test (women only)
   - On the next day following the clinic visit #1, the research staff will call the participant with eGFR < 50 and inform him/her of his/her ineligibility to participate this study due to the decreased renal function or positive pregnancy test if a participant is a woman (SCRIPT for POST CLINIC VISIT #1 INELIGIBILITY CALL, Appendix B).

B. Recruitment Procedures for patients with adenoma or/and hyperplastic polyp from the Vanderbilt University Hospital



To recruit more participants into the trial, we will expand the candidate pool by identifying the potential participants from patients with adenoma or/and hyperplastic polyp diagnosed at Vanderbilt University Hospital.

Recruitment Procedures for patients with colorectal adenoma or/and hyperplastic polyp from Vanderbilt University hospital are described as follows:

1. Identification of eligible candidates from patients with colorectal adenoma or/and hyperplastic polyps from Vanderbilt University Hospital.
   - A list of patients with colorectal adenoma or/and hyperplastic polyp will be created from the Vanderbilt Electronic Medical Record Database based on the screening inclusion criteria.

   The Research Derivative is a database of clinical and related data derived from the Medical Center's clinical systems and restructured for research. Data is repurposed from VU's enterprise data warehouse, which includes data from StarPanel, VPIMS, and ORMIS (Operating Room Management Information System), EPIC, Medipac, and HEO among others. The medical record number and other person identifiers are preserved within the database. Data types include reimbursement codes, clinical notes and documentation, nursing records, medication data, laboratory data, encounter and visit data, among others. Output may include structured data points, such as ICD 9 codes or encounter dates, semi-structured data such as laboratory tests and results, or unstructured data such as physician progress reports. The database is maintained by the Office of Research Informatics under the direction of Paul Harris, PhD.

   Screening Criteria:
   1. Age today between 40-85 inclusive
   2.
   3. SNOMED codes fitting both of the following criteria:
      a. Topography code of 67 or 68 (colon or rectum) T67000 or T68000
      b. Morphology code of one of the following:

M81400	adenoma (NOS)
M82100	adenomatous polyp (NOS)
M82110	tubular adenoma
M82610	villous adenoma (SNOMED-3)
M82611	villous adenoma (SNOMED-2)
M82630	tubulovillous adenoma
M74000, M74001, M74002, MGA373, MGF500	dysplastic adenoma
M82130, MZ0366	Serrated adenomatous polyp

   4. ICD-9/10 diagnosis code, CPT code or other code will also be used if needed.

5. No SNOMED or ICD-9/10 for a cancer other than non-melanoma skin cancer
6. Individual is not known deceased
7. Individual does not have a personal history of any of the following conditions:
    a. Chronic heart failure
    b. Chronic renal disease
    c. Hepatic cirrhosis
    d. Inflammatory bowel disease
    e. Colectomy
    f. Organ transplantation
    g. Type I diabetes mellitus
    h. Pituitary dwarfism
    i. Gastric bypass

- PPCCT research staff will initiate a participant eligibility form (ELIGIBILITY FORM 1, Appendix B).

The eligibility form will document:

- Name
- Date of birth
- Age
- Sex
- Race
- Medical record number
- Mailing address
- Telephone number

2. PPCCT research staff will review the participants' medical records to confirm their diagnosis of colorectal adenoma or/and hyperplastic polyp.

3. PPCCT research staff will review medical record and record the potential participant's current address and contact information (ELIGIBILITY FORM 2, Appendix B).
   Exclusion Criteria:
    - Intolerance to magnesium glycinate or microcrystalline cellulose (placebo)
    - Current breastfeeding
    - Current or planned pregnancy
    - Chronic renal diseases and hepatic cirrhosis
    - Chronic ischemic heart disease with unstable angina, chronic heart failure class III or IV, and myocardial infarction in the last 6 months
    - Chronic diarrhea
    - Type I diabetes mellitus
    - Pituitary dwarfism
    - Medication use
        - Current use of digoxin and licorice
        - Current use of blood anticoagulant drugs such as Dicumarol (Warfarin), Clopidogrel (Plavix), Prasugrel HCl (Efficent), Ticlopidine (Ticlid), Lovenox (Enoxaparin), Fragmin (Dalteparin), Innohep (Tinzaparin), Eptifibatide (Integrilin), Tyrofiban (Aggrastat), and Abciximab (Reopro)
        - Current use of lithium carbonate therapy (Eskalith, Lithobid, Lithonate, Lithotabs, Apo-Lithium carbonate, Apo-Lithium carbonate SR, Carbolth, Duralith, PMS-Lithium carbonate, PMS-Lithium citrate)
    - Individuals with a history of colon resection or colectomy due to any reason

- Individuals with any history of cancer other than non- melanoma skin cancer
- Individual with history of any organ transplantation
- Individual with history of gastric bypass
- Individuals with any history of cancer other than non- melanoma skin cancer
- Individuals with Inflammatory bowel disease

4. PPCCT research staff will mail a recruitment letter describing study and invitation to potential participants.
   - Mail eligible candidates an introductory letter from the study PI and the gastroenterologist (Introductory letter III, Appendix B) describing the study and inviting them to participate.
   - A study project telephone number and email address are included with the recruitment letter.
   - In the meanwhile the introductory letter will be sent to participants by email.

5. PPCCT research staff will place a follow-up phone call (*RECRUITMENT ELIGIBILITYFORM 3 for non-TCPS participants*, Appendix B) beginning seven days after the letter is sent to answer questions, evaluate interest and determine the participants' potential eligibility.
   - Attempts will be made to telephone each participant for the purposes of recruitment
     - Attempt includes leaving a message on an answering machine or with a household member to call regarding study.
   - Answer questions about the study
     - Verify preliminary contact information
     - Response of participant
   - Preliminary screening questions. The screening questionnaire (RECRUITMENT ELIGIBILITY FORM 3 for *non-TCPS participants*, Appendix B) will be administrated to determine the eligibility for the intervention.
     - Evaluate interest in participating
     - Possibility to come to Vanderbilt Clinic Research Center
     - Availability for the duration of the study
     - Ask preliminary eligibility questions to determine eligibility to the study
     - Review Inclusion / Exclusion Criteria

       Exclusion Criteria:
       - Intolerance to magnesium glycinate or microcrystalline cellulose (placebo)
       - Current breastfeeding
       - Current or planned pregnancy
       - Chronic renal diseases and hepatic cirrhosis
       - Chronic ischemic heart disease with unstable angina, chronic heart failure class III or IV, and myocardial infarction in the last 6 months
       - Chronic diarrhea
       - Type I diabetes mellitus
       - Pituitary dwarfism
       - Current use of digoxin, and licorice
       - Current use of blood anticoagulant drugs such as Dicumarol (Warfarin), Clopidogrel (Plavix), Prasugrel HCl (Efficent), Ticlopidine (Ticlid), Lovenox (Enoxaparin), Fragmin (Dalteparin), Innohep (Tinzaparin), Eptifibatide (Integrilin), Tyrofiban (Aggrastat), and Abciximab (Reopro)

- Current use of lithium carbonate therapy (Eskalith, Lithobid, Lithonate, Lithotabs, Apo-Lithium carbonate, Apo-Lithium carbonate SR, Carbolth, Duralith, PMS-Lithium carbonate, PMS-Lithium citrate)
- Individuals with a history of colon resection or colectomy due to any reason
- Individuals with any history of cancer other than non-melanoma skin cancer
- Individual with history of any organ transplantation
- Individual with history of gastric bypass
- Individuals with Inflammatory bowel disease
- Currently institutionalized
- Homeless individual (address, telephone etc.)
- Unable to provide informed consent
- Any condition that in the opinion of the investigator raises concerns about protocol compliance

  - If candidate is not eligible, no further contact is required. Eligibility status is recorded.
  - If the candidate does not consent to further contact, no further contact is required. Refusal status will be recorded.
  - If candidate is eligible after the Recruitment Eligibility Form_3 interview, continue with the following procedures.

6. PPCCT research staff will mail an envelope including a kit for collecting mouthwash sample, Instructions, Consent form and a letter to participants, and place phone calls (Script for mouthwash sample collection, Appendix B) beginning seven days after the letter is sent to collect mouthwash samples from participants for genetic analysis.

   - PPCCT staff will mail an envelope to the potential participants. Inside the envelope are a kit for collecting mouthwash sample, instructions, consent form, and a letter.
   - Participants will collect and mail the mouthwash samples to Vanderbilt Epidemiology Center.
   - SRSR interviewers will place a follow-up phone call and walk the participants through the mouthwash sample collection if they have not sent the samples to Vanderbilt Epidemiology Center.
   - Genetic analysis will be performed at Vanderbilt Molecular Epidemiology Center. It may take 6 to 8 months to complete the genetic analysis.

7. Once we have the genetic information for the potential participants recruited from Vanderbilt University hospital and find out they are eligible after the medical record review, continue with the procedure 6 on page 19 to the procedure 9 on page 22 in section A.

C. Recruitment Procedures for participants from other resources

To recruit more participants into the trial, we will recruit participants from other resources, including from those who contact us after they see the study advertisements such as the information posted on the ClinicTrials.gov website.

I). For those who contact us after they see the study advertisements such as the information posted on the ClinicTrials.gov website.

1. PPCCT research staff will contact the participants through phone or email to collect the following information.
    - Name
    - Date of birth
    - Age
    - Sex
    - Race
    - Mailing address
    - Telephone number
    - Whether the participants had adenoma or/and hyperplastic polyp
    - Whether the participant would like to release their medical records to our research team
2. After the initial contact, we will continue with the procedure 2 on page 24 till the procedure 7 on page 26 in section B.

C. Dietary Intake Assessment

Dietary Intake Assessment Protocol

We have collected a FFQ in the TCPS (IRB # 090235). In this study, we will perform two baseline 24-hour dietary recall assessments for each participant to update intake information during the introductory phase of the study prior to the supplementation of magnesium. The assessments will take place by telephone and will include one weekday and one weekend day where possible. The Ca/Mg intake ratio will be calculated from the 24-hour recalls to confirm the current ratio, to determine eligibility, and to determine the appropriate dose of magnesium supplementation in the treatment arm to reduce the ratio to < 2.6 or the number of placebo capsules in the placebo arm. They will also be used to verify the dietary Ca/Mg intake ratio, excluding any treatment magnesium, remains stable throughout the intervention period. Thus participants will also complete 4 additional 24-hr dietary assessments during the intervention phase; 2 times of diet recalls at week 1 - 6, and 2 times at week 7-12 throughout the course of the magnesium treatment.

Dietary intake will be measured using the 24-hour recall (24HR) assessment approach. The 24HR is the least biased method of dietary assessment during an intervention. Compared to a food record or food diary, the 24HR is less time consuming for the participant, and does not rely on their concentration and recording skills. The 24HR approach is interviewer based, and the interviewer is able to probe as needed. Therefore, dietary monitoring is not as reliant on the personality attributes of the study participant. Nutrient scores from multiple 24HRs are highly correlated with nutrient scores from a food record, and the total variance of nutrient scores from multiple 24HRs is smaller than the variance derived from food records.

The research staff in the Survey Research Shared Resource (SRSR) will administer the recall using the Minnesota Nutrient Data System (NDS) which is licensed to the SRSR. NDS uses a multiple-pass approach with prompts displayed throughout the interview to assist in data collection and review. In this phone-based dietary assessment, we will also obtain questionnaire information on the participant's use of medications, nutritional supplements, and other conditions. Nutrient scores are calculated from each 24HR (e.g., fat g/d, energy kcal/d, calcium mg/day, etc.) using the Nutritional Data System (University of Minnesota). Vitamin, mineral, fiber and other supplementation use are monitored by the 24HRs, and nutrients derived from supplementation are added to nutrients from dietary sources.

Two baseline 24 HR dietary recalls will be completed, using either a recall only method or a record-assisted recall method. Detailed procedures for each method are described as follows.

Procedures

The procedures for completion of two baseline 24 hour recalls, using a recall only method, prior to April, 2012.

- Participants will be told of the calls at initial contact and in informed consent document.
- Participants will receive a food amount booklet by mail (FOOD AMOUNT BOOKLET, Appendix C).
- Convenient windows of time to call participant are confirmed.
- Two baseline 24-hour dietary recall assessments for each participant will be made to obtain current intake information prior to the baseline treatment at one weekday and one weekend day where possible.
- If individuals have had an acute or short-term illness in the past 48 hours, which substantially changed their usual diet the day before SRSR interviewer placed the call, the

research staff would have to call again on the same day of the following week to complete the 24 hour dietary recall.

- If the weekend recall is not completed, the weekend recall will be replaced by the another available weekday recall. This procedure will be followed for weekday recalls.
- The dietary assessments will take place by telephone. Callbacks will be made until participant is contacted.
- A trained research member in the SRSR telephones the participant (Script for 24 Hour Dietary Recall, Appendix C) and asks what they consumed the day before.
    - Working through each meal, snack, beverage, and supplement information is entered directly into a computer
    - The Nutritional Data System will calculate nutrient scores
- Each 24HR requires about 30 minutes.
- Four additional 24-hr dietary assessments will be conducted during the intervention phase; 2 times of diet recalls at week 1 - 6, and 2 times at 7-12 throughout the course of the magnesium treatment.
- Nutrient scores are calculated from each 24HR (e.g., fat g/d, energy kcal/d, calcium mg/day, etc.) using the Nutritional Data System (University of Minnesota). Vitamin, mineral, fiber and other supplementation use are monitored by the 24HRs, and nutrients derived from supplementation are added to nutrients from dietary sources.

The procedures for completion of two baseline 24-hour recalls using a food-record assisted dietary recall approach (April, 2012)

It was very time-consuming to complete the two baseline 24-hour diet recalls by calling the participants twice at one weekday and at one weekend day, which might slow down the participant recruitment process. To speed up the participant recruitment, in April of 2012, we plan to use a new approach of food-record assisted 48-hour diet recall, utilizing the two-day food records to complete two 24 hour dietary recalls over one phone call interview. Based on the current 24-hour recall data we collected in our study, we found the weekday nutrient intakes are similar to those on the weekend day. Participants will be assigned to complete two-day food records of any one of the six combinations, Monday and Tuesday, Tuesday and Wednesday, Wednesday and Thursday, Thursday and Friday, Friday and Saturday, or Saturday and Sunday. The detailed procedures are described as follows.

- Participants will be told of the calls at initial contact and in informed consent document.
- Convenient windows of time to call participant are confirmed.
- Participants will receive a Food Amounts Booklet, two Food Record Forms, Food Record Instructions by mail (FOOD AMOUNT BOOKLET, FOOD RECORD FORMS, FOOD RECORD INSTRUCTIONS, Appendix C).
- Participants will complete two food records of any one of the six combinations of day of week, Sunday and Monday, Monday and Tuesday, Tuesday and Wednesday, Wednesday and Thursday, Thursday and Friday, Friday and Saturday, which will be randomly assigned to participants. The participants will record all the foods and drinks they have from the twelve midnight of the assigned day of week to the next twelve midnight. They will complete two food record forms in two consecutive days of the week. A separate form will be used for each day.
- If individuals have had an acute or short-term illness in the past two days (48 hours), which substantially changed their usual diet, the two food records should be completed over the two days after their diet changes back to normal.

- The two baseline 24-hour dietary recall assessments for each participant will be made to obtain current intake information prior to the baseline treatment by one telephone interview. The phone call will be placed within a couple of days after participants complete their food records.
- A trained research member in the SRSR telephones the participant (Script for 48 Hour Dietary Recall_2012, Appendix C) and asks what they consumed the two days before they are called.
    - Working through each meal, snack, beverage, and supplement information is entered directly into a computer
    - The Nutritional Data System will calculate nutrient scores
- Each 24HR requires up to 30 minutes.
- Four additional 24-hr dietary assessments will be conducted during the intervention phase; using the record-assisted method, two times of diet recalls at week 1-6 and two times at week 7-12 throughout the course of the magnesium treatment.
- Nutrient scores are calculated from each 24HR (e.g., fat g/d, energy kcal/d, calcium mg/day, etc.) using the Nutritional Data System (University of Minnesota). Vitamin, mineral, fiber and other supplementation use are monitored by the 24HRs, and nutrients derived from supplementation are added to nutrients from dietary sources.
- If missed, the phone call will be placed and completed immediately on the next two days.

Prior to enrollment, two baseline 24 hour dietary recalls are performed to estimate participants' current diet intake information for determining participants' eligibility and for calculating the dosage of magnesium/placebo supplementation. The time interval window between 24 hour recalls and the first clinical visit should be within approximately 3 months. Otherwise the two baseline 24 hour diet recalls should be conducted again.

D. Baseline Interview

Baseline Telephone Interview

The research staff in the Survey Research Shared Resource (SRSR) will administer a study questionnaire (Appendix D) at baseline. Questionnaire data to be collected include diet, medication use, health history, physical activity and other cancer risk factors.

E. Randomization and Intervention

Randomization Protocols

A randomized controlled study is one in which there are two groups, one treatment group and one control group. The treatment group receives the treatment under investigation, and the control group receives placebo. Assigning patients at random reduces the risk of bias and increases the probability that differences between the groups can be attributed to the treatment. Randomization is used to determine who will receive the magnesium supplement and who will receive placebo treatment.

This study will enroll 288 participants with colorectal hyperplastic polyp or/and adenoma, polyps free but being at high risk of colorectal polyps or cancer. 152 of whom have the GG genotype and 136 of whom have GA/AA genotype. Within the two genotype groups, a permuted-block randomization algorithm will be used to allocate the subjects into the treatment/placebo arm.

Finally, these participants will be randomized to either treatment or placebo using a double-blind two-factorial (Ca/Mg intake ratio and genotype) design so that each treatment/placebo arm will contain 60 participants with the GG genotype and 60 participants with at least one A allele.

Procedures

- The Principal Investigator will generate a list of the 288 participants, 152 of whom have the GG genotype and 136 of whom have GA/AA genotype.
- Dr. Yu, the co-PI, will conduct the randomization and provide a script to randomly allocate the participants into each treatment/placebo arm within the two genotype groups.
- Each treatment/placebo arm will contain 60 participants with the GG genotype and 60 participants with at least one A allele.
- Both the participants and the study staff will be blind to randomization status until the conclusion of the study. Pharmacists are un-blinded to randomization status and treatment assignment.

Intervention Protocol

The goal of this study is to evaluate the main effect on carcinogenesis related biomarkers, inflammatory biomarkers and magnesium status by reducing the dietary Ca/Mg intake ratio to under 2.6 by magnesium supplementation on carcinogenesis related biomarkers, inflammatory biomarkers and magnesium status and to estimate the potential interaction between magnesium treatment and the Thr1482Ile polymorphism (G→A) of the *TRPM7* gene. The study will enroll 288 participants with colorectal hyperplastic polyp or/and adenoma, polyps free but being at high risk of colorectal polyps or cancer, 152 of whom have the GG genotype and 136 of whom have GA/AA genotype. These participants will be randomized to either treatment or placebo using a double-blind two-factorial (Ca/Mg intake ratio and genotype) design so that each treatment/placebo arm will contain 60 participants with the GG genotype and 60 participants with at least one A allele. Based on the participant's Ca/Mg intake ratio (obtained from the two 24-hour recalls), he or she will be assigned to a dose that will reduce the Ca/Mg intake ratio to around 2.3 (less than 2.6 but no less than 2.0). The dose of magnesium supplementation is personalized based on the current intake ratio. The duration of the study from recruitment through the end of the intervention is approximately 14 weeks including a 12-week intervention. (See attached Figure E2)

The trial will be double-blind: neither participants nor research staff who interacts with the participants or their samples will be aware of the treatment assignments. The treatment period will last for 12 weeks.

Procedures:

1. Intervention layout
    - Based on the participant's Ca/Mg intake ratio (obtained from the two 24-hour recalls), the participant will be assigned to a dose that will reduce the Ca/Mg intake ratio to around 2.3 (less than 2.6 but no less than 2.0). Each dose will correspond to one or multiple capsules from three different sizes of magnesium supplementation or placebo.
    - Presented in Table E2 is the detailed treatment dosage based on baseline calcium/magnesium ratio and calcium intake level as well as resulting calcium/magnesium ratio after supplementation. Treatment or placebo capsules will be discarded if they are not used within 6-months.
    - Based on the participant's Ca/Mg intake ratio and a table provided by the PI of this study, the research staff will devise standard operating procedures for the Vanderbilt Investigational Drug Service (VIDS) to follow with regard to preparing, labeling, blinding, and dispensing study drug.
    - The VIDS will retain a secure set of sealed envelopes containing the treatment assignment. These will be opened in the event of a clinical scenario which necessitates unblinding, as determined by the PI and the safety reviewers.
2. Prescription
    - A detailed prescription for Pharmacists, including information on calcium intake, calcium and magnesium ratio, genotype, treatment assignment, administration etc, will be provided by Dr. Doug Seidner or Dr. Reid Ness.
3. Magnesium capsule and placebo preparation

- The magnesium supplementation and placebo capsules will be made by the Compounding Pharmacist of the Vanderbilt Investigational Drug Service.
- The Vanderbilt Investigational Drug Service (VIDS) will be responsible for the storage, preparation, and labeling of all investigational agents, and for maintaining accurate drug storage and dispensing logs.
- We have chosen to use magnesium glycinate, a form of magnesium that will avoid a laxative side effect. Three sizes of capsules will be used in this study: 515 mg delivering 77.25 mg elemental magnesium (Size 1) in blue opaque; 694 mg delivering 104.1 mg elemental magnesium (Size 00) in orange opaque; and 489 mg delivering 73.35 mg elemental magnesium (Size 1) in green opaque.
- Identical-appearing placebo (made from microcrystalline cellulose) will be made to match these three magnesium capsules (Sizes 00 orange opaque, 1 blue opaque, and 1 green opaque).

4. Dispensation

The whole intervention period lasts for 12 weeks. Participants will take either magnesium or placebo capsules once per day. Participants will be given the magnesium or placebo capsule at the Clinical Research Center (CRC) during the first clinic visit (week 0), and the second clinic visit at the trial, (week 6).

1st time to dispense the capsules (during the first clinic visit at the beginning of the intervention, week 0)

- Research staff notify Vanderbilt Investigational Drug Service (VIDS) to anticipate a prescription for the day of participant's baseline visit (week 0).
- Research staff provide the physician with the information necessary to complete a prescription.
- After the physician has signed the prescription and the participant has provided written informed consent, the research staff fax the prescription and copy of signed consent form to Vanderbilt Investigational Drug Services (VIDS) during the first clinic visit (day 1, week 0).
- VIDS Call the research staff and send the Mg/placebo capsules to CRC on the day of appointment. The research staff gives the participant capsules at the Clinical Research Center of Vanderbilt University Medical Center and an Instruction for taking pill sheet (Appendix E) will be given to the participant and taken home.
- Pharmacist will send magnesium or placebo capsules to participants by Fedex if the participant leaves clinic visit prior to dispense.

2nd time to dispense the capsules (During the second clinic visit, week 6)

- Research staff notify Vanderbilt Investigational Drug Service (VIDS) to anticipate a prescription for the day of participant's second visit (week 6)
- Research staff provides the physician with the information necessary to complete a prescription.
- After the physician has signed the prescription, the research staff fax the prescription to Vanderbilt Investigational Drug Services during the second clinic visit (week 6).
- VIDS Call the research staff and send the Mg/placebo capsules to CRC on the day of appointment.
- The research staff gives the participant capsules at the Clinical Research Center of Vanderbilt University Medical Center and an Instruction for taking pill sheet (Appendix E) will be given to the participant and taken home.

- Pharmacist will send magnesium or placebo capsules to participants by Fedex if the participant leaves clinic visit prior to dispense.

If needed, an extra supply of Magnesium/Placebo supplements will be sent to the participants by Fedex to make sure participants have enough pills until their clinic visit.

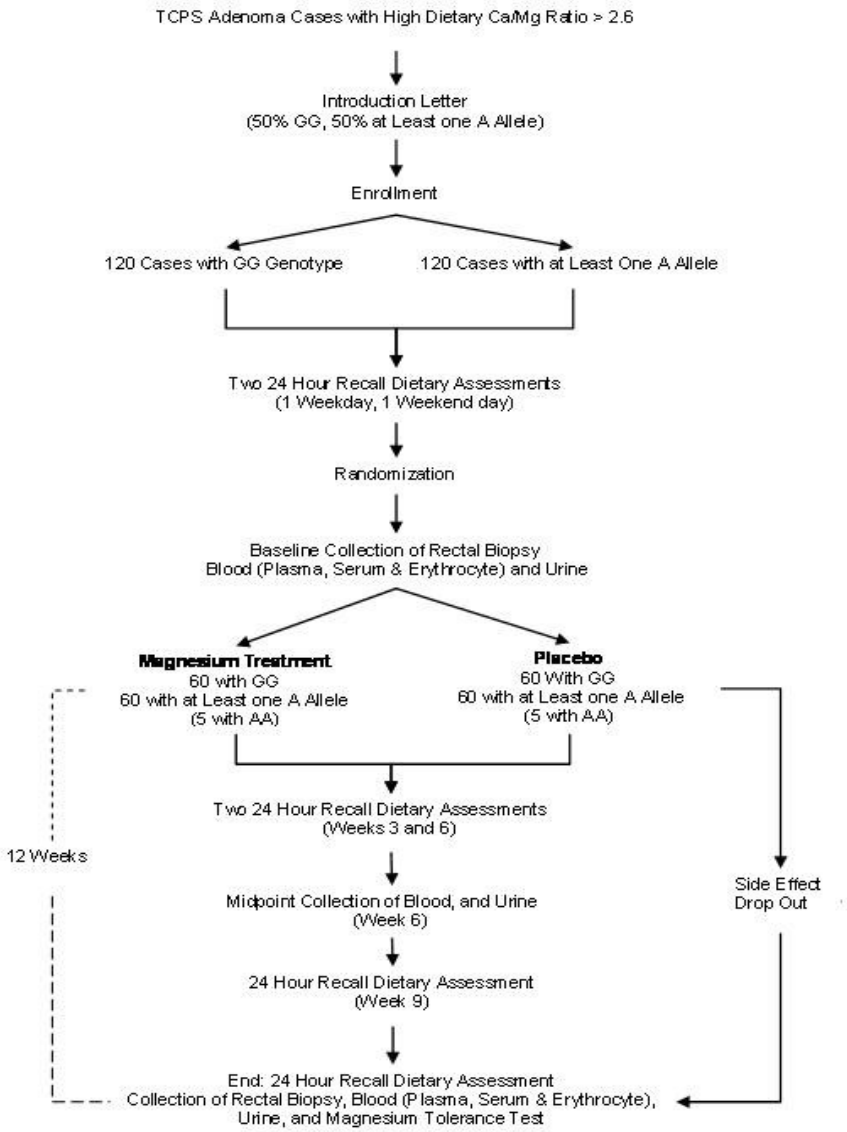


Figure E2. The planed study design

Table E2. Magnesium treatment dosage based on baseline Ca/Mg ratio and calcium intake level and estimated Ca/Mg ratio after use of magnesium supplementation.

Baseline Ca/Mg ratio	Ca intake (mg/day)	Treatment dosage (pill size * number)	Resulting ratio	Ca intake (mg/day)	Treatment dosage (pill size and number)	Resulting ratio
2.6-2.8	700	size 1 (blue) *1	2.08	1400	size 00 *1	2.25
2.6-2.8	800	size 1 (blue) *1	2.14	1500	size 00 *1	2.27
2.6-2.8	900	size 1 (blue) *1	2.19	1600	size 00 *1	2.3
2.6-2.8	1000	size 1 (blue) *1	2.23	1700	size 00*1	2.32
2.6-2.8	1100	size 1 (blue) *1	2.27	1800	size 00*1	2.34
2.6-2.8	1200	size 1 (blue) *1	2.3	1900	size 1 (green)*2	2.23
2.6-2.8	1300	size 00 *1	2.22	2000	size 00*1	2.37
2.8-3.0	700	size 1 (blue) *1	2.2	1400	size 1 (green)*2	2.22
2.8-3.0	800	size 1 (blue) *1	2.26	1500	size 1 (green)*2	2.26
2.8-3.0	900	size 00 *1	2.17	1600	size 1 (green)*2	2.3
2.8-3.0	1000	size 00 *1	2.23	1700	size 1 (blue) *1 & size 00 *1	2.22
2.8-3.0	1100	size 00 *1	2.27	1800	size 1(blue)*1	2.58
2.8-3.0	1200	size 1 (green)*2	2.14	1900	size 00*1	2.5
2.8-3.0	1300	size 1 (green)*2	2.18	2000	size 1(blue)*1	2.6
3.0-3.2	700	size 00 *1	2.12	1400	size 1 (blue) *1 & size 00 *1	2.21
3.0-3.2	800	size 00 *1	2.21	1500	size 1 (blue) *1 & size 00 *1	2.25
3.0-3.2	900	size 00 *1	2.28	1600	size 1 (blue) *1 & size 00 *1	2.29
3.0-3.2	1000	size 1 (green)*2	2.13	1700	size 00*2	2.25
3.0-3.2	1100	size 1 (green)*2	2.19	1800	size 1(blue)*2	2.45
3.0-3.2	1200	size 1 (green)*2	2.25	1900	size 1(blue)*2	2.48
3.0-3.2	1300	size 1 (green)*2	2.3	2000	size 1 (blue) *2	2.5
3.2-3.4	700	size 00 *1	2.21	1400	size 1 (blue) *1 & size 1 (green)*2	2.16
3.2-3.4	800	size 1 (green)*2	2.06	1500	size 1 (blue) *1 & size 1 (green)*2	2.21
3.2-3.4	900	size 1 (green)*2	2.15	1600	size 1 (blue) *1 & size 1 (green)*2	2.26
3.2-3.4	1000	size 1 (green)*2	2.22	1700	size 1 (blue) *1 & size 1 (green)*2	2.3
3.2-3.4	1100	size 1 (green)*2	2.29	1800	size 1(blue)*1 & size 00*1	2.48
3.2-3.4	1200	size 1 (blue) *1 & size 00 *1	2.2	1900	size 1(blue)*1 & size 00*1	2.51
3.2-3.4	1300	size 1 (blue) *1 & size 00 *1	2.26	2000	size 1(blue)*1 & size 00*1	2.54
3.4-3.6	700	size 1 (green)*2	2.02	1400	size 1 (blue) *1 & size 1 (green)*2	2.24
3.4-3.6	800	size 1 (green)*2	2.13	1500	size 1 (blue) *1 & size 1 (green)*2	2.23
3.4-3.6	900	size 1 (green)*2	2.23	1600	size 00 *1 & size 1 (green)*2	2.26
3.4-3.6	1000	size 1 (blue) *1 & size 00 *1	2.14	1700	size 1(blue)*2 & size 00*1	2.28
3.4-3.6	1100	size 1 (blue) *1 & size 00 *1	2.22	1800	size 00*2	2.49
3.4-3.6	1200	size 1 (blue) *1 & size 00*1	2.29	1900	size 00*2	2.53
3.4-3.6	1300	size 1 (blue) *1 & size 1 (green)*2	2.18	2000	size 00*2	2.56
3.6-3.8	700	size 1 (green)*2	2.08	1400	size 00 *1 & size 1 (green)*2	2.22
3.6-3.8	800	size 1 (green)*2	2.2	1500	size 00 *1 & size 1 (green)*2	2.28
3.6-3.8	900	size 1 (blue) *1 & size 00 *1	2.12	1600	size 1 (green)*4	2.2
3.6-3.8	1000	size 1 (blue) *1 & size 00 *1	2.21	1700	size 1 (green)*4	2.26
3.6-3.8	1100	size 1 (blue) *1 & size 00 *1	2.3	1800	size 1(blue)*3	2.51
3.6-3.8	1200	size 1 (blue) *1 & size 1 (green)*2	2.19	1900	size 1(blue)*3	2.55
3.6-3.8	1300	size 1 (blue) *1 & size 1 (green)*2	2.26	2000	size 1(blue)*3	2.59
3.8-4.0	700	size 1 (blue)*2	2.1	1400	size 1(blue)*2 & size 00*1	2.27
3.8-4.0	800	size 1 (blue)*2	2.22	1500	size 1(blue)*1 & size 00*2	2.24
3.8-4.0	900	size 1 (blue)*1 & size 00*1	2.18	1600	size 1(blue)*1 & size 00*2	2.3
3.8-4.0	1000	size 1 (blue)*1 & size 00*1	2.28	1700	size 00*3	2.27
3.8-4.0	1100	size 00*2	2.24	1800	size 1(blue)*1 & size 00*2	2.41
3.8-4.0	1200	size 1 (blue)*3	2.22	1900	size 1(blue)*1 & size 00*2	2.46
3.8-4.0	1300	size 1 (blue)*2 & size 00*1	2.2	2000	size 1(blue)*1 & size 00*2	2.51
4.0-4.2	700	size 1 (blue)*2	2.15	1400	size 1(blue)*1 & size 00*2	2.23
4.0-4.2	800	size 1 (blue)*2	2.29	1500	size 00*3	2.21
4.0-4.2	900	size 1 (blue)*1 & size 00*1	2.25	1600	size 00*3	2.28
4.0-4.2	1000	size 00*2	2.21	1700	size 1(blue)*3 & size 00*1	2.27
4.0-4.2	1100	size 1 (blue)*3	2.2	1800	size 1(blue)*1 & size 00*2	2.48
4.0-4.2	1200	size 1 (blue)*3	2.29	1900	size 1(blue)*1 & size 00*2	2.54
4.0-4.2	1300	size 1 (blue)*2 & size 00*1	2.26	2000	size 1(blue)*1 & size 00*2	2.59
4.2-4.4	700	size 1 (blue)*2	2.21	1400	size 1(blue)*1 & size 00*2	2.29
4.2-4.4	800	size 1 (blue)*1 & size 00*1	2.18	1500	size 00*3	2.27
4.2-4.4	900	size 00*2	2.16	1600	size 1(blue)*3 & size 00*1	2.26
4.2-4.4	1000	size 00*2	2.27	1700	size 1(blue)*2 & size 00*2	2.24
4.2-4.4	1100	size 1 (blue)*3	2.26	1800	size 00*3	2.46
4.2-4.4	1200	size 1 (blue)*2 & size 00*1	2.23	1900	size 00*3	2.52
4.2-4.4	1300	size 1 (blue)*1 & size 00*2	2.21	2000	size 00*3	2.57
4.4-4.6	700	size 1 (blue)*2	2.26	1400	size 00*3	2.25
4.4-4.6	800	size 1 (blue)*1 & size 00*1	2.23	1500	size 1(blue)*3 & size 00*1	2.24
4.4-4.6	900	size 00*2	2.2	1600	size 1(blue)*2 & size 00*2	2.23
4.4-4.6	1000	size 1 (blue)*3	2.2	1700	size 1(blue)*2 & size 00*2	2.3
4.4-4.6	1100	size 1 (blue)*2 & size 00*1	2.19	1800	size 00*3	2.53
4.4-4.6	1200	size 1 (blue)*2 & size 00*1	2.28	1900	size 00*3	2.59
4.4-4.6	1300	size 1 (blue)*1 & size 00*2	2.26	2000	size 1(blue)*3 & size 00*1	2.56
4.6-4.8	700	size 1 (blue)*1 & size 00*1	2.12	1400	size 00*3	2.29
4.6-4.8	800	size 1 (blue)*1 & size 00*1	2.28	1500	size 1(blue)*3 & size 00*1	2.29
4.6-4.8	900	size 00*2	2.25	1600	size 1(blue)*2 & size 00*2	2.28
4.6-4.8	1000	size 1 (blue)*3	2.25	1700	size 1(blue)*1 & size 00*3	2.26
4.6-4.8	1100	size 1 (blue)*2 & size 00*1	2.23	1800	size 1(blue)*3 & size 00*1	2.5
4.6-4.8	1200	size 1 (blue)*1 & size 00*2	2.22	1900	size 1(blue)*3 & size 00*1	2.57
4.6-4.8	1300	size 00*3	2.21	2000	size 1(blue)*2 & size 00*2	2.54
4.8-5.0	700	size 1 (blue)*1 & size 00*1	2.16	1400	size 1(blue)*3 & size 00*1	2.25
4.8-5.0	800	size 00*2	2.15	1500	size 1(blue)*2 & size 00*2	2.24
4.8-5.0	900	size 00*2	2.3	1600	size 1(blue)*1 & size 00*3	2.23
4.8-5.0	1000	size 1 (blue)*3	2.29	1700	size 1(blue)*1 & size 00*3	2.31
4.8-5.0	1100	size 1 (blue)*2 & size 00*1	2.28	1800	size 1(blue)*1 & size 00*3	2.38
4.8-5.0	1200	size 1 (blue)*1 & size 00*2	2.26	1900	size 1(blue)*2 & size 00*2	2.53
4.8-5.0	1300	size 00*3	2.25	2000	size 1(blue)*2 & size 00*2	2.59
5.0-5.2	700	size 1 (blue)*1 & size 00*1	2.2	1400	size 1(blue)*3 & size 00*1	2.29

5.0-5.2	800	size 00*2	2.19	1500	size 1(blue)*2 & size 00*2	2.28
5.0-5.2	900	size 1 (blue)*3	2.2	1600	size 1(blue)*1 & size 00*3	2.28
5.0-5.2	1000	size 1 (blue)*2 & size 00*1	2.2	1700	size 1(blue)*1 & size 00*3	2.35
5.0-5.2	1100	size 1 (blue)*1 & size 00*2	2.2	1800	size 1(blue)*1 & size 00*3	2.42
5.0-5.2	1200	size 00*3	2.19	1900	size 1(blue)*1 & size 00*3	2.49
5.0-5.2	1300	size 00*3	2.29	2000	size 1(blue)*1 & size 00*3	2.56
5.2-5.4	700	size 1 (blue)*1 & size 00*1	2.2	1400	size 1(blue)*2 & size 00*2	2.23
5.2-5.4	800	size 00*2	2.23	1500	size 1(blue)*1 & size 00*3	2.23
5.2-5.4	900	size 1 (blue)*3	2.24	1600	size 1(blue)*1 & size 00*3	2.31
5.2-5.4	1000	size 1 (blue)*2 & size 00*1	2.24	1700	size 1(blue)*1 & size 00*3	2.39
5.2-5.4	1100	size 1 (blue)*1 & size 00*2	2.23	1800	size 1(blue)*1 & size 00*3	2.47
5.2-5.4	1200	size 00*3	2.23	1900	size 1(blue)*1 & size 00*3	2.54
5.2-5.4	1300	size 1(blue)*3 & size 00*1	2.24	2000	size 1(blue)*1 & size 00*3	2.61
5.4-5.6	700	size 1 (blue)*1 & size 00*1	2.27	1400	size 1(blue)*2 & size 00*2	2.27
5.4-5.6	800	size 00*2	2.26	1500	size 1(blue)*1 & size 00*3	2.26
5.4-5.6	900	size 1 (blue)*3	2.28	1600	size 1(blue)*1 & size 00*3	2.35
5.4-5.6	1000	size 1 (blue)*2 & size 00*1	2.27	1700	size 1(blue)*1 & size 00*3	2.43
5.4-5.6	1100	size 1 (blue)*1 & size 00*2	2.27	1800	size 1(blue)*1 & size 00*3	2.51
5.4-5.6	1200	size 00*3	2.26	1900	size 1(blue)*1 & size 00*3	2.59
5.4-5.6	1300	size 1(blue)*3 & size 00*1	2.27	2000	size 1(blue)*1 & size 00*3	2.66
5.6-5.8	700	size 00*2	2.11	1400	size 1(blue)*1 & size 00*3	2.2
5.6-5.8	800	size 00*2	2.3	1500	size 1(blue)*1 & size 00*3	2.3
5.6-5.8	900	size 1 (blue)*2 & size 00*1	2.16	1600	size 1(blue)*1 & size 00*3	2.39
5.6-5.8	1000	size 1 (blue)*1 & size 00*2	2.17	1700	size 1(blue)*1 & size 00*3	2.47
5.6-5.8	1100	size 1 (blue)*1 & size 00*2	2.3	1800	size 1(blue)*1 & size 00*3	2.55
5.6-5.8	1200	size 00*3	2.3	1900	size 1(blue)*1 & size 00*3	2.63
5.6-5.8	1300	size 1(blue)*2 & size 00*2	2.2	2000	size 1(blue)*1 & size 00*3	2.7
5.8-6.0	700	size 00*2	2.14	1400	size 1(blue)*1 & size 00*3	2.23
5.8-6.0	800	size 1 (blue)*3	2.18	1500	size 1(blue)*1 & size 00*3	2.33
5.8-6.0	900	size 1 (blue)*2 & size 00*1	2.19	1600	size 1(blue)*1 & size 00*3	2.42
5.8-6.0	1000	size 1 (blue)*1 & size 00*2	2.2	1700	size 1(blue)*1 & size 00*3	2.51
5.8-6.0	1100	size 00*3	2.21	1800	size 1(blue)*1 & size 00*3	2.59
5.8-6.0	1200	size 1(blue)*3 & size 00*1	2.23	1900	size 1(blue)*1 & size 00*3	2.67
5.8-6.0	1300	size 1(blue)*2 & size 00*2	2.23	2000	size 1(blue)*1 & size 00*3	2.75
6.0-6.2	700	size 00*2	2.17	1400	size 1(blue)*1 & size 00*3	2.26
6.0-6.2	800	size 1 (blue)*3	2.2	1500	size 1(blue)*1 & size 00*3	2.36
6.0-6.2	900	size 1 (blue)*2 & size 00*1	2.22	1600	size 1(blue)*1 & size 00*3	2.45
6.0-6.2	1000	size 1 (blue)*1 & size 00*2	2.23	1700	size 1(blue)*1 & size 00*3	2.54
6.0-6.2	1100	size 00*3	2.23	1800	size 1(blue)*1 & size 00*3	2.63
6.0-6.2	1200	size 1(blue)*3 & size 00*1	2.25	1900	size 1(blue)*1 & size 00*3	2.71
6.0-6.2	1300	size 1(blue)*2 & size 00*2	2.26	2000	size 1(blue)*1 & size 00*3	2.79
6.2-6.4	700	size 00*2	2.19	1400	size 1(blue)*1 & size 00*3	2.29
6.2-6.4	800	size 1 (blue)*3	2.23	1500	size 1(blue)*1 & size 00*3	2.39
6.2-6.4	900	size 1 (blue)*2 & size 00*1	2.24	1600	size 1(blue)*1 & size 00*3	2.49
6.2-6.4	1000	size 1 (blue)*1 & size 00*2	2.25	1700	size 1(blue)*1 & size 00*3	2.58
6.2-6.4	1100	size 00*3	2.26	1800	size 1(blue)*1 & size 00*3	2.67
6.2-6.4	1200	size 1(blue)*3 & size 00*1	2.28	1900	size 1(blue)*1 & size 00*3	2.75
6.2-6.4	1300	size 1(blue)*2 & size 00*2	2.28	2000	size 1(blue)*1 & size 00*3	2.83
6.4-6.6	700	size 00*2	2.22	1400	size 1(blue)*1 & size 00*3	2.31
6.4-6.6	800	size 1 (blue)*3	2.25	1500	size 1(blue)*1 & size 00*3	2.42
6.4-6.6	900	size 1 (blue)*2 & size 00*1	2.27	1600	size 1(blue)*1 & size 00*3	2.52
6.4-6.6	1000	size 1 (blue)*1 & size 00*2	2.28	1700	size 1(blue)*1 & size 00*3	2.61
6.4-6.6	1100	size 00*3	2.28	1800	size 1(blue)*1 & size 00*3	2.7
6.4-6.6	1200	size 1(blue)*2 & size 00*2	2.19	1900	size 1(blue)*1 & size 00*3	2.79
6.4-6.6	1300	size 1(blue)*1 & size 00*3	2.21	2000	size 1(blue)*1 & size 00*3	2.87
6.6-6.8	700	size 00*2	2.24	1400	size 1(blue)*1 & size 00*3	2.34
6.6-6.8	800	size 1 (blue)*3	2.28	1500	size 1(blue)*1 & size 00*3	2.45
6.6-6.8	900	size 1 (blue)*2 & size 00*1	2.29	1600	size 1(blue)*1 & size 00*3	2.55
6.6-6.8	1000	size 00*3	2.17	1700	size 1(blue)*1 & size 00*3	2.64
6.6-6.8	1100	size 1(blue)*3 & size 00*1	2.2	1800	size 1(blue)*1 & size 00*3	2.73
6.6-6.8	1200	size 1(blue)*2 & size 00*2	2.21	1900	size 1(blue)*1 & size 00*3	2.82
6.6-6.8	1300	size 1(blue)*1 & size 00*3	2.23	2000	size 1(blue)*1 & size 00*3	2.91
6.8-7.0	700	size 00*2	2.26	1400	size 1(blue)*1 & size 00*3	2.36
6.8-7.0	800	size 1 (blue)*2 & size 00*1	2.14	1500	size 1(blue)*1 & size 00*3	2.47
6.8-7.0	900	size 1 (blue)*1 & size 00*2	2.16	1600	size 1(blue)*1 & size 00*3	2.57
6.8-7.0	1000	size 00*3	2.19	1700	size 1(blue)*1 & size 00*3	2.67
6.8-7.0	1100	size 1(blue)*3 & size 00*1	2.22	1800	size 1(blue)*1 & size 00*3	2.77
6.8-7.0	1200	size 1(blue)*2 & size 00*2	2.24	1900	size 1(blue)*1 & size 00*3	2.86
6.8-7.0	1300	size 1(blue)*1 & size 00*3	2.25	2000	size 1(blue)*1 & size 00*3	2.94
7.0-7.2	700	size 00*2	2.28	1400	size 1(blue)*1 & size 00*3	2.39
7.0-7.2	800	size 1 (blue)*2 & size 00*1	2.15	1500	size 1(blue)*1 & size 00*3	2.5
7.0-7.2	900	size 1 (blue)*1 & size 00*2	2.18	1600	size 1(blue)*1 & size 00*3	2.6
7.0-7.2	1000	size 00*3	2.21	1700	size 1(blue)*1 & size 00*3	2.7
7.0-7.2	1100	size 1(blue)*3 & size 00*1	2.24	1800	size 1(blue)*1 & size 00*3	2.8
7.0-7.2	1200	size 1(blue)*2 & size 00*2	2.26	1900	size 1(blue)*1 & size 00*3	2.89
7.0-7.2	1300	size 1(blue)*1 & size 00*3	2.27	2000	size 1(blue)*1 & size 00*3	2.98
7.2-7.4	700	size 1 (blue)*3	2.14	1400	size 1(blue)*1 & size 00*3	2.41
7.2-7.4	800	size 1 (blue)*2 & size 00*1	2.17	1500	size 1(blue)*1 & size 00*3	2.52
7.2-7.4	900	size 1 (blue)*1 & size 00*2	2.2	1600	size 1(blue)*1 & size 00*3	2.63
7.2-7.4	1000	size 00*3	2.23	1700	size 1(blue)*1 & size 00*3	2.73
7.2-7.4	1100	size 1(blue)*3 & size 00*1	2.26	1800	size 1(blue)*1 & size 00*3	2.83
7.2-7.4	1200	size 1(blue)*2 & size 00*2	2.28	1900	size 1(blue)*1 & size 00*3	2.92
7.2-7.4	1300	size 1(blue)*1 & size 00*3	2.29	2000	size 1(blue)*1 & size 00*3	3.01
7.4-7.6	700	size 1 (blue)*3	2.15	1400	size 1(blue)*1 & size 00*3	2.43
7.4-7.6	800	size 1 (blue)*2 & size 00*1	2.19	1500	size 1(blue)*1 & size 00*3	2.54
7.4-7.6	900	size 1 (blue)*1 & size 00*2	2.22	1600	size 1(blue)*1 & size 00*3	2.65
7.4-7.6	1000	size 00*3	2.24	1700	size 1(blue)*1 & size 00*3	2.76
7.4-7.6	1100	size 1(blue)*3 & size 00*1	2.28	1800	size 1(blue)*1 & size 00*3	2.86
7.4-7.6	1200	size 1(blue)*2 & size 00*2	2.3	1900	size 1(blue)*1 & size 00*3	2.96
7.4-7.6	1300	size 1(blue)*1 & size 00*3	2.31	2000	size 1(blue)*1 & size 00*3	3.05

7.6-7.8	700	size 1 (blue)*3	2.17	1400	size 1(blue)*1 & size 00*3	2.45
7.6-7.8	800	size 1 (blue)*2 & size 00*1	2.21	1500	size 1(blue)*1 & size 00*3	2.57
7.6-7.8	900	size 1 (blue)*1 & size 00*2	2.24	1600	size 1(blue)*1 & size 00*3	2.68
7.6-7.8	1000	size 00*3	2.26	1700	size 1(blue)*1 & size 00*3	2.79
7.6-7.8	1100	size 1(blue)*3 & size 00*1	2.3	1800	size 1(blue)*1 & size 00*3	2.89
7.6-7.8	1200	size 1(blue)*1 & size 00*3	2.2	1900	size 1(blue)*1 & size 00*3	2.99
7.6-7.8	1300	size 1(blue)*1 & size 00*3	2.33	2000	size 1(blue)*1 & size 00*3	3.08
7.8-8.0	700	size 1 (blue)*3	2.19	1400	size 1(blue)*1 & size 00*3	2.47
7.8-8.0	800	size 1 (blue)*2 & size 00*1	2.22	1500	size 1(blue)*1 & size 00*3	2.59
7.8-8.0	900	size 1 (blue)*1 & size 00*2	2.25	1600	size 1(blue)*1 & size 00*3	2.7
7.8-8.0	1000	size 00*3	2.28	1700	size 1(blue)*1 & size 00*3	2.81
7.8-8.0	1100	size 1(blue)*2 & size 00*2	2.19	1800	size 1(blue)*1 & size 00*3	2.92
7.8-8.0	1200	size 1(blue)*1 & size 00*3	2.22	1900	size 1(blue)*1 & size 00*3	3.02
7.8-8.0	1300	size 1(blue)*1 & size 00*3	2.35	2000	size 1(blue)*1 & size 00*3	3.11
8.0-8.2	700	size 1 (blue)*3	2.2	1400	size 1(blue)*1 & size 00*3	2.49
8.0-8.2	800	size 1 (blue)*2 & size 00*1	2.24	1500	size 1(blue)*1 & size 00*3	2.61
8.0-8.2	900	size 1 (blue)*1 & size 00*2	2.27	1600	size 1(blue)*1 & size 00*3	2.73
8.0-8.2	1000	size 00*3	2.29	1700	size 1(blue)*1 & size 00*3	2.84
8.0-8.2	1100	size 1(blue)*2 & size 00*2	2.21	1800	size 1(blue)*1 & size 00*3	2.94
8.0-8.2	1200	size 1(blue)*1 & size 00*3	2.23	1900	size 1(blue)*1 & size 00*3	3.04
8.0-8.2	1300	size 1(blue)*1 & size 00*3	2.36	2000	size 1(blue)*1 & size 00*3	3.14
8.2-8.4	700	size 1 (blue)*3	2.21	1400	size 1(blue)*1 & size 00*3	2.51
8.2-8.4	800	size 1 (blue)*2 & size 00*1	2.25	1500	size 1(blue)*1 & size 00*3	2.63
8.2-8.4	900	size 1 (blue)*1 & size 00*2	2.28	1600	size 1(blue)*1 & size 00*3	2.75
8.2-8.4	1000	size 1(blue)*3 & size 00*1	2.19	1700	size 1(blue)*1 & size 00*3	2.86
8.2-8.4	1100	size 1(blue)*2 & size 00*2	2.22	1800	size 1(blue)*1 & size 00*3	2.97
8.2-8.4	1200	size 1(blue)*1 & size 00*3	2.25	1900	size 1(blue)*1 & size 00*3	3.07
8.2-8.4	1300	size 1(blue)*1 & size 00*3	2.38	2000	size 1(blue)*1 & size 00*3	3.17
8.4-8.6	700	size 1 (blue)*3	2.23	1400	size 1(blue)*1 & size 00*3	2.53
8.4-8.6	800	size 1 (blue)*2 & size 00*1	2.27	1500	size 1(blue)*1 & size 00*3	2.65
8.4-8.6	900	size 1 (blue)*1 & size 00*2	2.3	1600	size 1(blue)*1 & size 00*3	2.77
8.4-8.6	1000	size 1(blue)*3 & size 00*1	2.21	1700	size 1(blue)*1 & size 00*3	2.88
8.4-8.6	1100	size 1(blue)*2 & size 00*2	2.24	1800	size 1(blue)*1 & size 00*3	2.99
8.4-8.6	1200	size 1(blue)*1 & size 00*3	2.26	1900	size 1(blue)*1 & size 00*3	3.1
8.4-8.6	1300	size 1(blue)*1 & size 00*3	2.4	2000	size 1(blue)*1 & size 00*3	3.2
8.6-8.8	700	size 1 (blue)*3	2.24	1400	size 1(blue)*1 & size 00*3	2.54
8.6-8.8	800	size 1 (blue)*2 & size 00*1	2.28	1500	size 1(blue)*1 & size 00*3	2.67
8.6-8.8	900	size 00*3	2.16	1600	size 1(blue)*1 & size 00*3	2.79
8.6-8.8	1000	size 1(blue)*3 & size 00*1	2.22	1700	size 1(blue)*1 & size 00*3	2.91
8.6-8.8	1100	size 1(blue)*2 & size 00*2	2.25	1800	size 1(blue)*1 & size 00*3	3.02
8.6-8.8	1200	size 1(blue)*1 & size 00*3	2.27	1900	size 1(blue)*1 & size 00*3	3.13
8.6-8.8	1300	size 1(blue)*1 & size 00*3	2.41	2000	size 1(blue)*1 & size 00*3	3.23
8.8-9.0	700	size 1 (blue)*3	2.26	1400	size 1(blue)*1 & size 00*3	2.56
8.8-9.0	800	size 1 (blue)*2 & size 00*1	2.3	1500	size 1(blue)*1 & size 00*3	2.69
8.8-9.0	900	size 00*3	2.18	1600	size 1(blue)*1 & size 00*3	2.81
8.8-9.0	1000	size 1(blue)*3 & size 00*1	2.23	1700	size 1(blue)*1 & size 00*3	2.93
8.8-9.0	1100	size 1(blue)*2 & size 00*2	2.26	1800	size 1(blue)*1 & size 00*3	3.04
8.8-9.0	1200	size 1(blue)*1 & size 00*3	2.29	1900	size 1(blue)*1 & size 00*3	3.15
8.8-9.0	1300	size 1(blue)*1 & size 00*3	2.43	2000	size 1(blue)*1 & size 00*3	3.26
9.0-9.2	700	size 1 (blue)*3	2.27	1400	size 1(blue)*1 & size 00*3	2.58
9.0-9.2	800	size 1 (blue)*1 & size 00*2	2.14	1500	size 1(blue)*1 & size 00*3	2.71
9.0-9.2	900	size 00*3	2.19	1600	size 1(blue)*1 & size 00*3	2.83
9.0-9.2	1000	size 1(blue)*3 & size 00*1	2.24	1700	size 1(blue)*1 & size 00*3	2.95
9.0-9.2	1100	size 1(blue)*2 & size 00*2	2.27	1800	size 1(blue)*1 & size 00*3	3.06
9.0-9.2	1200	size 1(blue)*1 & size 00*3	2.3	1900	size 1(blue)*1 & size 00*3	3.18
9.0-9.2	1300	size 1(blue)*1 & size 00*3	2.44	2000	size 1(blue)*1 & size 00*3	3.28
9.2-9.4	700	size 1 (blue)*3	2.28	1400	size 1(blue)*1 & size 00*3	2.59
9.2-9.4	800	size 1 (blue)*1 & size 00*2	2.15	1500	size 1(blue)*1 & size 00*3	2.72
9.2-9.4	900	size 00*3	2.2	1600	size 1(blue)*1 & size 00*3	2.85
9.2-9.4	1000	size 1(blue)*3 & size 00*1	2.26	1700	size 1(blue)*1 & size 00*3	2.97
9.2-9.4	1100	size 1(blue)*2 & size 00*2	2.29	1800	size 1(blue)*1 & size 00*3	3.09
9.2-9.4	1200	size 1(blue)*1 & size 00*3	2.31	1900	size 1(blue)*1 & size 00*3	3.2
9.2-9.4	1300	size 1(blue)*1 & size 00*3	2.46	2000	size 1(blue)*1 & size 00*3	3.31
9.4-9.6	700	size 1 (blue)*3	2.29	1400	size 1(blue)*1 & size 00*3	2.61
9.4-9.6	800	size 1 (blue)*1 & size 00*2	2.16	1500	size 1(blue)*1 & size 00*3	2.74
9.4-9.6	900	size 00*3	2.21	1600	size 1(blue)*1 & size 00*3	2.87
9.4-9.6	1000	size 1(blue)*3 & size 00*1	2.27	1700	size 1(blue)*1 & size 00*3	2.99
9.4-9.6	1100	size 1(blue)*2 & size 00*2	2.3	1800	size 1(blue)*1 & size 00*3	3.11
9.4-9.6	1200	size 1(blue)*1 & size 00*3	2.33	1900	size 1(blue)*1 & size 00*3	3.22
9.4-9.6	1300	size 1(blue)*1 & size 00*3	2.47	2000	size 1(blue)*1 & size 00*3	3.33
9.6-9.8	700	size 1 (blue)*2 & size 00*1	2.12	1400	size 1(blue)*1 & size 00*3	2.62
9.6-9.8	800	size 1 (blue)*1 & size 00*2	2.17	1500	size 1(blue)*1 & size 00*3	2.76
9.6-9.8	900	size 00*3	2.22	1600	size 1(blue)*1 & size 00*3	2.89
9.6-9.8	1000	size 1(blue)*3 & size 00*1	2.28	1700	size 1(blue)*1 & size 00*3	3.01
9.6-9.8	1100	size 1(blue)*1 & size 00*3	2.19	1800	size 1(blue)*1 & size 00*3	3.13
9.6-9.8	1200	size 1(blue)*1 & size 00*3	2.34	1900	size 1(blue)*1 & size 00*3	3.25
9.6-9.8	1300	size 1(blue)*1 & size 00*3	2.48	2000	size 1(blue)*1 & size 00*3	3.36
9.8-10.0	700	size 1 (blue)*2 & size 00*1	2.13	1400	size 1(blue)*1 & size 00*3	2.64
9.8-10.0	800	size 1 (blue)*1 & size 00*2	2.18	1500	size 1(blue)*1 & size 00*3	2.77
9.8-10.0	900	size 00*3	2.23	1600	size 1(blue)*1 & size 00*3	2.9
9.8-10.0	1000	size 1(blue)*3 & size 00*1	2.29	1700	size 1(blue)*1 & size 00*3	3.03
9.8-10.0	1100	size 1(blue)*1 & size 00*3	2.2	1800	size 1(blue)*1 & size 00*3	3.15
9.8-10.0	1200	size 1(blue)*1 & size 00*3	2.35	1900	size 1(blue)*1 & size 00*3	3.27
9.8-10.0	1300	size 1(blue)*1 & size 00*3	2.5	2000	size 1(blue)*1 & size 00*3	3.38
10.0-10.2	700	size 1 (blue)*2 & size 00*1	2.13	1400	size 1(blue)*1 & size 00*3	2.65
10.0-10.2	800	size 1 (blue)*1 & size 00*2	2.19	1500	size 1(blue)*1 & size 00*3	2.79
10.0-10.2	900	size 00*3	2.24	1600	size 1(blue)*1 & size 00*3	2.92
10.0-10.2	1000	size 1(blue)*3 & size 00*1	2.3	1700	size 1(blue)*1 & size 00*3	3.05
10.0-10.2	1100	size 1(blue)*1 & size 00*3	2.21	1800	size 1(blue)*1 & size 00*3	3.17
10.0-10.2	1200	size 1(blue)*1 & size 00*3	2.36	1900	size 1(blue)*1 & size 00*3	3.29

10.0-10.2	1300	size 1(blue)*1 & size 00*3	2.51	2000	size 1(blue)*1 & size 00*3	3.4
10.2-10.4	700	size 1 (blue)*2 & size 00*1	2.14	1400	size 1(blue)*1 & size 00*3	2.66
10.2-10.4	800	size 1 (blue)*1 & size 00*2	2.2	1500	size 1(blue)*1 & size 00*3	2.8
10.2-10.4	900	size 00*3	2.25	1600	size 1(blue)*1 & size 00*3	2.94
10.2-10.4	1000	size 1(blue)*2 & size 00*2	2.17	1700	size 1(blue)*1 & size 00*3	3.07
10.2-10.4	1100	size 1(blue)*1 & size 00*3	2.22	1800	size 1(blue)*1 & size 00*3	3.19
10.2-10.4	1200	size 1(blue)*1 & size 00*3	2.37	1900	size 1(blue)*1 & size 00*3	3.31
10.2-10.4	1300	size 1(blue)*1 & size 00*3	2.52	2000	size 1(blue)*1 & size 00*3	3.43
10.4-10.6	700	size 1 (blue)*2 & size 00*1	2.15	1400	size 1(blue)*1 & size 00*3	2.68
10.4-10.6	800	size 1 (blue)*1 & size 00*2	2.21	1500	size 1(blue)*1 & size 00*3	2.82
10.4-10.6	900	size 00*3	2.26	1600	size 1(blue)*1 & size 00*3	2.95
10.4-10.6	1000	size 1(blue)*2 & size 00*2	2.18	1700	size 1(blue)*1 & size 00*3	3.08
10.4-10.6	1100	size 1(blue)*1 & size 00*3	2.23	1800	size 1(blue)*1 & size 00*3	3.21
10.4-10.6	1200	size 1(blue)*1 & size 00*3	2.38	1900	size 1(blue)*1 & size 00*3	3.33
10.4-10.6	1300	size 1(blue)*1 & size 00*3	2.53	2000	size 1(blue)*1 & size 00*3	3.45
10.6-10.8	700	size 1 (blue)*2 & size 00*1	2.16	1400	size 1(blue)*1 & size 00*3	2.69
10.6-10.8	800	size 1 (blue)*1 & size 00*2	2.22	1500	size 1(blue)*1 & size 00*3	2.83
10.6-10.8	900	size 00*3	2.27	1600	size 1(blue)*1 & size 00*3	2.97
10.6-10.8	1000	size 1(blue)*2 & size 00*2	2.19	1700	size 1(blue)*1 & size 00*3	3.1
10.6-10.8	1100	size 1(blue)*1 & size 00*3	2.23	1800	size 1(blue)*1 & size 00*3	3.23
10.6-10.8	1200	size 1(blue)*1 & size 00*3	2.39	1900	size 1(blue)*1 & size 00*3	3.35
10.6-10.8	1300	size 1(blue)*1 & size 00*3	2.54	2000	size 1(blue)*1 & size 00*3	3.47
10.8-11.0	700	size 1 (blue)*2 & size 00*1	2.17	1400	size 1(blue)*1 & size 00*3	2.7
10.8-11.0	800	size 1 (blue)*1 & size 00*2	2.23	1500	size 1(blue)*1 & size 00*3	2.85
10.8-11.0	900	size 00*3	2.28	1600	size 1(blue)*1 & size 00*3	2.98
10.8-11.0	1000	size 1(blue)*2 & size 00*2	2.2	1700	size 1(blue)*1 & size 00*3	3.12
10.8-11.0	1100	size 1(blue)*1 & size 00*3	2.24	1800	size 1(blue)*1 & size 00*3	3.25
10.8-11.0	1200	size 1(blue)*1 & size 00*3	2.4	1900	size 1(blue)*1 & size 00*3	3.37
10.8-11.0	1300	size 1(blue)*1 & size 00*3	2.55	2000	size 1(blue)*1 & size 00*3	3.49
11.0-11.2	700	size 1 (blue)*2 & size 00*1	2.18	1400	size 1(blue)*1 & size 00*3	2.71
11.0-11.2	800	size 1 (blue)*1 & size 00*2	2.24	1500	size 1(blue)*1 & size 00*3	2.86
11.0-11.2	900	size 00*3	2.29	1600	size 1(blue)*1 & size 00*3	3
11.0-11.2	1000	size 1(blue)*2 & size 00*2	2.21	1700	size 1(blue)*1 & size 00*3	3.13
11.0-11.2	1100	size 1(blue)*1 & size 00*3	2.25	1800	size 1(blue)*1 & size 00*3	3.26
11.0-11.2	1200	size 1(blue)*1 & size 00*3	2.41	1900	size 1(blue)*1 & size 00*3	3.39
11.0-11.2	1300	size 1(blue)*1 & size 00*3	2.57	2000	size 1(blue)*1 & size 00*3	3.51
11.2-11.4	700	size 1 (blue)*2 & size 00*1	2.18	1400	size 1(blue)*1 & size 00*3	2.73
11.2-11.4	800	size 1 (blue)*1 & size 00*2	2.25	1500	size 1(blue)*1 & size 00*3	2.87
11.2-11.4	900	size 00*3	2.3	1600	size 1(blue)*1 & size 00*3	3.01
11.2-11.4	1000	size 1(blue)*2 & size 00*2	2.22	1700	size 1(blue)*1 & size 00*3	3.15
11.2-11.4	1100	size 1(blue)*1 & size 00*3	2.26	1800	size 1(blue)*1 & size 00*3	3.28
11.2-11.4	1200	size 1(blue)*1 & size 00*3	2.42	1900	size 1(blue)*1 & size 00*3	3.41
11.2-11.4	1300	size 1(blue)*1 & size 00*3	2.58	2000	size 1(blue)*1 & size 00*3	3.53
11.4-11.6	700	size 1 (blue)*2 & size 00*1	2.19	1400	size 1(blue)*1 & size 00*3	2.74
11.4-11.6	800	size 1 (blue)*1 & size 00*2	2.25	1500	size 1(blue)*1 & size 00*3	2.88
11.4-11.6	900	size 1(blue)*3 & size 00*1	2.17	1600	size 1(blue)*1 & size 00*3	3.03
11.4-11.6	1000	size 1(blue)*2 & size 00*2	2.22	1700	size 1(blue)*1 & size 00*3	3.16
11.4-11.6	1100	size 1(blue)*1 & size 00*3	2.27	1800	size 1(blue)*1 & size 00*3	3.3
11.4-11.6	1200	size 1(blue)*1 & size 00*3	2.43	1900	size 1(blue)*1 & size 00*3	3.42
11.4-11.6	1300	size 1(blue)*1 & size 00*3	2.59	2000	size 1(blue)*1 & size 00*3	3.55
11.6-11.8	700	size 1 (blue)*2 & size 00*1	2.2	1400	size 1(blue)*1 & size 00*3	2.75
11.6-11.8	800	size 1 (blue)*1 & size 00*2	2.26	1500	size 1(blue)*1 & size 00*3	2.9
11.6-11.8	900	size 1(blue)*3 & size 00*1	2.18	1600	size 1(blue)*1 & size 00*3	3.04
11.6-11.8	1000	size 1(blue)*2 & size 00*2	2.23	1700	size 1(blue)*1 & size 00*3	3.18
11.6-11.8	1100	size 1(blue)*1 & size 00*3	2.27	1800	size 1(blue)*1 & size 00*3	3.31
11.6-11.8	1200	size 1(blue)*1 & size 00*3	2.44	1900	size 1(blue)*1 & size 00*3	3.44
11.6-11.8	1300	size 1(blue)*1 & size 00*3	2.6	2000	size 1(blue)*1 & size 00*3	3.57
11.8-12.0	700	size 1 (blue)*2 & size 00*1	2.21	1400	size 1(blue)*1 & size 00*3	2.76
11.8-12.0	800	size 1 (blue)*1 & size 00*2	2.27	1500	size 1(blue)*1 & size 00*3	2.91
11.8-12.0	900	size 1(blue)*3 & size 00*1	2.19	1600	size 1(blue)*1 & size 00*3	3.05
11.8-12.0	1000	size 1(blue)*2 & size 00*2	2.24	1700	size 1(blue)*1 & size 00*3	3.19
11.8-12.0	1100	size 1(blue)*1 & size 00*3	2.28	1800	size 1(blue)*1 & size 00*3	3.33
11.8-12.0	1200	size 1(blue)*1 & size 00*3	2.45	1900	size 1(blue)*1 & size 00*3	3.46
11.8-12.0	1300	size 1(blue)*1 & size 00*3	2.61	2000	size 1(blue)*1 & size 00*3	3.59
12.0-12.2	700	size 1 (blue)*2 & size 00*1	2.21	1400	size 1(blue)*1 & size 00*3	2.77
12.0-12.2	800	size 1 (blue)*1 & size 00*2	2.28	1500	size 1(blue)*1 & size 00*3	2.92
12.0-12.2	900	size 1(blue)*3 & size 00*1	2.19	1600	size 1(blue)*1 & size 00*3	3.07
12.0-12.2	1000	size 1(blue)*2 & size 00*2	2.25	1700	size 1(blue)*1 & size 00*3	3.21
12.0-12.2	1100	size 1(blue)*1 & size 00*3	2.29	1800	size 1(blue)*1 & size 00*3	3.34
12.0-12.2	1200	size 1(blue)*1 & size 00*3	2.46	1900	size 1(blue)*1 & size 00*3	3.48
12.0-12.2	1300	size 1(blue)*1 & size 00*3	2.62	2000	size 1(blue)*1 & size 00*3	3.6
12.2-12.4	700	size 1 (blue)*2 & size 00*1	2.22	1400	size 1(blue)*1 & size 00*3	2.78
12.2-12.4	800	size 1 (blue)*1 & size 00*2	2.28	1500	size 1(blue)*1 & size 00*3	2.93
12.2-12.4	900	size 1(blue)*3 & size 00*1	2.2	1600	size 1(blue)*1 & size 00*3	3.08
12.2-12.4	1000	size 1(blue)*2 & size 00*2	2.25	1700	size 1(blue)*1 & size 00*3	3.22
12.2-12.4	1100	size 1(blue)*1 & size 00*3	2.3	1800	size 1(blue)*1 & size 00*3	3.36
12.2-12.4	1200	size 1(blue)*1 & size 00*3	2.46	1900	size 1(blue)*1 & size 00*3	3.49
12.2-12.4	1300	size 1(blue)*1 & size 00*3	2.62	2000	size 1(blue)*1 & size 00*3	3.62
12.4-12.6	700	size 1 (blue)*2 & size 00*1	2.23	1400	size 1(blue)*1 & size 00*3	2.79
12.4-12.6	800	size 1 (blue)*1 & size 00*2	2.29	1500	size 1(blue)*1 & size 00*3	2.94
12.4-12.6	900	size 1(blue)*3 & size 00*1	2.21	1600	size 1(blue)*1 & size 00*3	3.09
12.4-12.6	1000	size 1(blue)*2 & size 00*2	2.26	1700	size 1(blue)*1 & size 00*3	3.23
12.4-12.6	1100	size 1(blue)*1 & size 00*3	2.3	1800	size 1(blue)*1 & size 00*3	3.37
12.4-12.6	1200	size 1(blue)*1 & size 00*3	2.47	1900	size 1(blue)*1 & size 00*3	3.51
12.4-12.6	1300	size 1(blue)*1 & size 00*3	2.63	2000	size 1(blue)*1 & size 00*3	3.64
12.6-12.8	700	size 1 (blue)*2 & size 00*1	2.23	1400	size 1(blue)*1 & size 00*3	2.8
12.6-12.8	800	size 1 (blue)*1 & size 00*2	2.3	1500	size 1(blue)*1 & size 00*3	2.95
12.6-12.8	900	size 1(blue)*3 & size 00*1	2.21	1600	size 1(blue)*1 & size 00*3	3.1
12.6-12.8	1000	size 1(blue)*2 & size 00*2	2.27	1700	size 1(blue)*1 & size 00*3	3.25
12.6-12.8	1100	size 1(blue)*1 & size 00*3	2.31	1800	size 1(blue)*1 & size 00*3	3.39

12.6-12.8	1200	size 1(blue)*1 & size 00*3	2.48	1900	size 1(blue)*1 & size 00*3	3.52
12.6-12.8	1300	size 1(blue)*1 & size 00*3	2.64	2000	size 1(blue)*1 & size 00*3	3.66
12.8-13.0	700	size 1 (blue)*2 & size 00*1	2.24	1400	size 1(blue)*1 & size 00*3	2.81
12.8-13.0	800	size 00*3	2.14	1500	size 1(blue)*1 & size 00*3	2.97
12.8-13.0	900	size 1(blue)*3 & size 00*1	2.22	1600	size 1(blue)*1 & size 00*3	3.12
12.8-13.0	1000	size 1(blue)*2 & size 00*2	2.27	1700	size 1(blue)*1 & size 00*3	3.26
12.8-13.0	1100	size 1(blue)*1 & size 00*3	2.32	1800	size 1(blue)*1 & size 00*3	3.4
12.8-13.0	1200	size 1(blue)*1 & size 00*3	2.49	1900	size 1(blue)*1 & size 00*3	3.54
12.8-13.0	1300	size 1(blue)*1 & size 00*3	2.65	2000	size 1(blue)*1 & size 00*3	3.67
13.0-13.2	700	size 1 (blue)*2 & size 00*1	2.24	1400	size 1(blue)*1 & size 00*3	2.82
13.0-13.2	800	size 00*3	2.14	1500	size 1(blue)*1 & size 00*3	2.98
13.0-13.2	900	size 1(blue)*3 & size 00*1	2.22	1600	size 1(blue)*1 & size 00*3	3.13
13.0-13.2	1000	size 1(blue)*2 & size 00*2	2.28	1700	size 1(blue)*1 & size 00*3	3.27
13.0-13.2	1100	size 1(blue)*1 & size 00*3	2.32	1800	size 1(blue)*1 & size 00*3	3.42
13.0-13.2	1200	size 1(blue)*1 & size 00*3	2.49	1900	size 1(blue)*1 & size 00*3	3.55
13.0-13.2	1300	size 1(blue)*1 & size 00*3	2.66	2000	size 1(blue)*1 & size 00*3	3.69
13.2-13.4	700	size 1 (blue)*2 & size 00*1	2.25	1400	size 1(blue)*1 & size 00*3	2.83
13.2-13.4	800	size 00*3	2.15	1500	size 1(blue)*1 & size 00*3	2.99
13.2-13.4	900	size 1(blue)*3 & size 00*1	2.23	1600	size 1(blue)*1 & size 00*3	3.14
13.2-13.4	1000	size 1(blue)*2 & size 00*2	2.28	1700	size 1(blue)*1 & size 00*3	3.29
13.2-13.4	1100	size 1(blue)*1 & size 00*3	2.33	1800	size 1(blue)*1 & size 00*3	3.43
13.2-13.4	1200	size 1(blue)*1 & size 00*3	2.5	1900	size 1(blue)*1 & size 00*3	3.57
13.2-13.4	1300	size 1(blue)*1 & size 00*3	2.67	2000	size 1(blue)*1 & size 00*3	3.7
13.4-13.6	700	size 1 (blue)*2 & size 00*1	2.25	1400	size 1(blue)*1 & size 00*3	2.84
13.4-13.6	800	size 00*3	2.15	1500	size 1(blue)*1 & size 00*3	3
13.4-13.6	900	size 1(blue)*3 & size 00*1	2.24	1600	size 1(blue)*1 & size 00*3	3.15
13.4-13.6	1000	size 1(blue)*2 & size 00*2	2.29	1700	size 1(blue)*1 & size 00*3	3.3
13.4-13.6	1100	size 1(blue)*1 & size 00*3	2.34	1800	size 1(blue)*1 & size 00*3	3.44
13.4-13.6	1200	size 1(blue)*1 & size 00*3	2.51	1900	size 1(blue)*1 & size 00*3	3.58
13.4-13.6	1300	size 1(blue)*1 & size 00*3	2.68	2000	size 1(blue)*1 & size 00*3	3.72
13.6-13.8	700	size 1 (blue)*2 & size 00*1	2.26	1400	size 1(blue)*1 & size 00*3	2.85
13.6-13.8	800	size 00*3	2.16	1500	size 1(blue)*1 & size 00*3	3.01
13.6-13.8	900	size 1(blue)*3 & size 00*1	2.24	1600	size 1(blue)*1 & size 00*3	3.16
13.6-13.8	1000	size 1(blue)*2 & size 00*2	2.3	1700	size 1(blue)*1 & size 00*3	3.31
13.6-13.8	1100	size 1(blue)*1 & size 00*3	2.34	1800	size 1(blue)*1 & size 00*3	3.46
13.6-13.8	1200	size 1(blue)*1 & size 00*3	2.51	1900	size 1(blue)*1 & size 00*3	3.6
13.6-13.8	1300	size 1(blue)*1 & size 00*3	2.68	2000	size 1(blue)*1 & size 00*3	3.73
13.8-14.0	700	size 1 (blue)*2 & size 00*1	2.27	1400	size 1(blue)*1 & size 00*3	2.86
13.8-14.0	800	size 00*3	2.16	1500	size 1(blue)*1 & size 00*3	3.02
13.8-14.0	900	size 1(blue)*3 & size 00*1	2.25	1600	size 1(blue)*1 & size 00*3	3.17
13.8-14.0	1000	size 1(blue)*1 & size 00*3	2.17	1700	size 1(blue)*1 & size 00*3	3.32
13.8-14.0	1100	size 1(blue)*1 & size 00*3	2.35	1800	size 1(blue)*1 & size 00*3	3.47
13.8-14.0	1200	size 1(blue)*1 & size 00*3	2.52	1900	size 1(blue)*1 & size 00*3	3.61
13.8-14.0	1300	size 1(blue)*1 & size 00*3	2.69	2000	size 1(blue)*1 & size 00*3	3.75
14.0-14.2	700	size 1 (blue)*2 & size 00*1	2.27	1400	size 1(blue)*1 & size 00*3	2.86
14.0-14.2	800	size 00*3	2.17	1500	size 1(blue)*1 & size 00*3	3.02
14.0-14.2	900	size 1(blue)*3 & size 00*1	2.25	1600	size 1(blue)*1 & size 00*3	3.18
14.0-14.2	1000	size 1(blue)*1 & size 00*3	2.17	1700	size 1(blue)*1 & size 00*3	3.33
14.0-14.2	1100	size 1(blue)*1 & size 00*3	2.35	1800	size 1(blue)*1 & size 00*3	3.48
14.0-14.2	1200	size 1(blue)*1 & size 00*3	2.53	1900	size 1(blue)*1 & size 00*3	3.62
14.0-14.2	1300	size 1(blue)*1 & size 00*3	2.7	2000	size 1(blue)*1 & size 00*3	3.76
14.2-14.4	700	size 1 (blue)*2 & size 00*1	2.28	1400	size 1(blue)*1 & size 00*3	2.87
14.2-14.4	800	size 00*3	2.17	1500	size 1(blue)*1 & size 00*3	3.03
14.2-14.4	900	size 1(blue)*3 & size 00*1	2.26	1600	size 1(blue)*1 & size 00*3	3.19
14.2-14.4	1000	size 1(blue)*1 & size 00*3	2.18	1700	size 1(blue)*1 & size 00*3	3.34
14.2-14.4	1100	size 1(blue)*1 & size 00*3	2.36	1800	size 1(blue)*1 & size 00*3	3.49
14.2-14.4	1200	size 1(blue)*1 & size 00*3	2.53	1900	size 1(blue)*1 & size 00*3	3.64
14.2-14.4	1300	size 1(blue)*1 & size 00*3	2.71	2000	size 1(blue)*1 & size 00*3	3.78
14.4-14.6	700	size 1 (blue)*2 & size 00*1	2.28	1400	size 1(blue)*1 & size 00*3	2.88
14.4-14.6	800	size 00*3	2.18	1500	size 1(blue)*1 & size 00*3	3.04
14.4-14.6	900	size 1(blue)*3 & size 00*1	2.26	1600	size 1(blue)*1 & size 00*3	3.2
14.4-14.6	1000	size 1(blue)*1 & size 00*3	2.18	1700	size 1(blue)*1 & size 00*3	3.35
14.4-14.6	1100	size 1(blue)*1 & size 00*3	2.36	1800	size 1(blue)*1 & size 00*3	3.5
14.4-14.6	1200	size 1(blue)*1 & size 00*3	2.54	1900	size 1(blue)*1 & size 00*3	3.65
14.4-14.6	1300	size 1(blue)*1 & size 00*3	2.71	2000	size 1(blue)*1 & size 00*3	3.79
14.6-14.8	700	size 1 (blue)*2 & size 00*1	2.29	1400	size 1(blue)*1 & size 00*3	2.89
14.6-14.8	800	size 00*3	2.18	1500	size 1(blue)*1 & size 00*3	3.05
14.6-14.8	900	size 1(blue)*3 & size 00*1	2.27	1600	size 1(blue)*1 & size 00*3	3.21
14.6-14.8	1000	size 1(blue)*1 & size 00*3	2.19	1700	size 1(blue)*1 & size 00*3	3.37
14.6-14.8	1100	size 1(blue)*1 & size 00*3	2.37	1800	size 1(blue)*1 & size 00*3	3.52
14.6-14.8	1200	size 1(blue)*1 & size 00*3	2.55	1900	size 1(blue)*1 & size 00*3	3.66
14.6-14.8	1300	size 1(blue)*1 & size 00*3	2.72	2000	size 1(blue)*1 & size 00*3	3.81
14.8-15.0	700	size 1 (blue)*2 & size 00*1	2.29	1400	size 1(blue)*1 & size 00*3	2.9
14.8-15.0	800	size 00*3	2.19	1500	size 1(blue)*1 & size 00*3	3.06
14.8-15.0	900	size 1(blue)*3 & size 00*1	2.27	1600	size 1(blue)*1 & size 00*3	3.22
14.8-15.0	1000	size 1(blue)*1 & size 00*3	2.19	1700	size 1(blue)*1 & size 00*3	3.38
14.8-15.0	1100	size 1(blue)*1 & size 00*3	2.37	1800	size 1(blue)*1 & size 00*3	3.53
14.8-15.0	1200	size 1(blue)*1 & size 00*3	2.55	1900	size 1(blue)*1 & size 00*3	3.67
14.8-15.0	1300	size 1(blue)*1 & size 00*3	2.73	2000	size 1(blue)*1 & size 00*3	3.82
15.0-15.2	700	size 1 (blue)*2 & size 00*1	2.3	1400	size 1(blue)*1 & size 00*3	2.9
15.0-15.2	800	size 00*3	2.19	1500	size 1(blue)*1 & size 00*3	3.07
15.0-15.2	900	size 1(blue)*3 & size 00*1	2.28	1600	size 1(blue)*1 & size 00*3	3.23
15.0-15.2	1000	size 1(blue)*1 & size 00*3	2.19	1700	size 1(blue)*1 & size 00*3	3.39
15.0-15.2	1100	size 1(blue)*1 & size 00*3	2.38	1800	size 1(blue)*1 & size 00*3	3.54
15.0-15.2	1200	size 1(blue)*1 & size 00*3	2.56	1900	size 1(blue)*1 & size 00*3	3.69
15.0-15.2	1300	size 1(blue)*1 & size 00*3	2.73	2000	size 1(blue)*1 & size 00*3	3.83
15.2-15.4	700	size 1 (blue)*2 & size 00*1	2.3	1400	size 1(blue)*1 & size 00*3	2.91
15.2-15.4	800	size 00*3	2.19	1500	size 1(blue)*1 & size 00*3	3.08
15.2-15.4	900	size 1(blue)*3 & size 00*1	2.28	1600	size 1(blue)*1 & size 00*3	3.24
15.2-15.4	1000	size 1(blue)*1 & size 00*3	2.2	1700	size 1(blue)*1 & size 00*3	3.4

15.2-15.4	1100	size 1(blue)*1 & size 00*3	2.38	1800	size 1(blue)*1 & size 00*3	3.55
15.2-15.4	1200	size 1(blue)*1 & size 00*3	2.56	1900	size 1(blue)*1 & size 00*3	3.7
15.2-15.4	1300	size 1(blue)*1 & size 00*3	2.74	2000	size 1(blue)*1 & size 00*3	3.84
15.4-15.6	700	size 1 (blue)*1 & size 00*2	2.12	1400	size 1(blue)*1 & size 00*3	2.92
15.4-15.6	800	size 00*3	2.2	1500	size 1(blue)*1 & size 00*3	3.08
15.4-15.6	900	size 1(blue)*3 & size 00*1	2.28	1600	size 1(blue)*1 & size 00*3	3.25
15.4-15.6	1000	size 1(blue)*1 & size 00*3	2.2	1700	size 1(blue)*1 & size 00*3	3.41
15.4-15.6	1100	size 1(blue)*1 & size 00*3	2.39	1800	size 1(blue)*1 & size 00*3	3.56
15.4-15.6	1200	size 1(blue)*1 & size 00*3	2.57	1900	size 1(blue)*1 & size 00*3	3.71
15.4-15.6	1300	size 1(blue)*1 & size 00*3	2.75	2000	size 1(blue)*1 & size 00*3	3.86
15.6-15.8	700	size 1 (blue)*1 & size 00*2	2.12	1400	size 1(blue)*1 & size 00*3	2.92
15.6-15.8	800	size 00*3	2.2	1500	size 1(blue)*1 & size 00*3	3.09
15.6-15.8	900	size 1(blue)*3 & size 00*1	2.29	1600	size 1(blue)*1 & size 00*3	3.26
15.6-15.8	1000	size 1(blue)*1 & size 00*3	2.21	1700	size 1(blue)*1 & size 00*3	3.41
15.6-15.8	1100	size 1(blue)*1 & size 00*3	2.39	1800	size 1(blue)*1 & size 00*3	3.57
15.6-15.8	1200	size 1(blue)*1 & size 00*3	2.58	1900	size 1(blue)*1 & size 00*3	3.72
15.6-15.8	1300	size 1(blue)*1 & size 00*3	2.75	2000	size 1(blue)*1 & size 00*3	3.87
15.8-16.0	700	size 1 (blue)*1 & size 00*2	2.12	1400	size 1(blue)*1 & size 00*3	2.93
15.8-16.0	800	size 00*3	2.21	1500	size 1(blue)*1 & size 00*3	3.1
15.8-16.0	900	size 1(blue)*3 & size 00*1	2.29	1600	size 1(blue)*1 & size 00*3	3.26
15.8-16.0	1000	size 1(blue)*1 & size 00*3	2.21	1700	size 1(blue)*1 & size 00*3	3.42
15.8-16.0	1100	size 1(blue)*1 & size 00*3	2.4	1800	size 1(blue)*1 & size 00*3	3.58
15.8-16.0	1200	size 1(blue)*1 & size 00*3	2.58	1900	size 1(blue)*1 & size 00*3	3.73
15.8-16.0	1300	size 1(blue)*1 & size 00*3	2.76	2000	size 1(blue)*1 & size 00*3	3.88
16.0-16.2	700	size 1 (blue)*1 & size 00*2	2.13	1400	size 1(blue)*1 & size 00*3	2.94
16.0-16.2	800	size 00*3	2.21	1500	size 1(blue)*1 & size 00*3	3.11
16.0-16.2	900	size 1(blue)*3 & size 00*1	2.3	1600	size 1(blue)*1 & size 00*3	3.27
16.0-16.2	1000	size 1(blue)*1 & size 00*3	2.21	1700	size 1(blue)*1 & size 00*3	3.43
16.0-16.2	1100	size 1(blue)*1 & size 00*3	2.4	1800	size 1(blue)*1 & size 00*3	3.59
16.0-16.2	1200	size 1(blue)*1 & size 00*3	2.59	1900	size 1(blue)*1 & size 00*3	3.74
16.0-16.2	1300	size 1(blue)*1 & size 00*3	2.76	2000	size 1(blue)*1 & size 00*3	3.89
16.2-16.4	700	size 1 (blue)*1 & size 00*2	2.13	1400	size 1(blue)*1 & size 00*3	2.94
16.2-16.4	800	size 00*3	2.21	1500	size 1(blue)*1 & size 00*3	3.11
16.2-16.4	900	size 1(blue)*2 & size 00*2	2.15	1600	size 1(blue)*1 & size 00*3	3.28
16.2-16.4	1000	size 1(blue)*1 & size 00*3	2.22	1700	size 1(blue)*1 & size 00*3	3.44
16.2-16.4	1100	size 1(blue)*1 & size 00*3	2.41	1800	size 1(blue)*1 & size 00*3	3.6
16.2-16.4	1200	size 1(blue)*1 & size 00*3	2.59	1900	size 1(blue)*1 & size 00*3	3.75
16.2-16.4	1300	size 1(blue)*1 & size 00*3	2.77	2000	size 1(blue)*1 & size 00*3	3.9
16.4-16.6	700	size 1 (blue)*1 & size 00*2	2.13	1400	size 1(blue)*1 & size 00*3	2.95
16.4-16.6	800	size 00*3	2.22	1500	size 1(blue)*1 & size 00*3	3.12
16.4-16.6	900	size 1(blue)*2 & size 00*2	2.16	1600	size 1(blue)*1 & size 00*3	3.29
16.4-16.6	1000	size 1(blue)*1 & size 00*3	2.22	1700	size 1(blue)*1 & size 00*3	3.45
16.4-16.6	1100	size 1(blue)*1 & size 00*3	2.41	1800	size 1(blue)*1 & size 00*3	3.61
16.4-16.6	1200	size 1(blue)*1 & size 00*3	2.6	1900	size 1(blue)*1 & size 00*3	3.76
16.4-16.6	1300	size 1(blue)*1 & size 00*3	2.78	2000	size 1(blue)*1 & size 00*3	3.92
16.6-16.8	700	size 1 (blue)*1 & size 00*2	2.14	1400	size 1(blue)*1 & size 00*3	2.96
16.6-16.8	800	size 00*3	2.22	1500	size 1(blue)*1 & size 00*3	3.13
16.6-16.8	900	size 1(blue)*2 & size 00*2	2.16	1600	size 1(blue)*1 & size 00*3	3.3
16.6-16.8	1000	size 1(blue)*1 & size 00*3	2.23	1700	size 1(blue)*1 & size 00*3	3.46
16.6-16.8	1100	size 1(blue)*1 & size 00*3	2.42	1800	size 1(blue)*1 & size 00*3	3.62
16.6-16.8	1200	size 1(blue)*1 & size 00*3	2.6	1900	size 1(blue)*1 & size 00*3	3.77
16.6-16.8	1300	size 1(blue)*1 & size 00*3	2.78	2000	size 1(blue)*1 & size 00*3	3.93
16.8-17.0	700	size 1 (blue)*1 & size 00*2	2.14	1400	size 1(blue)*1 & size 00*3	2.96
16.8-17.0	800	size 00*3	2.22	1500	size 1(blue)*1 & size 00*3	3.14
16.8-17.0	900	size 1(blue)*2 & size 00*2	2.16	1600	size 1(blue)*1 & size 00*3	3.3
16.8-17.0	1000	size 1(blue)*1 & size 00*3	2.23	1700	size 1(blue)*1 & size 00*3	3.47
16.8-17.0	1100	size 1(blue)*1 & size 00*3	2.42	1800	size 1(blue)*1 & size 00*3	3.63
16.8-17.0	1200	size 1(blue)*1 & size 00*3	2.61	1900	size 1(blue)*1 & size 00*3	3.79
16.8-17.0	1300	size 1(blue)*1 & size 00*3	2.79	2000	size 1(blue)*1 & size 00*3	3.94
17.0-17.2	700	size 1 (blue)*1 & size 00*2	2.14	1400	size 1(blue)*1 & size 00*3	2.97
17.0-17.2	800	size 00*3	2.23	1500	size 1(blue)*1 & size 00*3	3.14
17.0-17.2	900	size 1(blue)*2 & size 00*2	2.17	1600	size 1(blue)*1 & size 00*3	3.31
17.0-17.2	1000	size 1(blue)*1 & size 00*3	2.23	1700	size 1(blue)*1 & size 00*3	3.48
17.0-17.2	1100	size 1(blue)*1 & size 00*3	2.42	1800	size 1(blue)*1 & size 00*3	3.64
17.0-17.2	1200	size 1(blue)*1 & size 00*3	2.61	1900	size 1(blue)*1 & size 00*3	3.79
17.0-17.2	1300	size 1(blue)*1 & size 00*3	2.79	2000	size 1(blue)*1 & size 00*3	3.95
17.2-17.4	700	size 1 (blue)*1 & size 00*2	2.15	1400	size 1(blue)*1 & size 00*3	2.98
17.2-17.4	800	size 00*3	2.23	1500	size 1(blue)*1 & size 00*3	3.15
17.2-17.4	900	size 1(blue)*2 & size 00*2	2.17	1600	size 1(blue)*1 & size 00*3	3.32
17.2-17.4	1000	size 1(blue)*1 & size 00*3	2.24	1700	size 1(blue)*1 & size 00*3	3.48
17.2-17.4	1100	size 1(blue)*1 & size 00*3	2.43	1800	size 1(blue)*1 & size 00*3	3.65
17.2-17.4	1200	size 1(blue)*1 & size 00*3	2.61	1900	size 1(blue)*1 & size 00*3	3.8
17.2-17.4	1300	size 1(blue)*1 & size 00*3	2.8	2000	size 1(blue)*1 & size 00*3	3.96
17.4-17.6	700	size 1 (blue)*1 & size 00*2	2.15	1400	size 1(blue)*1 & size 00*3	2.98
17.4-17.6	800	size 00*3	2.23	1500	size 1(blue)*1 & size 00*3	3.16
17.4-17.6	900	size 1(blue)*2 & size 00*2	2.17	1600	size 1(blue)*1 & size 00*3	3.33
17.4-17.6	1000	size 1(blue)*1 & size 00*3	2.24	1700	size 1(blue)*1 & size 00*3	3.49
17.4-17.6	1100	size 1(blue)*1 & size 00*3	2.43	1800	size 1(blue)*1 & size 00*3	3.66
17.4-17.6	1200	size 1(blue)*1 & size 00*3	2.62	1900	size 1(blue)*1 & size 00*3	3.81
17.4-17.6	1300	size 1(blue)*1 & size 00*3	2.8	2000	size 1(blue)*1 & size 00*3	3.97
17.6-17.8	700	size 1 (blue)*1 & size 00*2	2.15	1400	size 1(blue)*1 & size 00*3	2.99
17.6-17.8	800	size 00*3	2.24	1500	size 1(blue)*1 & size 00*3	3.16
17.6-17.8	900	size 1(blue)*2 & size 00*2	2.18	1600	size 1(blue)*1 & size 00*3	3.33
17.6-17.8	1000	size 1(blue)*1 & size 00*3	2.24	1700	size 1(blue)*1 & size 00*3	3.5
17.6-17.8	1100	size 1(blue)*1 & size 00*3	2.44	1800	size 1(blue)*1 & size 00*3	3.66
17.6-17.8	1200	size 1(blue)*1 & size 00*3	2.62	1900	size 1(blue)*1 & size 00*3	3.82
17.6-17.8	1300	size 1(blue)*1 & size 00*3	2.81	2000	size 1(blue)*1 & size 00*3	3.98
17.8-18.0	700	size 1 (blue)*1 & size 00*2	2.16	1400	size 1(blue)*1 & size 00*3	2.99
17.8-18.0	800	size 00*3	2.24	1500	size 1(blue)*1 & size 00*3	3.17
17.8-18.0	900	size 1(blue)*2 & size 00*2	2.18	1600	size 1(blue)*1 & size 00*3	3.34

17.8-18.0	1000	size 1(blue)*1 & size 00*3	2.25	1700	size 1(blue)*1 & size 00*3	3.51
17.8-18.0	1100	size 1(blue)*1 & size 00*3	2.44	1800	size 1(blue)*1 & size 00*3	3.67
17.8-18.0	1200	size 1(blue)*1 & size 00*3	2.63	1900	size 1(blue)*1 & size 00*3	3.83
17.8-18.0	1300	size 1(blue)*1 & size 00*3	2.81	2000	size 1(blue)*1 & size 00*3	3.99
18.0-18.2	700	size 1 (blue)*1 & size 00*2	2.16	1400	size 1(blue)*1 & size 00*3	3
18.0-18.2	800	size 00*3	2.24	1500	size 1(blue)*1 & size 00*3	3.18
18.0-18.2	900	size 1(blue)*2 & size 00*2	2.18	1600	size 1(blue)*1 & size 00*3	3.35
18.0-18.2	1000	size 1(blue)*1 & size 00*3	2.25	1700	size 1(blue)*1 & size 00*3	3.52
18.0-18.2	1100	size 1(blue)*1 & size 00*3	2.44	1800	size 1(blue)*1 & size 00*3	3.68
18.0-18.2	1200	size 1(blue)*1 & size 00*3	2.63	1900	size 1(blue)*1 & size 00*3	3.84
18.0-18.2	1300	size 1(blue)*1 & size 00*3	2.82	2000	size 1(blue)*1 & size 00*3	4
18.2-18.4	700	size 1 (blue)*1 & size 00*2	2.16	1400	size 1(blue)*1 & size 00*3	3
18.2-18.4	800	size 00*3	2.25	1500	size 1(blue)*1 & size 00*3	3.18
18.2-18.4	900	size 1(blue)*2 & size 00*2	2.19	1600	size 1(blue)*1 & size 00*3	3.35
18.2-18.4	1000	size 1(blue)*1 & size 00*3	2.25	1700	size 1(blue)*1 & size 00*3	3.52
18.2-18.4	1100	size 1(blue)*1 & size 00*3	2.45	1800	size 1(blue)*1 & size 00*3	3.69
18.2-18.4	1200	size 1(blue)*1 & size 00*3	2.64	1900	size 1(blue)*1 & size 00*3	3.85
18.2-18.4	1300	size 1(blue)*1 & size 00*3	2.82	2000	size 1(blue)*1 & size 00*3	4.01
18.4-18.6	700	size 1 (blue)*1 & size 00*2	2.17	1400	size 1(blue)*1 & size 00*3	3.01
18.4-18.6	800	size 00*3	2.25	1500	size 1(blue)*1 & size 00*3	3.19
18.4-18.6	900	size 1(blue)*2 & size 00*2	2.19	1600	size 1(blue)*1 & size 00*3	3.36
18.4-18.6	1000	size 1(blue)*1 & size 00*3	2.25	1700	size 1(blue)*1 & size 00*3	3.53
18.4-18.6	1100	size 1(blue)*1 & size 00*3	2.45	1800	size 1(blue)*1 & size 00*3	3.7
18.4-18.6	1200	size 1(blue)*1 & size 00*3	2.64	1900	size 1(blue)*1 & size 00*3	3.86
18.4-18.6	1300	size 1(blue)*1 & size 00*3	2.83	2000	size 1(blue)*1 & size 00*3	4.02
18.6-18.8	700	size 1 (blue)*1 & size 00*2	2.17	1400	size 1(blue)*1 & size 00*3	3.01
18.6-18.8	800	size 00*3	2.25	1500	size 1(blue)*1 & size 00*3	3.19
18.6-18.8	900	size 1(blue)*2 & size 00*2	2.19	1600	size 1(blue)*1 & size 00*3	3.37
18.6-18.8	1000	size 1(blue)*1 & size 00*3	2.26	1700	size 1(blue)*1 & size 00*3	3.54
18.6-18.8	1100	size 1(blue)*1 & size 00*3	2.45	1800	size 1(blue)*1 & size 00*3	3.71
18.6-18.8	1200	size 1(blue)*1 & size 00*3	2.64	1900	size 1(blue)*1 & size 00*3	3.87
18.6-18.8	1300	size 1(blue)*1 & size 00*3	2.83	2000	size 1(blue)*1 & size 00*3	4.03
18.8-19.0	700	size 1 (blue)*1 & size 00*2	2.17	1400	size 1(blue)*1 & size 00*3	3.02
18.8-19.0	800	size 00*3	2.26	1500	size 1(blue)*1 & size 00*3	3.2
18.8-19.0	900	size 1(blue)*2 & size 00*2	2.19	1600	size 1(blue)*1 & size 00*3	3.37
18.8-19.0	1000	size 1(blue)*1 & size 00*3	2.26	1700	size 1(blue)*1 & size 00*3	3.55
18.8-19.0	1100	size 1(blue)*1 & size 00*3	2.46	1800	size 1(blue)*1 & size 00*3	3.71
18.8-19.0	1200	size 1(blue)*1 & size 00*3	2.65	1900	size 1(blue)*1 & size 00*3	3.88
18.8-19.0	1300	size 1(blue)*1 & size 00*3	2.84	2000	size 1(blue)*1 & size 00*3	4.04
19.0-19.2	700	size 1 (blue)*1 & size 00*2	2.17	1400	size 1(blue)*1 & size 00*3	3.02
19.0-19.2	800	size 00*3	2.26	1500	size 1(blue)*1 & size 00*3	3.2
19.0-19.2	900	size 1(blue)*2 & size 00*2	2.2	1600	size 1(blue)*1 & size 00*3	3.38
19.0-19.2	1000	size 1(blue)*1 & size 00*3	2.26	1700	size 1(blue)*1 & size 00*3	3.55
19.0-19.2	1100	size 1(blue)*1 & size 00*3	2.46	1800	size 1(blue)*1 & size 00*3	3.72
19.0-19.2	1200	size 1(blue)*1 & size 00*3	2.65	1900	size 1(blue)*1 & size 00*3	3.89
19.0-19.2	1300	size 1(blue)*1 & size 00*3	2.84	2000	size 1(blue)*1 & size 00*3	4.05
19.2-19.4	700	size 1 (blue)*1 & size 00*2	2.18	1400	size 1(blue)*1 & size 00*3	3.03
19.2-19.4	800	size 00*3	2.26	1500	size 1(blue)*1 & size 00*3	3.21
19.2-19.4	900	size 1(blue)*2 & size 00*2	2.2	1600	size 1(blue)*1 & size 00*3	3.39
19.2-19.4	1000	size 1(blue)*1 & size 00*3	2.27	1700	size 1(blue)*1 & size 00*3	3.56
19.2-19.4	1100	size 1(blue)*1 & size 00*3	2.46	1800	size 1(blue)*1 & size 00*3	3.73
19.2-19.4	1200	size 1(blue)*1 & size 00*3	2.66	1900	size 1(blue)*1 & size 00*3	3.89
19.2-19.4	1300	size 1(blue)*1 & size 00*3	2.85	2000	size 1(blue)*1 & size 00*3	4.06
19.4-19.6	700	size 1 (blue)*1 & size 00*2	2.18	1400	size 1(blue)*1 & size 00*3	3.03
19.4-19.6	800	size 00*3	2.26	1500	size 1(blue)*1 & size 00*3	3.22
19.4-19.6	900	size 1(blue)*2 & size 00*2	2.2	1600	size 1(blue)*1 & size 00*3	3.39
19.4-19.6	1000	size 1(blue)*1 & size 00*3	2.27	1700	size 1(blue)*1 & size 00*3	3.57
19.4-19.6	1100	size 1(blue)*1 & size 00*3	2.47	1800	size 1(blue)*1 & size 00*3	3.74
19.4-19.6	1200	size 1(blue)*1 & size 00*3	2.66	1900	size 1(blue)*1 & size 00*3	3.9
19.4-19.6	1300	size 1(blue)*1 & size 00*3	2.85	2000	size 1(blue)*1 & size 00*3	4.06
19.6-19.8	700	size 1 (blue)*1 & size 00*2	2.18	1400	size 1(blue)*1 & size 00*3	3.04
19.6-19.8	800	size 00*3	2.27	1500	size 1(blue)*1 & size 00*3	3.22
19.6-19.8	900	size 1(blue)*2 & size 00*2	2.2	1600	size 1(blue)*1 & size 00*3	3.4
19.6-19.8	1000	size 1(blue)*1 & size 00*3	2.27	1700	size 1(blue)*1 & size 00*3	3.57
19.6-19.8	1100	size 1(blue)*1 & size 00*3	2.47	1800	size 1(blue)*1 & size 00*3	3.74
19.6-19.8	1200	size 1(blue)*1 & size 00*3	2.66	1900	size 1(blue)*1 & size 00*3	3.91
19.6-19.8	1300	size 1(blue)*1 & size 00*3	2.85	2000	size 1(blue)*1 & size 00*3	4.07
19.8-20.0	700	size 1 (blue)*1 & size 00*2	2.18	1400	size 1(blue)*1 & size 00*3	3.04
19.8-20.0	800	size 00*3	2.27	1500	size 1(blue)*1 & size 00*3	3.23
19.8-20.0	900	size 1(blue)*2 & size 00*2	2.21	1600	size 1(blue)*1 & size 00*3	3.4
19.8-20.0	1000	size 1(blue)*1 & size 00*3	2.27	1700	size 1(blue)*1 & size 00*3	3.58
19.8-20.0	1100	size 1(blue)*1 & size 00*3	2.47	1800	size 1(blue)*1 & size 00*3	3.75
19.8-20.0	1200	size 1(blue)*1 & size 00*3	2.67	1900	size 1(blue)*1 & size 00*3	3.92
19.8-20.0	1300	size 1(blue)*1 & size 00*3	2.86	2000	size 1(blue)*1 & size 00*3	4.08

Size 1 green opaque: 489 mg delivering 73.35 mg elemental magnesium
Size 1 blue opaque: 515 mg delivering 77.25 mg elemental magnesium
Size 00 orange opaque: 694 mg delivering 104.10 mg elemental magnesium
Row in green only for men

5. Treatment of side effects of magnesium supplements:
   - When a study participant reports a side effect of diarrhea from taking the study supplement, the participant will be instructed to take Loperamide (Imodium™), according to the manufacturer's directions for no more than 7 days.
   - If the Loperamide is not effective within 48 hours of beginning the dosing of Loperamide, the participant will call the study staff. The supplement dose reduction plan will be initiated (see attached table).
   - After 7 days of use the participant will be contacted. If the diarrhea has resolved while taking the Loperamide the participant will be instructed to continue to take the prescribed dosage of the study supplement.
   - When diarrhea recurs after stopping the initial effective use of Loperamide, the participant will be instructed to resume the manufacturer's dosing recommendations until diarrhea resolves and then decrease the dose of the Loperamide until the minimum effective dose is reached. The participant will remain at this minimum dose for the remaining time left in their study participation. If the diarrhea never resolves, the supplementation reduction plan will be initiated (see attached table).
   - A participant, who reports side effects of abdominal gas or cramping, will be advised to take simethicone for relief.

   Both Loperamide (Imodium™) and Simethicone (Gas-X™, Flatulex™, Genasyme™, and Mylicon™) are inexpensive over-the-counter medications that are safe for long-term use and do not alter intestinal absorption patterns.

6. Dose reduction plan:
   When a participant reports that the use of Loperamide is not effective within 48 hours or the diarrhea recurs after stopping the initial dose of Loperamide and never resolves by resuming the Loperamide (see the attached flowchart), the supplement dose reduction plan will be initiated (see attached table). Six reduction steps are included in the Dose Reduction Guidelines.
   - After 4 days of initiating the first reduction step, the participant will be contacted. If the reduction of supplement dosing is ineffective, subsequent reduction steps will be implemented and the participant contacted after 4 days of reduction until resolution of the diarrhea is achieved.
   - If diarrhea does not resolve within 4 reduction steps, the participant will be referred to the study physician for consultation.
   - When the diarrhea resolves during the use of the dose reduction step plan, the participant will be instructed to return to the use of the initial dosing of the supplement.

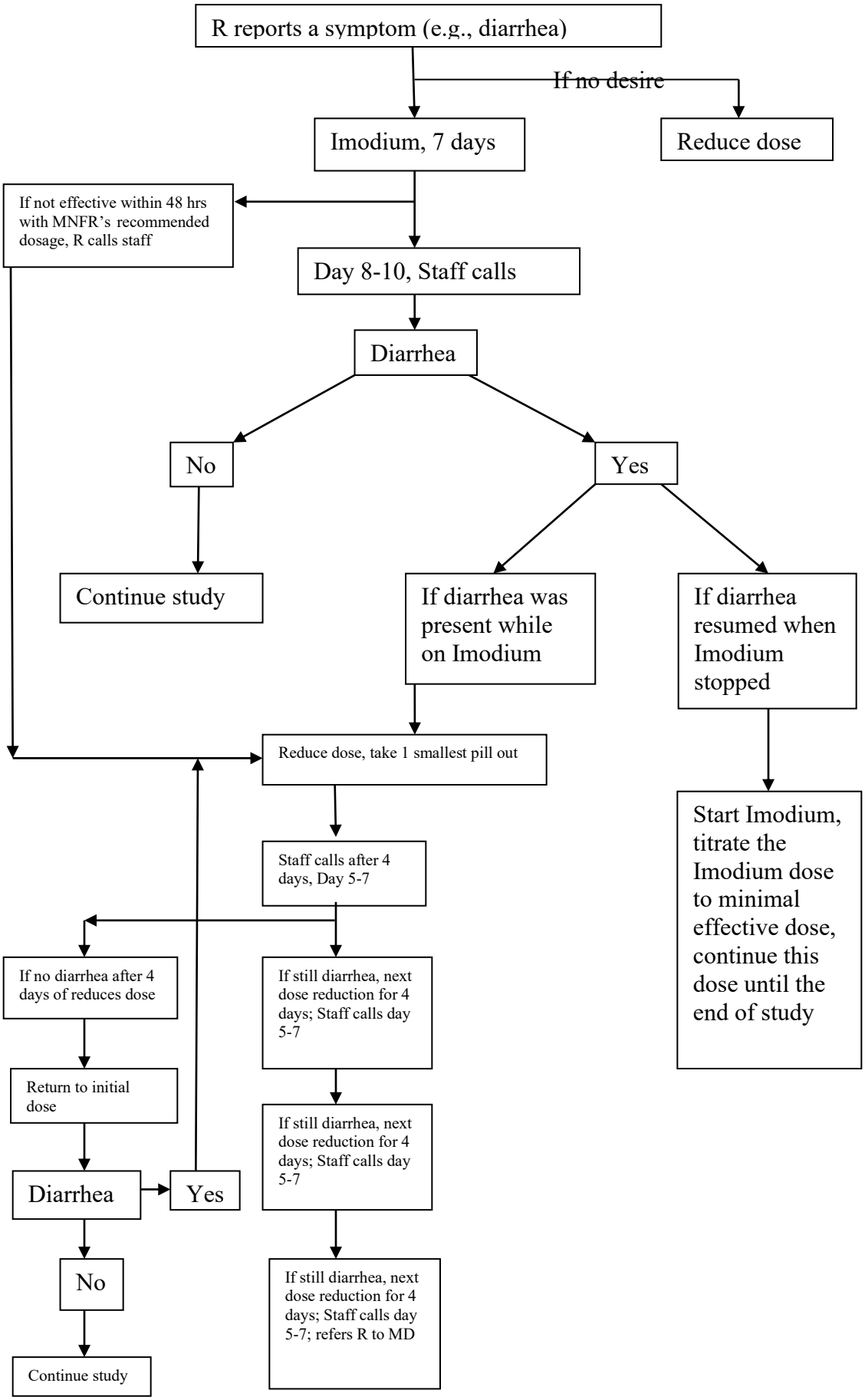

R reports a symptom (e.g., diarrhea)
If no desire
Imodium, 7 days
Reduce dose
If not effective within 48 hrs with MNFR's recommended dosage, R calls staff
Day 8-10, Staff calls
Diarrhea
No
Yes
Continue study
If diarrhea was present while on Imodium
If diarrhea resumed when Imodium stopped
Reduce dose, take 1 smallest pill out
Start Imodium, titrate the Imodium dose to minimal effective dose, continue this dose until the end of study
Staff calls after 4 days, Day 5-7
If no diarrhea after 4 days of reduces dose
If still diarrhea, next dose reduction for 4 days; Staff calls day 5-7
Return to initial dose
If still diarrhea, next dose reduction for 4 days; Staff calls day 5-7
Diarrhea
Yes
No
If still diarrhea, next dose reduction for 4 days; Staff calls day 5-7; refers R to MD
Continue study

Table Magnesium Dose Reduction Guidelines for PPCCT

Original dosage	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
B1	End							
77.25	100%							
O1	B1	End						
104.1	25.80%	100%						
G2	G1	End						
146.7	50%	100%						
B2	B1	End						
154.5	50%	100%						
B1O1	O1	B1	End					
181.35	42.60%	25.80%	100%					
O2	O1	End						
208.2	50%	100%						
B1G2	G2	G1	End					
223.95	34.50%	50%	100%					
O1G2	O1G1	G2	O1	G1	End			
250.8	29.20%	17.30%	29.04%	29.50%	100%			
B3	B2	B1	End					
231.75	33.30%	50%	100%					
B2O1	B1O1	B2	O1	B1	End			
258.6	29.90%	14.80%	32.62%	26%	100%			
B1O2	O2	B1O1	B2	O1	B1	End		
285.45	27.10%	12.90%	14.80%	32.62%	26%	100%		
G4	G3	G2	G1	End				
293.4	25%	33.33%	50%	100%				
O3	O2	O1	End					
312.3	33.33%	50%	100%					
B3O1	B2O1	B1O1	B2	O1	B1	End		
335.85	23%	29.90%	14.80%	32.62%	26%	100%		
B2O2	B1O2	O2	B1O1	B2	O1	B1	End	
362.7	21.30%	27.10%	12.90%	14.80%	32.62%	26%	100%	
B1O3	O3	B1O2	O2	B1O1	B2	O1	B1	End
389.55	19.83%	8.60%	27.10%	12.90%	14.80%	32.62%	26%	100%

Size 1 green opaque: 489 mg delivering 73.35 mg elemental magnesium
Size 1 blue opaque: 515 mg delivering 77.25 mg elemental magnesium
Size 00 orange opaque: 694 mg delivering 104.10 mg elemental magnesium

F. Clinical Visits

1. Clinic Visits

Eligible participants will be invited to the Clinic Research Center, Vanderbilt University Medical Center three times during the period of 12-week intervention, prior to the treatment (week 0), the midpoint (week 6) and the end of intervention (week 12). In addition, the participants will be invited to undergo magnesium tolerance test at the Vanderbilt CRC following the completion of the 12-week intervention with magnesium supplements.

--- Clinic Visit #1

Location

Clinic Visit #1 will be in the Clinical Research Center (CRC) at Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will be scheduled for clinic visit #1 prior to the intervention. All clinic visits on a given participant will be scheduled for the same time of day to avoid potential variability due to circadian rhythm.

Preparation

Documents

- Study Activity Calendar
- Informed Consent Form
- Payment Authorization Form
- Prescription Note
- Instruction Sheet for Taking Pills
- Biopsy Procedure Notes for Dr. Reid or Dr. Seidner
- Post Procedure Safety Handout
- Biological Specimen Collection Form H_1
- Specimen Transit Log Form
- CRC Order Sheet for Clinic Visit #1
- Specimen Labels
- Clinic Progress Note
- Montreal Cognitive Assessment Form for Clinic Visit #1 (Instructions attached)
- Health Assessment Form for Clinic Visit #1

Biopsy procedure supplies (see the Rectal Biopsy protocol)

Blood draw supplies (see the Blood Draw Protocol)

Nail collection supplies (see the Nail Collection Protocol)

Hair collection supplies (see the Hair Collection Protocol)

Skin swab collection supplies (see the Skin Swab Collection Protocol)

Patient packet (for the next sample collection at week 6)

- A copy of signed consent
- Instruction sheet for taking pill

- Magnesium/placebo pills
- Post procedure safety handout
- Instruction booklet for urine, stool and saliva collection at home
- Clinic visit questionnaire form
- Health questionnaire form for clinical visit #2
- Probiotics questionnaire form for clinical visit #2
- Urine sample collection kit
- Stool sample collection kit
- Saliva sample collection kit
- Cardboard box containing a styrofoam transportation cooler
- Freezer pack
- Zip lock bags
- Menus (to choose breakfast, lunch, and dinner at CRC clinic visit)

Procedures

1. Preparation on the day prior to the First Clinic Visit
    - The PPCCT research staff will place a reminder call (REMINDER CALL SCRIPT for CLINIC VISIT #1, Appendix F) on the day prior to the first clinic visit.
    - A reminder email will be sent to the recruited participant to remind him/her of the time, date of the clinical visit.
    - The PPCCT research staff will prepare all the materials, including documents and supplies, for the clinic visit.

2. Before obtaining the signed consent form, the PPCCT research staff will meet with the participants and discuss the study in lay terminology in the Clinic Research Center at Vanderbilt Medical Center on the day of the first clinic visit,.
    - The purpose of the study will be explained during the in-person interview.
    - The study design, magnesium supplementation, the biospecimen and data collection in this study will be described.
    - Participants will be invited to make three clinic visits at the Clinic Research Center, Vanderbilt University during the period of 12-week intervention and two additional clinic visits for the magnesium tolerance test after completion of the 12-week intervention.
    - Compensation
        - $250 for all clinic visits
        - Additional $50 for consistently providing fasting blood sample during the study
    - Magnesium supplementation trial
        - Magnesium/placebo pills orally taken with breakfast meal daily for 12 weeks
        - Magnesium supplementation safety and compliance will be closely monitored and carefully taken care of via phone calls, pill count report, serum magnesium measurement, and renal function test, etc.
    - Participants will be asked to provide the following biological samples at week 0, week 6 and week 12 during the study respectively.
        - Rectal biopsies × 2 (obtained at week 0 and week 12)
        - Blood samples × 3 (collected on biopsy days and at week 6)
        - Urine samples × 3 (collected on biopsy days and at week 6)
        - Stool samples × 3 (collected on biopsy days and week 6)
        - Saliva samples × 3 (collected on biopsy days and week 6)
        - Nail samples × 2 (collected on biopsy days)
        - Hair samples × 2 (collected on biopsy days)

        - Skin swab samples × 3 (collected on biopsy days and week 6)

- Participants who undergo magnesium tolerance test will be asked to provide the following samples prior to the test and following the initiation of magnesium tolerance test.
    - 24 hour urine samples × 2
    - Fasting blood samples × 2
- We will measure the body size and vital signs for the participants during each clinic visit.
    - Weight
    - Height
    - Waist-hip circumference
    - Blood pressure
- The risks and benefits of the study will be discussed.
- The participant will be told that participation is voluntary.
- Review the inclusion and exclusion criteria again.
- Answer participant's questions and concerns.
- Further evaluate the participant's interest.
- Obtain the signed Informed Consent Form and give a copy of signed consent to the participant
- Obtain the signed Payment Authorization Form

3. After obtaining the signed consent form, the PPCCT research staff will perform the following procedures with the help of the CRC nurse
    - Contact the pharmacist at the VIDS and fax the Prescription once the signed Informed Consent Form is obtained. The VIDS will deliver pills (Magnesium glycinate or placebo) to the CRC. The participant will be given magnesium/placebo pills and verbal instructions. An Instruction Sheet for Taking Pill will also be given and taken home (Appendix E).
    - Obtain the signed Payment Authorization Form (Appendix J).
    - Measure the body size and vital signs for each participant and record in the Biological Specimen Collection Form H_1 (Appendix H).
    - Obtain the urine, stool, and saliva samples and temporarily store the samples in the freezer at CRC. The completed Clinic Visit Questionnaire Form (Appendix F) will be reviewed by the research staff. Particularly, record medications and supplements currently used by the participant and the dosage.
    - The urine, stool, saliva sample collection information will be recorded in the Biological Specimen Collection Form H_1 by research staff.
    - Cognitive assessment will be performed and the collected information will be recorded in a Montreal Cognitive Assessment Form for Clinic Visit #1 (Appendix F) by a PPCCT research staff.
    - Health assessment will be performed and the collected information will be recorded in a Health Assessment Form for Clinic Visit #1 (Appendix F) by a PPCCT research staff. The procedure was only conducted during 6/18/2012 to 9/30/2012 because of the staff's departure. "
    - The blood draw, skin swab sample collection, and hair and nail sample collection procedures will be performed. Sample collection protocols for blood, hair, and nail will be applied and samples will be obtained respectively. The collection information will be recorded in the Biological Specimen Collection Form H_1. (Details in the Biological Specimen collection protocols).

- The rectal biopsy procedure will be performed by a well trained gastroenterologist. The rectal biopsies and rectal swabs will be obtained. The collection information will be recorded in the Biological Specimen Collection Form H_1. The participant will be closely observed for 30 minutes at the Clinical Research Center after the procedure. A Post Procedure Safety Handout will be given to the participant and taken home (Appendix F).
- Give participant a packet for next biological sample collection at week 6.
- Fill out and review the Biological Specimen Collection Form H_1.
- Complete the Clinic Progress Note (Appendix F)

4. Biological specimens handling and processing (See details in the Biological Specimen Collection Protocols)
   - *Rectal Biopsies and swabs*: Deliver the collected rectal biopsies and rectal swabs to the Molecular Epidemiology Lab. The Rectal biopsies handling and processing protocol will be applied.
   - *Blood samples*: Deliver the collected blood samples to the Molecular Epidemiology Lab. The blood sample handling and processing protocol will be applied.
   - *Urine samples*: Deliver the urine samples to the Molecular Lab. The Urine sample handling and processing protocol will be applied.
   - *Stool samples*: Deliver the stool samples to the Molecular Epidemiology Lab. The stool sample handling and processing protocol will be applied.
   - *Saliva samples*: Deliver the saliva samples to the Molecular Epidemiology Lab. The saliva sample handling and processing protocol will be applied.
   - *Hair samples*: Deliver the hair samples to the Molecular Epidemiology Lab. The hair sample handling and processing protocol will be applied.
   - *Nail samples*: Deliver the nail samples to the Molecular Epidemiology Lab. The hair sample handling and processing protocol will be applied.
   - *Skin Swabs*: Deliver the skin swab samples to the Molecular Epidemiology Lab. The skin swab sample handling and processing protocol will be applied.
   - *Serum creatinine test*: Deliver 1.5 -2 ml of plasma from the green top tube to the Vanderbilt Hospital Lab for renal function panel and serum magnesium assays.
   - *Pregnancy test*: Deliver 1 ml of plasma from the green top tube to the CRC laboratory for pregnancy test if the participant is a woman.
   - Complete the Biological Specimen Transit Log Form and Laboratory Log.
5. Eligibility Further confirmed by the renal creatinine clearance
   - The PPCCT research staff receive the results of pregnancy test for the female participants from CRC laboratory.
   - The PPCCT research staff get access to the StarPanel on the next day following the clinic visit #1, record the serum creatinine result, and enter it into the Redcap database. The estimated glomerular filtration rate (eGFR) will be calculated based on the serum creatinine level, age, gender, and race.
   - If either the creatinine clearance is < 50 or the pregnancy test is positive, a phone call will be placed to inform the participant of his/her ineligibility due to the decreased renal function or pregnancy. [Script for Post Clinic Visit #1 Ineligibility Call (Appendix B)]

--- Clinic Visit #2 (Week 6)

Location

Clinic Visit #2 will be in the Clinical Research Center (CRC) at Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will be scheduled for clinic visit #2 at week 6 of the magnesium supplementation trail. All clinic visits on a given participant will be scheduled for the same time of day to avoid potential variability due to circadian rhythm.

Preparation

Documents

- Prescription Note
- Pill Count Report
- Instruction Sheet for Taking Pills
- Biological Specimen Collection Form H_2
- Specimen Transit Log Form
- CRC Order Sheet for Clinic Visit #2
- Specimen Labels
- Clinic Progress Note
- Health Assessment Form for Clinical Visit #2

Blood draw supplies (see the Blood Draw Protocol)

Skin swab collection supplies (see the Skin Swab Collection Protocol)

Patient packet

- Instruction Sheet for Taking Pill
- Magnesium/placebo pills
- Instruction booklet for urine, stool and saliva collection
- Clinic Visit Questionnaire Form
- Diet Questionnaire for Clinic Visit #3
- Health Questionnaire for Clinic Visit #3
- Probiotocs Questionnaire for Clinical Visit #3
- Urine sample collection kit
- Stool sample collection kit
- Saliva sample collection kit
- Cardboard box containing a styrofoam transportation cooler
- Freezer pack
- Zip Lock bags
- Menus (to choose breakfast, lunch, and dinner at CRC clinic visit)

Procedures:

1. Preparation on the day prior to Clinic Visit #2
   - The PPCCT research staff will place a reminder call (REMINDER CALL SCRIPT for CLINIC VISIT #2, Appendix F) on the day prior to clinic visit #2.
   - A reminder email will be sent to the recruited participant to remind him/her of the time, date of the clinical visit.
   - The PPCCT research staff will prepare all the materials needed for clinic visit #2 on the day prior to the second clinic visit.

2. The PPCCT research staff will meet the participant at the Clinical Research Center on the day of Clinic Visit #2
    - Confirm continued willingness to participate in the study and address any issues or concerns.
    - Contact the pharmacist at VIDS and fax the prescription note. Magnesium supplement or placebo pills will be given to the participant at CRC. An Instruction Sheet for Taking Pill will be given to the participant and taken home.
    - Measure the body size and the vital signs with the help of CRC nurse and record the measurement in the Biological Specimen Collection Form H_2 (Appendix H)
    - Conduct pill counting and fill out the Pill Count Report (Appendix E)
    - Health assessment will be performed and the collected information will be recorded on a Health Assessment Form for Clinic Visit #2 (Appendix F) by a PPCCT research staff. The procedure was only conducted from 6/18/2012 to 9/30/2012 because of the staff's departure. "
    - Obtain the urine, stool, and saliva samples collected by the participant at home and temporarily store the samples in the freezer at CRC. Review the completed Clinic Visit Questionnaire Form. Record the sample collection information in the Biological Specimen Collection Form H_2.
    - The blood draw procedure and skin swab sample collection will be performed and blood sample will be collected. The collection information will be recorded in the Biological Specimen Collection Form H_2.
    - A packet for the next biological sample collection at week 12 will be given to participant.
    - Review and complete the Biological Specimen Collection Form H_2.
    - Complete the Clinic Research Progress Note (Appendix F).

3. Biological specimens handling and processing (see details in Biological Specimen Collection Protocols)
    - *Blood samples*: Deliver the collected blood samples to the Molecular Epidemiology Lab. The blood sample handling and processing protocol will be applied.
    - *Urine samples*: Deliver the urine samples to the Molecular Lab. The Urine sample handling and processing protocol will be applied.
    - *Stool Samples*: Deliver the stool samples to the Molecular Epidemiology Lab. The stool sample handling and processing protocol will be applied.
    - *Saliva samples*: Deliver the saliva samples to the Molecular Epidemiology Lab. The saliva sample handling and processing protocol will be applied.
    - *Skin Swabs*: Deliver the skin swab samples to the Molecular Epidemiology Lab. The skin swab sample handling and processing protocol will be applied.
    - Complete the Biological Specimen Transit Log Form and Laboratory Log.

--- Clinic Visit #3 (Week 12)

Location

Clinic Visit #3 will be in the Clinical Research Center (CRC) at Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will be scheduled for clinic visit #3 at week 6 of the magnesium supplementation trail. All clinic visits on a given participant will be scheduled for the same time of day to avoid potential variability due to circadian rhythm.

Preparation

Documents

- Pill Count Report
- Post Procedure Safety Handout
- Biological Specimen Collection Form H_3
- Specimen Transit Log Form
- CRC Order Sheet for Clinic Visit #2
- Specimen Labels
- Clinic Progress Note
- Montreal Cognitive Assessment Form for Clinic Visit #3
- Health Assessment Form for Clinic Visit #3

Rectal biopsy procedure supplies (see Rectal Biopsy Protocol)

Blood draw supplies (see the Blood Draw Protocol)

Nail collection supplies (see the Nail Collection Protocol)

Hair collection supplies (see the Hair Collection Protocol)

Skin swab sample collection supplies (see the Skin Swab Collection Protocol)

Participant packet

- Instruction for 24 hour urine collection
- 24 hour urine collection kit
- Clinic Visit Questionnaire Form
- Zip Lock bags
- Menus (to choose breakfast and lunch at CRC clinic visit)

Procedures:

1. Preparation on the day prior to Clinic Visit #3
   - The PPCCT research staff will place a reminder call (REMINDER CALL SCRIPT for CLINIC VISIT #3, Appendix F) on the day prior to clinic visit #3.
   - A reminder email will be sent to the recruited participant to remind him/her of the time, date of the clinical visit.
   - The PPCCT research staff will prepare all the materials for clinic visit #3 on the day prior to the third clinic visit.

2. The PPCCT research staff will meet the participant at the Clinical Research Center on the day of Clinic Visit #3
    - Measure the body size and the vital signs with the help of CRC nurse; record the measurements in the Biological Specimen Collection Form H_3 (Appendix H)
    - Obtain the urine, stool, and saliva samples collected by the participant at home and temporarily store the samples in the freezer at CRC. Review the completed Clinic Visit Questionnaire Form. Record the sample collection information in the Biological Specimen Collection Form H_3.
    - Conduct pill counting and fill out the Pill Count Report (Appendix E)
    - Cognitive assessment will be performed and the collected information will be recorded in a Montreal Cognitive Assessment Form for Clinic Visit #1 (Appendix F) by a PPCCT research staff.
    - Health assessment will be performed and the collected information will be recorded on a Health Assessment Form for Clinic Visit #3 (Appendix F) by a PPCCT research staff. The procedure was only conducted from 6/18/2012 to 9/30/2012 because of the staff's departure. "
    - The blood draw, skin swab sample collection, hair sample collection, and nail sample collection procedures will be performed. Samples will be collected and the collection information will be recorded in the Biological Specimen Collection Form H_3.
    - A light green top tube of blood will be collected and sent to the Vanderbilt Hospital for renal function test.
    - The rectal biopsy procedure will be performed by a well trained gastroenterologist. The rectal biopsies and rectal swabs will be collected. The collection information will be recorded in the Biological Specimen Collection Form H_3. The participant will be closely observed for 30 minutes after the procedure at the Clinical Research Center. A post procedure safety handout will be given to the participant and taken home (Appendix H).
    - Schedule a clinic appointment for Magnesium Tolerance Test. A packet for the 24 hour urine collection and a magnesium tolerance test instruction handout will be given to the participant.
    - Fill out and review the Biological Specimen Collection Form H_3.
    - Complete the Clinic Progress Note (Appendix F)

3. Biological specimens handling and processing (see details in the Biological Specimen Collection Protocols)
    - *Rectal Biopsies and swabs*: Deliver the collected rectal biopsies and rectal swabs to the Molecular Epidemiology Lab. The Rectal biopsies handling and processing protocol will be applied.
    - *Blood samples*: Deliver the collected blood samples to the Molecular Epidemiology Lab. The blood sample handling and processing protocol will be applied.
    - *Urine samples*: Deliver the urine samples to the Molecular Lab. The Urine sample handling and processing protocol will be applied.
    - *Stool samples*: Deliver the stool samples to the Molecular Epidemiology Lab. The stool sample handling and processing protocol will be applied.
    - *Saliva samples*: Deliver the saliva samples to the Molecular Epidemiology Lab. The saliva sample handling and processing protocol will be applied.
    - *Hair samples*: Deliver the hair samples to the Molecular Epidemiology Lab. The hair sample handling and processing protocol will be applied.
    - *Nail samples*: Deliver the nail samples to the Molecular Epidemiology Lab. The hair sample handling and processing protocol will be applied.

- *Skin Swabs*: Deliver the skin swab samples to the Molecular Epidemiology Lab. The skin swab sample handling and processing protocol will be applied.
- Complete the Biological Specimen Transit Log and Laboratory log

--- Clinic Visit #4 (Magnesium Tolerance Test)

Location

Clinic Visit #4 will be in the Clinical Research Center (CRC) at Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will receive a schedule of planned the magnesium tolerance test on two consecutive days. A 24 hour urine sample will be collected for determining the basal urinary magnesium excretion prior to the intravenous infusion of magnesium sufate. Another 24 hour urine sample will be obtained starting with the IV infusion.

Preparation

Documents

- Reminder Call Script for Clinic Visit #4
- Biological Specimen Collection Form H_4
- Clinic research progress note

Medications for magnesium tolerance test

- Magnesium sulfate, 2 g in 50 ml, 0.325 mEq/mL; Hospira, Inc., Lake Forest, IL
- 500 ml of 5% dextrose

Blood draw supplies (see the Blood Draw Protocol)

Participant packet

- Instruction for 24 hour urine collection
- 24 hour urine collection kit
- Clinic visit questionnaire form
- FedEx label

Procedures:

1. Preparation on the day prior to Clinic Visit #4
   - The PPCCT research staff will place a reminder call (REMINDER CALL SCRIPT for CLINIC VISIT #4, Appendix F) on the day prior to clinic visit #4.
   - A reminder email will be sent to the recruited participant to remind him/her of the time, date of the clinical visit.
   - The PPCCT research staff will prepare all the materials needed for clinic visit #4 on the day prior to the fourth clinic visit.

3. The PPCCT research staff will meet the participant at the Clinical Research Center on the day of Clinic Visit #4
   - Explain the purpose of the magnesium tolerance test with the participant again.
   - Obtain the 24 hour urine sample and temporarily store the samples in the freezer at CRC. Review the clinic visit questionnaire form. Record the information on 24 hour urine collection (pre magnesium tolerance test) in the Biological Specimen Collection Form H_4.

- Blood samples will be obtained prior to the IV. Infusion and the Blood draw protocol will be applied. The collection information will be recorded in the Biological Specimen Collection Form H_4.

4. Magnesium Tolerance Test (MTT)
   - Infuse 0.2mEq (2.4mg) elemental magnesium per kilogram of body weight in 500 ml of 5%dextrose over 4 hours, which will be conducted by the CRC nurses. (The infusion is no faster than 8meq of magnesium sulfate per hour. Participants with a body weight of 160kg or greater are ineligible for the magnesium tolerance test.)
   - Collect urine (starting with infusion) for magnesium and creatinine for 24 hours.

5. MTT safety monitoring
   - The infusion is no faster than 8meq of magnesium sulfate per hour. Participants with a body weight of 160kg or greater are ineligible for the magnesium tolerance test.
   - We will check and record the participant's blood pressure and heart rate before initiating the Magnesium sulfate intravenous drip infusion and monitor and record the same in the course of a four-hour infusion and within 1 hour following the infusion completion.

   - In addition, the participant's hemoglobin oxygen saturation levels (%), blood pressure, heart rate and respiration rate will be monitored continuously throughout the time of admission by experienced registered nurses at the CRC.

   - An attending physician, who is knowledgeable about the study and is part of the study team (a co-investigator), will be on call to accompany each participant during the Magnesium Tolerance Test.

   - In the event of a life-threatening situation, the participant will be sent to emergency room or a life-support team will be called to the CRC.

6. Percentage magnesium retained is calculated by the formula below

$$\left[1 - \frac{(\text{post infusion Mg excretion - basal Mg excretion}*)}{\text{total Mg infused}}\right] \times 100$$

$$*\text{basal Mg excretion} = \frac{\text{pre - infusion urine Mg}}{\text{pre - infusion urine Creatinine}} \times \frac{\text{post - infusion 24 hour urine}}{\text{Creatinine}}$$

7. Criteria for magnesium deficiency
   - ≥ 50% retention at 24 h: definite deficiency
   - 25% to < 50% retention at 24 h: probable deficiency
   - ≤ 25% retention at 24 h: normal

8. A letter will be mailed to the participant to inform them of his/her body magnesium status (Appendix K).

--- Clinic Visit #5 --- (Post Magnesium Tolerance Test)

Location

Clinic Visit #5 will be in the Clinical Research Center (CRC) at Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will receive a schedule for a clinic visit on the next day with magnesium tolerance test. Clinic visit #4 and clinic visit #5 will be scheduled for the same time of day to avoid potential variability due to circadian rhythm. The 24-hour urine sample obtained starting with the IV infusion of Magnesium tolerance test, will be delivered by the participant in person at clinic visit #5 or mailed in by FedEx, using the prepaid label provided at Clinic visit #4.

Preparation

Documents

- Biological Specimen Collection Form H_4
- Clinic research progress note

Blood draw supplies (see the Blood Draw Protocol)

Procedures:

1. The PPCCT research staff will meet the participant at the Clinical Research Center on the day of Clinic Visit #5
    - Obtain the 24 hour urine sample post-MTT and temporarily store the samples in the freezer at CRC. Review the clinic visit questionnaire form. Record the information on 24 hour urine collection (post MTT) in the Biological Specimen Collection Form H_4.
    - Blood draw protocol will be applied and blood samples (post-MTT) will be obtained. The collection information will be recorded in the Biological Specimen Collection Form H_4.
    - All samples will be delivered to the Molecular Epidemiology Lab. The urine and blood sample handling and processing protocols will be applied respectively (see details in the Biological Specimen Collection Protocols).

--- Health Survey for Chinese participants in Nashville area

We have conducted Chinese traditional Medicine assessments in our clinical trial for participants diagnosed with colorectal adenoma, who are mainly Caucasians.
We plan to conduct Chinese Traditional Medicine Assessments and two 24-hour dietary recalls among Chinese Americans in Nashville since Chinese or Chinese Americans have a lower incidence of colorectal cancer. We will compare the Chinese Traditional Medicine assessments and dietary intake pattern with those of Caucasians to identify possible protective effects against colorectal cancer or other diseases.

Location

Health survey will be conducted at the Vanderbilt Epidemiology Center or at the Nashville Chinese Baptist Church

Scheduling

Participants will receive a schedule for a health survey, including health examination, health questionnaire survey based on the Chinese Traditional Medicine Assessment, and two food record forms.

Preparation

Documents

- Consent form for Chinese participants
- Food amount booklet and two food record forms (stamped returned envelope provided as needed)
- Health assessment form for Chinese participants (Appendix F)
- Health questionnaire for Chinese participants and Chinese version attached (Appendix G)

Procedures:

1. An email (Appendix F) will be sent out to invite potential Chinese participants to participate in the Chinese Traditional Medicine assessment and Health Questionnaire survey.
2. The PPCCT research staff will meet the participant at the Vanderbilt Epidemiology Center or at the Nashville Chinese Baptist Church on the day scheduled for the health survey.
3. The PPCCT research staff member will describe the study protocol in lay terminology, including the purpose of the study and the study design.
4. The risks and benefits of the study are discussed prior to obtaining signatures on the consent form. The participant will be told that participation is voluntary.
5. The research staff will provide ample time for the participant to have questions answered prior to enrollment.
6. Obtain written informed consent.
7. The research staff will also provide the study participant with a copy of the consent form.
8. Health assessment will be performed and the collected information will be recorded on a Health Assessment Form for Chinese participants (Appendix F) by a PPCCT research staff.
9. Health questionnaire for Chinese Participants both in Chinese version and English version (Appendix G), two food record forms and a food amount booklet (Appendix F) will be administrated in person. A stamped return envelope will be provided if required.
10. A Thank you letter will be mailed to the participant after completion of the health survey.

4. Biological Specimen Collection Protocol

-- Rectal Biopsy

Rectal Biopsy Procedures and Initial Handling Protocol

Biomarkers measured from rectal biopsies will include Ki-67 (cellular proliferation), Bax (pro-apoptosis), COX2 (inflammation), and TRPM7 (Calcium/Magnesium absorption), pMLKL (necessary for the execution of necroptosis) and TUNEL assay will be performed for Apoptotic Index measurement. These biomarkers will be analyzed using immunohistochemical (IHC) techniques and the TUNEL method in rectal tissues obtained at baseline and the end of the intervention.

Procedures

Location

Biopsies will be collected at the baseline and final clinic visit in the Clinical Research Center (CRC) in Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will be scheduled for the biopsies. All biopsy visits on a given participant will be scheduled for the same time of day to avoid potential variability due to circadian rhythm. A gastroenterologist trained in the procedure will take the biopsies in CRC procedure rooms.

Preparation

Prior to the arrival of the patient, study staff will prepare the following:

Proctoscopy supplies

- ID labels with the patient's ID number, date, and visit number
- 1 collection bottle filled with 10% normal buffered formalin
- Specimen container
- Plastic proctoscope
- Biopsy forceps, Cook 230 cm flexible biopsy forceps (2.4 mm cup diameter).
- Bowl of sterile normal saline
- Wash cloths
- 4x4's
- K-y jelly
- Magnifying glass
- Bath towel
- Gooseneck light
- Headband LED light
- Purple (nitrile) Gloves
- Rectal swabs
- Suction
- Toothpicks
- Disposable plastic spoon

Fresh rectal biopsy (non-paraffin imbedded):

- Cryo vials
- Insulated container
- Liquid nitrogen
- Dry Ice
  - Dry ice will be placed in an insulated bucket or container.
  - Plastic cup will be placed in dry ice to hold fresh tissue specimen
  - Place a small amount of dry ice into plastic cup
  - Cap the plastic container, until the cryovial with specimen is inserted.

Rectal biopsy for paraffin embedment

- Vials containing 10% normal buffered formalin

Rectal swabs

- Culturette swab and transport medium

Patient Arrival

- Review medication list
- Review supplement list
- Verify continued eligibility
- Verify Empty Rectal Vault (Recent bowel movement)
- Measure body size i.e., height, weight, hip and waist circumferences
- Answer questions
- **Take vital signs**
- Obtain informed consent and give the participant a written copy of the consent form
- Escort patient to the examining room and have them undress from the waist down and cover with a drape or gown. Notify the physician the patient is ready

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

1. Patient is positioned in the Sims position
2. Insert culturette swab about 2 inches into rectum, rotate swab to collect fecal material, and carefully remove swab from rectum. Insert swab into the tube containing transport medium. (Do not use lubricant).
3. Biopsies are taken at 10 cm above the anal verge through a proctoscope using a large cup Cook flexible 230 cm biopsy forceps (2.4mm cup diameter).
4. Care is taken to place the fully open biopsy forceps at right angles to and against the mucosa in order to get a sample of sufficient area and depth.
    - At least **eight** adequate biopsies must be obtained
    - Study staff will examine biopsies with a magnifying glass to determine adequacy of biopsy
    - If a biopsy pinch is inadequate, another is obtained.
    - Do not remove the biopsy from the forceps using a needle or other instrument. Previously, we removed it with a pair of pincers, plastic (single use) or metal (sterilizable)
    - **Four to six** rectal biopsy specimens will be snap frozen for future analyses requiring fresh (non-paraffin imbedded) tissue. (**prepare the snap frozen tissue first !!)**
    - Place remaining biopsy specimens in labeled specimen bottle with 10% normal buffered formalin immediately.
    - If the biopsy cannot be teased off using the toothpick, shake it off in the normal saline, remove and put in formalin or in prepared green capped cryo vial
    - Biopsies can be retrieved from saline using a small plastic spoon
    - Record site of biopsy
        - Number of biopsies collected
        - Note any bleeding

Patient monitoring

After the biopsy, the patient is monitored for thirty minutes. The CRC staff will check blood pressure and have the patient go to the restroom to check for bleeding prior to leaving the clinic. Patient is told that they can expect some blood on the tissue on the day of the

biopsy. Bleeding heavier or more persistent than this should be reported. They should also report anal or lower abdominal pain, fever, a foul anal discharge, or anything else they think could be related to the biopsy. The gastroenterologists and the supporting medical team will handle emergencies. Any other problems discovered by the study team will be discussed at an appropriate level with the patient sufficient to affect referral to the patient's gastroenterologists or primary care physician who will also be informed after written consent by the patient.

Any unexpected anoscopy events (> Grade 1 pain events and any other complications to the biopsies) will be recorded on an Adverse Events Form; information will include a description of the complication, a rating of the severity, other essential information needed to evaluate the complication, an assessment of the likelihood that the event was due to a study procedure, and a statement of action taken. All potential complications of biopsies are rated as mild, moderate, or severe. Minor problems after the procedure are handled in consultation with the gastroenterologist, and recorded in the subject's study file. If undue complications of biopsies or other procedures arise, the study will be stopped ahead of schedule. In addition, in the event of a severe or life-threatening complication, the Vanderbilt Cancer Center Clinical protocol Review Committee will be immediately notified. Only participants with ongoing doctor/patient relationships will be used in this study. Patients will continue their usual care with the physician. Telephone numbers for the PI and study coordinator will be given to the participant. Emergencies will be handled by the gastroenterologist and supporting medical staff.

Rectal Swab processing

- At least 2 rectal swabs will be taken.
- Insert one swab into tube of transport media. Freeze the tube containing transport media and store the sample at -80˚C until future analysis.
- Place another swab into culture media. Swirl and drain to side of culture media. Discard swab.
- Record the time of collection on BIOLOGICAL SAMPLE COLLECTION FORM (Appendix H).
- Send the sample to lab for immediate culture.
- All samples will be logged into a log sheet for that day, and signed off by respective personnel along their movement from the participant (retrieval, placing into freezing medium, transportation to the lab)

Biopsy Processing

Biopsy Collection

- 1-2mm wide and 1mm thick
- At least eight biopsies will be taken
- Remove from forceps by teasing off with toothpick or shaking into saline bowl Biopsies can be retrieved from saline using a small plastic spoon
- Place fresh biopsy specimens into separate green capped vials and put vials into liquid nitrogen or dry ice. Place remaining biopsy specimens in labeled specimen bottle with 10% normal buffered formalin immediately.
- Enter the time the biopsies were dropped into formalin and / or snap frozen on the BIOLOGICAL SAMPLE COLLECTION FORM (Appendix H).

Laboratory Processing

Fresh Specimen (frozen):

- Rectal biopsy in the vials should freeze almost immediately when placed in the liquid nitrogen or dry ice.

- Frozen specimen should remain in the liquid nitrogen or dry ice until they can be placed in the -80° C freezer
- Study staff will then transport specimens to Dr. Qiuyin Cai, Molecular Epidemiology Core Laboratory, MCN, B-2188, ext # 6-1351
- Date, time and location of sample in the freezer will be recorded on the specimen log
- Samples will be stored at –80° C.
- Specimens will be stored until ready for analysis.
- All samples will be logged into a log sheet for that day, and signed off by respective personnel along their movement from the participant (retrieval, placing into freezing medium, transportation to the lab)

Fresh Specimen (cell/tissue culture):

- One of the fresh rectal biopsy will kept in 2 ml eppendorf tube with 1.5 ml regular culture medium at room temperature immediately.
- Study staff will then transport specimens to Dr. Qiuyin Cai, Molecular Epidemiology Core Laboratory, MCN, B-2188, ext # 6-1351 as soon as possible.

Embedded Biopsy Specimens:

Deliver specimen to Dr. Yinghao Su, Molecular Epidemiology Core Laboratory, MCN, B-2188, within one hour of collection

- Biopsy Orientation
- Biopsies are approximately 1 mm thick
- Core laboratory staff will orient specimen tissue samples are placed on a biopsy sponge and visualized under the dissecting microscope.
- Turn and orient the biopsy mucosal side up so that the specimen is stretched out as flat as possible.
- Mucosal side can be identified by its honeycomb-like appearance. Handle the specimen as little as possible.
- Do not touch the mucosal side of the biopsy except at the very edge of the biopsy.
- Do not worry if the biopsy is not completely flat as long as it is not twisted
- The laboratory/study staff must reorient biopsies within 30 minutes of being obtained.

Rectal Biopsy Laboratory Protocols
Procedure
Tissue Blocks

Embedding Biopsies

- To embed biopsy specimens as soon as possible after biopsy
  - Biopsies are fixed and processed for twenty-four hours in a tissue processor.
  - After 24h fixation in neutral buffered formalin, the biopsies are placed in a Shandon Hypercenter Processing Center (ThermoShandon, Pittsburgh, PA), a programmable, automated reagent-moving system used to dehydrate and infiltrate fixed tissues with paraffin.
  - Biopsies will be embedded in one paraffin block. Pay attention to embedding orientation of the tissues so that well-oriented longitudinal crypts can be sectioned.

- The cassettes are labeled with a # 3 pencil on the front side of each block with the participants ID # and visit date.
- Initial water residues are removed from tissues by flushing graded alcohols (70, 80, 95, and 100%) sequentially into the reaction chamber.
- Each alcohol remains in the tissue-containing chamber for approximately 45 minutes, and a 5-minute draining period is allowed between alcohol changes. Two xylene exchanges are followed by two 60C heated paraffin exchanges, each one hour in duration.
- The paraffin-infiltrated tissues are removed from the Hypercenter, embedded into paraffin blocks, and prepared for shipment.
- Blocks are then stored in the refrigerator in the core lab at 4 degrees C until ready for analysis.

Storage

- Blocks are stored in the core lab at 4 degrees C

Slide preparation

Biopsies will be sectioned with a microtome so that crypts will be longitudinally sectioned from base to lumen.

- Sections are then placed sequentially on positive charged slides and numbered from 1 to 26.
- Section levels are Five microns thick, and are taken approximately 50 microns apart. (Taking sections 50 microns apart to avoid double counting of crypts in biopsy specimens)
- The sections numbered 1, 11 and 21 will be used for H&E staining.
- Three slides per biomarker are prepared for processing each of the antibodies for KI-67, Bax, COX2, TRPM7, pMLKL and the TUNEL assay.
- Multiple biopsies are collected and multiple sections will be prepared. In case of staining run failures, one backup serial section per slide for staining is prepared.
- All blocks and slides will be stored in a vacuum chamber at 4 degree C in a cold room until analysis.

Immunohistochemistry

Slides are then subjected to immunohistochemical analysis for the antibody of interest in the Molecular Epidemiology Lab at Vanderbilt. Ki-67 (mib1), bax, COX2, TRPM7, pMLKL expression is detected in colon crypt cells by using immunohistochemical (IHC) technique that stains positive cells a brown color. This permits visual identification of cells expressing these genes.

Expression of Ki-67, Bax and COX-2 in colon epithelial cells will be detected following the standard IHC protocol of EnVision™+ System, HRP (DAKO). After deparaffinization of the tissue slides, antigen retrieval is performed by heating the slides with a pressure cooker in the optimized buffer solutions: Retrievagen A pH6.5 (BD Pharmingen) for Ki-67, R-buffer A (PickCell Lab.) for COX-2, and Epitope Retrieval Solution (IHCWorld.com) for Bax. The primary antibodies of mouse monoclonal anti-Ki-67(BD Biosciences Pharmingen), mouse monoclonal anti-COX-2 (Zymed), and rabbit polyclonal anti-Bax (Santa Cruz) are applied with proper work dilutions. The TUNEL (TdT-mediated dUTP Nick-End Labeling) assay is conducted to measure apoptosis of colon epithelium using DeadEnd Colorimetric TUNEL System (Promega). The expression and co-existence of TRPM7 is detected using a sequential double immunofluorescent staining proposed by Vector Laboratories (Protocol: Double Immunofluorescent Labeling Using Primary Antibodies from Different Species: http://www.vectorlabs.com/infopage.asp?dpID=53&locID=684325). In brief, antigen retrieval is performed by heating the slides with a pressure cooker in 0.01 M citrate buffer (pH 6.0). The

slides are blocked with Avidin/Biotin Blocking Kit (Vector) and 5% normal goat serum, incubated with 1st primary antibody (rabbit polyclonal anti-TRPM6, Abcam), biotinylated goat anti-rabbit IgG (Vector), and fluorescein avidin DCS (Vector). For staining of second antigen, the blocking step is repeated with Avidin/Biotin Blocking Kit (Vector) and 5% normal rabbit serum, the slides are incubated with the 2nd primary antibody (Sheep polyclonal anti-TRPM7 (Abcam), biotinylated rabbit anti-sheep IgG (Vector), and Texas red avidin DCS (Vector). The slides are washed and coverslipped with the anti-fade and anti-photobleaching VECTASHIELD HardSet Mounting Medium with DAPI (Vector). A lab-constructed tissue microarray block composed of lymph node, kidney, lung, small intestine and known positive and negative colon adenoma tissues for these biomarkers is used as control slide. The control slide is stained in parallel with each batch using an Autostainer Universal Staining System (DAKO).

Scoring Procedures:
All biomarkers will be scored using imaging analysis. We use a system composed of an Olympus BX40 microscope, a Retiga FAST 1394 color digital camera and BioQuant NOVA Prime imaging software (BioQuant, Nashville, TN). The video images are captured using a 10X objective lens under a constant state of exposure control. Approximately 24-30 well-oriented longitudinal crypts at three section levels per biopsy specimen are quantified. The distribution of positive cells in the upper, middle, and basal zones is evaluated separately by a zonal quantitative analysis procedure established in our lab. When each field is measured, region of interest tools are used to exclude blank, folding, hemorrhage, necrosis, poorly stained, and stromal areas, whereas threshold tools are used to precisely define and measure total epithelial area, total nuclear area, positively stained area, and average gray density[151]. After all fields of each sample are measured, the final immunoreaction indices are generated automatically by setting algorithms as ‘‘total positive area / total nuclear area’’ for nuclear staining markers (Ki-67, TUNEL), or ‘‘total positive area / total epithelial area X average density’’ for cytoplasmic/membranous staining markers (Bax, COX-2, TRPM7). Apoptotic activity is also scored using standard morphologic criteria applied to H&E stained sections[85,86]. Briefly, a total of 24 to 30 longitudinal crypts at three section levels are scored per biopsy. The cells are considered apoptotic if the shrinkage of the cell from its neighbors, chromatin condensation and nuclear fragmentation (karyorrhexis) are presented. Cells manifesting these criteria are included in apoptosis scores only when observed in isolated, single cells not associated with an inflammatory response. The number of apoptotic cells for each crypt is combined to calculate a mean apoptosis score per crypt for each specimen.

Quality Control
Dr. Su, the study pathologist, has had over 23 years of experience in conducting pathologic studies of cancers. He will be blinded to the treatment and genotyping status and will score all sections for all biomarkers (in total, 7200 biomarkers slides and 1440 H&E slides). Furthermore, the co-PI (Dr. Chang Yu) will randomly select 10% of slides (864 slides) for rescoring. For the rescoring, Dr. Su and a research staff will score the slides independently. Inter-scorer agreement will be compared and calculated.

Cell/Tissue culture
We will test the carcinogenesis-related biomarkers and use the vitro system to identify the most effective and optimized agents. (1) cell biological features: epithelial and stromal morphology; (2) immunophenotypes: CK5/14; CK8/18; Pan-CK; Vimentin; Smooth muscle actin detected by IF staining; (3) molecular signaling pathways; (4) organ-specific gene expression (5) carcinogenesis-related biomarkers; (6) stemness.

<u>Measurement of Microbiomial Markers</u>

Micorbiomial markers in rectal biopsy/swab samples will be measured at baseline, 3-month after the magnesium supplementation.

Measurement of other IHC biomarkers

IHC markers (e.g. CES1, CES2, DEC1, pMLKL) in rectal biopsy samples will be measured at baseline, 3-month after the magnesium supplementation.

Title of Study: Personalized Prevention of Colorectal Cancer Trial

IRB#:

Study Note

Date: Time:

Anoscopy with Rectal Biopsy for Study Protocol

Patient's Name:
Operator: Reid Ness, MD

Procedure: Anoscope was gently inserted into the anus. The obturator was then removed. Using a flexible biopsy forceps - model DBF-2.4-160-S (Cook Medical Inc.) , 8 specimens were obtained. Blood loss was minimal. The anoscope was removed. No complications noted. Patient was monitored per study protocol.

Reid Ness, MD

Title of Study: Personalized Prevention of Colorectal Cancer Trial

IRB#:

Study Note

Date: Time:

Anoscopy with Rectal Biopsy for Study Protocol

Patient's Name:
Operator: Douglas L. Seidner, MD

Procedure: Anoscope was gently inserted into the anus. The obturator was then removed. Using a flexible biopsy forceps - model DBF-2.4-160-S (Cook Medical Inc.), 8 specimens were obtained. Blood loss was minimal. The anoscope was removed. No complications noted. Patient was monitored per study protocol.

Douglas L. Seidner, MD

--- Blood Sample Collection

Blood Draw Protocol

In the study, erythrocyte magnesium (a candidate biomarker for marginal magnesium deficiency) will be used as the primary endpoint to monitor magnesium status and the compliance of treatment, complimented by the secondary endpoint, serum magnesium. Blood samples will be collected at weeks 0, 6 and 12 during the course of magnesium treatment.

In the study, serum magnesium, vitamin D and plasma C-reactive protein will be measured as secondary endpoints to evaluate the effects of the reduction of dietary Ca/Mg intake ratio by magnesium supplementation.

A fasting blood (15 ml for each person) will be drawn into serum, EDTA, and heparin tubes. A blood sample will be collected on the biopsy collection day at the beginning and the end of the intervention phase respectively, at week 0 and week 12. An additional blood sample will be collected at the midpoint of the intervention phase, at week 6.

Location

Clinical Research Center (CRC) Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will receive a schedule of planned blood draw, possibly fasting blood draw. Approved research staff with demonstrated competency will draw blood in CRC procedure rooms. A blood sample will be collected on the biopsy collection day at the beginning and the end of the intervention phase respectively, at week 0 and week 12. An additional blood sample will be collected at the midpoint of the intervention phase, at week 6. All visits on a given participant will be taken at the same time of day to avoid potential variability due to circadian rhythm.

Some participants, if willing, will provide two fasting blood samples in place of 1st and 3rd clinic visit collection. The first fast blood collection for the participant will take place less than or equal to 7 days prior to the baseline clinic visit. The last fasting blood collection will take place less than or equal to 7 days prior to the final clinic visit. Participants will receive $25 for each blood draw.

Preparation

- Tourniquet
- Non-latex disposable gloves
- Syringe or Evacuated Tubes
- Needle holder
- 70% alcohol pads
- 20 or 21 Gauge needle for the forearm or 25 Gauge needle for the wrist or hand
- Red, Lavender and Green top collection tubes
- Labels
- 2 x 2 Gauze pads
- Adhesive Tape or Bandages
- Biohazard sharps collection container

Procedure

- Wash your hands thoroughly and don non-latex disposable gloves to prevent cross-contamination.
- Ask the ambulatory patient to sit in a chair and support his arm securely on an armrest or tabletop.

- Assess the patient's veins to determine the best puncture site. Observe the skin for the vein's blue color, or palpate the vein for a firm rebound sensation.
- Tie a tourniquet 2" proximal to the area chosen. If the tourniquet fails to dilate the vein, have the patient open and close his fist repeatedly. Then ask him to close his fist as you insert the needle and to open it again when the needle is in place.
- Clean the venipuncture site with an alcohol pad using friction for 30 seconds. Wipe in a circular motion, spiraling outward from the site. Allow to dry before performing venipuncture.
- Immobilize the vein by pressing just below the venipuncture site with your thumb and drawing the skin taut.
- Position the needle holder or syringe with the needle bevel up and the shaft parallel to the path of the vein and at a 30-degree angle to the arm. Insert the needle into the vein. If you are using a syringe, venous blood will appear in the hub; withdraw the blood slowly, pulling the plunger of the syringe gently to create steady suction until you obtain the required sample. Pulling the plunger to forcibly may collapse the vein. If you're using a needle holder and an evacuated tube, grasp the holder securely to stabilize the vein, and push down on the collection tube until the needle punctures the rubber stopper. Blood will flow into the collection tube automatically.
- Remove the tourniquet as soon as the blood flows adequately to prevent stasis and hem concentration, which can impair test results. If blood flow is sluggish, leave the tourniquet in place longer, but always remove it before withdrawing the needle.
- Continue to fill the required tubes, removing one and inserting another. Gently rotate each tube as you remove it to help mix the additive.
- After you've drawn the sample, place a gauze pad over the puncture site, and slowly and gently remove the needle from the vein. When using and evacuated tube, remove it from the needle holder to release the vacuum before withdrawing the needle from the vein.
- Apply gentle pressure to the puncture site for 2 to 3 minutes or until bleeding stops. This prevents extravasation into the surrounding tissue, which causes hematoma.
- After the bleeding stops, apply an adhesive bandage.
- If you have used a syringe, transfer the sample to a collection tube. Detach the needle from the syringe, open the collection tube, and gently empty the sample into the tube, being careful to avoid foaming, which may cause hemolysis.
- Label the patient chart with appropriate blood tube labels and record the time of collection on BIOLOGICAL SAMPLE COLLECTION FORM (Appendix H).
- Place the red and purple top tubes into black wet ice bucket and leave the green top tube at room temperature.
- Finally, check the venipuncture site to make sure a hematoma has not developed. If it has, then apply warm soaks.
- Discard syringes, needles, and used gloves in the appropriate containers.

Special Considerations

- If the patient has large, distended, highly visible veins, perform venipuncture without a tourniquet to minimize the risk of hematoma
- If the patient has a clotting disorder or is receiving anticoagulant therapy, maintain firm pressure on the venipuncture site for no less than 5 minutes after withdrawing the needle to prevent formation of a hematoma.

Blood processing

- Blood specimens will be stored in CRC refrigerator.
- All specimen's will be labeled with date, participant ID #

- Specimens will be processed within 4-6 hours after collection
- Serum will be collected after coagulation.
- Whole blood (EDTA tube) will be processed within 4-6 hours and separated into plasma, buffy coats (white cells).
- Erythrocytes will be retained (heparin tube). After separation, erythrocytes will be washed twice with cold solution (about 10 ml of 0.9% NaCl at 4°C). The erythrocytes will be then transferred to two 2-ml tubes and stored for future analyses. Both pipette tips and tubes are plastic, ordered from SARSTEDT (Germany). The magnitude of metal contamination of the SARSTEDT tubes has been examined in a previous study by using flame atomic absorption spectroscopy
- 1-2 ml aliquots of the serum/plasma sample will be pipetted into each of 9-labeled cryovials.
- Vials will be placed in specimen box for storage
- Study staff will then transport specimens to Dr. Qiuyin Cai MCN, B-2104, ext # 6-1351
- Date, time and location of sample in the freezer will be recorded on the specimen log
- Samples will be stored at –80° C.
- Blood specimens will be stored until use in relevant assays.

Blood sample allocation

- One aliquot of erythrocytes will be assayed for total magnesium in NMS Labs, Inc.
- One aliquot of serum will be assayed for serum magnesium at the Vanderbilt Pathology Laboratory Services.
- One aliquot of plasma will be assayed for plasma vitamin D metabolite, 25(OH)D in Dr. Bruce Hollis's laboratory at Medical University of South Carolina.
- One aliquot of plasma will be assayed for plasma C-reactive protein at the Vanderbilt Pathology Laboratory Services.
- The remaining samples will be stored for additional assays in the Molecular Epidemiology core Laboratory freezer.

Laboratory Assay

Determination of Total Magnesium in Erythrocytes

Flame atomic absorption spectroscopy is the most widely used technique for measuring total magnesium. The sensitivity of magnesium measured by flame atomic absorption is sufficiently high so a graphite furnace is not required for determination. We will use erythrocytes which are washed twice using ice-cold solution before storage at -80°C. These assays are routinely conducted in NMS Labs, Inc. In brief, erythrocyte samples will be diluted with 0.5% Lanthanum Chloride Solution and analyzed by flame atomic absorption using a hollow cathode lamp source that emits light at a wavelength of 285.2 nm. The sample concentrations are determined by comparison of the absorbance of the sample with the calibration curve constructed from a series of standards. Magnesium calibrators are run at 0, 0.40, 1.0, 2.5 and 5.0 mg/dL. This assay is regularly validated through inclusion of external standards by using three levels of control: commercial serum at 1.8 and 4.7 mg/dL and in-house blood control at 4.0 mg/dL. The method has a coefficient of variation (CV) between runs of 3.0% at target concentrations of 1.8 and 4.7 mg/dL serum. The between run CV for the blood control is 2.4%. The means of the three controls are all within +/- 3.2%.

Serum magnesium

Serum magnesium will be determined by standard analytic method on the Beckman DXC 800 chemistry analyzer and 24-hour urine magnesium will be assayed by an atomic absorption spectrophotometer, both provided by the Vanderbilt Pathology Laboratory Services.

Measuring Plasma Vitamin D metabolite, 25-Hydroxyvitamin D (25(OH)D)

The radioimmunoassay (RIA) for plasma vitamin D metabolite, 25(OH)D, has been developed and validated in Dr. Bruce Hollis's laboratory. The RIA assay results compare well with those from a liquid-chromatographic procedure involving specific ultraviolet detection of 25(OH) D in plasma. This assay has been successfully applied in a number of epidemiological and clinical studies with excellent reproducibility, including all four previous studies on the association between blood 25(OH)D and risk of colorectal cancer or adenoma. Antibodies will be generated in a goat immunized with the vitamin D analog 23, 24, 25, 26, 27-pentanor-C(22)-carboxylic acid of vitamin D, coupled directly with bovine serum albumin. The ^{125}I-labeled tracer will be prepared by reacting a 3-amino-propyl derivative of vitamin D-C(22)-amide with Bolton-Hunter reagent. Calibrators will be prepared in vitamin D-stripped human serum. 25(OH) D will be quantitatively extracted from serum or plasma (50 µl) with acetonitrile. The assay will consist of a 90-min incubation at room temperature with primary antiserum, followed by a 20-min incubation with a second antiserum and separation of bound from free fractions by centrifugation. The detection limit of the assay will be 2.8 mg/L for 25(OH) D.

Measurement of blood Vitamin D metabolite

Assays of blood vitamin D metabolite including 24,25-dihydroxyvitamin D (24,25(OH)D), 25-Hydroxyvitamin D (25(OH)D), and 1,25-Dihydroxyvitamin (1,25(OH)D) and vitamin D binding protein will be performed.

Measurement of Plasma CRP

Assays of CRP will be performed using immuno turbidimetric immunoassay based commercial assay kits purchased from Pointe Scientific, Inc, Canton, MI, applying autoanalyzers available in the Vanderbilt Pathology Laboratory Services.

Measurement of Red Blood Cell Heavy Metal

Assays of heavy metal will be performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the Vanderbilt Mass Spectrometry Core laboratory.

Measurement of Serum Lipid Profile Markers

Assays of serum lipid profile markers (including uric acid, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol, triglycerides) will be performed in the Dr Sergio Fazio's lipid lab at the Vanderbilt University.

Measurement of One-carbon Metabolites

Assays of blood one-carbon metabolites will be performed.

Measurement **of** Methylomic biomarkers

Assay of methylomic biomarkers in biospecimen will be performed.

Measurement **of** miRNA profiling

Assay of miRNA in biospecimen will be performed.

Measurement **of** circulating inflammatory biomarkers

Assay of circulating inflammatory biomarkers (e.g. GGT, HBVs, HCV, TNF and others) in biospecimen will be performed.

--- Urine Sample Collection

Urine Collection and Handling Protocol

The fasting urine samples will be collected prior to the treatment (week 0), the midpoint (week 6) and the end of intervention (week 12). Each urine sample will be collected in a sterile urine specimen container containing 100 mg ascorbic acid as a preservative.

Location

Clinical Research Center (CRC) Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will receive a schedule of planned fasting urine collections. A urine sample will be collected on the biopsy collection day at the beginning and the end of the intervention phase respectively, at week 0 and week 12. An additional urine sample will be collected at the midpoint of the intervention phase, at week 6. All samples on a given participant will be taken at the same time of day to avoid potential variability due to circadian rhythm.

Preparation

Sterile specimen container 20 ml with 100 mg ascorbic acid
10-1.8ml cryovials vials
Specimen box
Labels
Permanent markers
Biohazard Bags

Procedure

Before the clinic visit, participants will be mailed:

- Sterile urine containers containing a preservative
- Zip-lock bags/ biohazard bags
- Insulated bag with ice packs
- Patients will be given written and verbal instructions for collection of urine samples
- Patients will be instructed to:
    1. Collect the fasting urine of the day.
    2. Place specimen in zip-lock bag / biohazard bag.
    3. Fill the urine collection questionnaire (Appendix G)
    4. Store specimen in the freezer until ready to leave for clinic.
    5. Place zip-locked bag in insulated bag with ice pack.
    6. Transport the sample to the clinic.
    7. Give specimen to study staff and receive clean container for next collection.

Urine Processing

- Urine specimens will be stored in CRC freezer.
- Specimens will be processed within 4-6 hours after collection.
- All specimen's will be labeled with date, participant ID #.
- 1-2 ml aliquots of the urine sample will be pipetted into each of 9-labeled cryovials.
- Vials will be placed in specimen box for storage.
- Remaining specimen will be discarded.
- Study staff will then transport specimens to Dr. Qiuyin Cai MCN, B-2104, ext # 6-1351.
- Date, time and location of sample in the freezer will be recorded on the specimen log.
- Samples will be stored at –80° C'

- Urine specimens will be stored until ready for analysis.

Urine Allocation

One aliquot will be assayed for prostaglandin E2 metabolite (PGE-M) at Vanderbilt by:

Dr. Milne
Light Hall, 502-A, Ext 6-5611

One aliquot will be assayed for creatinine at Vanderbilt Clinical Research Center

The remaining samples will be stored as back up and for addition assays in Dr. Cai's Molecular Epidemiology core Laboratory freezer.

Laboratory assay:

Quantification of Urinary metabolite of PGE2 (PGE-M)

Urinary PGE-M (11 alpha-hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid) level will be measured using a liquid chromatography/tandem mass spectrometric method described previously, at the Integrated Health Sciences Facility Core of the Vanderbilt Center in Molecular Toxicology, directed by Dr. Milne (see letter of support). Briefly, 0.75 mL urine will be acidified to pH 3 with HCl and endogenous PGE-M will be then converted to the *O*-methyloxime derivative by treatment with methyloxime HCl. The methoximated PGE-M will be extracted, applied to a C-18 Sep-Pak, and eluted with ethyl acetate. An [$^2H_6$]*O*-methyloxime PGE-M internal standard will be then added. Liquid chromatography will be performed on a Zorbax Eclipse XDB-C18 column attached to a ThermoFinnigan Surveyor MS Pump (Thermo Finnigan, San Jose, CA). For endogenous PGE-M, the predominant product ion *m/z* 336 representing [M-(OCH_3+H_2O)]$^-$ and the analogous ion, *m/z* 339 (M-OC[$^2H_3$+H_2O), for the deuterated internal standard, will be monitored in the selected reaction monitoring (SRM) mode. Quantification of endogenous PGE-M will utilize the ratio of the mass chromatogram peak areas of the *m/z* 336 and *m/z* 339 ions. The lower limit of detection of PGE-M was in the range of 40 pg, approximately 100-fold below levels in normal human urine. The coefficient of variation for samples analyzed in multiple batches was 7.2%.

Urinary Creatinine Measurement

Urinary creatinine levels will be determined spectrophotometrically using a kit from Sigma Chemical Co. (St. Louis, MO) as performed previously. This assay requires 0.01 ml of urine sample for each subject and uses a test kit from Sigma Company (Sigma, St. Louis, MO). The assay is based on a modification of the Jaffe reaction by measuring the color intensity difference of a creatinine picrate complex before and after acidification. In our previous studies, the mean CVs for intra- and inter-assay variability were found to be 4.1 % and 6.7%, respectively.

Urinary Heavy Metal Measurement

Urinary heavy metal concentration will be determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the Vanderbilt Mass Spectrometry Core laboratory.

Urinary Magnesium Measurement

Urinary magnesium concentration will be determined at the Vanderbilt University Hospital laboratory.

Urinary eicosanoids metabolites measurement

Assay of eicosanoids metabolites in biospecimen will be performed.

24 Hour Urine Collection for Magnesium Tolerance Test

Magnesium load test is a reliable indicator of magnesium status and considered as the gold standard for the diagnosis of magnesium deficiency. This test requires a pre-test 24 hour urinary magnesium content, followed by a second 24 hour urinary magnesium content, after initiating the intravenous magnesium sulfate infusion. Performing this test need well trained staff. It is not a method that can be used as a routine test for the evaluation of Mg status.

Location

Clinical Research Center (CRC) Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will receive a schedule of planned the magnesium load test on a two consecutive days. A 24 hour urine sample will be collected for determining the basal urinary magnesium excretion. Another 24 hour urine sample will be obtained following the load test.

Preparation

Sterile specimen containers with ascorbic acid
10-1.8ml cryovials vials
Specimen box
Labels
Permanent markers
Biohazard Bags

Procedure

1. Collect 24 hour urine the day before the load test for basal magnesium and creatinine ratio.
2. Infuse 0.2mEq (2.4mg) elemental magnesium per kilogram of body weight in 50 ml of 5%detrose over 4 hours, which will be conducted by CRC nurses.
3. Collect urine (starting with infusion) for magnesium and creatinine for 24 hours
4. We will check the blood pressure and heart rate for the participant before initiating magnesium sulfate IV. Infusion, and monitor the same vital signs in the course of infusion and within 1 hour following infusion completed.
5. Percentage magnesium retained is calculated by the formula below

$$\left[1 - \frac{(\text{post infusion Mg excretion - basal Mg excretion}*)}{\text{total Mg infused}}\right] \times 100$$

$$*\text{basal Mg excretion} = \frac{\text{pre - infusion urine Mg}}{\text{pre - infusion urine Creatinine}} \times \frac{\text{post - infusion 24 hour urine}}{\text{Creatinine}}$$

6. Criteria for magnesium deficiency
   ≥ 50% retention at 24 h: definite deficiency
   > 25% to < 50% retention at 24 h: probable deficiency
   ≤ 25% retention at 24 h: normal
7. A fasting 2h spot or shorter timed urine may be used

--- Stool Sample Collection

Stool Collection and Handling Protocol

The stool samples will be collected prior to the treatment (week 0), the midpoint (week 6) and the end of intervention (week 12). Each stool sample will be collected in a sterile stool specimen container.

Location

Clinical Research Center (CRC) Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will receive a schedule of planned stool collections. A stool sample will be collected within 7 days prior to the biopsy collection day at the beginning and the end of the intervention phase respectively, at week 0 and week 12. An additional stool sample will be collected at the midpoint of the intervention phase, at week 6. All samples on a given participant will be taken at the same time of day to avoid potential variability due to circadian rhythm.

Preparation

Sterile stool collection container with wide mouth labeled with Stool Sample
Sterile stool tubes with spoon × 5 (Polypropylene, 30 ml) labeled with Stool Sample
Specimen box
Permanent markers
Biohazard Bags

Procedure

Before the clinic visit, participants will be mailed:

- Sterile stool container with wide mouth
- Zip-lock bags/ biohazard bags
- Insulated bag with ice packs
- Patients will be given written and verbal instructions for collection of stool samples

Patients will be instructed to collect stool specimen.

- Collect the stool within 7 days prior to the biopsy collection day.
- Place specimen in zip-lock bag / biohazard bag.
- Place zip-locked bag in insulated bag with ice pack.
- Store specimen in the freezer until ready to leave for clinic.
- Transport the sample to the clinic.
- Give specimen to study staff and receive clean container for next collection.

Stool Processing

- Stool specimens will be stored in CRC freezer or taken to the Dr. Qiuyin Cai's Molecular Epidemiology Lab, MCN, B-2104, ext # 6-1351 immediately.
- A walnut size aliquot of stool (about 2 grams / 2 ml) stool will be transferred into 5 labeled plastic tubes with spoon.
- All specimen contains will be labeled with collection date and time, sample ID #, and participant ID #.
- Stool sample tubes will be placed in specimen box for storage.
- Date, time, sample ID #, participant ID #, and location of sample in the freezer will be recorded on the specimen log.
- Samples will be stored at –80° C immediately.
- Stool specimens will be stored until ready for analysis.

Laboratory assay:

Measurement of Stool Microbiomial Markers

Micorbiomial markers in stool samples will be measured at baseline and 6-week, 3-month after the magnesium supplementation. PPCCT staff will construct the data. De-identified stool samples will be measured.

--- Saliva Sample Collection

Saliva Collection and Handling protocol

The saliva samples will be collected prior to the treatment (week 0), the midpoint (week 6) and the end of intervention (week 12). Each saliva sample will be collected in a sterile saliva sample collection tube.

Location

Clinical Research Center (CRC) Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will receive a schedule of planned saliva collections. A saliva sample will be collected on the biopsy collection day at the beginning and the end of the intervention phase respectively, at week 0 and week 12. An additional saliva sample will be collected at the midpoint of the intervention phase, at week 6. All samples on a given participant will be taken at the same time of day to avoid potential variability due to circadian rhythm.

Preparation

Saliva sample collection tube labeled Saliva Sample (Polypropylene, 15ml)
Plastic drinking straws
Cryovials (Polypropylene, 2 ml x 5-10)
Labels
Specimen box
Permanent markers
Biohazard Bags

Procedure

Before the clinic visit, participants will be mailed:

- Polypropylene saliva collection cryovials
- Plastic drinking straws (2 inches)
- Zip-lock bags/ biohazard bags
- Insulated bag with ice packs
- Patients will be given written and verbal instructions for collection of saliva sample

Patients will be instructed to collect saliva sample

1. Collect saliva within 1 hour of awaking (approximately 15 minutes after getting out of bed).
2. Don't eat, brush or floss your teeth or drink anything other than water before collecting the saliva sample.
3. Wash hands thoroughly with soap and water.
4. Rinse mouth twice with cool water about 10 minutes prior to collection.
5. Allow saliva to pool in the mouth. (Some find it helpful to imagine eating their favorite food or gently press the tip of your tongue against your teeth)
6. Begin collecting saliva. Use a plastic drinking straw to funnel the saliva into the tube. Fill the tube to the halfway of the tube with saliva that is liquid (5 ml). To avoid foamy saliva, gently tap the tube with your finger or tap on a hard surface to reduce bubble.
7. Screw the caps on the tube tightly.
8. Mark the collection time and date on the label.
9. Wash hands again.
10. Place specimen in zip-lock bag / biohazard bag.
11. Store specimen in the freezer until ready to leave for clinic.

12. Place zip-locked bag in insulated bag with ice pack.
13. Transport the sample to the clinic.
14. Give specimen to study staff and receive another clean container for next collection.

Saliva Processing

- Saliva samples will be stored in CRC freezer or taken to the laboratory immediately.
- Study staff will then transport specimens to Dr. Qiuyin Cai MCN, B-2104, ext # 6-1351.
- Specimens will be processed within 4-6 hours after collection.
- All specimens will be labeled with collection time and date, sample ID #, and participant ID #.
- 1-1.5 ml aliquots of the saliva sample will be pipetted into each of the labeled cryovials (5 – 10 vials).
- All specimen's will be labeled with time and date, sample ID #, participant ID #, and will be further labeled as 1 of ID #, 2 of ID#, 3 of ID#, etc.
- Cryovials will be placed in a specimen box for storage.
- Date, time, sample ID #, participant's ID #, and location of sample in the freezer will be recorded on the specimen log.
- Samples will be stored at – 80° C immediately.
- Saliva samples will be stored until ready for future analysis.

--- Hair Sample Collection

Hair Sample Collection and Handling Protocol

The hair samples will be collected prior to the treatment (week 0) and the end of intervention (week 12). Each hair sample will be collected in a clean hair sample collection container during the participant's clinical visit.

Location

Clinical Research Center (CRC) Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will receive a schedule of planned hair collections. A hair sample will be collected on the biopsy collection day at the beginning and the end of the intervention phase respectively, at week 0 and week 12. All samples on a given participant will be taken at the same time of day to avoid potential variability due to circadian rhythm.

Preparation

Hair sample plastic container labeled with Hair Sample
Hair clip
Scissors
Alcohol wipe
Specimen box
Permanent marker
Biohazard Bags

Procedure:

- Clean the scissors with an alcohol wipe.
- Cut samples of hair from the back of the head (the lowest margin of hair on the nape of the neck). Cut hair as close to the scalp as possible. (It is better if small amounts of hair are cut from 5 to 6 areas in the back of the head.)
- As each piece of hair is cut from the head, save only 1-inch (2.5cm) of the hair closest to the scalp (new growth). Cut off and discard the rest.
- Collect about 0.25 - 0.5 grams of hair needed.
- Place all collected hair samples into the Hair Sample collection container.
- Screw the caps on the container tightly.
- Mark the collection time and date on the label.
- Place specimen in zip-lock bag / biohazard bag.
- Research Staff record the time of collection on BIOLOGICAL SAMPLE COLLECTION FORM (Appendix H).

Hair Sample Processing

- All specimen's container will be labeled with collection date and time, sample ID #, participants ID #.
- Study staff will then transport specimens to Dr. Qiuyin Cai MCN, B-2104, ext # 6-1351.
- Containers will be placed in a specimen box for storage.
- Collection date and time, sample ID #, participant's ID #, and location of sample in the freezer will be recorded on the specimen log
- Samples will be stored at – 20° C.
- Hair samples will be stored until ready for analysis.

Laboratory assay:

Hair Heavy Metal Measurement

Assay of hair heavy metal will be performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the Vanderbilt Mass Spectrometry Core laboratory.

--- Nail Sample Collection

Nail Sample Collection and Handling Protocol

The nail samples will be collected prior to the treatment (week 0) and the end of intervention (week 12). Each nail sample will be collected in a clean nail sample container.

Location

Clinical Research Center (CRC) Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will receive a schedule of planned nail collections. A nail (finger nails, toes or both) sample will be collected on the biopsy collection day at the beginning and the end of the intervention phase respectively, at week 0 and week 12. All samples on a given participant will be taken at the same time of day to avoid potential variability due to circadian rhythm.

Preparation

Nail sample container labeled with Nail Sample
Nail clippers
Specimen box
Permanent markers
Biohazard Bags

Procedure

At the clinic visit, participants will be given:

- Nail sample container
- Nail clippers
- Zip-lock bags/ biohazard bags
- Patients will be given verbal instructions for collection of nail sample

Patients will be instructed to collect nail sample

1. Wash hands thoroughly with soap and water.
2. Rub nails with alcohol swabs to clean thoroughly prior to cutting.
3. Nails should be clean of polish, dirt and debris.
4. Clip nails from all fingers and toes onto a clean paper. (Please include shavings or finger nail fillings along with nail clippings.)
5. Place all collected nail samples into the Nail Sample Container (at least 0.5 grams).
6. Screw the caps on the container tightly.
7. Mark the collection time and date on the label.
8. Place specimen in zip-lock bag / biohazard bag.
9. Give nail samples to study staff.
10. Research Staff record the time of collection on BIOLOGICAL SAMPLE COLLECTION FORM (Appendix H).

Nail Sample Processing

- Study staff will transport specimens to Dr. Qiuyin Cai MCN, B-2104, ext # 6-1351.
- All specimen containers will be labeled with date and time, sample ID #, participants ID #. Containers will be placed in a specimen box for storage
- Date and time of collection, sample ID #, participant's ID #, and location of sample in the freezer will be recorded on the specimen log.
- Nail Samples will be stored at –20° C until ready for analysis.

Laboratory assay:

Nail Heavy Metal Measurement

Assay of nail heavy metal will be performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the Vanderbilt Mass Spectrometry Core laboratory.

--- Skin Swab Sample Collection

Skin Swab Collection and Handling protocol

The skin swab samples will be collected prior to the treatment (week 0), the midpoint (week 6) and the end of intervention (week 12). Each skin swab sample will be collected in a sterile skin swab sample collection tube.

Location

Clinical Research Center (CRC) Medical Center North, 3rd floor on the Vanderbilt Campus

Scheduling

Participants will receive a schedule of planned skin swab collections. A skin swab sample will be collected on the biopsy collection day at the beginning and the end of the intervention phase respectively, at week 0 and week 12. An additional skin swab sample will be collected at the midpoint of the intervention phase, at week 6. All samples on a given participant will be taken at the same time of day to avoid potential variability due to circadian rhythm.

Preparation

Skin swab sample collection tube labeled Skin Swab Sample (cryovials, 2ml)
Labels
Gloves
Culturette swab
Solution (0.15 mol/L NaCl with 0.1% Tween 20)
Specimen box
Permanent markers

Procedure

1. Participants will be instructed not to wash with anything for an 8h interval prior to sampling.
2. At the clinic, research staff washes hands thoroughly with soap and water. A new pair of sterile glove will be used for each individual sampling process.
3. Samples were taken from 2X2 cm of skin surface from the central area of the forehead.
4. Swab the skin for a 1 min with a sterile cotton swab soaked in sterile solution.
5. Place the swab directly in the tube.
6. Break off the cotton tip of each swab directly into the tubes.
7. Screw the caps on the tube tightly.
8. At least two skin swab samples will be collected.

Skin swab sample Processing

- Skin swab samples will be stored in CRC freezer or taken to the laboratory immediately.
- Study staff will then transport specimens to Dr. Qiuyin Cai at MCN, B-2104, ext # 6-1351.
- All specimens will be labeled with sample ID # and participant ID #.
- Date, time, sample ID #, participant's ID #, and location of sample in the freezer will be recorded on the specimen log.
- Cryovials will be placed in a specimen box for storage.
- Date, time, sample ID #, participant's ID #, and location of sample in the freezer will be recorded on the specimen log.
- Samples will be stored at – 80° C immediately.
- Skin swab samples will be stored until ready for future analysis.

G. Data and Safety Monitoring Plan

Protocols for monitoring of safety and compliance

Because we wish to reduce participant burden, we will not conduct a placebo run-in period to assess participant compliance. We will also not conduct a run-in period for tolerance to magnesium supplementation because magnesium is a dietary supplement which has been well tolerated in previous studies with a low rate of reported adverse events. The dose of magnesium supplementation is personalized based on the current intake ratio, thus, the dose used in the proposed trial is generally smaller than that used in most previous studies, and provides additional assurance for the safety of participants. Thus, we predict the drop-out rate will be low based on previous clinical trials and our experience.
In the study, the research nurses or the research staff will telephone each participant for any adverse events as well as compliance monitoring. Furthermore, changes in serum and erythrocyte magnesium (baseline, the middle and the end of the study) will also be used to monitor compliance. We will follow the Vanderbilt Data Safety Monitoring (DSM) Plan

Procedures

- The day following each of the in-person visits (baseline, week 6, and week 12), the research staff or nurse will telephone each participant to monitor for any adverse events due to biospecimen collection as well as compliance monitoring.
- During the 12-week intervention, research staff from the SRSR or the research nurse will contact the participants to check their adherence to study treatment and to ask if there are any potential adverse events, 3 times of safety monitoring calls at week 1-6 and 3 times at week 7-12 until the twelfth week of trial has completed.
- Research staff will also check the participant's adherence to study treatment by undertaking pill counts during the in-person visits at week 6 and week 12.
- In addition, we will also assess compliance using 24-hour recalls, two diet recalls conducted at week 1 – 6 and another two at week 7-12, using food-record assisted 48-hour recalls conducted at weeks 5 and 11.

In all, the participant will be followed up by phone or in-person visit 13 times for compliance and safety monitoring during the 12 weeks of intervention.

Data and Safety Monitoring Plan

A Data and Safety Monitoring Plan is outlined to protect the participants' safety for this study, help assure that the quality of the research data is acceptable. Adverse events will be considered as any untoward medical occurrence in a subject, even those not necessarily having a causal relationship with the study. Serious adverse events (SAEs) will be classified as untoward medical occurrences that a) result in death, b) are life threatening, c) require inpatient hospitalization, or d) result in persistent or significant disability/incapacity. All AEs will be recorded on an Adverse Event Form and graded as Mild (no limitation of usual activities), Moderate (some limitation), or Severe (inability to carry out usual activities) and attributed according to the relationship to the study intervention and/or procedures (Not related, Unlikely, Possible, Probable, or Definite). Action taken by study staff will also be recorded on the form. These forms remain as part of the participant's file. Mild events will be recorded and reviewed as they occur by the Project Coordinator and Dr. Dai, in consultation with Drs. Seidner and Ness as needed. Participants with Moderate events may need to be removed temporarily or permanently from the study. AEs will be reviewed as a matter of course in monthly meetings of study investigators and staff.

Education

The interventions under study are believed to reduce the risk of adenoma recurrence and to have minimal, if any, toxic effects. Each participant provides informed consent prior to participation, and our monitoring program is designed to identify any adverse events.

Across all intervention arms, participants are instructed to monitor for any signs or symptoms, including dizziness, flushing, muscle paralysis, troubled breathing, diarrhea and others. They are instructed to report these symptoms to study staff. Participants have the contact information for the study line and the PI. Participants receive this information verbally during the initial counseling session and each telephone monitoring call. Instructions in writing are provided during the One-on-One clinic visit session. Participants are encouraged to call or e-mail with questions or concerns, and participants may receive additional counseling to address the specific event.

Patient monitoring after rectal biopsy After the biopsy, the patient is monitored for thirty minutes. The CRC staff will check blood pressure and have the patient go to the restroom to check for bleeding prior to leaving the clinic. Patient is told that they can expect some blood on the tissue on the day of the biopsy. Bleeding heavier or more persistent than this should be reported. They should also report anal or lower abdominal pain, fever, a foul anal discharge, or anything else they think could be related to the biopsy. The gastroenterologists and the supporting medical team will handle emergencies. Any other problems discovered by the study team will be discussed at an appropriate level with the patient sufficient to affect referral to the patient's gastroenterologists or primary care physician who will also be informed after written consent by the patient.Any unexpected anoscopy events (> grade 1 pain events and any other complications to the biopsies) will be recorded on an Adverse Events Form; information will include a description of the complication, a rating of the severity, other essential information needed to evaluate the complication, an assessment of the likelihood that the event was due to a study procedure, and a statement of action taken. All potential complications of biopsies are rated as mild, moderate, or severe. Minor problems after the procedure are handled in consultation with the gastroenterologist, and recorded in the study file. If undue complications of biopsies or other procedures arise, the study will be stopped ahead of schedule. In addition, in the event of a severe or life-threatening complication, the Vanderbilt Cancer Center Clinical protocol Review Committee will be immediately notified. Only participants with ongoing doctor/patient relationships will be used in this study. Patients will continue their usual care with

the physician. Telephone numbers for the PI and study line will be given to the participant. Emergencies will be handled by the gastroenterologist and supporting medical staff.

Monitoring of other adverse events Throughout the trial, we will closely monitor for adverse events through telephone calls, monitoring following a clinic visit, and questionnaire. Participants will be called the day following the rectal biopsy collection. We will call participants once a week for the first two weeks and once every two weeks thereafter, during which they are asked about nausea, diarrhea, cramping or abdominal pain, or other discomforts or adverse effects potentially related to the intervention protocols. Our research objectives include identifying these problems, such that the value of these interventions might be assessed. Reported adverse events are recorded on an Adverse Report Form, and reported to the Project Coordinator and PI, who will contact these participants for further evaluation. Each questionnaire contains a list of potential symptoms, and participants report the frequency of each symptom during the prior week. Participants may write-down other symptoms that they have experienced during this period as well. Participants also are queried for changes in health status or medication use, which may lead to ineligibility and study exclusion. These questionnaires will be reviewed, and the Project Coordinator will be informed of those participants reporting any symptom.

Data collection, Storage of Data/Specimens and Issues of Confidentiality All materials and data will be collected for research purposes, and include:

1) Questionnaires to determine eligibility, demographics, medical, lifestyle, family cancer history, physical activity and dietary intake
2) Medical record review, including size and location of prior hyperplastic polyp or/and adenoma
3) Urine for PGE-M and other biomarkers
4) Blood for biomarker assays

Only research staff and investigators who must have direct contact with participants or with their medical record will have access to identifiable private information. All staff and investigators will complete and maintain current training in human subjects protection (e.g. CITI training) and HIPAA training. All data and specimens to be collected will be coded with a unique identification number. Only staff requiring direct contact with participants or with their medical records will have access to the link between the code and identifying information.

Throughout the study, standard measures to ensure privacy of information on study participants will be maintained. Participants will be informed that all information will be confidentially held, and assured that data will be used only for statistical/research purposes. Individuals will not be identified in the analyses. No data beyond what is stated in the informed consent will be sought without authorization from the participant or next of kin. Information on hospitalization or illness will not be sought from hospitals or doctors without a signed medical release from the participant. All participants will have assigned code numbers. All completed hard copy forms will be kept in locked files in locked rooms to which only study personnel have access. Study databases are housed in the VUMC data center and require password access. No information on any individual will be released to anyone other than study personnel without the written approval of the individual or, where appropriate, the next of kin or physician in the case of a life-threatening situation. All study personnel will receive training in ethical conduct, HIPAA regulations, and must receive additional IRB training. Staff is instructed not to discuss any participants with persons other than appropriate study personnel.

Procedure

- Identification number will be assigned prior to the first Clinic Visit
- Each patient will be assigned a numerical identification number for confidentiality purposes

    - The numbers will be assigned in sequence 1,2,3, etc
    - Patients will retain the same number throughout the study
    - A alpha designation will be made to denote clinic visits
        - A= clinic visit 1
        - B= clinic visit 2
        - C= clinic visit 3
    - All specimens will have the patient id # and an alpha code
        - Example, id # 1a would denote patient 1, 1st clinic visit
        - All specimens would be labeled using this identification system
- All completed forms will be kept in locked files in locked rooms to which only study personnel have access.
- All computers require double-password access.
- Human subjects approval for this study has been obtained.

H. Payment

Compensation protocol

Request for Payment to Volunteer participants

Participants will be given $300 for undergoing biopsy procedures, blood draw and urine collections during the study if all the procedures are completed. This is provided for the inconvenience of the biopsy protocol, and to compensate for any financial costs due to travel.

Procedure

- Participant will complete a payment authorization form at the first clinic visit.
- Principal Investigator will sign form.
- Payment will be provided at the conclusion of participation in the study.

I. Data Analysis

Protocols for statistical analysis

Data analysis plan

This study will adopt a 2X2 factorial design. The first factor is genotype (GG vs. GA/AA or GG vs. GA vs. AA) and the second factor is intervention (magnesium treatment: modulating dietary Ca/Mg intake ratio versus placebo). The primary endpoints, which are all continuous variables, include apoptosis biomarkers (e.g. TUNEL and Bax), and expression of TRPM7, pMLKL, COX-2 (inflammation) and Ki-67 (proliferation index) in colorectal mucosa. . The secondary endpoints include serum level magnesium, serum vitamin D, plasma concentration of C-reactive protein, and other biomarkers as well as magnesium status (magnesium tolerance test), and urinary excretion of prostaglandin E_2 metabolite (PGE-M).

Procedures

Standard graphing and screening techniques will be used to detect outliers and to ensure data accuracy. Distributions of continuous outcomes will be assessed for normality. If normality is violated, proper data transformation will be applied or non-parametric analysis methods will be considered. Summary statistics for both continuous (mean ± standard deviation) and categorical variables (count and percent) will be provided by randomization groups to describe the study sample. Specific inferences on effects of interest will be made by reporting a point estimate along with a 95% confidence interval and the p value. Hypotheses will be tested at the level of α=0.05. This data analysis plan will be carried out using statistical software SAS® (Cary, North Carolina) or statistical analysis package R (R Development Core Team, 2008). These basic statistical rules will be applied to all study aims.

Univariate analyses will be conducted for these ten primary and secondary end points (continuous variables) in relationship to magnesium treatment and placebo using between-group t-test or non-parametric tests within genotype groups. The difference in the study endpoints (primary+secondary) between magnesium supplement and placebo will be estimated for the genotype groups (GG, GA, AA and GA and AA combined) along with their 95% confidence intervals. The interaction between the genotype and intervention will be estimated as the difference of the within-genotype magnesium supplement versus placebo differences.

Two-way ANOVA or ANCOVA analyses will be conducted to analyze the primary and secondary endpoints with intervention and genotype in the model (ANOVA) or other potential confounders including polyp type, size, and location for primary hyperplastic polyp or/and adenoma as well as body mass index, age, total energy intake, and intakes of other nutrients (average of six 24-hour recalls) in the model (ANOCOVA). Overall intervention effect and genotype effect and their interactions will be tested.

Administrative Interim analyses Administrative interim analyses may be performed as needed prior to the completion of the clinical trial over the 5 year period. One example of such administrative interim analyses is to generate preliminary data for a new grant submission. The study team does not even have any intention to stop the trial at these interim analyses, thus there is no need to establish a stopping rule. A biostatistician from outside the study team will perform such analyses on unblinded data. All study team members remain blinded until the completion of the study. However, summarized study group-level data from such interim analyses may be communicated to the study PI's solely for the purpose of planning other studies.

Routine data monitoring will be conducted to ensure data quality. All the statistical analyses will be conducted by or under the guidance of Dr. Yu Chang, one PI of the study.

7.0 Risks

Physical Risks

Adverse events associated with oral magnesium supplementation include nausea, cramping abdominal pain, and diarrhea. These effects are caused by the osmotic action of unabsorbed magnesium salts. The magnesium supplement that has been selected for this study is magnesium glycinate. This magnesium salt readily dissolves within the intestinal tract and is less likely to cause gastrointestinal symptoms when compared to other forms of magnesium supplementation. Participants will be generally healthy and able to take all supplementation by mouth. Magnesium supplementation is not a prescribed medication, instead, it is considered a dietary supplement.

Magnesium toxicity should not occur in normal healthy subjects enrolled in this intervention trial. Excess oral intake of magnesium through dietary sources and supplement use is readily excreted by the kidneys. Magnesium supplements should be avoided in individuals with severe renal insufficiency as elevated blood levels of magnesium and toxicity can occur in these individuals. Subjects with renal insufficiency will be excluded from this investigation. Should a study subject developed acute renal insufficiency the development of magnesium toxicity is possible. However, if this were to occur it is much more likely that the study subject will present with symptoms of renal insufficiency rather than magnesium toxicity. Having said this, the oral intake of 30 g of magnesium daily can lead to metabolic alkalosis and hypokalemia. A single dose of over 400 g of magnesium can result in paralytic ileus and cardiorespiratory arrest. It is hightly unlikely that any of these events will occur during the course of the study as the dosage form of the supplement is far below this amount.

Magnesium salts, especially those in antacids, may decrease the bioavailability and absorption of digoxin, chlordiazepoxide, and several antibiotics, including tetracycline, aminoquinolone and nitrofurantoin. It may also increase the absorption of dicumarol. Subjects who are on these medications will be instructed to take the study supplement at least two hours before or after one of these medications. Individuals currently receiving dicumarol will be excluded from participation in the study.

Magnesium has been widely used in clinical practice and tested in previous clinical trials; it has been well tolerated in previous clinical trials and the reported rate of adverse events has been low. No severe adverse event has been observed even when it was administered intravenously among pregnant women. On the other hand, magnesium supplementation improves insulin resistance and reduces insulin level. Growing evidence from studies conducted in Western societies has linked low intake of magnesium to insulin resistance and systemic inflammation and, thus, risk of diseases common in Western countries, such as type II diabetes, coronary heart disease, and recently colorectal cancer. Magnesium supplementation improves insulin resistance and reduces insulin level. According to data from the US National Health and Nutrition Examination Survey (NHANES), 1999-2000, 79% of US adults do not meet the Recommended Dietary Allowances (RDA) of magnesium. In our proposed study, the dose of magnesium supplementation is personalized based on the current intake ratio; thus, the dose used is generally smaller than that used in most previous studies. Each dose will not surpass the sex- and age-specific DRI for magnesium by more than 100mg/day. Furthermore, we use additional exclusion criteria to avoid potential side effects for subgroups of participants, including potential drug-magnesium interactions, providing additional assurance for the safety of participants. Our protocol enrolls patients believed to be healthy. As an alternative, participants may choose to temporarily or permanently stop supplementation if they feel a complication is unacceptable.

A possible complication of venipuncture is bleeding or the development of a small hematoma at

the point of needle insertion. Blood will be collected from the arm using standard sterile techniques and equipment, and direct pressure at the point of injection will stop this bleeding. Occasionally blood collection will cause a small hematoma, which will heal without permanent damage.

The dietary assessment (i.e., 24-hour recall), and measurement of height, weight, and body circumferences, and urine collection, present no physical risks.
Physical risks related to rectal biopsy For rectal biopsy, no fasting or bowel cleansing preparations is required. Participants are asked to have a bowel movement prior to arriving at clinic. Rectal biopsy has been used in a number of previous studies conducted by other investigators as well as one study conducted by our research nurse and our co-investigator (Dr. Reid, gastroenterologist). In general, the risk is low. However, rectal biopsy may lead to rectal bleeding, infections or other complications, including severe or life-threatening complications. Any unexpected anoscopy events (> Grade 1 pain events and any other complications to the biopsies) will be recorded on an Adverse Events Form. Furthermore, we have developed a comprehensive procedure to monitor and report adverse events, prevent and treat complications.

Psychological Risks
Psychological risks could occur if participants feel manipulated or coerced by study personnel. Further risks are that participants may feel that study personnel judge participants based on reported behaviors. The likelihood of these events is very small. Informed consent is obtained before any counseling regarding the intervention, and all counselors are trained and monitored to present information in a non-judgmental manner.

Social Risks
Social risks could occur if confidentiality is violated. As well, dissemination of information obtained from genetic research has the potential of adversely affecting employability or insurability. The likelihood of such adverse events is very small, since identifying information will be rigorously protected. Only coded ID numbers will be used to label samples and other research materials.

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Education
Across all intervention arms, participants are instructed to monitor for any signs or symptoms, including dizziness, flushing, muscle paralysis, troubled breathing, diarrhea etc. They are instructed to report these symptoms to study staff. Participants have the contact information for the Project Director and the PI. Participants receive this information verbally during the initial counseling session and each telephone monitoring call. Participants are encouraged to call or e-mail with questions or concerns, and participants may receive additional counseling to address the specific event.

Monitoring
Patient monitoring after rectal biopsy After the biopsy, the patient is monitored for thirty minutes. The CRC staff will check blood pressure and have the patient go to the restroom to check for bleeding prior to leaving the clinic. Patient is told that they can expect some blood on the tissue on the day of the biopsy. Bleeding heavier or more persistent than this should be reported. They should also report anal or lower abdominal pain, fever, a foul anal discharge, or anything else they think could be related to the biopsy. The gastroenterologists and the supporting medical team will handle emergencies. Any other problems discovered by the study team will be discussed at an appropriate level with the patient sufficient to affect referral to the patient's

gastroenterologists or primary care physician who will also be informed after written consent by the patient.

Any unexpected anoscopy events (> Grade 1 pain events and any other complications to the biopsies) will be recorded on an Adverse Events Form; information will include a description of the complication, a rating of the severity, other essential information needed to evaluate the complication, an assessment of the likelihood that the event was due to a study procedure, and a statement of action taken. All potential complications of biopsies are rated as mild, moderate, or severe. Minor problems after the procedure are handled in consultation with the gastroenterologist, and recorded in the subject's study file. If undue complications of biopsies or other procedures arise, the study will be stopped ahead of schedule. In addition, in the event of a severe or life-threatening complication, the Vanderbilt IRB will be immediately notified. Patients will continue their usual care with the physician. Telephone numbers for the PI and study director will be given to the subject. Emergencies will be handled by the gastroenterologist and supporting medical staff.

Monitoring of other adverse events Throughout the trial, we will closely monitor for adverse events through telephone calls, monitoring following a clinic visit, and questionnaire. Participants will be called the day following the rectal biopsy collection. We will call participants once a week for the first two weeks and once every two weeks thereafter, during which they are asked about dizziness, flushing, muscle paralysis, troubled breathing, diarrhea, or other discomforts or adverse effects potentially related to the intervention protocols. Our research objectives include identifying these problems, such that the value of these interventions might be assessed. Reported adverse events are recorded on an Adverse Report Form, reported to the Project Director and PI, who will contact these participants for further evaluation, and submitted to the IRB. Questionnaires are administered at baseline and each follow-up. Each questionnaire contains a list of potential symptoms, and participants report the frequency of each symptom during the prior week. Participants may write-down other symptoms that they have experienced during this period on the questionnaire, as well. Participants also are queried for changes in health status or medication use, which may lead to ineligibility and study exclusion. These questionnaires will be reviewed, and the Project director will be informed of those participants reporting any symptoms.

Adverse events will be considered as any untoward medical occurrence in a subject, even those not necessarily having a causal relationship with the study. Serious adverse events (SAEs) will be classified as untoward medical occurrences that a) result in death, b) are life threatening, c) require inpatient hospitalization, or d) result in persistent or significant disability/incapacity. All AEs will be recorded on an Adverse Event Form and graded as Mild (no limitation of usual activities), Moderate (some limitation), or Severe (inability to carry out usual activities) and attributed according to the relationship to the study intervention and/or procedures (Not related, Unlikely, Possible, Probable, or Definite). Action taken by study staff will also be recorded on the form. These forms remain as part of the participant's file. Mild events will be recorded and reviewed as they occur by the Project Director and the Principal Investigator, Dr. Dai, in consultation with Drs. Seidner and Ness as needed. AEs will be reviewed as a matter of course in monthly meetings of study investigators and staff. All Moderate and Severe AEs will be reported to the Vanderbilt IRB within 5 working days, and severe events will be reported to NIH within 5 working days. On the anniversary date of the initial IRB approval for investigator-initiated clinical trials, the principal investigator will submit to the Vanderbilt IRB an annual data safety and monitoring report summarizing the study’s safety experience and efficacy over the preceding year and to date.

8.0 Study Withdrawal/Discontinuation

Participants may be withdrawn from the study if they are not able to provide blood, urine or rectal samples, or they are not able to take their supplements as instructed.

If the intervention proves clearly harmful, the study will be stopped ahead of schedule. Furthermore, a single life-threatening condition related to the intervention, or a single life-threatening event from biospecimen collection, would cause consideration for study termination. Moderate complications occurring in more than one subject from any protocol would likewise cause consideration for study termination.

9.0 Statistical Considerations

Power analysis: Our primary endpoints include apoptosis biomarkers (e.g. TUNEL and Bax), and expression of TRPM7, COX-2 (inflammation) and Ki-67 (proliferation index), pMLKL in colorectal mucosa as well as magnesium status (total erythrocyte magnesium, magnesium tolerance test), and urinary excretion of prostaglandin E_2 metabolite (PGE-M). No study has ever reported the effects of magnesium supplementation on the primary endpoints in the mucosa or any other tissues of the colorectum. Our study will be the first study of this kind. However, all the anti-carcinogenesis effect of magnesium supplementation will be related to the change in the level of body magnesium status. Therefore, we based our power analysis on the pilot data of total erythrocyte magnesium, one of the primary study endpoints.

We have conducted a pilot study of total erythrocyte magnesium with 50 colorectal adenoma cases and 50 matched controls. Presented in the table are the means and standard deviations by the *TRPM7* genotype among colorectal adenoma patients with a high dietary ratio of calcium to magnesium (ratio>2.6). Even with such a small sample size, we still found a statistically significant (p=0.04) difference in the mean of erythrocyte magnesium between the GG and the GA/AA combined group (only one subject with the AA genotype). On the other hand, we did not find a significant difference by genotype among adenoma patients with a low dietary ratio of calcium to magnesium. These results are consistent with those we recently reported on the interaction between calcium/magnesium intake ratio and the *TRPM7* polymorphism in relation to both colorectal adenoma and hyperplastic polyps (e.g. increased risk of colorectal adenoma associated with the GA or AA genotype only appeared in those with a high calcium/magnesium ratio, but not in those with a low ratio). Based on these findings, we expect the primary treatment effect (modulating high calcium/magnesium ratio from above 2.6 to below 2.6) will be a reduction in the difference in erythrocyte magnesium between genotypes. The normal reference range for erythrocyte magnesium is from 4.0 to 6.5 mg/dL. Thus, we expect treatment will increase the mean erythrocyte magnesium among the GA or AA group to the median mid-point of the normal range, (i.e. 5.25 mg/dL,) which is also the observed mean for the GG genotype group,

Table D3. Mean (standard deviations) for total erythrocyte magnesium among colorectal adenoma patients with a high Ca/Mg intake ratio, according to the Thr1482Ile *TRPM7* polymorphism.

TRPM7 genotype	Ca/Mg intake ratio>2.6 (placebo)
GG	5.25 ± 1.16 (n=16)
GA/AA	4.49 ± 0.50 (n=7)
GA	4.61 ± 0.40 (n=6)
AA*	3.7 ± 0.40 (n=1)

* There is only one patient possessing the AA for Ca/Mg ≤2.6; thus, no standard deviations can be estimated. We used the standard deviations for the GA genotype in the power calculation.

Since the study has a 2 by 2 factorial design, we calculated the study power based on an ANOVA model which has two factors: treatment (treatment vs. placebo) and genotype (GG vs. GA and AA combined). There are 4 groups with 60 patients each by study design. At a 5% two-sided significance level, a two-way analysis of variance will have 94% power to detect the main treatment effect, the main genotype effect, and their interaction, assuming that the common

standard deviation is 0.830 (average of 1.16 and 0.50). In this calculation, we expect both the treated GG genotype group and the treated A allele carrier (GA and AA combined) group will have mean total erythrocyte magnesium of 5.25 mg/dL. If we conservatively use a standard deviation of 1.16, the power will be 71%. This power calculation is conducted using nQuery®, a commercial software.

Base on our recently reported findings, the AA genotype would likely benefit the most from the magnesium supplementation. It is important to estimate the treatment effect within the three genotype groups and to compare the effects between the genotype groups. However, the AA genotype frequency is low (1.6%). We will be making every effort to enroll AA genotypes and expect to recruit 10 subjects during the 4 year recruiting period. Five each will be randomized to the treatment and the placebo group by stratifying on genotype groups. Due to the unequal number of subjects in the 2 by 3 ANOVA analysis, we conducted a simulation study to calculate the power for the interaction between the treatment and the GA genotype and the interaction between the treatment and the AA genotype. We used GG as the reference group. The power is 68% and 65%, respectively, with alpha=0.05. These separate powers are lower than the 0.94 power when GA and AA are combined since, besides the sample size difference, the simulation study allows us to use different standard deviations for the different subgroups of subjects, as opposed to a common standard deviation that has to be assumed by the method in the nQuery® software.

Data analysis plan: This study will adopt a 2X2 factorial design. The first factor is genotype (GG vs. GA/AA or GG vs. GA vs. AA) and the second factor is intervention (magnesium treatment: modulating dietary Ca/Mg intake ratio versus placebo). The primary endpoints, which are all continuous variables, include apoptosis biomarkers (e.g. TUNEL and Bax), and expression of TRPM7, COX-2 (inflammation) and Ki-67 (proliferation index), pMLKL in colorectal mucosa. The secondary endpoints include serum level magnesium, serum vitamin D, and plasma concentration of C-reactive protein as well as magnesium status (magnesium tolerance test), and urinary excretion of prostaglandin E_2 metabolite (PGE-M).

Standard graphing and screening techniques will be used to detect outliers and to ensure data accuracy. Distributions of continuous outcomes will be assessed for normality. If normality is violated, proper data transformation will be applied or non-parametric analysis methods will be considered. Summary statistics for both continuous (mean ± standard deviation) and categorical variables (count and percent) will be provided by randomization groups to describe the study sample. Specific inferences on effects of interest will be made by reporting a point estimate along with a 95% confidence interval and the p value. Hypotheses will be tested at the level of α=0.05. This data analysis plan will be carried out using statistical software SAS® (Cary, North Carolina) or statistical analysis package R (R Development Core Team, 2008). These basic statistical rules will be applied to all study aims.

Two-way ANOVA or ANCOVA analyses will be conducted to analyze the primary and secondary endpoints with intervention and genotype in the model (ANOVA) or other potential confounders including polyp type, size, and location for primary hyperplastic polyp or/and adenoma as well as body mass index, age, total energy intake, and intakes of other nutrients (average of six 24-hour recalls) in the model (ANOCOVA). Overall intervention effect and genotype effect and their interactions will be tested.

Univariate analyses will be conducted for these ten primary and secondary end points (continuous variables) in relationship to magnesium treatment and placebo using between-group t-test or non-parametric tests within genotype groups. The difference in the study endpoints (primary+secondary) between magnesium supplement and placebo will be estimated for the genotype groups (GG, GA, AA and GA and AA combined) along with their 95%

confidence intervals. The interaction between the genotype and intervention will be estimated as the difference of the within-genotype magnesium supplement versus placebo differences.

Administrative Interim analyses Administrative interim analyses may be performed as needed prior to the completion of the clinical trial over the 5 year period. One example of such administrative interim analyses is to generate preliminary data for a new grant submission. The study team does not have any intention to stop the trial at these interim analyses, thus there is no need to establish a stopping rule. A biostatistician from outside the study team will perform such analyses on unblinded data. All study team members remain blinded until the completion of the study. However, summarized study group-level data from such interim analyses may be communicated to the study PI's solely for the purpose of planning other studies.

10.0 Privacy/Confidentiality Issues

Throughout the study, standard measures to ensure privacy of information on study participants will be maintained. Participants will be informed that all information will be confidentially held, and assured that data will be used only for statistical/research purposes. Individuals will not be identified in the analyses. No data beyond what is stated in the informed consent will be sought without authorization from the subject or next of kin. Information on hospitalization or illness will not be sought from hospitals or doctors without a signed medical release from the subject. All participants will have assigned code numbers. All completed forms will be kept in locked files in locked rooms to which only study personnel have access. All computers are kept in locked rooms, and require password access. All biological samples are assigned a numeric code. Laboratory staff has no access to the database linking the original code to the study participant.

No information on any individual will be released to anyone other than study personnel without the written approval of the individual or, where appropriate, the next of kin or physician in the case of a life-threatening situation. All study personnel will receive training in ethical conduct, HIPPA regulations, and must receive additional IRB training. Staff is instructed not to discuss any cases with persons other than appropriate study personnel.

All materials and data will be collected for research purposes, and include:

1) Questionnaires to determine eligibility, demographics, medical, lifestyle, family cancer history, physical activity and dietary intake
2) Medical record review, including size and location of prior hyperplastic polyp or/and adenoma
3) Urine for PGE-M and other biomarkers
4) Blood for biomarker assays
5) Rectal biopsies for biomarker assays
6) Stool and Rectal swab for gut flora status
7) Hair and Nail samples for metal assays
8) Skin swab samples for microbiotia analysis

All data and specimens to be collected will be coded with a unique identification number. The link between this identification number and identifying information is located in restricted access files. All biological samples are assigned a numeric code. Laboratory staff has no access to the database linking the original code to the study participant. Only staff requiring direct contact with participants or with their medical records will have access to the link between the code and identifying information.

Only research staff and investigators who must have direct contact with participants or with their medical record will have access to identifiable private information. All staff and investigators will

complete and maintain current training in human subjects protection (e.g. CITI training) and HIPAA training.

11.0 Follow-up and Record Retention

Our proposed study will last 5 years (See Appendix A, Study Calendar).

Hard copies of all records will be stored in locked filing cabinets located in locked office space. Electronic databases are located in restricted access folders within the Vanderbilt University Medical Center computer network. Access to database is restricted by password or permission-based access. All identifying information will be removed and/or deleted from the participant records at 30 years following the conclusion of the investigation. Biological samples are coded and retained in a locked location with access limited to specific study personnel. DNA extracted will be stored for 30 years. After this period DNA samples will be destroyed.