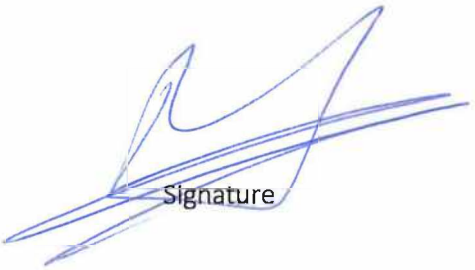
Statistical analysis plan

Study: Functional Improvement of non-infarcT related coronary artery stenosis by Extensive LDL-C Reduction with a PCSK9 Antibody

FITTER study

Date: 28-03-2024

Approved by:



Signature

29 March 2024

Date

Prof. Dr. Robert-Jan van Geuns

Contents

1 Study synopsis

In a large number of patients presenting with acute coronary syndrome (ACS) multivessel disease is identified. Mechanical treatment of the infarct related artery (IRA) is indisputable, yet mechanical treatment of other bystander lesions in non-infarct related arteries (non-IRAs) is controversial. Some randomized studies have favored preventive complete revascularization during invasive coronary angiography (ICA) over conservative medical treatment with deferred percutaneous intervention (PCI). Yet patient selection and medical treatment in the conservative medical treatment groups were suboptimal. Revascularization of lesions in the non-IRA can be guided by fractional flow reserve (FFR). In current practice, a value of 0.80 or lower is often used for FFR to mark a functionally significant stenosis at a stabilized moment after initial hyperemic response. However, recent evidence suggests that hyperemic response to adenosine is impaired in patients with ACS, which could underestimate how flow-limiting a non-culprit lesion is as measured by FFR. A large patient-level meta-analysis of multiple FFR trials showed that FFR values below 0.67 most evidently identify those at risk of MI or death. Thus, in patients with values above 0.67, mechanical revascularization has less apparent benefit as compared to patients with values below 0.67. The threshold of 0.67 could be a lower safety margin applied for non-IRA lesions, with percutaneous intervention (PCI) as treatment. For values between 0.67 and 0.85, medical treatment could be optimized using the latest generation LDL-C lowering agents on top of current high-intensity statin therapy (HIST) before directly stenting the lesion. PCSK9-inhibitors have been shown to induce regression of coronary atherosclerotic plaque volume (PV) in patients with coronary artery disease (CAD). As high-risk lesions with large plaque burden (PB) and lipid content are frequently present in ACS, a rapid response on PB and PV can be expected when starting PCSK9-inhibitors on top of HIST. In addition to plaque size, plaque morphology is important in determining residual risk. Lipid-rich plaques have recently again shown to increase the risk of major adverse cardiac events. Lipid rich plaque can be identified using NIRS. The amount of lipid is represented in the lipid core burden index (LCBI) and is an independent risk factor for future coronary events. A recent study demonstrated the effect on plaque composition in 52 weeks. In this study, an effect in 12 weeks will be evaluated as a potential independent explanation of reduced events in long-term clinical follow-up studies. The change in plaque volume might be closely related to FFR changes.

In addition, it is now well-appreciated that an ACS, a result of atherosclerotic plaque destabilization, initiates a temporary acceleration of atherogenesis in itself. An ACS induces rapid activation of the bone marrow hematopoietic stem- and progenitor cells resulting in monocytosis and activation of innate immune cells, which subsequently accelerate atherosclerosis progression throughout the body. Hypercholesterolemia also activates the innate immune system bone marrow progenitors resulting in long-term activation of the innate immune system. In patients with familial hypercholesterolemia (FH), PCSK9 treatment reduced monocyte CCR2 expression and ex-vivo migratory capacity. Therefore, in the first weeks after an ACS occurred, there is an optimal time window for preventing atherosclerosis progression by powerful lowering of plasma cholesterol. This pharmaco-invasive strategy with a combination of HIST and a PCSK9-inhibitor could possibly prevent mechanical revascularization (PCI or CABG) in a large cohort of patients.

In this study we want to investigate the effect of maximal LDL-C reduction by evolocumab and HIST compared to placebo on functional impairment of non-IRA lesions, measured by FFR, and we want to evaluate the change in Near-InfraRed Spectroscopy (NIRS) derived lipid core burden index at the 4mm maximal segment ($maxLCBI_{4mm}$) from baseline to follow-up in the non-IRA. Secondly, we want to evaluate the change in plaque characteristics, measured by IVUS. Thirdly, we will evaluate the change in microvascular function and investigate correlations between on treatment LDL-C, LCBI, and plaque characteristics, with non-culprit FFR. Finally, the study will investigate the relation between LDL-C reduction and change in pro- inflammatory monocyte phenotypes.

2 Study objective

2.1 Primary objectives

1A. To evaluate the effect of maximal LDL-C reduction by evolocumab on top of high intensity lipid-lowering therapy, initiated immediately after invasive ACS treatment on functional impairment of non-infarct related artery (non-IRA) lesions, measured by FFR, in patients presenting with MVD-ACS.

1B. To evaluate the effect of maximal LDL-C reduction by evolocumab on top of high intensity lipid-lowering therapy, initiated immediately after invasive ACS treatment on the lipid core burden of non-infarct related artery (non-IRA) lesions, measured by NIRS, in patients presenting with MVD-ACS.

2.2 Secondary objectives

2A. To evaluate the effect of maximal LDL-C reduction by evolocumab on top of high intensity lipid-lowering therapy, initiated immediately after invasive ACS treatment on plaque characteristics of non-infarct related artery (non-IRA) lesions, measured by IVUS, in patients presenting with MVD-ACS.

2B. To evaluate the relation between baseline lipid core burden and changes in functional impairment of non-IRA lesions during treatment by evolocumab on top of high intensity lipid-lowering therapy.

2C. To evaluate the effect of maximal LDL-C reduction by evolocumab on top of high intensity lipid-lowering therapy, initiated immediately after invasive ACS treatment on microvascular circulation in patients presenting with MVD-ACS.

2D. To investigate the relationship between LDL-C reduction post-ACS and change in pro-inflammatory monocyte phenotypes.

2.3 Assessment of objectives

Functional impairment, measured by FFR, and intracoronary imaging, using IVUS-NIRS, are used in a serial fashion (baseline and 12 weeks) to assess primary and secondary endpoints.

CFR and IMR are measurements are performed during coronary angiography at baseline and 12 weeks, when possible.

Lipid levels are measured at local laboratories. Assessment of inflammatory biomarkers and monocyte phenotype will be performed at the central laboratory located at the Radboudumc.

2.4 Changes of the primary objective during the conduct of the study

To evaluate the effect of maximal LDL-C reduction by evolocumab on top of high intensity lipid-lowering therapy, initiated immediately after invasive ACS treatment on the lipid core burden was upgraded from secondary objective to a primary objective.

3 Study design

3.1 General design and plan

The study is designed as a randomized, double-blind, placebo controlled multi-center clinical trial to evaluate the effect of the PCSK9 inhibitor evolocumab on functional impairment and plaque composition at baseline and following 12 weeks of treatment in patients presenting with ACS and multivessel disease.

3.2 Sample size

Primary hemodynamic parameter
This study is powered to detect a difference in the primary endpoint (FFR of lesions in non-IRA).

In a previous trial (YELLOW), FFR level at follow-up of 7 weeks was 0.75±0.1 in the intervention group (high intensity statin therapy) and 0.73±0.1 in the control group (normal statin therapy).
Since "high intensity statin therapy" is our control, we expect that effect on FFR will be higher in our intervention group (high intensity statin therapy plus evolocumab). We expect FFR levels to be 0.78±0.1 and 0.75±0.1 in the intervention and control group, respectively. Based on ANCOVA, at a two-sided alpha level of 0.05, a total sample size of 127 would result in 80% power to detect this difference. We assumed a correlation of 0.8 between FFR at baseline and FFR at follow-up based on previous FFR studies. To compensate for dropouts of about 15%, a total of 150 patients should be included at baseline.

$k = n_2 / n_1 = 1$
$n_1 = (\sigma_1^2 + \sigma_2^2) / K)(z_{1-\alpha/2} + z_{1-\beta})^2 / \Delta^2$
$n_1 = (0.1^2 + 0.1^2 / 1)(1.96 + 0.84)^2 / 0.03^2$
$n_1 = 174$
$n_2 = K * 174 = 174$
$n_{tot} = 174 * 2 = 384$

Total sample size corrected for using ANCOVA = $348 * (1 - \rho^2) + 2$
where ρ = correlation coefficient between baseline FFR and follow-up FFR = 0.8

$348 * (1- 0.8^2) \approx 125$

corrected total sample size = 125 + 2 = 127

Primary imaging parameter
To investigate the primary invasive imaging endpoint on plaque composition, IVUS-NIRS image acquisition is only necessary in a smaller group. In the YELLOW trial [11], intensive statin treatment resulted in a reduction of LDL-C from 79.1 mg/dl to 58 mg/dl (26% relative reduction of baseline LDL-C). Continuation of this therapy for six to eight weeks reduced the $maxLCBI_{4mm}$ by a median of 32.2% (95% CI: −40.4 to −12.4), theoretically equivalent to a mean change of 28.33% with a SD of 20.7. We expect that the additional PCSK9-inhibitor treatment will reduce LDL-C to 36.6 mg/dl (= 54% relative reduction to baseline LDL-C) as seen in the GLAGOV [8] and FOURIER [16] trials. This additional reduction of LDL-C should result in a larger decrease in $maxLCBI_{4mm}$. This decrease will probably not be equal to the decrease in LDL-C levels, but we assume an additional effect of 50% on $maxLCBI_{4mm}$ reduction.

This would implicate a $maxLCBI_{4mm}$ reduction of 42.49 mean percentage change in the PCSK9-inhibitor treated group with a similar SD of 20.7.
Based on ANCOVA, at a two-sided alpha level of 0.025, a total sample size of 55 would result in 80% power to detect this difference. We assumed a correlation of 0.6 between $maxLCBI_{4mm}$ at baseline and $maxLCBI_{4mm}$ at follow-up based on previous NIRS studies. To compensate for dropouts of about 20%, a total of 66 patients should be included at baseline.
To reach 90% power, we would need a total of 84 included patients at baseline.

$k = n_2 / n_1 = 1$
$n_1 = (\sigma_1^2 + \sigma_2^2) / K)(z_{1-\alpha/2} + z_{1-\beta})^2 / \Delta^2$
$n_1 = (20.7^2 + 20.7^2 / 1)(2.24 + 0.84)^2 / 14.16^2$
$n_1 = 41$
$n_2 = K * 174 = 41$
$n_{tot} = 174 * 2 = 82$

Total sample size corrected for using ANCOVA = $82 * (1 - \rho^2) + 2$
where ρ = correlation coefficient between baseline $maxLCBI_{4mm}$ and follow-up $maxLCBI_{4mm} = 0.6$

$82 * (1- 0.6^2) \approx 53$

corrected total sample size = 53 + 2 = 55

3.3 Randomization

Randomization will be performed once the culprit lesion in the IRA has been treated successfully, eligibility is confirmed (after all inclusion and exclusion criteria have been checked), written informed consent has been obtained, and after baseline parameters during coronary angiography has been performed successfully.

Patients are randomized after the initial procedure into two groups (evolocumab or placebo). The randomization will take place in 1:1 fashion. Randomization is stratified per study center. Allocation sequences will be based on computer-generated random numbers, using CastorEDC. To ensure a balanced allocation of treatment and control over time, randomization lists will be generated in blocks of 2, 4, or 6 patients. Block size will be generated at random.

Each patient will receive one randomization number and each randomization number will be assigned to a single patient. A patient is considered randomized once randomization to evolocumab of placebo is completed. evolocumab or matching placebo will be administered on day 1 or 2 through week 12. Participants will receive a dosage of evolocumab or placebo every 2 weeks (2QW). The first dose of evolocumab or placebo will preferably be given within 24 hours after the index procedure but must be given within 48 hours after index procedure.

3.4 Blinding

Both the patient and all study personnel (including investigators, imaging assessors and monitors will remain blinded after assignment of the treatments. Importantly, any LDL-C measurements during the study (except LDL-C at screening and after follow-up) will be blinded for the researchers.

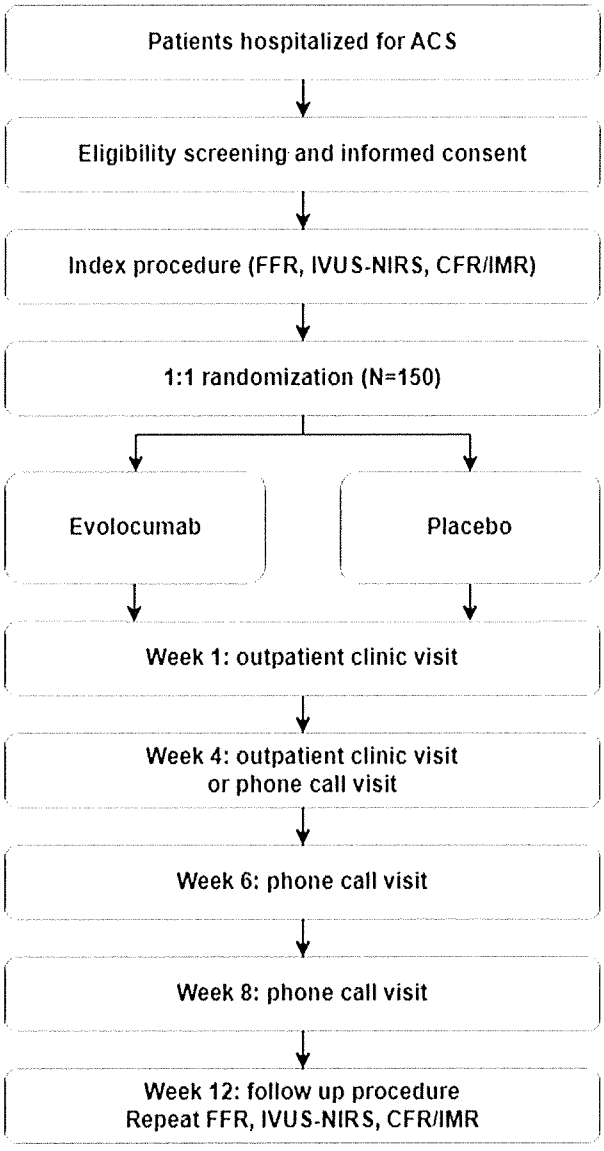
After all queries have been resolved and after the data has been locked, the trial statistician will produce all tables with the unblinded information, correctly assigning the patients to treatment evolocumab or placebo.

3.5 Study assessments

After signing the consent form, patients will be enrolled in the study. Following screening, enrollment and randomization, the total study duration for each individual patient will amount to 12 weeks. The final study visit should take place at week 12 (+/- 2 weeks).

If a patient undergoes a clinically indicated repeat coronary angiography prior to the planned week 12 visit, the study parameters will be allowed to be performed as early as 8 weeks after baseline imaging, but not earlier. Patients with coronary angiography before 8 weeks of treatment should have the per protocol follow-up as planned, except when an intervention on the study vessel is performed before final follow-up. In this case, it will be recommended to perform the study procedures prior to revascularization, if technically and clinically feasible. The derived information will be used for the primary endpoint measures. Figure 1 shows the visit plan for each patient:

Figure 1. FITTER study flowchart



4 Data management

4.1 Data export

Data are captured inside the CastorEDC system (https://castoredc.com) and data will be exported using the CastorEDC export tool. Data will be exported as SPSS.dat files.

Imaging data will be received directly as text or comma delimited files or any other standardized data export, according to the specifications of the imaging software provider; and separately for each patient identifier. The imaging Core Laboratory is blinded to the patient's treatment arm. The independent expert analyzing the IVUS-NIRS data is also blinded to whether the images are from a Baseline or Week 12 follow-up visit.
Similarly, biomarker data will be received directly as text or comma delimited files or any other standardized data export, according to the specifications of the central laboratory; and separately for each patient identifier.

4.2 Data validation

All tables with sample sizes per item will be checked by the coordinating research team for plausibility of missing data.

5 Study populations

5.1 Patient flow

Only randomized patients are reported.

Whether the patient received a first and final injection (evolocumab or placebo) is reported in the flowchart, see for details of compliance the section Evaluation of treatment compliance and exposure.

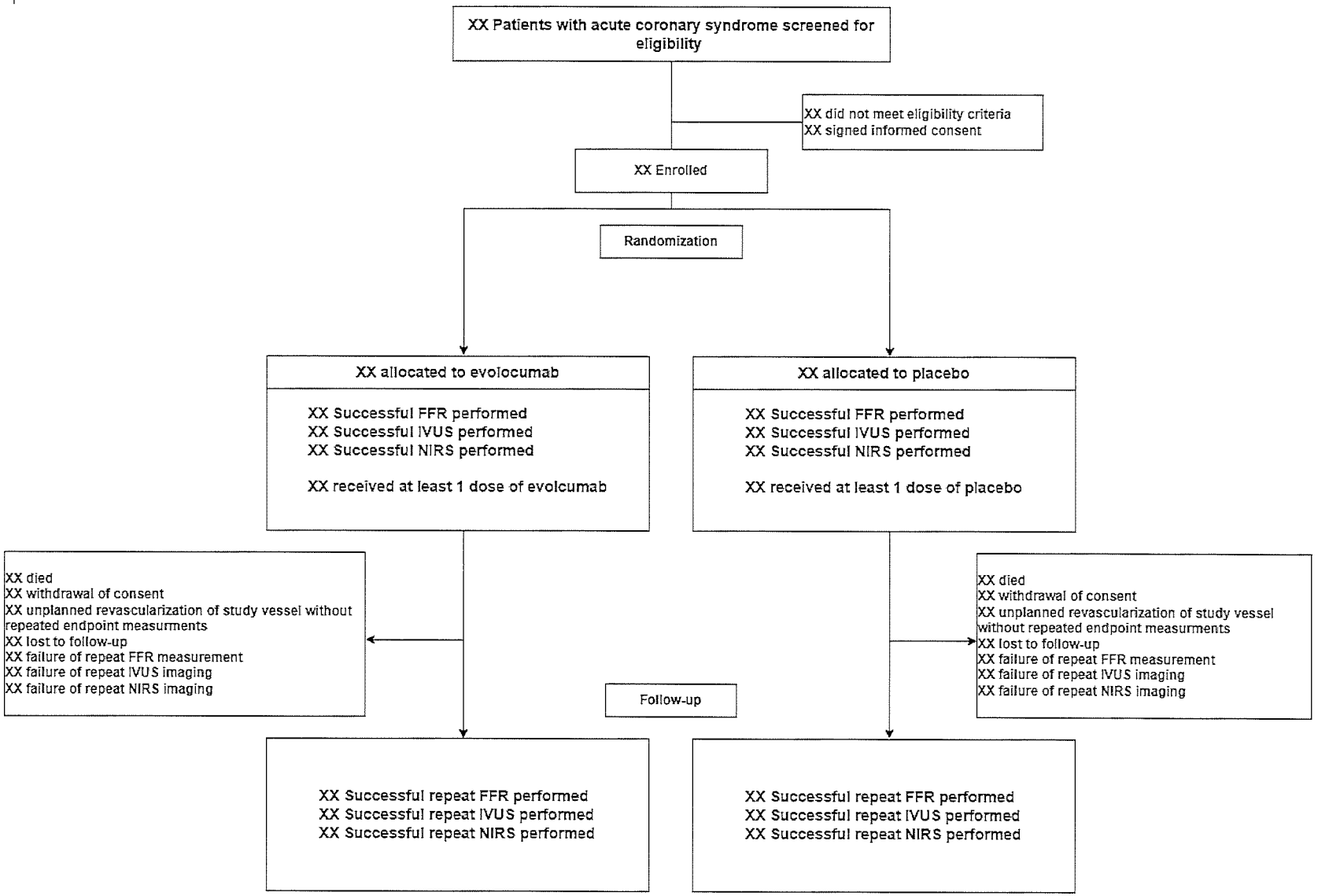
Deaths are reported in the Adverse Events with date of death, confirmed in follow-up visit as patient died item, and end of study.

Withdrawal of consents are reported in the follow-up visit as patient withdrew consent/explicitly refuses follow-up, and end of study.

Lost-to-follow-up are reported in the follow-up visit as patient alive, follow-up not performed / vital status unclear, and end of study.

If applicable, patient removed from study as determined by study investigator will be reported separately in the flowchart.

Figure 2. patient flow



5.2 Definition of populations for analysis

The primary and secondary endpoints will be analyzed using the full analysis set and the IVUS-NIRS set. A secondary per-protocol analysis will also be performed.

5.3 Full analysis set (FAS)

The full analysis set (FAS) includes all randomized subjects who received at least 1 dose of IP and who had a baseline and follow-up assessment of the FFR. In efficacy analyses, subjects will be grouped according to their randomized treatment group assignment, regardless of the treatment received, following the intention-to-treat principle.

5.4 IVUS-NIRS set

The IVUS-NIRS analysis set includes subjects in the FAS with a baseline and follow-up IVUS-NIRS measurements.

5.5 Per-protocol

The Per-protocol analysis set (PP) will include all randomized patients who had a baseline and follow-up assessment of the primary endpoint and also had all the injections from randomization up to the last needed before follow-up imaging (last injection at 10 weeks, or before coronary angiography if coronary angiography was performed before a revascularization or if coronary angiography was performed at an unplanned angiography between 8 and 12 weeks).

Subjects in the PP set will be analyzed according to the treatment they are assigned to at randomization (evolocumab vs placebo).

5.6 Safety population

The safety analysis set includes all randomized subjects who have taken at least 1 dose of investigational product. For safety analyses, the subjects will be grouped according to actual treatment group.

5.7 Definition of sub-group populations in different analyses

Randomized patients are analyzed together for all primary and secondary endpoints. There will be exploratory subgroup analyses (see point 7.6.7.).

6 Study endpoints

6.1 Primary endpoints

1A. The primary physiological study endpoint is the change in FFR from baseline to follow-up in non-IRA lesions.

1B. The primary invasive imaging endpoint is the change in lipid core burden index at the 4mm maximal segment (maxLCBI4_{mm}) from baseline to follow-up of the non-IRA as performed in sites capable of Near-InfraRed Spectroscopy (NIRS).

6.2 Secondary endpoints

Secondary invasive imaging (IVUS) endpoints are:

2a. The change in percent atheroma volume (PAV, mm^3)

2b. The change in normalized total atheroma volume (TAV, mm^3)

2c. The change in maximum plaque burden (%)

2d. The change in minimum lumen area (MLA, mm^2)

6.3 Exploratory endpoints

1. The correlation between achieved on-treatment LDL-C and the change in FFR, the change in LCBI, and the change in PAV.

2. The correlation between baseline NIRS derived maxLCBI$_{4mm}$ and change in FFR of the non-IRA.

3. The correlation between change in IVUS-derived plaque characteristics and change in FFR of the non-IRA

4. Change of microvascular resistance as measured by CFR and IMR

The immunological side study will investigate the relation between LDL-C reduction and reduction of pro-inflammatory monocyte phenotypes.

Clinical endpoints will be tabulated and listed in the final study report; among which percentage of lesions with a FFR ≤0.80 at follow-up and patient-oriented composite endpoint (POCE): composite of all-cause death, any stroke, any MI and any revascularization, unplanned ischemia driven PCI of the target lesion, any unplanned ischemia driven PCI in the total study population.

Table 1. Lipid table

Lipid levels	Evolocumab (N=)	Placebo (N=)	Mean difference (95% CI)	p-value
HDL at baseline – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
HDL at follow-up – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
HDL change – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
LDL at baseline – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
LDL at follow-up – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
LDL change – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Triglycerides at baseline – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
Triglycerides at follow-up – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Triglycerides change – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Total cholesterol at baseline – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
Total cholesterol at follow-up – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Total cholesterol change – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Non-HDL at baseline – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
Non-HDL at follow-up – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Non-HDL change – mmol/L (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx

Table 2a. Vessel-based parameters baseline to follow-up complete group

Complete group	Baseline (N=)	Follow-up (N=)	Mean difference (95% CI)	p-value
FFR performed – no. (%)	x (x.x%)	x (x.x%)		
LAD – no. (%)	x (x.x%)	x (x.x%)		
Cx – no. (%)	x (x.x%)	x (x.x%)		
RCA – no. (%)	x (x.x%)	x (x.x%)		
FFR – index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
FFR > 0.80 – no. (%)	x (x.x%)	x (x.x%)		x.xx
Study vessels with increase in FFR at follow-up – no. (%)		x (x.x%)		
Patients with PCI at FU – no. (%)		x (x.x%)		
Patients with PCI at FU with baseline FFR ≤ 0.80 – no. (%)		x (x.x%)		
Patients without PCI at FU because FFR > 0.80 – no. (%)		x (x.x%)		
Patients without PCI at FU and FFR ≤ 0.80 but no complaints – no. (%)		x (x.x%)		
NIRS performed – no. (%)	x (x.x%)	x (x.x%)		
LAD – no. (%)	x (x.x%)	x (x.x%)		
Cx – no. (%)	x (x.x%)	x (x.x%)		
RCA – no. (%)	x (x.x%)	x (x.x%)		
$MaxLCBI_{4mm}$ – index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Regressors: decrease in $maxLCBI_{4mm}$ – no. (%)		x (x.x%)		
$MaxLCBI_{4mm}$ ≥324·7 – no. (%)	x (x.x%)	x (x.x%)		x.xx
$MaxLCBI_{4mm}$ >400 – no. (%)	x (x.x%)	x (x.x%)		x.xx
LCBI total – index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
IVUS performed – no. (%)	x (x.x%)	x (x.x%)		
LAD – no. (%)	x (x.x%)	x (x.x%)		
Cx – no. (%)	x (x.x%)	x (x.x%)		
RCA – no. (%)	x (x.x%)	x (x.x%)		
Percent atheroma volume — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Regressors: decrease PAV — no. (%)		x (x.x%)		
Maximum plaque burden — % (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
Plaque burden ≥ 70% — no. (%)	x (x.x%)	x (x.x%)		x.xx
Minimum lumen area — mm^2 (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
Total atheroma volume — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Normalized total atheroma volume — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Regressors: normalized total atheroma volume — no. (%)		x (x.x%)		

Table 2b. Vessel-based parameters baseline to follow-up evolocumab group

Evolocumab group	Baseline (N=)	Follow-up (N=)	Mean difference (95% CI)	p-value
FFR performed – no. (%)	x (x.x%)	x (x.x%)		
LAD – no. (%)	x (x.x%)	x (x.x%)		
Cx – no. (%)	x (x.x%)	x (x.x%)		
RCA – no. (%)	x (x.x%)	x (x.x%)		
FFR – index (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
FFR > 0.80 – no. (%)	x (x.x%)	x (x.x%)		x.xx
Study vessels with increase in FFR at follow-up – no. (%)		x (x.x%)		
Patients with PCI at FU – no. (%)		x (x.x%)		
Patients with PCI at FU with baseline FFR ≤ 0.80 – no. (%)		x (x.x%)		
Patients without PCI at FU because FFR > 0.80 – no. (%)		x (x.x%)		
Patients without PCI at FU and FFR ≤ 0.80 but no complaints – no. (%)		x (x.x%)		
NIRS performed – no. (%)	x (x.x%)	x (x.x%)		
LAD – no. (%)	x (x.x%)	x (x.x%)		
Cx – no. (%)	x (x.x%)	x (x.x%)		
RCA – no. (%)	x (x.x%)	x (x.x%)		
$MaxLCBI_{4mm}$ – index (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
Regressors: decrease in $maxLCBI_{4mm}$ – no. (%)		x (x.x%)		
$MaxLCBI_{4mm}$ ≥324·7 – no. (%)	x (x.x%)	x (x.x%)		x.xx
$MaxLCBI_{4mm}$ >400 – no. (%)	x (x.x%)	x (x.x%)		x.xx
LCBI total – index (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
IVUS performed – no. (%)	x (x.x%)	x (x.x%)		
LAD – no. (%)	x (x.x%)	x (x.x%)		
Cx – no. (%)	x (x.x%)	x (x.x%)		
RCA – no. (%)	x (x.x%)	x (x.x%)		
Percent atheroma volume — % (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
Regressors: decrease PAV – no. (%)		x (x.x%)		
Maximum plaque burden — % (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
Plaque burden ≥ 70% – no. (%)	x (x.x%)	x (x.x%)		x.xx
Minimum lumen area — mm^2 (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
Total atheroma volume — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
Normalized total atheroma volume — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
Regressors: normalized total atheroma volume – no. (%)		x (x.x%)		

Table 2c. Vessel-based parameters baseline to follow-up placebo group

Placebo group	Baseline (N=)	Follow-up (N=)	Mean difference (95% CI)	p-value
FFR performed – no. (%)	x (x.x%)	x (x.x%)		
LAD – no. (%)	x (x.x%)	x (x.x%)		
Cx – no. (%)	x (x.x%)	x (x.x%)		
RCA – no. (%)	x (x.x%)	x (x.x%)		
FFR – index (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
FFR > 0.80 – no. (%)	x (x.x%)	x (x.x%)		x.xx
Study vessels with increase in FFR at follow-up – no. (%)		x (x.x%)		
Patients with PCI at FU – no. (%)		x (x.x%)		
Patients with PCI at FU with baseline FFR ≤ 0.80 – no. (%)		x (x.x%)		
Patients without PCI at FU because FFR > 0.80 – no. (%)		x (x.x%)		

	Patients without PCI at FU and FFR ≤ 0.80 but no complaints – no. (%)		x (x.x%)		
NIRS performed – no. (%)		x (x.x%)	x (x.x%)		
	LAD – no. (%)	x (x.x%)	x (x.x%)		
	Cx – no. (%)	x (x.x%)	x (x.x%)		
	RCA – no. (%)	x (x.x%)	x (x.x%)		
	$MaxLCBI_{4mm}$ – index (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
	Regressors: decrease in $maxLCBI_{4mm}$ – no. (%)		x (x.x%)		
	$MaxLCBI_{4mm}$ ≥324·7 – no. (%)	x (x.x%)	x (x.x%)		x.xx
	$MaxLCBI_{4mm}$ >400 – no. (%)	x (x.x%)	x (x.x%)		x.xx
	LCBI total – index (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
IVUS performed – no. (%)		x (x.x%)	x (x.x%)		
	LAD – no. (%)	x (x.x%)	x (x.x%)		
	Cx – no. (%)	x (x.x%)	x (x.x%)		
	RCA – no. (%)	x (x.x%)	x (x.x%)		
	Percent atheroma volume — % (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
	Regressors: decrease PAV – no. (%)		x (x.x%)		
	Maximum plaque burden — % (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
	Plaque burden ≥ 70% – no. (%)	x (x.x%)	x (x.x%)		x.xx
	Minimum lumen area — mm^2 (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
	Total atheroma volume — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
	Normalized total atheroma volume — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x ± x.x	x.xx
	Regressors: normalized total atheroma volume – no. (%)		x (x.x%)		

Table 3. Vessel-based endpoints

Vessel based analysis	Evolocumab (N=)	Placebo (N=)	Mean difference (95% CI)	p-value
FFR performed at baseline and follow-up – no. (%)	x (x.x%)	x (x.x%)		
LAD – no. (%)	x (x.x%)	x (x.x%)		
Cx – no. (%)	x (x.x%)	x (x.x%)		
RCA – no. (%)	x (x.x%)	x (x.x%)		
FFR at baseline – index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
FFR at follow-up – index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
FFR change – index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
FFR > 0.80 at follow-up – no. (%)	x (x.x%)	x (x.x%)		x.xx
Study vessels with increase in FFR at follow-up – no. (%)	x (x.x%)	x (x.x%)		x.xx
NIRS performed at baseline and follow-up – no. (%)	x (x.x%)	x (x.x%)		
LAD – no. (%)	x (x.x%)	x (x.x%)		
Cx – no. (%)	x (x.x%)	x (x.x%)		
RCA – no. (%)	x (x.x%)	x (x.x%)		
$MaxLCBI_{4mm}$ at baseline — index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
$MaxLCBI_{4mm}$ at follow-up — index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
$MaxLCBI_{4mm}$ change – index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Regressors: decrease in $maxLCBI_{4mm}$ – no. (%)	x (x.x%)	x (x.x%)		x.xx
No. of vessels with $maxLCBI_{4mm}$ ≥324·7 at baseline – no. (%)	x (x.x%)	x (x.x%)		
No. of vessels with $maxLCBI_{4mm}$ ≥324·7 at follow-up – no. (%)	x (x.x%)	x (x.x%)		x.xx
LCBI total at baseline — index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
LCBI total at follow-up — index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
LCBI total change — index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
IVUS performed at baseline and follow-up – no. (%)	x (x.x%)	x (x.x%)		
LAD – no. (%)	x (x.x%)	x (x.x%)		
Cx – no. (%)	x (x.x%)	x (x.x%)		
RCA – no. (%)	x (x.x%)	x (x.x%)		
Percent atheroma volume at baseline — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
Percent atheroma volume at follow-up — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Percent atheroma volume change — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Regressors: decrease PAV – no. (%)	x (x.x%)	x (x.x%)		x.xx

	Total atheroma volume at baseline — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
	Total atheroma volume at follow-up — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
	Total atheroma volume change — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
	Regressors: normalized total atheroma volume – no. (%)	x (x.x%)	x (x.x%)		x.xx
	Normalized total atheroma volume at baseline — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
	Normalized total atheroma volume at follow-up — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
	Normalized total atheroma volume change — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
	Maximum plaque burden at baseline — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
	Maximum plaque burden at follow-up — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
	Maximum plaque burden change — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
	Vessels with plaque burden at baseline ≥ 70% – no. (%)	x (x.x%)	x (x.x%)		
	Vessels with plaque burden at follow-up ≥ 70% – no. (%)	x (x.x%)	x (x.x%)		x.xx
	Minimum lumen area at baseline — mm^2 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
	Minimum lumen area at follow-up — mm^2 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
	Minimum lumen area change — mm^2 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx

Table 4. Lesion-based endpoints

Lesion based analysis	Evolocumab (N=)	Placebo (N=)	Mean difference (95% CI)	p-value
IVUS-NIRS performed at baseline and follow-up – no. (%)	x (x.x%)	x (x.x%)		
Total number of ROI's – no. (%)	x (x.x%)	x (x.x%)		
LAD – no. (%)	x (x.x%)	x (x.x%)		
Cx – no. (%)	x (x.x%)	x (x.x%)		
RCA – no. (%)	x (x.x%)	x (x.x%)		
$MaxLCBI_{4mm}$ at baseline — index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
$MaxLCBI_{4mm}$ at follow-up — index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
$MaxLCBI_{4mm}$ change — index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
No. of lesions with $maxLCBI_{4mm}$ ≥324·7 at baseline – no. (%)	x (x.x%)	x (x.x%)		
No. of lesions with $maxLCBI_{4mm}$ ≥324·7 at follow-up – no. (%)	x (x.x%)	x (x.x%)		x.xx
LCBI total at baseline — index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
LCBI total at follow-up — index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
LCBI total change — index (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Percent atheroma volume at baseline — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
Percent atheroma volume at follow-up — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Percent atheroma volume change — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Total atheroma volume at baseline — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
Total atheroma volume at follow-up — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Total atheroma volume change — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Normalized total atheroma volume at baseline — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
Normalized total atheroma volume at follow-up — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Normalized total atheroma volume change — mm^3 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Maximum plaque burden at baseline — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
Maximum plaque burden at follow-up — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Maximum plaque burden change — % (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Lesions with plaque burden ≥ 70% – no. (%)	x (x.x%)	x (x.x%)		
Lesions with plaque burden ≥ 70% – no. (%)	x (x.x%)	x (x.x%)		x.xx
Minimum lumen area at baseline — mm^2 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	
Minimum lumen area at follow-up — mm^2 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx
Minimum lumen area change — mm^2 (±SD)	x.x ± x.x	x.x ± x.x	x.x (x.x - x.x)	x.xx

7 Statistical analysis

7.1 General

To be able to calculate change, longitudinal measurements for all endpoints with a change score are needed. If any endpoints are not based on change, measurement at the specific time point are sufficient.

Multiplicity correction for primary endpoints will be done to maintain the overall familywise error rate at 0.05 using a Hochberg correction. A hierarchical testing method will be used for the secondary endpoints.

All calculations will be generated by statistical package for social sciences software (SPSS) or R. Descriptive statistics will be expressed as mean ± SD, median and (Q1-Q3) (continuous data) or as frequencies and proportions (categorical data). Furthermore, scatterplots or boxplots will be used to visualize the data per group. For subgroup analysis, forest plots will be used to visualize the results from all subgroups.

Analysis of other continuous outcome parameters (if not described in the analysis of primary, secondary or exploratory outcomes sections) will be done using t-tests or Mann Whitney U test. For the analysis of binary outcome variables Chi-square test or Fisher exact test will be used to compare the groups. If needed, analysis including center (stratification factor) might be performed.

7.2 Analysis of primary, secondary, and exploratory endpoints

7.2.1 Analysis of primary endpoints

Analysis of the primary outcome will be done using an ANCOVA model as primary analysis using Full Analysis Set (FAS) and IVUS-NIRS analysis set population. The ANCOVA model will include treatment group and randomisation stratification center as a fixed factor, and will be corrected for the baseline value of the primary outcome variable.
A secondary per-protocol analysis will also be performed using the same model.

7.2.2 Analysis of secondary endpoints

For continuous secondary outcome parameters, the approach applied for the primary parameter will be followed. Lesion based analysis will include a random effect for lesion, accounting for the correlation among lesions within a subject.

7.2.3 Analysis of exploratory endpoints

For exploratory purpose, correlation coefficients will be calculated.
For binary exploratory parameters a Cochran Mantel Hansel test can be used with study centers as strata. Alternatively, logistic regression might be used, and it would also allow for correcting for differences in baseline characteristics if deemed necessary.

7.3 Interim analysis

No interim analysis is planned for this study.

7.4 Time-points for analysis

Time-points for analysis are Baseline (randomization, coronary angiography, blood sampling), Week 1 (blood sampling), week 4 (blood sampling) and week 12 (coronary angiography, blood sampling).

7.5 Methods for handling missing data and drop-out

For the FAS analysis, there will be no data imputation; as baseline and follow-up endpoint data is needed to calculate the endpoints.

For other sensitivity analyses, missing primary endpoint data at baseline visit can be imputed using mean imputation (if percentage of missings is less than 5%). For missing data other than baseline data, multiple imputation might be used.

For multiple imputation, fifty imputations may be performed. Recorded patient characteristics (age, sex, BMI, hypertension, dyslipidaemia, family history of CAD, smoker history, diabetes, stroke/TIA, peripheral artery disease, prior MI, prior PCI, premature CVD, ACS type at index), or baseline endpoint data (FFR at baseline; plaque characteristics data), can be utilized in multiple imputations to impute primary outcome. Results obtained in different imputed datasets will be summarized using Rubin's rule.

If the data is missing due to image quality, other mechanical problems, data reading, or loss to follow-up for reasons other than efficacy, then it can be considered as missing at random. For other reasons of missings, different assumptions might be needed in the imputation.

7.6 Statistical analytical issues

7.6.1 Assessment of statistical assumptions

For checking the fit of the ANCOVA model, two assumptions should be checked at minimum: Normality of the residuals and homogeneity of variance. For checking the normality of residuals, residual plots (QQ plots and histograms) are visually explored. For checking the homogeneity of variance, visual inspection of residuals versus predicted values can be created. Additionally, Levene's test can be used.
Additionally, linearity of the dependent variable for the levels of independent variables could also be checked.
For the logistic regression analysis, significance of the model should be checked before the fitted model can be used (Omnibus test of model coefficients). Additionally, Hosmer-Lemeshow test will be checked if logistic regression is adequate for the data. If the logistic regression has problem with fitting due to quasi-complete separation, exact logistic regression or Firth's logistic regression might be considered.

Furthermore, checking the residual plots from both regression models for outliers would help to see if there are some extreme observations that might influence the conclusion of the study. In such a case, analyses without outliers will be also performed as sensitivity analysis.

7.6.2 Adjustments for covariates

For covariates used in the ANCOVA model, please see chapter 7.2.1. No further adjustment for covariates are intended for the primary and secondary endpoints.

7.6.3 Vessel-based analysis

For our vessel-based analysis of the primary and secondary endpoints, only one vessel per patient can be included. When multiple vessels have a valid FFR measurement at baseline and follow-up, then the vessel with an IVUS-NIRS (at baseline and follow-up) will be included in the analysis. If no IVUS-NIRS was performed or if IVUS-NIRS was also performed in multiple vessels, then the vessel with the lowest FFR at baseline will be included. Similarly, if IVUS-NIRS was successfully performed in multiple vessels, then the IVUS-NIRS of the vessel with de lowest FFR at baseline will be included for the vessel-based analysis.

7.6.4 Lesion-based analysis

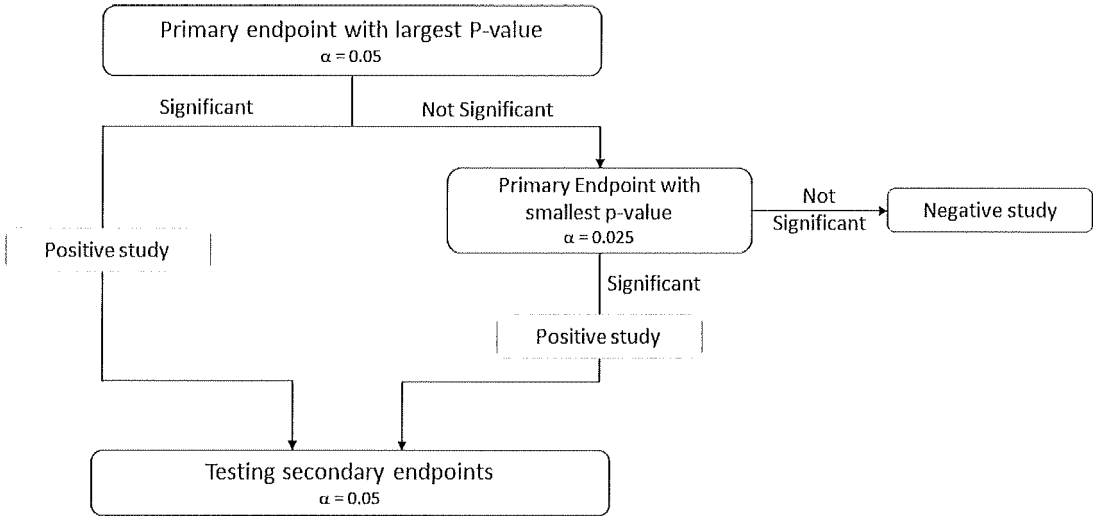
For our lesion-based analysis, multiple separate lesions within the same vessel or patient can be included. A random effect for lesion in the ANCOVA will be used to account for the correlation among the lesions within patient.

7.6.5 Multicenter studies

The analysis model will include a center effect to be able to account for any potential differences among the centers.

7.6.6 Multiple comparisons

The two primary endpoints will be tested using the Hochberg procedure. The strategy will work as follows: The p-values for the primary endpoints are sorted in descending order. Then the largest p-value is compared with alpha value of 0.05; if it is smaller than 0.05, both null hypotheses are rejected. If the largest p-value is not smaller than 0.05, then the smaller p-value is compared with alpha = 0.025. If the second p-value is smaller than 0.025, then the null hypothesis corresponding to that primary outcome variable will be rejected. If this is the case, the overall study result for the primary outcome can be considered as being positive.
The p-values for the secondary endpoints will only be interpreted (i.e., the subsequent null hypotheses can only be rejected), if at least one of the null hypotheses of the primary endpoints is rejected.



The secondary endpoints (change in PAV, change in normalized TAV, change in maximum plaque burden and change in MLA) will be tested using a hierarchical testing procedure. A p-value of 0.05 will be used to test H0 for the secondary endpoints. For example, endpoint 2b. will only be tested if endpoint 2a. has a p-value <0.05 etc.

7.6.7 Examination of subgroups

Prespecified stratified analyses of the primary and secondary endpoints will be performed according to the following characteristics: age, sex, diabetes mellitus, ACS type, vessel localization, FFR at baseline, statin use at baseline, LDL-level at baseline, LDL reduction throughout study period, $maxLCBI_{4mm}$ at baseline.

Hypotheses:

1. Change in FFR will be larger in patients with high LDL-C at baseline
2. Change in FFR will be larger in patients who are statin naive at baseline
3. Change in FFR will be larger in younger patients
4. Change in FFR will be larger in patients with high $maxLCBI_{4mm}$ at baseline
5. Change in $maxLCBI_{4mm}$ will be higher in patients with high LDL-C at baseline
6. Change in $maxLCBI_{4mm}$ will be higher in statin naive patients

7.6.8 Sensitivity analysis due to COVID-19

Due to the COVID-19 several patients might have a delay of several weeks before their week 12 follow-up visit. The number of subjects reporting Protocol Deviations due to COVID-19 Impact will be summarized in a table. If applicable, a sensitivity analyses will be conducted. All the available post-baseline primary endpoint values will be considered in the analysis, irrespective of the analysis visit window (i.e., the post-baseline primary endpoint values after week 12 will also be considered for the analysis).

8 Evaluation of demographics, baseline characteristics and medications during the study

Baseline characteristics are shown in Table 5. Randomization will occur after the angiography and after the quality of FFR assessments has been confirmed.

Baseline values for background statin therapy, lipid-modifying concomitant medication usage, other concomitant medication and medical history are defined as the assessment measured at screening.

Table 5. Baseline characteristics FITTER population

	Evolocumab (N=)	Placebo (N=)
Age – years (±SD)	x.x ± x.x	x.x ± x.x
Sex, male – no. (%)	x (x.x%)	x (x.x%)
BMI – kg/m^2 (±SD)	x.x ± x.x	x.x ± x.x
Hypertension – no. (%)	x (x.x%)	x (x.x%)
Dyslipidemia – no. (%)	x (x.x%)	x (x.x%)
Family history of CAD – no. (%)	x (x.x%)	x (x.x%)
Smoker History – no. (%)	x (x.x%)	x (x.x%)
Current Smoker – no. (%)	x (x.x%)	x (x.x%)
Diabetes – no. (%)	x (x.x%)	x (x.x%)
Insulin-treated – no. (%)	x (x.x%)	x (x.x%)
Stroke or TIA – no. (%)	x (x.x%)	x (x.x%)
Peripheral artery disease – no. (%)	x (x.x%)	x (x.x%)
Prior myocardial infarction – no. (%)	x (x.x%)	x (x.x%)
Prior PCI – no. (%)	x (x.x%)	x (x.x%)
Premature CVD (CAD/CVA/TIA/PAD) – no. (%)	x (x.x%)	x (x.x%)
Killip class at presentation		
- Killip I – no. (%)	x (x.x%)	x (x.x%)
- Killip II – no. (%)	x (x.x%)	x (x.x%)
- Killip III – no. (%)	x (x.x%)	x (x.x%)
- Killip IV – no. (%)	x (x.x%)	x (x.x%)

Table 6. ACS type at hospitalization and time to PCI, randomization and first

	Evolocumab (N=)	Placebo (N=)
STEMI – no. (%)	x (x.x%)	x (x.x%)
NSTEMI – no. (%)	x (x.x%)	x (x.x%)
UAP – no. (%)	x (x.x%)	x (x.x%)

Time from admission to PCI – days (±SD)	x.x ± x.x	x.x ± x.x
Time from admission to randomization – days (±SD)	x.x ± x.x	x.x ± x.x
Time from admission to first dose of investigational product – days (±SD)	x.x ± x.x	x.x ± x.x
Time from PCI to first medication – days (±SD)	x.x ± x.x	x.x ± x.x

Table 7. Medication before hospitalization at index

	Evolocumab (N=)	Placebo (N=)
Aspirine – no. (%)	x (x.x%)	x (x.x%)
ADPRI (ticagrelor/clopidogrel/prasugrel) – no. (%)	x (x.x%)	x (x.x%)
DAPT – no. (%)	x (x.x%)	x (x.x%)
Statins use – no. (%)	x (x.x%)	x (x.x%)
- High intensity statin therapy – no. (%)	x (x.x%)	x (x.x%)
Other lipid lowering drugs – no. (%)	x (x.x%)	x (x.x%)
- Ezetimibe – no. (%)	x (x.x%)	x (x.x%)
- Fibrates – no. (%)	x (x.x%)	x (x.x%)
- Niacin – no. (%)	x (x.x%)	x (x.x%)
- Resins – no. (%)	x (x.x%)	x (x.x%)
ACE inhibitor – no. (%)	x (x.x%)	x (x.x%)
ARB – no. (%)	x (x.x%)	x (x.x%)
Bèta-blocker – no. (%)	x (x.x%)	x (x.x%)

Table 8. Medication at discharge

	Evolocumab (N=)	Placebo (N=)
Aspirine – no. (%)	x (x.x%)	x (x.x%)
ADPRI (ticagrelor/clopidogrel/prasugrel) – no. (%)	x (x.x%)	x (x.x%)
DAPT – no. (%)	x (x.x%)	x (x.x%)
Statins use – no. (%)	x (x.x%)	x (x.x%)
- High intensity statin therapy – no. (%)	x (x.x%)	x (x.x%)
Other lipid lowering drugs – no. (%)	x (x.x%)	x (x.x%)
- Ezetimibe – no. (%)	x (x.x%)	x (x.x%)
- Fibrates – no. (%)	x (x.x%)	x (x.x%)
- Niacin – no. (%)	x (x.x%)	x (x.x%)
- Resins – no. (%)	x (x.x%)	x (x.x%)
ACE inhibitor – no. (%)	x (x.x%)	x (x.x%)
ARB – no. (%)	x (x.x%)	x (x.x%)

Bèta-blocker – no. (%)	x (x.x%)	x (x.x%)

Table 9. Medication at follow-up procedure

	Evolocumab (N=)	Placebo (N=)
Aspirine – no. (%)	x (x.x%)	x (x.x%)
ADPRI (ticagrelor/clopidogrel/prasugrel) – no. (%)	x (x.x%)	x (x.x%)
DAPT – no. (%)	x (x.x%)	x (x.x%)
Statins use – no. (%)	x (x.x%)	x (x.x%)
- High intensity statin therapy – no. (%)	x (x.x%)	x (x.x%)
Other lipid lowering drugs – no. (%)	x (x.x%)	x (x.x%)
- Ezetimibe – no. (%)	x (x.x%)	x (x.x%)
- Fibrates – no. (%)	x (x.x%)	x (x.x%)
- Niacin – no. (%)	x (x.x%)	x (x.x%)
- Resins – no. (%)	x (x.x%)	x (x.x%)
ACE inhibitor – no. (%)	x (x.x%)	x (x.x%)
ARB – no. (%)	x (x.x%)	x (x.x%)
Bèta-blocker – no. (%)	x (x.x%)	x (x.x%)

9 Evaluation of treatment compliance and exposure

9.1 Compliance to study drug and treatment

Compliance is reported in Table 10 (see below). Whether and when the patient received the randomized injection (containing either evolocumab or placebo) is captured for each visit with the item:

Double blind injection of study drug at study site (baseline, recorded in Discharge Medication eCRF, as injection is allowed after the PCI).

Has patient taken all doses of study drug as planned up to current visit (at each follow-up by either telephone call or during clinical visit, recorded in Follow-up Medication eCRF):

- Compliant are considered patients who answered yes, or answered no with one or more injections delayed by less than or equal to 7 days and scheduled dose administered. Considering the wash-out time, the latter patient are regarded as sufficiently compliant.
- Not compliant are considered patients who answered no with: one or more injections delayed by more than 7 days and scheduled dose not administered or completely stopped taking the study drug.
- Completely stopped taking the study drug answer is attributed to the visit and all visits beyond until alive at end of study (restarts are not expected).

Details of IMP injection compliance per patient with respect to the total number of injections needed are showed in Table 10 as well.

Table 10. Details of IMP injection compliance (with respect to the total number of injections needed)

		Evolocumab (N = xx)	Placebo (N = xx)	p-value
Total	Injected with all doses of IMP until follow-up angiography — no. (%)	x (x.x%)	x (x.x%)	x.xx
	Injected with all doses of IMP – no. (%)	x (x.x%)	x (x.x%)	x.xx
Baseline	Compliant injection of IMP — no. (%)	x (x.x%)	x (x.x%)	x.xx
	One or more injections delayed >7 days or skipped — no. (%)	x (x.x%)	x (x.x%)	x.xx
	Cumulative IMP discontinuation— no. (%)	x (x.x%)	x (x.x%)	x.xx
Week 1	Compliant injection of IMP — no. (%)	x (x.x%)	x (x.x%)	x.xx
	One or more injections delayed >7 days or skipped — no. (%)	x (x.x%)	x (x.x%)	x.xx
	Cumulative IMP discontinuation— no. (%)	x (x.x%)	x (x.x%)	x.xx
Week 4	Compliant injection of IMP — no. (%)	x (x.x%)	x (x.x%)	x.xx
	One or more injections delayed >7 days or skipped — no. (%)	x (x.x%)	x (x.x%)	x.xx
	Cumulative IMP discontinuation— no. (%)	x (x.x%)	x (x.x%)	x.xx
Week 6	Compliant injection of IMP — no. (%)	x (x.x%)	x (x.x%)	x.xx
	One or more injections delayed >7 days or skipped — no. (%)	x (x.x%)	x (x.x%)	x.xx
	Cumulative IMP discontinuation— no. (%)	x (x.x%)	x (x.x%)	x.xx
Week 8	Compliant injection of IMP — no. (%)	x (x.x%)	x (x.x%)	x.xx
	One or more injections delayed >7 days or skipped — no. (%)	x (x.x%)	x (x.x%)	x.xx
	Cumulative IMP discontinuation— no. (%)	x (x.x%)	x (x.x%)	x.xx
Week 12	Compliant injection of IMP — no. (%)	x (x.x%)	x (x.x%)	x.xx
	One or more injections delayed >7 days or skipped — no. (%)	x (x.x%)	x (x.x%)	x.xx
	Cumulative IMP discontinuation— no. (%)	x (x.x%)	x (x.x%)	x.xx

9.2 Exposure to study drug

9.2.1 Extent, dose and duration of exposure

Treatment with the investigational product (IP) (140 mg evolocumab or placebo) will start at Baseline (Week 0) and finish on Week 10. Patients get an injection with the IP every second week. In total, 6 doses of evolocumab or placebo will be administered. The last dose will be given in week 10, which is 2 weeks before the follow-up procedure.
This treatment will be on top of HIST (e.g. Atorvastatin 40mg – 80mg or Rosuvastatin 20mg – 40mg) throughout the entire study period.

9.2.2 Drug concentrations

Evolocumab (PCKS9 inhibitor) will be administered as a sterile solution in a single-use, disposable, prefilled autoinjector pen for fixed-dose subcutaneous injection (SureClick™ auto-injector). The prefilled pen contains 1.0 ml deliverable volume of 140 mg/mL evolocumab in 220 mM proline, 20 mM acetate, 0.01% (w/v) polysorbate 80, pH 5.0. 2.
Placebo will be prepared in the same formulation as evolocumab without the addition of protein and will be administered in an identical prefilled pen containing 1.0 ml of deliverable volume. Both preparations will be manufactured and packaged by Amgen B.V. and will be stored refrigerated and protected from light.

9.2.3 Drug injection

Health care professionals at the participating sites are trained to administer the IMP. An auto-injector training guide is also provided to the sites. A diary with instructions for use of the auto-injector pen is provided to the patient. The IMP will be kept outside the refrigerator at room temperature for about 30 minutes immediately prior to administration. The first dose of IMP is administered by a health care professional, and patients are first trained to administer the IMP themselves, before boxes of IMP are provided to the patient. If indicated (e.g., wish patient), patients have the option to return to the site for IMP administration by a health care professional.
Each administration will consist of 1.0 mL SC injection in the abdomen, thigh, or outer area of upper arm (deltoid region). If another concomitant drug is being injected the patient should be advised to use different injection sites. The used prefilled pens will be returned to the site at the next follow-up visit.

9.2.4 Drug dose adjustment and withdrawal

There will be no dose adjustment of the IP in this study. If in the judgment of the investigator a patient cannot tolerate the IP, administration will be discontinued but the patient will be asked to return for all protocol-required visits and study procedures until completion of the study.

10 Intracoronary procedures and laboratory measurements

10.1 Imaging of coronary arteries

During the index procedure, after coronary angiography and PCI of the culprit lesion, patients will be checked for eligibility to participate in the study. If eligible and informed consent is obtained, patients will undergo a FFR measurement of the non-culprit vessel. The FFR measurement is preferably performed during initial angiography but can be performed as a second staged procedure (for example, in case of STEMI or unstable patients with culprit-only PCI). If the FFR measurement is in the eligible range (0.67 – 0.85), patients are included in the study and intracoronary imaging of the non-culprit vessel is performed, if available and feasible. Baseline intracoronary imaging is performed in the same procedure as the FFR measurement.
After 12 weeks, patients will be readmitted for repeat coronary angiography with FFR measurement of the same non-culprit vessel. If IVUS-NIRS was performed at baseline, repeat IVUS-NIRS of the same non-culprit vessel is also performed. Revascularization of the non-culprit vessel at the follow-up procedure is based on the repeat FFR measurements and the symptoms of the patient.

If a patient undergoes a clinically indicated repeat coronary angiography prior to the planned week 12 visit, the study parameters will be allowed to be performed as early as 8 weeks after baseline imaging, but not earlier. Patients with coronary angiography before 8 weeks of treatment should have the per protocol follow-up as planned, except when an intervention on the study vessel is performed before final follow-up. In this case, it will be recommended to perform the study procedures prior to revascularization, if technically and clinically feasible.

10.2 Measurement of fractional flow reserve (FFR)

A fractional flow reserve (FFR) measurement of the non-culprit vessel has to be performed for patients to be included in the study. FFR measurements are done using a FFR-wire (preferably PressureWire™ X Guidewire by Abbott Cardiovascular or OmniWire™ by Philips). A standard operating procedure (SOP) for a correct execution of the FFR measurement can be found in the study protocol (appendix 4). The FFR-wire is advanced distal to the non-culprit lesion of interest. Position of the FFR-wire will be captured. During follow-up procedure, this image is used to place the FFR-wire in the exact same position as the baseline measurement. Resting Pd/Pa is captured. Hyperemia is achieved by infusion of 100-200 mcg adenosine intracoronary or 140 mcg/kg/min adenosine administered through a central or peripheral vein. Either intracoronary or intravenous adenosine administration can be chosen per the interventional cardiologists discretion, however, administration method must be the same for baseline and follow-up measurements. After the FFR measurement, drift is captured. If drift is above 0.02, FFR measurement is repeated and drift is measured again, until acceptable drift is achieved (≤0.02 drift).

10.3 IVUS-NIRS imaging

IVUS-NIRS imaging will be performed in a subset of participating centers after FFR measurement (to make sure patients are eligible for the study). The combined NIRS-IVUS catheter (3.2-F rapid exchange catheter, InfraReDx, Burlington, Massachusetts) will be positioned distal in the study vessel. Angiographic recordings of the anatomical landmarks will be made. Image acquisition will be performed by a motorized catheter pullback at a speed of 0.5 mm/s and 240 rpm. The system performs 1,000 chemical measurements per 12.5 mm, in which each measurement interrogates 1 to 2 mm2 of vessel wall from a depth of approximately 1 mm in the direction from the luminal surface toward the adventitia. NIRS Spectra will be transformed into a probability of lipid core that will be

mapped to a red-to-yellow color scale, with the low probability of lipid shown as red and the high probability of lipid shown as yellow. The measurement of the probability of lipid core is displayed as a NIRS 'chemogram', a color-coded map of the location and intensity of lipid core, with the X-axis indicating the pullback position in millimeters and the Y-axis indicating the circumferential position. IVUS images are simultaneously acquired by a transducer at a frequency between 30 and 70 MHz and with an axial resolution of 100 µm, together with co-registered NIRS measurements. Thus, the NIRS spectra data are mapped and paired with corresponding cross-sectional IVUS frames, presented as a ring around the IVUS image.

NIRS and IVUS images will be analyzed offline by an independent core laboratory (Imaging Core Laboratory at Cardiovascular Research Institute (CVRI) Dublin, Mater Private Network, Dublin, Ireland). This core laboratory is blinded to all patient data, outcome data and whether the imaging was performed at baseline or follow-up. The arterial lumen and external elastic membrane (EEM) borders will be segmented spaced every 1 mm. All regions of interests at baseline will be matched with follow-up pullbacks, based on the anatomical locations of readily visible IVUS-derived landmarks.

Percent atheroma volume (PAV) will be measured according to the following equation:
$PAV = \Sigma(EEM_{area} - Lumen_{area}) / \Sigma EEM_{area} x100$

Normalized total atheroma volume will be measured to the following equation
$TAV_{normalized} = (EEM_{area} - Lumen_{area})$/Number of Images in Pullback × Median Number of Images in Cohort

Single slice plaque burden will be calculated as follows: $(EEM_{area} - Lumen_{area}) / EEM_{area} x100$. The highest plaque burden is referred to as the highest value within the region of interest.

The smallest minimum lumen area (MLA) is referred to as the smallest $Lumen_{area}$ within the region of interest.

LCBI is computed as the fraction of valid pixels within the study region that exceed a lipid-core plaque (LCP) probability of 0.6, multiplied by 1000. Thus, LCBI is measured on a scale from 0 to 1000. For each vessel, we will calculate the LCBI over the total length of the region of interest ($LCBI_{total}$) and also for the 4-mm region with maximum LCBI from any 4-mm segment within the region of interest ($maxLCBI_{4mm}$).

For our lesion-based analysis, multiple separate lesions per patient can be identified on baseline pullbacks and matched with follow-up pullbacks.

10.4 Measurement coronary flow reserve (CFR) and index of microcirculatory resistance (IMR)

CFR and IMR measurements are performed in a smaller number of patients for exploratory analysis. Microvascular function can be measured by integrating pressure and temperature measured simultaneously using thermodilution-based measurements of coronary artery flow and pressure. The PressureWire™ X Guidewire by Abbott Cardiovascular will be used. A standard operating procedure (SOP) for a correct execution of the CFR and IMR measurements can be found in the study protocol (appendix 4).
Ideally, the CFR and IMR measurements are performed in the same wire position as the FFR measurement. For resting values, 3 ml bolus injections of room temperature saline are performed in resting state. A temperature decline of at least 2 °C should typically be obtained. These injections are repeated until three similar curves are obtained. Transit time, aortic pressure and distal coronary pressure are recorded. These bolus injections are repeated in steady hyperemic state after

administering 140 mcg/kg/min adenosine through a central or peripheral vein for at least two minutes.
CFR is measured by dividing the mean transit time during maximal hyperemia by the mean transit time at rest.
The IMR is calculated by multiplying the distal coronary pressure by the mean transit time during maximal coronary hyperemia. Since a coronary stenosis may be associated with a recruitable collateral supply, the coronary wedge pressure and venous pressure should be used to estimate IMR when IMR is measured in an obstructed coronary artery, according to the following equation: IMRc = [(Pa – Pv) x Tmn] x [(Pd – Pw) / (Pa – Pw)]. When wedge and venous pressure are not available, IMR may be estimated using this equation: IMR = Pa x Tmn x FFRcor where FFRcor = 1.35 x FFRmyo - 0.32.

10.5 Laboratory measurements

Lipid levels during the study are measured at local laboratories. Importantly, any lipid measurements during the study (except lipid values at screening and after follow-up) will be blinded for the researchers.

Assessment of inflammatory biomarkers and monocyte phenotype will be performed at the central laboratory located at the Radboudumc. Assessment of monocyte phenotype will be performed in a subset of patients included at the Radboudumc. Blood samples for inflammatory biomarker measurement and monocyte phenotyping will be stored at the Radboudumc laboratory of experimental internal medicine.

Blood sampling will occur at:

- Baseline (complete blood count, lipid values (total cholesterol, LDL-C, HDL-C, triglycerides), kidney function, liver panel, glucose, liver panel, cardiac biomarkers, inflammatory biomarkers, blood samples for monocyte phenotyping)
- Week 1 (lipid values, inflammatory biomarkers)
- Week 4 (lipid values, inflammatory biomarkers, blood samples for monocyte phenotyping)
- Follow-up (lipid values, inflammatory biomarkers, blood samples for monocyte phenotyping)

11 Evaluation of safety parameters

11.1 Adverse events and patient-oriented composite endpoints

11.1.1 Brief summary of adverse events

Adverse events (AEs), serious adverse events (SAEs), and suspected unexpected serious adverse events (SUSARs) are collected. AEs will be recorded from time of signature of informed consent till 30 days after participant received the last dose of study medication (IMP).

All adverse events are adjudicated by a Clinical Event Committee (CEC) and reported as patient-oriented composite endpoint (POCE): composite of all-cause death, any stroke, any MI and any revascularization, unplanned ischemia driven PCI of the target lesion, any unplanned ischemia driven PCI in the total study population, if applicable.

11.1.2 Display of adverse events

See table 11, 12 and 13 for display of POCE and for (S)AEs.

Table 11. Safety of deferred procedure

	Complete group (N=xx)	Evolocumab (N=xx)	Placebo (N=xx)	Rate Ratio Evolocumab/Placebo (95% CI)	p-value
Any revascularization of study vessel before anticipated follow-up date — no. (%)	x (x.x%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
Any MI with culprit in study vessel before anticipated follow-up date — no. (%)	x (x.x%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
Any cardiovascular death before anticipated follow-up date — no. (%)	x (x.x%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx

Table 12. POCE endpoint table

	Evolocumab (N=xx)	Placebo (N=xx)	Rate Ratio Evolocumab/Placebo (95% CI)	p-value
POCE	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- All cause death — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- Any stroke — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- Any MI — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- Any revascularization (not mentioned: revascularization of study vessel at planned follow-up) — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx

- Unplanned ischemia driven PCI of the target lesion — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- Any unplanned ischemia driven PCI — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx

Table 13. Adverse event table

	Evolocumab (N=xx)	Placebo (N=xx)	Rate Ratio Evolocumab/Placebo (95% CI)	p-value
Any AE — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
Any SAE — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
Neurocognitive events — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- SAE only — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- AE only — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
Symptomatic overdose — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- SAE only — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- AE only — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
General allergic reaction — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- SAE only — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- AE only — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
Local injection site reaction — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- SAE only — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- AE only — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
(Serious) Adverse event other than any of the above — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- AE — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx
- SAE — no. (%)	x (x.x%)	x (x.x%)	x.x (x.x – x.x)	x.xx

11.1.3 Analysis of adverse events

The number of (serious) adverse events and number of subjects who experience at least one adverse event per treatment arm will be reported. Proportion of subjects with at least one adverse event will be compared using binomial proportions and 95% Clopper-Pearson confidence intervals. Additionally, number of (serious) adverse events might be compared evolocumab vs placebo with rate ratios (95% confidence interval) from Poisson regression with the time to end of study as the offset. Forest plots of the SOC might be created to visualise the adverse events.

11.1.4 Listing of adverse events by patient

Listing of adverse events by patient or case histories will be provided from the eCRF on request.

11.2 Clinical laboratory evaluations

Baseline laboratory values will be measured in local labs at each participating center and will include complete blood count, lipid values (total cholesterol, LDL-C, HDL-C,

triglycerides), kidney function, liver panel, glucose, liver panel, cardiac biomarkers, and inflammatory cytokines in selected centers. The lipid panel and inflammatory cytokines are repeated on week 1, week 4, and week 12, and are blinded during the execution of the study.

11.3 Concomitant medications

All patients will receive effective statin therapy consisting of high intensity statin therapy (rosuvastatin 20mg-40mg/day or atorvastatin 40-80mg/day) throughout the study period, starting on Day 1 (randomization). Between randomization and end of study, the background statin therapy should not be changed. If during the study follow-up period treating physicians strongly wish to discontinue or alter high-intensity statin therapy, physicians will strongly be advised to switch to another high-intensity regimen which is equipotent. If physicians want to change statin dose, it is advised to contact the local study representative. It will be advised to reduce the statin dose as clinically indicated.

11.4 Vital signs and physical examination

Dietary recommendations will be communicated to patients at baseline, and at scheduled clinical visits. Lifestyle and dietary habits as well as level of physical exercise should be maintained stable if possible.

During clinical visits, vital signs will be checked and physical examination will be performed. Based on the circumstances and if necessary, adequate measures will be taken. Change in concomitant medications will be noted.

12 Planned substudies

Table 14. Planned substudies

	Substudy	Year
1	Lesion based analysis of IVUS-NIRS parameters	2024
2	Analysis of change in monocyte phenotype	2024
3	Analysis of inflammatory response after myocardial infarction	2024
4	Matching of inflammatory response, monocyte phenotype and IVUS-NIRS imaging	2024/2025
5	Extended follow-up	2024/2025