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CHORD – **CHO**lesterol Lowering and **R**esidual Risk in **D**iabetes

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1. Synopsis

Cardiovascular disease (CVD) is the leading cause of death among women and men with type 2 diabetes mellitus (T2D) in the United States. Despite optimization of evidence-based medicine aimed at hyperglycemia and hyperlipidemia, diabetes associates with a markedly increased risk of heart attack, stroke, and limb events (known as residual risk). Consistent with clinical observations from randomized trials and large population cohort studies, mechanistic studies from our group and others have demonstrated the negative impact of diabetes on atherosclerotic plaque repair/regression. Several contributory mechanisms have been proposed including heightened inflammation, immune dysregulation and macrophage polarization – all of which are affected by platelets. We and others have reported that patients with T2D have a particularly hyperactive platelet activity profile, platelets exacerbate atherogenesis and thrombosis, and that the association between T2D and incident cardiovascular events is mediated by platelet activity.

Sex-specific differences have emerged in the pathogenesis, presentation and outcomes of T2D. Diabetes is associated with a higher risk of prevalent CVD and incident cardiovascular events in women than in men. Several contributory mechanisms have been proposed for sex differences in cardiovascular outcomes, including immune dysregulation, heightened inflammation, estrogens and oxidative stress, all of which may be partly mediated by platelets. Among healthy controls without T2D, women have increased platelet activity compared with men and a proinflammatory platelet transcriptome. However, whether sex differences in platelet activity contribute to increased adverse cardiovascular outcomes in women with T2D is unknown.

Pathological and clinical studies consistently demonstrate that platelets are major culprits in the pathogenesis of CVD. Anuclear platelets play a major role in thrombosis, atherosclerosis, and inflammation. Platelets, which contain transcripts and the necessary molecular machinery to conduct translation, are intercellular regulators of vascular homeostasis, inflammation, and immune activity. There is increasing evidence that platelet activity can identify individuals at increased cardiovascular risk. The platelet transcriptome has been used to identify individuals with metabolic, inflammatory, and CVD. Preliminary data from our group also demonstrate that platelets skew macrophage polarization towards a more inflammatory phenotype and play a significant role in atherogenesis. Thus, through their complex network of effect, platelets may be a significant mediator of diabetic complications of CVD.

We propose a complementary set of studies, some of which we have developed, to fully evaluate the role of platelets in women and men with T2D, including investigating the effect of marked reduction in LDL-Cholesterol (LDL-C) on the platelet phenotype (activity and transcriptome; Aim 1), and studies investigating the platelet transcriptome in T2D with established CVD with (clinical progression) versus without (non-clinical progression; repair) incident cardiovascular events (Aim 2). Our aims will be powered to investigate sex-specific differences, and will improve our understanding of why women with T2D are at greater risk for CVD than men. Altogether, these studies will assess the influence of sex and cholesterol reduction on the platelet phenotype in women and men with T2D, and define the association between the platelet transcriptome and incident cardiovascular events in patients with T2D.

2. Background and significance

Diabetes and Cardiovascular Disease

It is estimated that over 35 million United States (US) adults have diabetes, of which 90-95% of cases are type 2 diabetes (T2D).[1] Alarmingly, >90 million US adults have prediabetes.[1] It is estimated that by 2050, 1 in 3 US individuals will have T2D.[2] Compared to those without disease, T2D is associated with double the risk for death and a 10-fold increase in hospitalizations for coronary heart disease.[1, 3] Cardiovascular disease (CVD) accounts for up to 80% of deaths in diabetic patients, most of which are due to ischemic heart disease.[4] Therefore, the American Heart Association (AHA) has concluded that “diabetes is a CVD equivalent”.[5] Because the prevalence of T2D continues to rise, because diabetes markedly increases the risk of CVD, and because cardiovascular events occur at younger ages in patients with diabetes,[3,6] it is critical to understand how diabetes increases CVD risk and how cardiovascular events can be prevented.

Although the CVD burden associated with T2D is obvious, the targetable causal pathways are incompletely understood. A number of fundamental mechanisms contribute to the increased cardiovascular risk observed in T2D including chronic hyperglycemia, insulin resistance, and dyslipidemia.[7, 8] Hyperglycemia has long been the major focus of clinical studies on CVD risk in diabetic patients. However, clinical trials with a number of glucose-reducing therapies, including insulin, confirm that glucose lowering does not, by itself, attenuate diabetes-accelerated atherosclerosis and subsequent clinical progression of cardiovascular events.[9] Recent studies with newer agents suggest that CVD is reduced in patients with diabetes but via glucose-independent mechanisms.[10] Clinical trials with cholesterol reduction have demonstrated a significant decrease in cardiovascular events following aggressive cholesterol lowering (e.g. statins, PCSK9 inhibitors).[11, 12] Recent data suggest that even with statin treatment and aggressive cholesterol reduction, T2D associates with a markedly increased risk of heart attack, stroke, and limb events (termed residual risk).[13, 14] Our group and others demonstrate that in T2D platelet activity is increased and the platelet transcriptome altered, and we hypothesize that platelets mediate the pathogenesis of residual risk in T2D. Moreover, these changes in platelets are likely to alter macrophages and drive them to a more inflammatory phenotype.

Diabetes, Sex and Cardiovascular Disease

Although younger women experience relative protection from CVD compared with men in the general population, diabetes blunts the sex-specific benefit observed in the female population.[15] In fact, there is persistent evidence of clinically meaningful sex differences in the pathogenesis, treatment, and prognosis of women with diabetes. Women have a greater risk of coronary artery disease, myocardial infarction (MI), and stroke from T2D compared with their male counterparts.[15] Significant knowledge gaps remain in understanding sex differences in the pathogenesis of T2D macrovascular complications. Understanding sex differences in cardiovascular pathogenesis in T2D will be important to guide prevention targets to reduce cardiovascular health disparities. Several contributory mechanisms have been proposed for sex differences, including immune dysregulation, heightened inflammation, estrogens and oxidative stress,[16] all of which may be partly mediated by platelets.[17-19]

As summarized in a scientific statement for the AHA,[15] there is a clear knowledge gap in understanding sex differences in the pathogenesis of diabetic complications of CVD. By investigating how T2D, sex and cholesterol reduction alter platelet activity and transcriptome in a prospective clinical study this project will address the knowledge gap for sex differences in platelets and residual risk in T2D. This information will directly address diagnostic markers, pathogenesis and therapeutic targets in cardiovascular risk factor management and outcomes for women and men with T2D.

Cholesterol and Platelet Activity

Elevated cholesterol is associated with increased platelet activity,[20] and patients with elevated cholesterol are at increased risk for platelet-mediated events.[21] In hypercholesterolemic patients, LDL-C levels correlate with platelet activity.[22] Cholesterol reduction with statins has a significant effect on decreasing platelet activity.[13,22-24] Simvastatin reduced platelet aggregation, plasma platelet biomarkers, subclinical inflammation, and improved endothelial dysfunction. Three days of atorvastatin also reduced platelet activity.[25] Following statin discontinuation, platelet activity increased within 2 weeks and correlated with LDL-C.[26] The proposed clinical project will determine if very potent cholesterol reduction will normalize platelet activity and alter the platelet transcriptome in women and men with T2D. Our studies may identify a major cause of residual risk in T2D.

3. Specific Aims

3.1 To prospectively investigate the effect of maximal cholesterol reduction on platelet activity and platelet transcriptome in women and men with T2D.

3.2 To investigate the association between platelet transcriptome in women and men with T2D compared to those without incident cardiovascular events.

4. Research Design

4.1 Recruitment

We will recruit individuals with diabetes who meet one of the following criteria: on glucose lowering medication or A1C ≥ 6.5% or FPG ≥ 126 mg/dl or OGTT ≥ 200 mg/dl. We will also recruit controls without known diabetes.

Participants will be enrolled from NYU Langone Health, including the faculty group practice, (e.g. NYU Center for the Prevention of CVD, Division of Endocrinology, and Diabetes Clinic at Winthrop).

4.2 Inclusion/Exclusion Criteria for Diabetes Patients

Inclusion Criteria

Group 1: Subjects with diabetes

1. Age ≥ 18 & < 90
2. LDL-C >100mg/dl
    a. Previous clinical labs will be used for eligibility assessment/screening.*
3. Able and willing to provide written informed consent for the study

*Individuals with an LDL-C >100mg/dl noted at assessment/screening who sign consent and then are found to have an LDL-C <100mg/dl at baseline will not be excluded.

Exclusion Criteria

1. Established cardiovascular disease on antithrombotic therapy
2. Triglycerides >400mg/dl
3. Use of a PCSK9 inhibitor
4. Recent infection in the past 30 days
5. Any hospitalization in the past 30 days
6. Use of Immunosuppressive therapy
7. Use of any antithrombotic therapy
8. Use of aspirin
9. Use of NSAID within the past 72 hours
10. Pregnancy
11. Anemia (hemoglobin < 9 g/dl) or thrombocytopenia (Platelet count <75), or thrombocytosis (Platelet count >600)
12. Known hemorrhagic diathesis
13. Chronic kidney disease (CrCl < 30ml/min)

4.3 Inclusion/Exclusion Criteria for Controls

Inclusion Criteria

Group 2: Subjects without known diabetes

1. Age ≥ 18 & < 90
2. **Either: LDL-C >100mg/dl, or Lp(a) > 50 mg/dl**
    a. Previous screening labs will be used for eligibility assessment/screening.*
3. Able and willing to provide written informed consent for the study

*Individuals with an LDL-C >100mg/dl noted at assessment/screening who sign consent and then are found to have an LDL-C <100mg/dl at baseline will not be excluded.

Exclusion Criteria

1. Diabetes (type 1 or type 2)
2. All other exclusions are identical to the diabetes group.

Recruitment goals are listed in the schedule of assessments below. Both female and males will be recruited. Subjects will be recruited from the outpatient general medicine, cardiology, and endocrinology clinics at NYU Langone Health (including the Manhattan, Brooklyn and Long Island campuses). We will also recruit from the Bellevue Hospital Adult Clinics and in-patient service, and the VA hospital. Patients fulfilling inclusion and exclusion criteria identified either by research staff who will pre-screen select clinic schedules or directly by the patient's health care professional, who will obtain verbal consent for contact by a member of the research staff.

Study information will be left at the front desk of these practices in the event that subjects would like more information from study staff. Patients will be approached after their visit with the health care provider in the clinic setting. Subjects and controls will also be recruited from posted fliers across the medical center, and by advertisements or other referrals (word of mouth, etc.). Additionally, subjects who have previously enrolled in any study (10-00607, S12-03123, S14-00531, s14-01418, and 15-00725) by the co - PI (Jeffrey Berger) and who checked off the box that they may be contacted by a member of the study staff for potential enrollment will be included.

Additional recruitment will be done through posted fliers and/or approved brochures at identified clinics with office staff permission. Where available, we may use TV monitors and/or computer screensavers in office waiting rooms to display a slide advertising the study. We may also recruit through social media postings including Craigslist, Facebook, Twitter, diabetes support groups and diabetes society patient advocacy groups as well the NYU approved research match and iConnect, and the health-union diabetes advocacy website. Similarly, we may post approved fliers/brochures at community health and outreach events with the permission of event organizers. The advertisement for these postings will contain the same content as noted on the IRB approved flyer and/or other IRB approved text such as the research match advertisement. We will also place a brief description of the study in our quarterly Prevention newsletter (please see attachments).

Proposed recruitment methods include direct recruitment to identify and reach potential subjects in EPIC based on diagnostic codes and demographic criteria that meets the study's inclusion and exclusion criteria. We will be using Epic's Reporting Workbench tool to identify potential subjects that have diabetes and present to NYU Langone Health (Manhattan, Brooklyn and Long Island Campus) and the FGP and other CV risk clinics. Additionally, we will take the following approaches:

1) DATACORE (FOR USING EPIC)
DataCore is the access point for querying the electronic health records of patients seen by an NYU Langone Health healthcare provider. The types of data in Epic that can be queried include demographic characteristics, health conditions, behaviors (e.g., smoking), vital signs, labs, procedures, medications, free text notes, and problem lists.

DataCore will provide the study team with a list of patients with identifiable protected health information for research-related purposes. Lists of actual patients can only be provided after the study team has received IRB approval and has submitted a formal data request to DataCore.

The Research team will screen the list of patients and reach out to patients who are confirmed to meet eligibility requirements via either letter, email, telephone, or contacting them in MyChart. Either the PI, co-investigator, study investigators, or study coordinator may contact the primary care or treating physician in advance of contacting the patient if appropriate. The patient will be approached after confirmation with the treating physician.

2) USING EPIC VIA MYCHART FOR RESEARCH RECRUITMENT
We will follow the workflow in place for study teams to send recruitment messages within Epic to Active MyChart patients. These messages allow patients the ability to respond if they are interested or not in potentially participating in a study.

Patients who have opted out of research contact in EPIC will not be included in the DATACORE results and will not be contacted. Other patients in EPIC who have not opted out of research contact have already provided consent to be contacted for research participation.
Listing of applicable forms:
1. Text for advertisement letter or email
2. MyChart recruitment message
3. Telephone recruitment script

Only subjects that have the capacity to consent will be asked to consent. Any recruitment information sent by email will utilize Send Safe email. If a subject requests information regarding opting out of further recruitment for all research, subjects will be directed to contact study coordinator or have subjects contact research-contact-optout@nyumc.org or 1-855-777-7858.We will not be enrolling vulnerable populations.

4.4 Informed consent

If a subject wishes to participate, a member of the research team will review the informed consent documents, answer any questions, and obtain written consent for participation in the study. A member of the research team will conduct the baseline interview with the subject and review the clinical history, demographic information and medication use with the individual. Part of the clinical history review will include questions related to social health, including depression and stress, and a pregnancy/reproductive questionnaire for female participants. The questionnaires will be administered at the study visit after consent for study participation is obtained. While the primary goal of the CHORD study is to look at lipid lowering, this is in the context of cardiovascular risk. Over the past decade, we have learned that pregnancy history and stress have a major impact of CVD. Thus, just as we want to know an individual's traditional risk factors for CVD (e.g. weight, hypertension, etc.) – it would be important for us to know an individual's pregnancy history and history of depression and stress. Obtaining this information is important to understand the participant's risk of cardiovascular disease – thus, would be very important to obtain.

Participants will consent to complete the baseline assessments (see table 1). Participants may opt to decline participation in any baseline assessment they feel uncomfortable with or time constraints do not allow. Follow-up assessments will be completed in those who agree to follow-up for the longitudinal part of the analysis. The consent process will take place in private offices at the NYU FGP, NYU Winthrop, NYU Lutheran, CTSI, ambulatory care center, Bellevue adult clinics and the VA. Subjects will be given the opportunity to be contacted for future studies that they may qualify for as well. All information including the consent will be kept under lock and key retained by research staff.

At the time of this writing, the CHORD study has enrolled >50 female participants who have completed the study. We would like to re-contact the female subjects who agreed to future contact on Page 11 of the ICF to obtain verbal consent to administer the reproductive questionnaire by telephone. As indicated in the verbal script submitted, we will inform the participant why we are calling and ask if they would like to proceed with the questionnaire. If the subject indicates they are not sure or need more time to decide, we will ask them if we can call back at another time and will document this. The research team member who contacts the patient will document the outcome of the call either manually on a written form and/or electronically in the study's existing secure RedCap. If there are any written forms documenting verbal consent, these will be stored in the regulatory binder along with the subject's initial consent forms. Regardless of format, documentation will include subject's study ID #, date and time of the call, who obtained verbal consent, whether the subject consented or not, and any other relevant notes.

4.5 Methods

4.5.1 Schedule of assessments

A baseline assessment is required for all study participants where both a blood draw of up to 90ccs (for the use of platelet and WBC analysis, DNA, RNA, and biospecimen collection) will be performed. Non-invasive vascular function testing (e.g. glycocalyx) and endothelial vein harvesting will be offered (table 1). A subject can choose to (or not to) participate in any vascular function baseline testing they feel uncomfortable with but must participate in the baseline blood collection to be included in the study. Please note while we are working with a co-investigator at Winthrop Hospital for the purpose of identifying potential subjects, all study procedures will take place at NYU Langone in Manhattan.

4.5.2 Longitudinal component

After baseline assessment, participants will be provided atorvastatin and a PCSK9 inhibitor (evolocumab). For 1 month, subjects enrolled will be provided with evolocumab 140 mg (2 injections, one administered at baseline visit and another self-administered 2 weeks later and a month's supply of atorvastatin up to 80mg dose (1 tab per day, starting at baseline visit post-assessment) The initial injection of evolocumab will be administered by qualified NYU Langone staff. The participant will receive instructions about how and when to self-administer the second injection at home, and will also receive instructions about use of atorvastatin. A member of the research staff will call to remind the patient about the required injection in advance and answer any questions the participant may have. Study staff will be in touch with participants weekly during the study period and patients will be asked to fill out a study drug diary. If the participant expresses a preference to return to CTSI for 2nd Repatha injection rather than self-administer at home, they will be given the option.

This study was designed to investigate maximal cholesterol reduction to better understand how cholesterol reduction lowers the risk of heart disease and to understand why patients with type 2 diabetes remain at high risk despite robust cholesterol reduction. Thus, we need to use a high intensity statin to achieve those aims. Moreover, many clinical studies randomized patients to atorvastatin (without any leading in dose) and there was no excess risk with this approach. In some cases (e.g.: participant(s) with statin intolerance or unwilling to go on a statin), we may consider the use of evolocumab alone with or without ezetimibe 10mg (1 tab per day, starting at baseline visit post-assessment)). The use of evolocumab together with ezetimibe has been studied and found to be well tolerated and effective in reducing LDL-cholesterol in patients at risk for cardiovascular disease, including patients with type 2 diabetes[27,28].

The use of statin (atorvastatin) and PCSK9 inhibitor (evolocumab) have been studied together and are routinely used and FDA-approved in medical practice for the prevention of cardiovascular events in patients at high-risk for cardiovascular events. Statins have been used in patients with diabetes for more than three decades and more recently when more aggressive lipid lowering is needed, a PCSK9 inhibitor is added. Use of these medications are based on national guidelines when cardiovascular risk is elevated. Evolocumab is very effective at reducing LDL-cholesterol and lowering cardiovascular risk. Because of its price, it was approved in a small group of patients (as described in package insert). Our subject population includes patients with diabetes and elevated LDL-C. The purpose of our study is to test the effect of robust LDL reduction on cardiovascular risk factors in this patient population. We plan on using both atorvastatin and evolocumab so we can achieve a robust LDL reduction in a short period of time. Research cited in this protocol (articles attached in IRB submission) indicates that the use of evolocumab with statins does not increase risks in this patient population. We added a comparator group to see if the difference observed in T2D is specific for diabetes. We chose controls without diabetes who had elevated LDL-C or elevated Lp(a) – 2 cohorts of patients where lipid lowering is strongly considered to prevent cardiovascular events (similar in subjects with T2D).

Atorvastatin will be administered on-label. Evolocumab will be administered off-label, however, its use in this study meets the requirements for IND exemption as the drug product is lawfully marketed in the US, there is no intent to use study results for new or changed indications, changes in labeling, or changes in advertising, and the use of evolocumab in this study population does not involve any factor that significantly increases the risk with the use of the drug product. The study is conducted in compliance with an IRB and consent is obtained. The study is also conducted in compliance with 21 CFR 312.7 (no intention to promote or commercialize the drug product). Ezetimibe will be administered on-label. These medications will be obtained and dispensed by the outpatient pharmacy at Kimmel. Research staff will pick up the subject's medications on the day of baseline visit/blood draw. Subjects will be advised not to pick up medication themselves directly from pharmacy and that research staff will provide explicit instructions on when to begin taking medication.
An initial follow up assessment will be planned (table 1) at 4 weeks +/- 5 days and will include repeat blood draw up to 85ccs (for the use of platelet and WBC analysis, DNA, RNA, and biospecimen collection). As in the baseline assessment, non-invasive vascular function testing (e.g. glycocalyx) and endothelial vein harvesting will be offered to subjects who consented to and completed the procedures at baseline (Table 1).
Subjects will also be given the option to 'opt in' to a second follow up assessment at 8 weeks (range 7 to 12 weeks) from baseline assessment, which will include a repeat blood draw of up to 85 ccs (for the use of platelet and WBC analysis, DNA, RNA and biospecimen collection) without any additional procedures. The additional

follow-up visit will only be offered to future subjects not yet enrolled. With accumulating data suggesting an adverse effect of lipid variability (changing lipid values over time), this component will allow us to investigate potential mechanistic memory effect of certain cell types (including platelets, leukocytes and endothelial cells). With limited power, this preliminary/pilot data will enable hypothesis-generating evidence that can then be followed up in larger prospectively designed clinical studies.
The end of the study is considered when the subject completes the initial follow up assessment or when the subject completes the optional second follow up assessment. All study drugs will be stopped at initial follow-up assessment (4 weeks +/- 5 days). There is no risk to stopping use of study medication. If the subject wishes to continue the study drugs, we will refer them to their primary provider and recommend that they discuss their trial involvement and options for maintenance therapy. As the use of statins in this population is evidence-based and concordant with guidelines, we will also provide the participant a notice to share with their treating physician (please see attached note). Participation in this study will not impact planned treatment of the subjects by their primary provider. Please note while we are working with a co-investigator at Winthrop Hospital for the purpose of identifying potential subjects, all study procedures will take place at NYU Langone in Manhattan.

All subjects may be asked to provide additional information about their medical history and update any interim history. Health information will be monitored over time by review of the medical chart. Additional questions may be asked by phone or during routine clinical follow-up, as appropriate.

Table 1. **Patient Group**	**Baseline Visit**	**Longitudinal Analysis**	**Initial Follow-up Visit (at 4 weeks +/- 5 days)**	**Second Follow-up Visit (at 7-12 weeks from baseline) *Optional***
1) n = up to 75 participants with diabetes	-Blood draw -Endothelial vein harvesting (optional) -Glycocalyx (optional) -Questionnaire(s)	-Cholesterol lowering medication(s)	-Blood draw -Endothelial vein harvesting (optional) -Glycocalyx (optional)	-Blood draw
2) n = up to 75 participants without diabetes (controls)	-Blood draw -Endothelial vein harvesting (optional) -Glycocalyx (optional) -Questionnaires(s)	-Cholesterol lowering medication(s)	-Blood draw -Endothelial vein harvesting (optional) -Glycocalyx (optional)	-Blood draw

We plan to enroll up to 155 participants (up to 80 participants with diabetes and up to 75 controls without diabetes).

1.1.1 Biospecimen collection

Study blood will be collected by trained staff in the outpatient clinics, phlebotomy center, or in the CTSI. We will request that patients do not eat for at least 4 hours prior. If the subject declined endothelial vein harvesting but agrees to a blood draw, then standard techniques of blood drawing will be used as follows. After cleansing of the venipuncture site with an alcohol wipe and removal of excess alcohol with sterile gauze, a tourniquet will be applied to the patient's bicep region. A *19 or 21G butterfly needle or less will be inserted into the peripheral vein, the tourniquet will be removed, and free-flowing blood will be collected with minimal trauma and stasis. The needle will be removed when blood collection is complete and sufficient pressure will be applied using sterile gauze at the puncture site until cessation of bleeding. A sterile band-aid will be applied to cover the venipuncture site.

*In the case of an unusually challenging blood draw, we may use a smaller needle (e.g. 23G or 25G) to finish collecting all tubes or reapply the tourniquet if needed. If multiple sticks are required to complete the draw, we will get verbal consent from the patient to continue with the blood draw. We will try to avoid these scenarios by encouraging patients to hydrate well prior to the visit and offering them water prior to blood draw.

For each patient, no more than 90 ml (≈6 tablespoons and about 1/6 of a standard blood donation) of blood will be collected at the baseline visit in red (no anticoagulant) top, lavender (EDTA anticoagulant) top tube(s), light green (lithium heparin) top, gold (serum collection) top tube(s) and blue (sodium citrate anticoagulant) top tubes. We believe this amount of blood presents low risk for the patient. Approximately 45 cc of blood will be used for the different measurements of platelet function, metabolic control, coagulation, inflammation, and approximately 45 cc of blood will be used for isolation of RNA, microRNA and storage of DNA at -80C. A full lipid panel, hs-CRP, and Lp(a) blood tests will be included at baseline and follow-up as part of the research and these tests will be run by the Tisch outpatient lab. We may also include hemoglobin A1C testing at baseline when necessary (e.g. if a recent clinical test has not been done), at the discretion of the PI. Hemoglobin A1C tests will be run by the Tisch outpatient lab. The samples will be stored in the PI's lab at the NYU Science building 7th floor without any identifying information other than a code number. Only study investigators will have access to stored samples. These samples will be barcoded system and lab vantage and stored in a freezer with back-up power supply.

Baseline labs will not be used as a screening assessment since lab values (including lipid values) have normal variability between tests. Since a subject would be given the PCSK9 inhibitor at the baseline visit on the day of the baseline lab assessment, there is a small possibility that the LDL-C would be higher than 100mg/dl at screening and < 100mg/dl at baseline. These individuals would not be excluded from our analyses.

1.1.2 Markers of cardiovascular risk may be measured at New York University Health Langone or our collaborators with expertise in laboratory sample analysis in areas related to cardiovascular risk, including inflammation. We are unable to list all study collaborators since new hypotheses are constantly emerging with review of preliminary data and/or new relevant studies/articles. In all cases samples will have no protected or identifiable information.

1.1.3 Vascular function assessment

Vascular function may be assessed using the glycocalyx (and/or with endothelial cell harvesting). Evaluation of sublingual microvasculature with a clinical video microscope with sidestream darkfield imaging provides a direct and noninvasive modality to assess microvascular health. All agreeing participants will undergo imaging of the sublingual microvasculature using a handheld sidestream darkfield microscan video microscope (KK Technology, England). Analyses will be performed using GlycoCheck Glycocalyx Measurement Software (GlycoCheck, Maastricht, the Netherlands). Dedicated processing software allows evaluation of microvascular density, microvascular percent RBC filling, and the perfused boundary region (PBR), an indirect measurement of endothelial glycocalyx thickness and luminal penetration. As an increased perfused boundary region (PBR) indicates deeper erythrocyte penetration into the glycocalyx, it suggests a loss of glycocalyx barrier properties and may be a marker of reduced microvascular health. Sidestream darkfield imaging measurements are determined based on a sample of >3000 sublingual vascular segments with diameters ranging from 5 to 25 µm. This approach has demonstrated good reproducibility and has been proposed as a valid method to assess endothelial integrity by the European Society of Cardiology Working Group on Peripheral Circulation.

The video microscope is not FDA approved and will be used on a research basis to collect physiologic data. This poses a non-significant risk for the device according to 21 CFR 812.3 (m) because: 1) There is NO implantation of the device. 2) This device will not be used to sustain human life and does not pose a serious risk to health or safety of the subject. 3) This device will not be used to diagnose, cure, mitigate or treat a disease. 4) This device does not pose a serious risk to health, safety or welfare of the subject.

1.1.4 Endothelial cell harvesting

Endothelial harvesting will take place in the CTSI (Bellevue clinical research center or at the satellite space in Tisch Hospital – 5B/C). This center has access to nursing support and ancillary support staff including trained phlebotomists. All subjects will have their blood drawn and endothelial harvesting after informed consent and completion of the screening interview. We will request that patients do not eat for at least 4 hours prior. They will

be in a reclined position with feet elevated. An angiocatheter ≤ 21 gauge will be inserted into a peripheral vein on the upper extremity using aseptic technique. A 0.018in. diameter J-shaped wire (Arrow, Reading, PA) will be then advanced into the angiocatheter, to a distance of 4cm beyond the end of the angiocatheter. Angiocatheters and J shaped wire described above are routinely used for intravenous fluids and medication administration in this hospital. Venous endothelial cells will be scraped from the intimas of superficial forearm veins by repeated (3 times) insertion and removal of the wire. This procedure will be done by Drs. Manila Jindal, Michael Garshick, Brenda Dorcely and/or Adedoyin Akinlonu as described below. Dr. Garshick has spent considerable time learning and mastering the endothelial harvesting technique where it was developed at Columbia University Medical Center. At the NYU CTSI, he has performed this previously IRB approved research technique over 100 times in collaborative with the NYU CTSI nurses without any complication. Dr. Jindal has both shadowed and had comprehensive training with Dr. Garshick to learn this technique, and has since performed this technique independently many times without issue. Drs. Brenda Dorcely and Adedoyin Akinlonu are currently undergoing training for endothelial cell harvesting procedure with Dr. Michael Garshick and will be delegated to perform the procedure for the study once training has been completed. The distal portion of the wire will be transferred to a 50ml conical tube containing dissociation buffer (0.5% bovine serum albumin, 2 mM EDTA, and 100 microg/ml heparin in PBS, pH 7.4) and maintained at 4°C until study. Retrieved endothelial cells will be used for RNA extraction and protein measurements.

The angiocatheter used will be a ≤ 21 gauge (typically 20 gauge) INSYTE autoguard IV catheter which is routinely used for clinical care, IV hydration and administration of medications. In this case we will be using this angiocatheter off label as a method to safely access the peripheral vein.

This poses a non-significant risk for the device according to 21 CFR 812.3 (m) because: 1) There is NO implantation of the device. 2) This device will not be used to sustain human life and does not pose a serious risk to health or safety of the subject. 3) This device will not be used to diagnose, cure, mitigate or treat a disease. 4) This angiocatheter device is routinely used across the country in clinics and hospitals and does not pose a serious risk to health, safety or welfare of the subject.

The J – wire used will be a 0.018in. diameter J-shaped wire (Arrow, Reading, PA). This device will be used off label as a means of accessing and collected endothelial cells. In clinical practice these devices are typically used in hospital settings as a guidewire for accessing human veins and threading other catheters into the vein. These wires therefore routinely come into contact of vein walls during clinical care. HOWEVER, our use is to harvest endothelial cells that come into contact with these wires. Therefore, this is considered off label.

This poses a non-significant risk for the device according to 21 CFR 812.3 (m) because: 1) There is NO implantation of the device. 2) This device will not be used to sustain human life and does not pose a serious risk to health or safety of the subject. 3) This device will not be used to diagnose, cure, mitigate or treat a disease. 4) This j-wire device is routinely used across the country in clinics and hospitals and does not pose a serious risk to health, safety or welfare of the subject.

Longitudinal Follow-up Data Collection

Follow-up biospecimen collection(s) will allow us to assess how cholesterol reduction affects platelets, leukocytes, inflammation and vascular health. Follow-up component(s) will consist of additional blood samples of no more than 85 ml (~5.75 tablespoons or less) that will be collected for different measurements of platelet function, lipid levels, coagulation, inflammation, biomarkers of cardiovascular risk, isolation of RNA, microRNA and storage of DNA at -80C. The samples will be stored in the PI's lab at the NYU Science building 7th floor without any identifying information other than a code number. Repeat vascular health measures are optional and may be performed as described above.

Overall, code numbers used for collection of samples will not be based on any information that could be used to identify the subject (for example, social security number, initials or birth date). The master list linking names to code numbers will be kept in a locked file cabinet, under double lock and key, and may also be kept electronically, under double lock and key.

The total amount of blood withdrawn (baseline plus both initial follow up and optional secondary follow up) will be no more than 260 ml over the course of about 2 months (≈18 tablespoons and about 1/2 of a standard blood donation). This amount of blood collection is within established safety guidelines of research blood collection in adults. Blood collection at baseline and at the follow up time points will allow us to examine how robust lipid lowering affects 1) platelet activity, 2) platelet and leukocyte transcriptome, 3) inflammation, 4) coagulation and other markers of residual risk, and 5) vascular function.

For all cases and controls, we will call weekly during the longitudinal component and 1 week after follow-up to assess how patients are feeling after endothelial vein harvesting (if performed). Adverse events will be managed as mentioned below.

1.1.5 Genetic Profiling

The blood and endothelial specimens will be analyzed for RNA, microRNA, and DNA profiling upon completion of the study or once a year if sufficient samples are obtained.We will perform a detailed molecular profile study, describing genetic arrays of patterns of platelet and endothelial vein analysis associated with atherosclerotic heart disease and diabetes. We will be looking at specific transcripts in platelets, WBCs, and endothelial cells associated with cardiovascular diseases. Platelet-rich plasma (PRP) will be isolated for platelet purification and subsequent genetic analysis will be performed. We will not be performing whole genome sequencing, only genetic testing as it relates to cardiovascular risk described above. Because there is no potential for clinically actionable genetic incidental findings as a result of this testing, genetic information will not be disclosed to anyone, including the participants and their physicians. The samples will be stored in dedicated freezer space in the Marc and Ruti Bell Program for Vascular Biology (the NYU Science building 7th floor) for the PI without any identifying information other than a unique code number. Or if additional space is need, in locked freezers within research space of the Division of Endocrinology, Science Building 6th floor. The code number will not be based on any information that could be used to identify the subject (for example, social security number, initials, birth date, etc.). The master list linking names to code numbers will be kept in a locked file cabinet, separate from all research information. All confidential data will be stored with a unique code as an identifier and will be protected by a double electronic lock. All physical data will be kept under double physical lock. Access to the data will be given to the study personnel only.

1.2 Future Sample Storage

By participating in this study, it is mandatory that we store the samples collected for continued analyses of this study, for studies as new scientific discoveries are made, to allow for retesting if necessary and for future research related to cardiovascular risk. We prefer that subjects who participate in the study do not have the option to opt out as there are many unknowns about cardiovascular risk in patients with diabetes, and being able to maintain the samples will allow us to build upon the knowledge we gain from this initial study. Patients will be informed of this during the consent process, and because future research will not stray from the purpose of the original application, we believe it should be part of the original protocol.

Blood samples for future analysis will be assigned a unique code number and stored in the dedicated freezer storage in the NYU Science Building (6th and 7th floors) by the PI without any identifying information other than a code number. The unique code number will not be based on any information that could be used to identify the subject (for example, social security number, initials, or birth date). We will use freezer-safe labels and store samples in freezers with emergency backup power. The master list linking names to code numbers will be kept in a locked file cabinet, separate from all research information, under double lock and key. Only the NYU PI will have access to the linking key between subject ID and subject identity. All confidential data will be stored with a unique code as an identifier and will be protected by a double electronic lock. All physical data will be kept under double physical lock. Only the PI and Co-Is will have access to the banked samples.

Samples will be stored for no more than 20 years. The specimens for future analysis may be analyzed upon completion of the study for RNA, microRNA, and DNA as described above. As described in the above section, we will not be performing whole genome sequencing, only genetic testing as it relates to cardiovascular risk. True genetic testing will not be done on samples. Genetic information will not be disclosed to anyone, including

the participants. Samples could also be used for testing other potential biomarkers in patient plasma and/or serum related to diabetes and CVD that may occur with exception of those which are already part of the protocol. Results of the future research will not be shared with the subjects or their study doctor, and will not become part of the medical records.

In the future, other institutions or future collaborators at or outside of NYU Medical Center may want to study a portion of these samples, too. If that happens, samples would be sent to other places so other people interested in studying these diseases and conditions could do that. The samples sent to other researchers will be de-identified without PHI. Samples will be stored for no more than 20 years following completion of the study. At the end of the 20-year period, all samples will be destroyed.

Subjects can request for withdrawal of samples at any time by contacting PI Dr. Berger in writing. His mailing address is Faculty Practice Tower 9 R, 530 First Ave NEW YORK 10016. Withdrawing Authorization only affects uses and sharing of information after written request has been received, and subject may not withdraw his/her Authorization for uses or disclosures that have previously been made or must continue to complete analyses or report data from the research.

2. RISKS

The potential risks of phlebotomy are pain, bruising, fainting, or small infection at puncture site. The procedure to perform endothelial vein harvesting is identical to the placement of an IV and routine phlebotomy blood draw. These risks are pain, bruising, fainting or small infection at the puncture site.

Sterile gloves and sterile wires are used to harvest the endothelial cells. The wires are advanced < 5 inches. Our lab is experienced with this technique and has published on the technique[14]. In our experience patients complain only on the routine discomfort of the IV insertion. There is a theoretical risk of vein thrombosis. In our collaborators experience, in over 10 years of sampling, not one thrombosis has occurred[14]. To minimize this risk, we will encourage arm mobilization post procedure. We will be using our collaborators' protocols and their consent language to ensure adequate patient knowledge on the risks of the procedure and we will train extensively prior to carrying out this low risk minimally invasive procedure to harvest endothelial veins. Their lab has also agreed to help mentor and guide us to help with the appropriate and safe procurement of endothelial cells when performing this testing on our initial subject cohort.

Sidestream darkfield imaging (non-invasive vascular testing as described above) is performed with a clinical video microscope positioned under the tongue. Image acquisition takes 2-3 minutes. Holding a microscope under the tongue for 3 minutes may be associated with mild participant discomfort. The video microscope is not expected to confer any additional risks to participants.

Treatment risks:

Atorvastatin and evolocumab are standard medications used for the prevention of CVD. Nevertheless, there are risks associated with being on atorvastatin. With atorvastatin use, the most common risks experienced are nasopharnygitis, arthralgia (joint pain), diarrhea, pain in extremity, and urinary tract infection. Other known side effects to short term therapy include hepatotoxicity, myositis, muscle soreness, stomach irritation and interaction with other medications (such as antifungals) and foods (such as grapefruit). Exceedingly rare from short term atorvastatin use includes risks such as major liver problems and muscle breakdown. If these reactions do occur, hospitalization may be required. We are using a statin (up to 80mg of atorvastatin) which has been widely studied with a very low rate of interactions, hepatotoxicity and incidence of myositis. In order to minimize risks of being on a high-intensity statin, we will educate subjects on potential adverse effects, minimize drug interactions that are known to inhibit the metabolism of atorvastatin, and maintain our exclusion criteria of patients who are taking immunosuppressive medications and patients with chronic kidney disease.

There is also a risk of being on evolocumab. With evolocumab use, the most common risks experienced include nasopharnygitis (common cold), upper respiratory tract infection, influenza, back pain, and injection site reactions. Other less common side effects include muscle pain, limb pain, joint pain, headache, fatigue,

dizziness, diarrhea, hypertension, gastroenteritis, urinary tract infection and other cold or flu-like symptoms. In very rare cases, serious allergic reaction may occur. Our study uses the recommended dosage of evolocumab (140 mg every 2 weeks).

Use of statins is indicated in persons with T2D and high CV risk. Evolocumab (a PCSK9 inhibitor) is FDA-approved as an adjunct to other lipid-lowering therapies (ex: statins) to lower cholesterol in patient populations with established cardiovascular disease and elevated cholesterol and those with inherited forms of high LDL cholesterol. The use of evolocumab with statins has also been investigated in many studies in patients with type 2 diabetes with no increased safety risk.[27,29]

The most common risks experienced with use of ezetimibe include joint pain, headache, diarrhea, indigestion, abdominal pain, back pain, sinus infection, cold or flu-like symptoms and feeling tired. Other less common side effects include nausea, pancreatitis, skin rash, muscle aches, dizziness, depression and gallstones. Rarely, hypersensitivity reactions such as angioedema (swelling of the skin and underlying tissues of the head and neck that can be life-threatening) can occur. Severe muscle problems that can lead to muscle breakdown are exceedingly rare from short-term ezetimibe use.

Genetic risks:
Genetic testing can generate information about a subject's personal health risks and can cause or increase anxiety, damage family relationships, and/or compromise insurability, employability and can even lead to discrimination. In general, results from studies that use data collected as part of this research will be preliminary, and the clinical implications of any findings may not be understood for years. Therefore, individual study results will not be shared with participants or their physicians.

Potential loss of confidentiality: Confidentiality will be preserved to the fullest extent by the research team but absolute confidentiality can't be guaranteed. All data will be stored with a unique code that does not identify the participant. Furthermore, data will be saved using a double electronic lock. All physical data will also be kept under double physical lock. Access to the data will be given to the study personnel only, thereby greatly reducing the possibility of psychological or social risks that could arise from knowledge of this genetic information, such as risk for employability or insurability or the risk of discrimination.

6. BENEFITS
The proposed study will examine the relationships between diabetes, cholesterol reduction and residual risk by assessing endothelial and vascular function and markers of thrombotic and CVD risk. Further, we will be able to examine how cholesterol reduction will improve markers of inflammation and vascular function in those with diabetes disease. The long-term goal is to develop a better understanding of residual risk in patients with diabetes. This study may lead to improved personalized medicine. There may be no direct benefit to enrolled subjects. While the medication is meant to lower cholesterol for the short-term duration of the study, there is no evidence to suggest this will have a long-term clinical effect once the medication is stopped.

7. COST
There is no cost to the study participants. There is also no direct benefit expected from the participation in this study. This study may provide valuable information to medical/cardiovascular physician scientists with minimal risk to study subjects. It is hoped that knowledge gained will be of benefit to others in the future.

8. Reimbursement:
All subjects will receive reimbursement for transportation or parking costs related to study site visits, as needed. Subjects must provide receipts to the study staff. In addition, subjects who complete all required visits will receive compensation for their time as follows:

- After completing initial follow-up visit: $100 (no endothelial cell harvesting) or $150 (if subject completed endothelial cell harvesting).
- Subjects who complete the optional second follow-up visit will receive an additional $50 payment.

Those who choose to leave or are withdrawn from the study for any reason before finishing the study (i.e. they do not complete any follow-up visits) will only receive reimbursement for applicable transportation and parking costs.

9. Statistical Analysis

Specific Power Analysis:

Sample Size Estimation for Platelet Activity: We will assess the difference in platelet activity using two co-primary measures: LTA and MPA. The comparisons will be performed in T2D before and after cholesterol reduction. Next, these measures will be stratified by sex. We expect a 25% between-group detectable difference, which is calculated at 80% power with Bonferroni adjustment. Based on published.[30, 31] and preliminary data, we believe these difference are reasonable and of clinical significance. For MPA, a 25% reduction will achieve a power of >95%. We have 80% power to detect a 20% reduction in MPA when stratified by sex. For LTA, a 25% reduction will achieve a power of >97%. We have 80% power to detect a 15% reduction in LTA when stratified by sex. We have 82% power to detect an interaction by sex, where we hypothesize that platelet activity will be decreased by 40% in men and decreased by 20% in women.

Sample Size Estimation for Platelet Transcriptome: For sample size estimation for the platelet transcriptome, differential expression analysis, including Benjamini-Hochberg adjusted p-values, was performed using DESeq2.[32] Based on preliminary data from our published and preliminary data of platelet RNAseq in T2D, a sample of 50 T2D patients before and following cholesterol reduction (Aim 1) would provide >90% power to detect a clinically meaningful effect size of 1.5 fold change following cholesterol reduction at adjusted α=0.05. When stratified by sex (25 women, 25 men), sequencing data pre- and post-cholesterol reduction would provide 85% power (α=0.05) to detect a clinically meaningful effect size of 1.5-fold change in women and men, respectively. Within the cohort of pre- or post-cholesterol reduction, a sample of 25 subjects in each group (e.g. women vs. men) would provide 82% power to detect a clinically meaningful effect size of 1.7-fold change between groups (α=0.05).

10. Data Analysis

All eligible subjects will be assigned a unique identification number upon enrollment in the study. Initial study data will be recorded on a printed form, which will be later converted to electronic database (Redcap). All potential identifiers will be kept separately in a safe location by the PI in a locked file cabinet in a locked room. After verification of entered data at completion of the study all potential identifiers will be stripped from the database.

Statistical Analysis Plan for Platelet Activity Measurements

Descriptive statistics, including frequency tables, measures of center and variability, and graphical displays will present baseline participant characteristics. Similar analyses will present values of platelet activity measures. We will assess the normality assumption for each measure. Non-parametric alternatives or transformations (e.g., Box-Cox) are applied to platelet activity measures that deviate from normal distributions.

We will compare platelet data between groups (event vs. no event; women vs. men) by *t*-test on normally distributed platelet activity measure or transformed measures, or the Mann-Whitney test if the transformed data still deviates from normal distribution. We will also investigate the variance of platelet data for each group to assess sample heterogeneity in each group, defined by sex and clinical event. Platelet data will also be analyzed in multivariable regressions. The dependent variable will be the change in platelet activity, and the candidates of variables include sex and sociodemographic/clinical characteristics, such as those gleaned from stress assessments. Interactions between sociodemographic and clinical characteristics will be considered for inclusion.

The difference in platelet activity before and after cholesterol reduction will be compared using paired t-test or Wilcoxon signed-rank test. We will also perform a linear mixed model for the multivariate analysis; the primary outcome will be the change in platelet activity (MPA and LTA) before and after cholesterol reduction. All tests will be 2-tailed, and a $P < 0.05$ will be considered as statistically significant.

Statistical Analysis Plan for Platelet Transcriptomics

The quality of the RNA-seq will be assessed at the sequence level using FastQC (http://www.bioinformatics.-babraham.ac.uk/projects/fastqc/) which reports multiple quality metrics, including the distribution of average per-base and per-sequence quality, per-base and per-sequence GC content, sequence length distribution, and sequence duplication levels. Read alignment and transcriptome assembly will be performed using the Cufflinks protocol. The quality of the alignments will be assessed based on the percentage of high-quality alignments obtained (MAPQ>30) and the percentage of duplicate alignments. Principal component analysis (PCA) and hierarchical clustering will be used to verify that the expression profiles of the sequenced samples cluster as expected. Gene set enrichment analysis of differentially expressed genes will be performed using GSEA.[33]

11. Safety and Adverse Events

11.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event

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

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

Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

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

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious will be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events will be reported is the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 5 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality will be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events will be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator will notify the IRB of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study.

Abnormal Laboratory Values
A clinical laboratory abnormality will be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

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

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

11.2 Recording of Adverse Events

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period will be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed

up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

11.3 *Reporting of Serious Adverse Events and Unanticipated Problems*

Investigators and the protocol sponsor will conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others (see definitions, section 11.1).

For Narrative Reports of Safety Events

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

11.3.1 Investigator reporting: notifying the study sponsor

The following describes events that must be reported to the study sponsor in an expedited fashion.

Initial Report: within 24 hours:

The following events must be reported to the study sponsor by telephone within 24 hours of awareness of the event:

- Unanticipated problems related to study participation,
- Serious adverse events, regardless of whether they are unexpected.

Follow-up report: within 48 hours:

As a follow-up to the initial report, within the following 48 hours of awareness of the event, the investigator will provide further information, as applicable, on the unanticipated device event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Unanticipated Problem form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing unanticipated adverse device effects shall be provided promptly to the study sponsor.

Other Reportable events:

- **Deviations from the study protocol**
  Deviations from the protocol must receive the investigator's IRB approval before they are initiated. Any protocol deviations initiated without Sponsor and the investigator's IRB approval that may affect the scientific soundness of the study, or affect the rights, safety, or welfare of study subjects, must be reported to the Sponsor and to the investigator's IRB as soon as a possible, but ***no later than 5 working days*** of the protocol deviation.

- ***Withdrawal of IRB approval***
  An investigator shall report to the sponsor a withdrawal of approval by the investigator's reviewing IRB as soon as a possible, but ***no later than 5 working days*** of the IRB notification of withdrawal of approval.

11.3.2 Investigator reporting: notifying the IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the NYULMC IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

Report Promptly, but no later than 5 working days:
Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

- ***Unanticipated problems including adverse events* that are unexpected and related**
  - *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
  - *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*
  - *Harmful: either caused harm to subjects or others, or placed them at increased risk*

Other Reportable events:
The following events also require prompt reporting to the IRB, though ***no later than 5 working days***:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - *one or more participants were placed at increased risk of harm*
  - *the event has the potential to occur again*
  - *the deviation was necessary to protect a subject from immediate harm*
- ***Breach of confidentiality***
- ***Incarceration of a participant*** when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- ***New Information indicating a change to the risks or potential benefits*** of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

Reporting Process
The reportable events noted above will be reported to the IRB using the form: "Reportable Event Form" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

11.4 Stopping Rules
In this study, if we encounter any serious adverse advents or > 5 adverse events in general we will interrupt the study and monitor for etiology and reasons behind the study. These events will be reported to the IRB as noted above.

11.5 Data and Safety Monitoring Plan
It is the responsibility of the Principal Investigator to oversee the data and safety monitoring plan. The PI as well as sub-investigator Jonathan Newman will be conducting the data safety monitoring review. We will review all endothelial sampling procedures for any adverse events after each one is performed. Important reviewable events include excess pain, significant bruising, or vagal responses in addition to those listed above in the adverse events section. Important reviewable events related to study drug medication include muscle pain and weakness and infections.

All serious adverse events will immediately be reported to the IRB along with a decision and plan on how to prevent these events in the future as detailed above in the reporting of adverse events section. A routine summary report will be sent to the IRB every three months to ensure adequate safety.

Safety will be monitored throughout the longitudinal phase of the study. For all cases and controls, we will call weekly during the longitudinal component and 1 week after each procedure to assess how patients are feeling after endothelial vein harvesting (if performed) and to ensure safety and tolerability of cholesterol lowering medications in diabetes cohort.

There are no predefined stopping rules. If there are any serious adverse events, we will notify the IRB immediately and will discuss whether or not the study should continue.

On the consent form, all participants will have access to several phone numbers with the ability to leave a message to talk to study members including the PI, Dr. Jeff Berger's office, and sub-investigator Jonathan Newman's telephone number. Subjects will be counseled on the potential complications of cholesterol lowering medication use as detailed above in the consent form and that for medical questions or problems they deem as emergencies, including severe bleeding, they should phone their own physician or report to the nearest emergency room.

12. Data Handling and Record Keeping

12.1 Confidentiality

This study is for research purposes only. Participants will be able to view clinical labs reported in NYU MyChart by the clinical lab (lipid panel, hs-crp, lipoprotein(a), and hemoglobin A1C (if applicable)) but otherwise individuals result will not be given back to study participant. This will include information from final results of the study, and interim results of the study. Confidentiality will be preserved to the fullest extent by the research team. All data will be stored with a unique code that does not identify the subject. Initial study data will be recorded on a printed form, which will be later converted to an IRB approved electronic database, REDCap. Only study personnel will have access to this database. Furthermore, data will be saved using a double electronic lock. All physical data will also be kept under double physical lock.

12.2 Confidentiality and HIPAA

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). We are providing a consent form informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
    - Name, date of birth, MRN
- Who will have access to that information and why
    - Only IRB approved study investigators. Statisticians will have access to de-identified datasets.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts will be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Source Documents

Source documents will be stored for a minimum of 2 years after study completion. Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete,

microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

Case Report Forms

The study case report form (CRF) will be the primary data collection instrument for the study. All data requested on the CRF will be recorded. All missing data will be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, “N/D” will be recorded. If the item is not applicable to the individual case, “N/A” will be noted. All entries will be printed legibly in black ink. If any entry error has been made, to correct such an error, a single straight line will be drawn through the incorrect entry and enter the correct data above it. All such changes will be initialed and dated. ERRORS WILL NOT BE ERASED OR WHITED OUT. For clarification of illegible or uncertain entries, clarification above the item will be printed then initial and dated.

Signature and Delegation Responsibility Log

A signature and delegation of responsibility log will be kept. This log will list all of the study personal as well as their individual responsibilities, study – related tasks, and a description of these activities. This log will also hold a record of the signatures and initials of various research staff. This log will be kept in the regulatory binder.

Records Retention

We will retain case report forms and source documents for 2 years after study completion. After that, primary study documents will be discarded and stored in electronic form.

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