

Pilot Clinical Trials in CKD

**EFFECTS OF NICOTINAMIDE AND LANTHANUM
CARBONATE ON SERUM PHOSPHATE AND FGF23 LEVELS
IN PATIENTS WITH STAGE 3-4 CHRONIC KIDNEY DISEASE**

**The CKD Optimal Management with Binders and Nicotinamide:
COMBINE STUDY**

**COMBINE Protocol Version 1.2
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EFFECTS OF NICOTINAMIDE AND LANTHANUM CARBONATE ON SERUM PHOSPHATE AND FGF23 LEVELS IN PATIENTS WITH STAGE 3-4 CHRONIC KIDNEY DISEASE

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1 INTRODUCTION

1.1 Executive Summary

Chronic kidney disease (CKD) is a major public health problem¹ that impairs functional status, shortens lifespan and consumes healthcare resources. CKD is complicated by disordered mineral metabolism, characterized by abnormalities in calcium and phosphate homeostasis, calcitriol deficiency and increased parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) levels.^{2,3} Observational studies report strong associations between disordered mineral metabolism and risks of end-stage renal disease (ESRD), cardiovascular disease (CVD), fractures, and death.⁴⁻⁶ Experimental data suggest that arterial stiffness and left ventricular hypertrophy (LVH) may serve as useful intermediate endpoints for CVD outcomes in CKD patients. Phosphate excess promotes vascular calcification leading to arterial stiffness,^{7,8} and FGF23 excess exerts hypertrophic effects on the myocardium, contributing to LVH.^{9,10} Thus, interventions that lower phosphate and FGF23 may reduce risks of morbidity and mortality in CKD.

Prior to embarking on a large randomized controlled trial to test the impact of phosphate and FGF23 reduction on hard clinical endpoints in CKD, additional data are needed on the effects of proposed interventions on serum phosphate and FGF23 levels. In this pilot study, a 12-month, randomized, double-blinded, four arm parallel study in 200 CKD patients with eGFR between 20 and 45 ml/min/1.73m² will be conducted. Participants will be randomized to one of the four groups: 1) lanthanum carbonate + nicotinamide; 2) lanthanum carbonate + nicotinamide placebo; 3) lanthanum carbonate placebo + nicotinamide; 4) lanthanum carbonate placebo + nicotinamide placebo.

The Specific Aims for this protocol are:

Specific Aim 1: Determine the efficacy of nicotinamide and lanthanum carbonate on serum phosphate and FGF23 levels in CKD stage 3-4:

Primary hypothesis: Compared to placebo, monotherapy with nicotinamide, monotherapy with lanthanum carbonate, and combined nicotinamide and lanthanum carbonate therapy will lower serum phosphate and FGF23 levels.

Secondary hypothesis: Combined active therapy with nicotinamide and lanthanum carbonate will lower serum phosphate and FGF23 levels more than nicotinamide alone or lanthanum carbonate alone.

Specific Aim 2: Determine the safety and tolerability of nicotinamide and lanthanum carbonate in CKD stage 3-4:

Hypothesis: Combined treatment with nicotinamide and lanthanum carbonate will be safe, well-tolerated, and acceptable to CKD participants, demonstrating feasibility of long-term compliance in a subsequent phase 3 clinical trial.

The secondary objectives of this pilot clinical trial are to evaluate the following:

1. The effects of active therapy with nicotinamide and lanthanum carbonate compared to placebo on bone and mineral metabolism markers in patients with Stage 3-4 CKD, as assessed by changes in PTH, calcitriol, klotho, N terminal propeptide of Type 1 procollagen (P1NP) and tartrate-resistant acid phosphatase (TRAP) 5b levels over 12 months.
2. The effects of active therapy with nicotinamide and lanthanum carbonate compared to placebo on surrogate measures of CVD risk in patients with Stage 3-4 CKD, as assessed by gadolinium-free Cardiac Magnetic Resonance Imaging (MRI)-measured changes in left ventricular (LV) mass index, LV end diastolic volume, left atrial volume, and levels of brain natriuretic peptide (BNP), troponin T, cholesterol, and asymmetric dimethylarginine (ADMA) over 12 months.
3. The effects of active therapy with nicotinamide and lanthanum carbonate compared to placebo on surrogate measures of CKD progression and inflammation with Stage 3-4 CKD, as assessed by changes in intra-renal oxygenation and fibrosis as measured by gadolinium-free blood oxygenation

level dependent (BOLD) MRI and diffusion-weighted MRI and changes in GFR, albuminuria and CRP and IL-6 over 12 months.

1.2 Background

Disordered mineral metabolism is a near-universal complication of CKD^{2,3} and is strongly linked to risks of ESRD, CVD, fractures, and death.⁴⁻⁶ Phosphate excess induces arterial calcification,⁷ which is an important phenotype of CVD in CKD. Elevated FGF23 maintains serum phosphate in the normal range in CKD, but FGF23 excess contributes mechanistically to LVH.⁹ Both phosphate and FGF23 excess are associated with CVD and CKD progression,⁶ and data in laboratory animals suggest causal links.^{7,9} Therefore, our over-arching hypothesis is that interventions targeting phosphate and FGF23 excess in CKD stages 3–4 will reduce risks of ESRD, CVD, fractures, and death.

Small studies suggest that phosphate binders and dietary phosphate modification may lower FGF23 and phosphate, and improve other indices of mineral metabolism and vascular biology in CKD.¹¹ Nicotinamide (vitamin B3, also called niacinamide) lowers serum phosphate levels in animals¹² and in patients with ESRD.¹³⁻¹⁶ Rather than binding dietary phosphate, nicotinamide reduces expression of NPT2b,¹² the major sodium-phosphate co-transporter in the small bowel. Animal studies demonstrate that NPT2b is upregulated when dietary phosphate intake is decreased or when phosphate binders are administered.^{12,17} As a result, attempts to reduce dietary phosphate absorption using phosphate binders alone may be limited by upregulation of sodium-phosphate transporters and resultant increases in dietary phosphate absorption at times when phosphate binders are not present in the intestinal lumen. Therefore, we hypothesize that use of nicotinamide combined with phosphate binders will provide the most effective method to reduce phosphate and FGF23 levels and related risks of ESRD, CVD, fractures, and death in CKD patients.

1.3 Goals/Aims/Hypotheses

Our long-term goal is to conduct a placebo-controlled, randomized trial to target phosphate and FGF23 excess. Hard end-points will include a combination (to be determined) of ESRD, CVD events, fractures and death. The purpose of this pilot study is to determine whether intervention with both nicotinamide and phosphate binders, in either isolation or in combination will provide an efficacious, safe, well tolerated and feasible strategy to advance into the large outcomes trial.

A 12-month, randomized, double-blinded, four-arm parallel study in 200 CKD stage 3–4 patients will be conducted. Participants will be randomized to one of the four groups: 1) lanthanum carbonate + nicotinamide; 2) lanthanum carbonate + nicotinamide placebo; 3) lanthanum carbonate placebo + nicotinamide; 4) lanthanum carbonate placebo + nicotinamide placebo.

The following hypotheses will be tested:

1. Compared to placebo, monotherapy with nicotinamide, monotherapy with lanthanum carbonate, and combined nicotinamide and lanthanum carbonate therapy will lower serum phosphate and FGF23 levels.
2. Combined treatment with nicotinamide and lanthanum carbonate will be safe, well-tolerated, and acceptable to CKD participants, demonstrating feasibility of long-term compliance in a subsequent phase 3 clinical trial.

Secondary hypotheses:

- 1) Combined active therapy with nicotinamide and lanthanum carbonate will lower serum phosphate and FGF23 levels more than nicotinamide alone or lanthanum carbonate alone.
- 2) Compared with placebo, active therapy with nicotinamide and lanthanum carbonate will blunt the slope of PTH rise, attenuate the decline of calcitriol and klotho levels, and improve bone turnover markers, P1NP and Trap-5b.

- 3) Compared with placebo, active therapy with nicotinamide and lanthanum carbonate will reduce or blunt the increase in LV mass index; attenuate LV diastolic dysfunction, indicated by increased LV end diastolic volume and decreased left atrial volume (all measures assessed by gadolinium-free Cardiac MRI); and reduce levels of biomarkers that are associated with CVD: BNP, troponin T, cholesterol, and ADMA.
- 4) Compared with placebo, active therapy with nicotinamide and lanthanum carbonate will blunt the slope of decline in GFR and the rise in proteinuria and the inflammatory markers, CRP and IL-6, improve intra-renal oxygenation and stabilize or reduce progression of renal fibrosis, as assessed by gadolinium-free renal BOLD MRI.

1.4 Description of the treatments being tested

Nicotinamide: Animal studies¹² and pilot studies in humans show that nicotinamide substantially lowers serum phosphate levels, and can do so with only 1-2 pills/day.¹³⁻¹⁶ Nicotinamide reduces serum phosphate levels by blocking intestinal phosphate absorption through down-regulation of NPT2b.¹² The lipid drug niacin contains both nicotinamide and nicotinic acid, and it has been shown that it lowers serum phosphate in CKD patients.¹⁸ However, nicotinamide alone may have advantages over niacin. Unlike niacin, nicotinamide does not cause flushing and is thought to be less likely to cause liver test abnormalities, hyperuricemia, or insulin resistance. Nicotinamide has considerable long-term safety data in the general population and is available over the counter as a dietary supplement in the US. The use of nicotinamide at a dose of 750 mg once daily during the first month following randomization, followed by up-titration to 750 mg twice daily thereafter is proposed.

Phosphate binders: Several studies suggest that calcium-based binders do not lower FGF23.^{19,20} Therefore, active therapy will consist of fixed doses of lanthanum carbonate (3000 mg/day), which will be off patent by 2017. Use lanthanum carbonate at a dose of 500 mg three times daily with meals during the first month following randomization and a 1000 mg three times daily with meals thereafter is proposed. This fixed dose was chosen because it has been demonstrated that this dose safely reduced urinary phosphate, signifying effective phosphate binding, in CKD stages 3–4.^{20,21} Importantly, lanthanum carbonate has been used in the hemodialysis population, with 6-year long safety data demonstrating a good safety profile.²² Participants who do not eat regular meals will be instructed to take full doses with larger meals and half with snacks.

An IND exemption from the FDA has been applied and received for the use of both of these agents alone and in combination for this trial.

Dietary phosphate restriction: In all study arms, during the run-in phase, dietary information will be provided to participants to reduce dietary phosphate intake. The study coordinator will provide this information, which will include: a) instructions on how to read food labels in order to avoid foods with phosphate additives²³; b) advice to reduce serving sizes of animal protein; c) encouragement of consumption of vegetarian sources of protein instead of animal protein; d) advice to reduce consumption of dairy, and e) encouragement of consumption of egg whites instead of egg yolks. Prior proof-of-concept data demonstrated feasibility of this approach and the importance of this adjunctive intervention to potentiate the efficacy of phosphate binders in lowering serum phosphate and FGF23 levels.¹¹

1.5 Design of this Pilot Clinical Trial

This is a 12-month, randomized, double-blinded, four arm parallel study in 200 CKD stage 3–4 patients (50 in each of the four treatment groups) investigating the effects of the interventions on changes in serum phosphate and FGF23 levels. Participants will be randomized to one of the four groups: 1) lanthanum carbonate + nicotinamide; 2) lanthanum carbonate + nicotinamide placebo; 3) lanthanum carbonate placebo + nicotinamide; 4) lanthanum carbonate placebo + nicotinamide placebo.

The COMBINE Study is a multi-center clinical trial being carried out by the NIDDK-funded Pilot Clinical Trials in CKD Consortium. Participants will be enrolled at each of the core clinical centers

(George Washington University; Northwestern University; University of California San Diego/Denver Nephrology; University of Utah).

1.6 Sample Size for this Pilot Clinical Trial

Assumptions based on prior studies:

- In our pilot trial in CKD,¹⁸ niacin lowered serum phosphate by 0.42 mg/dL relative to placebo. This magnitude of effect occurred within 2 months and was stable for the remainder of the 6-month study.¹⁸ Thus, it is anticipated that a similar magnitude of effect in the proposed study despite the longer follow-up time proposed in this study. The within group standard deviation of change in serum phosphate over 6 months was 0.55 mg/dL
- In our prior unpublished data, the within group standard deviation for the change in FGF23 levels in untreated individuals over 2 years was 18.65 pg/ml.

Power justification:

This pilot study is powered to demonstrate both a decrease in serum phosphate and a decrease in FGF23 levels. Specifically, the primary hypotheses for these two primary end points will compare each of the three treatment groups to placebo. To control for three comparisons and two primary end points, Bonferroni correction is applied by dividing the standard α of 0.05 by 6 to yield a two-sided α of 0.0083. Using this conservative method for correction of multiple testing, an estimated sample size of 43 participants per group will yield 80% power to detect a difference of 0.42 mg/dl in serum phosphate, at $\alpha=0.0083$, assuming a within group SD of the change in serum phosphate over time of 0.55 mg/dL. The same sample size of 43 participants per group will allow detection of a difference of 14.3 pg/ml in FGF23, with 80% power, at $\alpha=0.0083$, assuming a SD of the change of 18.65 pg/ml.

Fifty participants per arm will be enrolled to provide a reasonable buffer from drop-outs (~14% drop out rate). Importantly, our power calculations are based on t-tests that compare pre to post intervention changes across two groups. Because the repeated measures analyses employed using linear mixed models will reduce within-subject variability, there will be greater power than is tabulated above and the presented power calculations are conservative estimates.

Sample size for the secondary objectives:

To maximize power for the secondary objectives analyses, change over time in the outcome measures in all actively treated vs. placebo-treated participants (3:1) will be compared. With this approach, we anticipate having sufficient power for all end points proposed under secondary objectives.

In a previous study of CKD patients with comparable GFR,¹⁹ PTH levels declined by 35.5%, from 107 pg/ml to 69 pg/ml by 6 weeks among the 21 participants treated with sevelamer hydrochloride. In our previous study, the within group standard deviation (SD) for % change in PTH over 3 months was 42%. With our planned analysis being the comparison of all actively treated vs. placebo-treated participants (3:1), assuming a conservative SD of the change of 45%, 150 actively-treated and 50 placebo-treated individuals will allow us to detect a difference in % change in PTH as small as 21% in response to active treatment with 80% power at a two-sided $\alpha=5\%$. Keeping all assumptions the same but changing the SD to 50% yields a minimum detectable difference of 23%, which will decrease to 22.2% for a possible 14% drop out rate. Importantly, our power calculations are based on t-tests that compare pre to post intervention changes across two groups. Because the repeated measures analyses employed using linear mixed models will reduce within-subject variability, there will be greater power than is tabulated above and view the presented power calculations as conservative estimates.

It is anticipated that the **sample size for the MRI studies** will be approximately 70% of the total sample size (n=140) because of contraindications (pacemakers, metal fragments, and claustrophobia) and participant refusal. It is believed that this estimate is conservative, given that > 70% of enrolled participants in the NHLBI-sponsored Multi-Ethnic Study of Atherosclerosis successfully completed the MRI measurements.²⁴ A 2-sided t-test to calculate the power to detect a significant difference from

baseline to end of study in change in LVMI between 105 actively-treated and 35 placebo-treated participants was used. For our estimate of the within-subject SD for change in LVMI over time, data on repeated measures of LVMI by MRI from the PRIMO study was used, which was a randomized trial of paricalcitol in patients with a similar range of eGFR as in the COMBINE study. In the 91 placebo-treated PRIMO participants, the SD for the change in LVMI over 48 weeks was $2.4 \text{ g/m}^{2.7}$.²⁵ Assuming a SD of the change of $2.4 \text{ g/m}^{2.7}$, 105 actively-treated and 35 placebo-treated individuals will allow us to detect a difference in change in LVMI as small as $1.3 \text{ g/m}^{2.7}$ in response to active treatment with 80% power at a two-sided $\alpha=5\%$. Eighty percent power for change in LVMI if 124 participants (93 active-treated and 31 placebo) are enrolled and provide at least one baseline and one follow-up MRI examination will be maintained. Keeping all assumptions the same but allowing for a more conservative SD of $3.0 \text{ g/m}^{2.7}$ will enable us to detect a difference in change in LVMI as small as $1.7 \text{ g/m}^{2.7}$. A difference of $\sim 3.0 \text{ g/m}^{2.7}$ is considered to be a clinically meaningful between-group difference in LVMI and corresponds to a mean between-group difference of $\sim 10 \text{ g}$ in LVM.^{25,26} The study will have $>90\%$ power to detect a difference of this magnitude, assuming a SD of the change of $2.4 - 3.0 \text{ g/m}^{2.7}$.

Since there are no studies with repeated measures of **BOLD MRI in CKD**, we relied on collaborators' cross-sectional data to calculate the power for our proposed analyses in Aim 3. Assuming a common SD of 10 s^{-1} , a conservative correlation of repeated $R2^*$ measures of 0.5, and a two-sided α of 5%, 105 participants in the actively-treated arm and 35 participants in the placebo arm will allow us to detect a difference between the groups in $R2^*$ as small as 4.5 s^{-1} with 80% power. Keeping all assumptions the same, but changing the SD to 15 s^{-1} yields a minimal detectable difference of 6.4 s^{-1} .

1.7 Timeline for this Pilot Clinical Trial

The study will include the following 3 phases:

1. Screening Period
2. Baseline and Run-in Period
3. Follow-up Period

A 2-week run-in will be included in the 3-week long Baseline Period. During the run in, compliance with the nicotinamide placebo and lanthanum carbonate placebo, visits, and procedures will be assessed. During the run-in, a first visit will occur to dispense placebo pills, followed by 2 visits one week apart. After participants meet all inclusion criteria, screening requirements and MRI safety criteria, pre-randomization cardiac and BOLD renal MRI will be obtained. These baseline imaging studies should be performed anytime between Baseline Visit 0 (B0) and prior to Baseline Visit 2 (B2). The optimal timing of the baseline MRI scans is prior to B2, but the studies may occur up to two calendar months after B2.

Thereafter, follow-up visits will take on the following schedule:

- monthly visits for the first 3 months (months 1-3)
- visits every 3 months from month 3 (months 3-12), with an extra visit at month 11

A timeline of the key events in the study is shown in Table 1 below.

1.8 Patient Timeline (Key events of Screening, Baseline, Randomization, Follow-up) – Table 1

Periods	SCREENING	BASELINE/RUN-IN			FOLLOW-UP						
Visits	S0	B0	B1	B2/F0	F1	F2	F3	F6	F9	F11	F12
Months (unless otherwise specified)	-3 weeks	-2 weeks	-1 weeks	0 weeks	1	2	3	6	9	11	12
Pregnancy testing	X										
Distribute placebos for run in and measure height		X									
Distribute drug/placebo (X = 30 day supply)				X	X	X	XXX	XXX	XX	X	
Demographics, medical history	X										
BP, pulse, weight	X		X	X			X	X	X		X
Dietary information and adherence reinforcement			X	X	X	X	X	X	X	X	X
PTH	X		X	X			X	X	X		X
Serum calcium			X	X			X	X	X		X
Spot urine phosphate, calcium, creatinine, and albumin			X	X			X	X			X
24-hr urine phosphate, calcium, creatinine, and urea nitrogen			X	X			X	X			X
Renal parameters											
Chem 7 (Na, K, Cl, HCO ₃ , BUN, Cr, glucose)	X		X	X	X	X	X	X	X		X
Serum uric acid			X					X			X
Whole blood HbA1c			X								
Blood samples for FGF23			X	X	X	X	X	X	X		X
Biorepository blood samples			X	X			X	X			X
Biorepository urine samples			X	X			X	X			X
Safety assessments											
Serum phosphate	X				X	X	X	X	X		X
Hemoglobin, Platelets, LFTs, CK (Local) ¹	X										
CBC, LFTs, CK (Central)					X	X	X	X	X		X
Adverse events assessment			X	X	X	X	X	X	X	X	X
Compliance questions			X	X	X	X	X	X	X	X	X
Pill Counts			X	X	X	X	X				X
GI symptoms questionnaire		X	X	X	X	X	X	X	X	X	X
Cardiac and BOLD renal MRI			X								X
Bone and mineral metabolism markers²			X					X			X
CVD biomarkers³			X					X			X
Inflammation biomarkers⁴			X					X			X

Notes for Table 1:

S0: screening visit

B: baseline visit; baseline visits are 1-2 weeks apart

F: follow-up visits

X: This evaluation/procedure applies at this visit

¹With the exception of screening tests, which will be performed locally, all other tests will be performed at a central lab.

FGF23, serum phosphate, PTH, CBC, serum calcium, 24-hr and spot urinary phosphate, serum creatinine, UACR, 24-hr urine creatinine and urea, uric acid, and HbA1c will be performed centrally as received or in batches.

²Bone and mineral metabolism markers include calcitriol, klotho, P1NP and Trap-5b (performed centrally in batches).

³CVD biomarkers include BNP, troponin T, cholesterol, and ADMA (performed centrally in batches).

⁴Inflammation markers include CRP and IL-6 (performed centrally in batches).

Please see appendix for volumes of blood drawn at each visit.

2 PATIENT SELECTION

2.1 Introduction

CKD stage 3b–4 patients will be recruited. All inclusion and exclusion criteria will be based on measurements and questionnaires obtained at the screening visit. Labs will be run in the local lab to allow final determination of eligibility with rapid turn-around time.

2.2 Inclusion and Exclusion Criteria

Inclusion criteria:

1. Patients with eGFR 20-45 ml/min/1.73m²
2. Age \geq 18 years
3. Serum phosphate \geq 2.8 mg/dL
4. Platelet count \geq 125,000/mm³
5. Able to provide consent
6. Able to travel to study visits
7. Able to eat at least two meals a day
8. In the opinion of the site investigator, willing and able to follow the study treatment regimen and comply with the site investigator's recommendations.

Exclusion criteria:

1. History of allergic reaction to nicotinamide, niacin (excluding flushing), multivitamin preparations, or lanthanum carbonate
2. Liver disease, defined as known cirrhosis by imaging or physician diagnosis, documented alcohol use > 14 drinks/week, or aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, or total bilirubin concentrations > 2 times the upper limit of the local laboratory reference range
3. Creatine kinase (CK) concentrations > 2 times the upper limit of the local laboratory reference range
4. Major hemorrhagic event within the past six months requiring in-patient admission
5. Blood or platelet transfusion within the past six months
6. Secondary hyperparathyroidism (PTH > 5 times the upper limit of normal range for the laboratory) or currently taking cinacalcet (Sensipar)
7. Current, clinically significant malabsorption, as determined at the discretion of the site investigator
8. Anemia (screening Hg < 9.0 g/dl)
9. Serum albumin < 2.5 mg/dl
10. Anticipated initiation of dialysis or kidney transplantation within 12 months as assessed by and at the discretion of the site investigator.
11. Use of immunosuppressive medications. (This criterion excludes anyone who has received a solid organ transplant. Stable oral steroids \leq 10 mg of prednisone/day or inhaled steroids are exempted.)
12. In the opinion of the site investigator, active abuse of alcohol or drugs
13. Recent (within the last 14 days) initiation or change in dose of treatment with 1,25 (OH)₂ vitamin D or active vitamin D analogues (paricalcitol or hectorol). Patients on stable doses of these agents initiated more than 14 days prior to screening are eligible to participate.
14. Current or recent treatment (within the last 14 days) with phosphate binder or niacin/nicotinamide > 100 mg/day
15. Current participation in another clinical trial or other interventional research
16. Currently taking investigational drugs
17. Institutionalized individuals, including prisoners and nursing home residents
18. Malignancy requiring therapy within 2 years (basal or squamous cell skin carcinoma and localized prostate cancer are exempted)

19. Pregnancy or planning to become pregnant or currently breast-feeding. Women of childbearing potential (pre-menopausal and not surgically sterilized) will have pregnancy test before enrollment.
20. Life expectancy < 12 months as determined by the site investigator.
21. Hospitalization within the past 30 days prior to Screening visit (24 hour observation admissions are exempted)
22. Plans to leave the immediate area within 12 months
23. Eat less than 2 meals a day
24. Routinely leaves town for multiple weeks each year such that protocol visits would be missed

In addition to the inclusion/exclusion criteria, at the Screening Visit participants will also be asked questions listed on the MRI safety screening form. These questions will also be reviewed immediately before MRI scanning on the days of the MRI visits. Participants who meet all of the inclusion criteria and none of the exclusion criteria and who have a contraindication for the MRI will not undergo the imaging studies but will proceed with all other study procedures.

2.3 Identification of participants for screening

Sites may use some or all of the following recruitment strategies: clinic-based recruitment; referrals from providers; community-based recruitment campaigns. Brief descriptions of steps taken with each method are listed below.

Clinic-based recruitment:

- After HIPAA waivers and waivers of Informed Consent are obtained, if required by local Institutional Review Boards, electronic medical records will be reviewed to identify potential patients who are eligible for participation.
- Study staff will approach patients during their clinic sessions and invite them to learn more about the study. Recruitment letters may also be used instead of verbal contact during the course of providing medical care. Following introduction, study staff will communicate with interested individuals and set up a screening visit.

Referral from providers:

Providers will be introduced to the study and will be provided with list of inclusion and exclusion criteria. The primary providers will be encouraged to discuss the study with potentially eligible participants, and to either provide the participant with contact information for the study, or alternatively ask if study personnel may contact the participant to discuss the study in more detail. Study staff will initiate contact with interested and potentially eligible patients and describe the study to them. Recruitment letters may also be used instead of phone contact. Following introduction, study staff will set up screening visits with interested individuals.

Community-based recruitment:

The site investigators will initiate contact with community leaders and introduce them to the study. Community leaders will suggest possible ways to disseminate information regarding the study to potential participants in the community. Study staff will respond to contact initiated by interested individuals and briefly describe the study to them. Following introduction, study staff will communicate with interested individuals and set up a screening visit. Additional strategies, including targeted mailings, advertisements within healthcare settings and external promotions will also be considered.

2.4 Informed Consent Procedures

2.4 Informed Consent Procedures

All participants will provide informed consent. Participants will be given ample time to review the consent and ask questions and will be told that participation in the study is entirely voluntary. They will also be told that if they choose not to participate in the study their care will in no way be affected.

Consent discussions:

Discussions regarding consent will occur in either a private exam room or in private research offices. In either case, these discussions will occur behind closed doors for both privacy and to allow the participant time to focus on the consent without distractions. Participants will be asked to describe what they understand about the study protocol after their review to assure accurate understanding, and will be given the opportunity to take the consent home to read and/or discuss with family members or other health care providers before consenting, if they so choose.

Consent and Withdrawal of Consent for the NIDDK Biosample Repository:

Participants may consent or withdraw consent for the repository at any time during the COMBINE Study. If a participant consents to the NIDDK biosample repository, his or her samples will be stored at the NIDDK biosample repository indefinitely. A participant may withdraw consent for the repository during the study by notifying the COMBINE physician at his site. This physician will notify the DCC staff of this request in writing. The DCC will then notify the repository. The repository will first obtain approval from NIDDK to destroy the samples and then discard the samples with its biological and laboratory waste. The DCC will follow through to make sure the samples have been destroyed. After the COMBINE study ends, participants may no longer withdraw consent for the biosample repository, as there will no longer be a way to link any individual with any stored specimen.

2.5 Privacy and security

Local clinical sites will store patient information in a secure manner, and HIPAA privacy rules will be followed. For purposes of this study, patients will be identified only by an assigned identification number and a randomly generated alphanumeric code. The study database will be password protected, and clinical site personnel will be restricted from seeing data for patients from centers other than their own. The study database will reside on a password-protected computer at the Data Coordinating Center (DCC). No individual identifiers (patient name, SSN, or device serial number) will be stored. Data will be entered with strong encryption and a Thawte, Inc., system will be used for secure transmission of data from the clinical sites to the Data Coordinating Center. The DCC ensures accuracy and reliability of computer systems used for this study with detailed edit checks and tests of data interface screens, reports, and procedures before implementation. Computer-generated, time-stamped audit trails are kept. The database is incrementally backed up daily, with full back ups performed weekly. Backups are stored at a secure location separate from the site of the database server.

In those instances where a patient document (such as a hospital discharge summary or death certificate) is sent to the DCC, the site study coordinator will de-identify this prior to providing it to the DCC. The DCC will store these de-identified patient documents in locked file cabinets.

Clinical site and DCC staff members will be required to change their passwords every 75 days, and staff who are no longer active in the study will be blocked from database access.

3 BASELINE PERIOD EVALUATION

3.1 Introduction

Participants who successfully complete the screening visit and are deemed eligible will proceed to the Baseline Period. The Baseline Period will consist of a 2-week run in period with 3 weekly visits (B0, B1 and B2, Table 1).

3.2 Baseline collection of concurrent meds at study entry

Participants will be asked to bring their medications, including all current prescription medications, over-the-counter medicines, with them to the study visit. Study staff will record all concomitant medications that the participant is taking.

3.3 Comorbidity assessment

Study staff will record medical history, including history of tobacco and alcohol use.

3.4 Description of run in

The study will include a 2-week run-in, during which compliance with the two placebos and adherence to requirements for visits and procedures will be assessed. Placebo treatment will consist of one nicotinamide 750 mg placebo daily and three lanthanum 500 mg placebos daily (one taken with each meal) reflecting similar dosing from the F0 to F1 period. During this run-in, 2 weekly visits will take place.

The following adjustments in lanthanum carbonate placebo will be made during run-in for participants who eat fewer than 3 meals per day:

- Participants who eat only 2 meals a day and no snacks will take one 500 mg lanthanum carbonate placebo with each meal
- Participants who eat 2 meals a day and one snack will take one 500 mg lanthanum carbonate placebo with each meal and one with a snack

For calculation of compliance, all participants will be assumed to be taking two lanthanum carbonate placebo per day.

Patients will bring 24-hour urine collections to B1 and B2 visits. Study staff will record the 24-hour urine volume and participants will be asked whether all urine was collected. Aliquots of the 24-hour urine will be collected and submitted to the core lab.

At each visit, participants will receive education from the trained coordinators regarding efforts to reducing dietary phosphate intake. The information will include a) instructions on how to read food labels in order to avoid foods with phosphate additives²³; b) advice to reduce serving sizes of animal protein; c) encouragement of consumption of vegetarian sources of protein instead of animal protein; d) advice to reduce consumption of dairy, and e) encouragement of consumption of egg whites instead of egg yolks.

At each baseline study visit, blood samples will be collected. Whenever blood is drawn, time of day will be recorded. When two core lab measures of serum phosphate, FGF23 level, and mineral metabolites are obtained during the baseline period, the mean of these two will be used as the baseline values in the analyses.

Height will be measured at screening or at the baseline 0 visit.

Prior studies have shown that assessment of the effects of dietary phosphate restriction on serum phosphate levels were masked if serum phosphate measurements were done in the early morning.²⁷ In contrast, assessments in the mid-afternoon allowed for detection a change in serum phosphate levels following interventions.²⁷ Because of this, it is recommended that the trial's two baseline serum collections are both conducted between noon and 6:00 pm. It is also recommended that subsequent clinic appointments be held in the afternoon to maximize detection of the effect of the intervention on serum phosphate levels and minimize influence of circadian changes. Ability to come in for afternoon appointments is not an absolute requirement for study participation. Participants who conduct both of their baseline visits in the morning due to necessity will be categorized as morning participants. Study coordinators will schedule their follow-up visits at about the same time as their baseline visits were held to minimize effects of circadian rhythm on longitudinal changes in phosphate and FGF23.

After participants meet all inclusion criteria and screening requirements, pre-randomization cardiac and BOLD renal MRI scans will be obtained. These imaging studies could occur anytime between Baseline Visit 0 (B0) and prior to Baseline Visit 2 (B2). The optimal timing for the baseline MRI scans is prior to B2, but the studies may occur up to two weeks after B2. Participants who have a contraindication to the MRI (i.e. pacemaker) will not undergo MRI studies but will continue to undergo all other study procedures.

Each participating clinical center will be provided with a detailed imaging protocol and will be required to submit qualifying studies for certification by the MRI Cores prior to enrolling participants. All cardiac and BOLD renal MRI results will be captured according to pre-specified protocols, sent to and stored at the Cardiac MRI Core at Northwestern University. All cardiac MRI Core results will be read by the Cardiac MRI Core at Northwestern University. All BOLD renal MRI results will read by the Renal MRI Core at NorthShore.

3.5 Compliance assessment

Compliance with study procedures will be defined as attendance at Baseline visits 0 and 1 and pill count results showing > 80% adherence to baseline placebo medications during Baseline, as assessed at least once (either B1 or B2).

3.6 Maximum time allowed in baseline

The maximum time between the screening and the B0 visits will be 1 month. Baseline officially begins when the patient is given his or her study medications at the B0 visit. It is expected that patients will be randomized within 3 weeks of the B0 visit. If necessary, patients can be randomized up to 6 weeks (42 days) after B0. If more than 42 days pass, the patient is excluded but may restart baseline, repeating screening and baseline measures to assure that the participant still meets all inclusion and exclusion criteria.

3.7 Collecting reasons for baseline drop out

Study staff will document reasons for baseline exclusion or drop out.

3.8 Assessment of readiness for randomization

Study staff will run the "Ready to Randomize Report" checklist for each participant to check that the participant meets criteria.

4 RANDOMIZATION AND RECRUITMENT MONITORING

4.1 Randomization in an intent-to-treat clinical trial

The study site Principal Investigator or a designated site investigator will review the screening and baseline materials and sign off on randomization before randomization is completed. Randomization marks the participant's official and irrevocable entry into the Follow-up period. Once a participant has been randomized, efforts will be made to conduct all evaluations irrespective of whether the participant starts the study treatment regimen, how long the participant continues on the study treatment regimen or not, and how well the participant complies with the study treatment regimen.

Routine data collection will end when a patient begins dialysis, dies, is deemed lost to follow-up or withdraws consent. Otherwise, all efforts will be made to continue data collection through the end of follow-up even if a participant's medication never begins, the participant is not compliant, or the participant must stop randomized medications for any reason. The primary analysis of outcome will be analyzed as intent-to-treat, by randomized treatment group.

4.2 Logistics of randomization

The DCC will prepare randomization schedules prior to the start of recruitment. Randomization will be stratified by participating site. Randomly permuted blocks of random sizes will be used to help balance numbers of participants assigned to each treatment regimen. This method guarantees that at no time during randomization will the number of participants in any arm be grossly imbalanced and ensures that the sites will be unable to predict assignments of future patients based on knowledge of assignments of past patients. All randomization schedules will remain confidential and known only by members of the DCC staff.

Baseline data that have been categorized as essential must be in the database and support eligibility in order for a subject to be randomized. The study coordinator can run the Ready to Randomize Report at any time during baseline to check the patient's status with respect to meeting eligibility requirements.

When the site investigator signs off that a participant should be randomized and the participant's Ready to Randomize Report shows eligibility criteria have been met, the Study Coordinator will use his or her current database password to access an on-line interactive randomization program. The program will verify eligibility and Baseline criteria to confirm that the participant is eligible and ready to be randomized and a randomized treatment assignment will be recorded for that participant, based upon his or her stratum. The study is blinded, so treatment assignment will not be displayed. The computer will display the participant's initial bottle numbers for the Nicotinamide/Placebo and for the Lanthanum/Placebo. Confirmation of randomization will be displayed on the screen and emailed to the Participating Clinical Center. The treatment assignment will be used to assign the patient with his or her coded packages of study drug.

4.3 How recruitment will be monitored

The DCC will email study-wide weekly recruitment reports showing study-wide weekly and cumulative enrollment in baseline and randomization. The reports will also include each site's current baseline goal, achievement of baseline goal, randomization goal, and achievement of randomization goal. Clinical site investigators will meet regularly with their study teams to discuss progress on weekly goals, identify barriers and problems, and revise recruitment plans as necessary. The Investigators from multiple sites will meet by conference call at regular scheduled intervals to review each site's recruitment progress, share problems and successes, and revise recruitment plans as necessary.

5 TREATMENT ARMS

5.1 Initiation of treatment

Participants meeting eligibility criteria, having given their informed consent and successfully collected required run in data and completed the run-in period will be eligible for randomization. Baseline cardiac and BOLD renal MRI studies should preferably take place prior to randomization. However, the studies may occur up to two weeks after B2. As soon as Baseline data, including cardiac and BOLD renal MRI, if available, have been collected and required data show that the patient has met criteria for eligibility for randomization, the study coordinator will randomize the patient. The patient should receive the first bottles of blinded study medication as soon as possible post-randomization.

Participants will be randomized to one of the following 4 groups:

- Lanthanum carbonate + nicotinamide
- Lanthanum carbonate + nicotinamide placebo
- Lanthanum carbonate placebo + nicotinamide
- Lanthanum carbonate placebo + nicotinamide placebo

During the first month following randomization (F0 to F1), randomized treatments will be started at lower doses. All participants will take one 750-mg nicotinamide or matched placebo daily.

Lanthanum carbonate or its placebo will be prescribed as follows:

Participants who eat 3 meals a day will take one 500 mg lanthanum carbonate or matched placebo with each meal (1500 mg).

Those who eat fewer than 3 meals a day will receive adjusted doses:

- Participants who eat 2 meals a day with no snacks will take one 500 mg lanthanum carbonate or matched placebo with each meal (1000 mg).
- Participants who eat 2 meals a day and one or more snacks will take one 500 mg lanthanum carbonate or matched placebo with each meal and one 500 mg lanthanum carbonate or matched placebo with a snack (1500 mg).

For the remainder of the study (F1 through F12), randomized treatments will be taken at full doses. All participants will take nicotinamide 750 mg or matched placebo twice daily (1500 mg).

Participants who eat 3 meals a day will take two 500-mg lanthanum carbonate or matched placebo with each meal (3000 mg).

Those who eat fewer than 3 meals a day will receive adjusted doses:

- Participants who eat 2 meals a day with no snacks will take two 500-mg lanthanum carbonate or matched placebo with each meal (2000 mg).
- Participants who eat 2 meals a day and one snack will take two 500-mg lanthanum carbonate or matched placebo with each meal and one 500 mg lanthanum carbonate or matched placebo with the snack (2500 mg).
- Participants who eat 2 meals a day and two or more snacks will take two 500-mg lanthanum carbonate or matched placebo with each meal and one 500-mg lanthanum carbonate or matched placebo with 2 snacks (3000 mg).

5.2 Dose adjustments and treatment stops related to serum phosphate levels

5.2.1 Dose adjustment due to hypophosphatemia

If a study participant has a core lab serum phosphate level lower than 2.8 mg/dl at any visit, an extra visit should be held as soon as possible so the serum phosphate test can be repeated. This repeat lab will be the "action lab" for dose adjustments, regardless of the core lab serum phosphate level. The local action lab should be obtained as soon as possible, but must be obtained within 15 days of the core lab blood draw. In the event that the local action lab cannot be obtained, or is not available within 15 days, then the available core lab serum phosphate level becomes the action lab for dose adjustments.

If the action lab test demonstrates a serum phosphate level of 2.8 mg/dL or higher, the participant should continue on full dose medications until the next protocol visit. If the serum phosphate level on the action lab test is between 1.5 and 2.7mg/L, both study medications should be prescribed at half dose until the next protocol visit. If the serum phosphate level is ≤ 1.4 mg/dL on the action lab test, both study medications should be stopped until the next protocol visit.

Participants with an action lab test of 1.5 mg/dL or higher can enter into the same algorithm summarized above at any subsequent protocol visit. In contrast, participants with an action lab test of 1.4 mg/dL or lower require special consideration through the remainder of the trial. In such an individual, if a core lab serum phosphate is 2.8 mg/dL or higher at a subsequent protocol visit, both study medications should be restarted at half dose, and the doses of these medications should not be titrated above half dose throughout the remainder of the trial. If the serum phosphate level on a subsequent protocol visit core lab is 2.7 mg/dL or lower, the participant should return for a local action lab draw within 15 days, as summarized above. If the action lab is 2.7 mg/dL or lower, the participant should remain off both study medications until such time as their serum phosphate is 2.8 mg/dL or higher at a subsequent core lab measurement. Last, if a participant has previously had an action lab test ≤ 1.4 mg/dL resulting in the need to hold their study medications at any point during the trial, and has a subsequent action lab test ≤ 1.4 mg/dL again at any point during the remainder of the trial, this individual should be maintained off both study medications for the remainder of the trial. In such an individual, even if subsequent core or local action lab tests return to levels of 2.8 mg/dL at future visits, the study medications should not be restarted. Additional recommendations for managing COMBINE participants with hypophosphatemia are included in the Manual of Operations (MOP).

5.2.2 Treatment safety medication stop due to hyperphosphatemia

If a study participant has a central serum phosphate level of 5.9 or greater, an extra visit should be held as soon as possible so the test can be repeated locally. If the local test confirms a serum phosphate level over 5.9 mg/dl, this is a treatment safety medication stop for both study medications. All visits and measurements will continue. If the patient has a primary nephrologist or primary care physician, it is recommended that he or she be notified about the hyperphosphatemia and management will be deferred to these providers.

5.2.3 Dose adjustments due to intolerable GI or other side effects

Calcium carbonate tablets such as Tums, may be taken for indigestion if used once per day or less. However, patients who report using Tums (calcium carbonate) often will be encouraged to try over-the-counter Pepcid (famotidine), Tagamet (cimetidine), or Zantac (ranitidine).

If a participant develops GI or other side effects that he or she considers to be intolerable or that are considered intolerable by the site investigators, dose adjustment will be at the discretion of the site investigator. Appropriate actions may include reduction of the dose of lanthanum carbonate or its placebo and/or reduction of the dose of nicotinamide or its placebo, as described in detail in the MOP.

5.3 Management of dose adjustments of study drugs in the presence of other medications

Use of any 1,25(OH)₂ vitamin D preparations and active vitamin D analogues is strongly discouraged during the study. Use of 1,25(OH)₂ vitamin D and/or active vitamin D analogues will be recorded.

5.4 Supply of treatment

The COMBINE Study Drug Distribution Center will send COMBINE participating sites kit boxes of either bottles of blinded lanthanum and lanthanum placebo or bottles of nicotinamide and nicotinamide placebo.

5.5 Resupply of treatment

Treatment bottles will be sufficient to supply 30 days of study agents plus 10 days of extra medication to account for variability in dates of scheduling for follow-up visits (80 tablets per bottle for nicotinamide and its placebo, and 240 tablets per bottle for lanthanum carbonate and its placebo). For follow-up visits separated by multiple months, participants will receive the appropriate number of treatment kits to provide sufficient drug/placebo (e.g., 3 kits for a 3 month interval between visits). Dose reductions procedures are described in detail in the MOP.

5.6 Maintaining blinding

Throughout the Follow-up Period, all efforts will be made to keep study coordinators, investigators and participants blinded to treatment group. Coordinator and investigator blinding will be assessed quarterly; patient blinding will be assessed at the end of the study.

5.7 Unblinding

In the situations previously described, a participant will stop his or her blinded nicotinamide and lanthanum carbonate. In this situation where appropriate clinical management of the patient is dependent on knowledge of whether the patient had been taking active nicotinamide or active lanthanum carbonate, the site investigator will explain the necessity to the study Executive Committee, and the Executive Committee will unblind the site physician to the necessary arm.

6 FOLLOW-UP PERIOD EVALUATION

6.1 Follow-up visit schedule and schedule of tests done during follow-up

6.1.1 Overview

In person follow-up visits will occur monthly for first 3 months after Randomization and every 3 months from month 3 until month 9. There will be a two month interval between month 9 and 11, and finally a 1 month interval between month 11 and 12 (Table 1). Additional visits will be held as needed at the discretion of the site investigator and study team members. All visits will be documented in the study database.

6.1.2 Description of procedures during in-person visits

At in-person visits, patient compliance to study drugs will be assessed either by questionnaire or pill count depending upon the visit (Table 1). The study coordinator will reinforce instructions on dietary phosphate restriction. Concomitant medication therapy will be reviewed and updated at baseline, F3 and every 3 months thereafter. Information on adverse events (serious and non-serious) will be collected. The schedule of laboratory tests to be obtained during the in-person visits is listed in Table 1. Additional blood samples will be collected and stored for future measurements. A GI symptoms form will be

completed by questionnaire at each protocol visit. Blood pressure will be measured by trained personnel at all follow-up visits. Twenty-four hour urine specimens will be collected at some visits (Table 1) and will be measured phosphate, calcium, urea, and creatinine. The 24 hour urine phosphate will be used as an indicator of dietary phosphate absorption and the 24 hour urea will be used as an indicator of dietary protein intake.

At each follow-up visits, participants will be counseled that they must not take any phosphate binders or multivitamins containing vitamin B3 in the form of niacin or nicotinamide in quantities greater than 100 mg/day. If a participant is taking a standard multi-vitamin tablet with < 100 mg/day of nicotinamide, the dose of the multivitamin may not be changed during the study period.

Between F11 and F12 or upon withdrawal from study (for dialysis initiation or preemptive transplantation), participants will undergo cardiac and BOLD renal MRI. The cardiac and BOLD renal MRI may occur anytime between F11 and 2 weeks after F12 or at the time of early discontinuation prior to initiation of renal replacement therapy. If a participant withdraws from the study within 6 weeks of their baseline MRI studies, discontinuation MRI studies will not be performed. MRIs should not be obtained in participants who have started dialysis or have been transplanted. Participants who have a contraindication to the MRI (i.e. pacemaker) will not undergo MRI studies but will continue to undergo all other study procedures.

6.2 Monitoring compliance to treatment

In order to document compliance with the treatment regimen, participants will be instructed to return all drug containers (even if empty) to study staff at all visits. Pill counts will be done at B1, B2, F1, F2, F3, and F12. To decrease the chance of a participant forgetting to bring pills at pill-count visits, participants will be asked to bring remaining pills and empty containers to each visit during the study.

Study staff will record the number of each type of pill returned, and the study database will calculate percent of medications taken. Participants will also be asked a semi-quantitative question for each medication: whether or not they are taking each medication regularly (in every intended instance or almost every intended instance), irregularly, or not at all.

Compliance with each study drugs will be defined as use of $\geq 80\%$ of expected drugs based on pill count data. Data from the semi-quantitative questions will be used to enhance adherence and to provide a secondary measure of adherence.

6.3 Procedures for patients who start dialysis or undergo kidney transplantation

Participants will be withdrawn from the study if they initiate renal replacement therapy or undergo kidney transplantation. MRIs should be obtained in these participants prior to their first renal replacement therapy.

6.4 Procedures for patients who become pregnant

Participants will be withdrawn from the study if they become pregnant.

7 CHARACTERIZATION OF THE MEASUREMENTS AND PROCEDURES DURING SCREENING, BASELINE, AND FOLLOW-UP AND METHODS OF MEASUREMENT TO BE USED

7.1 GFR Calculation

GFR will be estimated from Core Biochemistry Lab serum creatinine values using the CKD-Epidemiology Collaboration equation.²⁷

7.2 List of laboratory measurements

The following lab tests will be performed at the COMBINE Primary Core Lab at Spectra:

Serum Tests

sodium, potassium, chloride, bicarbonate, urea nitrogen, creatinine, glucose, calcium, phosphate, aspartate aminotransferase, alanine aminotransferase, total bilirubin, alkaline phosphatase, albumin, creatine kinase, uric acid, and PTH

Whole Blood Tests

hemoglobin A1c, white blood cell count, hemoglobin, hematocrit, and platelets.

Urine Tests

calcium, creatinine, phosphorus, and urea nitrogen

The following lab tests will be performed at a central FGF23 laboratory:

Serum Tests

FGF23

Levels of calcitriol, klotho, P1NP, Trap-5b, BNP, troponin T, cholesterol, ADMA, CRP and IL-6 will also be measured at the central FGF23 laboratory (performed in batches, in either plasma or serum).

8 ADVERSE EVENTS, ADVERSE EVENT REPORTING

8.1 Introduction

Adverse events include any untoward medical occurrence in a participant administered the study drugs and which does not necessarily have a causal relationship with the study drugs. AEs can include abnormal laboratory findings, symptoms, or disease temporally associated with the use of study drugs, whether or not the event is considered causally related to the use of the study drugs. AEs can result from use of the study drugs as stipulated in the protocol, and from accidental or intentional overdose, drug abuse, or drug discontinuation. An AE may meet the criteria for a serious adverse event (SAE).

8.2 Expected Study Adverse Events

8.2.1 Types of AEs that may be treatment or procedure side effects

Lanthanum carbonate may lower serum phosphate levels and is associated with gastrointestinal side effects including nausea, vomiting, constipation and diarrhea.

Nicotinamide may lower serum phosphate levels and may be associated with other laboratory abnormalities including thrombocytopenia, abnormalities in serum liver function tests and CK. Gastrointestinal side effects, such as diarrhea and heartburn may also occur.

The following laboratory results will be flagged as **expected study adverse events** in this trial:

- **Hypophosphatemia:** Serum phosphate level <1.5 mg/dl
- **Hyperphosphatemia:** Serum phosphate >5.9 mg/dl
- **Thrombocytopenia:** Platelet count <100,000
- **Liver function test abnormalities:** AST, ALT, total bilirubin, alkaline phosphatase > 4 times the upper limit of normal

Elevated Creatine Kinase: Creatinine kinase > 4 times upper limit of normal.

The following symptoms will be flagged as **expected study adverse events** in this trial.

- **Bruising**
- **Bleeding**
- **Severe diarrhea**
- **Severe nausea**

- **Flushing**
- **Hives**
- **Heartburn.**

The following are **expected adverse events related to the MRI scans**:

- **Risks related to undetected metal**
- **Distress from incidental findings that may necessitate additional work up**
- **Risks from furosemide administration in participants with unknown or unrecorded allergy to furosemide.**

8.2.2 How AEs will be reported

Laboratory tests will be performed periodically to monitor safety, as indicated in Table 1. Laboratory measurement based AEs will be detected. Other adverse effects will be assessed at each follow-up visit during symptom questionnaire discussions. Adverse events related to MRI scans will be recorded after each MRI visit and summary reports will be made available to the DSMB.

8.2.3 Follow-up of AEs

Hypo- and hyperphosphatemia will be treated as described in the MOP.
GI side effects will be treated as described in the MOP.

Some AEs, if confirmed, lead to discontinuation of one or both blinded study medications. Details are provided in Protocol Section 9.1

8.3 SAE, Hospitalization, and Death reporting

Standard SAEs listed as follows will be reported in detail and reviewed by the Event Review Committee.

- Death
- Life-threatening event
- Hospitalization
- Prolongation of hospitalization
- Congenital anomaly
- Persistent or significant disability/incapacity
- Important medical event requiring medical or surgical intervention to prevent serious outcome
- Spontaneous abortion

8.3.1 Local Categorization of SAEs as Unanticipated, Related

When an SAE is documented, the local Clinical Center's Investigator will report on the SAE Detail Form whether the SAE was unanticipated. An unanticipated SAE, as defined by DHHS 45 CFR part 46, meets following three criteria: 1) unexpected, in terms of nature, severity, or frequency, given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the study population; 2) related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and 3) suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized will determine use the following definitions to assess the relationship of the adverse event to the use of study treatment.

The site investigator will also classify the SAE as not related, probably not related, possibly related, probably related, or related to study procedures and treatments.

8.3.2 Event Committee Review of SAEs

The COMBINE Study Event Committee will review the first 10 SAEs occurring at each site in the COMBINE Study. After that, the Event Committee will review all deaths, all SAEs that the site investigators categorized as possibly related, probably related, or related and a 10% QC subset of the

SAEs that the site investigator categorized as unrelated. The primary Event Committee reviewer for each reviewed SAE will be chosen from members of the Event Committee who are from sites other than the site where the SAE occurred. When SAE data are presented to the study's DSMB for safety review, both the local site and Event Committee categorization will be presented.

9 STOPPING RANDOMIZED TREATMENT

9.1 Stopping randomized treatment for Adverse Events

Participants will stop treatment with both nicotinamide and lanthanum carbonate due to either hypo- or hyperphosphatemia. Data collection will continue as usual.

Participants will stop treatment with nicotinamide, but continue on the blinded lanthanum carbonate, if the following occur:

- On two consecutive measures, participant is found to have liver function test (AST, ALT, total bilirubin, or alkaline phosphatase) or CK levels > 4 times the upper limit of normal without other identifiable cause.
- On two consecutive measures, participant is found to have a platelet count <100,000 with clinical signs of platelet dysfunction (bruising or bleeding noted at a study visit and documented on the symptom form) or <75,000 without clinical signs

9.2 Stopping randomized treatment for Adverse Events

Participants will stop treatment with nicotinamide and/or lanthanum carbonate, but data collection will continue as usual, if the site investigator and the Event Committee agree that an SAE was possibly, probably, or definitely related to treatment and that it is unsafe for the patient to continue on the specified treatment (nicotinamide and/or lanthanum carbonate).

Procedures for dose adjustment in the case of gastrointestinal side effects are described in the MOP.

9.3 Discontinuation of study medications due to patient preference/non-compliance

Participants who wish to discontinue study medications will be encouraged by the Site PI and study coordinator to continue with visits, lab tests, and data collection so data will be available for intent to treat analysis of the trial. The site PI will encourage passive data collection such as hospitalization documentation for participants who no longer want to attend visits.

9.4 The role of Study Committees in review of lab data and adverse events

Lab data from the Core Lab will be reported to each site investigator and the site team as soon as the results are available to the study database. The Quality Control Committee will review lab data. The Event Review committee will review SAEs and any events that the clinical centers categorize as requiring treatment discontinuation for safety reasons.

10 DSMB

NIDDK will name a DSMB for this clinical trial. The DSMB will include clinicians, statisticians, and other experts as needed, and will include experts on research and clinical expertise in CKD-mineral bone disorders. Acting as a protocol review committee, the DSMB will review and approve the study protocol. As the study progresses, NIDDK will schedule routine meetings and conference calls of the DSMB. At these meetings, the DSMB will review reports of study progress in the areas of recruitment, retention, quality control, and safety related to study medications and to MRI scans. The DSMB can ask for additional reports or additional meetings at their discretion.

11 ANALYSIS PLAN

11.1 Primary analysis

Specific Aim 1 of this clinical trial will evaluate changes over time in absolute levels of serum phosphate and FGF23 levels, with each active treatment group compared to the double placebo group. Based on prior data, both serum phosphate and FGF23 levels are expected to decline from baseline within the first three months and then to be stable thereafter.

Because the distribution of FGF23 is expected to be skewed, FGF23 values will be log transformed. A piecewise mixed-model, repeated-measures analysis will be performed to assess separately changes in two primary endpoints within the first 3 months and in the period thereafter (months 3 – 12). The analysis will be stratified by clinical center. Covariates included in the model will include baseline eGFR and gender.

When two measures of serum phosphate and FGF 23 are available, the average of these two baseline values of serum phosphate (for models evaluating phosphate as the outcome variable) and FGF23 (for models evaluating FGF23 as the outcome variable) levels will be used as the baseline value for analyses of treatment comparison (Table 1).

Specific Aim 2 will address tolerability and safety evaluations in the intention to treat population.

Tolerability: The primary measure of tolerability will be the percentage of persons who come off study drug across treatment. The percentages will be summarized by treatment group and differences will be evaluated using the Fisher's exact test. Compliance by pill count and questionnaire will be evaluated as secondary outcomes.

Safety: The number and percentage of the participants who report at least one adverse event will be summarized by treatment group and overall. The treatment group difference in the percentages will be evaluated using Fisher's exact test. Adverse events will also be summarized descriptively with counts and percentages.

11.2 Secondary Analyses

A secondary objective is to determine whether or not combined treatment with lanthanum carbonate and nicotinamide lowers serum phosphate or FGF23 more so than either nicotinamide alone or lanthanum alone. If no significant interaction between nicotinamide and lanthanum carbonate is seen, the main effects of each active treatment as in a factorial design study will be evaluated. As in analysis for the primary outcome, we will stratify for clinical center and use averages of baseline serum phosphate (in models evaluating phosphate as the outcome variable) and averages of baseline FGF23 (for models evaluating FGF23 as the outcome variables) as the baseline values in the analyses. Models will be adjusted for eGFR and gender, as in the primary analysis.

To address expected cross-over from on-protocol to off-protocol management due to hypo- and hyperphosphatemia and off-protocol binder use, “as-treated” exploratory analyses will be included.

For analyses of the secondary objectives, linear mixed models to analyze effects of all active-treatment groups combined vs. placebo group will be used. In pre-specified secondary analyses, differences between the four treatment groups, perform as treated analyses and test the association between the magnitude of reduction in phosphate and FGF23 and changes in the secondary objective end points will be used.

11.3 Analyses of other follow-up data

Mixed-model, repeated-measures analyses will be used for comparisons of changes of PTH, calcium, eGFR, and urine ACR. When analyses of 24-hour urine are performed, the only collections that will be used will be those in which the measured 24-hour urine creatinine excretion is within 30% of estimated creatinine excretion using the CKD-EPI creatinine excretion rate estimation equation.²⁴

11.4 Safety analyses

Standard analyses will be used for safety evaluations conducted in the intention-to-treat population. As-treated analyses will be performed if there are treatment crossovers due to non-compliance or if study medications are stopped due to adverse or other events.

11.5 Planned analyses of feasibility/logistics including rates of missing data, missing forms, missing visits, and of non-compliance.

This 12-month study will provide important feasibility data on long-term tolerability, safety, and adherence. Standard analyses of recruitment, retention, and compliance will be used for feasibility evaluations

11.6 What will it take to say that this pilot study is feasible?

The COMBINE Study will be judged to be feasible if 1) any of the primary efficacy analyses on serum phosphorus or FGF23 is significant at the .05/6 level and 2) the intervention(s) associated with the outcome(s) met the following criterion for compliance and tolerability: The majority of randomized patients are able to complete 12 months of follow-up without having to discontinue either the lanthanum arm or the nicotinamide arm.

11.7 Test run of the analysis plan for the full scale study

Although this pilot study is not powered to detect changes in CKD progression, longitudinal data on serum creatinine and spot urinary albumin-creatinine ratio (UACR) will be considered. At the end of the study, changes in eGFR over time and rates of ESRD, CVD events, fractures and death by randomized treatment group will be done.

12 PLANS FOR THE STUDY DESIGN OF THE FULL SCALE TRIAL

12.1 Current Plans for Primary Outcome of the Full Scale Trial

Our long-term goal is to conduct a placebo-controlled, randomized trial to target hard clinical endpoints hypothesized as down-stream consequences of phosphate and FGF23 excess. Hard end-points will include a combination (to be determined) of ESRD, CVD events, fractures and death.

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14 REFERENCES

1. Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P, Van Lente F, Levey AS. Prevalence of chronic kidney disease in the United States. *JAMA* 2007; 298:2038-47.
2. Isakova T, Wahl P, Vargas GS, Gutierrez OM, Scialla J, Xie H, Appleby D, Nessel L, Bellovich K, Chen J, Hamm L, Gadegbeku C, Horwitz E, Townsend RR, Anderson CA, Lash JP, Hsu CY, Leonard MB, Wolf M. Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. *Kidney Int* 2011; 79:1370-8.
3. Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, Andress DL. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. *Kidney Int* 2007; 71:31-8.
4. Isakova T, Xie H, Yang W, Xie D, Anderson AH, Scialla J, Wahl P, Gutierrez OM, Steigerwalt S, He J, Schwartz S, Lo J, Ojo A, Sondheimer J, Hsu CY, Lash J, Leonard M, Kusek JW, Feldman HI, Wolf M. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. *JAMA* 2011; 305:2432-9.
5. Palmer SC, Hayen A, Macaskill P, Pellegrini F, Craig JC, Elder GJ, Strippoli GF. Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. *JAMA* 2011; 305:1119-27.
6. Scialla JJ, Astor BC, Isakova T, Xie H, Appel LJ, Wolf M. Mineral metabolites and CKD progression in African Americans. *J Am Soc Nephrol* 2013; 24:125-35.
7. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM. Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res* 2000; 87:e10-7.

8. Scialla JJ, Lau WL, Reilly MP, Isakova T, Yang HY, Crouthamel MH, Chavkin NW, Rahman M, Wahl P, Amaral AP, Hamano T, Master SR, Nessel L, Chai B, Xie D, Kallem RR, Chen J, Lash JP, Kusek JW, Budoff MJ, Giachelli CM, Wolf M. Fibroblast growth factor 23 is not associated with and does not induce arterial calcification. *Kidney Int* 2013; 83:1159-68.
9. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T, Gutierrez OM, Aguillon-Prada R, Lincoln J, Hare JM, Mundel P, Morales A, Scialla J, Fischer M, Soliman EZ, Chen J, Go AS, Rosas SE, Nessel L, Townsend RR, Feldman HI, St John Sutton M, Ojo A, Gadegbeku C, Di Marco GS, Reuter S, Kentrup D, Tiemann K, Brand M, Hill JA, Moe OW, Kuro OM, Kusek JW, Keane MG, Wolf M. FGF23 induces left ventricular hypertrophy. *J Clin Invest* 2011; 121:4393-408.
10. Gutierrez OM, Januzzi JL, Isakova T, Laliberte K, Smith K, Collerone G, Sarwar A, Hoffmann U, Coglianese E, Christenson R, Wang TJ, deFilippi C, Wolf M. Fibroblast growth factor 23 and left ventricular hypertrophy in chronic kidney disease. *Circulation* 2009; 119:2545-52.
11. Isakova T, Barchi-Chung A, Enfield G, Smith K, Vargas G, Houston J, Xie H, Wahl P, Schiavenato E, Dosch A, Gutierrez OM, Diego J, Lenz O, Contreras G, Mendez A, Weiner RB, Wolf M. Effects of Dietary Phosphate Restriction and Phosphate Binders on FGF23 Levels in CKD. *Clin J Am Soc Nephrol* 2013; 8:1009-18.
12. Sabbagh Y, O'Brien SP, Song W, Boulanger JH, Stockmann A, Arbeeney C, Schiavi SC. Intestinal npt2b plays a major role in phosphate absorption and homeostasis. *J Am Soc Nephrol* 2009; 20:2348-58.
13. Cheng SC, Young DO, Huang Y, Delmez JA, Coyne DW. A randomized, double-blind, placebo-controlled trial of niacinamide for reduction of phosphorus in hemodialysis patients. *Clin J Am Soc Nephrol* 2008; 3:1131-8.
14. Shahbazian H, Zafar Mohtashami A, Ghorbani A, Abbaspour MR, Belladi Musavi SS, Hayati F, Lashkarara GR. Oral nicotinamide reduces serum phosphorus, increases HDL, and induces thrombocytopenia in hemodialysis patients: a double-blind randomized clinical trial. *Nefrologia* 2011; 31:58-65.
15. Takahashi Y, Tanaka A, Nakamura T, Fukuwatari T, Shibata K, Shimada N, Ebihara I, Koide H. Nicotinamide suppresses hyperphosphatemia in hemodialysis patients. *Kidney Int* 2004; 65:1099-104.
16. Young DO, Cheng SC, Delmez JA, Coyne DW. The effect of oral niacinamide on plasma phosphorus levels in peritoneal dialysis patients. *Perit Dial Int* 2009; 29:562-7.
17. Schiavi SC, Tang W, Bracken C, O'Brien SP, Song W, Boulanger J, Ryan S, Phillips L, Liu S, Arbeeney C, Ledbetter S, Sabbagh Y. Npt2b Deletion Attenuates Hyperphosphatemia Associated with CKD. *J Am Soc Nephrol* 2012; 23:1691-700.
18. Ix JH, Ganjoo P, Tipping D, Tershakovec AM, Bostom AG. Sustained hypophosphatemic effect of once-daily niacin/laropiprant in dyslipidemic CKD stage 3 patients. *Am J Kidney Dis* 2011; 57:963-5.
19. Oliveira RB, Cancela AL, Gracioli FG, Dos Reis LM, Draibe SA, Cuppari L, Carvalho AB, Jorgetti V, Canziani ME, Moyses RM. Early control of PTH and FGF23 in normophosphatemic CKD patients: a new target in CKD-MBD therapy? *Clin J Am Soc Nephrol* 2010; 5:286-91.
20. Block GA, Wheeler DC, Persky MS, Kestenbaum B, Ketteler M, Spiegel DM, Allison MA, Asplin J, Smits G, Hoofnagle AN, Kooienga L, Thadhani R, Mannstadt M, Wolf M, Chertow GM. Effects of Phosphate Binders in Moderate CKD. *J Am Soc Nephrol* 2012; 23:1407-15.
21. Isakova T, Gutierrez OM, Smith K, Epstein M, Keating LK, Juppner H, Wolf M. Pilot study of dietary phosphorus restriction and phosphorus binders to target fibroblast growth factor 23 in patients with chronic kidney disease. *Nephrol Dial Transplant* 2011; 26:584-91.
22. Hutchison AJ, Barnett ME, Krause R, Kwan JT, Siami GA. Long-term efficacy and safety profile of lanthanum carbonate: results for up to 6 years of treatment. *Nephron Clin Pract* 2008; 110:c15-23.

23. Sullivan C, Sayre SS, Leon JB, Machekano R, Love TE, Porter D, Marbury M, Sehgal AR. Effect of Food Additives on Hyperphosphatemia Among Patients With End-stage Renal Disease: A Randomized Controlled Trial. *JAMA* 2009; 301:629-35.
24. Ix JH, Katz R, Peralta CA, de Boer IH, Allison MA, Bluemke DA, Siscovick DS, Lima JA, Criqui MH. A high ankle brachial index is associated with greater left ventricular mass MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol* 2010; 55:342-9.
25. Thadhani R, Appelbaum E, Pritchett Y, Chang Y, Wenger J, Tamez H, Bhan I, Agarwal R, Zoccali C, Wanner C, Lloyd-Jones D, Cannata J, Thompson BT, Andress D, Zhang W, Packham D, Singh B, Zehnder D, Shah A, Pachika A, Manning WJ, Solomon SD. Vitamin D therapy and cardiac structure and function in patients with chronic kidney disease: the PRIMO randomized controlled trial. *JAMA* 2012; 307:674-84.
26. Pitt B, Reichek N, Willenbrock R, Zannad F, Phillips RA, Roniker B, Kleiman J, Krause S, Burns D, Williams GH. Effects of eplerenone, enalapril, and eplerenone/enalapril in patients with essential hypertension and left ventricular hypertrophy: the 4E-left ventricular hypertrophy study. *Circulation* 2003; 108:1831-8.
27. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150:604-12.