PCSK9 Inhibition After Heart Transplantation

Study Protocol and Statistical Analysis Plan

NCT03537742

January 14, 2021

CLINICAL PROTOCOL SYNOPSIS

Brief Title:
PCSK9 Inhibition after Heart Transplantation

Focus of the Study:
The focus of this study is to test the safety and efficacy of the PCSK9 inhibitor, alirocumab when administered early after heart transplantation (HT).

Objectives:
The main objective of this project is to test the safety and impact on cardiac allograft vasculopathy (CAV) of alirocumab when given early after HT. In this process, we will also characterize the pleiotropic effects of alirocumab, in particular focusing on its effects on lipoprotein (a) [Lp(a)], apolipoprotein B (apoB), triglyceride rich lipoproteins (TRL), and C-reactive protein (CRP) and their relationship to development of CAV and abnormal coronary physiology and endothelial function. A secondary objective is to demonstrate the value of invasive coronary physiologic and functional parameters for predicting development of CAV, independent of intravascular ultrasound (IVUS) findings.

Study Design:
This is a prospective, randomized, placebo-controlled, double-blind phase II study.

Intervention to be Tested:
Alirocumab will be compared to placebo starting within the first 8 weeks after HT and continued for one year. Cardiac transplant recipients will undergo baseline laboratory studies, coronary angiography, IVUS, coronary physiology (fractional flow reserve (FFR), coronary flow reserve (CFR), and the index of microcirculatory resistance(IMR)), and coronary endothelial function assessments within the first 8 weeks after HT. Subjects will then be randomized to alirocumab or placebo. Laboratory studies will be performed at 3, 6 and 12 months. At one year coronary angiography, IVUS, coronary physiology, and coronary endothelial function assessments will be repeated.

Primary and Important Secondary Endpoints:
The primary endpoint of this study is the change in plaque volume from baseline to one year as measured by IVUS in the alirocumab treated patients compared to the placebo treated patients. The effect of alirocumab on LP(a), apoB, TRL and CRP measured at one year and its relationship to CAV and abnormal coronary physiology and function will also be assessed. A key secondary endpoint is the ability of invasive coronary physiology, namely FFR and IMR, and coronary endothelial dysfunction to predict development of CAV, independent of IVUS findings.

Detailed Milestones:

We are currently preparing our IRB submission, including the informed consent document.

R61 Phase:

Month 1-
- Receive IRB approval if not already received.
- Register trial at ClinicalTrials.gov website.
- Begin construction of REDCap database.
- Begin Manual of Operations.
- Begin to create electronic case report form.

Month 2-
- Finalize case report form on REDCap database.
- Finalize Manual of Operations.
- Finalize DSMB.
- Pharmacy and Sanofi/Regeneron to finalize drug/placebo delivery and storage.

Month 3- ● Steering Committee to begin scheduled meetings to review progress of the study and address any delays.
● Finalize training of the study staff and meet with adult and pediatric heart transplant clinical teams to review protocol and site performance plan.
● Begin screening for enrollment.

Month 4- ● Enroll first patient.

Month 10- ● Prepare communication with NIH regarding status of the study.

Month 12- ● Enroll 20th patient. Goal of enrolling 15% of patients.
● Present status of the study to NIH.

R33 Phase:

Year 2- ● Perform 1 year follow-up evaluations on patients enrolled in Year 1.
● Recruit and enroll 35 patients.
● Assessment of protocol implementation and performance.
● Prepare and submit annual review for NIH.
● Goal of enrolling 50% of patients.

Year 3- ● Perform 1 year follow-up evaluations on patients enrolled in Year 2.
● Recruit and enroll 35 patients.
● Assessment of protocol implementation and performance.
● Prepare and submit annual review for NIH.
● Goal of enrolling 80% of patients.

Year 4- ● Perform 1 year follow-up evaluations on patients enrolled in Year 3.
● Recruit and enroll 30 patients.
● Assessment of protocol implementation and performance.
● Goal to complete enrolment before month 6.
● Prepare and submit annual review for NIH.

Year 5- ● During the first 6 months, perform 1 year follow-up evaluations on patients enrolled in Year 4.
● During months 6-9, complete, clean, and lock the database.
● During months 9-12, analyze the data for each specific aim.
● Prepare abstracts to present at national meetings.
● Prepare manuscripts to publish the primary findings.
● During month 12, prepare and submit final report to NIH.
● Submit final results to ClinicalTrials.gov.

Schedule of Clinical and Laboratory Evaluations:

Time post HT	**Event**
0-8 Weeks	Recruitment and enrollment
1-8 Weeks	Angiogram, endothelial function, FFR, IMR, IVUS and laboratory studies
1-8 Weeks	Randomization to alirocumab or placebo
3, 6,12 Months	Lipid and other laboratory studies
One Year	Angiogram, endothelial function, FFR, IMR and IVUS studies

Study Population:
One hundred and twenty subjects will be recruited soon after HT at Stanford University. The goal is to recruit an equal number of men and women, however more men tend to undergo transplantation. In order to be

included in this study, subjects must be 12 years or older, be within the first 8 weeks of HT, and provide informed, written consent. Younger patients will not be eligible because their coronary arteries are too small for IVUS examination. We will exclude patients who remain hospitalized because of complications from their HT. The Stanford University transplant program has performed approximately 65 heart transplants per year during the past 3 years, with 85% adult recipients and 10-15% recipients who are children over 12 years old. The age, gender and ethnic distribution (%) of heart transplant donors and recipients is summarized below.

	Age	**Gender (F/M)**	**White**	**Black**	**Hispanic**	**Asian/Pac. Isl.**
Donors	2 – 56	30% / 70%	80%	6%	10%	4%
Recipients	12 - 69	35% / 65%	70%	14%	8%	8%

Group Assignment:
A stratified randomization scheme will be applied by age group (≤ 18 years of age vs > 18 years of age) and by gender. Within each age/gender stratum, subjects will be randomized using randomized block sizes of 2, 4, or 6 in an equal and double-blind fashion to either alirocumab (initial dose: 75 mg subcutaneously every 2 weeks) or to placebo and begin treatment the day after their baseline angiogram procedure. Both the patient and the investigators/treating physicians will be blinded to treatment assignment. Implementation of the randomization scheme will be performed by the senior study statistician within the Quantitative Sciences Unit (QSU) at Stanford University under the direction of the statistical investigator, Dr. Desai. The QSU has extensive experience in designing and implementing randomization schemes and has performed this task for numerous clinical trials. Randomization will occur after enrollment and baseline assessment. The alirocumab and placebo will be identical so that the patient and investigators/treating physicians will remain blinded to treatment assignment. Regeneron/Sanofi, the maker of alirocumab will provide the study drug and matching placebo (see attached letter). After a subject has been on alirocumab/placebo for four weeks, the LDL-C cholesterol will be checked. If the LDL-C response is inadequate (LDL-C>70 mg/dL) and there have been no adverse effects, the dosage will be increased to the maximum dosage of 150 mg administered every 2 weeks. The patient/family will be instructed on how to administer the medication while an inpatient. This will be reviewed at the time of clinic visits. The patient/family will administer the medication at home when the patient is discharged from the hospital. Total cholesterol, LDL-C, high density lipoprotein, triglycerides, Lp(a), apoB and TRL will be measured at 3, 6, and 12 months post transplantation during clinic visits, but both the treating physicians, investigators and patients will be blinded to the results until the patient completes the one year invasive study. Patients will be closely monitored for any adverse effects of alirocumab as detailed below. Dr. Josh Knowles is a Co-Investigator on this project who directs Stanford's Familial Hypercholesterolemia (FH) clinic and is the Chief Medical Advisor for the *FH Foundation,* a patient-founded and patient-led, non-profit organization for individuals with FH. He has extensive experience managing patients on PCSK9 inhibitors and will oversee this aspect of our proposal.

Statistical Design and Power:
We will perform an analysis based on ***intent-to-treat principles*** to evaluate our primary outcome. This means we will analyze all subjects randomized and according to their randomized treatment assignment. The primary outcome is plaque volume, and our primary objective is to evaluate ***change in volume of plaque at 1 year post-HT relative to baseline between the two study arms.*** To address the primary objective, we will employ generalized linear mixed effects regression methods. The primary hypothesis will be tested using a two-sided Wald test at the 0.05 level of significance. A secondary goal will be to assess the effect of alirocumab versus placebo on coronary physiology, FFR and IMR, and on endothelial function. Another important secondary aim is to evaluate the mechanisms through which the treatment acts, where potential mechanisms include LDL-C, LP(a), apo(B), TRL and CRP. A final secondary aim is to evaluate the ability of FFR, IMR and endothelial dysfunction to predict clinically meaningful increases in plaque volume one year post-transplant relative to that of plaque volume measured via IVUS at baseline. Secondary analyses will be considered hypothesis generating. Dr. Manisha Desai, a Co-Investigator on this proposal and the Director of Stanford's Quantitative Sciences Unit (QSU), is an experienced clinical trialist and biostatistician who will oversee the statistical analyses.

Primary and Secondary Outcomes

The primary objective of this project is to determine whether PCSK9 inhibition with alirocumab early after HT impacts the development of CAV. For this purpose, we define the primary endpoint as plaque volume, measured by IVUS, in order to compare changes at 1 year post HT relative to baseline between patients in the two study arms. Plaque volume as determined by IVUS is used to assess CAV because we and others feel that a volumetric evaluation is the most accurate assessment of intimal thickening and changes in plaque have been associated with increased long-term mortality after HT.[35,36,67] Secondary endpoints include differences in LDL-C and other lipid particle values between the two arms at 3, 6 and 12 months, differences in coronary physiology (FFR and IMR) and endothelial function between the two groups from baseline to 1 year, and adverse events including mortality and retransplantation.

Specific Aim 1. Measure the safety and impact on CAV of the PCSK9 inhibitor, alirocumab after heart transplantation.

Descriptive Statistics

We will present descriptive statistics on the full analysis set (FAS) by study arm such as means, medians, standard deviations, and interquartile ranges for continuous variables like plaque volume at each time point and plaque change. Frequency distributions for categorical variables such as sex and race will also be provided by study arm. Graphical tools such as histograms and boxplots will be used to assess distributional properties of continuous variables.

Analytic Tools

Using intent-to-treat principles, we will address our primary aim by regressing plaque volume on treatment arm, days since randomization (time) and an interaction between time and treatment arm on the FAS using generalized linear mixed effects regression techniques, where the parameter for the interaction term is of interest and represents the differential progression in plaque volume at one year post-HT between study arms. Such a model will include a subject-specific random effect to allow for correlation of outcome within a subject over time. Using a two-sided Wald test at the 0.05 level of significance, we will assess whether the change in plaque volume differs at 1 year by treatment arm. ***It is important to note that a significant advantage to employing this particular modeling strategy is that it enables us to adhere to intent-to-treat principles by including the full analysis sample and using robust assumptions about the missing data (described in greater detail below).*** Thus, even those who do not provide change in plaque volume are included in this analysis, where the treatment effect on plaque change is estimated for the sample by borrowing strength from those who do contribute data on all time points (no imputation is used in the generalized linear mixed effects model). Our model is expressed as:

$$Plaque\ Volume_{ij} = \beta_0 + \delta_i \quad + \beta_1 Treatment_i + \beta_2 Time_{\ ij} + \beta_3 Treatment_i \times Time_{\ ij} + \varepsilon_{ij}$$

where $Plaque\ Volume_{ij}$ is the plaque volume measurement for the i^{th} subject at the j^{th} time point; $Treatment_i$ is an indicator for whether the i^{th} subject is assigned to the experimental treatment arm; and $Time_{\ ij}$ is the number of days since randomization for the i^{th} subject at the j^{th} time point. This approach has become standard in cardiovascular clinical trials involving longitudinal repeated measures over time such as this one.[61-63] However, as a secondary analysis we will employ a simple t-test to compare the change in plaque volume between study arms among those who complete the study and provide outcomes of change using the modified intent-to-treat analysis set (MITT).

Hypothesis Tests

We will test the primary hypothesis using a two-sided Wald test at the 0.05 level of significance.

Power Considerations

Although there will be some attrition in measures collected over the follow-up period, the initial sample size (i.e., those randomized at baseline) will be maintained in the proposed analysis, as we will adhere to intent-to-treat principles. There may however be an impact of attrition on our power due to the loss in the efficiency (or precision) of the estimate as the variation in our estimate of change in plaque may increase. We will therefore

account for the impact of extra variation in our measure of change by considering a more conservative sample size.

Table 4. Power for addressing Aim 1 assuming 54 subjects per arm

Scenario	Average difference in log-plaque volume progression	Standard deviation of change in log-plaque volume progression	Power
1	0.06	0.10	0.87
2	0.10	0.10	> 0.95
3	0.15	0.10	> 0.95
4	0.06	0.25	0.24
5	0.10	0.25	0.54
6	0.15	0.25	0.87

We have excellent power to address our primary aim. We will randomize 120 patients. While all 120 patients will be included in the analysis according to intent-to-treat principles, we anticipate that 108 patients will complete the study without withdrawal or loss to follow-up. ***To incorporate the impact of attrition on the standard error we considered a smaller sample size of 108 or 54 per treatment arm, yielding conservative estimates of power.*** Based on larger studies in the literature and from our most recent work evaluating 96 patients who had serial IVUS at baseline and at 1 year after HT, we find that the common logarithm (base 10) of plaque volume at either time point to be approximately normally distributed.[50] Assuming a standard deviation of change in plaque volume (on the log-scale) of 0.10, based on empirical evidence, the effective sample size of 54 per arm gives over 85% power to detect a significant difference of change in plaque volume of at least 0.06 on the log scale assuming a type I error rate of 0.05. If the standard deviation of change in plaque volume is considerably higher than previously indicated, say 0.25, we have the same power to detect a difference in change in plaque progression of at least 0.15 on the log-scale.

Specific Aim 2: Identify the pleiotropic effects of PCSK9 inhibition after heart transplantation.

Descriptive Statistics

As in Aim 1, descriptive statistics will be provided on the FAS by study arm such as means, medians, standard deviations, and interquartile ranges for continuous variables including all biomarkers such as LDL-C, lipoprotein (a), apolipoprotein B, triglyceride rich lipoproteins and CRP. Frequency distributions of clinically meaningful categories of these biomarkers will also be presented.

Analytic Tools

To evaluate mechanisms of action, we will continue to rely on the framework of generalized linear mixed effects regression techniques. Importantly, we will test for mediation through the fitting of multiple regression models. Specifically, using the statistical method by MacArthur that modifies the seminal work in this area by Baron and Kenney and is described for a broader audience by Kraemer and others, we will evaluate the interrelationships among treatment, plaque changes and biomarkers such as LDL-C to evaluate whether changes in plaque are mediated by LDL-C, lipoprotein (a), apolipoprotein B, triglyceride rich lipoproteins and CRP.[64,65] The approach involves fitting a series of models to evaluate associations between the treatment and outcome, between treatment and the potential mediator, between the potential mediator and the outcome, and finally, between the treatment and outcome in the presence of the mediator. The presence of these various associations are necessary criteria in concluding that treatment is mediated through a factor. Essentially, for each biomarker or potential mediator of interest, evidence for mediation involves comparing the effect of treatment on plaque progression (b_3) derived from a full model of plaque volume as a function of treatment arm, time, an interaction between treatment arm and time, and the potential mediator of interest (written below with LDL-C as the mediator) to the effect of treatment on plaque progression derived from a model that does not include the mediator (β_3). If the association of treatment arm by time is attenuated in the presence of LDL-C, this will suggest that PCSK9 affects plaque volume by lowering LDL-C. The full model used to evaluate a potential mediator can be described as follows:

$$Plaque\ Volume_{ij} = b_0 + \delta_i + b_1 Treatment_i + b_2 Time_{ij} + b_3 Treatment_i \times Time_{ij} + b_4 LDL_{ij} + \varepsilon_{ij}$$

Analogous comparisons of such models involving the other three mediators will be drawn using these methods. Attenuation in the beta coefficient representing the effect of alirocumab on plaque volume over time of 15% or greater in the presence of changes in a biomarker will suggest a potential pathway through which alirocumab acts. We will then perform identical analyses evaluating models of coronary physiology and endothelial dysfunction.

Power considerations
A sample size of 108 achieves 90% power to detect an R-Squared of as low as 0.07 attributed to each potential mediator we consider, assuming a type I error of 0.05. The term involving the mediator to be tested is adjusted for an additional 4 variables assuming a large R-Squared of 0.20.

Specific Aim 3: Compare the prognostic ability of invasive coronary physiologic and functional parameters to IVUS in cardiac transplant recipients.

Descriptive Statistics
Using the MITT, we will present descriptive statistics on the residuals from our two competing model fits. Using graphical tools, such as histograms and boxplots we will assess the distributional properties of the residuals for assessment of model fit. Further, scatterplots will be used to evaluate whether residuals are systematically related to patient-level characteristics such as age, treatment arm, and other features utilized in the model(s).

Analytic Tools
Using standard linear regression techniques we will fit and compare two competing models that predict plaque volume at 1 year post HT on the MITT (i.e., on the set of subjects who provide an outcome at 1 year). More specifically, the first model will evaluate the ability of FFR, IMR, endothelial dysfunction, and IVUS plaque volume all measured within 8 weeks of HT as well as treatment arm to predict IVUS plaque volume at 1 year post HT. Note that these models differ from the framework above, in that there is only one outcome per subject, and thus a simple regression framework can be employed. The model that includes all features will be expressed as:

$$Plaque\ Volume\ at\ 1\ year_i = \gamma_0 + \gamma_1 FFR_i + \gamma_2 IMR_i + \gamma_3 ED_{ij} + \gamma_4 Plaque\ Volume\ at\ HT_i + \varepsilon_i$$

Using the same techniques, we will evaluate the ability of IVUS plaque volume measured within 8 weeks of HT in the presence of treatment arm to predict IVUS plaque volume at 1 year post HT:

$$Plaque\ Volume\ at\ 1\ year_i = g_0 + g_1 Plaque\ Volume\ at\ HT_i + \varepsilon_i$$

We will compare the predictive ability of the two models using the Akaike Information Criterion or AIC.[66] Such analyses will provide insight into the ability of FFR, IMR and endothelial function to contribute to prediction of clinically meaningful plaque development within a relatively small window after HT, allowing for earlier and more aggressive intervention for a subset of patients. As we anticipate that the treatment will play a large role in arresting plaque progression, we have included it as a term in the predictive model.

Power considerations
We have excellent power to achieve our aims. A sample size of 108 provides 90% power to detect an R-Squared of only 0.05 attributed to the plaque volume measured at baseline alone compared to a model that additionally utilizes FFR, IMR and endothelial dysfunction, assuming a model with all four variables can explain 40 percent of the total variation in plaque volume at 1 year.

Missing Data:
As we propose a prospective clinical trial, we expect (and plan for) a small proportion of subjects to be lost to follow-up, and this will have impact for all three of our aims. Specifically, this poses an issue for adhering to intent-to-treat principles, the gold standard for producing unbiased treatment effects, which stipulate that *all* subjects randomized be included in the analysis and analyzed by their randomized treatment assignment. Because our interest lies in comparing change in plaque volume or plaque progression between study arms, patients who do not provide an IVUS measurement at 1 year will not have a measure of change in plaque

volume. Our approach to handling this missing outcome measure, therefore, is to define the primary outcome as plaque volume at a time point and to model change between arms as described above, using mixed effects models that estimate treatment effects on change by borrowing strength from those individuals who provide data on all time points, making robust assumptions about the data that are missing. Specifically, we assume the reason for missing data on plaque volume at 1 year is related only to observed variables including the patient's initial plaque volume measurement and treatment assignment and further that the reason for missingness is not related to the actual plaque volume measurement at 1 year. Based on our understandings, we believe this to be a reasonable assumption. Under this condition, the mixed effects modeling framework proposed above will yield unbiased and efficient estimates of the treatment effect. We will test departures to this assumption using multiple imputation techniques that vary the specification of the missing data mechanism. Dr. Desai is a leading expert in missing data and currently PI of a PCORI-sponsored award to study multiple imputation techniques of longitudinal measures.

Study Participation Duration:
Each subject will participate for one year after HT.

Study Duration:
The study duration from the first enrolment to the last follow-up will be 54 months. The final data analyses will be completed 6 months after the last patient follow-up.

Concomitant Medical Treatment:
Adult patients will receive induction therapy with methylprednisolone, 500 mg IV when coming off bypass and rATG at 1 mg/kg (maximum dose 125 mg) given on post-operative days 1, 2, and 3; daclizumab will be administered at 1 mg/kg to the pediatric population. Two additional doses of methylprednisolone will be given over the next 24 hours; pediatric patients will receive daclizumab every other week for a total of 5 doses after transplantation. The daily maintenance immunosuppressive regimen includes cyclosporine (2-4 mg/kg/day) in children; tacrolimus (0.5 mg twice daily) in adults, and mycophenolate mofetil (1000 mg twice daily); dose adjustments are made as indicated by adverse effects and/or acute rejection; prednisone is initiated at 1 mg/kg/day, and tapered to 0.5 mg/kg/day by month 6, and to 0.1 mg/kg/day by month 12. Concomitant medications are initiated as soon as the patient is able to tolerate oral intake. All patients receive co-trimoxazole (one double strength tablet daily) for pneumocystis pneumonia prophylaxis, except for patients with sulfa allergy who received atovaquone instead of co-trimoxazole. The HMG-CoA reductase inhibitor rosuvastatin will be initiated in all adult patients, within one week of transplant at 10 mg per day, regardless of plasma cholesterol or triglyceride concentration; pediatric patients will receive 5 mg per day. If after 4 weeks, the adult/pediatric patient is tolerating the rosuvastatin without any adverse effects, it will be increased to 20/10 mg per day, respectively.

All patients in whom either the donor or recipient is cytomegalovirus positive by serologic testing receive the standard ganciclovir prophylaxis, consisting of valganciclovir (900 mg twice daily) for one month initiated as soon as the patient can tolerate oral medications, followed by 900 mg daily until the end of the third post-operative month. Thereafter, all patients remain on valganciclovir 450 mg daily for the remainder of the first year post HT. Patients presenting with signs or symptoms of cytomegalovirus infection will undergo testing for confirmation and treatment. Patients will be seen in clinic and monitored for acute rejection by routine surveillance endomyocardial biopsy and echocardiography to evaluate allograft function at two weeks and 1, 2, 3, 4, 6 months after HT; the AlloMap test will be performed after that time period. At one year patients will be seen in clinic and then undergo their one year invasive studies. Other follow-up will be via phone call.

STUDY ORGANIZATION PLAN

Dr. Fearon, a Professor of Medicine and Director of Interventional Cardiology at Stanford University is the Principle Investigator of the proposed study and will oversee all aspects of the study conduct and management. He is an experienced clinical trialist with a long track record of successfully completing impactful trials. He will be assisted by Helen Luikart, a Cardiac Transplant Research nurse. Dr. Fearon and Nurse Luikart have worked closely and successfully together on a previous NIH sponsored clinical trial evaluating the role of angiotensin converting enzyme inhibitors early after heart transplantation.[50] They have an excellent working relationship and communicate well with each other.

Dr. Fearon will establish a steering committee consisting of the other Co-Investigators and critical personnel participating in the trial. The following will serve on the Steering Committee with Dr. Fearon:

- Dr. Kiran Khush, an Associate Professor of Medicine and the Director of the Adult Cardiac Transplant Research at Stanford
- Dr. Manisha Desai, a Professor of Medicine (Research) - Biomedical Informatics Research, and (By courtesy), Health Research & Policy, and the Director of the Stanford University Quantitative Sciences Unit
- Dr. Joshua Knowles, an Assistant Professor of Medicine, Director of the Familial Hypercholesterolemia clinic and a recognized expert in PCSK9 inhibitors
- Helen Luikart, RN, MS and clinical research nurse with over 20 years of experience coordinating the Stanford Cardiac Transplant research program

The Steering Committee will meet in person on a monthly basis to monitor the trial's progress and address issues with enrolment, compliance with the protocol, completeness of data, the budget, and safety. Informal ad hoc meetings between individuals will occur on a weekly basis. In this manner, the PI and the Steering Committee will pro-actively evaluate and prioritize issues that jeopardize study goals and necessitate corrective responses. Any disputes will be resolved by a vote of the Steering Committee members with each member retaining an equal vote. Minutes from the Steering Committee meetings will be taken by Helen Luikart and a summary report will be provided on a quarterly basis to the NHLBI to provide budgetary and enrolment updates.

Helen Luikart will serve as the point person interacting with Stanford's Internal Review Board and with the Data Safety and Monitoring Board (DSMB) for this project. She will notify both of any adverse events which occur during the course of the study. Data will be monitored in a blinded fashion until the end of the study. She will also coordinate study closure with Stanford's Internal Review Board at the end of the study.

Dr. Fearon will perform the cardiac catheterization procedures on the enrolled patients. He is an experienced interventional cardiologist who has performed more than 500 such evaluations in transplant recipients over the past 15 years. Nurse Luikart will oversee the communication with the Stanford Internal Review Board and be responsible for assuring that the consent forms are updated appropriately and the protocol is renewed in a timely fashion.

Dr. Khush, a Co-Investigator on this proposal is the Director of the Adult Heart Transplantation Research Program at Stanford and an experienced clinical investigator. She will oversee the recruitment and treatment of the cardiac transplant recipients. Nurse Luikart will assist her with recruiting and consenting patients. Dr. Khush will be responsible for evaluating patients during their clinical follow-up. She will report all adverse events to Nurse Luikart who will review them with Dr. Fearon and the DSMB. Dr. Fearon and Dr. Khush will communicate regularly in an ad hoc fashion and during the scheduled Steering Committee meetings. Dr. Fearon and Dr. Khush have collaborated on a number of previous studies and have an excellent working relationship.[6,7,37,38,50]

Dr. David Rosenthal, a Professor in Pediatrics, the Director of the Pediatric Cardiac Transplant program will serve as a Co-Investigator and oversee recruitment and enrolment of the pediatric participants in this clinical trial. He will monitor the children during follow-up for any adverse events and report these to Nurse Luikart, who will report them to Dr. Fearon and the DSMB. Dr. Fearon and Dr. Rosenthal have worked together on a

previous NIH sponsored clinical trial evaluating the role of angiotensin converting enzyme inhibitors early after heart transplantation and have an excellent working relationship.[50]

Dr. Manisha Desai, a Professor of Medicine (Research)- Biomedical Informatics Research, and (By courtesy), Health Research & Policy, and the Director of the Stanford University Quantitative Sciences Unit is a Co-Investigator and will oversee the data management and statistical analyses performed in this clinical trial. Dr. Desai has extensive experience designing, capturing, and analyzing data from clinical trials. She and Dr. Fearon have worked together on previous projects and communicate extremely well with each other.[68] Dr. Desai has assigned a staff biostatistician, Lingyao Yang to assist with establishing the database, creating the electronic case report form, evaluating the data for accuracy and completeness, and performing the analyses.

Dr. Joshua Knowles, an Assistant Professor of Medicine, Director of the Familial Hypercholesterolemia clinic and a recognized expert in PCSK9 inhibitors will serve as a Co-Investigator and provide expertise regarding monitoring and treating the enrollees with alirocumab/placebo.

Dr. Francois Haddad, a Clinical Associate Professor of Medicine, adult cardiac transplant cardiologist, the Director of the Stanford Cardiovascular Institute Biomarker and Phenotypic Core Laboratory, and a Co-Investigator on this trial will provide assistance coordinating the serologic analyses testing for the anti-inflammatory and pleiotropic effects of alirocumab.

The DSMB will be chaired by Michael Pham, MD, MPH, a heart transplant physician with more than 10 years of experience now practicing at the California Pacific Medical Center (see letter of acceptance in appendix). Other members of the DSMB will include a biostatistician and an ethicist. Meetings will occur every 3 months for the first year, and every 6 months thereafter to assess the rate of recruitment, completeness of data, completeness of follow-up, and for any major disparities in adverse clinical events between the two groups (presented to the DSMB as “group A” and “group B”), in a blinded fashion. If the DSMB identifies issues with recruitment or data management, it will notify the PI and Steering Committee and an action plan to correct the issue will be prepared and presented to the DSMB which will determine if it adequately addresses the issue(s). If there is concern regarding a major difference in adverse clinical events between the two groups, then the DSMB will be unblinded by an independent statistician from the Quantitative Sciences Unit at Stanford who is not involved in the study to determine if the active treatment group or the placebo group has the higher event rate and decide if the study needs to be stopped prematurely. Helen Luikart, RN will receive data from the statistical team and report all of the adverse events to the DSMB. Ad hoc meetings will be convened to review any serious adverse events felt to possibly be related to study drug or procedure. There will be no formal stopping rules for the trial. However, should the DSMB feel that patient safety is being compromised in any way by the study, it will be their prerogative to stop the trial.

STATISTICAL ANALYSIS AND PLAN

We will perform an analysis based on ***intent-to-treat principles*** to evaluate our primary outcome. This means we will analyze all subjects randomized and according to their randomized treatment assignment. The primary outcome is plaque volume, and our primary objective is to evaluate ***change in volume of plaque at 1 year post-HT relative to baseline between the two study arms.*** To address the primary objective, we will employ generalized linear mixed effects regression methods. The primary hypothesis will be tested using a two-sided Wald test at the 0.05 level of significance. A secondary goal will be to assess the effect of alirocumab versus placebo on coronary physiology, FFR and IMR, and on endothelial function. Another important secondary aim is to evaluate the mechanisms through which the treatment acts, where potential mechanisms include LDL-C, LP(a), apo(B), TRL and CRP. A final secondary aim is to evaluate the ability of FFR, IMR and endothelial dysfunction to predict clinically meaningful increases in plaque volume one year post-transplant relative to that of plaque volume measured via IVUS at baseline. Secondary analyses will be considered hypothesis generating. Dr. Manisha Desai, a Co-Investigator on this proposal and the Director of Stanford's Quantitative Sciences Unit (QSU), is an experienced clinical trialist and biostatistician who will oversee the statistical analyses.

Specific Aim 1. Measure the safety and impact on CAV of the PCSK9 inhibitor, alirocumab after heart transplantation.

Primary and Secondary Outcomes: The primary objective of this project is to determine whether PCSK9 inhibition with alirocumab early after HT impacts the development of CAV. For this purpose, we define the primary endpoint as plaque volume, measured by IVUS, in order to compare changes at 1 year post HT relative to baseline between patients in the two study arms. Plaque volume as determined by IVUS is used to assess CAV because we and others feel that a volumetric evaluation is the most accurate assessment of intimal thickening and changes in plaque have been associated with increased long-term mortality after HT.[35,36,67] Secondary endpoints include differences in LDL-C and other lipid particle values between the two arms at 3, 6 and 12 months, differences in coronary physiology (FFR and IMR) and endothelial function between the two groups from baseline to 1 year, and adverse events including mortality and retransplantation.

Descriptive Statistics: We will present descriptive statistics on the full analysis set (FAS) by study arm such as means, medians, standard deviations, and interquartile ranges for continuous variables like plaque volume at each time point and plaque change. Frequency distributions for categorical variables such as sex and race will also be provided by study arm. Graphical tools such as histograms and boxplots will be used to assess distributional properties of continuous variables.

Analytic Tools: Using intent-to-treat principles, we will address our primary aim by regressing plaque volume on treatment arm, days since randomization (time) and an interaction between time and treatment arm on the FAS using generalized linear mixed effects regression techniques, where the parameter for the interaction term is of interest and represents the differential progression in plaque volume at one year post-HT between study arms. Such a model will include a subject-specific random effect to allow for correlation of outcome within a subject over time. Using a two-sided Wald test at the 0.05 level of significance, we will assess whether the change in plaque volume differs at 1 year by treatment arm. ***It is important to note that a significant advantage to employing this particular modeling strategy is that it enables us to adhere to intent-to-treat principles by including the full analysis sample and using robust assumptions about the missing data.*** Thus, even those who do not provide change in plaque volume are included in this analysis, where the treatment effect on plaque change is estimated for the sample by borrowing strength from those who do contribute data on all time points (no imputation is used in the generalized linear mixed effects model). Our model is expressed as:

$$Plaque\ Volume_{ij} = \beta_0 + \delta_i \quad + \beta_1 Treatment_i + \beta_2 Time_{\ ij} + \beta_3 Treatment_i \times Time_{\ ij} + \varepsilon_{ij}$$

where $Plaque\ Volume_{ij}$ is the plaque volume measurement for the i^{th} subject at the j^{th} time point; $Treatment_i$ is an indicator for whether the i^{th} subject is assigned to the experimental treatment arm; and $Time_{\ ij}$ is the number of days since randomization for the i^{th} subject at the j^{th} time point. This approach has become standard

in cardiovascular clinical trials involving longitudinal repeated measures over time such as this one.[61-63] However, as a secondary analysis we will employ a simple t-test to compare the change in plaque volume between study arms among those who complete the study and provide outcomes of change using the modified intent-to-treat analysis set (MITT).

Power Considerations: Although there will be some attrition in measures collected over the follow-up period (See Methods for Handling Missing Data below), the initial sample size (i.e., those randomized at baseline) will be maintained in the proposed analysis, as we will adhere to intent-to-treat principles. There may however be an impact of attrition on our power due to the loss in the efficiency (or precision) of the estimate as the variation in our estimate of change in plaque may increase. We will therefore account for the impact of extra variation in our measure of change by considering a more conservative sample size.

Table 4. Power for addressing Aim 1 assuming 54 subjects per arm

Scenario	Average difference in log-plaque volume progression	Standard deviation of change in log-plaque volume progression	Power
1	0.06	0.10	0.87
2	0.10	0.10	> 0.95
3	0.15	0.10	> 0.95
4	0.06	0.25	0.24
5	0.10	0.25	0.54
6	0.15	0.25	0.87

We have excellent power to address our primary aim. We will randomize 120 patients. While all 120 patients will be included in the analysis according to intent-to-treat principles, we anticipate that 108 patients will complete the study without withdrawal or loss to follow-up. ***To incorporate the impact of attrition on the standard error we considered a smaller sample size of 108 or 54 per treatment arm, yielding conservative estimates of power.*** Based on larger studies in the literature and from our most recent work evaluating 96 patients who had serial IVUS at baseline and at 1 year after HT, we find that the common logarithm (base 10) of plaque volume at either time point to be approximately normally distributed.[50] Assuming a standard deviation of change in plaque volume (on the log-scale) of 0.10, based on empirical evidence, the effective sample size of 54 per arm gives over 85% power to detect a significant difference of change in plaque volume of at least 0.06 on the log scale assuming a type I error rate of 0.05. If the standard deviation of change in plaque volume is considerably higher than previously indicated, say 0.25, we have the same power to detect a difference in change in plaque progression of at least 0.15 on the log-scale.

Specific Aim 2: Identify the pleiotropic effects of PCSK9 inhibition after heart transplantation.

Descriptive Statistics: As in Aim 1, descriptive statistics will be provided on the FAS by study arm such as means, medians, standard deviations, and interquartile ranges for continuous variables including all biomarkers such as LDL-C, lipoprotein (a), apolipoprotein B, triglyceride rich lipoproteins and CRP. Frequency distributions of clinically meaningful categories of these biomarkers will also be presented.

Analytic Tools: To evaluate mechanisms of action, we will continue to rely on the framework of generalized linear mixed effects regression techniques. Importantly, we will test for mediation through the fitting of multiple regression models. Specifically, using the statistical method by MacArthur that modifies the seminal work in this area by Baron and Kenney and is described for a broader audience by Kraemer and others, we will evaluate the interrelationships among treatment, plaque changes and biomarkers such as LDL-C to evaluate whether changes in plaque are mediated by LDL-C, lipoprotein (a), apolipoprotein B, triglyceride rich lipoproteins and CRP.[64,65] The approach involves fitting a series of models to evaluate associations between the treatment and outcome, between treatment and the potential mediator, between the potential mediator and the outcome, and finally, between the treatment and outcome in the presence of the mediator. The presence of these various associations are necessary criteria in concluding that treatment is mediated through a factor. Essentially, for each biomarker or potential mediator of interest, evidence for mediation involves comparing the effect of treatment on plaque progression (b_3) derived from a full model of plaque volume as a function of treatment arm, time, an interaction between treatment arm and time, and the potential mediator of interest

(written below with LDL-C as the mediator) to the effect of treatment on plaque progression derived from a model that does not include the mediator (β_3). If the association of treatment arm by time is attenuated in the presence of LDL-C, this will suggest that PCSK9 affects plaque volume by lowering LDL-C. The full model used to evaluate a potential mediator can be described as follows:

$$Plaque\ Volume_{ij} = b_0 + \delta_i + b_1 Treatment_i + b_2 Time_{ij} + b_3 Treatment_i \times Time_{ij} + b_4 LDL_{ij} + \varepsilon_{ij}$$

Analogous comparisons of such models involving the other three mediators will be drawn using these methods. Attenuation in the beta coefficient representing the effect of alirocumab on plaque volume over time of 15% or greater in the presence of changes in a biomarker will suggest a potential pathway through which alirocumab acts. We will then perform identical analyses evaluating models of coronary physiology and endothelial dysfunction.

Power considerations: A sample size of 108 achieves 90% power to detect an R-Squared of as low as 0.07 attributed to each potential mediator we consider, assuming a type I error of 0.05. The term involving the mediator to be tested is adjusted for an additional 4 variables assuming a large R-Squared of 0.20.

Specific Aim 3: Compare the prognostic ability of invasive coronary physiologic and functional parameters to IVUS in cardiac transplant recipients.

Descriptive Statistics: Using the MITT, we will present descriptive statistics on the residuals from our two competing model fits. Using graphical tools, such as histograms and boxplots we will assess the distributional properties of the residuals for assessment of model fit. Further, scatterplots will be used to evaluate whether residuals are systematically related to patient-level characteristics such as age, treatment arm, and other features utilized in the model(s).

Analytic Tools: Using standard linear regression techniques we will fit and compare two competing models that predict plaque volume at 1 year post HT on the MITT (i.e., on the set of subjects who provide an outcome at 1 year). More specifically, the first model will evaluate the ability of FFR, IMR, endothelial dysfunction, and IVUS plaque volume all measured within 8 weeks of HT as well as treatment arm to predict IVUS plaque volume at 1 year post HT. Note that these models differ from the framework above, in that there is only one outcome per subject, and thus a simple regression framework can be employed. The model that includes all features will be expressed as:

$$Plaque\ Volume\ at\ 1\ year_i = \gamma_0 + \gamma_1 FFR_i + \gamma_2 IMR_i + \gamma_3 ED_{ij} + \gamma_4 Plaque\ Volume\ at\ HT_i + \varepsilon_i$$

Using the same techniques, we will evaluate the ability of IVUS plaque volume measured within 8 weeks of HT in the presence of treatment arm to predict IVUS plaque volume at 1 year post HT:

$$Plaque\ Volume\ at\ 1\ year_i = g_0 + g_1 Plaque\ Volume\ at\ HT_i + \varepsilon_i$$

We will compare the predictive ability of the two models using the Akaike Information Criterion or AIC.[66] Such analyses will provide insight into the ability of FFR, IMR and endothelial function to contribute to prediction of clinically meaningful plaque development within a relatively small window after HT, allowing for earlier and more aggressive intervention for a subset of patients. As we anticipate that the treatment will play a large role in arresting plaque progression, we have included it as a term in the predictive model.

Power considerations: We have excellent power to achieve our aims. A sample size of 108 provides 90% power to detect an R-Squared of only 0.05 attributed to the plaque volume measured at baseline alone compared to a model that additionally utilizes FFR, IMR and endothelial dysfunction, assuming a model with all four variables can explain 40 percent of the total variation in plaque volume at 1 year.

Data and Safety Monitoring Plan:

Safety data will be captured at the time of study visits (Baseline angiogram, Months 1, 2, 3, 4, 6, 9, and 1 Year angiogram), and at any time point when an adverse event (AE) occurs. AEs will be managed by the treating physicians in conjunction with the study investigators using best clinical practice. The Principal Investigator and the study nurse coordinator will monitor data and safety internally. Each adverse event will be assessed by the Principal Investigator and the study coordinater for designation as serious AE (SAE) or AE, and determination of whether it was study-related (either drug or procedure). The specific AEs of alirocumab that will be monitored are described in the Protection of Human Subjects attachment and include injection site reaction, neurocognitive events and cataracts. Assessment of AEs and SAEs by the Principal Investigator and study coordinator will take place on a weekly basis during a scheduled research meeting designed to review and discuss progress of the study, and to evaluate AEs. The results of these regular assessments by the Principal Investigator and study coordinator will be captured in the database as AEs. All AEs will be reported to the IRB (Stanford University Committee for the Protection of Human Subjects) and to the Data Safety Monitoring Board (DSMB). Once reviewed, all AEs and any actions taken by the IRB including changes or amendments to the protocol or consent form will be reported to the appropriate NIH funding institute. The potential risks and benefits to the subjects are outlined in the Protection of Human Subjects section.

The DSMB will be chaired by Dr. Michael Pham, the Medical Director of the Advanced Heart Failure, Transplant and Mechanical Circulatory Support Program at the California Pacific Medical Center, and a physician with 13 years of clinical and research experience in the field of heart transplantation (see letter of acceptance in appendix). Other members of the DSMB will include an independent biostatistician and an independent ethicist. All AEs will be reported and reviewed by the DSMB on a quarterly basis. Ad hoc meetings will be convened to review any SAEs felt to possibly be related to study drug or procedure. There will be no formal stopping rules for the trial. However, should the DSMB feel that patient safety is being compromised in any way by the study, it will be their prerogative to stop the trial. Interim analyses will occur every 3 months for the first year, and every 6 months thereafter to assess the rate of recruitment, completeness of data, completeness of follow-up, and for any major disparities in adverse clinical events between the two groups (presented to the DSMB as “group A” and “group B”), in a blinded fashion. If the DSMB identifies issues with recruitment or data management, it will notify the PI and Steering Committee and an action plan to correct the issue will be prepared and presented to the DSMB which will determine if it adequately addresses the issue(s). If there is concern regarding a major difference in adverse clinical events between the two groups, then the DSMB will be unblinded by an independent statistician from the Quantitative Sciences Unit at Stanford who is not involved in the study to determine if the active treatment group or the placebo group has the higher event rate and decide if the study needs to be stopped prematurely. If adequate recruitment is not being achieved, the study will be expanded to include another center. For example, we have a good collaboration with Dr. Jon Kobashigawa and Cedars Sinai Medical Center in Los Angeles, CA, a very high volume transplant center. We will establish a subaward with the other center and the PI and our research staff will travel to the other center to review the objectives of the study and the protocol in detail. Periodically, Helen Luikart, our research nurse will travel to the site to ensure data are being captured completely and appropriately. This model has worked successfully for us in previous trials.[50]