Original Article

Assessing the impact of PCSK9 inhibition on coronary plaque phenotype with optical coherence tomography: rationale and design of the randomized, placebo-controlled HUYGENS study

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Background: Technological advances in arterial wall imaging permit the opportunity to visualize coronary atherosclerotic plaque with sufficient resolution to characterize both its burden and compositional phenotype. These modalities have been used extensively in clinical trials to evaluate the impact of lipid lowering therapies on serial changes in disease burden. While the findings have unequivocally established that these interventions have the capacity to either slow disease progression or promote plaque regression, depending on the degree of lipid lowering achieved, their impact on plaque phenotype is less certain. More recently optical coherence tomography (OCT) has been employed with a number of studies demonstrating favorable effects on both fibrous cap thickness (FCT) and the size of lipid pools within plaque in response to statin treatment.

Methods: The phase 3, multi-center, double-blind HUYGENS study will assess the impact of incremental lipid lowering with the proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitor, evolocumab, on plaque features using serial OCT imaging, in statin-treated patients following an acute coronary syndrome (ACS). Subjects with non-ST-elevation ACS (n=150) will be randomized 1:1 into two groups to receive monthly injections of evolocumab 420 mg or placebo.

Results: The primary endpoint is the effect of evolocumab on coronary atherosclerotic plaques will be assessed by OCT at baseline and at week 50.

Conclusions: The HUYGENS study will determine whether intensified lipid lowering therapy with evolocumab in addition to maximally tolerated statin therapy will have incremental benefits on high-risk features of coronary artery plaques.

Trial registration: This study was registered on Clinicaltrials.gov (NCT03570697).

Keywords: Imaging; lipids; clinical trials; proprotein convertase subtilisin kexin type 9 (PCSK9); risk factors

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Introduction

Randomized controlled trials have unequivocally established that lowering levels of low-density lipoprotein cholesterol (LDL-C) reduces cardiovascular event rates in both the primary and secondary prevention setting (1). Meta-analyses have subsequently demonstrated a direct association between the degree of LDL-C reduction and cardiovascular benefit (1). As a result, treatment guidelines for cardiovascular prevention have increasingly emphasized the use of intensive statin therapy for higher risk patients (2). However, the observation of a considerable residual clinical risk in statin-treated patients related to insufficient LDL-C targets (3,4). And the emerging reality of statin intolerance has supported the need to develop additional LDL-C lowering strategies for use in clinical practice (5).

Lipid lowering beyond statins

A number of therapeutic agents have been developed for patients who are not able to achieve satisfactory LDL-C lowering despite use of maximally tolerated statin therapy. Ezetimibe is a cholesterol absorption inhibitor, which lowers LDL-C by 15–25% when administered as monotherapy or in addition to statin therapy (6). Two large clinical outcomes trials have demonstrated that ezetimibe administration on top of statin therapy results in a reduction in cardiovascular events in patients with end stage kidney disease and on long term follow up after an acute coronary syndrome (ACS) (7,8). Bempedoic acid inhibits ATP citrate lyase, a factor which similarly to statins is involved in the hepatic cholesterol synthesis pathway (9,10). As a result, this agent has the ability to lower LDL-C levels by 16–25% (11,12). The effect of this agent on cardiovascular outcomes is currently being evaluated in a large clinical trial of high-risk patients with statin intolerance and hypercholesterolemia.

Proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors present the opportunity to profoundly lower LDL-C. Genetic studies associate gain of function PCSK9 mutations with hypercholesterolemia (13) and loss of function polymorphisms with both low LDL-C levels and a lower prevalence of cardiovascular disease (14,15). Subsequent genetic and biochemical studies have established that PCSK9 plays an important role in the regulation of cholesterol metabolism, by lowering expression of the LDL receptor on the hepatocyte surface (13,16,17). Development of inhibitory monoclonal antibodies have been demonstrated to effectively reduce PCSK9 activity and to lower LDL-C in a dose dependent fashion on average to 60% when administered as monotherapy or in combination with statin therapy (18-20). This provides the opportunity to achieve effective lipid lowering, particularly in patients with statin intolerance and familial hypercholesterolemia, but also to reduce LDL-C to very low levels in those patients already treated with a statin (21,22). Two large outcomes trials have demonstrated that lowering LDL-C to much lower levels than previously observed reduced cardiovascular events in patients with either stable atherosclerotic cardiovascular disease (23) or in those with an ACS in the prior 4–52 weeks (24). It is therefore likely that the patient at elevated cardiovascular risk warrants efforts to lower LDL-C to very low levels.

Effect of statins on atherosclerotic plaque using arterial wall imaging

Technological advances in arterial wall imaging have produced a range of modalities with the ability to not only localize plaque in a range of vascular beds, but also to quantify its burden and characterize its composition. Early studies demonstrating the benefit of statins on disease progression using coronary angiography (25,26) and carotid intima-medial thickness (27,28) have been extended to intravascular ultrasound (IVUS), which examines the full burden of coronary atherosclerosis. Clinical trials employing serial IVUS imaging have demonstrated that increasingly intensive statin therapy slows disease progression and promotes plaque regression, with the degree of benefit proportional to the extent of LDL-C lowering achieved (29-31). Additional studies have revealed that C-reactive protein (CRP) lowering with statins independently associates with their impact on plaque progression, supporting reports that statins possess anti-inflammatory properties (32,33).

With increasing interest in the potential for plaque composition to be an important determinant of the translation of atherosclerotic disease to clinical manifestation, considerable attempts have been made to characterize the effects of statins on plaque morphology, beyond its size. Pathology studies have demonstrated that statin treatment prior to endarterectomy produces atheroma that contains less lipid and inflammatory material, supporting a potential role for plaque stabilization (34). While IVUS imaging lacks the resolution to effectively visualize plaque components, serial studies have reported

that statin use associates with progressive plaque calcification (35). Studies using different imaging modalities have reported that statins may also reduce plaque lipid on near infrared spectroscopy (NIRS) (36), necrotic core on magnetic resonance imaging (MRI) (37) and the degree of inflammatory activity using positron emission tomography (PET) (38). While these findings align with the observations from pathology studies, these modalities have been less commonly used in serial imaging studies of anti-atherosclerotic therapies.

Intracoronary optical coherence tomography (OCT) is a catheter-based imaging technique using coherent near infrared light to generate images with micrometer spatial resolution from optical backscatter, thus enabling detailed imaging of the intimal aspects of the coronary artery wall. As a result, OCT has been established to visualize the fibrous cap and underlying lipid pools with high resolution, permitting their measurement *in vivo*. Numerous reports have emerged from OCT-based studies demonstrating the presence of hallmark features of vulnerable plaque, including a thin fibrous cap, large lipid pool, cholesterol crystals, spotty calcification, neovascularization and potentially macrophage collections in culprit lesions (39-42) and other sites within the vasculature (43,44) of ACS patients. Furthermore, the presence of a lipid rich plaque, as evidenced primarily by a thin fibrous cap, but also with wide lipid arc and presence of macrophages on OCT, has been reported to associate with a greater rate of cardiovascular events on long term follow-up (45).

This technique has also been employed to study the effects of LDL-C levels and statins on atherosclerotic plaque. Observational studies have demonstrated that higher LDL-C levels associate with a thinner fibrous cap and large lipid pool (46), while use of more intensive statin therapy associates with a greater fibrous cap thickness (FCT) and less evidence of neovascularization (47). Clinical trials using serial OCT imaging have demonstrated that statin therapy has a favorable effect on plaque, as evidenced by an increase in FCT and a reduction in the size of the lipid pool (47-53). These findings provide an important contribution to the literature understanding the mechanistic effects of statins on plaque.

Effect of the PCSK9 inhibitor, evolocumab, on atherosclerotic plaque using arterial wall imaging

In parallel with the outcomes trials, the effect of the PCSK9 inhibitor, evolocumab, on coronary atherosclerosis has been studied in the Global Assessment of Plaque Regression with a PCSK9 Antibody as Measured by Intravascular Ultrasound (GLAGOV) study. This study compared the effects of treatment with evolocumab or placebo on plaque progression in statin-treated patients with angiographic coronary artery disease (CAD). Addition of evolocumab reduced LDL-C from 93.0 to 36.6 mg/dL, and was associated with a reduction in percent atheroma volume (PAV) by 0.95% and a greater proportion of patients demonstrating any degree of plaque regression over 18 months (54).

A virtual histology substudy reported progressive increases in plaque calcium with both statin monotherapy and when used in combination with evolocumab. The inverse association between achieved LDL-C levels and plaque calcification, suggests that this effect is a result of lipid lowering, rather than a pleiotropic effect of statins (55). A study in 129 subjects assessed the effect of evolocumab compared to placebo on arterial wall inflammation, in patients with elevated Lp(a) but did not meet the primary endpoint, as measured by fluorodeoxyglucose (FDG) PET uptake (56). Whether this reflects a lack of impact of evolocumab on plaque inflammatory activity, resistance to modification in the setting of elevated Lp(a) levels or a limitation of FDG scanning as a tool to evaluate medical therapies is uncertain. Nevertheless, there is an ongoing interest to further characterize the effects of PCSK9 inhibition on atherosclerotic plaque.

HUYGENS study objective

The High-Resolution Assessment of Coronary Plaques in a Global Evolocumab Randomized Study (HUYGENS) will employ serial OCT imaging to evaluate the impact of PCSK9 inhibition with evolocumab on coronary atheroma phenotype. The primary objective is to evaluate the effect of evolocumab on changes in FCT in patients with a non-ST-elevation ACS taking maximally tolerated statin therapy. Additional objectives are to evaluate the impact of evolocumab on other measures of plaque phenotype and the safety and tolerability of evolocumab when administered to patients in the post ACS setting. The primary hypothesis of the study is that achieving a low LDL-C with evolocumab in combination with statin therapy will result in a greater increase in the minimum FCT compared with statin monotherapy. We present the protocol in accordance with the SPIRIT reporting checklist (available at http://dx.doi.org/10.21037/cdt-20-684).

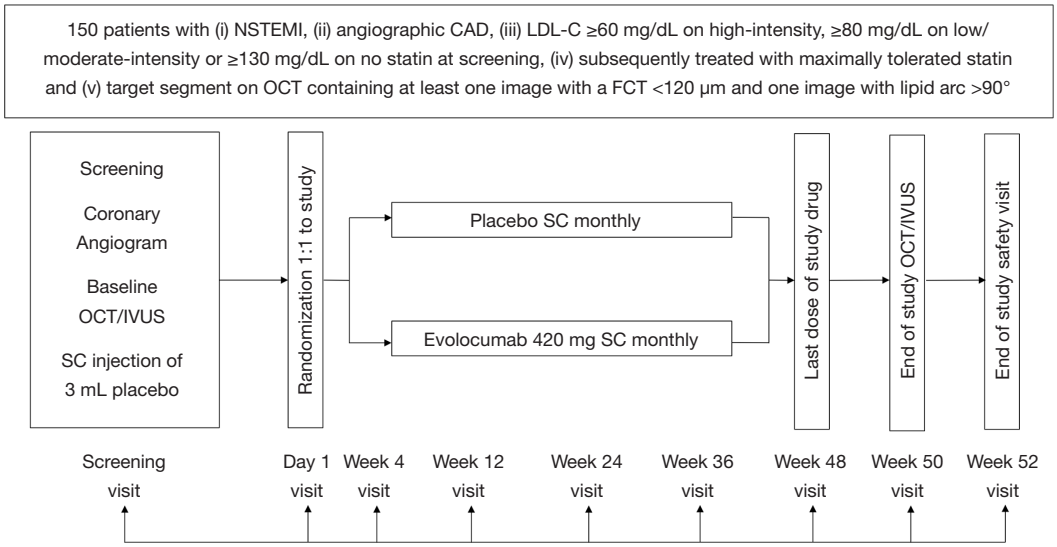

Figure 1 HUYGENS study design. NSTEMI, non-ST-elevation myocardial infarction; CAD, coronary artery disease; LDL-C, low-density lipoprotein cholesterol; FCT, fibrous cap thickness; IVUS, intravascular ultrasound; OCT, optical coherence tomography; SC, subcutaneous.

Methods

All procedures in this study will be conducted in accordance with the Declaration of Helsinki (as revised in 2013), International Conference on Harmonization, Good Clinical Practice guidelines, and applicable regulatory requirements. The final protocol was reviewed and approved by Ethics Committees and Institutional Review Boards at each study site and by Amgen Inc. (No. RES-18-0000-283A). All study participants will provide informed consent before participating in this study.

HUYGENS study design and inclusion criteria

HUYGENS (ClinicalTrials.gov Identifier: NCT03570697) is an ongoing (study commenced November 2018) randomized, double-blind, placebo-controlled, global, clinical trial to evaluate the impact of evolocumab on plaque phenotype. Eligible patients will include those who are (I) at least 18 years of age, (II) able to provide written, informed consent, (III) undergoing clinically indicated coronary angiography during admission due to non-ST-segment elevation ACS with interventional treatment of culprit plaque, (IV) have a qualifying LDL-C level at the time of screening depending on their use of either no statin (LDL-C ≥130 mg/dL), low or moderate intensity statin (LDL-C ≥80 mg/dL) or high intensity statin (LDL-C ≥60 mg/dL) and (V) on maximally tolerated statin therapy in accordance with standard of care per local guidelines prior to randomization. In addition, patients must have evidence of (I) an angiographic stenosis of ≥20% in addition to the culprit plaque, (II) no left main coronary artery stenosis >50%, (III) a target vessel for imaging which cannot be deemed to be the culprit artery for the index or prior MI, has not undergone or intended to undergo revascularization and must be accessible by an OCT imaging catheter and (IV) an arterial segment containing no stenosis >50%, be at least 40-mm in length and containing at least one image with a FCT ≤120 μm and one image with a lipid arc >90°. Exclusion criteria for the study include the presence of an ST-elevation MI, triglyceride levels ≥400 mg/dL, moderate to severe renal dysfunction (eGFR <30 mL/min/1.73m^2), malignancy, statin intolerance, prior or current use of PCSK9 inhibitors, women who are pregnant or breastfeeding or intend to become pregnant and any other condition deemed by the treating physician to impair the ability of the patient to comply with all study related procedures. All patients who meet all of the inclusion criteria, none of the exclusion criteria and who tolerate the placebo run-in injection at the time of screening will be deemed eligible and proceed to randomization within 1 week of providing informed consent.

Patients will be randomized to treatment with evolocumab 420 mg or matching placebo administered by subcutaneous (SC) injection monthly for 48 weeks (*Figure 1*). Randomization will be stratified by current statin use

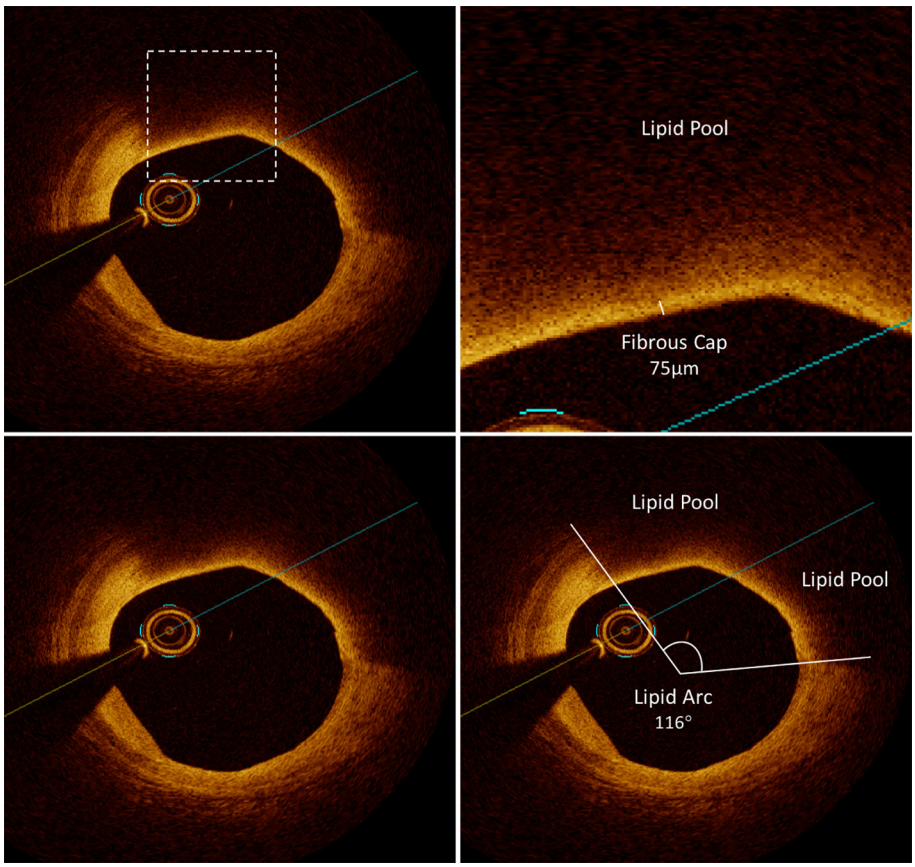

Figure 2 OCT plaque measurements. Cross-sectional plaque images acquired with OCT and demonstration of measurements of FCT (upper panels) and lipid arc (lower panels). OCT, optical coherence tomography; FCT, fibrous cap thickness.

(>4 or ≤4 weeks duration) at screening. Study visits will be performed in the clinic at weeks 4 and 24 and by telephone at weeks 12 and 36. Imaging of the target artery by OCT and IVUS will be performed at the time of the screening visit coronary angiography and then repeated at week 50. A final end-of-study telephone call will be performed at week 52 to complete the safety evaluation.

Image acquisition and analysis

Intravascular imaging with OCT and IVUS will be performed within the target artery selected for investigation at both baseline and end of study. OCT imaging will be acquired by placement of an OCT imaging catheter (DragonFly Optis: Abbott) as distally as possible and withdrawn to the aorta by automatic pullback at a speed of 36 mm/second. The IVUS imaging will be acquired in a similar fashion, using an IVUS imaging catheter (OptiCross 40 MHz: Boston Scientific; Revolution 45 MHz: Phillips) at an automatic pullback speed of 0.5 mm/second. All imaging performed will be electronically transferred to the central core laboratory at the South Australian Health and Medical Research Institute. Screening OCT imaging will be assessed for image quality and to determine that the patient meets all eligible imaging inclusion criteria. Once a patient has completed the study and has evaluable imaging at both time points, measurements of plaque phenotype and burden will be performed. The segment selected for analysis will be defined by proximal and distal side branches and by the presence of at least one image containing a FCT ≤120 µm and one image with a lipid arc >90°. The same segment will undergo measurements of both OCT and IVUS imaging by analysts who are blinded to the treatment status of the patient and to imaging timepoint (baseline/follow-up). For the OCT imaging, cross-sectional images, spaced 0.2 mm apart will be selected for analysis. For each image, where plaque is present, measurements of minimum FCT and the lipid arc will be performed (*Figure 2*). Each image will

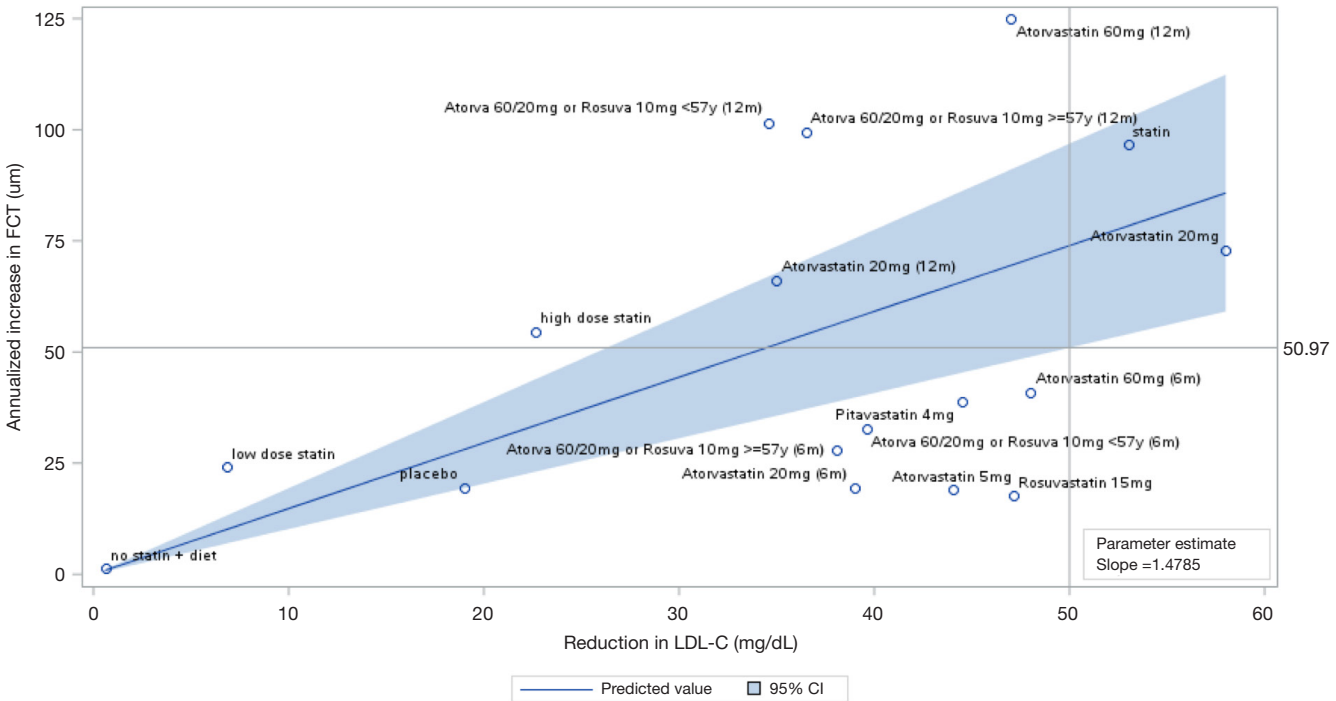

Figure 3 LDL-C reduction and change in FCT. Relationship between reduction in LDL-C and annualized change in FCT in trials that have employed serial OCT imaging to evaluate the effect of statin therapy. Given the invasive nature of the imaging performed in this study, it is assumed that 16% of patients will not have evaluable imaging at both time points. Accordingly, 150 patients or more will be randomized 1:1 to treatment with evolocumab 420 mg or placebo to ensure there will be at least 125 patients completing the study. LDL-C, low-density lipoprotein cholesterol; FCT, fibrous cap thickness; OCT, optical coherence tomography.

be graded for the presence of macrophage and calcium accumulation (40). For IVUS imaging, cross-sectional images, spaced 0.5 mm apart, will be selected for analysis. The leading edges of the external elastic membrane (EEM) and lumen will be defined by manual planimetry. Plaque area in each image will be calculated as the area between the EEM and lumen. PAV will be calculated as the average of the proportion of plaque to EEM area in all images. Total atheroma volume (TAV) will be calculated as the average plaque area and normalized by the median number of images in the cohort. Regression will be defined as any decrease in PAV or TAV from baseline.

Statistical analysis

The primary and secondary endpoints will be analyzed by analysis of covariance (ANCOVA) with the covariates of treatment group, patient demographics, tobacco usage, type 2 diabetes mellitus, stratification factor of statin use at screening, baseline LDL-C, and baseline FCT. For missing post-baseline FCT measurements, values to be used for the primary and secondary endpoints will be imputed by multiple imputation. In addition, non-parametric Quade test will be used for the sensitivity analysis on the primary endpoint.

The assumptions in the sample size calculation are based on a linear regression meta-analysis of seven studies that have employed serial OCT imaging to evaluate the effect of statin therapy (47-53). The meta-analysis suggests that each 1 mg/dL reduction in LDL-C associates with an increase in FCT by 1.48 μm during 12-month follow-up (*Figure 3*). Assuming a 50 mg/dL reduction in LDL-C, the predicted mean increase (95% CI) in FCT from the meta-analysis was 73.92 (50.97, 96.88) μm. For this study, the assumed treatment effect is at least 50.97 μm increase, which is approximated from the lower bound 95% confidence interval. The standard deviation of change in FCT was only reported in two of the seven trials, ranging from 22 to 86 μm. To be conservative, the assumed common standard deviation for this study is 86 μm. 125 completers will

be needed to reach 90% power to detect this difference in FCT.

Results

HUYGENS endpoints

The primary endpoint of the study is the absolute change in minimum FCT in a matched arterial segment from baseline to week 50. Secondary endpoints include the (I) percent change in minimum FCT, (II) absolute change in the average of the minimum FCT for all images and (III) absolute change in the maximum lipid arc. An additional plaque-based analysis will determine the absolute change in minimum FCT, maximum lipid arc and lipid core length in lipid rich plaques, defined as the presence of a minimum FCT ≤120 μm and a lipid arc >90° in at least three consecutive images. Measurements of plaque burden by IVUS and lipid parameters will be studied for an exploratory analysis to determine their relationship with changes in plaque phenotype on OCT imaging. Additional OCT imaging exploratory endpoints include the number of microchannels, thought to reflect plaque neovascularization, and macrophage presence and extension.

Discussion

Lipid lowering has had a major influence on cardiovascular outcomes. The use of arterial wall imaging has permitted the opportunity to characterize the effect of lipid lowering interventions on atherosclerotic plaque *in vivo*. HUYGENS will extend these observations to determine the impact of achieving very low LDL-C levels with a PCSK9 inhibitor on vulnerable coronary plaque features in patients with an ACS. The findings have the potential to further understand the potential link between the effects of evolocumab on the biology within the artery wall and how that may translate to a reduction in cardiovascular risk.

Registration details

This clinical trial is registered on Clinicaltrials.gov with the number NCT03570697.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures in this study will be conducted in accordance with the Declaration of Helsinki (as revised in 2013), International Conference on Harmonization, Good Clinical Practice guidelines, and applicable regulatory requirements. The final protocol was reviewed and approved by Ethics Committees and Institutional Review Boards at each study site and by Amgen Inc. (No. RES-18-0000-283A). All study participants will provide informed consent before participating in this study.

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