

Effect of Evolocumab on Coronary Endothelial Function

NCT03500302

August 25, 2017

1. Objectives (include all primary and secondary objectives)

To evaluate the effect of the PCSK9 inhibitor evolocumab on coronary endothelial function, systemic biomarkers of inflammation, and LDL cholesterol in people living with HIV (PLWH). Potential participants will be asked to undergo a screening MRI exam. Those who have evidence of coronary endothelial dysfunction on the MRI exam will receive evolocumab 420 mg sq (the dose that is approved for treatment of hypercholesterolemia) following the screening exam and again at one month. The evolocumab will be administered by the Johns Hopkins Investigational Drug Service in the HIV+ group. Repeat MRI measures of coronary endothelial function, and serum markers of endothelial function and inflammation will be obtained at one and six weeks following the first administration of evolocumab.

We will test the hypotheses that PCSK9 inhibition improves endothelial function measured non-invasively on MRI and systemic markers of inflammation at one week and six weeks after initiation of the PCSK9 antibody in PLWH.

Primary Endpoint:

Coronary endothelial function at 1 week and 6 weeks following initiation of PCSK9 inhibitor administration; specifically, change in coronary artery cross sectional area (CSA) from that at rest to that during isometric handgrip exercise (IHE) stress (as mm² and as % rest) at 1 week and 6 weeks. We will be evaluating the change in endothelial function from baseline to one week and from baseline to six weeks.

Secondary Endpoints:

1. Change in coronary blood flow (CBF) from rest to that during IHE stress (% rest and as ml/min) from baseline to one week and from baseline to six weeks.
2. Change in inflammatory markers (e.g. hsCRP, IL-6, soluble) and lipids from baseline to one week and from baseline to six weeks.

Also, the relationship between change in inflammatory markers/lipids over time and change in CEF will be quantified and compared.

2. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Increased systemic inflammation and altered lipid metabolism are two factors which play important roles in atherogenesis, both in the general population and in HIV+ individuals. Lipid abnormalities are common in HIV infected individuals and may contribute to the increased cardiovascular risk in this population. HIV infection itself and its treatment with antiretroviral therapy can alter lipid metabolism and both are associated with higher LDL cholesterol and triglyceride levels. In addition, HIV infection is marked by increased systemic inflammation, which correlates with the presence and extent of

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atherosclerosis, and although systemic inflammatory markers are reduced by ART, they are not reduced to normal levels¹⁵⁻¹⁸. Although statins have antiinflammatory properties and lower CV mortality in the general population, their use is often problematic in the HIV population due to frequent drug interactions and concurrent liver disease. Importantly, CV event rates remain high in HIV+ patients on statin therapy^{19, 20} and statins alone often do not adequately suppress inflammation in many patients^{20, 21}. Therefore, new therapies are needed to target the fundamental underlying pathophysiologic mechanisms, namely increased inflammation and altered lipid metabolism contributing to HIV-mediated atherosclerotic disease. Finally, although traditional imaging methods document the anatomic extent of coronary atherosclerosis, there was no prior safe and non-invasive way to probe the critical early mechanisms contributing to CAD, namely coronary endothelial dysfunction. However, recent advances by our research group now make it possible to non-invasively quantify coronary endothelial function (CEF) and therefore better understand the role of inflammation in coronary atherosclerosis.

3. Study Procedures

- a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

The following are research procedures:

HIV positive subjects:

All of the procedures described below are research procedures.

Research study of the effect of PCSK9 inhibition in 20 HIV+ subjects (with LDL cholesterol > 70 mg/dl) involving (1) subcutaneous administration of two doses of evolocumab, (420 mg, one following baseline measures of coronary endothelial function and inflammatory mediators and the second one month following the first dose). For this population, we had an FDA exemption for the off label use of evolocumab in this study, and the medication will be provided and administered by the Johns Hopkins Investigational Drug Service. HIV+ participants on stable, clinically-guided ART and meeting entry criteria will undergo baseline assessment of serum inflammatory mediators, as well as PCSK9 activity, lipids, MRI quantification of local coronary endothelial function (CEF). Those subjects with abnormal CEF, defined by an increase in coronary CSA during IHE of $\leq 5\%$ from the resting value, will qualify and enter the study. Subjects will then receive evolocumab 420 mg sq once after baseline studies and will return for repeat clinical assessment, repeat imaging, and blood draw 1 week after receiving the dose. Four weeks after receiving the first dose, they will receive a second evolocumab dose, with repeat imaging, blood draw, and safety analysis performed at week 6 after the first dose (2 weeks after the second administration). There will be a final safety assessment performed at week 10 (6 weeks after final dose given).

Initial evaluation: A careful history and physical examination will be performed. Blood samples will be acquired for complete blood cell count, routine chemistry panel including hepatic transaminases and creatinine, LDL and HDL cholesterol and triglycerides. Serum markers reflective of inflammation and endothelial function will include serum hsCRP, IL-6, TNF α , IFN- γ , and PCSK9 level. Patients will also be tested for hepatitis C

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(Hepatitis C Antibody test) if Hep C status is unknown from clinical records. If the patient is found to have positive reactivity to Hep C Antibody or is previously known to be Hep C positive (from clinical chart review using same criteria), then HCV RNA level in blood will be quantified as part of research procedure.

MRI methods for Coronary Vasoreactivity: an index of CEF: Patients will undergo a detailed baseline MRI study of CEF in the fasting state using MRI methodology at rest and during continuous IHE as previously described²⁸. The MRI study will be used to measure coronary cross-sectional area (CSA), coronary flow velocity (CFV), and coronary blood flow (CBF) changes in response to IHE stress (continuous isometric handgrip for 4-8 min at 30% of each subject's maximum, determined prior to entering the MRI), as previously reported^{29, 28}.

Fasting: Subjects will be asked to fast after midnight and delay eating breakfast and taking morning cardiovascular medicines until after the study procedure, which will be completed by noon.

MRI analysis: MR images will be analyzed in blinded fashion without operator knowledge of time or treatment group for CEF (e.g. changes in CBF, CBV, and CSA) as previously validated and described²⁸. Baseline, 1-week and 6-week images will be analyzed at the same time for each subject by investigators blinded to time point of study to reduce variability and assure identical segments are analyzed at each time.

Blood Draw and Analysis: Blood samples will be obtained from a peripheral vein using standard venipuncture techniques and will be sent for routine chemistries (basic metabolic panel and liver function tests, CBC) and lipids. Biomarkers will be analyzed from blood specimens collected into collection tubes without anticoagulant and centrifuged within 1 hour after collection (to allow time for clotting) using a centrifuge with an integrated refrigeration system at 4°C/1000 g for 15 min and stored at -80°C. Analyses will be performed with ELISA or multiplexed ELISA including the following biomarkers of inflammation/activation/clotting: hsCRP, IL-6, TNF α , IFN- γ , PCSK9 activity and CD163.³² In addition, comprehensive lipid panel will be measured. To minimize variability of assays of longitudinal markers, the baseline, 1- and 6-week specimens of each individual will be analyzed on the same plate.

Safety surveillance: Patients will undergo surveillance safety monitoring at 1 week, 4 weeks (before 2nd dose of PCSK9 inhibitor is given to HIV+ participants) and 6 weeks that will include detailed history, physical exam and complete blood count. In the HIV+ group, liver and renal function tests will additionally be performed, and there will be a 10 week safety follow up performed by phone 6 weeks after the last dose of drug is given. Members of the research team will review laboratory results from a safety standpoint.

Follow up End-point Evaluation: After 1 week of the first dose and at 6 weeks after the first dose (and two weeks after the second dose) of PCSK9 inhibitor administration, subjects will undergo repeat clinical evaluation, lipid and inflammatory biomarker analysis, as well as coronary MRI with the same protocols used at baseline. In terms of MRI follow up exams (at weeks 1 and 6) coronary MRI will be repeated with an identical protocol and special attention taken to interrogate the same coronary segments as those studied at

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baseline, using anatomic landmarks of coronary ostia and branch vessels- as we have done in the past.

List of study procedures (HIV+ group):

1) **Visit 1 (Screening Visit):** includes Informed Consent, History and physical examination, vital signs (temperature, blood pressure, heart rate, weight, height), blood draw (CBC, CMP, Lipid panel, Hepatitis C Antibody Test if HCV status is unknown, HCV Quant PCR if known HCV positive, serum biomarkers of inflammation (above). MRI Screening questionnaire, cardiac MRI, If patient qualifies (based on MRI and entry criteria), the subject will receive PCSK9 inhibitor (this will be administered by a qualified study team member)

Visit 2 (Baseline Visit) Subject will return for medical history reviewed for changes, concomitant medications, vital signs, 1st Dose of Evolocumab will be given by study team.

Visit 3 (1 Week after Evolocumab is started): Subject will return for physical examinations, concomitant medications, vital signs (temperature, blood pressure, heart rate, weight), monitor for adverse events, blood draw (CBC, CMP, Lipid panel, serum biomarkers of inflammation/activation/clotting: hsCRP, IL-6, TNF α , IFN- γ , PCSK9 activity), MRI Screening questionnaire, cardiac MRI

Visit 4: Subject will return 4 weeks after initial dose for safety assessment and to receive 2nd and final dose of evolucumab, review concomitant medications, and monitor for adverse events, measurement of vital signs (temperature, blood pressure, heart rate, weight).

Visit 5 (6 Weeks after Evolocumab started) Subject will return 6 weeks after initial PCSK9 inhibitor given for physical examination, concomitant medications, vital signs (temperature, blood pressure, heart rate, weight), monitor for adverse events, blood draw (CBC, CMP, Lipid panel, serum biomarkers of inflammation), cardiac MRI. Subjects will receive a phone call follow for safety assessment at week 10.

Early stopping rules

Participants who experience significant symptoms or who develop significant laboratory abnormalities will be evaluated and the scheduled second (final) study drug dose withheld. Participants who experience significant symptoms will be evaluated and those or others who develop new laboratory abnormalities will be reviewed and labs possibly repeated. If any of the following criteria are met (CBC<2000, platelets<50,000, Hct<27%, GFR <30ml/min or a >50% reduction in GFR compared to baseline values, and LFTs >3x upper limit of normal), the test would be repeated. If confirmed on repeat, the second (and final) dose of the drug would not be given (HIV+ group). If the abnormality resolves, study drugs would be given at the same or lower dose. If the abnormality does not resolve, then the case will be reviewed for discontinuation from the study. Based on the clinical status, severity of the problem, and reversal of symptoms/laboratory findings, a decision will be made as to whether the study drug (2nd and final dose) can be started under close observation (weekly surveillance) or the patient withdrawn from the study. For every study participant who withdraws, we plan to recruit an additional subject so that the overall sample size of those completing the study is close to 20-25 subjects in each group.

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Participants who wish to end participation in the trial at any time may do so upon request without penalty.

- b. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.

Participants withdrawn from receiving the scheduled second (final) dose of evolocumab due to an adverse experience will be followed by the Investigator until the outcome is determined. Every effort will be made to follow the subject for the full study period as per the schedule of study visits.

4. Inclusion/Exclusion Criteria

Inclusion Criteria (HIV positive):

- A. Participants of either gender who are ≥ 21 years of age (no upper age limit),
- B. HIV positive and taking stable ART (no change in ART regimen in last 3 months)
- C. Clinically controlled HIV viral load (plasma HIV RNA concentration ≤ 100 copies/mL)
- D. Abnormal CEF on MRI at baseline ($\leq 5\%$ change in CSA during IHE as compared to resting value).
- E. Lipids at screening visit: Fasting LDL-C > 70 mg/dL; fasting TG < 500 mg/dL
- F. Permission of attending physician

Exclusion criteria:

- A.** Patients unable to understand the risks, benefits, and alternatives of participation and unable to give meaningful consent,
- B.** Patients with contraindications to MRI such as implanted metallic objects, cardiac pacemakers, and cerebral clips,
- C.** History of a recent cardiovascular or cerebrovascular event or procedure (e.g. myocardial infarction, stroke, transient ischemic attack, angioplasty, CABG surgery) during the past 90 days.
- D.** Subjects with prior exposure to evolocumab or another PCSK9 inhibitor.
- E.** Pregnant women or breastfeeding women. Women of childbearing potential (even if using oral contraceptive agents) or intention to breastfeed. Pregnancy status will be determined by performing a urine pregnancy test.
- F.** History of alcoholism or drug addiction according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria within 12 months prior to screening. Use of any recreational drugs within 6 months prior to screening.
- G.** Renal impairment defined by estimated glomerular filtration rate < 45 ml/min.
- H.** Moderate-severe hepatic disease (elevation in hepatic transaminases > 3 x upper limit of normal) or direct bilirubin > 3.0 X ULN at screening.
- I.** CD4 < 200 cell/mm³ in the HIV+ subject group

- J.** Congestive heart failure, New York Heart Association functional class IV.
- K.** Poorly controlled hypertension at screening visit (defined as the average of two systolic blood pressure (BP) measurements greater than 180 mm Hg or the average of two diastolic BP measurements greater than 110 mm Hg).
- L.** History of allergic or anaphylactic reaction to any therapeutic or diagnostic monoclonal antibody (IgG protein) or molecules made of components of monoclonal antibodies.
- M.** Active phase hepatitis. Stable patients with hepatitis B or C infection >2 years before randomization are eligible.
- N.** Subjects who are HCV antibody positive that have detectable HCV RNA levels.
- O.** Latex allergy

5. Study Statistics

- a. Primary outcome variable. Change in endothelial function, specifically, change in coronary cross sectional area (CSA) from rest to that during IHE stress, from baseline to one week and from baseline to six weeks.
- b. Secondary outcome variables.
 - (i) Change in coronary artery blood flow (CBF) (from rest to IHE stress (as ml/min and as % rest) from baseline to one week and from baseline to six weeks.
 - (ii) Inflammatory markers (hsCRP), LDL cholesterol at 1 and 6 weeks and change in these measures between baseline and 1 week and baseline and 6 weeks.

To make full use of the data, changes in CEF from baseline to 1- and 6-weeks will be analyzed, changes in inflammatory biomarkers and lipids (hsCRP and LDL cholesterol) from baseline to 1- and 6-weeks. Also, the relationship between change in inflammatory markers and lipids from baseline to 1 and to 6 weeks and change in CEF between baseline and 1 and 6 weeks will be quantified.

Safety endpoints: metabolic panel and complete blood count, withdrawal due to sideeffects SAEs and all AEs

- c. Statistical plan including sample size justification and interim data analysis.

This is a pilot study to understand the effect size, if any, of the initiation of PCSK9 antibody on coronary endothelial function (CEF) and the relationship between any change in CEF and change in inflammatory mediators. However, it is possible, based on prior studies conducted by us and others that a significant change in CEF will be observed and it is very likely that we will observe changes in inflammatory markers and mediators over the six week study period. The principal outcome of this observational study of the effects

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of evolocumab on CEF and inflammatory mediators in subjects with abnormal CEF is the change in CSA from rest to IHE at 1 and 6 weeks. We chose to study the change in CSA because it 1.) reflects both macrovascular changes related to the endothelial-dependent IHE stressor, 2.) CEF reflected by CSA change is significantly depressed in HIV+ subjects compared to healthy subjects 3.) is reproducible over this proposed time period, and 4.) because endothelial function is an independent predictor of atherosclerotic progression and clinical events³³⁻³⁷. The 6 week time was chosen to minimize confounding events occurring over longer times and because improvements in endothelial function are present in as short as 1 day to 6 weeks following the initiation of statin³⁸. We will also collect CEF measures at 1 week to evaluate safety and detect whether a short term acute CEF effect is present. Drugs that improve cardiovascular outcomes improve endothelial function in fewer than 8 weeks³⁹. An increase in the IHECSA response in subjects to approximately 30% of that of healthy subjects does not assume complete normalization of CEF in participants with evolocumab but, instead, an increase that would be biologically significant and consistent with the changes in endothelial function observed with medications shown to reduce cardiovascular outcomes. For example, a landmark study demonstrated that statins improve CEF in CAD patients to approximately half of normal/maximal responses⁴⁰. In studies of subjects with CAD risk factors, comparisons are typically made with baseline or placebo measures and such studies consistently show that statins and ACE inhibitors improve abnormal endothelial function by ~50% or more in CAD patients⁴¹⁻⁴⁴. A similar 50% increase in endothelial function was also observed in HIV+ people treated for 8 weeks with statins⁴⁵. Thus based on the existing literature of the impact of established cardiovascular medications (statins, ACE-I) on endothelial function and based on our observations of differences in coronary endothelial function between HIV+ and HIV- individuals (CSA increases during IHE in healthy subjects $+12\% \pm 5\%$ (mean \pm SD) and does not increase in HIV+ or CAD patients, change of $-2\% \pm 6\%$ (mean \pm SD), we propose that there will be an increase to 4% CSA change with IHE (i.e. approximately 33% of the normal 15% increase in CSA with IHE we observe in healthy individuals) in the evolocumab-administered subjects. We assume that at 6 weeks:

- (1) Mean CSA will increase from -2% change from rest to IHE in subjects at baseline to +4% CSA change on evolocumab at 1- and 6 weeks.
- (2) Mean CBF will increase from -3% (CBF change from rest to IHE) in subjects at baseline to +15% (from rest to IHE, which represents improvement of CBF to about 1/3 of normal "healthy" CBF response) in subjects on evolocumab at week 1 and week 6.
- (3) Standard deviation (variability) of 6% for study subjects

The variability is conservatively based on the mean standard deviation in CSA response observed in the HIV+ population and in CAD subjects. With a sample size of 18, we will have power of 90% ($\alpha=0.05$, two-sided test) to detect such a difference in CSA-IHE response between the baseline and study follow up time points (1- and 6 weeks)⁴⁶. We will conservatively assume a 10% dropout rate over 6 weeks due to unwillingness to repeat MRI study. Therefore, we plan to study 20 participants.

d. Early stopping rules.

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Participants who experience significant symptoms will be evaluated and those or others who develop laboratory abnormalities will have the study drug withheld. Based on the clinical status, severity of the problem, and reversal of symptoms/laboratory findings, a decision will be made as to whether the study drug can be restarted under close observation (weekly surveillance) or the patient withdrawn from the study. Participants who wish to end participation in the trial at any time may do so upon request without penalty. Because this is a short pilot study, we will attempt to recruit an additional subject for any that withdraws so that our target sample size of n=18 of those completing the study is met.