

Alirocumab in Patients with Acute Myocardial Infarction

A Randomized Controlled Double-Blinded Study

The VCU Alirocumab Response Trial



STUDY PROTOCOL

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Sponsor: Virginia Commonwealth University

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TABLE OF CONTENTS

- 1) PERSONNEL**
- 2) PROTOCOL SUMMARY**
- 3) BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE**
- 4) OBJECTIVES**
 - 4.1 Study objectives
 - 4.2 Study outcome measures
 - 4.3 Study endpoints
- 5) STUDY DESIGN**
- 6) ENROLLMENT IN THE STUDY**
 - 6.1 Inclusion criteria
 - 6.2 Exclusion criteria
- 7) RANDOMIZATION**
- 8) TREATMENT WITH THE INVESTIGATIONAL DRUG**
- 9) STUDY ASSESSMENT**
- 10) POTENTIAL BENEFITS AND RISKS**
 - 10.1 Alirocumab
 - 10.2 Social and psychological risks
 - 10.3 Testing Risks
 - 10.4 Direct benefits to study participants
- 11) ASSESSMENT OF SAFETY**
 - 11.1 Reporting of Adverse Events
- 12) DISCONTINUATION OF TREATMENT AND WITHDRAWAL**
- 13) STATISTICAL CONSIDERATIONS**
- 14) REFERENCES**

1) PERSONNEL

Name	Role	Institution/ Division
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Benjamin Van Tassell, PharmD	Investigator	VCU, School of Pharmacy
Salvatore Carbone, MS	Investigator	VCU, Cardiology
Leo Buckley, PharmD	Investigator	VCU, School of Pharmacy
Justin Canada, MS	Investigator	VCU, Cardiology
Dinesh Kadariya, MD	Investigator	VCU, Internal Medicine
Robin Sculthorpe, PharmD	Investigational Pharmacy	VCU, School of Pharmacy

2) PROTOCOL SUMMARY

- Title:** **Alirocumab in Patients with Acute Myocardial Infarction**
- Population:** 20 patients aged ≥ 21 years presenting with an acute spontaneous non-ST elevation myocardial infarction (NSTEMI) who are already taking at least moderate intensity statin therapy at time of hospital admission
- Description:** Phase IV clinical trial of the alirocumab, an inhibitor of the proprotein convertase subtilisin/kexin (PCSK9), or placebo added to high-intensity statin (atorvastatin 80 mg) in LDL cholesterol lowering during NSTEMI.
- Objectives:** To determine the effectiveness of alirocumab in further lowering cholesterol LDL and inflammatory biomarkers when initiated acutely in patients with NSTEMI, in addition to high intensity statin, atorvastatin 80 mg.
- Primary Endpoint:** Placebo-corrected percentage change in calculated LDL cholesterol from baseline to day 14.
- Secondary Endpoints:** Placebo-corrected percentage change in calculated LDL cholesterol from baseline to 72 hours; placebo-corrected percentage change in PCSK9 levels from baseline to 72 hours and 14 days; placebo-corrected percentage change in lipoprotein(a) levels from baseline to 72 hours and 14 days; placebo-corrected percentage change in inflammatory markers (high-sensitivity CRP, IL-6, IL-10, and TNF- α) from baseline to 72 hours and 14 days; and safety and tolerability of alirocumab when initiated in the inpatient setting soon after an NSTEMI.
- Study Design:** Randomized, double-blinded, placebo-controlled phase IV clinical trial of a single dose of Alirocumab 150 mg or matched placebo in a 1:1 ratio. Patients will be assessed at enrollment, 72 hours and 14 days after treatment.

3) BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

Acute myocardial infarction (AMI) remains a leading cause of mortality and morbidity across the nation and worldwide. While major advances in reperfusion strategies over the past several decades have significantly reduced early mortality rates after AMI, patients who survive the index event are increasingly at risk for adverse cardiac remodeling and the sequelae of heart failure and sudden cardiac death¹. The aging population accelerates this problem, making heart failure a major public health concern. Heart failure, indeed, currently affects approximately five million Americans with increasing rates of prevalence and incidence.

Anticoagulants, antiplatelet agents, and neurohormonal blockers have become standard of care in the treatment of patients with AMI, given their established effects on mortality. Cholesterol-lowering drugs, primarily HMG-coA reductase inhibitors – statins – have been studied as a means not only to reduce LDL cholesterol (and hence the incidence of a first or recurrent AMI) but also as an adjunct to acute AMI therapy. In the MIRACL trial, atorvastatin 80mg was administered within 24 to 96 hours after presentation with acute coronary syndrome (ACS). When compared to placebo, the atorvastatin group had a reduction in the primary endpoint (composite of death, nonfatal AMI, cardiac arrest with resuscitation, or recurrent symptomatic myocardial infarction) from 17.4% to 14.8% (relative risk = 0.84 [0.70 – 1.00], p=0.048)².

The ARMYDA trial showed that loading with high dose statins for 7 days prior to elective percutaneous intervention (PCI) significantly reduced periprocedural myocardial infarction (MI) rates from 18% to 5% (p=0.025)³. This was followed by the ARMYDA-ACS trial, showing that initiation of high dose statin therapy in patients presenting with non-ST elevation ACS sent to early (<48 hours) angiography reduced the composite primary endpoint of death, MI, or target vessel revascularization from 17% to 5% compared to placebo (p=0.010) at 30 days⁴.

Proprotein convertase subtilisin/kexin 9 (PCSK9) inhibitors are emerging as a promising new treatment strategy for LDL cholesterol lowering, demonstrating potent LDL lowering effects in addition to or compared to statins, in stable patients in primary or secondary prevention⁵⁻⁹. PCSK9 is predominantly produced in the liver, intestine, and kidney, where it is secreted into the plasma and binds the extracellular component of LDL receptors (LDLR). Once bound, the LDLR-PCSK9 complex is internalized and directed to the lysosome for degradation¹⁰. PCSK9 inhibitors have been formulated as monoclonal antibodies, designed to bind PCSK9 and prevent this series of events. As an effect, LDLR concentrations on the surface of hepatocytes are increased, and more LDL is removed from the circulation.

Alirocumab (Praluent) is the first FDA-approved PCSK9 inhibitor, approved for primary prevention in patients with heterozygous familial hypercholesterolemia or secondary prevention in patients with clinically stable atherosclerotic cardiovascular disease who require additional lowering of LDL-cholesterol¹¹.

To date, there have been no studies of alirocumab or any other PCSK9 inhibitor given acutely in patients with acute myocardial infarction (AMI).

Considering the residual risk of complications and death in patients with AMI, there is an urgent clinical need to investigate new strategies for risk reduction in AMI. A rapid reduction of LDL cholesterol within 2-4 weeks of an AMI may provide additional benefit on top of standard care, including high-dose statins.

Moreover, a cross-talk between lipoprotein metabolism and systemic inflammation exists. In multiple in vitro studies, PCSK9 has demonstrated influence on receptors other than LDLR, including the LDLR-related protein 1 (LRP-1)¹² and CD36¹³. LRP-1 has been shown to have anti-inflammatory effects, at least in part due to its indirect control on the IKK/NF- κ B pathway by downregulation of the cell surface expression of tumor necrosis factor-1¹⁴. PCSK9 appears to have a similar effect on LRP-1 as it does on LDLR (i.e. facilitating LRP-1 degradation)¹⁵, suggesting that PCSK9 inhibition would prevent this process and could have an anti-inflammatory effect.

Macrophages play an important role in atherosclerosis formation and the associated inflammatory pathways. Increased levels of plasma LDL lead to oxidative modification of the LDL particles by macrophages in the arterial intima (forming oxLDL), which is then recognizable to scavenger receptors (SR), principally SR-CD36 and SR-A¹⁶. Once CD36 binds oxLDL, it interacts with Toll-like receptor 4 (TLR4) and TLR6 to activate NF- κ B and ultimately the inflammasome complex¹⁷. PCSK9 has been shown not only to increase levels of circulating LDL, which then promotes CD36 stimulation/upregulation¹⁸, but also to directly increase the expression of surface CD36^{19,20}. Thus, CD36 appears to have a net proinflammatory effect that is stimulated by PCSK9, and in this manner, PCSK9 inhibition could again have anti-inflammatory effects.

Overlapping pathways between lipoprotein metabolism and inflammation has a theoretical basis in the immune response to pathologic lipoproteic signals (i.e. cell debris during myocardial injury or lipopolysaccharide in Gram negative bacteria). Indeed, in a mouse model of septic shock, PCSK9 knockout mice or mice receiving PCSK9 modulators had a more favorable outcome than their counterparts. Accordingly, retrospective evidence from a large clinical study of septic shock showed a correlation between estimated PCSK9 activity (extrapolated from detected gain of function or loss of function genetic mutations), the measured inflammatory biomarker levels, and survival during septic shock²¹.

PCSK9 inhibitors have not been tested in acute settings such as AMI. The effects of the PCSK9 inhibitors on LDL cholesterol and on the systemic inflammation in AMI are unknown. It has been shown that persistently high levels of IL-6 and CRP during admission for acute coronary syndrome and at discharge are associated with worse outcomes^{21,22}. In the PROVE-IT trial, despite optimal medical care including high intensity statin therapy during the time of an ACS, more than 40% of patients had CRP levels >2mg/L at 30 days from the index events, and such patients were at increased risk of adverse outcomes²³.

4) OBJECTIVES

4.1 Study Objectives

We propose to study the effects of early initiation of alirocumab in addition to high intensity statin therapy in patients who have previously been treated with moderate-high intensity statins, who present with a type I (spontaneous) acute non-ST segment elevation MI (NSTEMI). Particular attention will be paid to additional LDL lowering effects, as well as the effects on PCSK9 levels and inflammatory biomarkers.

Biomarkers will be collected at baseline, 3 days, and 14 days after randomization.

4.2 Study Outcome Measures

LDL cholesterol levels. We will administer one dose of alirocumab 150 mg subcutaneously in addition to atorvastatin 80 mg given as standard of care, then monitor the change in LDL cholesterol at baseline, 3 days, and 14 days when compared to control subjects who receive placebo in addition to atorvastatin 80 mg.

PCSK9 levels. PCSK9 levels will also be measured at each time point, to monitor the effectiveness of alirocumab in lowering those levels when initiated acutely during an NSTEMI. We expect PCSK9 levels to rise with high intensity statin treatment alone but be lowered by treatment with alirocumab.

Lipoprotein(a). Levels of lipoprotein(a) [Lp(a)], another well-validated cardiovascular risk marker, will be measured at each time point. We expect a marked reduction in Lp(a) levels, as was observed in prior trials using alirocumab.

Inflammatory biomarkers. High-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), IL-10, and tumor necrosis factor- α (TNF- α) will be measured at each time point. We expect an inflammatory response after an NSTEMI, with a peak in many of the markers at 72 hours after the onset of symptoms. We suspect that treatment with alirocumab may blunt this inflammatory response.

Safety and tolerability. Although Alirocumab is already approved as an adjunct to diet and maximally tolerated statins for the treatment of patients with familial hypercholesterolemia and for patients with clinical atherosclerotic cardiovascular disease who require additional lowering of LDL-cholesterol, the current study represents the first clinical trial in which alirocumab will be given in patients with acute NSTEMI in the inpatient setting. Subjects will be screened for any adverse side effects or events thought potentially related to alirocumab treatment immediately after administration of the study injection, at 3 days, and at 14 days after the dose. A complete blood count with differential and complete metabolic panel will be drawn at each time point, as well.

4.3 Study Endpoints

Primary Endpoint:

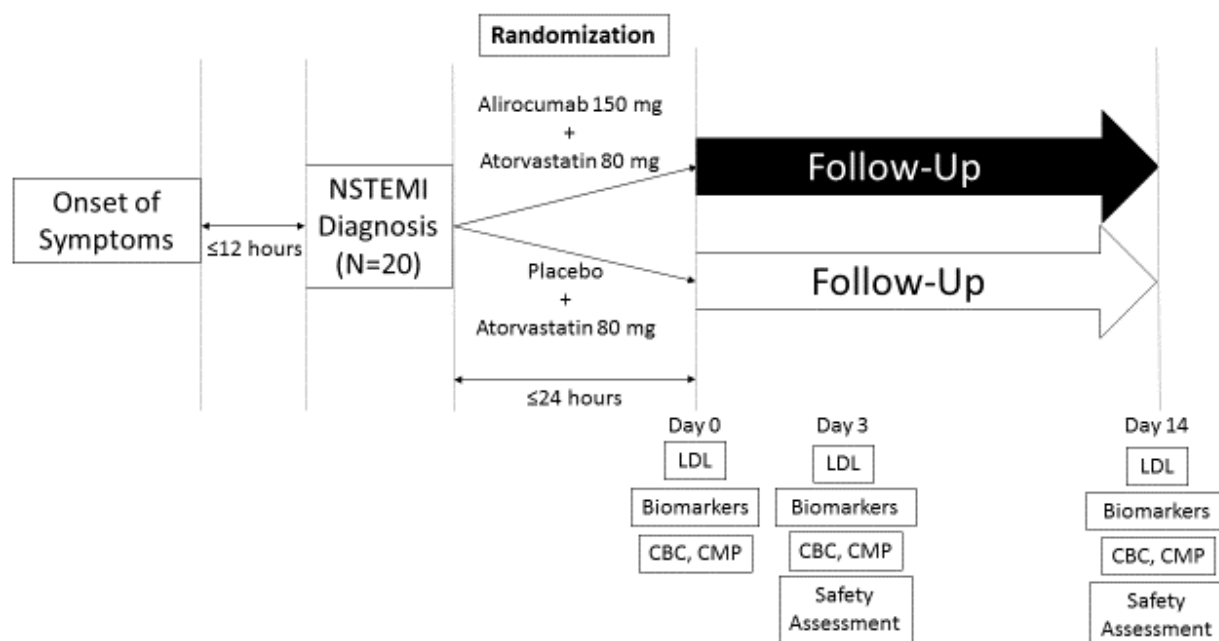
- Placebo-corrected percentage change in calculated LDL cholesterol from baseline to day 14.

Secondary Endpoints:

- Placebo-corrected percentage change in calculated LDL cholesterol from baseline to 72 hours;
- Placebo-corrected percentage change in PCSK9 levels from baseline to 72 hours and 14 days;
- Placebo-corrected percentage change in Lp(a) levels from baseline to 72 hours and 14 days;
- Placebo-corrected percentage change in inflammatory markers (hsCRP, IL-6, IL-10, and TNF- α) from baseline to 72 hours and 14 days;
- Safety and tolerability of alirocumab when initiated in the inpatient setting soon after an NSTEMI.

5) STUDY DESIGN

We designed an active-controlled, double-blinded study of alirocumab versus placebo in addition to high intensity statin, atorvastatin 80 mg, in patients who present with acute type I (spontaneous) NSTEMI and who are already taking a moderate-high intensity statin prior to admission.



The study is composed of two treatment arms exploring:

- a) Alirocumab 150 mg given as a single dose subcutaneously + Atorvastatin 80 mg orally daily;
- b) Matching placebo injection subcutaneously + Atorvastatin 80 mg daily.

6) ENROLLMENT IN THE STUDY

Patients who are admitted to the Pauley Heart Center in the VCU Medical Center will be screened for inclusion and exclusion criteria based on a cursory evaluation of the information available in their medical charts. This approach is supported by a partial waiver of informed consent for authorization to access protected health information to allow identification of the subjects. After identifying the potential subjects, the investigators will approach the subjects in a private manner and review the informed consent form approved by the Institutional Review Board. Any additional information about their medical history that is unclear or incomplete from the medical charts will be clarified by speaking with the patients and obtaining confirmatory outside medical records, with the consent of the patients, if deemed necessary.

Patients agreeing to participate in the study will sign the informed consent form and will be given a copy of the consent form signed by the investigator performing the informed consent process. No study procedures will occur prior to the subject signing the approved informed consent.

6.1 Inclusion Criteria

Both criteria must be met for enrollment of the patient into the study

- 1) Acute type I (spontaneous) non-ST elevation AMI defined as chest pain (or equivalent) with an onset of symptoms within 12 hours of presentation, a duration of >15 minutes, and elevated cardiac troponin I levels, with or without electrocardiographic changes [with the exclusion of ST elevation];
- 2) On medical therapy with moderate-high intensity statin prior to admission (either atorvastatin 20, 40 or 80 mg, or simvastatin 20, 40 or 80 mg or rosuvastatin 20 or 40 mg) as documented by hospital or pharmacy records and with known LDL cholesterol ≥ 70 mg/dL within the prior 12 months.

6.2 Exclusion Criteria

Subjects will not be eligible if they meet any of the following 11 exclusion criteria

1. Age <21 years of age (NIH standard);
2. Inability to give informed consent;
3. Previous, current or planned treatment with a PCSK9 inhibitor;
4. Known history of loss of function of PCSK9 (i.e., genetic mutation or sequence variation);
5. Patient with homozygous FH (clinically or by previous genotyping);

6. Recent (<14 days) or active use of immunosuppressive drugs (including but not limited to high-dose corticosteroids [$>1\text{mg/kg}$ of prednisone equivalent], TNF- α blockers, cyclosporine) not including NSAIDs or corticosteroids used for IV dye allergy or corticosteroids used as replacement therapy for pituitary/adrenal disease with a stable regimen for at least 6 weeks prior to randomization (note: topical, intra-articular, nasal, inhaled, and ophthalmic steroid therapies are not considered “systemic” and are allowed);
7. Chronic auto-immune or auto-inflammatory disease (including but not limited to rheumatoid arthritis, systemic lupus erythematosus);
8. History of cancer within the past 5 years, except for adequately treated basal cell skin cancer, squamous cell skin cancer, or *in situ* cervical cancer;
9. Known chronic hepatitis B or C infection (excluding patients with a positive antibody who were successfully treated or who have demonstrated no viral load);
10. Known human immunodeficiency virus infection.
11. Use of fibrates other than fenofibrate within 6 weeks of the screening visit.
12. Uncontrolled hypothyroidism. Note: patients on thyroid replacement therapy can be included if the dosage of thyroxin has been stable for at least 12 weeks prior to screening.
13. Known history of a hemorrhagic stroke.
14. Has been previously treated with at least 1 dose of alirocumab or any other anti-PCSK9 monoclonal antibody in other clinical studies.
15. Conditions/situations such as:
 - a. Any clinically significant abnormality identified at the time of screening that in the judgment of the investigator or any sub-investigator would preclude safe completion of the study or constrain assessment of endpoints, such as major systemic diseases or patients with short life expectancy.
 - b. Patients considered by the investigator or any sub-investigator to be inappropriate for this study for any reason, eg:
 - i. Those patients deemed unable to meet specific protocol requirements, such as scheduled visits.
 - ii. Those patients the investigator deems unable to administer or tolerate long-term injections.
 - c. Investigator or any sub-investigator, pharmacist, study coordinator, other study staff, or relative thereof directly involved in the conduct of the protocol.
 - d. Presence of any other conditions (eg, geographic or social), actual or anticipated, that the investigator feels would restrict or limit the patient’s participation for the duration of the study.
16. Thyroid-stimulating hormone (TSH) $<$ lower limit of normal (LLN) or $>$ upper limit of normal (ULN); if TSH is abnormal due to controlled hypothyroidism (patient is on a stable dose of thyroid replacement therapy), the patient may be enrolled into the study;

17. Exclusion Criteria Related to the Active Comparator and/or Mandatory Background Therapies: All contraindications to the background therapies or warnings/precautions of use (when appropriate) as displayed in the respective national product labeling.
18. Exclusion Criteria Related to the Current Knowledge of Alirocumab
 - a. Known hypersensitivity to monoclonal antibody therapeutics
 - b. Pregnant or breastfeeding women
19. Women of childbearing potential who are not protected by highly effective method(s) of birth control (as defined in the informed consent form [ICF] and/or in a local protocol addendum) and/or who are unwilling or unable to be tested for pregnancy. Note: Women of childbearing potential must have a confirmed negative pregnancy test at the screening and randomization visits. They must use an effective contraceptive method throughout the entire duration of study treatment and for 10 weeks after the last dose of study drug, and agree to repeat urine pregnancy testing at designated visits. The applied methods of contraception must meet the criteria for a highly effective method of birth control according to International Conference on Harmonisation (ICH) guideline M3(R2) “Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals” (EMA/CPMP/ICH/286/95), which is available at the following Internet address: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002720.pdf . Postmenopausal women must be amenorrheic for at least 12 months.

7) RANDOMIZATION

The Investigational Pharmacist (Robin Sculthorpe, RPh) who will not be involved in patient care, data gathering, or data analysis, will be in charge of randomization. Patients will be randomized 1:1 to Alirocumab 150 mg or placebo.

Access to randomization log will be restricted and allowed only on an emergency basis, or at the end of the study including all data collection. In case of an emergency, a physician treating any patient enrolled in the study may request un-blinding of that individual patient if the physician determines that un-blinding is necessary to make a treatment decision. The PI will contact the VCU Investigational Pharmacy to provide the treatment allocation to the PI, who will then relay the information to the treating physician. Upon completion of the study (including completion of all data collection and event adjudication), the PI will request the complete randomization log from the VCU Investigational Pharmacy.

8) TREATMENT WITH THE INVESTIGATIONAL DRUG

After signing of the informed consent, verification of the inclusion/exclusion criteria, obtaining baseline laboratory samples, and randomization, patients will receive their

injection of either alirocumab 150 mg or matching placebo administered subcutaneously. The dose will be administered by a nurse or an investigator appropriately using a dedicated auto-injector. The syringes and auto-injectors are prepared in a manner in which the active drug will be indistinguishable from the placebo, to ensure blinding of the providers and subjects.

To avoid prescribers prescribing additional PCSK9 inhibitors during the study, we will write a note in the clinical chart of each study subject informing providers about the enrollment in the study and the recommendation to not prescribe PCSK9 inhibitors until completion of the trial protocol (2 weeks). This will avoid patients being treated with two different PCSK9 inhibitors or with overlapping doses of alirocumab.

9) STUDY ASSESSMENTS

	Baseline (Day 0)	Day 3 (Or Discharge)	Follow Up (14 ± 3 days)
Screening	X		
Consent	X		
Administer Med	X		
History	X		X
Physical Exam	X		X
Medication List	X	X	X
Biomarkers	X	X	X

Patient will have blood samples (approximately 30 mL) drawn at baseline after signing the informed consent form but before receiving the study injection. The panel of biomarkers will include a lipid panel (total cholesterol, LDL-C, HDL-C, triglyceride levels, and Lp[a]), PCSK9 levels, inflammatory biomarkers (including hsCRP, IL-6, IL-10, and TNF- α), complete blood count (CBC), and complete metabolic panel (CMP).

After 3 days, or at the time of patient discharge (whichever occurs first), blood samples (approximately 30 mL) will be drawn again to re-measure the biomarkers listed above, and the patient will be given an appointment for a visit 14±3 days from enrollment. At their follow up visit, patients will undergo a repeat history and physical exam, and blood samples (approximately 30 mL) will again be drawn to re-measure the biomarkers listed above. This will complete the participation of the subjects in the clinical trial. The patient's clinical care will then continue on a regular basis, according to standard of care.

Biomarkers will be measured by LabCorp (Cincinnati, OH). The tubes will be collected by trained personnel, and immediately inverted 8-10 times after being drawn. Samples are then processed, refrigerated and shipped within 24 hours. The results from LabCorp will be sent securely to the PIs and designated investigators by email and/or fax.

For the measurement of the PCSK9 levels, not available commercially, we will collect samples, process them, and store them in a freezer located our lab for single batch analysis by Regeneron Pharmaceuticals, Inc. and Sanofi U.S.

10) POTENTIAL BENEFITS AND RISKS

10.1 Alirocumab

Alirocumab is a PCSK9 inhibitor which has been shown to significantly lower LDL-cholesterol, both in patients with heterozygous familial hypercholesteremia (HeFH) and in patients without HeFH who have clinical atherosclerotic disease. It is subcutaneously administered by means of an auto-injector. Its safety and efficacy have been broadly investigated multiple clinical trials in patients with or without HeFH who are already on maximally tolerated statins, with or without additional lipid-lowering agents⁵⁻⁸. Patients in this study will similarly be required to have been on at least moderate-intensity statin therapy prior to presentation for acute MI, and they will receive a single dose of alirocumab 150 mg subcutaneously or placebo. There are no dose-adjustments needed based on age or weight. Drug-to-drug interactions are minimal and not expected to be clinically relevant.

Side effects have been reported with alirocumab. Adverse reactions recorded in the current reference document for alirocumab are: injection site reactions (including erythema/redness, itching, swelling, pain, tenderness), upper respiratory tract signs and symptoms (including mainly oropharyngeal pain, rhinorrhea, sneezing), pruritis, hypersensitivity, eczema nummular, urticarial, and hypersensitivity vasculitis.

10.2 Social and psychological risks

There is a social/psychological risk in this study of breaching confidentiality and having a patient's diagnosis discovered. The likelihood of this occurring is very low, and will be further lowered by creating a database that does not link patients' identity with their clinical data. Loss of confidentiality is a potential risk. However, except when required by law, patients will not be identified by name, social security number, address, or any other personal identifier.

10.3 Testing risks

The only study-related testing risks will be those of a venous blood draw, which involves minor bleeding (rare) and infection (extremely rare) at the puncture site.

10.4 Potential benefits to study participants

The hypothesis being tested is that alirocumab will be well tolerated when initiated in the inpatient setting during an acute MI, and that it will be effective in further lowering LDL-C levels in addition to standard of care, possibly with the additional effect of attenuating the inflammatory response during acute myocardial infarction. There is no guarantee, however, that there is a benefit of using alirocumab in the setting of acute myocardial

infarction. There is however, also, no guarantee that the subject will receive alirocumab as 50% of subjects will receive placebo. Finally, we also cannot exclude the possibility, although highly unlikely, that alirocumab will provide harm in the investigated population. In the ODYSSEY Long-Term analysis, indeed, fewer patients experienced an acute myocardial infarction on alirocumab vs placebo (0.9% vs 2.3%, $P=0.01$), and there is no evidence suggesting that the patients who experienced an acute myocardial infarction while on alirocumab had a worse outcome than those on placebo, as shown by a trend toward reduced cardiac mortality with alirocumab (0.3% vs 0.9%)⁵.

11) ASSESSMENT OF SAFETY

Safety parameters will include data deriving from history and physical examination performed at each encounter, laboratory data, and results of functional and imaging tests obtained as part of standard clinical care. The patient will be encouraged to contact the research team at any time with any concern or change.

Disease-related data will be assessed including change in symptoms (or new symptoms), change in functional capacity, vital signs, and significant changes in medications.

Data specific to the treatment will also be assessed. The patient will be asked about symptoms. Changes to treatment for side-effects or unanticipated problems will be performed without breaking the randomization code, unless deemed necessary for the treatment of the individual patient by the physician caring for him, in which case the physician will be made aware while the remainder of the team, especially the investigators performing and interpreting the tests, will be maintained blinded, if possible.

The risks of the tests performed have been described above. In order to reduce risk, the procedures will be performed by skilled practitioners in the standard clinical fashion.

11.1 Reporting of Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product.

The occurrence of an AE may come to the attention of study personnel during study encounters and interviews of a study recipient for medical care, or upon review by an investigator and/or study coordinator. **All events considered AEs including local and systemic reactions will be recorded on the appropriate AE form.** Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study intervention (assessed only by those with the training and authority to make a diagnosis, which would include a physician) and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution. All AEs must be graded for severity and relationship to study product.

Severity of Event. All AEs will be assessed by the clinician as:

- Mild: events require minimal or no treatment and do not interfere with the daily activities.
- Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning and may require local or systemic drug therapy.
- Severe: events interrupt a patient's usual daily activity and will likely require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Life threatening: any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred (it does not include a reaction that had it occurred in a more severe form, might have caused death).

Relationship to the intervention. All suspected AEs must have their relationship to study intervention assessed using the terms: associated or not associated.

- Definitely related: The event is temporally related to the administration of the study intervention and, in the opinion of the investigator, no other etiology explains the event.
- Probably related: The event is temporally related to the administration of the study intervention and represents, in the opinion of the investigator, the most plausible explanation of the event.
- Possibly related: The event is temporally related to the administration of the study intervention but, in the opinion of the investigator, it does not represent the most likely explanation of the event.
- Definitely Unrelated: The event is temporally independent of study intervention and/or the event appears, in the opinion of the investigator, to be explained by another etiology.

An **unexpected event** means an event, the nature or severity of which is not consistent with the applicable product information. An expected adverse drug reaction with a fatal outcome should be considered unexpected unless the local/regional product labelling specifically states that the event might be associated with a fatal outcome.

A serious adverse event (SAE) is any adverse event/experience occurring between baseline assessments and the patients final study visit that results in any of the following outcomes and is considered by the investigator(s) to be unexpected or not consistent with the natural history of the disease:

- Death;
- Life threatening (subject at immediate risk of death);
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in a persistent or significant disability or incapacity;
- Is a congenital anomaly/birth defect;
- Important medical events that may not result in death, be life threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical

judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

For reports of hospitalization, it is the sign, symptom or diagnosis which led to the hospitalization, that is, the serious event for which details must be provided.

Any event requiring in-patient hospital admission (or the prolongation of hospitalization) must be reported as a SAE.

Admissions to the hospital that do not meet the criteria for SAE reporting are:

- Social reasons, e.g. overnight stay because of distance between home and hospital
- Surgery or procedure planned and documented prior to entry into the study

All serious adverse events that have not resolved by the end of the study, or that have not resolved upon discontinuation of the subject's participation in the study, must be followed until any of the following occurs:

- The event resolves
- The event stabilizes
- The event returns to baseline, if a baseline value/status is available
- The event can be attributed to agents other than the study drug or to factors unrelated to study conduct
- It becomes unlikely that any additional information can be obtained (subject or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts)

Adverse Event of Special Interest (AESI): An adverse event of special interest (AESI) is an adverse event (serious or non-serious) of scientific and medical concern specific to the investigational medicinal product (IMP), for which ongoing monitoring and rapid communication by the Investigator to the Marketing Administration Holder (MAH) may be appropriate. Such events may require further investigation in order to characterize and understand them. AESIs may be added or removed during a study by protocol amendment.

For this study, the AESI are:

- Increase in alanine aminotransferase (ALT):
 - ALT >3 x the ULN unless associated with aspartate aminotransferase (AST) increase (AST>ALT) and clearly attributed to cardiac cause.
- Allergic events.
 - Allergic drug reactions and/or local injection site reactions deemed to be allergic by the Investigator (or have an allergic component), that require consultation with another physician for further evaluation of hypersensitivity/allergy as per the Investigator's medical judgment should be reported as an AESI.
- Pregnancy:
 - Pregnancy occurring in a female patient enrolled in the study will be recorded as a pre-specified AE with immediate notification in all cases, and IMP should

- be discontinued in all cases. It will be qualified as an SAE only if it fulfills the SAE criteria. The follow-up of the pregnancy will be mandatory until the outcome of the pregnancy has been determined.
- Pregnancy occurring in the female partner of a male patient included in the clinical trial: if permitted by the female partner and by local regulatory policies, it will be recorded as a pre-specified AE with immediate notification (SAE if it fulfills the SAE criteria), and pregnancy should be followed-up until the outcome has been determined.
 - Symptomatic overdose with IMP:
 - An overdose (accidental or intentional) is an event suspected by the Investigator or spontaneously notified by the patient (not based on systematic injection counts) and defined as at least twice of the intended dose within the intended therapeutic interval (ie, 2 or more injections are administered in <7 calendar days), to be reported using the Term “symptomatic OVERDOSE (accidental or intentional), indicating the circumstance in parentheses (eg, “symptomatic overdose [accidental]” or “symptomatic overdose [intentional]”). The patient should be monitored and appropriate symptomatic treatment instituted.
 - The circumstances of the overdose should be clearly specified in the verbatim.
 - Neurologic events:
 - Neurologic events that require additional examinations/procedures and/or referral to a specialist should be reported as an AESI. If the event does not require additional examinations/procedures and/or referral to a specialist, it should be reported as a standard AE.
 - Neurocognitive events
 - All neurocognitive events will be considered as AESI
 - **New Safety Finding** means any (other than reportable individual case safety report (ICSR)) safety issue that may require expedited reporting because providing information that may lead to a change in the known risk-benefit balance for the product and as mentioned, but not limited to, in the following regulatory texts: *Europe: Volume 9A of the Rules Governing Medicinal products in the European Union (September 2008) Section 4.1*; and *US: FDA: 21 CFR Parts 312 Investigational New Drug Application- Section 312.32, (c) (1) IND safety reports*.

Instructions:

- All Serious Adverse Events (SAEs) regardless of the relationship to a Regeneron/Sanofi product and all Adverse Events of Special Interest (AESIs) must be reported to Regeneron/Sanofi within 24 hours of becoming aware of the event

Reports by E-MAIL should be sent to: CL-CPV-Receipt@sanofi.com or Fax: 908-547-8000, within 24 hours of receipt by investigator/sponsor. E-Mail or Fax transmission should include the following:

Investigator-Sponsored (IST #) study number: _____

Study Title: _____

Name of Principal Investigator: _____

- The Sponsor must provide to Regeneron/Sanofi upon request, results of any relevant complementary exams performed to obtain the final diagnosis of any SAE or AESI (e.g. hospital discharge summary, autopsy, consultation).
- The sponsor shall be responsible for ensuring submission of required expedited and periodic reports to the appropriate Regulatory Authority (RA), the Ethics Committee and investigators of each country participating in the Investigator-Sponsored Study (ISS, based on applicable regulations).
- Any Periodic reports (e.g. Development Safety Update Report (DSUR)), submitted to Regulatory Authority must first be transmitted to Regeneron/Sanofi for review and comment
- The Sponsor must provide to Regeneron/Sanofi any New Safety Findings pertaining to safety of the Investigational product within one (1) business day (e.g. Data Safety Monitoring Board recommendations).

12) DISCONTINUATION OF STUDY PARTICIPATION AND WITHDRAWAL

Patients may withdraw from the study at any time. The patient can decide to forgo any study procedures that makes him/her uncomfortable or wish not to complete. The study doctor may however stop the participation of the patient in this study at any time for many reasons including (*but not limited to*) the study doctor thinks it is necessary for patient health or safety, the patient has not followed study instructions, or administrative reasons requiring withdrawal.

Loss to follow-up can occur due to patients' withdrawal or death. If patients are lost to follow up and their clinical condition cannot be established (alive vs dead, hospitalized vs not), they will be excluded from the initial analysis, and then reintroduced for sensitivity analysis considering all potential outcomes. A 10% attrition rate is a reasonable assumption.

13) STATISTICAL CONSIDERATIONS

13.1 Sample Size

In the ODYSSEY Long Term trial, the addition of alirocumab 150 mg to maximally tolerated statin therapy resulted in a reduction of LDL cholesterol of approximately 60% at 4 weeks⁵. Similar LDL reductions were seen even when used in statin-intolerant

patients, as demonstrated in the ODYSSEY Alternative trial²⁴. We anticipate that patients will have baseline LDL levels of approximately 124 mg/dL on first presentation², and that a >40% in LDL reduction will be observed after treatment with alirocumab + atorvastatin 80 mg on top of what is seen with placebo + atorvastatin 80 mg at 2 weeks (primary endpoint), with a standard deviation of approximately 25%. These estimates lead to a predicted power >99% ($\alpha=0.01$) with a sample size of 20 patients, and of >95% with a 25% loss to follow up at 14 days. We also expect that patients with an acute myocardial infarction to have increased CRP levels, peaking at 72 hours^{22,23}. A sample size of 20 patients will also provide a power of 65-85% to detect a reduction of 33-50% in CRP or IL-6 plasma levels at 72 hours or 14 days with alirocumab + atorvastatin 80 mg on top of placebo + atorvastatin 80 mg (secondary endpoint).

13.2 Analysis of the data

Continuous variables will be reported as median and interquartile, because of potential deviation from Gaussian distribution. Discrete variables will be reported as N and %. For the primary endpoint, the comparison in the interval change in LDL cholesterol between alirocumab and placebo will be completed using an ANOVA for repeated measures and assessing the P value for time_x_group allocation. P values <0.05 will be considered significant. Missing data will not be input. For the purpose of safety analysis, all patients receiving the investigational drug will be considered, independent of missing data.

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