The effects of Patiromer on serum potassium level and gut microbiome of ESRD patients with hyperkalemia

Protocol #:	R-01
Principal Investigator:	Dominic Raj
Version #:	1.4
Version Date:	July 25, 2017

STATEMENT OF COMPLIANCE

STUDY TITLE	*The effects of Patiromer on serum potassium level and gut microbiome of ESRD patients with hyperkalemia*
PROTOCOL NUMBER	R-01
PROTOCOL VERSION	v1.4
VERSION DATE	July 01, 2017
STUDY SPONSOR	Relypsa

DECLARATION OF THE INVESTIGATOR:

The study will be conducted in accordance with the International Conference on Harmonization (ICH) document "Guidance for Industry – E6 Good Clinical Practice: Consolidated Guidance" dated April 1996 and the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the study subjects. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

Further, I will conduct the study in keeping with local legal and regulatory requirements.

NAME (Print): _____

SIGNATURE: _____

DATE: _____

PROTOCOL SYNOPSIS

Study Title:	The effects of Patiromer on serum potassium level and gut microbiome of ESRD patients with hyperkalemia
Phase:	Phase 2, safety and efficacy trial
Primary Objective:	To examine the tolerability, safety, and efficacy of Patiromer in lowering serum potassium levels in ESRD patients that are on maintenance dialysis with hyperkalemia (K>5.0 mEq/L).
Secondary Objective:	To assess the changes in gut microbiome and serum metabolom profiles of hyperkalemic ESRD patients treated with Patiromer.
Study Design:	This is a non-randomized, crossover study. 25 ESRD patients with hyperkalemia (K>5.0 mEQ/L) will be enrolled in an open-label, pilot clinical trial with 3 sequential phases of (a) 2 weeks of no intervention, (b) 12 weeks of Patiromer treatment, and (c) 6 weeks of no intervention. Treatment with Patiromer will be initiated at a dose of 8.4 gram, once daily and observed for a week, then uptitrated to 16.8 g once daily. Eligible subjects will collect stool samples and provide blood and urine samples.
Study Duration:	The total anticipated study duration is approximately 2 years, with anticipated enrollment duration of 9 months, and anticipated study conduct duration upon full enrollment of 3 months. The remaining 12 month will needed for data analysis.
Study Population	The study will enroll approximately 25 adult male or female subjects between the ages of 18-85 years. Subjects with diabetes or HIV will also be enrolled.
Study Products:	Patriomer is an FDA-approved orally administered drug used for the treatment of hyperkalemia. It is a non-absorbed polymer that binds potassium throughout the gastrointestinal tract and leads to

lowering serum potassium levels (1). Prior Patiromer clinical trials have demonstrated the drug's utility in treating hyperkalemia in patients with diabetic kidney disease (1; 2).

Key Roles

Principal Investigator (responsible for study conduct and all site-related medical decisions)	Dominic Raj, MD 2150 Pennsylvania Ave NW, Suite 3-438, Washington DC, 20037 Phone#: (202) 741-2283 Email: draj@mfa.gwu.edu
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1 Introduction: Background and Scientific Rationale

1.1 Background

1.1.1 Gut Microbiota

Human gut harbors a complex community of over 100 trillion microbial cells, which constitute the microbiota (3). The gut microbiome encodes about 3.3 million genes, which is 150 times that of our own genome (4). The symbiotic gut microbiota provides complementary biological and metabolic functions that cannot be performed by humans (5). Disruption to the normal balance between the gut microbiota and the host (dysbiosis) is associated with diverse disease states such as obesity, inflammatory bowel disease, neurological disorders, cancer, and cardiovascular disease (CVD) (6-11).

Findings from the human microbiome project (HMP) revealed that the gut microbiome is highly specific, personalized and functionally relevant to health (12). Gut microbiota is altered in patients with chronic kidney disease (CKD). Preliminary evidence indicates that the dysbiotic gut microbiota could be the source for a plethora of uremic toxins (13), many of which have been shown to be positively correlated with progression of CKD and increased risk for major adverse cardiovascular events defined as death, myocardial infarction, or stroke (14-16). Therefore, correcting the gut microbiota dysbiosis might be a therapeutic option for reducing both the progression as well as the cardiovascular burden of CKD.

Hyperkalemia, a frequent and serious electrolyte abnormality in patients with kidney failure, can lead to life threatening cardiac arrhythmias (17; 18). Even relatively mild (5.5 to 6.0 mEq/L) hyperkalemia is associated with an increased risk of mortality (19). Compensatory increase in fecal potassium excretion occurs in patients with renal failure (20; 21). Normally, the gut is responsible for only 5% of total potassium excretion; however, in patients with end-stage renal disease (ESRD), this can increase substantially to account for 30% to 50% of potassium excretion (22).

Preliminary evidence indicates that the growth rate of certain bacteria is affected by the increasing concentration of potassium in the growth medium (23). *E. coli* populations exhibited higher growth rates in the presence of additional potassium (23). However, potassium may also

adversely affect the growth of commensal methanogens. At increased concentrations, potassium decreases methanogenesis by entering cells through membrane leaks or via a potassium transport carrier, thus decreasing the transmembrane electron potential (24). This has been shown to have a significant impact on the pH regulation of the bacteria (25).

Patiromer for oral suspension is an FDA-approved orally administered drug used for the treatment of hyperkalemia. It is a nonabsorbed polymer that binds potassium throughout the gastrointestinal tract and leads to lowering serum potassium levels (1). Prior Patiromer clinical trials have demonstrated the drug's utility in treating hyperkalemia in patients with diabetic kidney disease (1; 2).

1.1.2 Rationale for Study and Study Design

This study will add to the body of data and expand the knowledge about the tolerability, safety, and efficacy of Patiromer in lowering serum potassium levels in ESRD patients with hyperkalemia. Furthermore, the study will examine changes in gut microbiome and serum metabolome profiles of hyperkalemic ESRD patients treated with Patiromer in order to determine the impact of lowering gut potassium levels using Patiromer on gut microbiome dysbiosis and generation of uremic toxins. We anticipate that Patiromer will improve the metabolomics profile and gut permeability through binding to and removing endotoxin and bacteria-generated uremic toxins, and by altering the microbiome profile in ESRD patients. In addition, this study will unravel the effect of hyper/normo-kalemia on plasma metabolom.

1.2 Potential Risks and Benefits

1.2.1 Known Potential Risks

Patiromer is fairly safe and approved by the FDA. Most commonly observed side effects are hypomagnesaemia and constipation. The other study related risks is associated with the study procedures, which is the blood draws.

1.2.2 Known Potential Benefits

There are no benefits for study participants.

The study will add to the knowledge on the gut dysbiosis in subjects with ESRD on hemodialysis, the safety and efficacy of Patiromer, and changes in gut microbiome and serum metabolic profiles of hyperkalemic ESRD patients treated with Patiromer.

1.3 Study Objectives

1.3.1 Primary Objective

- Examine the tolerability, safety, and efficacy of Patiromer in lowering serum potassium levels in ESRD patients with hyperkalemia (K>5.0 mEq/L). In a non-randomized, crossover study, 25 ESRD patients with hyperkalemia (K>5.0 mEQ/L) will be enrolled in an open-label, pilot clinical trial with 3 sequential phases of (a) 2 weeks of no intervention, (b) 12 weeks of Patiromer treatment at 8.4-16.8 g/day, and (c) 6 weeks of no intervention. Blood and stool samples will be collected throughout the study period.

1.3.2 Secondary Objectives

- Determine the changes in gut microbiome and serum metabolome profiles of hyperkalemic ESRD patients treated with Patiromer. Blood and stool samples collected from the ESRD patients enrolled in the above study will be collected at pre-specified time points and analyzed by metagenomics for gut microbiome profiles, and untargeted and targeted metabolomics for stool and serum metabolome profiles, in order to determine the impact of lowering gut potassium levels using Patiromer on gut microbiome dysbiosis and generation of uremic toxins.

2 STUDY DESIGN AND ENDPOINTS

2.1 Overview of Study Design

This is an intervention study in subjects with ESRD on hemodialysis. After a screening visit, all eligible subjects will collect stool and provide blood samples according to Table 1.

2.2 Study Endpoints

2.2.1 Primary Endpoint

- For Specific Aim 1, the primary safety end point is adverse events during the 12 weeks of intervention.
- For Specific Aim 2, the primary efficacy end points will be change in microbiome, metabolome and markers of inflammation.

2.2.2 Secondary Endpoints

- For Specific Aim 1, the Secondary efficacy end points included mean change in serum potassium level through 12 weeks.

2.3 Blinding

This sampling study will not be blinded.

3 STUDY ENROLLMENT AND WITHDRAWAL

This is a non-randomized, crossover study. 25 ESRD patients with hyperkalemia (K>5.0 mEQ/L) will be enrolled in an open-label, pilot clinical trial with 3 sequential phases of (a) 2 weeks of no intervention, (b) 12 weeks of Patiromer treatment, and (c) 6 weeks of no intervention.

3.1 Inclusion Criteria

Subjects must meet all of the following criteria in order to be enrolled into the study:

1. Subjects on stable hemodialysis for more than 90 days
2. Age 18-85 years
3. Persistent hyperkalemia, defined as elevated serum potassium >5.0 mEq/L in more than 2 occasions during the previous 3 months.
4. Ability to provide informed consent

3.2 Exclusion Criteria

Subjects must not meet any of the following criteria in order to be enrolled into the study:

1. Use of pre- or probiotics during the past 2 months
2. Use of antibiotics within the past 2 months, if the patient received a single course of antibiotic.
3. Presence of chronic wound infection and osteomyelitis
4. Inflammatory bowel disease, chronic diarrhea, current *C. difficile* infection
5. Liver cirrhosis or chronic active hepatitis
6. Treatment with immunosuppressive medications in the past 6 months or more than a week of treatment with prednisone >10 mg in the last 3 months
7. Anticipated kidney transplant within 9 months
8. Expected survival < 9 months
9. Pregnancy, anticipated pregnancy, or breastfeeding
10. Incarceration
11. Participation in another intervention study
12. Severe anemia defined as hemoglobin <8.0 g/dl any time during the last 2 months

3.3 Strategies for Recruitment

Subjects will be recruited through a search of the dialysis unit database. Potentially eligible subjects resulting from this database search may then be approached and offered a Screening Visit if interested to participate. It is anticipated that approximately 120 potential subjects will be screened in order to enroll 25 eligible subjects, reflecting an expected screen failure rate of ~80%. 36 weeks is the anticipated timeframe for recruitment.

3.4 Subject Withdrawal or Termination

3.4.1 Reasons for Withdrawal or Termination

Upon enrollment, a subject may withdraw consent to participate in the study at any point without prejudice or consequence. Participation in this study is entirely voluntary. Likewise, a subject may also be withdrawn if the Investigator deems a subject unfit to continue with or complete the study.

Subject withdrawal or termination may occur due to any of the following reasons:

- Subject independently withdraws consent from the study
- Investigator determines that the subject has developed an intercurrent illness, condition, becomes pregnant or experiences adverse event in which continued participation in the study is considered potentially harmful to the subject and discontinuation is in the best interest of the subject
- Subject non-compliance (e.g. failure to comply with other protocol-specific assessments)
- Subject is lost to follow-up

3.4.2 Handling of Subject Withdrawals or Termination

If a subject withdraws or is discontinued from the study before completion, every effort should be made to complete the assessments. The reason for subject withdrawals from the study will be documented in the subject's case notes.

Subjects will be replaced until the targeted number of subjects has been enrolled.

4 STUDY PRODUCTS

4.1 Safe Use of Study Agent

Patriomer is an FDA-approved orally administered drug used for the treatment of hyperkalemia. It is a non-absorbed polymer that binds potassium throughout the gastrointestinal tract and leads to lowering serum potassium levels (1). Prior Patiromer clinical trials have demonstrated the drug's utility in treating hyperkalemia in patients with diabetic kidney disease (1; 2). Adverse events and other safety data will be obtained at each visit. The most commonly reported treatment related adverse events have been hypomagnesemia (7.2%), constipation (4.6%), and diarrhea (2.7%).

This study incorporates several design elements to minimize adverse events related to the study agent including:

- Eligibility criteria that exclude individuals with normal or low blood potassium levels. Only ESRD patients with hyperkalemia (K>5.0 mEQ/L) will be enrolled
- A maximum daily Patiromer dose of 16.8 g once daily
- Frequent monitoring of gastrointestinal symptoms
- Regular monitoring of concomitant medications and antibiotic use
- Pre-dialysis serum potassium will be monitored at regular intervals. Appropriate adjustment in dialysate potassium will be made to avoid hypokalemia.
- Follow-up of serum magnesium levels

4.2 Study Agent Dose Adjustments

4.2.1 Protocolized Dosing of Study Agent

Participants will orally self-administer Patiromer at a dose of 8.4 gram, once daily and observed for a week, during which serum potassium and gastrointestinal symptoms will be evaluated. If tolerated and in the absence of hypokalemia (serum potassium less than 4 meq/L), the dose will be up-titrated to 16.8 g once daily. Participants will remain on the 16.8 g daily dosage for another 11 weeks, for a total of 12 weeks of treatment. Study agent kits will contain 4 weeks'

worth of doses, and will be distributed three times during the course of the study. Pharmaco-adherence will be ensured and confirmed through frequent communications and pill count.

5 ADVERSE EVENTS

This section describes the requirements and processes for reporting adverse events and unanticipated problems that occur during the study to Relypsa and the IRB.

5.1 Definitions

Definitions are per the January 2007 Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Participants or Others and Adverse Events, Office on Human Research Protection (OHRP) Guidance. http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm. The requirements and processes for reporting adverse events are described in the corresponding NIH Guidelines.

Adverse Event (AE): An AE is any untoward or unfavorable medical occurrence in a human study participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participant's involvement in the research, whether or not considered related to the participant's participation in the research.

Internal adverse event: From the perspective of one particular institution engaged in a multicenter clinical trial, internal adverse events are those adverse events experienced by participants enrolled by the investigator(s) at that institution.

5.2 Collecting Adverse Event Information

The Investigator and research team is obligated to monitor participants so that their participation in the clinical trial is as safe as possible.

The center staff will collect adverse event data at each study visit by checking several different sources of information by:

- asking participants directly
- evaluating medical record information
- reviewing all laboratory values
- examining reports generated by the data management system based on data entered into logs and forms

AE information may also be reported between scheduled study visits via regular contacts if the participant reports health related problems or concerns. The study period during which adverse events must be reported is defined as the period from initiation of the study procedures to the end of the treatment follow-up. In other words, AE documentation should begin when the patient signs the informed consent form.

Encourage participants to contact the investigator after the conclusion of the trial if subsequent medical events occur which the participant, or the participant's physician, believes may be related to participation in the research study.

Other medical information

A participant may report that he or she had a medical test or procedure. If so, inquire as to why this was done as it may reveal pertinent information about a medical condition or diagnosis. In addition, if a participant reports starting a new medication, it may also indicate a new diagnosis or a change in a pre-existing medical condition that should be discussed and documented on the AE form.

5.2.1.1 Serious Adverse Events

A Serious Adverse Event **(SAE)** is defined as any AE that results in any of the following outcomes:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- results in congenital anomalies or birth defects
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. SAE reporting requirements are based on meeting the outcome definition outlined above.

Note that for this trial, hospitalized is defined as staying overnight in the hospital. Time spent in the emergency department may or may not be reported as an SAE, depending on the reason for the ED visit.

5.3 Reporting Adverse Events and Serious Adverse Events

The Investigator must report all SAEs from the signing of the informed consent until 14 days after the last dose of Patiromer to Relypsa's Drug Safety and Pharmacovigilance (DSPV) Department within 24 hours of becoming aware. SAE follow up information must also be provided as requested by Relypsa.

All SAE reports will be sent to Relypsa's Drug Safety by email to drugsafety@relypsa.com or via fax to (844) 867-7597. The Relypsa Generic IST SAE Form will be used for reporting SAEs to Relypsa's DSPV Department. All pertinent SAE information as indicated on the reporting form or as requested by Relypsa will be provided to facilitate safety monitoring for all patients taking Patiromer. These reports will be submitted by the Investigator or the research team to Relypsa even if it is not felt to be drug related. Prior to the start of the study, the Investigator and research team will complete SAE reporting training provided by Relypsa and document completion of the training.

The research team will also report any pregnancy that occurs in a female study patient or the male participant's female partner or any lactation exposure occurring to an infant or child by a female study patient within 24 hours of become aware to Relypsa's Drug Safety by email or fax. All pregnancy or lactation exposures will be reported on the Relypsa Generic IST Pregnancy or Lactation Report Form.

6 STUDY PROCEDURES AND SCHEDULE

6.1 Study Procedures/Evaluations

6.1.1 Study Specific Assessments and Procedures

This section describes the study specific assessments and procedures that will be conducted. Results from study specific procedures (e.g., laboratory evaluations or questionnaire results) will not be provided to subjects, unless the results are necessary in order to effectively communicate follow-up care for any adverse events that may occur as a result of participating in the study.

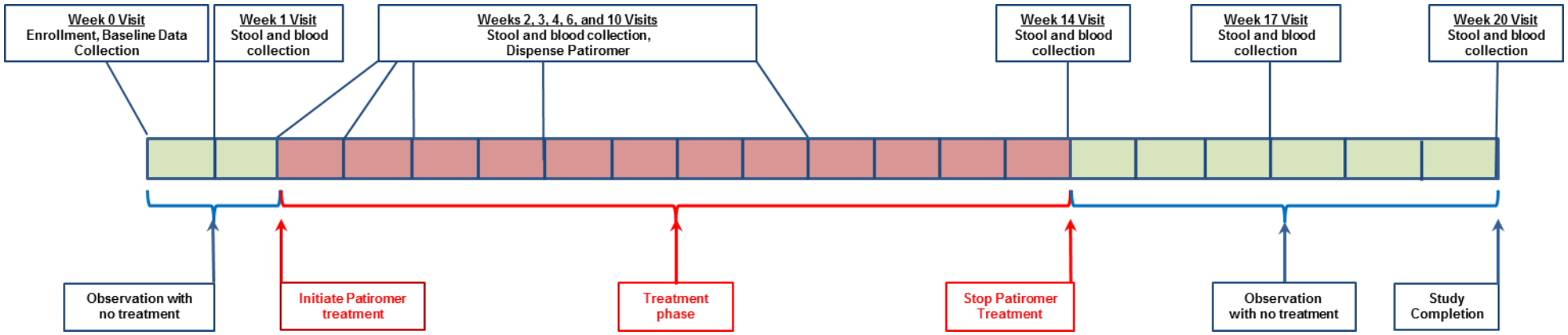

Figure 1. Sample collection. Stool and blood samples will be collected at weeks 1, 2, 3, 4, 6, 10, 14, 17 and 20 of the study. We will obtain information regarding dietary pattern and gastrointestinal health. Adverse events related to study drugs, and clinical events will also be collected. Patients will be requested to complete gastrointestinal (GI) health assessment survey at baseline, during treatment, and during post-treatment phase. Detailed information about infection and type of antibiotic used (if any) will be collected.

6.1.1.1 Medical History

A detailed medical history will be obtained. Subject's medical history will be obtained by chart review and interview.

6.1.1.2 Medication History

Subject's medication history will be obtained during each visit, including prescription, over-the-counter medications, and dietary supplements.

6.1.1.3 Dietary Assessment

Dietary assessment using the Block Food Frequency Questionnaire (https://nutritionquest.com) will be administered at the beginning (Week 1), during (Week 8) and end of the study (Week 20).

6.1.1.4 Physical Examination

A physical examination will be conducted, which will include body measurements (including height, weight, and calculation of BMI) and an assessment of vital signs (blood pressure, pulse, body temperature).

6.1.1.5 Biological Specimen Collection and Laboratory Evaluations

Stool and blood specimens will be collected from all subjects. In addition, data from routine laboratory investigations required for standard of care will be included into the clinical database as deemed necessary for annotation of the stool samples to the course of clinical disease and treatment.

6.1.2 Standard of Care Study Procedures

All subjects for this study are receiving the study specific assessments solely due to study participation. Therefore, there are no applicable standard of care procedures associated with this study.

6.2 Laboratory Procedures/Evaluations

6.2.1 Clinical Laboratory Evaluations

6.2.1.1 Serum Pregnancy Test

A serum/urine pregnancy test will be conducted at the Screening Visit for women of childbearing potential.

6.2.1.2 Hematology

Blood samples collected at weeks 0, 2, 4, 10, 14, 17, and 20 will be tested for CBC.

6.2.1.3 Biochemistry

Blood samples collected at weeks 0, 2, 4, 10, 14, 17, and 20 will be tested for the comprehensive metabolic panel (CMP). Blood phosphorous and PTH will be tested at week 0.

6.2.1.4 Metabolic and inflammatory biomarker studies

Blood and stool samples will be stored for metabolomics and biomarker studies. Both non-biased and targeted metabolomics profiling of the stool and blood will be performed at the West Coast Metabolomics Center at the University of California Davis. Filtered fecal water or plasma samples will be used for the mass spectrometry-based metabolomics profiling. The process of metabolite extraction will include internal standards and follow standard protocol. The resulting extract will be divided into a liquid chromatography fraction and a gas chromatography fraction.

6.2.1.5. Measurement of biomarkers of inflammation: Measurement of endotoxin, sCD14, hs-CRP, IL-6 and TNF-α will be performed at weeks 1, 2, 6, 10, 14, 17 and 20 of the study. Plasma LPS levels will be measured using a commercially-available kinetic chromogenic limulus amebocyte lysate assay kit (Lonza Walkersville, Walkersville, MD). High sensitivity sandwich ELISAs will be used to measure plasma sCD14, hs-CRP, IL-6, and TNFα. The samples will be stored at -80°C and assayed at the time of initial thawing to prevent degradation. All cytokine assays will be performed in duplicates. If funds are available, we will also measure IL-1β, IL-2, IL-4, IL-17, IL-10, and IL-22.

6.2.1.6 Measurement of specific uremic toxins and bacterial metabolites in plasma:
Measurement of plasma p-cresol sulfate, indoxyl sulfate, TMAO and SCFA will be performed at 2, 4, 10, 14 and 20 weeks of the study. Plasma will be separated from whole blood samples collected in tubes containing EDTA as anti-coagulant. Extracted plasma will be frozen immediately after collection and store at -80°C until further use. The chromatographic separation of metabolites will be performed using either reverse phase separation or normal phase online with QQQ mass spectrometers (Agilent Technologies). For this, we will employ a combination of solvents including gradients at varying concentrations of ammonium acetate, methanol, acentonitrile adjusted for specific metabolites. Metabolites will be separated by controlled flow rates on either a Luna Phenyl Hexyl column (3um, 2x150mm, Phenominex), a Luna Amino (NH2) column (4um, 100A 2.1x150mm, Phenominex), or an orbax Eclipse XDB-C18 column (50 × 4.6 mm i.d.; 1.8 µm, Agilent Technologies, CA), while adjusting the temperature of the chamber. All the columns used in this study will be washed and reconditioned after every 50 injections.

6.2.1.7 Measurement of intestinal barrier dysfunction using plasma zonulin: Commensal gut microbes maintain functional integrity of gut by several mechanisms, including restoration of tight junction protein structure.(26) Dysbiotic gut microbiome may be an important contributor to intestinal barrier dysfunction by altering structure and localization of TJs. Zonulin. Human zonulin is a <47-kDa protein that increases intestinal permeability in small intestine by modulating intercellular TJs.(27) Circulating zonulin in serum is considered as a useful marker of intestinal permeability.(27) To measure the impact of Patiromer on intestinal permeability, we will measure circulating zonulin levels in our study participants at 1, 2, 6, 10, 14, 17 and 20 weeks of the study. Plasma zonulin levels will be measured using a commercially-available ELISA kit.

6.2.2 Other Specimen Analyses/Assays

6.2.2.1 Gut microbiota taxonomic analysis

The structure of the microbiota from frozen stool samples will be assessed by DNA sequencing and computational analysis. Stool samples will be collected for DNA extraction and shotgun metagenomic sequencing. DNA will be extracted using the MoBio PowerFecal DNA Isolation kit. Quantification and quality control of DNA samples will be run on a BioAnalyzer 2100. All samples will be run on an Illumina MiSeq or NextSeq, which will provide us with ~10 million reads per sample to allow for accurate characterization of the microbiome. Reads will be pre-processed using PRINSEQ-lite 0.20.4 (trimming reads and bases < 25 PHRED, removing exact duplicates, and reads with undetermined bases). We will construct a 'target' genome library containing all bacterial, fungal, and viral sequences from the Human Microbiome Project Reference Database (http://www.hmpdacc.org/reference_genomes/reference_genomes.php) using the PathoLib module from PathoScope 2.0. We will align the reads to these libraries using the Bowtie2 algorithm, and then filter any reads that also aligned to the human genome (hg19) as implemented in PathoMap (very-sensitive-local -k 100 --score-min L,20,1.0). We will then apply PathoScope 2.0, specifically the PathoID module, to obtain accurate read counts for downstream analysis, including mapping against both a microbial database to identify species/strains and calculate relative abundances to determine the impact of Patriomer on the gut microbiome. Analyses, including testing of abundance of target species in treated patients and tests of correlations of changes in abundance with changes in metabolomics will be performed in R and Bioconductor using packages xlsx, gtools, CHNOSZ, plyr, ggplot2, reshape2, gplots, Phyloseq, and DESeq2.

6.3 Study Schedule

6.3.1 Schedule of Events Table

Table 1: Study procedures and time-line

				Intervention 2 to 14 weeks													
Weeks	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**17**	**20**
Physical examination	•																
Serum/Urine Pregnancy Test[1]	•																
Serum K				•		•		•	•	•		•	•	•			
Serum Mg++ Test			•				•								•	•	
Comprehensive metabolic panel	•		•		•						•				•	•	•
CBC	•		•		•						•				•	•	•
Study medication dispensing			•				•				•						
Blood sample collection		•	•	•	•		•				•				•	•	•
Endotoxin, sCD14, Zonulin and inflammatory biomarkers*†		•	•				•				•				•	•	•
Stool sample collection		•	•	•	•		•				•				•	•	•
Microbiome studies (Metagenomics)†		•	•		•		•				•				•	•	•
Metabolomics†			•		•						•				•		•
Gastrointestinal health assessment survey		•							•						•		•
Dietary assessment using food frequency questionnaire		•							•								•
Phosphorous and PTH‡	•																
Dialysis prescription and medication reconciliation‡	•	•	•				•				•				•	•	•

† *A purposeful approach to analyzing the stored blood and stool samples will be used*.
‡ Extracted from dialysis unit medical record periodically when new results are available
*hs-CRP, IL-6, and TNF-α
[1] For women of child bearing potential

The following will be collected monthly from the dialysis unit:

- Routine labs from dialysis unit including comprehensive metabolic panel, Mg++, hematological parameters, iron profile and dialysis adequacy
- Medication use including EPO, iron, proton pump inhibitor, phosphate binders, use of other potassium lowering agents, and vitamin D
- Data about infection, antibiotic use and adverse reaction to study drug will be collected by the study coordinator from personal interview with the patient, dialysis unit and inpatient/outpatient records

6.3.2 Screening Visit (Week 0)

Patients who appear eligible based on pre-screening will be approached in person to determine interest in participation and confirm eligibility. Study personnel will discuss the study goals and procedures with the potential participant in detail. Patients should have an understanding of the importance of providing frequent stool samples and what the process entails. If the patient agrees to participate in the study, study personnel will review and assess understanding of the entire informed consent form prior to obtaining written informed consent from the participant. The consenting process will be performed by a qualified investigator or study site designee. Informed consent will be obtained and documented before any study procedures are performed.

The following activities may take place over one or two visits.

- Determination of eligibility
- Informed consent process
- Collection of baseline data including demographics, medical history, and medication use including recent and current use of prescription products, over-the-counter products, herbal supplements, vitamins, and prebiotic and probiotic use in any form. In addition, medical records from the clinic facility will be reviewed to capture any non-reported data on recent medication use.

- Blood draw (13mL): Blood samples collected at Screening will be tested for the CMP, CBC, phosphorous and PTH.
- Serum pregnancy test for women of childbearing potential and tested at local lab.
- Instruction in stool sample collection and storage
- Instruct enrolled participants **not to change their dietary patterns**, if possible, during the study

6.3.3 Assigning a Participant ID

After the participant has signed the informed consent form, assign a unique 5-digit Participant Identification number (PID). Each participant should be assigned the next available PID.

6.3.4 Stool Sample Collection

Subjects will be provided with detailed instructions on how to collect and store their stool samples. Subjects will be instructed to collect samples from bowel movements at weeks 1, 2, 3, 4, 6, 10, 14, 17, and 20.

7 Evaluations and Follow Up

Participants will have in-person study visits at Weeks 1, 2, 3, 4, 6, 10, 14, 17 and 20. At each study visit, participants will be asked about emergency room visits, hospital admissions or any significant clinical events that occurred since the last visit. RCs will have weekly contact with participants to query them on antibiotic use in the previous week and medical records will be reviewed for blood potassium levels and antibiotics administered.

Dietary assessment using the Block Food Frequency Questionnaire (https://nutritionquest.com) will be administered at Baseline (W1), W8 and W20.

7.1 Pre-Treatment Observation Phase [Week 1 and 2]

The following activities will be performed during in-person visits at weeks 1 and/or 2:

- Study Visit Assessment
- Blood Specimen Collection
- Stool Sample Collection
- Antibiotic use review
- Study medication dispensing (Week 2 only)
- Review of adverse events
- Dietary assessment using food frequency questionnaire (Week 1 only)
- Gastrointestinal symptom assessment questionnaire (Week 1 only)
- Serum Mg++ (week 2 only)

Treatment Phase [Weeks 3-14]

The following activities will be completed during in-person visits at weeks 3, 4, 6, 10 and 14:

- Study Visit Assessment
- Review of information on clinical events and current use of medications
- Blood K+ levels (weeks 3, 5, 7, 8, 9, 11, 12, and 13)

- Serum Mg++ (weeks 2, 6, 14 and 17)
- Blood Specimen Collection
- Stool Sample Collection
- Gastrointestinal symptom assessment questionnaire administration (Weeks 8 and 14)
- Review of adverse events
- Study medication dispensing (Weeks 2, 6 and 10 only)

7.1.1 Post-Treatment Observation Phase (Weeks 15 to 20)

The following activities will be completed during Weeks 17 and 20

- Study Visit Assessment
- Antibiotic use review
- Blood Specimen Collection
- Stool Sample Collection
- Review adverse events
- Review of information of clinical events and current use of medications
- Gastrointestinal symptom assessment questionnaire administration (Week 20 only)
- Food Frequency Questionnaire (Week 20 only)

8 ETHICS/PROTECTION OF HUMAN SUBJECTS

8.1 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented subjects need to be re-consented.

8.2 Informed Consent Process

8.2.1 Consent and Other Informational Documents Provided to Subjects

Consent forms describing in detail the study agent, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to start of sampling of biological specimens. Any study related, subject facing materials will also be submitted to the IRB for approval.

8.2.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the subjects. Consent forms will be IRB-approved and the subject will be asked to read and review the document. The investigator or designee will explain the research study to the subjects and answer any questions that may arise. All subjects will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research subjects. Subjects will have the opportunity to carefully review the written consent form and ask questions prior to signing. The subjects will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subject will sign the informed consent document prior to any procedures being done specifically for the study. The rights and welfare of the subjects will be protected by emphasizing to them that the

quality of their medical care will not be adversely affected if they decline to participate in this study. The subjects may withdraw consent at any time throughout the course of the study.

The original signed informed consent form will be retained in the subject's records. A copy of the informed consent document will be given to the subject for their records. Documentation of the informed consent process will be captured in the source documents.

8.3 Subject and Data Confidentiality

The investigator will ensure to comply with all applicable federal, state, and local laws and regulations relating to the privacy of subjects' health information, including, but not limited to, the Standards for Individually Identifiable Health Information, 45 CFR Parts 160 and 164 (the Health Insurance Portability and Accountability Act of 1996 privacy regulation [HIPAA]). The investigator shall ensure that study subjects authorize the use and disclosure of protected health information in accordance with the privacy regulations of HIPAA and in a form acceptable satisfactorily to the sponsor. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The study subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location until records no longer need to be retained.

Study subject research data, will not include the subject's contact or identifying information. Subjects will be identified by their initials and an assigned unique subject identification number on CRFs, SAE reports, and other documents submitted to the Sponsor or Sponsor designated representative.

8.4 Use of Stored Samples and Data Derived from Samples

With the subject's approval and as approved by local IRBs, de-identified biological samples will be stored at the GWU. These samples could be used for research into various research and development activities and the study of clinical indications/diseases of interest as identified by the PI.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biological storage will not be possible after the study is completed.

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