Hemodialysis.-Induced Hypotension Therapy for End Stage Kidney Disease (Hinder)

NCT05297786

Prevention of Dialysis-Induced Hypotension by Inhibiting Plasma Kallikrein

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1.0 Background

A. Definition of DIH. Various definitions have been used for intradialytic hypotension. The National Kidney Foundation Kidney Disease Outcome Quality Initiative (K/DOQI) has defined DIH as a reduction in systolic blood pressure or mean arterial pressure during hemodialysis, which it is associated with symptoms such as muscle cramps, anxiety, yawning, dizziness, fainting, or abdominal discomfort.[1] The reduction in blood pressure should be equal to or greater than 20 mmHg for systolic blood pressure and equal to or greater than 10 mmHg for mean arterial blood pressure. Most of the reduction of blood pressure during hemodialysis occurs during the first quarter of treatment with a gradual decrease during the remaining treatment time (**Figure 1**).[2] Some investigators use a nadir-based DIH definition—when the systolic blood pressure falls below 90 or 100 mmHg.,[3] or the need for nursing intervention in addition to experiencing a fall in blood pressure and clinical symptoms.[4] The reduction in blood pressure can also be asymptomatic. Thus, it is important to recognize the occurrence of repeated episodes of hypotension that will damage organs including the brain, heart, gut, and liver as well as skeletal muscle. Furthermore, it is important to identify patients who are susceptible to intradialytic hypotension to prevent the undesirable consequences of DIH.

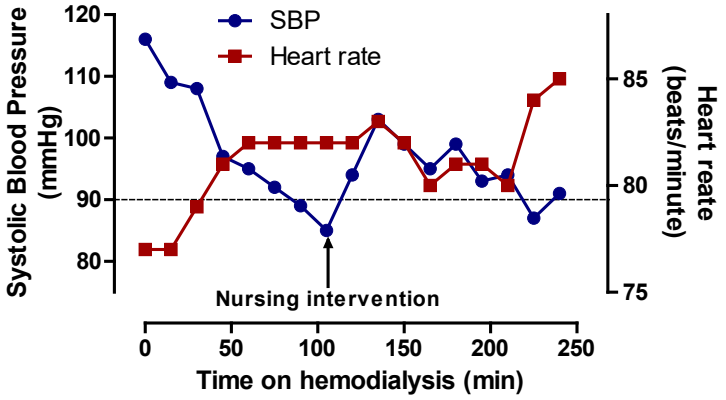


Figure 1. Blood pressure reduction during hemodialysis

B. Morbidity and mortality of DIH. Approximately 75% of patients suffer at least one episode of hypotension during hemodialysis,[5] and DIH occurs in approximately 20 to 30% of all regular hemodialysis treatments.[5, 6] DIH is a common clinical complication of hemodialysis, and it is associated with increased morbidity and mortality.[7] It has been shown that DIH may induce myocardial dysfunction due to reduced myocardial blood flow.[8] Recurrent episodes of hypotension are also associated with frontal lobe atrophy in the brain,[9] and with mesenteric ischemia.[10] Previous studies have identified an association between DIH and cardiovascular morbidity-mortality,[11] and all-cause mortality.[12] Two more recent studies, using large databases, also showed the association between DIH and mortality.[3, 13]

Nursing interventions during hemodialysis are usually initiated by patient-reported symptoms. Among them, nausea, dizziness, cramps, and fatigue are common during hemodialysis.[14, 15] DIH is associated with some of these symptoms, particularly dizziness and cramps.[16] Furthermore, DIH and a higher ultrafiltration rate have been associated with longer hemodialysis recovery time,[17] a measure of the quality of life (QOL) that has been validated in patients on hemodialysis that correlates well with longer and more complex QOL questionnaires.[18]

C. Multiple factors contribute to the pathophysiology of DIH. The pathogenesis of DIH and intradialytic hypotension have been attributed to the rapid removal of plasma volume during hemodialysis, a high rate of ultrafiltration and the inadequate mechanisms

to respond to hypovolemia in patients with ESRD.[19] Thus, the occurrence of DIH would depend on the rate of fluid removal and the compensatory mechanism for refilling the plasma volume (**Figure 2**). The rate of fluid removal can be controlled by limiting the ultrafiltration rate or increasing the hemodialysis time. Conversely, the compensatory mechanisms to hypovolemia are more difficult to control, and they depend on the patient's comorbidities—such as impaired cardiac contractility, reduced cardiac reserve, autonomic dysfunction, and reduced venous compliance.[19] Cardiac compensations include increasing the heart rate and the contractility.[20] Venous tone is also an important compensatory mechanism since more than three liters of plasma volume is located within the veins.[21] The increase of arteriolar vasoconstriction is another normal response to hypovolemia and hypotension.[19] Thus, it has been proposed that patients with DIH have impaired compensatory mechanisms.[20]

The autonomic nervous system controls many of the compensatory mechanisms to hypovolemia, such as heart rate, venous compliance, and arteriolar tone. Thus, it has been proposed that autonomic dysfunction plays a major role in DIH.[22] In fact, although patients on hemodialysis frequently present with dysfunction of the autonomic nervous system,[23, 24] the real prevalence in this population is unknown. Due to fluid removal, hemodialysis activates the sympathetic nervous system; thus, inappropriate activation of this system contributes to DIH.

The lack of vasoconstriction in response to hypovolemia has been implicated as one of the impaired responses during DIH. This may be due to the removal of vasoconstrictors or the increased production of vasodilators. Vasopressin, a vasoactive peptide that increases during hypovolemia, seems to have a blunted response during hemodialysis in patients with DIH.[25] Nitric oxide, another potent vasodilator, has been found elevated in patients with DIH.[26-28] Adenosine has also been implicated in the occurrence of DIH through the inhibitory effect on norepinephrine release.[29] Besides, hypo-perfused tissues may release adenosine that may have a local effect on vasodilation, which may worsen blood pressure reduction.[20] Accordingly, a recent study showed that blocking adenosine A_1 receptor may improve DIH.[30] Bradykinin is another vasoactive substance that is released during hypoperfusion and may have a role in the pathogenesis of DIH. It is noteworthy to mention that bradykinin may also act locally by stimulating cardiac vagal afferent chemoreceptors leading to sympathetic inhibition.[31]

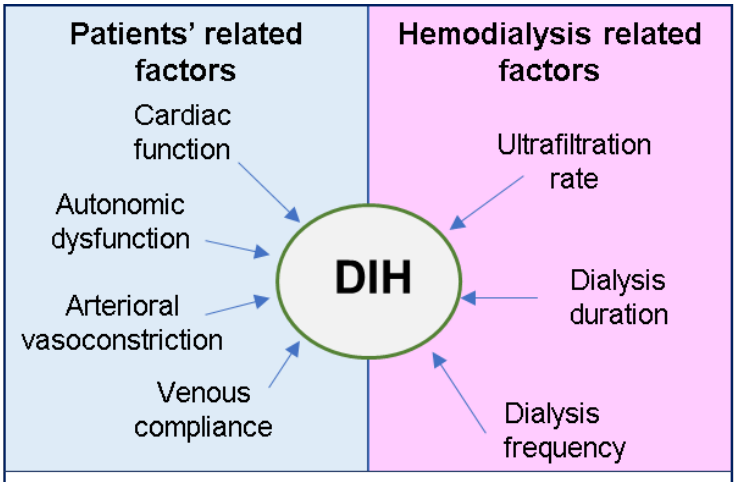


Figure 2. Factors associated with dialysis-induced hypotension (DIH)

D. Activation of the kallikrein-kinin system during hemodialysis increases bradykinin and may contribute hypotension. Bradykinin, a potent vasoactive peptide, results from the cleavage of high molecular kininogen (HMWK) by tissue and plasma kallikreins.[32] Bradykinin induces vasodilation by stimulation of the bradykinin B_2 receptor.[33] Hemodialysis induces the activation of the kallikrein-kinin system and the

production of bradykinin.[34] During hemodialysis, contact of blood with the dialyzer also activates the contact system,[35] which in turn also increases bradykinin levels.[36] Bradykinin levels are also increased during cardiopulmonary bypass,[37] and one of the complications of cardiopulmonary bypass is protamine-induced hypotension. Our group has previously shown that the hypotensive events coincide with increased levels of bradykinin.[38] Furthermore, blocking the bradykinin B_2 receptor with icatibant attenuates the hypotensive response to protamine. Thus, bradykinin may also induce hypotension during hemodialysis by inducing vasodilation. We have previously reported that bradykinin B_2 receptor blockade does not affect blood pressure reduction during hemodialysis,[39] but we did not assess the contribution of bradykinin to DIH.
A novel therapeutic alternative to inhibit the activation of the kallikrein-kinin system is lanadelumab, a human monoclonal antibody against plasma kallikrein, that prevents the cleavage of H<WK and the subsequent increase in bradykinin. Lanadelumab is an FDA approved drug against hereditary angioedema; however, the role of lanadelumab on dialysis induced hypotension has not been explored.

2.0 Rationale and Specific Aims

A. Specific Aims

In **Specific Aim 1** we will test the hypothesis that **in patients with DIH, inhibition of plasma kallikrein system with lanadelumab prevents the reduction of blood pressure and maintains hemodynamic stability.** For this purpose, we will monitor heart rate and blood pressure during hemodialysis. Importantly, we will conduct the study in an outpatient clinic, using the patients' usual hemodialysis dose and settings as for a regular hemodialysis session. This approach will minimize the risk that our findings were biased by conducting the study in a clinical research setting.

In **Specific Aim 2** we will test the hypothesis that **in patients with DIH, inhibition of plasma kallikrein system with lanadelumab prevents symptoms associated with DIH such as cramps, dizziness, and nausea and improve recovery time after hemodialysis.** Hemodialysis is associated with many complications, including DIH, that negatively affect the QOL of patients and their families. Any intervention that prevents the occurrence of DIH will result in a faster recovery from hemodialysis. The present study will evaluate the impact of lanadelumab on preventing symptoms associated with DIH and reducing recovery time after hemodialysis.

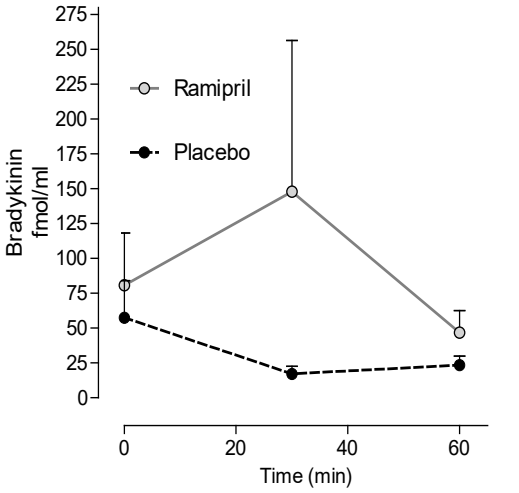


Figure 3. Effect of one- week treatment with ramipril or placebo on bradykinin concentrations in response to hemodialysis

3.0 PRELIMINARY RESULTS

Bradykinin increases during hemodialysis

Hemodialysis activates the kallikrein-kinin system and increases the levels of bradykinin. Accordingly, we have previously shown that bradykinin levels increase during the first 30 minutes of hemodialysis in 15 subjects that received 5 mg of ramipril for seven days (**Figure 3**).[40]

Bradykinin B2 receptor blockade prevents an excessive decrease in blood pressure

Another method for evaluating the effects of bradykinin during hemodialysis is by blocking its receptor. Thus, we evaluated the effect of icatibant on blood pressure during hemodialysis. For this purpose, we performed a secondary analysis in a randomized, double-blind, placebo-controlled study designed to examine the effect of icatibant on mitochondrial function during hemodialysis (**NCT03177798**). Icatibant or placebo was continuously IV-infused 30 minutes before the initiation of hemodialysis and throughout the whole hemodialysis session. Icatibant was infused at a dose of 100 ug/kg/h before hemodialysis, which was then changed to 50 ug/kg/h for the duration of hemodialysis. As we previously reported, we found that icatibant has no overall effect on blood pressure or heart rate during hemodialysis. We then stratified the analysis based on a reduction of systolic blood pressure (SBP) equal to or greater than 20 mmHg during hemodialysis. We found that in those patients with a reduction in blood pressure, icatibant prevented the decrease in SBP compared to placebo (**Figure 4A-B**). We also found that icatibant blunted the rise in heart rate during hemodialysis in patients whose blood pressure reduction was equal to or greater than 20 mmHg (**Figure 4C**). Icatibant did not affect either blood pressure or heart rate in patients who did not have a significant blood pressure reduction during hemodialysis.

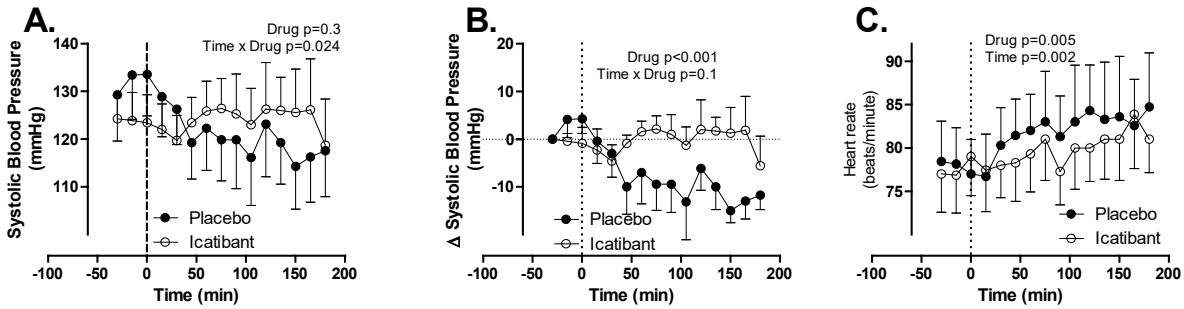


Figure 4. Effect of icatibant on blood pressure and heart rate during hemodialysis in patients with a reduction of blood pressure ≥ 20 mmHg (n=7).

4.0 Inclusion/Exclusion Criteria

Study population

Participants will be recruited from Vanderbilt Dialysis Outpatient Clinics. We will enroll patients who are on maintenance hemodialysis for at least six months, with pre-dialysis blood pressure between 110 and 170 mmHg, who are being adequately dialyzed (Kt/V >1.2), and who are between 18 and 85 years old, who have experienced dialysis-induced hypotension (DIH) episodes at least four times in a period of 12 weeks prior to enrollment. We define DIH as the drop of systolic blood pressure (SBP) of more than 30 mmHg with associated symptoms such as nausea, dizziness, cramps, yawning, and abdominal discomfort. We will also look for nursing intervention during the hypotensive episode, but it will not be used as an inclusion criterion. Nursing intervention is defined as a temporary interruption of ultrafiltration, Trendelenburg positioning, or administration of intravenous fluids. We will exclude patients who suffered severe DIH episodes that required the use of vasopressors and the termination of hemodialysis treatment. Patients who have been hospitalized during the last three months or with clinical evidence of inflammatory or infectious disease or with severe anemia (hemoglobin less than 8 g/dl) will be excluded. Institutional review board approval and written informed consent will be

obtained from all study subjects. We expect that 60% of our subjects will be African American and 40% will be women.

Inclusion criteria

- Subjects age 18 to 85 years
- On thrice-weekly hemodialysis for at least six months
- Clinically stable, adequately dialyzed (single-pool Kt/V > 1.2), with polysulphone membrane for at least three consecutive months before the study
- Subjects with pre-dialytic systolic blood pressure between 110 and 170 mmHg.
- Subjects with a reduction of systolic blood pressure during hemodialysis equal to or greater than 30 mmHg, with associated symptoms such as nausea, vomiting, muscle cramps, dizziness, or anxiety.
- Hypotensive episodes should occur four times or more in four weeks (12 hemodialysis sessions).

Exclusion criteria

- Subjects with intradialytic hypotension that require the use of pharmacological intervention such as midodrine or vasopressin
- Subjects with pre-dialytic systolic blood pressure greater than 170 mmHg or diastolic blood pressure greater than 110 mmHg
- History of myocardial infarction or cerebrovascular event within 3 months
- History of serious hemorrhage (including cerebral hemorrhage) in the past 12 months
- INR outside of the therapeutic range in the last 6 months before enrollment
- Advanced liver disease
- Ejection fraction less than 30%
- Anticipated live donor kidney transplant
- History of poor adherence to hemodialysis or medical regimen
- Severe anemia (hemoglobin less than 8 g/dl) requiring blood transfusions
- Use of immunosuppressive drugs within one month before study enrollment
- Active connective tissue disease
- History of acute infections disease within one month before study enrollment
- Inability to provide consent
- Pregnancy

5.0 Enrollment/Randomization

This is a parallel study where participants will be assigned to either taking the study drug (lanadelumab) or taking the placebo (saline pre-filled syringes.) Patients will be randomly assigned to the treatment order using a permuted-block randomization algorithm. The study drug, lanadelumab, will be shipped from Takeda as pre-filled syringes along with matching placebo, also in prefilled syringes.The Vanderbilt Investigational Drug Services (IDS) will be responsible for the storage, preparation, dispensing, and labeling of the investigational agents. The Vanderbilt IDS will also maintain accurate drug storage and dispensing logs. Subjects will be randomized to receive a placebo or 300 mg of lanadelumab (Takeda Pharmaceutical, Cambridge, MA). Subjects randomized who do

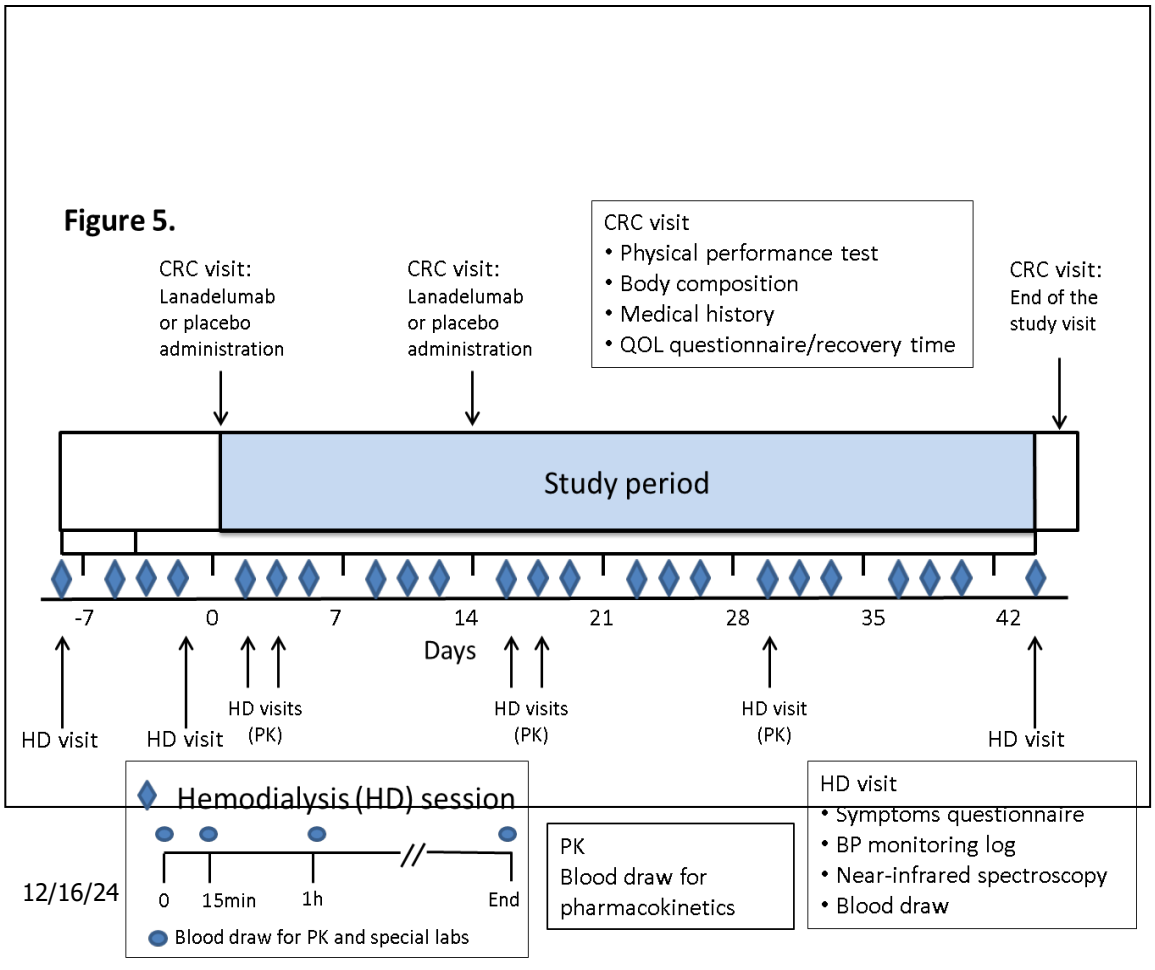
not complete the whole protocol for any reason will be replaced, and their data will be analyzed separately. The half-life of lanadelumab is 14 days, and the time to reach maximum concentration is five days. For this reason, we will monitor blood pressure and the occurrence of DIH for three weeks after the administration of the second dose of the study drug.

6.0 Study Procedures

Study protocol
After obtaining consent, study subjects will undergo the following procedures.

COVID-19 precautions prior to and during visits. Pre-visit phone call – we will contact the patient before all of their study visits to ask a series of COVID-19-related screening questions. Depending on their answers, we will continue with the study visit or postpone for another time. The patient will be asked to wear a face covering during all portions of their study visits in which they are in the presence of hospital staff. We will also encourage frequent hand washing and/or provide alcohol-based hand sanitizer.

Subjects will participate in a randomized, parallel-group, double-blind, placebo-controlled study in which they will receive two doses of placebo or 300 mg of lanadelumab subcutaneously. Participants will continue their usual medications throughout the study. One week (Day -7) before the administration of the first dose of the study drug, we will conduct a study visit at the hemodialysis unit. During this visit, we will give the participants a physical activity monitor to be worn for at least 5 consecutive days and will obtain baseline blood samples. During a second study visit at the dialysis unit (Day -1), we would obtain a second baseline blood sample one to three days before the administration of the first dose of the study drug. In this visit, we will also measure



muscle oxygenation using near-infrared spectroscopy (NIRS). Patients will be then asked to come to the Vanderbilt Clinical Research Center (CRC) on a non-dialysis day. During this visit (Day 0), we will obtain a detailed history, perform a physical examination, administer a quality of life (QOL) questionnaire, obtain the dialysis recovery time, and evaluate body composition and physical performance. We will then administer the first dose of the study drug and monitor the appearance of any Type I (immediate) hypersensitivity reaction for two hours before discharging patients from the CRC. We will repeat the same protocol, as outlined for Day 0 and administer the second dose of the study drug two weeks later at the CRC (Day 14).

The next two consecutive dialysis treatments after each drug administration (days 1 and 3, and days 15 and 17), we will conduct follow-up visits at the dialysis unit to collect information regarding symptoms after discharge from the CRC. During these visits, at the end of every hemodialysis session, we will also inquire, through a standardized questionnaire, about the presence of any symptoms associated with hypotension. This questionnaire will include symptoms such as cramps, dizziness, nausea, and will be scored according to the severity from 0 to 4 (none to severe). We will also collect blood samples at the dialysis unit for pharmacokinetics and special laboratory analysis. During the six-week length of the study, we will monitor the occurrence of DIH episodes and changes in blood pressure in every dialysis session. For this purpose, we will use the dialysis clinic blood pressure monitor logs. We will also conduct two additional visits at the dialysis units (Day 29 and 43) to draw blood, administer the symptomatology questionnaire, and measure blood flow with NIRS. In the final hemodialysis visit (day 43) we will also give the patients an activity monitor that will be collected after five consecutive days of continuous use. During this last visit, we will also administer the QOL questionnaire, obtain the dialysis recovery time, and perform body composition and physical performance tests (except for the six-minute walk test). Details of the study protocol can be found in Figure 5, Tables 1 and 2, and Appendix 1

Hemodialysis

All subjects will be dialyzed with their usual prescription. The duration of the dialysis treatment will vary between subjects, but it will be the same as the usual standard of care. Dialysis will be performed with biocompatible polysulfone membranes (Fresenius F80-A, Walnut Creek, CA). We will use new dialyzers on each study day. Dialyzers will be stored for further analysis of the proteins adsorbed to the membrane. Crit-Lines®, which monitors hematocrit, percent change in blood volume, and oxygen saturation during hemodialysis, will be applied every hemodialysis session during the study if the device is available and the nursing staff is trained appropriately.

Table 1. HD study visit
• Activity monitor (Day -7 and 43) • NIRS (Days -1, 15, 29, and 43) • Symptomatology questionnaire • Blood draw at the **venous and arterial sites**: t=0, t=15min, t=1h, t=end of HD for markers of kallikrein-kinin system and PK

Collection of blood samples

Blood samples will be collected for clinical laboratory analysis in the pre-dialysis blood sample. Blood samples at the dialysis unit will also be obtained at the arterial and venous sites before dialysis, 15 minutes and one hour after dialysis initiation, and at the end of hemodialysis for research labs (e.g., markers of the kallikrein-kinin system) and pharmacokinetics analysis.

Hemodynamic measurements

During the baseline visit, we will obtain three supine blood pressure measurements. During hemodialysis study days, blood pressure will be taken in the arm contralateral to the access for dialysis using an automated oscillometric recording device. We will obtain three measurements during a period of 15 minutes before starting dialysis. The average of these measurements will be considered the baseline blood pressure. During hemodialysis, we will obtain blood pressure every 15 minutes. At the end of hemodialysis, we will obtain two more blood pressure measurements that will be average to obtain the post-dialysis blood pressure

Table2. CRC study visit
• Physical exam and history • Body composition - physical performance tests • QOL questionnaire/recovery time • Study drug administration

Physical performance tests

Physical performance tests will include the six-minute walk test (6MWT), physical battery (gait speed and chair stand tests), and the handgrip strength, and they will be performed as previously described.[41-43] Briefly, the 6MWT consists of instructing the patients to walk back and forth on a 30-meter corridor. The distance will be recorded in meters. Handgrip strength will be assessed in both hands using a dynamometer. The mean from three consecutive efforts will be used for the analysis.

Repeated chair stands: The participants will be asked to move from a sitting position to a standing position on a 42-cm high chair as quickly as possible five times. Time to complete the five repetitions will be recorded.

Gait speed: it will be performed on a 20-meter straight path, with 5 m for acceleration, 10 m for steady state walking, and 5 m for deceleration. Time will be measured by a stopwatch as the patient reaches the markers and the end of the path. The physical battery will be evaluated using standardized tests, and the results will be scored accordingly to complete the assessment of physical performance.

Quality of life (QOL) questionnaire

In addition, we will evaluate the overall QOL (physical function and health status) using the Kidney Disease Quality of Life-Short form (KDQOL-SF) version 1.3.[44] The KDQOL-SF is a self-report questionnaire of 24 items designed for individuals with CKD on MHD. Each answer was scored on a 0 to 100 range; a higher score correlates with a better health state and physical function.

Body composition

We will measure body composition using the bioelectrical impedance analysis (BIA) and skinfold thickness, as previously described.[45] BIA will be measured using a standard single frequency (50 KHz) bioelectrical impedance analyzer. For this purpose, four electrodes will be placed on the foot after cleaning the skin with alcohol pads. A small current (~800 uA) will be passed through the electrodes and the body. Total body water and the percent of body fat will be calculated by the analyzer according to the predetermined regression equations. Mid-upper arm muscle circumference (MUAMC) measured. This estimates the circumference of the bone and muscle portions of the upper arm; which in turn helps estimate body fat. Skinfold calipers (Lange) will be used to measure the tricipital skin folds. The circumference of the upper arm will also be measured.

Near-infrared spectroscopy

Skeletal muscle tissue oxygenation will be measured, when possible, using near-infrared spectroscopy (NIRS), as previously described.[46] For this purpose, a rigid emitter-detector device will be placed over the belly of the quadriceps muscle. The device transmits near-infrared light at two wavelengths (735 and 810 nm) and will detect the reflected light to measure oxygenated hemoglobin and deoxygenated hemoglobin. The regional oxygen saturation, which is a measurement of tissue oxygenation, will be calculated as the ratio of oxygenated hemoglobin to the total hemoglobin. Skeletal muscle oxygenation will be measure before and during the hemodialysis session.

Dialysis Recovery Time

We will ask the patients "How long does it take to recover from a hemodialysis session", as described in a previous study.[18] This information will be collected during the follow-up phone call or the visit at the next regular hemodialysis session. The answer will be recorded in minutes. We will convert the following answers to numbers:1. One day = 1440 minutes; 2. More than one day = 2160 minutes, or 3. Half-day or next day = 720 minutes

Symptoms questionnaire

We will conduct a post-dialysis symptoms questionnaire at the next regular dialysis session at the Vanderbilt Dialysis Clinic, as previously described.[16] The symptoms will include, but not limited, to complaints associated with a reduction in blood pressure such as cramps, dizziness, nausea, vomiting, yawning. We will score the severity of the symptoms using a scale from 0 to 4 (0=none, 1=minor, 2=mild, 3= moderate, 4=severe).

7.0 Risks

a. Risks to human subjects

1. Redness, swelling, burning, rash, or discomfort may occur at the site of the lanadelumab injection. Should this occur we will put ice on the site.
2. Lanadelumab may increase the risk of upper respiratory infections. Cold symptoms such as runny nose, sore throat, and sneezing are commonly observed. We will closely monitor the occurrence of those symptoms, and if necessary, we will stop the study.
3. Other side effects of lanadelumab include headache, muscle pain, and diarrhea. These side effects are uncommon but will be monitored during the study. If any of them persist, the patient will be withdrawn from the study.
4. Lanadelumab may induce elevation of transaminases, which is rare and transient. We will closely monitor the transaminase levels, particularly after the administration of the study drugs.
5. Lanadelumab may pose unforeseeable risks to the subject, an embryo or fetus, or a pregnant woman. Pregnant women are excluded.
6. Should new information become available during the study that may influence a subject's willingness to participate, we will make that information available and amend the consent form appropriately.
7. The collection and storage of DNA for genotyping creates the risk of release of information that could link subjects to stored samples and genotyping results.

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

Protection against risk

- Investigators will obtain a detailed medical history to look for the exclusion and inclusion criteria. Patients will also undergo a complete physical examination prior to being included in the study. The assessment will include a thoughtful evaluation of the cardiovascular system, including blood pressure.
- Patients will be monitored for the presence of Type I hypersensitivity reaction after the administration of the first study drug. For this purpose, the patient will remain in the CRC for two hours after the administration of the study. If the patient presents this type of reaction, proper treatment will be administered in the CRC. Also, the patient will not receive the second dose of the study drug and will be withdrawn from the study.
- Baseline laboratory analysis and metabolic panels will be obtained to screen for any hematological or biochemistry abnormality.
- A resting electrocardiogram (ECG) will be obtained during the CRC visits to look for the presence of abnormal ST segments, T-wave inversion, or cardiac arrhythmias.
- Diagnosis of myocardial infarction will require the presence of at least two of the following criteria: typical chest pain with or without radiation, electrocardiogram compatible with myocardial infarction, and elevation of cardiac enzymes (creatine kinase MB or troponin). Stroke will be defined as new and sudden onset of neurological deficits that lasts longer than 24 hours.
- We will review blood pressures obtained at the dialysis center. If at any time the pre-dialysis systolic blood pressure exceeds 189 mmHg or diastolic blood pressure exceeds 114 mmHg, blood pressure will be closely monitored. In the case of uncontrolled high blood pressure, subjects will be discontinued from the study.
- A serum **pregnancy test** will be obtained the day of the study drug administration in all women with childbearing potential to exclude pregnancy.
- Serum electrolytes will be measured before study visits in blood draws for clinical labs (See Appendix A). Also, we will monitor potassium levels using the monthly labs obtained in the hemodialysis unit. For serum potassium from 5.5-5.9 mmol/L, the dietary restriction will be emphasized, and the potassium content of the dialysate will be adjusted to decrease serum potassium. Predialysis serum potassium of 6.0-6.4 mmol/L, if confirmed and not corrected with adjustment of dialysate potassium concentration, will result in discontinuation of the study. Confirmed predialysis serum potassium of 6.5 mmol/L or greater will result in immediate discontinuation. All potassium measurements will be obtained before dialysis.
- Transaminases levels will be monitor after the administration of the first study drug. If the levels increase four times the upper limit of normal the patient will be excluded from the study and will not receive the second dose of the study drug.

Also, if the levels of the transaminases do not return to normal within one week after the elevation, the patient will not receive the second dose and will be excluded from the study.

- There are many safeguards in place to prevent the release of information from this study. All research samples are coded with the subject's unique identifier. Data sets used for the analysis also only contain this identifier. The key to the code is protected. Only the investigator, co-investigators, and research nurse have access to information that identifies subjects as participating in the study. The results of tests run on research samples will not be recorded in any subject's medical record, and neither the subject nor his or her doctor will be told of the results, except in the case of unsuspected significant arrhythmia or myocardial infarction. Access to the Vanderbilt computer network is protected at the level of firewalls, TCP wrappers, and University assigned user IDs. Data are secured with encryption algorithms, and the network is maintained by the Medical Center's Network Computer Service.

Data and Safety Monitoring Plan

The principal investigator will be responsible for ensuring the data integrity and safety of all the study participants. All the adverse events will be recorded and reported to the Vanderbilt University Medical Center Institutional Review Board (IRB). The study progress report will be prepared every 6 months to describe: the screening and recruitment process, demographic of the participants, status and occurrence of adverse events (AEs), drug toxicities, treatment adherence, and any incident of non-compliance with the protocol. These reports will be prepared by the principal investigator. The reports will be presented to the Data and Safety Monitor (DSM) committee. The DSM may choose to perform an interim analysis; however, it is expected that this would not occur without reasonable concern related to either patient safety or data validity. The DSM may choose to become unblinded; however, it is expected that such unblinding would not occur without reasonable concern related to either patient safety or data validity.

Protocol changes will be reported to the IRB and will not be implemented until approval is obtained.

The DSM will objectively review the treatment results as they relate to human safety and data quality. Drs. Italo Biaggioni, Kerri Cavanaugh, Jonathan Mosley, and Thomas Stewart have agreed to be members of the DSM committee. Dr. Italo Biaggioni, Professor of Medicine and Pharmacology and Director of the Vanderbilt Autonomic Dysfunction Center, with extensive experience in human research and clinical trials, has agreed to take the role of DSM committee chair. Dr. Cavanaugh is Associate Professor in the Division of Nephrology who focuses on patient education and awareness regarding chronic kidney disease. Dr. Mosley is Assistant Professor in the Division of Clinical Pharmacology with background in epidemiology and bioinformatics. Dr. Stewart is a biostatistician with extensive experience as a consultant with the Department of Medicine. None of the members of the DSM committee have any conflict of interest, whether financial or intellectual with the investigator or the sponsor of the study.

All protocols have been or will be reviewed and approved by the Vanderbilt University Medical Center IRB before any subject is enrolled.

The principal investigator and co-investigators will closely oversee the protocol in

conjunction with the dedicated research nurse. Any AEs or toxicities will be reported to the IRB as per IRB guidelines. Any untoward medical event will be classified as an AE, regardless of its causal relationship with the study. An adverse event will be classified as serious if it a) results in death, b) is life-threatening, c) requires inpatient hospitalization or prolongation of existing hospitalization, d) results in persistent or significant disability or incapacity, or e) is a congenital anomaly or birth defect. Serious adverse events will be reported to the DSM, the IRB, the National Institutes of Health (NIH), and the FDA within 7 days of the PI's notification of the event. Non-serious, unexpected adverse events and instances of non-compliance with the protocol will be reported to the DSM and IRB at the time of continuing review.

9.0 Study Withdrawal/Discontinuation

Subjects who develop an adverse event that is not transient (such as persistent nausea, diarrhea, headache, etc.) will have any study drug discontinued and will be withdrawn from the study. Subjects who do not tolerate the first dose of lanadelumab will be withdrawn from the study. Subjects who are withdrawn will be treated and/or followed as appropriate until any symptoms are resolved. If in the opinion of the investigator a subject is non-compliant, the subject will be withdrawn from the study. Subjects may withdraw from the study at any time.

10.0 Statistical Considerations

a. Sample size

The primary outcome will be the change in blood pressure during hemodialysis. For this, we will average blood pressure during hemodialysis of three hemodialysis sessions within the second week of treatment. Secondary endpoints will also be recorded during the second week of treatment and will include the number of hypotensive events, frequency of nursing interventions, average of dialysis recovery times, and average symptoms severity score. Based on our preliminary studies with icatibant and assuming a standard deviation of differences of 13.6 mmHg (pooled SD of the delta), a sample size of 16 in each arm will have 80% power to detect a difference in means of -13.7 mmHg, using a paired t-test with a 0.05 two-sided significance level.

b. Data analysis plan

Standard graphing and screening techniques will be used to detect outliers and to ensure data accuracy. Distributions of the continuous outcomes will be assessed for normality. If normality is violated, proper data transformation will be applied, or non-parametric analysis methods will be considered. The primary endpoints are SBP and DBP. The secondary endpoints include heart rate, and the R-R interval (autonomic function), the time to recover after HD, symptoms associated with DIH, and the distance walked during the six-minute walk test.

Mixed effect models will be used to analyze the primary endpoint (blood pressure) with a random subject effect and with the treatment (lanadelumab vs placebo) and the time trend (measurements every 15 minutes) as fixed effects. The focus of this study will be the treatment effect and the time trend of the endpoints; however, mixed effect models also provide the flexibility of controlling for and evaluating covariates. However, we will be careful not to over-fitting the model due to the limited sample size.

Besides the above evaluation of the treatment effect and the time trend through the regression models, the direct treatment effect will also be estimated as within-subject mean difference along with their 95% confidence intervals. For the secondary endpoints, a paired t-test will be performed (lanadelumab vs placebo). If the normality of the data is violated, we will use non-parametric tests.
For binary endpoints (e.g., occurrence of hypotensive events), we will use the McNemar test to compare lanadelumab and placebo.
Missing Data. Based on our previous studies, we anticipate a drop-out rate of 10% or less. Subjects who drop out will be replaced. Specific inferences on effects of interest will be made by reporting a point estimate along with a 95% confidence interval and the p-value. Hypotheses will be tested at the level of $\alpha=0.05$. We will use SPSS (version 25.0, SPSS, Chicago, IL) and the open-source statistical package R (R Core Team, 2017) for analyses.[47]

11.0 Privacy/Confidentiality Issues

We will use the web-based Vanderbilt Research Electronic Data Capture (REDCap) system to design electronic data-collection forms in all Aims. These forms will be pilot tested before use. Data will be input into a protected, web-based case report form (which can be readily downloaded into SAS, STATA, R, or SPSS). The form allows for direct data entry by investigators and is designed to minimize errors and erroneous values. Expected ranges are pre-specified to prevent errors such as the shifting of decimal points. The program includes a computerized audit trail so that the identity of individuals entering or changing data and, in the case of changes, both original and revised data are saved. Data are backed up daily.

A unique identification case number will be used to protect the confidentiality of the study participants. Only case numbers will be included in spreadsheets used for the statistical analysis.

12.0 Follow-up and Record Retention

All research records will be accessible for inspection and copying by authorized representatives of the IRB, federal regulatory agency representatives, and the department or agency supporting the research. All study documents will be retained for at least six years after the closure of the study with the IRB.

Appendix A Summary of study procedures

	+/-3 days				+/-4days						
	Day-7	Day -1	Day 0	Day 1	Day 3	Day 13	Day 14	Day 15	Day 17	Day 29	Day 43
CRC visit			X				X				
HD visit	X	X		X	X	X		X	X	X	X
Blood draw PK		X		X	X			X	X	X	X
Blood draw clinical labs	X				X	X			X	X	
Blood draw research labs		X		X				X			X
Serum pregnancy test			X				X				
Drug administration			X				X				
Medical history /PE			X								
QOL questionaire/ recovery time			X				X				X
Symptoms questionnaire/ BP monitoring log				X	X			X	X	X	X
NIRS		X						X		X	X
Physical performance tests - Body composition***			X				X				X***
Collect dialyzer		X		X	X			X	X	X	X
activity monitor*	X*										X*

* monitor ends after at least 5 days of consecutive use
**It includes: physical performance tests (six-minute walk test, physical battery, and handgrip strength), bioelectrical impedance analysis, and MUAMC
***Physical performance will only include handgrip strength

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