Protocol #: 22-0187
Project Title: Targeting Leukotrienes in Kidney Disease: A Pilot Study
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Version Date: 9/23/2022

I. **Hypotheses and Specific Aims**: Chronic kidney disease (CKD) is highly prevalent in the United States and is associated with significant morbidity. Diabetic kidney disease (DKD), is pathologically characterized by renal fibrosis, tubular epithelial atrophy, and intra-renal inflammation. Progression of DKD to end stage kidney disease (ESKD) leads to a dramatic worsening in patients' quality of life as well to a high rate of cardiovascular disease (CVD) and mortality. Due to the high disease burden of DKD, therapies that prevent kidney disease progression are urgently needed. Equally important are therapies that improve cardiovascular risk, the leading cause of mortality, in patients with DKD.

Currently approved therapies to prevent DKD progression include agents that block the renin angiotensin aldosterone system (RAAS) and sodium-glucose cotransport 2 inhibitors (SGLT2i). While both RAAS antagonists and SGLT2i are effective treatments for patients with DKD, residual risks for CKD progression remain. Additionally, SGLT inhibitors may not be safe in all DKD groups. **Hence, identifying new treatments for CKD to be used alone or in combination with other therapies is a high priority**. Since inflammation is a unifying pathway in most forms of CKD, targeting intrarenal macrophages and lymphocytes may represent a promising target. Experimental CKD models have shown that macrophages are crucial mediators of renal epithelial damage, fibroblast activation, and CKD progression.

Our earlier studies have shown that macrophages promote tubular damage and renal fibrosis through activation of the **5-lipoxygenase (5-LO) pathway**. The 5-LO pathway is responsible for the synthesis of pro-inflammatory lipid mediators, known as leukotrienes. Leukotrienes have been implicated in the etiology of CKD and CVD. We showed that leukotrienes promote chronic inflammation and maladaptive renal tubular repair in several mouse models of renal fibrosis. We have recently found that short term inhibition of cysteinyl leukotrienes with montelukast (a leukotriene antagonist) in mice with acute kidney injury potently prevents CKD. This is associated with decreased chronic inflammation and improved tubuloepithelial repair. Leukotrienes also cause endothelial dysfunction which increases glomerular permeability to albumin and causes vascular dysfunction leading to CVD. Short-term treatment with a 5-LO inhibitor reduces proteinuria in patients with glomerulonephritis. Additionally, montelukast use is associated with reduced cardiovascular events in the general population. **However, to date, no interventional studies have examined the effect of montelukast on kidney or cardiovascular outcomes in patients with DKD**. Montelukast is currently used for asthma treatment and has an excellent safety profile for long-term use in both pediatric and adult patients, making it an attractive and safe option for chronic therapy in patients with DKD.

Our overall hypothesis is that cysteinyl leukotrienes promote DKD progression and vascular disease by increasing inflammation, tubular damage, endothelial dysfunction and

fibrosis. We will conduct a prospective, open-label pilot clinical trial examining the effect of oral montelukast for 3 months in 20 patients with DKD (eGFR 30-59 ml/min/1.73m^2) with urine albumin to creatinine ratio of 200-5000 mg/g and use of a RAAS inhibitor (may also be on stable dose of SGLT2i) to test the following aims:

Hypothesis 1: Treatment with montelukast will reduce proteinuria in patients with DKD.
Specific Aim One: To determine changes in 24-hour urine proteinuria before and after 3 months of montelukast therapy.

Hypothesis 2: Treatment with montelukast will improve vascular endothelial function and arterial stiffness in patients with DKD.
Specific Aim 2: To determine vascular endothelial function (measured by EndoPat) and large elastic artery stiffness (measured by the gold standard aortic pulse wave velocity) before and after 3 months of montelukast therapy.

Hypothesis 3: Treatment with montelukast will reduce kidney and vascular inflammation in patients with DKD.
Specific Aim 3: To determine kidney macrophage infiltration (measured by urinary sCD163) and cytokine production (measured by urinary MCP-1 and plasma TNFR-1) before and after 3 months of montelukast therapy.

This novel study will be conducted by experienced PIs and investigative team with expertise in cysteinyl leukotrienes, vascular function and clinical trials. Results from this study will inform the design of a larger, randomized controlled trial examining whether montelukast may be a safe and promising option to use for treatment of kidney and vascular disease in patients with CKD.

II. Background and Significance:

1. **Critical need for novel therapeutic strategies in chronic kidney disease (CKD).** CKD is highly prevalent in the United States and is associated with significant morbidity and mortality. In the US, almost 15% of Medicare beneficiaries have significant CKD.[1] Patients with advanced CKD have dramatically higher mortality rates compared to the general population. Preservation of kidney function should therefore improve health outcomes. Blood pressure control and antagonists of the renin angiotensin system (RAAS) are the recommended therapies to preserve kidney function. Recently, inhibitors of the sodium glucose cotransporter (SGLT2) have been shown to reduce disease progression in patients with diabetes and CKD.[2,3] However, residual albuminuria remains present during SGLT2 inhibitor treatment and is associated with adverse kidney and heart outcomes.[4] Despite the availability of SGLT2 inhibitors, over 120,000 patients progress to end stage kidney disease (ESKD) yearly. Additionally, the adoption of SGLT2 inhibitors in patients with diabetic and nondiabetic CKD remains low.[5] Furthermore, SGLT2 inhibitors may not be safe in all CKD groups. For example, in type 1 diabetes there may be an increased risk of euglycemic diabetic ketoacidosis with SGLT2 inhibitors.[6] Data from animal models suggests an increased risk of cyst burden in animals with polycystic kidney disease treated with SGLT2 inhibitors.[7] Finally, SGLT2 inhibitors may also be associated with an increased risk of infections in kidney transplant recipients.[8] **Identifying new treatments for CKD to be used alone or in combination with other therapies is a high priority.**

2. **Inflammation plays a key role in CKD progression and cardiovascular disease.** Most forms of CKD are characterized pathologically by inflammation in the kidney.[9] Renal macrophage and lymphocyte infiltration are seen in CKD and macrophage number correlates with the severity of CKD.[10] Experimental models have shown that macrophages promote CKD progression through induction of chronic inflammation, tubular injury, and fibrosis.[10,11] Studies in diabetic kidney disease have shown increasing proinflammatory cytokine levels with kidney disease progression.[12] In the Chronic Renal Insufficiency Cohort, elevated levels of inflammatory markers such as TNF-α, are independent predictors of CKD progression and are linked with rapid loss of kidney function.[13] Atherosclerosis is also driven by chronic inflammation. Significant inflammatory cell infiltrates have been found in atherosclerotic plaques.[14] Proinflammatory cytokines released by macrophages lead to endothelial dysfunction and microcalcification.[15] Systemic markers of inflammation (e.g. C-reactive protein) are strong predictors of future cardiovascular events.[16] CVD is the leading cause of death in patients with CKD.[17] Two of the greatest contributors to CVD in CKD are the development of vascular endothelial dysfunction, most commonly assessed as brachial artery flow mediated dilation (FMD_{BA}) and stiffening of the large elastic arteries (aorta and carotid arteries).[18] Hence, treatments that target chronic inflammation may have a significant impact on CVD outcomes in CKD. Despite widespread treatment with RAAS antagonists and SGLT2 inhibitors, there remains an unacceptably high rate of CKD progression and cardiovascular complications of CKD. **Treatments that specifically target inflammatory pathways may improve outcomes in patients with CKD and may synergistically decrease progression with RAAS antagonists and SGLT2 inhibitors.**

3. **Leukotrienes are potent lipid mediators of chronic inflammatory diseases.** Lipid mediators produced through the action of 5-lipoxygenase (5-LO) play a critical role in inflammation and disease progression in many settings.[19,20] In many tissues, macrophages and neutrophils exert biological effects through 5-lipoxygenase (**Figure 1**). These macrophage-derived leukotrienes promote chronic inflammation and fibrosis. After arachidonic acid is liberated from membrane phospholipids by group IVA cytosolic phospholipase A_2 ($cPLA_2$), 5-lipoxygenase (5-LO) and 5-lipoxygenase-activating protein (FLAP) catalyze the synthesis of a class of eicosanoids, known as leukotrienes. After synthesis of leukotriene A_4 by 5-LO, LTA_4 hydrolase (LTA_4H) catalyzes production of leukotriene B_4 (LTB_4) while LTC_4 synthase (LTC_4S) is responsible for the synthesis of leukotriene C_4, D_4, and E_4 (LTC_4, LTD_4, LTE_4), collectively known as the cysteinyl leukotrienes.

   LTB_4 binds to the cell surface receptors BLT1 and BLT2 while cysteinyl leukotrienes bind to separate cell surface receptors (CysLTR1, CysLTR2, and GPR99). Cysteinyl leukotrienes are sequentially metabolized from LTC_4 to LTD_4 to LTE_4. After activating the specific cell surface receptors, leukotrienes regulate a wide range of biological processes, including inflammation and epithelial damage. In animal models, leukotrienes promote allergic diseases, pulmonary fibrosis, and liver fibrosis.[21-23]

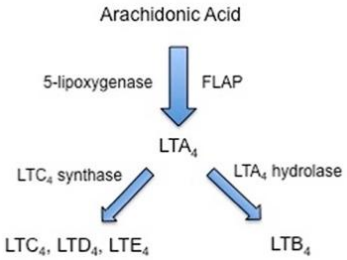
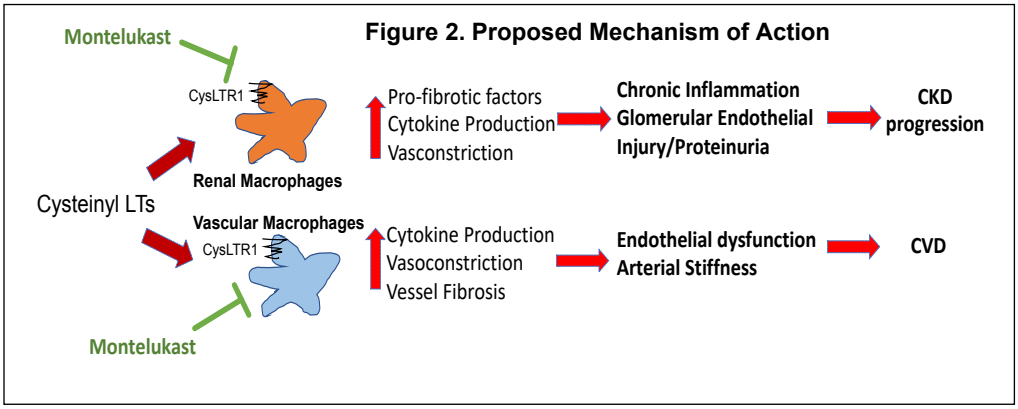


Figure 1. The 5-lipoxygenase pathway. After activation of cytosolic phospholipase A_2 ($cPLA_2$) membrane phospholipids are released as arachidonic acid, a 20-carbon lipid. Leukocytes predominantly express 5-lipoxygenase and synthesize the short-lived LTA_4. LTA_4 hydrolase and LTC_4 synthase are expressed in many cell types, including renal tubular epithelial cells and catalyze production of LTB_4 and the cysteinyl leukotrienes (LTC_4, LTD_4, and LTE_4), respectively.

4. **Leukotrienes play a key role in the pathogenesis of CKD.** Elevated levels of LTB_4 are associated with several models of kidney disease as it is a potent chemoattractant for neutrophils.[24,25] Cysteinyl leukotrienes trigger vasoconstriction resulting in reduced renal blood flow (RBF) and glomerular filtration rate (GFR).[26] Humans with CKD have increased tubulointerstitial expression of 5-LO.[27] The increased expression of 5-LO correlates with the severity of proteinuria and with serum creatinine levels.[27] In animal models of glomerulonephritis, inhibition of 5-LO results in reduced proteinuria and preservation of GFR.[28,29] As shown in **our preliminary data**, inhibition of 5-LO strongly reduces renal fibrosis in animal models of CKD.[25] Additionally, we have shown that inhibition of leukotrienes with montelukast (a leukotriene antagonist) in mice with acute kidney injury prevents progression to CKD (**preliminary data section 1**). In a small study of 11 adult patients with glomerulonephritis, short-term (4 days) administration of a 5-LO antagonist (MK-591) resulted in a significant decrease in proteinuria.[30] In children with steroid dependent minimal change disease, treatment with montelukast for 12 months significantly reduced relapse rates.[31] **However, to date, no randomized trials examining the effect of montelukast on kidney outcomes in patients with CKD have been performed and thus is the *primary aim* of the current study.**

5. **Leukotrienes mediate cardiovascular disease**. Increased expression of 5-LO, LTB_4 and cysteinyl leukotrienes are found in human atherosclerotic lesions.[32] This expression increases as lesions progress from early to late stage of atherosclerosis.[33] 5-LO expression is mostly localized to macrophages.[32,33] In patients with chronic obstructive pulmonary disease, high levels of cysteinyl leukotrienes correlate strongly with cardiovascular disease.[34,35] Increased intracoronary production of cysteinyl leukotrienes has also been shown in patients undergoing coronary angioplasty.[36] In animal models, montelukast has beneficial effects on vascular endothelial cell function and myocardial remodeling.[37,38] In a randomized, placebo-controlled crossover trial of a 5-LO inhibitor in patients with myocardial infarction, 4 weeks of treatment resulted in significant reduction is LTB_4 and myeloperoxidase.[39] Patients with asthma treated with montelukast for 6 months had lower levels of C-reactive protein and lipid levels compared to patients treated with placebo.[40] A recent observational study of 800 adult asthmatic patients found that montelukast use was associated with a significant independent reduction in cardiovascular events.[41] Thus, targeting the 5-LO pathway may be a strategy for reducing cardiovascular events. ***To date, no randomized controlled trials have been performed examining the effect of montelukast on surrogate markers of CVD and thus represents an aim of the current study.***

6. **Rigor of Prior Research**:



Leukotrienes play a significant role in kidney disease progression and cardiovascular disease in preclinical models. Observational studies in humans suggest a significant role for leukotrienes in cardiovascular and kidney disease. Small studies show that montelukast, a leukotriene antagonist) results in reduced proteinuria, less relapse of nephrotic syndrome and lower levels of LTB_4 and inflammatory markers. However, no randomized placebo-controlled trials have been performed in CKD examining the effect of montelukast on proteinuria, renal hemodynamics or surrogate markers of cardiovascular disease and inflammation. While SGLT2 inhibitors are becoming standard of care for patients with CKD, these medications may not be safe in all patients with CKD. Additionally, these medications can be cost prohibitive. This highlights the need for novel, inexpensive treatments for CKD progression and CVD in CKD, such as montelukast. Montelukast has been used extensively for the treatment of other diseases for decades and has an excellent safety profile in both adults and children. We hypothesize that montelukast will reduce cytokine production, pro-fibrotic factors and vasoconstriction leading to improvements in albuminuria, endothelial function and arterial stiffness (**Figure 2**). Positive results from the proposed study would provide evidence needed for a larger, multicenter, randomized controlled trial examining the effect of montelukast on hard clinical outcomes. Results from this study may also improve our ability to offer personalized treatments for patients with CKD. If montelukast does effectively decrease intrarenal inflammation in patients, that would support further study of montelukast in subgroups of patients with inflammatory CKD (high urinary biomarkers of inflammation or LTE_4).

III. Preliminary Studies/Progress Report:

1. Preliminary Data:

a. Montelukast decreases inflammation and CKD progression in a mouse model of CKD. We have previously showed that inhibition of 5-LO and LTC_4 synthase reduced fibrosis in two mouse models of renal fibrosis.[25] We next tested whether targeted inhibition of the cysteinyl leukotriene receptor 1 (CysLTR1) with montelukast would also reduce renal injury in mice using the folate nephropathy model. In the folate nephropathy model, mice develop

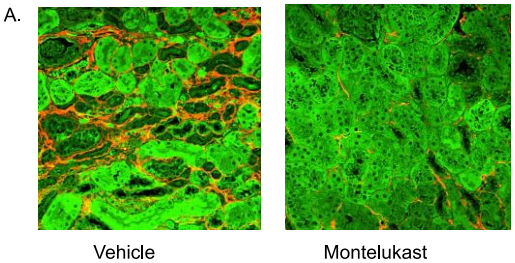


Figure 3. Montelukast decreases renal fibrosis and CKD progression. A. Renal fibrosis was assessed using second harmonic generation microscopy. Montelukast improved tubular architecture post-folate (green) and decreased collagen deposition (red). **B.** Montelukast was associated with improved plasma BUN. * p <0.05

acute kidney injury after administration of high dose folic acid. This is followed by a period of renal inflammation, inadequate renal tubular repair, and eventually development of CKD. After mice had established AKI, we began treatment with montelukast daily for two weeks. After an additional 75 days, mice were collected to assess the effects on CKD progression. We hypothesized that short-term inhibition of CysLTR1 during the critical period of renal inflammation and renal repair would prevent chronic inflammation and CKD progression.

We first found that mice treated with a short-term course of montelukast had significantly less renal fibrosis and improved renal function (**Figure 3**). To determine which pathways were regulated by montelukast in CKD, we next performed transcriptomic analysis of injured kidneys using RNA-Seq. Gene ontology enrichment analysis showed that montelukast regulated several pathways involved in immune function (**Figure 4**). To test whether this was associated with diminished chronic inflammation, we performed immunofluorescence for the macrophage marker CD68 in injured kidneys. 75 days after stopping montelukast, we found that montelukast had dramatically reduced the number of interstitial macrophages in the kidney (**Figure 5). In summary, these data show that short-term, low dose montelukast exerts very potent anti-inflammatory and anti-fibrotic effects in a mouse model of CKD.**

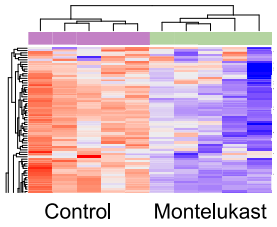


Figure 4. Montelukast prevents inflammatory gene transcription post-folate. Homogenized kidneys were analyzed from vehicle- and montelukast-treated mice using RNA-Seq. Gene set enrichment analysis (GSEA) using Hallmark-Inflammatory Responses is shown.

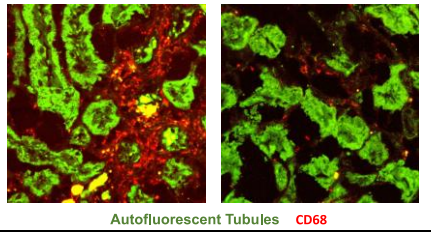


Figure 5. Montelukast decreases macrophage infiltration in injured kidneys. Immunofluorescence for CD68 showed that short term montelukast treatment significantly reduced CD68+ cells. * p< 0.05.

b. **Feasibility of the proposed study is demonstrated by the MPI Dr. Kendrick's experience in the NIH sponsored Vitamin D and Arterial Function in CKD Trial[42] and Bicarbonate Administration in CKD Trial (NCT0291560).** The Vitamin D trial conducted by Dr. Kendrick (Co-PI) was a single-center study examining the effects of oral cholecalciferol vs. calcitriol for 6 months on vascular function in 128 CKD stage 3b-4 patients. Recruitment was accomplished in 36 months with an attrition rate of 10%.[42] The Bicarbonate Administration in CKD Trial is an ongoing 12 month trial of patients with CKD stage 3-4 examining the effect of oral sodium bicarbonate on vascular function and left ventricular mass with a current attrition rate of 11.0%. 24-hour urines were collected at 3 time points in the Bicarbonate Administration in CKD showing that it is feasible to collect 24-hour urines for albuminuria in patients with CKD.

c. **Feasibility of recruitment is demonstrated by the large pool of patients available for enrollment**. At the University of Colorado Hospital and Denver Health Medical Center, we have access to over 4,000 patients with CKD.

Additionally, we have a recruitment database of patients with CKD stage 3 willing to participate in clinical trials. Feasibility of recruitment is demonstrated by prior recruitment experience of the investigators in several CKD interventional trials.

IV. Research Methods

Subjects. After obtaining their written informed consent, 20 patients with DKD (defined as estimated GFR (eGFR) 30-59 ml/min/1.73m^2) with albuminuria of 200-5000 mg/g will serve as subjects. This range of albuminuria was chosen as macroalbuminuria is associated with more adverse outcomes in patients with CKD and a previous small study found a greater reduction in albuminuria with a 5-LO inhibitor in participants with greater albuminuria at baseline.[30] **Relevant biological variables:** Men and women age 30-70 years of all races/ethnicities will be included. Major inclusion/exclusion criteria are presented in the table below (Table 1). Participants with GFR <30 ml/min/1.73m^2 are excluded as this is advanced kidney disease when complications become more common and vascular damage may not be reversible. Participants will undergo screening for eGFR at the Kidney Disease Research Center (KDRC) at the University of Colorado Anschutz Medical Campus. Estimated GFR will be calculated using the new 2021 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) prediction equation.[43] Participants will be recruited from nephrology clinics at the University of Colorado and Denver Health Medical Center. Additionally, patients will be recruited from our CKD database, which contains over 300 CKD patients willing to participate in clinical trials. Feasibility of recruitment is demonstrated by prior recruitment experience of the investigative team in several CKD interventional trials[42] (NCT02915601, NCT04600323). For this study

Table 1

Inclusion Criteria	Exclusion Criteria
• Age 30-70 years • Urine albumin to creatinine ratio 200-5000 mg/g on first morning void • CKD stage 3 at time of screening (eGFR 30-59 ml/min/1.73m^2) • Blood pressure <140/90 mm Hg prior to baseline visit • Use of angiotensin converting enzyme inhibitor or angiotensin receptor blocker or mineralocorticoid receptor antagonist with stable dose for 4 weeks • History of diabetes type 1 or type 2 • ~~BMI < 40 kg/m^2 (FMD measurements can be inaccurate in severely obese patients).~~ • Stable anti-hypertensive regimen for at least one month prior to enrollment • Stable diabetes regiment for at least one month prior to enrollment • Sedentary or recreationally active (≤2 days of vigorous aerobic exercise as vigorous exercise may affect vascular function measurements) • Able to provide consent	• Significant comorbid conditions that lead the investigator to conclude that life expectancy is less than 1 year • Uncontrolled hypertension • Factors judged to limit adherence to interventions (e.g. history of medication noncompliance, noncompliance with follow up visits, significant cognitive impairment, etc.) • Anticipated initiation of dialysis or kidney transplantation within 3 months • Current participation in another research study • Pregnancy or planning to become pregnant or currently breastfeeding • Allergy to aspirin • Severe hepatic impairment (Child-Pugh Class C) • History of major psychiatric disorder • Use of inhaled or systemic corticosteroids or long-acting beta agonists (higher risk of neuropsychiatric reaction) • Current use of phenobarbital, rifampin or carbamazepine.

Rigorous Experimental Design. A 3-month **open-label, prospective pilot study** with oral montelukast will be conducted. The study will include 3phases: screening, baseline, and follow-up.

- Screening: Subjects will undergo screening for inclusion/exclusion criteria during a 1-2 week period. Participants will have urine albumin/creatinine performed and basic metabolic panel to determine eligibility.
- Baseline: During the baseline phase the following will be measured: 1) 24-hour albuminuria; 2) vascular function (EndoPat, aPWV); 2) serum chemistry panel; 3) urine MCP-1, sCD163 and LTE_4; and 4) plasma TNFR-1.
- Follow-up: The follow-up schedule includes assessments at 3 months with monthly safety visits (either via phone, in person, or survey link). An additional safety check will be performed via phone, in person, or survey link 2 weeks after the last study visit.

Study Drug Dosing: Study drug will be received from the University of Colorado Hospital Pharmacy. Each bottle will contain 90 tablets of montelukast 10mg. Participants will receive 1 tablet daily (10mg) for the entire 3 months. The tablet will be taken in the evening per manufacturer recommendations. The dose of montelukast may be adjusted.

Rationale for study duration: From the study team's experience with other interventional studies evaluating albuminuria and vascular endothelial function changes can be seen in 4-6 weeks. Changes in aPWV can be seen after 12 weeks. Changes in patients with CKD stage 3 may require longer periods of time for these endpoints and published data use a period of 3-6 months.[42,44] Hence, we are proposing a 3-month intervention for this pilot study.

Data Collection

- Demographics, medical history and physical examination will be performed at screening or baseline visit.
- Circulating measures: Subjects will report fasted for standard blood chemistry analysis at baseline and 3 months. A urine pregnancy test will be performed if clinically indicated at the screening visit.
- Outcome measures:
    - 24-hour albuminuria will be measured at baseline and 3 months
    - EndoPat and aPWV will be measured at baseline and 3 months.
    - Urinary LTE4, MCP-1, and CD163 will be measured at baseline and 3 months.
    - Plasma TNFR-1 and basic metabolic panel will be measured at baseline and 3 months.
- Resting BP: Arterial BP will be measured in triplicate while seated at rest using an automated oscillometric machine (Dinamap) at all in-person study visits.
- Measures of adherence and safety:
    - Pill counts will be assessed at the 3-month visit to document subject adherence.
    - A neuropsychiatric assessment will be performed monthly to assess for any changes in mood, depression or anxiety symptoms via phone, in person, or survey link. We will utilize the Patient Health Questionnaire-9 (PHQ-9) and the General Anxiety Disorder-7 (GAD-7). These questionnaires will assess depression and anxiety symptoms, as well as any suicidal ideations and are routinely used in patients with CKD.[45,46] We chose these questionnaires as they are validated in patients with CKD and can be completed in 10 minutes.[45,46] Any participant that has a significant change in score from the

previous month or that reports suicidal ideations, will be referred immediately to Dr. Ritchie, Co-I. Dr. Ritchie is a clinical psychologist. She will contact the patient and do a risk assessment to determine if the participant needs urgent mental health treatment. Participants that require urgent psychiatric care will be discontinued on study drug and removed from the study. We will also assess sleep including insomnia, sleepwalking etc. Finally, we will assess whether the participant is experiencing any memory problems, tremor or uncontrolled muscle movements. Participants that report these issues will be instructed to stop the study drug immediately. Participants will also be informed at study initiation to stop the drug and call immediately should they experience any change in mood, depression, aggression, agitation, sleep disturbances, or suicidal thoughts.

- Adverse event assessment will be performed monthly either via telephone, in person, or survey link. Additional telephone calls and PRN follow-up visits will occur at the discretion of the PIs.

Safety monitoring and expected adverse events: Reporting of Side Effects. Subjects will be instructed to report serious or worrisome side effects to the investigators and their primary care physician (who will have been informed of their patient's participation in and the nature of the study). A subject experiencing side effects during off hours will contact Dr. Kendrick, who is available 24 hours a day. Dr. Furgeson will be on call during any period when Dr. Kendrick is not available. Thus, the physicians on the study team will work together to provide 24/7 medical coverage to subjects. Any unexpected adverse events will be reported immediately to the Colorado Institutional Review Board.

1. **Montelukast** is well tolerated and has been used in pediatric and adult patients for many years. The most common adverse reactions (incidence ≥ 5% and greater than placebo) include upper respiratory infection, fever, headache, pharyngitis, cough, abdominal pain, diarrhea, otitis media, influenza, rhinorrhea, sinusitis and otitis. These side effects are minor and will be assessed on a monthly basis via phone, in person, or survey link. **A serious side effect** of montelukast is a neuropsychiatric adverse reaction. This reaction has been reported in patients with and without a history of psychiatric illness. This has been added as a Black Box Warning by the FDA. Reactions include agitation, aggressive behavior, anxiety, depression, restlessness, suicidal ideations or tendencies. Some patients also develop sleep disturbances (abnormal dreams, insomnia, sleep walking). Additionally disturbances in attention or memory impairment, tremor or involuntary movements may occur (up to 1 in 1,000 people). Sleep disturbances, depression and agitation may affect up to 1 in 100 people taking montelukast. Suicidal behavior and hallucinations occur very rarely (up to 1 in 10,000 people). We will assess for these serious adverse events monthly through the use of questionnaires regarding depression, anxiety and sleep via phone, in person, or survey link. We will also assess for any new tremors or involuntary movements. Dr. Ritchie, Co-Investigator, is a clinical psychologist and researcher and will immediately do a risk assessment on any participant presenting with symptoms to determine if urgent psychiatric treatment is required and will arrange for treatment. Additionally, study drug will be stopped immediately. Most of these reactions resolve with stopping montelukast. We will exclude patients with a history of major depressive disorder, or that are on corticosteroids (inhaled or systemic) or long-acting beta-agonists as this may increase the risk of neuropsychiatric reactions.

2. **Fasting:** There is a small risk that blood sugars will be lower than normal in participants with diabetes while fasting. Participants will also be asked to check their blood sugars while fasting. If participants are unable to be fasting for 12 hours, they will be allowed to have a small snack prior to the study measurements. The fasting instructions for this study is similar to what we have prescribed in our other studies in participants with CKD with and without diabetes.

3. **Blood pressure, heart rate and oxygen saturation:** There are no known risks associated with collecting these vitals.

4. **Physical exam and 24 hour urine samples:** There are no known risks associated with the physical exam or urine collections. The 24-hour urine collections may be cumbersome for participants but the protocol used in this study is similar to what we have done in our other studies in participants with CKD.

5. **Venous blood draw:** discomfort associated with insertion of the needle; local bleeding and a small hematoma (~10% of cases); risk of infection of a hematoma or significant external blood loss (<1 in 1000), risk of fainting. Participants with CKD may have plans in place for future vascular access for hemodialysis and thus we will draw blood in the arm not designated for future vascular access.

6. **Vascular function measurements:** inflating the blood pressure cuff below the elbow during this procedure may cause a mild to moderate intensity "pins and needles or numbing" sensation that goes away as soon as the cuff is deflated. If participants have a fistula or graft, EndoPat will be performed in the opposite arm.

7. **Confidentiality:** There is a risk that people outside of the research team will see the research information. While we will do all that we can to protect participant information, it cannot be guaranteed. Our study will follow HIPAA guidelines to protect anyone who participates in this research study. All study procedures will be conducted in a private room with study-related personnel only.

8. **Pregnancy:** There are no adequate and well-controlled studies in pregnant women. Montelukast should be used during pregnancy only if clearly needed. No teratogenicity was observed in rats and rabbits at doses approximately 100 and 110 times, respectively, the maximum recommended daily oral dose in adults based on AUCs. Congenital limb defects have been rarely reported in the offspring of women being treated with montelukast during pregnancy. A causal relationship between these events and montelukast has not been established.

Outcome Measures

a) Primary Outcome

1. **24-hour albuminuria:** Participants will perform a 24-hour urine collection and return it the day of their visit at baseline and 3 months. Participants will be given the containers to collect the 24-hour urine samples at the screening visits.

2. **Justification for primary outcome:** Albuminuria is a marker of kidney damage and is an early and sensitive marker in many types of kidney disease.[47] The gold

standard for assessing albuminuria is a 24-hour urine collection.[48] Dr. Kendrick, MPI, has extensive experience in collections of 24-hour urine in participants with CKD (NCT0291560).

b) Secondary Outcome

Vascular endothelial function. All measurements will be made after a 12 hour fast (water allowed), 12 hours after abstaining from tobacco and caffeine, and 24 hours after abstaining from alcohol and exercise. No new over the counter medications within 48 hours. To control for menstrual cycle, all premenopausal women will be tested in the early follicular phase (days 1-6 of the menstrual cycle). Measurements will be made at baseline and 3 months.

Vascular endothelial function will be measured using EndoPat 2000. Testing will be performed in a quiet room with the patient in a supine position. EndoPat bio sensor is placed on the patient's right and left index fingers. A blood pressure cuff is wrapped around the non-dominant arm and records baseline data for 5 minutes. The cuff then inflates and occludes the brachial artery for another 5 minutes. The cuff is then released to measure the dilatory response. EndoPat's software performs an automatic analysis of the data obtained from both fingers and provides a post-occlusion to pre-occlusion ratio. The RHI score is provided in a logarithmic scale. A score less than 7 indicates endothelial dysfunction. Testing can be completed in 20 minutes. EndoPat has been used in numerous studies accessing endothelial function.

Large Elastic Artery Stiffness and Compliance: Aortic PWV, the gold standard measurement of large elastic artery stiffness, will be determined using the SphygmoCor device at baseline and 3 months.[49] Measurement of pulse wave analysis: The cuff of the SphygmoCor device will be placed on the non-dominant arm. BP will be measured involving an automatic recording of brachial BP immediately followed by reinflation of the cuff. The cuff is held at inflation for a period of 5 seconds during which time volumetric waveforms are recorded. Measurement of aPWV: Briefly, a transcutaneous custom tonometer will be positioned at the carotid artery and a cuff will be placed at the femoral artery to measure PWV. The distance between the carotid and the femoral cuff are measured as is the distance between the femoral artery and the femoral cuff. These distances are entered into the SphygmoCor software to determine the carotid-femoral distance used for calculation of PWV.[49] The tonomoter is positioned over the marked location with the strongest carotid pulse detected by the operator. The device automatically captures the pulse wave recording and will be attempted for a full cuff inflation/deflation cycle, pluse the first 20 s of the second cuff inflation. SphygmoCor software will perform the velocity calculation.[49]

Laboratory Measurements:

a) **Plasma comprehensive metabolic panel (CMP):** A CMP will be performed by the University of Colorado Hospital Clinical laboratory at UC-AMC using standard procedures at baseline and 3 months.
b) **Urinary sCD163:** Urinary sCD163 levels will be assayed using Human CD163 DuoSet ELISA kit in Dr. Furgeson's lab as has been previously described.[50]
c) **Urinary MCP-1:** Urinary MCP-1 will be measured using the R&D Systems Human CCL2/MCP-1 Quantikine ELISA kit as has been previously described.[51]
d) **Plasma TNFR-1:** Plasma TNFR-1 will be measured using Human TNFR1/TNFRSF1A Quantikine ELISA kit as has been described.[52]

e) **Urinary LTE_4**: After lipid extraction from urine samples, urinary LTE_4 will be measured by liquid chromatography/mass spectrometry.

Justification for secondary outcomes: Vascular endothelial dysfunction and arterial stiffness are key pathophysiological antecedents to CVD in patients with CKD.[53,54] EndoPat ia a common, non-invasive technique used to assess vascular endothelial function. Aortic PWV is the gold standard measurement of arterial stiffness and is non-invasive. Dr. Kendrick, MPI and the University of Colorado investigative team have expertise in these vascular function measurements.[42,55] 3) Increased inflammation may play a critical role in leukotriene activation and subsequent kidney and vascular dysfunction. The effect of montelukast on urinary markers of inflammation and macrophage activation and endovascular markers of inflammation has not been evaluated. Plasma levels of TNFR-1 (a biomarker of systemic inflammation) exhibit a robust association with CKD progression in a variety of patient populations, including those with diabetic kidney disease and hypertensive nephrosclerosis.[56,57] Urinary MCP-1, a biomarker of systemic and renal inflammation, is also strongly associated with CKD progression in patients with acute kidney injury and hypertension.[58,59] Although there are limited non-invasive methods to measure macrophage number within kidneys; urinary soluble CD163 has been shown to correlate well with inflammation on renal biopsies.[50,60] Since LTC_4 and LTD_4 are rapidly metabolized to LTE_4, urinary LTE_4 measurements are a very sensitive and specific way to measure changes in total body cysteinyl leukotrienes.[61] Dr. Furgeson, MPI has experience with these laboratory measurements.

Data Management: Data will be collected and managed using Research Electronic Data Capture (REDCap), a secure web application designed to support data capture for research studies. The database is hosted at the University of Colorado Development and Informatics Service Center (DISC), which will be used as a central location for data processing and management. Data quality checks will be ongoing, but will formally occur (e.g., missing data, duplicate entries) at least annually when progress reports are prepared. Blood and urine samples that are not assayed immediately will be stored for later analysis in locked -80˚ freezers managed by the PI and study staff. All samples will be labeled with the least amount of information necessary to accurately identify the samples (e.g., study number, subject identification number, visit date, and test time-point). Participants who drop from the study may request destruction of samples.

Power Calculations: The goal of this pilot study is to provide parameters to allow for a more accurate estimation of sample size for a larger clinical trial. Our sample size was determined based on feasibility and availability, not a specified statistical power.

Statistical Analysis: Descriptive statistics will be calculated for all variables. For example, mean and standard deviation will be calculated for a continuous variable, and count and proportion will be provided for a categorical or ordinal variable. For a continuous variable that is not normally distributed, median and interquartile range will be provided, and natural log transformation will be performed before further analysis. The 95% confidence interval will be calculated as well if appropriate.

For the primary outcome 24-hour albuminuria, its log value will be used in analysis. Analysis with a linear regression model will be performed to assess the montelukast effect

by regressing the albuminuria at end of study on baseline albuminuria. Furthermore, analysis with adjustment for age, sex will be performed. For the secondary outcomes for the linear regression model also is applicable, and the same analysis will be performed.

Potential Problems and Alternative Strategies

- Although subject recruitment and retention are always challenging, we should be able to complete the study in the proposed timeline given our group's excellent track record in recruiting, enrolling and retaining participants in clinical trials. The investigative team has access to over 4,000 patients in the CKD clinics at the University of Colorado and the Denver Health Medical Center.
- We should have few difficulties with the proposed experimental procedures and protocols as they are already establised in Dr. Kendrick's vascular laboratories and Dr. Furgeson's laboratory at the UC-AMC. The investigative team has extensive experience with the proposed study measurements.
- Systolic BP is an important determinant of proteinuria and endothelial function and could be a theoretical confounder when interpreting the mechanisms by which montelukast improves proteinuria and vascular function. All participants must have their BP controlled prior to entering the study.
- We recognize that ACEi/ARB reduce proteinuria and kidney disease progression in patients with CKD. It is possible montelukast has a synergistic effect with these medications. Participants must be on an ACEi/ARB to participate in the study.
- We acknowledge there are other markers of inflammation and it possible that urinary CD163 levels are low in our patient population. Additionally, we acknowledge that measurement of leukotrienes and other inflammatory cytokines in the blood may also be important. For this reason, we are creating a biobank and blood obtained at visits will be stored for future research purposes.

Potential Benefits of the Proposed Research to the Subjects and Others:
Participants will receive benefits associated with overall knowledge of their health from the extensive testing performed for screening purposes and the established subject characteristics (i.e. blood pressure, blood chemistries, etc.). The findings we will generate will answer important questions about montelukast administration in patients with DKD and help investigators design future clinical trials in this population. Thus, the new knowledge generated from this proposal will yield potential benefit to DKD patients in general and to society as a whole.

4. Importance of the Knowledge to be Gained: The new knowledge generated from this proposal will facilitate planning of future clinical trials in DKD patients that will aim to reduce the morbidity and mortality of DKD.

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