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Clinical Research Protocol

Clearance of 25-hydroxyvitamin D in cystic fibrosis

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LIST OF ABBREVIATIONS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
CRF	case report form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	intravenous
PI	Principal Investigator
PK	pharmacokinetic
SAE	serious adverse experience
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamate pyruvate transaminase

PROTOCOL SYNOPSIS

TITLE	Clearance of 25-hydroxyvitamin D in cystic fibrosis
SPONSOR	Ian H. de Boer, MD, MS
FUNDING ORGANIZATION	National Institute of Diabetes and Digestive and Kidney Diseases (P30DK089507, R01DK099199)
NUMBER OF SITES	Single-site study
RATIONALE	Cystic fibrosis (CF) is among the most common autosomal recessive disorders and leads to serious, life threatening respiratory, infectious, and bone complications. Vitamin D deficiency is highly prevalent among CF patients, is unusually resistant to standard vitamin D supplementation regimens, and may contribute to osteoporosis and immune dysfunction. The prevalence of vitamin D deficiency, determined by circulating concentrations of 25-hydroxyvitmain D (25(OH)D), remains as high as 90% in CF patients despite modern practices of vitamin D supplementation and pancreatic enzyme replacement. A number of plausible hypotheses may explain the marked vitamin D deficiency intrinsic to CF. Pancreatic insufficiency, the most common gastrointestinal CF complication, leads to malabsorption of fat soluble vitamins A, D, E, and K. Hepatobiliary disease may impair the uptake of vitamin D_2 and D_3 into the liver for conversion into 25(OH)D, or may interfere with enterohepatic recirculation to enhance loss of vitamin D metabolites. Small intestinal bacterial overgrowth may further obstruct the gastrointestinal absorption of vitamin D. Finally, urinary losses of vitamin D binding protein and albumin, the major carriers of vitamin D metabolites, may contribute to lower circulating concentrations of vitamin D metabolites. Proposed mechanisms to explain vitamin D deficiency in CF lack empiric supportive data. In this study, we will determine specific vitamin D metabolic disturbances that are intrinsic to CF patients and to explore their biologic sequelae. Identifying the causes of vitamin D deficiency in CF could suggest alternative treatments, such as intramuscular vitamin D preparations, activated vitamin D agents, or higher doses of supplements, to improve clinical outcomes that have been linked with vitamin D deficiency, such as bone disease and infection.
STUDY DESIGN	This is a single-dose, open-label pharmacokinetic study that uses intravenous administration of a stable deuterium-labeled $25(OH)D_3$ to evaluate the metabolic clearance of $25(OH)D_3$.

	Allocation: Non-Randomized **Intervention Model**: Single Group Assignment **Masking**: Open Label
PRIMARY OBJECTIVE	The goal of this study is to define 25(OH)D_3 catabolism in CF patients using gold standard pharmacokinetics studies. Specifically, we will evaluate the metabolic clearance of 25(OH)D_3 among participants with CF and matched control subjects. The goal of this work is to provide the first comprehensive characterization of vitamin D metabolism in CF patients and promote novel hypotheses for subsequent studies.
SECONDARY OBJECTIVES	• To explore the biochemical pathways through which 1,25(OH)D_3 is metabolized, and the effects of CKD and race on these pathways, by evaluating the appearance and disappearance of labeled metabolites of 25(OH)D_3. • To determine clinical and biochemical factors that are correlated with the metabolic clearance of 25(OH)D_3. • To evaluate biomarkers of 25(OH)D_3 clearance. • To compare monocyte expression of vitamin D-related genes in CF patients *versus* healthy controls
NUMBER OF SUBJECTS	Up to 20
SUBJECT SELECTION CRITERIA	Inclusion Criteria: • Age ≥ 18 years • Serum total 25(OH)D 10-50 ng/mL Exclusion Criteria: • Primary hyperparathyroidism • Gastric bypass • Tuberculosis or sarcoidosis • Current pregnancy • Use of vitamin D_3 or vitamin D_2 supplements exceeding a mean daily dose of 400 IU within 3 months (wash-out allowed) • Use of 1,25$(OH)_2D_3$ or an analogue, calcimimetics, or medications known to induce CYP24A1 within 3 months (wash-out allowed) • Serum calcium > 10.1 mg/dL • Hemoglobin < 10 g/dL
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	We will administer deuterated 25(OH)D_3-d6 [26,26,26,27,27,27-d6] intravenously. The labeled 25(OH)D_3 will be prepared in a co-solvent formulation of ethanol (10%) and propylene glycol (40%). To ensure reliable detection of circulating deuterated 25(OH)D_3, without administering a dose that might alter the underlying vitamin D metabolism, we aim to

	administer a dose that results in a peak deuterated 25(OH)D_3 concentration of approximately 5 ng/mL. The administered dose will be calculated as the targeted peak serum deuterated 25(OH)D_3 (5 ng/mL) multiplied by blood volume.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	There is no control product.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for approximately 12 weeks **Screening:** approximately 4 weeks **Treatment:** 1 day (subjects to the University of Washington Clinical Research Center) **Follow-up:** 8 weeks The total duration of the study is expected to be up to 3 years for subject recruitment and an additional 12 weeks for final subject follow-up.
CONCOMMITANT MEDICATIONS	**Allowed:** • Vitamin D supplements (cholecalciferol, ergocalciferol) not to exceed a mean daily dose of 400 IU **Prohibited** (within last 3 months, wash-out allowed): • 1,25(OH)$_2$$D_3$ or an analogue (e.g. paricalcitol, hectorol) • Calcimimetics (e.g. cinaclacet) • Medications known to potently induce or inhibit CYP24A1 or CYP3A4
EFFICACY EVALUATIONS	None
PRIMARY ENDPOINT	• Metabolic clearance of labeled 25(OH)D_3 (administered dose/AUC)
SECONDARY ENDPOINTS	• AUC of labeled 25(OH)D_3 • Terminal half-life of labeled 25(OH)D_3 • Volume of distribution in the central compartment of labeled 25(OH)D_3 • Monocyte transcription of CYP27B1, CYP24A1, VDR, and cathelicidin
OTHER EVALUATIONS	Metabolic formation clearance (metabolite/parent AUC ratio) for metabolites of labeled 25(OH)D_3
SAFETY EVALUATIONS	Change in the serum concentrations of calcium, creatinine, AST, and ALT and from baseline to 7 days after 25(OH)D_3 administration Incidence of adverse events

PLANNED INTERIM ANALYSES	This is not a clinical trial with an efficacy outcome, and it is neither randomized nor blinded. For interim analyses are not planned.
STATISTICS **Primary Analysis Plan**	We will examine the distribution of $25(OH)D_3$ clearance by CF status using box plots and summary statistics. We will test associations of CF status with $25(OH)D_3$ clearance (continuous outcome variable) using linear regression. Models will be adjusted for age, gender, body size, baseline 25(OH)D concentration, and other covariates strongly related to $25(OH)D_3$ clearance.
Rationale for Number of Subjects	For this descriptive study, our sample size of 20 was based on feasibility and funding considerations.

1 BACKGROUND

Cystic fibrosis (CF) is among the most common autosomal recessive disorders and leads to serious, life threatening respiratory, infectious, and bone complications. Vitamin D deficiency is highly prevalent among CF patients, is unusually resistant to standard vitamin D supplementation regimens, and may contribute to osteoporosis and immune dysfunction. The prevalence of vitamin D deficiency, determined by circulating concentrations of 25-hydroxyvitmain D (25(OH)D), remains as high as 90% in CF patients despite modern practices of vitamin D supplementation and pancreatic enzyme replacement. A number of plausible hypotheses may explain the marked vitamin D deficiency intrinsic to CF. Pancreatic insufficiency, the most common gastrointestinal CF complication, leads to malabsorption of fat soluble vitamins A, D, E, and K. Hepatobiliary disease may impair the uptake of vitamin D_2 and D_3 into the liver for conversion into 25(OH)D, or may interfere with enterohepatic recirculation to enhance loss of vitamin D metabolites. Small intestinal bacterial overgrowth may further obstruct the gastrointestinal absorption of vitamin D. Finally, urinary losses of vitamin D binding protein and albumin, the major carriers of vitamin D metabolites, may contribute to lower circulating concentrations of vitamin D metabolites. Proposed mechanisms to explain vitamin D deficiency in CF lack empiric supportive data. In this study, we will determine specific vitamin D metabolic disturbances that are intrinsic to CF patients and to explore their biologic sequelae. Identifying the causes of vitamin D deficiency in CF could suggest alternative treatments, such as intramuscular vitamin D preparations, activated vitamin D agents, or higher doses of supplements, to improve clinical outcomes that have been linked with vitamin D deficiency, such as bone disease and infection.

1.1 Overview of Clinical Studies

Intravenous deuterated 25(OH)D_3 has been used previously in humans in one published study, and intravenous tritiated 25(OH)D_3 has been used in humans in several previous studies.[1-5] In addition deuterium is used extensively in the study of metabolism and is considered safe.[6]

2 STUDY RATIONALE

Vitamin D deficiency is overwhelmingly prevalent in cystic fibrosis. Vitamin D deficiency is the most common and persistent nutritional inadequacy in CF.[7] Vitamin D is normally generated in the skin as vitamin D_3 (cholecalciferol) or consumed in the diet as either vitamin D_3 or vitamin D_2 (ergocalciferol).[8] Vitamin D_3 and vitamin D_2 are next converted in the liver to 25-hydroxyvitamin D_3 (25(OH)D_3) and 25-hydroxyvitamin D_2 (25(OH)D_2), respectively, which together are referred to as total 25-hydroxyvitamin D (25(OH)D). Circulating 25(OH)D concentrations are the accepted clinical markers of vitamin D status and are markedly reduced in CF patients. The prevalence of vitamin D deficiency in CF is as high as 90%, depending on the patient population and 25(OH)D threshold used to define deficiency (20-30 ng/mL).[9-12] Given the role of vitamin D in calcium homeostasis and bone health, the measurement and treatment of vitamin D deficiency is an integral component of clinical CF care. However, CF patients inadequately respond to vitamin D supplementation, such that 25(OH)D concentrations remain insufficient in many CF patients despite treatment.[12-14]

The cause of vitamin D deficiency in cystic fibrosis is unclear. Malabsorption of fat soluble vitamins, including vitamin D, is likely a major contributor to vitamin D deficiency in CF.[15,16]

Pancreatic insufficiency is the most plausible mechanism of fat malabsorption; the chloride channel cystic fibrosis transmembrane conductance regulator (CFTR), which is present in enterocytes, does not appear to directly contribute to malabsorption. CF patients have variable and often poor plasma 25(OH)D responses to dietary vitamin D supplementation, which could be due in part to reduced bioavailability.[12-16] However, in studies administering parenteral vitamin D_2 and ultraviolet light (one study each), plasma 25(OH)D response was also markedly impaired.[7,17] In addition, higher than usual antibiotic doses are often required in CF patients, suggesting induction of metabolizing enzymes that may overlap with those which metabolize vitamin D.[18-21] Together, these findings suggest additional defects in vitamin D metabolism in CF patients. For full hormonal activity, 25(OH)D must be converted to the active vitamin D hormone, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). In addition, 25(OH)D can undergo a series of catabolic steps leading to its elimination (**Figure 1**).[22] The most common route of $25(OH)D_3$ catabolism begins with conversion to 24,25-dihydroxyvitamin D_3 by the cytochrome P450 enzyme CYP24A1. Drs. Lin and Thummel (Co-investigators) have discovered that $25(OH)D_3$ also undergoes hydroxylation at the 4-position and sulfation at the 3-O-position. The resulting metabolites, $4\beta,25(OH)_2D_3$ and $25(OH)D_3$-3-sulfate, respectively, are generated in enterocytes and hepatocytes by CYP3A4 and SULT2A1.[23,24] Vitamin D metabolic enzymes are regulated by parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF-23) as well as $1,25(OH)_2D$ itself. Our group has contributed to an expanding knowledge of dysregulated vitamin D metabolism in chronic kidney disease, in which both vitamin D activation and catabolism are impaired.[22,25] However, little is known about the vitamin D metabolic axis in CF in terms of vitamin D activation, vitamin D catabolism, or the hormonal response to vitamin D deficiency. Clearance of 25(OH)D may be affected in CF by altered enterohepatic function of CYP3A4 and SULT2A1. In addition, enterohepatic vitamin D circulation, in which sulfated 25-hydroxyvitamin D is secreted in bile and delivered to target enterocytes, may be disrupted, leading to loss of conjugated vitamin D in the stool and altered enteric metabolism. Small bowel bacterial overgrowth due to impaired gastrointestinal mobility may further reduce absorption of vitamin D. Finally, vitamin D binding protein (DBP) and albumin, which carry circulating vitamin D metabolites, may be lost in the urine.[26] Defining which of these potential metabolic defects is clinically relevant may lead to improved methods of vitamin D deficiency diagnosis and treatment.

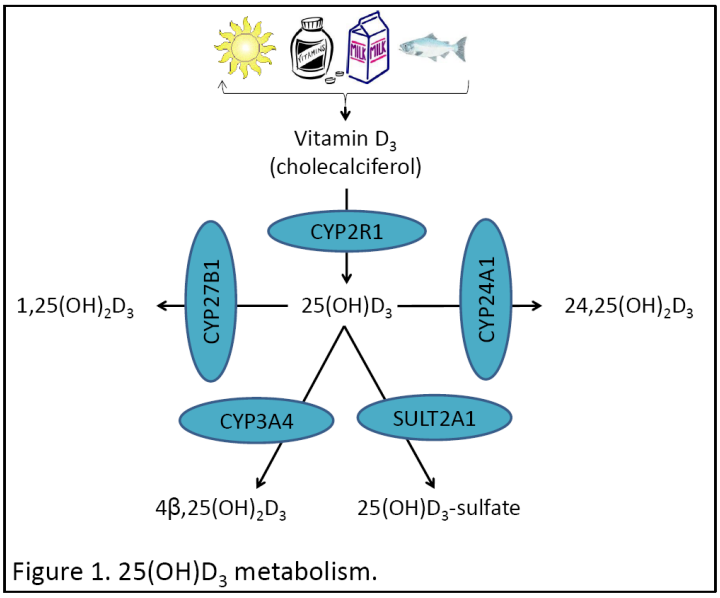

Figure 1. $25(OH)D_3$ metabolism.

Impaired vitamin D metabolism may have broad adverse health effects in cystic fibrosis. $1,25(OH)_2D_3$ binds to the intracellular vitamin D receptor to modulate transcription of genes involved in bone health, inflammation, and blood pressure. Vitamin D is a central determinant of calcium and phosphate absorption from gastrointestinal tract to provide mineral content for growing bones.[27] Moreover, vitamin D deficiency may directly impair muscle strength, thereby

indirectly reducing bone density. Monocytes metabolize vitamin D and require $1,25(OH)_2D_3$ for robust immune function. For example, within monocytes, autocrine metabolism of $25(OH)D_3$ to $1,25(OH)_2D_3$ by CYP27B1 is required for the expression of cathelicidin and resulting activity against *mycobacterium tuberculosis*.[28,29] Addition of $1,25(OH)_2D_3$ to cultured monocytes reduces expression of pro-inflammatory cytokines IL-1, IL-6, IL-8, and TNF-α, and promotes generation of regulatory Th2 lymphocytes. Disruption of the vitamin D receptor directly stimulates the renin-angiotensin-aldosterone system, promoting hypertension and arteriosclerosis. In large population studies, vitamin D deficiency, defined by lower circulating 25(OH)D concentrations, is associated with cardiovascular disease events, fractures, and cancer. The clinical consequences of vitamin D deficiency in CF patients are not yet clearly defined; however, bone disease and infections represent major clinical complications of CF that are plausibly influenced by vitamin D deficiency. A more detailed understanding of the relationships between vitamin D metabolism and bone disease and infections in CF may provide new opportunities to reduce these important clinical complications.

Multiple vitamin D interventions are widely available, but their proper use in CF awaits identification of specific vitamin D metabolic disturbances. Supplementation with vitamin D_3 or D_2 may be improved by administration of higher oral dosage programs or intramuscular injections. Ultraviolet light exposure has been used in small studies of CF patients and may augment concurrent vitamin D supplementation. If identified, cytochrome P-450 enzymes with altered activities in CF could be targeted for intervention. Finally, vitamin D receptor agonists ($1,25(OH)_2D$ and its analogues) are widely used in patients with kidney disease and could play a role in the treatment of vitamin D deficiency in CF.

2.1 Risk / Benefit Assessment

This study will not provide any direct benefit to the study subjects. Individual subjects may have a modestly reduced chance of adverse health events because their care will be monitored more closely than it might otherwise. Future patients may benefit from these studies by virtue of knowledge gained about the pathophysiology and ascertainment of impaired vitamin D metabolism.

The risks of administering deuterated $25(OH)D_3$ in tracer quantities are low. $25(OH)D_3$ is a naturally occurring substance and generally circulates in concentrations of 10-50 ng/mL. The deuterated $25(OH)D_3$ we propose to administer differs from the naturally occurring form only by the substitution of 6 hydrogen atoms with deuterium. This isotope is stable and has metabolic and biologic characteristics identical to the naturally occurring form. We will administer quantities intended to make small changes in total circulating $25(OH)D_3$ concentration, i.e., an increase of 5 ng/mL.

There is some risk of hypercalcemia with the administration $25(OH)D_3$, but this risk is minimized by excluding participants with baseline 25(OH)D > 50 ng/mL and by administering quantities that raise 25(OH)D by only approximately 5 ng/mL. We will monitor for hypercalcemia during our research. If instances of hypercalcemia are observed, we will change our study protocol accordingly to prevent hypercalcemia in future participants. This study also includes placement of peripheral intravenous catheters and blood draws. Risks include discomfort and minor bleeding associated with catheter placements and anemia due to blood sampling.

3 STUDY OBJECTIVES

3.1 Primary Objective

The goal of this study is to define 25(OH)D_3 catabolism in CF patients using gold standard pharmacokinetics studies. Specifically, we will evaluate the metabolic clearance of 25(OH)D_3 among participants with CF and matched control subjects. The goal of this work is to provide the first comprehensive characterization of vitamin D metabolism in CF patients and promote novel hypotheses for subsequent studies.

3.2 Secondary Objectives

- To explore the biochemical pathways through which 25(OH)D3 is metabolized, and the effects of CF on these pathways, by evaluating the appearance and disappearance of labeled metabolites of 25(OH)D3.
- To determine clinical and biochemical factors that are correlated with the metabolic clearance of 25(OH)D_3.
- To evaluate potential biomarkers of 25(OH)D_3
- To compare monocyte expression of vitamin D-related genes in CF patients *versus* healthy controls

4 STUDY DESIGN

This is a cross-sectional, observational study that uses the intravenous administration of stable isotope-labeled 25(OH)D_3 to evaluate the metabolic clearance of 25(OH)D3. It is a single-dose, open-label PK study of 25(OH)D_3.

We will recruit a total of up to 20 study subjects: 10 with and 10 without CF. Subjects who provide written informed consent and qualify at screening will receive a single dose of intravenous labeled 25(OH)D_3. Subsequent blood draws and urine collections will be used to determine the metabolic clearance of 25(OH)D_3 and related parameters.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

The primary outcome of interest is the metabolic clearance of labeled 25(OH)D_3.

We will measure serum concentrations of 24,25$(OH)_2D_3$, 25(OH)D_3, 25(OH)D_2, 1,25$(OH)_2D_3$ and 1,25$(OH)_2D_2$ at the University of Washington by mass spectrometry For each participant and time point, a single serum aliquot is used to measure this panel of vitamin D metabolites using by immunoaffinity extraction and HPLC-mass spectrometry (Xevo TQ, Waters Corp., Milford, MA) with deuterated internal standards.[25,30-32]

For each subject, non-compartmental analysis of plasma concentration versus time data will be performed using Phoenix software (Pharsight, Cary, NC). Clearance will be calculated as administered 25(OH)D3 dose divided by 25(OH)D3 AUC. We focus on clearance as our primary outcome because it reflects the metabolism of 25(OH)D_3 accounting for circulating 25(OH)D_3 concentration (units of volume/time, akin to creatinine clearance). Clearance is independent of volume of distribution, in contrast to $t_{1/2}$, which will be evaluated as a secondary outcome. Clearance will be evaluated with and without adjustment for body size.

5.2 Secondary Efficacy Endpoints

- AUC of labeled 25(OH)D_3
- Terminal half-life of labeled 25(OH)D_3
- Volume of distribution in the central compartment of labeled 25(OH)D_3
- Metabolic formation clearance (metabolite/parent AUC ratio) for metabolites of labeled 25(OH)D_3
- Monocyte transcription of CYP27B1, CYP24A1, VDR, and cathelicidin

5.3 Safety Evaluations

An abnormal clinical laboratory value will be documented as an adverse event if one of the following applies:

- The abnormality is not contradicted by a repeat test to confirm the abnormality.
- The abnormality suggests a disease and/or organ toxicity.
- The abnormality is of a degree that requires active management (e.g., requires a medication change, more frequent follow-up, or further diagnostic evaluation).

Change in clinical laboratory findings (if there are specific labs, then why they are appropriate to measure, e.g., BUN or Creatinine for an aminoglycoside)

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

A serious adverse event is any AE that is:

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

Important medical events are those that do not fit the other definitions of serious adverse events, but the event may jeopardize the patient and may require treatment to prevent one of the listed serious adverse events.

Adverse events will be reported if they occur between the time of informed consent and 30 days after the last study visit. All unresolved adverse events will be followed by the PI until resolution, the adverse event is otherwise explained, or the participant is lost to follow-up. At the last study visit, the investigator will instruct each participant to report any subsequent event that

the participant, or participant's personal physician, reasonably believes may be related to the study. The investigator will notify the study sponsor of any death or adverse event occurring after the participant has discontinued participation if the event can be reasonably related to the study.

6 SUBJECT SELECTION

6.1 Study Population

We will target enrollment of 10 patients with CF and 10 healthy controls matched on age, gender, and race. To recruit the 10 CF patients, we will first turn to observational studies of CF for which participants have already granted consent for contact for additional research studies. If sufficient participants are not identified through this route, we will recruit directly from the University of Washington CF clinic. The 10 matched healthy controls will be drawn from the Healthy Kidney Study. Clinical data and contact information for these control subjects are contained in the Kidney Research Institute biorepository.

6.2 Inclusion Criteria

We will study only adults (Table 6.2). We will limit to a range of total serum 25(OH)D.

Table 6.2. Participant Eligibility – Inclusion criteria
Age ≥ 18 years
Serum 25(OH)D concentration 10 – 50ng/ml

6.3 Exclusion Criteria

Exclusion criteria will be inability to give informed consent, pregnancy, active hepatitis or cirrhosis, hemoglobin <10mg/dL, serum calcium >10mg/dL, use of ergocalciferol or cholecalciferol (> 400 IU/day) within 3 months, use of active vitamin D receptor agonists (e.g. calcitriol) or cinacalcet within 4 weeks, or use of a cytochrome P-450 (CYP) inhibitor or inducer within 4 weeks (table 2 and table 3). If people do not meet eligibility criteria based on medication use, they will be allowed to participate in the study after an appropriate washout period if their primary physician agrees. For ergocalciferol and cholecalciferol, the washout period will be 3 months. For active vitamin D compounds, cinacalcet, and CYP inhibitors and inducers the washout period will be 4 weeks.

Table 6.3. Patient Eligibility – Exclusion criteria
Ergocalciferol in last 3 months, cholecalciferol >400IU/day in last 3 months, active vitamin D receptor agonist (e.g. calcitriol) or cinacalcet in last 4 weeks,
Active hepatitis or cirrhosis
Hemoglobin < 10mg/dl
Serum calcium concentration > 10.1 mg/dl

Pregnancy
Inability to give informed consent
Medications known to strongly induce or suppress the CYP enzymes which metabolize vitamin D (see example list below)

Table 6.4. CYP inhibitors and inducers

CYP Inhibitors		CYP Inducers
Amprenavir	Imatinib	Avasimibe
Aprepitant	Indinavir	Bosentan
Atazanivir	Itraconazole	Carbamazepine
Casopitant	Ketoconazole	Efavirenz
Cimetidine	Lopinavir	Etravirine
Ciprofloxacin	Nefazodone	Modafinil
Clarithromycin	Nelfinavir	Nafcillin
Conivaptan	Posaconazole	Phenobarbital
Darunavir	Ritonavir	Phenytoin
Diltiazem	Saquinavir	Rifabutin
Dronedarone	Shisandra	Rifampin
Elvitegravir	Telithromycin	St. John's Wort
Erythromycin	Tipranavir	
Fluconazole	Verapamil	
Grapefruit Juice	Voriconazole	

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies. Specific allowed and disallowed medications are detailed in Section 6 (Eligibility Criteria).

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

All consenting and qualified participants will be assigned to the single treatment arm.

8.2 Blinding

Neither participants nor study investigators will be blinded to the study intervention.

8.3 Formulation of Test and Control Products

We will administer labeled 25(OH)D_3 intravenously. Deuterated 25-dihydroxyvitamin D_3 (26, 26, 26, 27, 27, 27 – d6) will be manufactured under Good Manufacturing Practice by the Sigma-Aldrich ISOTEC Stable Isotope Division (Miamisburg, OH). The deuterated 25(OH)D_3 produced by Sigma-Aldrich will be provided in solid form and formulated for IV administration by the University of Iowa Pharmaceuticals.

8.3.1 Formulation of Test Product

The labeled 25(OH)D_3 prepared by Sigma-Aldrich will be formulated in a co-solvent of ethanol (10%) and propylene glycol (40%) by University of Iowa Pharmaceuticals. The formulated drug product will be aliquoted into single-use vials and frozen for storage.

8.3.2 Packaging and Labeling

Individual doses of formulated 25(OH)D_3 will be prepared for intravenous administration by the University of Washington Investigational Drug Services. The prepared dose of 25(OH)D_3 will be calculated according to body size and labeled with the participant's full name, date of birth, and medical record number along with the drug contents.

8.4 Supply of Study Drug at the Site

Single-use vials of formulated 25(OH)D_3 will be stored at the University of Washington Investigational Drug Services. Additional vials of formulated 25(OH)D_3 will be kept at University of Iowa Pharmaceuticals for stability testing.

8.4.1 Dosage/Dosage Regimen

Each participant will receive a single intravenous dose of deuterated 25(OH)D_3. We will use body size-based dosing to target a peak achieved deuterated 25(OH)D_3 concentration of 5 ng/mL. The target of 5 ng/mL was selected because it will allow precise tracking of circulating deuterated 25(OH)D_3 concentration throughout follow-up (based on known limits of detection for our assay) without substantially perturbing underlying vitamin D status. Theoretically, the dose required to achieve a 5 ng/mL increment in deuterated 25(OH)D_3 concentration can be calculated as the targeted peak concentration multiplied by the volume of distribution, which for 25(OH)D_3 is expected to be equal to blood volume. We will use the formula of Nadler et al (1962) to estimate blood volume for each participant. We will begin the study by administering to each participant a dose of 5 ng/mL x estimated blood volume. A typical dose will be 25-30 mcg. We will monitor achieved 25(OH)D_3 concentrations real time. If our initial approach fails to achieve peak 25(OH)D3 concentrations near 5 ng/mL, we will revise our approach for future participants, as indicated. For example, if mean peak achieved 25(OH)D_3 concentrations are near 2.5 ng/mL with our initial approach, we will increase our dose by a factor of two. We will not exceed a dose of 100 mcg for any participant.

8.4.2 Dispensing

All study drug will be dispensed by the University of Washington Investigational Drug Services.

8.4.3 Administration Instructions

The study drug will be administered intravenously on a single occasion over a period of 5 minutes.

8.5 Supply of Study Drug at the Site

Single-use vials of formulated 25(OH)D_3 will be stored at the University of Washington Investigational Drug Services. The vials will be labeled as investigational product by the University of Iowa Pharmaceuticals, the manufacturer of formulated product.

8.5.1 Storage

Study drug will be stored at a temperature <-60°C at the University of Washington Investigational Drug Services.

8.6 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an ongoing basis by the University of Washington Investigational Drug Services. The number of study drug dispensed and returned by the subject will be recorded on the Investigational Drug Accountability Record.

8.7 Measures of Treatment Compliance

Each study drug administration will be directly observed by study staff.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is provided in Section 10 (Evaluations by Visit) below and diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject or subject's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Screening, Baseline (Study Day 0), and the final Study Visit (Study Visit 10). Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

9.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at Screening.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent history, and information regarding underlying diseases will be recorded at Baseline (Study Day 0).

9.1.4 Physical Examination

Height and weight will be measured as the Screening visit and at Baseline (Study Day 0). When prompted by reports or suspicion of potential adverse effects during follow-up, physical exam findings will be documented by qualified staff (MD, NP, RN, or PA) and will be followed by a physician or other qualified staff immediately or at the next scheduled visit, as indicated.

9.1.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be measured after resting for 5 minutes at Baseline (Study Day 0), prior to administration of study drug.

9.1.6 Other Clinical Procedures

Physical activity will be ascertained by questionnaire and accelerometry between Study days 1 and 7. Vitamin D intake will be estimated by food frequency questionnaire at Baseline. Serum and plasma will be collected at Baseline to measure kidney and liver function, calcium and phosphorus, parathyroid hormone, fibroblast growth factor-23, and vitamin D binding protein. Whole blood will also be collected at baseline to isolate monocytes for RNA expression quantification. Two 24-hour urine collections will be obtained, immediately preceding and following deuterated $25(OH)D_3$ administration, to quantify urinary excretion of albumin and the metabolites of deuterated $25(OH)D_3$, respectively. Adiposity will be measured by Dual energy X-ray absorptiometry at the baseline visit.

9.1.7 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

9.2 Clinical Laboratory Measurements

9.2.1 Hematology

A complete blood count will be obtained at the Screening Visit.

9.2.2 Blood Chemistry Profile

Serum sodium, potassium, chloride, bicarbonate, random glucose, BUN, creatinine, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), albumin, calcium, and phosphorous will be obtained at the Screening Visit.

Repeat serum sodium, potassium, chloride, bicarbonate, random glucose, BUN, creatinine, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), albumin, calcium, and phosphorous will be obtained at Baseline and Study Day 7.

Intact parathyroid hormone (PTH) will be measured at Visits 2 and 5 (Day 0 and 7 gauged from administration of deuterated 25(OH)D3).

9.2.3 Pregnancy Test

A urine pregnancy test will be obtained from female subjects who are of childbearing age at the Baseline Visit prior to administration of the study drug.

9.3 Pharmacokinetic Measurements

Blood for determination of serum concentrations of deuterated $25(OH)D_3$ and its metabolites will be collected at Study Visits 2-10.

At Baseline, blood will be drawn prior to administration of study drug as well as 5 minutes and 4 hours after administration.

9.4 Research Laboratory Measurements

Novel products of $25(OH)D_3$ catabolism, including $4\beta,25(OH)_2D_3$ and $25(OH)D_3$-3-sulfate will be measured using LC-MS/MS, as previously described.[33] $25(OH)D_3$-3-sulfate will be extracted from plasma.

10 EVALUATIONS BY VISIT

10.1 Screening Visit (Study Visit 1)

The following activities will occur during the screening visit:

- Collect signed consent form
- Perform focused medical history restricted to study eligibility criteria
- Complete medication inventory through EMR and patient report.
- Record height and weight of patient
- Collect non-fasting blood sample for measurement of complete blood count and total 25(OH)D.
- Collect non-fasting blood sample for serum sodium, potassium, chloride, bicarbonate, random glucose, BUN, creatinine, AST/SGOT, ALT/SGPT, albumin, calcium, and phosphorous.
- Collect urine for measurement of albumin and creatinine (except for hemodialysis patients).
- Collect urine for pregnancy test for women of childbearing potential.
- Participants who meet eligibility criteria at the screening visit will be invited to return for the remaining study visits.
- Provide selected subjects with supplies required for first 24-hour urine collection

10.2 Baseline Visit (Study Visit 2, Day 0)

Visit 2 will take place at the University of Washington Clinical Research Center (CRC) or a suitable alternate facility and will last approximately 5 hours. Visit 2 will take place no more than 90 days after Visit 1. If more than 90 days elapse between Visits 1 and 2, screening procedures may be repeated to verify continued eligibility. The following activities will occur during the baseline visit:

- Perform a complete medical history

- Review current medications.
- Complete physical activity and food frequency questionnaires.
- Collect blood **prior to administration of study drug** for measurement of kidney and liver function, basic chemistries, vitamin D metabolites, parathyroid hormone (PTH), and other measurements related to vitamin D metabolism and for isolation of monocytes for RNA expression analyses.
- Collect urine sample from first 24-hour collection period. Sample will be used for measurement of albumin, creatinine, vitamin D binding protein, and vitamin D metabolites:
- Perform dual energy x-ray absorptiometry (DEXA) to measure adiposity.
- Administer deuterated 25(OH)D_3 intravenously as a 5 mL IV bolus.
- Collect blood 5 minutes and 4 hours post-infusion for measurement of vitamin D metabolites.
- Distribute supplies for second 24-hour urine collection.

10.3 Study Visit 3 (Day 1)

This visit is expected to last less than one hour. The following activities will occur during Visit 3:

- Perform an abbreviated medical history
- Collect urine sample from second 24-hour collection period.
- Collect blood for measurement of serum vitamin D metabolite concentrations.
- Distribute Accelerometer for measurement of physical activity

10.4 Study Visit 4 (Day 4)

This visit is expected to last less than one hour. The following activities will occur during the Visit 4:

- Perform an abbreviated medical history
- Collect blood for measurement of serum vitamin D metabolite concentrations.

10.5 Study Visit 5 (Day 7)

This visit is expected to last less than one hour. Clinical activities will occur in the following order:

- Perform an abbreviated medical history
- Collect blood for measurement of serum vitamin D metabolite concentrations.
- Collect blood for serum sodium, potassium, chloride, bicarbonate, random glucose, BUN, creatinine, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), albumin, calcium, and phosphorous at Study Visit 5 (Day 7) to monitor study safety.
- Collect Accelerometer

10.6 Study Visits 6-10 (Day 14, 21, 28, 42, and 56)

Each of these visits is expected to last less than one hour. Clinical activities will occur in the following order:

- Perform an abbreviated medical history
- Collect blood for measurement of serum vitamin D metabolite concentrations.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

11.1.1 AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 11.1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 11.1 AE Severity Grading

Severity (Toxicity Grade)	**Description**
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

11.1.2 AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 11.2.

Table 11.2 AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

A serious adverse event is any AE that is:

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

Important medical events are those that do not fit the other definitions of serious adverse events, but the event may jeopardize the patient and may require treatment to prevent one of the listed serious adverse events.

Adverse events will be collected if they occur between the time of informed consent and up to 30 days after the last study visit. All unresolved adverse events will be followed by the PI until resolution, the adverse event is otherwise explained, or the participant is lost to follow-up. At the last study visit, the investigator will instruct each participant to report any subsequent event that the participant, or participant's personal physician, reasonably believes may be related to the study. The investigator will notify the study sponsor of any death or adverse event occurring after the participant has discontinued participation if the event can be reasonably related to the study.

11.3 Abnormal lab values

An abnormal clinical laboratory value will be documented as an adverse event if one of the following applies:

1. The abnormality is not contradicted by a repeat test to confirm the abnormality.
2. The abnormality suggests a disease and/or organ toxicity.
3. The abnormality is of a degree that requires active management (e.g., requires a medication change, more frequent follow-up, or further diagnostic evaluation).

11.4 Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery will be documented as an adverse event if the condition meets the criteria for and adverse event. Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

1. Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
2. Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
3. Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

11.2.1 Serious Adverse Experience Reporting

The PI will document all SAEs that occur (whether or not related to study drug) on an SAE Report Form. The collection period for all SAEs will begin after informed consent is obtained until all procedures for the final study visit have been completed.

All SAE Report Forms will be reviewed by the PI. SAE reports that are both related and unexpected will be sent to FDA within one business day of learning of the event. SAE reports that are both related and unexpected will also be forwarded to the UW Institutional Review Board, in accordance with UW Standard Operating Procedures. Because this is an observational

study, no data safety and monitoring board will be created for this trial. Adverse events and recruitment will be monitored by the PI and reported to the UW IRB, as described above.

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent
- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up
- Sponsor request for early termination of study
- Positive pregnancy test (females)

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents Refer to Section 10 for early termination procedures.

12.3 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Subjects who withdraw after Visit 2 but prior to Visit 10 will be encouraged to come in for a final visit (and the procedures to be followed would include those for their next scheduled visit).

12.4 Replacement of Subjects

Subjects who withdraw from the study treatment will be replaced.

Subjects who withdraw from the study will be replaced.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject or Sponsor-Investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication
- Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor-Investigator will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the Sponsor-Investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor-Investigator. A copy of the form will be maintained in the regulatory binder and in the Sponsor-Investigator's files.

14 STATISTICAL METHODS AND CONSIDERATIONS

14.1 Data Sets Analyzed

All participants who receive the study drug (deuterated vitamin D) will be included in the safety analysis.

14.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be summarized by CKD status and race: age, gender, medical comorbidities, medications used, height, weight, body mass index, laboratory values (including calcium, phosphorus, albumin, vitamin D binding protein, parathyroid hormone, fibroblast growth factor-23), and adiposity measured by DXA.

14.3 Analysis of Primary Endpoint

We will examine the distribution of 25(OH)D_3 clearance by CF status using box plots and summary statistics. We will explore the distribution of 25(OH)D_3 clearance by key covariates (age, gender, and baseline concentrations of 25(OH)D, PTH, FGF-23, and vitamin D binding protein) using locally weighted scatterplot smoothing (LOWESS), cubic splines, and box plots. We will pay particular attention to the associations of PTH and FGF-23 with 25(OH)D_3 clearance, including multivariable modeling to determine independent associations. We will test associations of CF status with 25(OH)D_3 clearance (continuous outcome variable) using linear regression. Models will be adjusted for age, gender, body size, baseline 25(OH)D concentration, and other covariates strongly related to 25(OH)D_3 clearance.

14.4 Analysis of Secondary Endpoints

Analyses of secondary endpoints will parallel those of the primary endpoint.

14.5 Interim Analysis

This is not a clinical trial with an efficacy outcome, and it is neither randomized nor blinded. No formal interim analyses are planned.

14.6 Sample Size and Randomization

For this descriptive study, our sample size of 20 was based on feasibility and funding considerations.

15 DATA COLLECTION, RETENTION AND MONITORING

15.1 Data Collection Instruments

The PI will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug. Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a subject number. The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator.

15.2 Data Management Procedures

The data will be entered into a validated database. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

15.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Queries are entered, tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

15.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

15.5 Availability and Retention of Investigational Records

The PI will make study data accessible to the UW monitor, other authorized representatives of the University of Washington IRB, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject will be maintained that includes the signed Informed Consent and HIPAA Authorization and copies of all source documentation related to that subject. The Sponsor-Investigator will ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) will be maintained for at least two years after the study is completed.

15.6 Monitoring

Study Monitoring Plan. This study will be monitored according to the safety guidelines outlined above. The PI will allocate adequate time for such monitoring activities. The PI will also ensure that any compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. diagnostic laboratory), and has adequate space to conduct a monitoring visit, if requested.

Monitoring visits will be conducted by representatives of the University of Washington according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6).

15.7 Subject Confidentiality

In order to maintain subject confidentiality, only a subject number will identify all study subjects on CRFs and other documentation submitted to the Sponsor.

16 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

16.1 Protocol Amendments

Any amendment to the protocol will be written by the Investigator-Sponsor. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

16.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the University of Washington Institutional Review Board prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB"s written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRBs unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

16.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/IEC. The consent form generated by the Sponsor-Investigator must be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects must be given ample opportunity to inquire about details of the study. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject and the original will be maintained with the subject's records.

16.4 Publications

The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

16.5 References

1. Vicchio D, Yergey A, O'Brien K, Allen L, Ray R, Holick M. Quantification and kinetics of 25-hydroxyvitamin D3 by isotope dilution liquid chromatography/thermospray mass spectrometry. Biol Mass Spectrom. 1993;22(1):53-8. doi: 10.1002/bms.1200220107. PubMed PMID: 8431502.

2. Compston JE, Merrett AL, Ledger JE, Creamer B. Faecal tritium excretion after intravenous administration of 3H-25-hydroxyvitamin D3 in control subjects and in patients with malabsorption. Gut. 1982;23(4):310-5. PubMed PMID: 7076008; PubMed Central PMCID: PMCPMC1419735.

3. Davies M, Heys SE, Selby PL, Berry JL, Mawer EB. Increased catabolism of 25-hydroxyvitamin D in patients with partial gastrectomy and elevated 1,25-dihydroxyvitamin D levels. Implications for metabolic bone disease. J Clin Endocrinol Metab. 1997;82(1):209-12. PubMed PMID: 8989260.

4. Batchelor AJ, Compston JE. Reduced plasma half-life of radio-labelled 25-hydroxyvitamin D3 in subjects receiving a high-fibre diet. Br J Nutr. 1983;49(2):213-6. doi: S0007114583000318 [pii]. PubMed PMID: 6299329.

5. Gray RW, Weber HP, Dominguez JH, Lemann J. The metabolism of vitamin D3 and 25-hydroxyvitamin D3 in normal and anephric humans. J Clin Endocrinol Metab. 1974;39(6):1045-56. PubMed PMID: 4372244.

6. Speakman JR. The history and theory of the doubly labeled water technique. Am J Clin Nutr. 1998;68(4):932S-8S. PubMed PMID: 9771875.

7. Mailhot G. Vitamin D bioavailability in cystic fibrosis: a cause for concern? Nutrition reviews. 2012;70(5):280-93. doi: 10.1111/j.1753-4887.2012.00471.x. PubMed PMID: 22537214.

8. Rosen CJ. Clinical practice. Vitamin D insufficiency. The New England journal of medicine. 2011;364(3):248-54. Epub 2011/01/21. doi: 10.1056/NEJMcp1009570. PubMed PMID: 21247315.

9. Rovner AJ, Stallings VA, Schall JI, Leonard MB, Zemel BS. Vitamin D insufficiency in children, adolescents, and young adults with cystic fibrosis despite routine oral supplementation. Am J Clin Nutr. 2007;86(6):1694-9. PubMed PMID: 18065588.

10. Wolfenden LL, Judd SE, Shah R, Sanyal R, Ziegler TR, Tangpricha V. Vitamin D and bone health in adults with cystic fibrosis. Clin Endocrinol (Oxf). 2008;69(3):374-81. doi: 10.1111/j.1365-2265.2008.03216.x. PubMed PMID: 18284636; PubMed Central PMCID: PMC2851223.

11. Stephenson A, Brotherwood M, Robert R, Atenafu E, Corey M, Tullis E. Cholecalciferol significantly increases 25-hydroxyvitamin D concentrations in adults with cystic fibrosis. Am J Clin Nutr. 2007;85(5):1307-11. PubMed PMID: 17490967.

12. Boyle MP, Noschese ML, Watts SL, Davis ME, Stenner SE, Lechtzin N. Failure of high-dose ergocalciferol to correct vitamin D deficiency in adults with cystic fibrosis. Am J Respir Crit Care Med. 2005;172(2):212-7. doi: 10.1164/rccm.200403-387OC. PubMed PMID: 15860755; PubMed Central PMCID: PMC2718468.

13. West NE, Lechtzin N, Merlo CA, Turowski JB, Davis ME, Ramsay MZ, Watts SL, Stenner SP, Boyle MP. Appropriate goal level for 25-hydroxyvitamin D in cystic fibrosis. Chest. 2011;140(2):469-74. Epub 2011/03/12. doi: 10.1378/chest.10-2114. PubMed PMID: 21393390.

14. Green DM, Leonard AR, Paranjape SM, Rosenstein BJ, Zeitlin PL, Mogayzel PJ, Jr. Transient effectiveness of vitamin D2 therapy in pediatric cystic fibrosis patients. J Cyst Fibros. 2010;9(2):143-9. Epub 2010/02/02. doi: 10.1016/j.jcf.2010.01.002. PubMed PMID: 20117062.

15. Lo CW, Paris PW, Clemens TL, Nolan J, Holick MF. Vitamin D absorption in healthy subjects and in patients with intestinal malabsorption syndromes. Am J Clin Nutr. 1985;42(4):644-9. PubMed PMID: 4050723.

16. Lark RK, Lester GE, Ontjes DA, Blackwood AD, Hollis BW, Hensler MM, Aris RM. Diminished and erratic absorption of ergocalciferol in adult cystic fibrosis patients. Am J Clin Nutr. 2001;73(3):602-6. PubMed PMID: 11237938.

17. Khazai NB, Judd SE, Jeng L, Wolfenden LL, Stecenko A, Ziegler TR, Tangpricha V. Treatment and prevention of vitamin D insufficiency in cystic fibrosis patients: comparative efficacy of ergocalciferol, cholecalciferol, and UV light. J Clin Endocrinol Metab. 2009;94(6):2037-43. doi: 10.1210/jc.2008-2012. PubMed PMID: 19336509; PubMed Central PMCID: PMC2690417.

18. Hutabarat RM, Smith AL, Unadkat JD. Disposition of drugs in cystic fibrosis. VII. Acetylation of sulfamethoxazole in blood cells: in vitro-in vivo correlation and characterization of its kinetics of acetylation in lymphocytes. Clin Pharmacol Ther. 1994;55(4):427-33. Epub 1994/04/01. PubMed PMID: 8162669.

19. Hutabarat RM, Unadkat JD, Kushmerick P, Aitken ML, Slattery JT, Smith AL. Disposition of drugs in cystic fibrosis. III. Acetaminophen. Clin Pharmacol Ther. 1991;50(6):695-701. Epub 1991/12/01. PubMed PMID: 1752114.

20. Hutabarat RM, Unadkat JD, Sahajwalla C, McNamara S, Ramsey B, Smith AL. Disposition of drugs in cystic fibrosis. I. Sulfamethoxazole and trimethoprim. Clin Pharmacol Ther. 1991;49(4):402-9. Epub 1991/04/01. PubMed PMID: 2015729.

21. Wang JP, Unadkat JD, al-Habet SM, O'Sullivan TA, Williams-Warren J, Smith AL, Ramsey B. Disposition of drugs in cystic fibrosis. IV. Mechanisms for enhanced renal clearance of ticarcillin. Clin Pharmacol Ther. 1993;54(3):293-302. Epub 1993/09/01. PubMed PMID: 8375124.

22. Bosworth C, de Boer IH. Impaired vitamin D metabolism in CKD. Seminars in nephrology. 2013;33(2):158-68. Epub 2013/03/08. doi: 10.1016/j.semnephrol.2012.12.016. PubMed PMID: 23465502; PubMed Central PMCID: PMC3592201.

23. Wang Z, Lin YS, Dickmann LJ, Poulton EJ, Eaton DL, Lampe JW, Shen DD, Davis CL, Shuhart MC, Thummel KE. Enhancement of hepatic 4-hydroxylation of 25-hydroxyvitamin D3 through CYP3A4 induction in vitro and in vivo: implications for drug-induced osteomalacia. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2013;28(5):1101-16. Epub 2012/12/06. doi: 10.1002/jbmr.1839. PubMed PMID: 23212742; PubMed Central PMCID: PMC3609874.

24. Wang Z, Schuetz EG, Xu Y, Thummel KE. Interplay between vitamin D and the drug metabolizing enzyme CYP3A4. The Journal of steroid biochemistry and molecular biology. 2013;136:54-8. Epub 2012/09/19. doi: 10.1016/j.jsbmb.2012.09.012. PubMed PMID: 22985909; PubMed Central PMCID: PMC3549031.

25. Bosworth CR, Levin G, Robinson-Cohen C, Hoofnagle AN, Ruzinski J, Young B, Schwartz SM, Himmelfarb J, Kestenbaum B, de Boer IH. The serum 24,25-dihydroxyvitamin D concentration, a marker of vitamin D catabolism, is reduced in chronic kidney disease. Kidney international. 2012;82(6):693-700. Epub 2012/06/01. doi: 10.1038/ki.2012.193. PubMed PMID: 22648296; PubMed Central PMCID: PMC3434313.

26. Thrailkill KM, Jo CH, Cockrell GE, Moreau CS, Fowlkes JL. Enhanced excretion of vitamin D binding protein in type 1 diabetes: a role in vitamin D deficiency? The Journal of clinical endocrinology and metabolism. 2011;96(1):142-9. Epub 2010/10/15. doi: 10.1210/jc.2010-0980. PubMed PMID: 20943786; PubMed Central PMCID: PMC3038488.

27. Ott SM, Aitken ML. Osteoporosis in patients with cystic fibrosis. Clin Chest Med. 1998;19(3):555-67. Epub 1998/10/06. PubMed PMID: 9759556.

28. Fabri M, Stenger S, Shin DM, Yuk JM, Liu PT, Realegeno S, Lee HM, Krutzik SR, Schenk M, Sieling PA, Teles R, Montoya D, Iyer SS, Bruns H, Lewinsohn DM, Hollis BW, Hewison M, Adams JS, Steinmeyer A, Zugel U, Cheng G, Jo EK, Bloom BR, Modlin RL. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. Sci Transl Med. 2011;3(104):104ra2. Epub 2011/10/15. doi: 10.1126/scitranslmed.3003045. PubMed PMID: 21998409; PubMed Central PMCID: PMC3269210.

29. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zugel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science (New York, NY. 2006;311(5768):1770-3. PubMed PMID: 16497887.

30. Laha TJ, Strathmann FG, Wang Z, de Boer IH, Thummel KE, Hoofnagle AN. Characterizing antibody cross-reactivity for immunoaffinity purification of analytes prior to multiplexed liquid chromatography-tandem mass spectrometry. Clin Chem. 2012;58(12):1711-6. doi: 10.1373/clinchem.2012.185827. PubMed PMID: 22968104; PubMed Central PMCID: PMC3731945.

31. Strathmann FG, Laha TJ, Hoofnagle AN. Quantification of 1alpha,25-dihydroxy vitamin D by immunoextraction and liquid chromatography-tandem mass spectrometry. Clinical Chemistry. 2011;57(9):1279-85. Epub 2011/07/20. doi: 10.1373/clinchem.2010.161174. PubMed PMID: 21768219; PubMed Central PMCID: PMC3261234.

32. de Boer IH, Sachs MC, Chonchol M, Himmelfarb J, Hoofnagle AN, Ix JH, Kremsdorf RA, Lin YS, Mehrotra R, Robinson-Cohen C, Siscovick DS, Steffes MW, Thummel KE, Tracy RP, Wang Z, Kestenbaum B. Estimated GFR and circulating 24,25-dihydroxyvitamin D3 concentration: a participant-level analysis of 5 cohort studies and clinical trials. Am J Kidney Dis. 2014;64(2):187-97. doi: 10.1053/j.ajkd.2014.02.015. PubMed PMID: 24703961; PubMed Central PMCID: PMC4111986.

33. Wang Z, Senn T, Kalhorn T, Zheng XE, Zheng S, Davis CL, Hebert MF, Lin YS, Thummel KE. Simultaneous measurement of plasma vitamin D(3) metabolites, including 4beta,25-dihydroxyvitamin D(3), using liquid chromatography-tandem mass spectrometry. Anal

Biochem. 2011;418(1):126-33. Epub 2011/07/26. doi: 10.1016/j.ab.2011.06.043. PubMed PMID: 21784054; PubMed Central PMCID: PMC3164754.

APPENDIX 1. SCHEDULE OF STUDY PROCEDURES

Procedure	VISIT 1 SCREENING	VISIT 2 BASELINE (DAY 0)	VISIT 3 DAY 1	VISIT 4 DAY 4	VISIT 5 DAY 7	VISIT 6 DAY 14	VISIT 7 DAY 21	VISIT 8 DAY 28	VISIT 9 DAY 42	VISIT 10 DAY 56
Informed Consent	X									
Focused Medical History	X		X	X	X	X	X	X	X	X
Medical History		X								
Physical Exam and Vital Signs	X	X								
Medications Inventory	X	X								X
Blood Samples*	X	X	X	X	X	X	X	X	X	X
Urine collections	X									
24-hour urine collections		X	X							
DNA collection		X								
Physical activity and food frequency questionnaires		X								
Accelerometer			X	X	X					
DXA		X								
Study Drug Administration		X								

*Blood Draws Detailed in Appendix 2.

APPENDIX 2. DRAFT BLOOD DRAW TYPES AND VOLUMES

Blood sample	VISIT 1 SCREENING	VISIT 2 BASELINE (DAY 0)	VISIT 3 DAY 1	VISIT 4 DAY 4	VISIT 5 DAY 7	VISIT 6 DAY 14	VISIT 7 DAY 21	VISIT 8 DAY 28	VISIT 9 DAY 42	VISIT 10 DAY 56	TOTAL VOLUME (ML)
Clinical lab: CBC (3 mL EDTA-plasma [purple top])	**X**										**3**
Clinical lab: chemistries (3 mL serum [lime green top, PST])	**X**	**X**			**X**						**9**
Clinical lab: total 25(OH)D (3 mL serum [lime green top, PST])	**X**										**3**
Clinical lab: PTH*		**X**			**X**						**0***
Vitamin D metabolites (10 mL serum [red top])		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**90**
Stored blood (10 mL serum [red top], 20 mL EDTA-plasma [purple top], 4 mL citrate-plasma [blue top])		**X**									**34**
DNA**		**X**									**0****
RNA		**3**									
Total volume (mL)	**9**	**50**	**10**	**10**	**13**	**10**	**10**	**10**	**10**	**10**	**142**

Notes: * PTH measured with chemistries, requires no additional volume; **DNA extracted from EDTA plasma vacutainers, requires no additional volume