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ORIGINAL RESEARCH

Changes in non-culprit coronary lesions with PCSK9 inhibitors: the randomised, placebo-controlled FITTER trial

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ABSTRACT

BACKGROUND: Prolonged lipid-lowering therapy has demonstrated its ability to induce plaque regression and improve the plaque morphology of mild atherosclerotic lesions.

AIMS: This trial aimed to assess the short-term effect of evolocumab in addition to high-intensity statin therapy (HIST) on relevant non-culprit coronary artery lesions using fractional flow reserve (FFR) measurements and multimodality intracoronary imaging.

METHODS: Patients with an acute coronary syndrome (ACS) and relevant multivessel disease were randomised to receive either evolocumab or placebo for 12 weeks in addition to HIST. Patients underwent serial FFR and intravascular ultrasound (IVUS)-near-infrared spectroscopy imaging of a non-culprit vessel. The primary endpoints were the differences in the change in FFR and in the maximum lipid core burden index within any 4 mm segment ($maxLCBI_{4mm}$). The secondary endpoints were the differences in the change in IVUS-derived atheroma volume parameters.

RESULTS: Among 150 patients (mean age 64.2±8.5 years; 27 [18.0%] female) randomised to evolocumab (n=74) or placebo (n=76), 143 underwent follow-up coronary angiography. After 12 weeks of treatment, the adjusted mean change in FFR was 0.00 (95% confidence interval [CI]: −0.02 to 0.02) with evolocumab versus 0.01 (95% CI: −0.01 to 0.03) with placebo (adjusted mean difference: −0.01, 95% CI: −0.03 to 0.01; p=0.6). The adjusted mean change in the $maxLCBI_{4mm}$ was −27.8 (95% CI: −72.2 to 16.6) for evolocumab-treated patients versus −35.6 (95% CI: −82.5 to 11.4) for placebo-treated patients (adjusted mean difference: 7.8, 95% CI: −40.9 to 56.4; p=0.8). No between-group differences in any IVUS-derived parameter were found.

CONCLUSIONS: In patients with ACS and relevant non-culprit coronary artery lesions, the addition of evolocumab to HIST for 12 weeks, compared to placebo, did not result in improvement of FFR or $maxLCBI_{4mm}$. (ClinicalTrials.gov: NCT04141579)

KEYWORDS: acute coronary syndrome; fractional flow reserve; intravascular ultrasound; lipid-lowering therapy; multivessel disease; near-infrared spectroscopy

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The risk of recurrent major adverse cardiac events (MACE) after acute coronary syndrome (ACS) remains high[1]. After initial treatment of the culprit lesion with percutaneous coronary intervention (PCI), the majority of recurrent myocardial infarctions (MIs) originate from other pre-existing, non-culprit atherosclerotic lesions[2]. The presence of severe non-culprit lesions (e.g., >70% diameter stenosis) is the strongest predictor of recurrent ischaemic events after MI[3]. A high plaque volume, assessed via intravascular ultrasound (IVUS), and a lipid-rich composition, assessed via near-infrared spectroscopy (NIRS), in less severe non-culprit lesions have also been shown to identify lesions at risk of new events[3,4].

Immediate adjunctive pharmacotherapy with hydroxymethylglutaryl-CoA reductase inhibitors (statins) reduces recurrent events and has been shown to induce plaque regression and to improve plaque composition over time[5-10]. The introduction of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors leads to a further reduction in low-density lipoprotein cholesterol (LDL-C) levels within weeks after ACS[11,12]. Multiple trials have demonstrated that the addition of PCSK9 inhibitors to high-intensity statin therapy (HIST) has favourable effects on atherosclerotic plaque by improving plaque dimensions and reducing lipid content[13-15]. While these trials included non-target lesions with only mild visual obstruction, the effect on more severe lesions might be more pronounced. Consequently, short-term effects might influence the decision on additional PCI of these lesions. Fractional flow reserve (FFR) as a haemodynamic assessment of coronary lesions has served as an objective measurement to guide treatment decisions on PCI of visually indeterminate lesions[16]. Therefore, the "Functional Improvement of Non-infarcT relaTed Coronary Artery Stenosis by Extensive LDL-C Reduction With a PCSK9 Antibody" (FITTER) trial sought to evaluate the effect of 12 weeks of maximal LDL-C reduction by evolocumab in addition to HIST compared to placebo on non-culprit vessel FFR and on the plaque composition of haemodynamically relevant lesions in patients with ACS and multivessel disease.

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Impact on daily practice

In this multicentre, double-blind, placebo-controlled, randomised clinical trial of patients presenting with acute coronary syndrome and relevant non-culprit lesions, immediate introduction of intensive lipid-lowering therapy resulted in significant non-culprit plaque lipid regression in only 12 weeks. No short-term additional reduction of plaque lipid content by proprotein convertase subtilisin/kexin type 9 inhibition was found. Non-culprit plaque volume and coronary physiology showed no significant improvement after 12 weeks. Further studies with longer follow-up are needed to assess the effect and clinical outcomes of very high-intensity lipid-lowering therapy on significant non-critical, non-culprit coronary artery lesions.

Methods

TRIAL DESIGN

The FITTER trial (ClinicalTrials.gov: NCT04141579) was an investigator-initiated, multicentre, double-blind, placebo-controlled, randomised clinical trial conducted at seven centres in the Netherlands. The medical ethical committee (METC Oost-Nederland) approved the study protocol, and all patients provided written informed consent. The study protocol and statistical analysis plan are available in **Supplementary Appendix 1** and **Supplementary Appendix 2**, respectively, and the study design has been previously described[17]. Patients 18 years or older hospitalised with ST-segment elevation myocardial infarction (STEMI), non-STEMI (NSTEMI), or unstable angina pectoris (UAP) were screened. In short, patients were deemed eligible if successful PCI of the infarct-related artery (IRA) was performed and if at least one epicardial coronary artery stenosis with an FFR of 0.67-0.85 amenable for PCI was present. Lesions in the non-IRA with a visually estimated angiographic stenosis exceeding 30% were considered suitable for FFR measurement. Major exclusion criteria were prior coronary artery bypass grafting, untreated functional left main stem stenosis (FFR ≤0.80), or severe kidney dysfunction. For detailed inclusion/exclusion criteria, see **Supplementary Table 1**. Written informed consent was preferably obtained before the index procedure. However, in some emergency cases (i.e., STEMI), oral informed consent was given for invasive study procedures during the index procedure, with full written informed consent for the entire study acquired afterwards. In preselected centres with the ability to perform additional IVUS-NIRS, baseline imaging acquisition was achieved after FFR measurement in a subset of the overall study population. After the index study procedure, patients were randomised in a 1:1 fashion into two groups (evolocumab or placebo) using a 2:4:6 random block randomisation algorithm. Randomisation was stratified

Abbreviations

ACS	acute coronary syndrome	**$LCBI_{total}$**	total segment lipid core burden index	**PAV**	percent atheroma volume
ANCOVA	analysis of covariance			**PB**	plaque burden
EEM	external elastic membrane	**LDL-C**	low-density lipoprotein cholesterol	**PCI**	percutaneous coronary intervention
FFR	fractional flow reserve	**$MaxLCBI_{4mm}$**	maximum lipid core burden index within any 4 mm segment	**PCSK9**	proprotein convertase subtilisin/kexin type 9
HIST	high-intensity statin therapy				
IRA	infarct-related artery	**MLA**	minimum lumen area	**STEMI**	ST-segment elevation myocardial infarction
IVUS	intravascular ultrasound	**NIRS**	near-infrared spectroscopy		
LCBI	lipid core burden index	**NSTEMI**	non-ST-segment elevation myocardial infarction	**TAV**	total atheroma volume
				UAP	unstable angina pectoris

per study site. The first study drug (biweekly 140 mg evolocumab or matching placebo) dose was given as soon as possible after randomisation, preferably within 24 hours after the index procedure. Patients received HIST as background therapy, e.g., atorvastatin 40 mg daily or rosuvastatin 20 mg daily. During the follow-up phase of the study, patients were contacted regularly (at weeks 1, 4, 6, and 8) to monitor clinical status, evaluate treatment adherence, and to screen for potential adverse events. At week 12, repeat coronary angiography with FFR measurement and IVUS-NIRS imaging of the non-IRA lesions was performed. Patients, treating physicians, and the research team were blinded to LDL-C measurements throughout the study.

FFR MEASUREMENT AND IVUS-NIRS IMAGING ACQUISITION

Details about FFR measurements, as well as the acquisition and analysis of IVUS-NIRS imaging, have been described in the protocol and statistical analysis plan. At week 12, FFR measurements were repeated with the pressure wire at the exact same position as baseline. Hyperaemia was achieved similarly for baseline and follow-up measurements. When IVUS-NIRS imaging was performed at baseline, follow-up imaging of the same artery was also performed at week 12. IVUS and NIRS images were analysed offline by an independent core laboratory (Cardiovascular Research Institute, Dublin, Ireland). Core laboratory personnel were blinded to all other patient data, outcome data, and the sequence of imaging (baseline vs follow-up). For IVUS, frames were analysed every 1 mm in matched coronary artery segments. The arterial lumen and external elastic membrane (EEM) borders were delineated from IVUS images. For NIRS, the 4 mm segment with the maximum lipid core burden index ($maxLCBI_{4mm}$) was identified within the same segments used for IVUS analyses. IVUS outcome parameters were derived as follows:

- Percent atheroma volume (PAV) was calculated according to the following equation:
  $[\Sigma(EEM_{area} - lumen_{area}) / \Sigma EEM_{area}] \times 100$
- Normalised total atheroma volume (TAV) was calculated according to the following equation:
  $[\Sigma(EEM_{area} - lumen_{area})$ / number of images in pullback] × median number of images in cohort
- The maximum plaque burden (PB) was defined as the highest single-slice PB within the coronary artery segment:
  $[(EEM_{area} - lumen_{area}) / EEM_{area}] \times 100$
- The minimum lumen area (MLA) refers to the smallest lumen area within the coronary artery segment.

OUTCOMES

The two primary endpoints of this trial were the differences in the change in FFR (primary physiological endpoint) and in $maxLCBI_{4mm}$ (primary imaging endpoint) from baseline to follow-up in the non-IRA between evolocumab- and placebo-treated patients.

The secondary endpoints of this trial were the differences in change in IVUS-derived plaque characteristics of the non-IRA:

- percent atheroma volume
- normalised total atheroma volume
- maximum plaque burden
- minimum lumen area

A detailed list of all study endpoints is presented in **Supplementary Table 2**.

STATISTICAL METHODS

The study was originally designed with a single primary endpoint (the change in FFR) and a powered secondary endpoint (the change in $maxLCBI_{4mm}$). During the execution of the study, the importance of plaque composition as a predictor of non-culprit MACE and as a target for PCSK9 inhibitors was further recognised in contemporary publications[3,13]. Therefore, before completion of the trial and prior to unblinding, the powered secondary endpoint was upgraded to a second primary endpoint in an official amendment to the study protocol (version 8.0), which included a correspondingly updated statistical analysis plan.

Statistical comparisons of baseline to follow-up between the two groups were performed using an analysis of covariance (ANCOVA) model including the treatment and randomisation stratification centre as fixed factors, corrected for the baseline value of that specific outcome. The analysis of the first primary endpoint (the change in FFR) was performed on the full analysis set, which included all patients with available serial FFR data. The analyses of the second primary endpoint (the change in $maxLCBI_{4mm}$) and IVUS-derived secondary endpoints included all patients in the full analysis set with available serial NIRS or IVUS data, respectively. Participants were grouped according to their randomised treatment group assignment. Analyses of adverse events included patients who had received at least one administration of the study drug.

The study was considered positive in the presence of a statistically significant difference in at least one primary endpoint. Both primary endpoints were tested independently. A Hochberg correction was performed to maintain the overall familywise error rate at 0.05. In short, if the largest p-value was <0.05, both null hypotheses were rejected; if the largest p-value was ≥0.05, the smaller p-value was compared with alpha=0.025. If the smallest p-value was <0.025, then the null hypothesis corresponding to that primary outcome variable was rejected. The p-values for the secondary endpoints were only interpreted (i.e., the subsequent null hypotheses can only be rejected) if at least one of the null hypotheses of both primary endpoints was rejected. The secondary endpoints were tested using a hierarchical procedure, and a p-value of <0.05 was considered statistically significant.

The overall changes from baseline to follow-up were also examined using paired t-tests. Analysis of the LDL-C measurements over time was carried out using a repeated measures model with an unstructured variance-covariance matrix. All reported p-values are two-sided. Statistical analyses were performed using SPSS Statistics, version 29.0 (IBM).

SAMPLE SIZE: POWER ANALYSIS OF THE PRIMARY ENDPOINTS

Details about the sample size calculation are provided in the statistical analysis plan **(Supplementary Appendix 2)**. For our first primary endpoint (FFR), based on ANCOVA, a total sample size of 127 would provide 80% power to detect an expected between-group difference at follow-up of 0.03, using a 2-sided alpha level of 0.05. To compensate for a dropout rate of about

15%, a total of 150 patients were to be included at baseline. After upgrading the powered secondary endpoint to a second primary endpoint, no change was made to the initial sample size. In case the FFR had to be tested with an alpha of 0.025, this would result in less power (approximately 76%, under similar conditions and considering our eventual lower dropout ratio of 5.3%). For our second primary endpoint ($maxLCBI_{4mm}$), based on ANCOVA, an expected 14.2% larger decrease in the evolocumab group, at a 2-sided alpha level of 0.025, and to compensate for a dropout rate of about 20%, a total of 84 patients were to be included at baseline to reach 90% power.

Results

PATIENT CHARACTERISTICS

Between 10 November 2020 and 17 August 2023, a total of 150 patients (35.3% STEMI, 60% NSTEMI, 4.7% UAP) were included and randomised to receive treatment with evolocumab (n=74) or placebo (n=76). The patient flowchart is presented in **Figure 1**. Overall, 143 patients underwent coronary angiography for follow-up endpoint measurements. At baseline, successful IVUS and NIRS pullbacks were performed in 95 and 94 patients, respectively (1 IVUS-NIRS catheter failed to record the NIRS signal). At follow-up, IVUS-NIRS was successfully repeated in 86 patients. All patients received at least one study drug administration, and a total of 138 patients received all study drug injections per protocol. The clinical characteristics of all randomised patients are presented in **Table 1**. At admission, 41 patients (27.3%) were receiving any statin therapy, of whom 15 patients (10.0%) were on HIST. At discharge and follow-up, 141 (94.6%) and 136 (93.8%) patients were on HIST, respectively **(Supplementary Table 3, Supplementary Table 4)**. Overall, 142, 85, and 86 patients were included in the paired analyses of FFR, $maxLCBI_{4mm}$, and IVUS-derived parameters, respectively. Patients with additional IVUS imaging at baseline were similar to the overall group of patients **(Supplementary Table 5)**. Of the 143 patients who

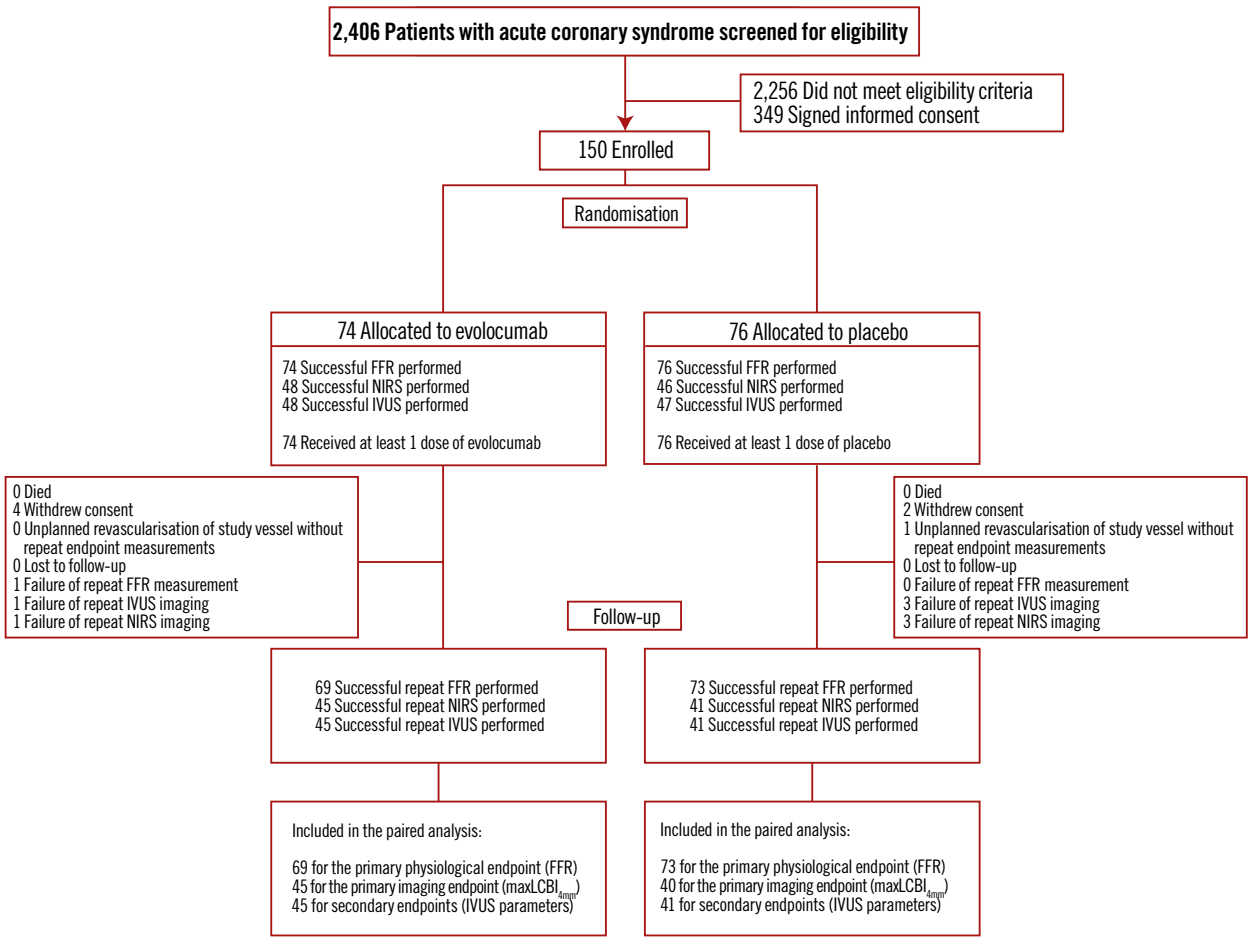


Figure 1. *Flow of patients in the FITTER trial. Overall, 143 patients underwent coronary angiography for follow-up endpoint measurements. At baseline, successful IVUS and NIRS pullbacks were performed in 95 and 94 patients, respectively (one IVUS-NIRS catheter failed to record the NIRS signal). At follow-up, IVUS-NIRS was repeated in 86 patients. In 9 patients, repeat IVUS-NIRS was not available: 4 patients withdrew consent, the IVUS-NIRS catheter was unable to cross the lesion in 2 patients, repeat IVUS-NIRS was not possible in 2 patients due to a defective device, and 1 patient was revascularised at the request of the treating physician because of poor left ventricular function 2 weeks after inclusion (unplanned revascularisation of the study vessel without repeat endpoint measurements). FFR: fractional flow reserve; IVUS: intravascular ultrasound; $maxLCBI_{4mm}$: maximum lipid core burden index within a 4 mm segment; NIRS: near-infrared spectroscopy*

Table 1. Baseline characteristics of all patients randomised in the FITTER trial.

	Evolocumab (n=74)	Placebo (n=76)
Demographics		
Age, years	63.5±8.3	65.0±8.8
Male sex	58 (78.4)	65 (85.5)
Female sex	16 (21.6)	11 (14.5)
BMI, kg/m^2	27.3±4.1	27.4±3.9
Cardiovascular risk factors		
Hypertension	29 (39.2)	30 (39.5)
Dyslipidaemia	29 (39.2)	34 (44.7)
Family history of premature CAD	25 (34.7)	32 (42.1)
Smoking history	54 (73.0)	56 (73.7)
Current smoker	24 (32.4)	21 (27.6)
Diabetes mellitus	6 (8.1)	9 (11.8)
Insulin-treated diabetes mellitus	1 (1.4)	3 (3.9)
Medical history		
Stroke or TIA	4 (5.4)	3 (3.9)
Peripheral artery disease	3 (4.1)	0 (0)
Prior myocardial infarction	7 (9.5)	13 (17.1)
Prior PCI	11 (14.9)	13 (17.1)
Premature CVD (CAD/stroke/TIA/PAD)	5 (6.8)	3 (3.9)
Baseline lipid-lowering therapy		
Any statins	18 (24.3)	23 (30.3)
High-intensity statin therapy[a]	8 (10.8)	7 (9.2)
Ezetimibe	2 (2.7)	3 (3.9)
Fibrates	1 (1.4)	0 (0)
Niacin	0 (0)	0 (0)
Resins	0 (0)	0 (0)
Other cardiac medications		
Aspirin	14 (18.9)	19 (25.0)
ADPRI (ticagrelor/clopidogrel/prasugrel)	3 (4.1)	5 (6.6)
DAPT	1 (1.4)	3 (3.9)
ACE inhibitor	10 (13.5)	8 (10.5)
ARB	6 (8.1)	7 (9.2)
Beta blocker	11 (14.9)	16 (21.1)
Type of ACS		
STEMI	26 (35.1)	27 (35.5)
NSTEMI	45 (60.8)	45 (59.2)
UAP	3 (4.1)	4 (5.3)
Study vessel		
LAD	60 (81.1)	49 (64.5)
RCA	5 (6.8)	9 (11.8)
Cx	9 (12.2)	18 (23.7)

Data are given as mean±SD or n (%). [a]Atorvastatin ≥40 mg, rosuvastatin ≥20 mg or simvastatin ≥80 mg. Note: BMI calculated as weight in kilograms divided by height in metres squared. ACE: angiotensin-converting enzyme; ACS: acute coronary syndrome; ADPRI: adenosine diphosphate receptor inhibitor; ARB: angiotensin receptor blocker; BMI: body mass index; CAD: coronary artery disease; CVD: cardiovascular disease; Cx: circumflex artery; DAPT: dual antiplatelet therapy; LAD: left anterior descending artery; NSTEMI: non-STEMI; PAD: peripheral artery disease; PCI: percutaneous coronary intervention; RCA: right coronary artery; SD: standard deviation; STEMI: ST-segment elevation myocardial infarction; TIA: transient ischaemic attack; UAP: unstable angina pectoris

underwent repeat coronary angiography, additional PCI was performed in 60 (42.0%) patients.

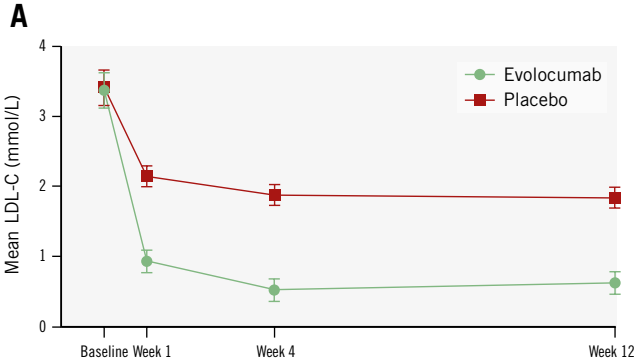
BIOCHEMICAL MEASUREMENTS

The change in lipid levels for all patients who completed clinical follow-up of the study are summarised in **Supplementary Table 6**. As the majority of patients were not on any statin therapy at baseline, both the placebo and evolocumab group showed significant improvement in their lipid levels. After 12 weeks of treatment, evolocumab-treated patients demonstrated greater reductions in levels of triglycerides (adjusted mean difference: –0.2 mmol/L, 95% confidence interval [CI]: –0.4 to –0.0; p=0.03), total cholesterol (adjusted mean difference: –1.3 mmol/L, 95% CI: –1.5 to –1.0; p<0.001), non-high-density lipoprotein cholesterol (adjusted mean difference: –1.3 mmol/L, 95% CI: –1.5 to –1.0; p<0.001) and LDL-C (adjusted mean difference: –1.2 mmol/L, 95% CI: –1.4 to –1.0; p<0.001). **Figure 2** emphasises the faster and larger reduction of LDL-C in the evolocumab group. After just 1 week, LDL-C was already significantly lower compared to the placebo group (between-group difference: –1.2 mmol/L, 95% CI: –1.4 to –1.0). This difference was maintained throughout the 12-week period.

PRIMARY AND SECONDARY ENDPOINTS

PRIMARY HAEMODYNAMIC ENDPOINT: FFR

At baseline, the mean FFR was 0.78±0.04 in the evolocumab group and 0.78±0.05 in the placebo group. After 12 weeks of treatment, the adjusted mean change in FFR was 0.00 (95% CI: –0.02 to 0.02) with evolocumab versus 0.01 (95% CI: –0.01 to 0.03) with placebo (adjusted mean



B

	Baseline	Week 1	Week 4	Week 12
Mean difference compared to placebo, mmol/L (95% CI)	0.0 (–0.4 to 0.3)	–1.2 (–1.4 to –1.0)	–1.3 (–1.5 to –1.1)	–1.2 (–1.4 to –1.0)

Figure 2. *A) Mean LDL-C values in the two study groups over time; error bars indicate 95% CIs. B) Mean difference between the evolocumab and placebo groups. To convert LDL-C values to mg/dL, divide by 0.0259. CI: confidence interval; LDL-C: low-density lipoprotein cholesterol*

difference: –0.01, 95% CI: –0.03 to 0.01; p=0.6) **(Table 2, Figure 3, Central illustration)**. The overall difference in FFR from baseline to follow-up is listed in **Supplementary Table 7**. Thirty patients (12 in the evolocumab group and 18 in the placebo group) with an impaired FFR at baseline (≤0.80) improved to a level >0.80 at follow-up, which often resulted in cancelled PCI **(Figure 3)**. Ten patients with a negative FFR at baseline had a positive FFR at follow-up.

PRIMARY IMAGING ENDPOINT: MAXLCBI$_{4MM}$

The adjusted mean change in maxLCBI$_{4mm}$ was –27.8 (95% CI: –72.2 to 16.6) for patients treated with evolocumab versus –35.6 (95% CI: –82.5 to 11.4) for patients treated with placebo (adjusted mean difference: 7.8, 95% CI: –40.9 to 56.4; p=0.8) **(Table 2, Figure 4, Central illustration)**. In line with this, no difference in the change in LCBI$_{total}$ was found (adjusted mean difference: 4.2, 95% CI: –11.7 to 20.2) **(Table 2)**. **Supplementary Table 7** provides a summary of the

Table 2. Primary and secondary outcome parameters of the FITTER trial.

Intracoronary physiology	Evolocumab (n=69)	Placebo (n=73)	*p*-value
Fractional flow reserve			
Baseline	0.78±0.04	0.78±0.05	
Follow-up	0.77±0.06	0.79±0.08	
Adjusted mean change	0.00 (–0.02 to 0.02)	0.01 (–0.01 to 0.03)	
Adjusted mean difference in change compared to placebo	–0.01 (–0.03 to 0.01)		0.6
Near-infrared spectroscopy parameters	**Evolocumab (n=45)**	**Placebo (n=40)**	***p*-value**
MaxLCBI$_{4mm}$			
Baseline	357.4±177.2	359.9±175.7	
Follow-up	324.2±184.8	318.0±155.1	
Adjusted mean change	–27.8 (–72.2 to 16.6)	–35.6 (–82.5 to 11.4)	
Adjusted mean difference in change compared to placebo	7.8 (–40.9 to 56.4)		0.8
LCBI$_{total}$[a]			
Baseline	86.5±52.8	88.8±69.4	
Follow-up	73.6±47.8	70.8±56.0	
Adjusted mean change	–14.9 (–29.2 to –0.5)	–19.1 (–34.4 to –3.8)	
Adjusted mean difference in change compared to placebo	4.2 (–11.7 to 20.2)		
Intravascular ultrasound parameters	**Evolocumab (n=45)**	**Placebo (n=41)**	***p*-value**
Percent atheroma volume, %			
Baseline	48.3±6.8	47.0±7.7	
Follow-up	47.6±5.9	46.7±7.7	
Adjusted mean change	–0.5 (–1.7 to 0.6)	–0.4 (–1.5 to 0.8)	
Adjusted mean difference in change compared to placebo	–0.2 (–1.4 to 1.0)		
Normalised total atheroma volume, mm^3			
Baseline	381.7±135.1	370.7±123.6	
Follow-up	370.5±127.1	364.1±116.9	
Adjusted mean change	–7.5 (–23.5 to 8.6)	–3.9 (–20.8 to 13.1)	
Adjusted mean difference in change compared to placebo	–3.6 (–21.1 to 13.9)		
Maximum plaque burden, %			
Baseline	71.2±6.8	70.4±7.3	
Follow-up	70.2±6.7	69.8±7.2	
Adjusted mean change	–0.6 (–2.1 to 0.9)	–0.3 (–1.8 to 1.3)	
Adjusted mean difference in change compared to placebo	–0.3 (–1.9 to 1.3)		
Minimum lumen area, mm^2			
Baseline	3.7±1.1	3.7±0.7	
Follow-up	3.6±1.2	3.6±0.7	
Adjusted mean change	0.0 (–0.2 to 0.3)	–0.0 (–0.3 to 0.2)	
Adjusted mean difference in change compared to placebo	0.1 (–0.2 to 0.3)		

Data are presented as mean±SD or as mean (95% CI). [a]Serial LCBI$_{total}$ values were missing for two evolocumab- (n=43) and two placebo-treated (n=38) patients. CI: confidence interval; LCBI$_{total}$: total segment lipid core burden index; maxLCBI$_{4mm}$: maximum lipid core burden index within any 4 mm segment; SD: standard deviation

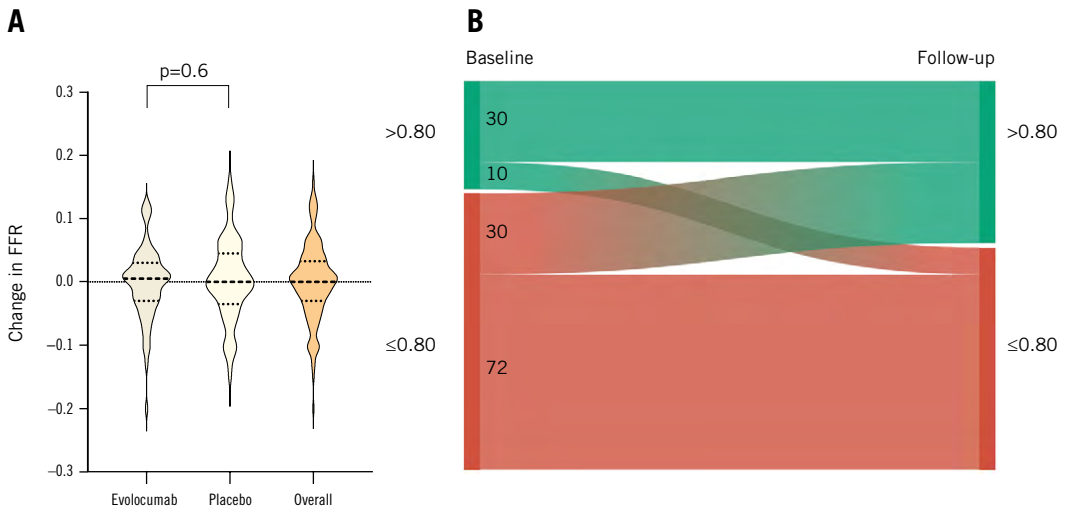


Figure 3. *Changes in FFR (primary haemodynamic endpoint) and FFR reclassification of the patients in the FITTER trial. A) The violin plot displays the observed changes in FFR values from baseline to follow-up. Dotted lines within the violin plots present the median, 25th, and 75th percentiles. No difference in change between evolocumab- and placebo-treated patients was found (adjusted mean difference: –0.01, 95% CI: –0.03 to 0.01; p=0.6). Also, no overall change in FFR was observed (overall change: 0.00, 95% CI: 0.01 to –0.01). B) The Sankey diagram shows the overall change in the FFR group (>0.80 or ≤0.80) from baseline to follow-up. CI: confidence interval; FFR: fractional flow reserve*

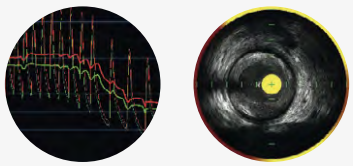
EuroIntervention — Central Illustration

Efficacy of 12 weeks of evolocumab treatment in addition to high-intensity statin therapy to improve the functional and morphological characteristics of relevant non-culprit coronary artery stenosis.

A Population

ACS patients undergoing PCI of the culprit lesion with an important non-culprit lesion (FFR 0.67-0.85)

B Non-culprit lesion measurements



FFR IVUS-NIRS

C Intervention

150 patients included and randomised

74 Evolocumab + High-dose statin

76 Placebo + High-dose statin

Repeat FFR and IVUS-NIRS after 12 weeks

D Results

FFR

	Evolocumab	Placebo
Baseline	0.78	0.78
Follow-up	0.77	0.79
Adjusted mean change (95% CI)	0.00 (–0.02 to 0.02)	0.01 (–0.01 to 0.03)

Difference compared to placebo: –0.01 (95% CI: –0.03 to 0.01; p=0.6)
Overall change: 0.00 (95% CI: –0.01 to 0.01)

maxLCBI$_{4mm}$

	Evolocumab	Placebo
Baseline	357.4	359.9
Follow-up	324.2	318.0
Adjusted mean change (95% CI)	–27.8 (–72.2 to 16.6)	–35.6 (–82.5 to 11.4)

Difference compared to placebo: 7.8 (95% CI: –40.9 to 56.4; p=0.8)
Overall change: –37.3 (95% CI: –63.2 to –11.3)

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A) Trial population; (B) non-culprit lesion assessment: FFR and IVUS-NIRS; (C) trial design; (D) results. In this double-blind, placebo-controlled, randomised clinical trial among patients presenting with ACS and relevant multivessel disease, the addition of evolocumab to high-intensity statin therapy for 12 weeks, compared to placebo, did not result in the improvement of FFR or plaque lipid content. ACS: acute coronary syndrome; CI: confidence interval; FFR: fractional flow reserve; IVUS-NIRS: intravascular ultrasound-near-infrared spectroscopy; maxLCBI$_{4mm}$; maximum lipid core burden index within a 4 mm segment; PCI: percutaneous coronary intervention

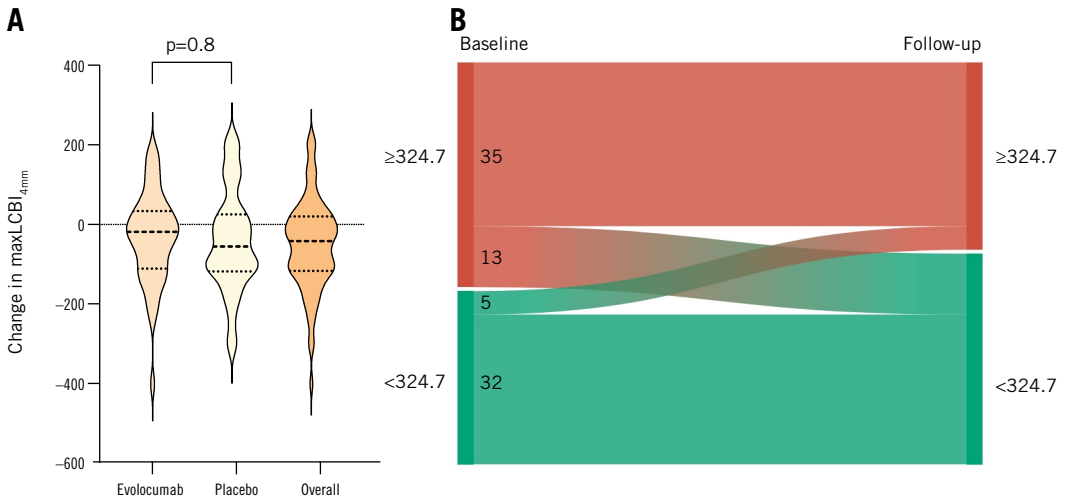

Figure 4. *Changes in maxLCBI$_{4mm}$ (primary imaging endpoint) and maxLCBI$_{4mm}$ reclassification of the patients in the FITTER trial. A) The violin plot displays the observed changes in maxLCBI$_{4mm}$ values from baseline to follow-up. Dotted lines within the violin plots present the median, 25th, and 75th percentiles. There was no significant difference between the change in evolocumab- and placebo-treated patients (adjusted mean difference: 7.8, 95% CI: −40.9 to 56.4; p=0.8). An overall reduction in maxLCBI$_{4mm}$ was observed (overall change: −37.3, 95% CI: −63.2 to −11.3). B) The Sankey diagram shows the overall transition of lipid-rich plaque (maxLCBI$_{4mm}$ ≥324.7) to non-lipid rich (maxLCBI$_{4mm}$ <324.7) from baseline to follow-up. CI: confidence interval; maxLCBI$_{4mm}$: maximum lipid core burden index within any 4 mm segment*

overall differences observed in LCBI values. After 12 weeks of treatment, 13 out of 48 vessels (27.1%) displaying lipid-rich regions (in 7 and 6 patients randomised to evolocumab and placebo, respectively) were reclassified as non-lipid rich according to the previous reported cutoff of 324.7 **(Figure 4)**[3].

SECONDARY ENDPOINTS: IVUS PARAMETERS

At baseline, the mean PAV was 48.3±6.8% in the evolocumab group and 47.0±7.7% in the placebo group. At follow-up, the adjusted mean change in PAV was −0.5% (95% CI: −1.7 to 0.6) for evolocumab-treated patients versus −0.4% (95% CI: −1.5 to 0.8) for placebo-treated patients (adjusted mean difference: −0.2%, 95% CI: −1.4 to 1.0). Similarly, no significant differences between patients treated with evolocumab or placebo were found in normalised TAV (adjusted mean difference: −3.6 mm^3, 95% CI: −21.1 to 13.9), maximum PB (adjusted mean difference: -0.3%, 95% CI: −1.9 to 1.3), or MLA (adjusted mean difference: 0.1 mm^2, 95% CI: −0.2 to 0.3) **(Table 2)**. The overall changes from baseline to follow-up are presented in **Supplementary Table 7**.

SAFETY AND CLINICAL EVENTS

During the execution of the study, clinical events were scarce. No myocardial infarction due to a culprit lesion in the study vessel occurred. One patient experienced a stroke after the index procedure. Two patients had an expedited follow-up procedure due to progressive chest pain. However, in one of the patients, the chest pain was likely of non-cardiac origin, as the FFR results were not significant. One patient died due to an unknown cause nine days after the follow-up procedure and PCI of the study vessel.

Discussion

The FITTER trial aimed to investigate the full potential of intensive lipid-lowering therapy on relevant non-culprit lesions in ACS patients at very short follow-up. A more profound reduction in LDL-C was already achieved after 1 week of evolocumab therapy compared to the placebo group. Regarding the trial's primary and secondary outcomes, no between-group differences were found between evolocumab- and placebo-treated patients. Deferral of PCI of non-culprit lesions with an FFR of 0.67-0.85 did not result in safety issues in this trial.

Few studies have examined the impact of lipid-lowering therapy on change in intracoronary physiology. In the YELLOW trial, patients with chronic stable angina and a non-target lesion with an FFR ≤0.80 were imaged with IVUS-NIRS and randomised to HIST or a moderate statin-therapy dose[7]. After 6-8 weeks, FFR and IVUS-NIRS were repeated. A non-significant increase in FFR was observed in patients on HIST, while no improvement was noted in those treated with moderate statin therapy. The non-randomised FORTE trial assessed the effect of 12-month atorvastatin therapy on non-significant lesions in 95 patients[18]. Overall, no significant change in FFR was found. However, patients who achieved optimal LDL-C targets did demonstrate a significant increase in FFR. Furthermore, an inverse correlation between achieved LDL-C and change in FFR was found. In a substudy of the PACMAN-AMI trial, in which ACS patients were also randomised to PCSK9 inhibitors or placebo in addition to HIST, no significant improvement of quantitative flow ratio (QFR) in any group was found after 52 weeks of therapy[19]. Theoretically, LDL-C lowering reduces non-culprit plaque size, which in turn could increase FFR. In our study, a substantial fraction of non-culprit lesions improved from an impaired FFR to a non-significant FFR at follow-up. However, since the baseline FFR was close

to the normal cutoff value, slight improvements and minimal variability might have contributed to this transition. Plaque size did not significantly differ after 12 weeks of therapy in either group, which may partially explain the absence of any observed differences on a continuous scale. Yet, patients in the FORTE trial and in the QFR substudy of PACMAN-AMI demonstrated no physiological improvement despite significant plaque size reduction. Therefore, greater plaque size reductions appear to be necessary to achieve improvements in intracoronary physiology. In addition, variability in non-culprit physiology between acute and late stages have been reported before[20]. It is hypothesised that the adenosine response is blunted to some degree in STEMI patients at presentation[20]. In addition, myocardial oedema and elevated left ventricular filling pressures might decrease initial hyperaemic non-culprit flow in the acute setting[21,22]. However, data are conflicting. Multiple trials have reported stable non-culprit FFR measurements in STEMI and NSTEMI patients between the acute and stabilised phases[23-25]. Therefore, the impact of ACS on non-culprit FFR seems to be reserved for patients presenting with large STEMI at very early stages. This appears to apply only minimally to the FITTER trial population, as only 35.3% of the patients presented with STEMI, and study vessel assessment was often performed during a second coronary angiography at the index hospitalisation. Nevertheless, the physiological differences between the acute and chronic phases after ACS might have masked slight changes.

The overall decreases of $maxLCBI_{4mm}$ and $LCBI_{total}$ align with previous trials investigating the effect of lipid-lowering therapy on plaque composition[7,13,26]. The reduction of intraplaque lipid occurs rapidly after intensification of lipid-lowering therapy[7,26]. In the YELLOW trial, the median change in $maxLCBI_{4mm}$ was 149.1 points in patients treated with HIST, while the moderate statin-therapy group demonstrated no improvement[7]. Moreover, a recent, small, single-arm trial by Kataoka et al observed a significant $maxLCBI_{4mm}$ reduction, from 387 to 315, in only 2 to 6 weeks after a single dose of a PCSK9 inhibitor[26]. In the FITTER trial, $maxLCBI_{4mm}$ decreased by 37.3 overall, which represents a markedly smaller reduction compared to the other trials assessing the short-term impact of LDL-C reduction[7,26]. The FITTER trial differs from other trials by including ACS patients in whom atherosclerotic disease has become destabilised, potentially featuring more vulnerable plaques that are less likely to show improvement[27]. Surprisingly, no between-group differences were found in $maxLCBI_{4mm}$ or $LCBI_{total}$ in the FITTER trial. The short timeframe conceivably plays a major role. Also, only 41 patients (27.3%) were on any statin therapy at baseline. This is notably lower compared to the YELLOW trial and the study by Kataoka et al, in which approximately 82% and 85% of the patients, respectively, were on statin therapy at baseline[7,26]. Our findings may suggest a maximum speed of "lipid washout" when HIST is initiated. Over time, prolonged LDL-C reduction through PCSK9 inhibition has been shown to lead to a more profound decrease in $maxLCBI_{4mm}$, as observed in the PACMAN-AMI trial[13].

The GLAGOV, PACMAN-AMI, and HUYGENS trials reported incremental plaque regression when patients were treated with PCSK9 inhibitors in addition to HIST compared to HIST alone[13-15]. Moreover, the HUYGENS and PACMAN-AMI trials observed a greater decline in PAV than the GLAGOV study, possibly due to a higher PAV at baseline[13-15]. Since these trials only included patients with ≤50% visual lumen obstruction, we hypothesised that an even greater effect could be expected when significant lesions were included. Despite focusing on relevant lesions, baseline PAV was only modestly higher (47.6%) compared to PACMAN-AMI and HUYGENS (approximately 42% and 45%, respectively). On the other hand, baseline normalised TAV was notably greater (376.5 mm^3 vs approximately 256 mm^3 and 245 mm^3 in PACMAN-AMI and HUYGENS, respectively), suggesting longer diseased arterial segments assessed by the FITTER trial. Moreover, vessels undergo positive remodelling in response to plaque growth, which preserves lumen area and limits initial PAV increase[28]. We observed an overall trend toward a reduction in normalised TAV and maximum PB; however, this was not statistically significant. Also, no between-group differences were found. In line with our results, no significant improvement of plaque volume parameters were reported in the YELLOW trial or the study by Kataoka et al, which also investigated the immediate impact on plaque volume[7,26].

In view of current results and contemporary related trials, plaque stabilisation seems to precede plaque volume reduction when lipid-lowering therapy is intensified[7,13,26]. The short-term overall reduction of plaque lipid content observed in the FITTER trial reinforces the fundamental importance of implementing lipid-lowering therapy immediately after ACS to mitigate future risk associated with vulnerable lipid-rich lesions. Our findings suggest that continuous treatment is required to induce significant plaque regression and further lipid content reduction. The potential of improving FFR within a very short timeframe seems limited. Further research with extended follow-up is needed to explore the long-term effects of an aggressive lipid-lowering therapy regimen on non-critical but relevant coronary artery lesions.

Limitations

This study has some limitations. First, baseline LDL-C values were lower compared to the PACMAN-AMI and HUYGENS trials (3.4 mmol/L vs approximately 4.0 mmol/L and 3.7 mmol/L, respectively), reducing treatment potential[13,14]. On the other hand, the lack of LDL-C thresholds in the FITTER trial indicates that the current population represents a typical ACS population. Second, non-culprit FFR measurements might be overestimated in the ACS setting, particularly in patients presenting with large STEMI, potentially obscuring small effects on the changes in FFR. Third, despite focusing on relevant coronary artery lesions, baseline PAV was only moderately higher compared to other trials, curtailing therapeutic efficacy. Fourth, quantitative coronary analysis was not performed, which could have been useful in comparing current lesions with those from other trials. Finally, although the target sample size for the primary imaging endpoint was achieved, the cohort with serial IVUS imaging was still relatively small, limiting power to demonstrate significant overall and between-group differences.

Conclusions

Among patients presenting with ACS and relevant multivessel disease, the addition of evolocumab to HIST for 12 weeks,

compared to placebo, did not result in the improvement of FFR or plaque lipid content. Further studies with extended follow-up are necessary to evaluate the impact of prolonged very high-intensity lipid-lowering therapy.

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Conflict of interest statement

M.M. Reda Morsy reports funding from the European Association of Percutaneous Cardiovascular Interventions (EAPCI) Education and Training Grants Programme. R.M. Oemrawsingh reports speaker fees from Abbott and Terumo. C. von Birgelen reports institutional research grants from Abbott, Boston Scientific, Biotronik, and Medtronic, outside the current study. A.J.J. IJsselmuiden reports institutional fees from Medtronic, Meril Life Sciences, and Abbott; and consulting fees from Meril Life Sciences, Angiocare, Abbott, Philips, and Translumina. P.C. Smits reports institutional research grants from Abbott and SMT; and consulting or speaker fees from Abbott, MicroPort, SMT, and Terumo; he participates on a data safety monitoring board or advisory board of the LEGACY trial, PROCTOR trial, and on the global coronary advisory board of Abbott; he is a minor shareholder of the European Cardiovascular Research Center. V. Paradies reports institutional grants from Abbott; and personal consulting or speaker fees from Abbott, Boston Scientific, Elixir, and Novo Nordisk; she participates on advisory boards or committees of Boston Scientific, EAPCI Chair Congress Committee, and is an ESC CPC member. C. Camaro reports institutional speaker fees from AstraZeneca and from regional interventional cardiology meetings. P. Damman reports research grants from Abbott, Philips, Pie Medical Imaging, and AstraZeneca; and consulting fees from Philips and Abbott. M.H. van Wely reports consulting fees from Boston Scientific and Abbott. R.A. Byrne reports grants received by the institutions of employment for research or education from Abbott, Biosensors, Boston Scientific, and Translumina, without impact on personal remuneration, and he does not accept personal payments from the medical device or pharmaceutical industry. N. van Royen reports research grants from Biotronik, Abbott, Medtronic, and Philips; and speaker fees from Abbott, RainMed, MicroPort, and Bayer. R.-J.M. van Geuns reports consulting and speaker fees from Abbott and AstraZeneca; and has received institutional research grants from Amgen, InfraRedx, AstraZeneca, and Sanofi. The other authors have no conflicts of interest to declare.

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Supplementary data

Supplementary Table 1. Inclusion and exclusion criteria.
Supplementary Table 2. Study endpoints.
Supplementary Table 3. Medication at discharge.
Supplementary Table 4. Medication at follow-up.
Supplementary Table 5. Baseline characteristics of all patients and of patients who underwent baseline intravascular ultrasound imaging.
Supplementary Table 6. Change in lipid levels of all patients from baseline to 12-week follow-up.
Supplementary Table 7. Overall changes in FFR, max$LCBI_{4mm}$, and atheroma volume parameters.
Trial sponsor
Data availability statement
Author statement
Supplementary Appendix 1. FITTER study protocol.
Supplementary Appendix 2. FITTER statistical analysis plan.

The supplementary data are published online at: https://eurointervention.pcronline.com/doi/10.4244/EIJ-D-24-01065

Supplementary data

Supplementary Table 1. Inclusion and exclusion criteria.

General inclusion criteria
Acute coronary syndrome (ACS) with percutaneous coronary intervention (PCI) of the infarct-related artery (IRA)
Multivessel disease
FFR of the non-IRA lesion: 0.67 – 0.85
Age ≥ 18 years at screening
General exclusion criteria
Refusal or inability to provide informed consent
Prior coronary artery bypass graft
Known left ventricular ejection fraction (LVEF) < 30%
Untreated functional left main stem stenosis (FFR ≤ 0.80)
Contra-indication for antithrombotic therapy according to European Society of Cardiology guidelines
Non-IRA stenosis not amenable for PCI treatment (operator's decision)
Complicated IRA treatment, with one or more of the following:
- Extravasation
- Permanent no re-flow after IRA treatment (thrombolysis in myocardial infarction (TIMI) flow 0-1)
- Inability to implant a stent
Known severe cardiac valve dysfunction that will require surgery in the follow-up period.

Severe kidney disease defined as an estimated glomerular filtration rate (eGFR) < 30 ml/min.
Known severe liver disease defined as Child-Pugh score of 10-15.
Female patient is pregnant, breastfeeding or planning to become pregnant or planning to breastfeed during treatment and for an additional 15 weeks after the last dose of investigational product. Females of childbearing potential should only be included in the study after a confirmed menstrual period and a negative highly sensitive serum pregnancy test.
Female patients of childbearing potential unwilling to use 1 acceptable method of effective contraception during treatment and for an additional 15 weeks after the last dose of investigational product.
Female patient who has not used an acceptable method(s) of birth control for at least 1 month prior to screening, unless the female patient is sterilized or postmenopausal.

Abbreviations: ACS, acute coronary syndrome; PCI, percutaneous coronary intervention; FFR, fractional flow reserve; IRA, infarct-related artery; LVEF, left ventricular ejection fraction; TIMI, thrombolysis in myocardial infarction; eGFR, estimated glomerular filtration rate.

Supplementary Table 2. Study endpoints.

Primary endpoints	1A. The primary physiological study endpoint is the difference in change in fractional flow reserve (FFR) from baseline to follow-up in the non-infarct-related artery (IRA). 1B. The primary invasive imaging endpoint is the difference in change in maximum lipid core burden index within a 4 mm segment ($maxLCBI_{4mm}$) from baseline to follow-up of the non-IRA as performed in sites capable of near-infrared spectroscopy (NIRS).
Secondary endpoints	Difference in change in intravascular ultrasound (IVUS)-derived plaque characteristics of the non-IRA: 2a. The change in percent atheroma volume (PAV, %) 2b. The change in normalised total atheroma volume (TAV, mm^3) 2c. The change in maximum plaque burden (PB, %) 2d. The change in minimum lumen area (MLA, mm^2)
Exploratory endpoints	1. The correlation between achieved on-treatment low-density lipoprotein cholesterol (LDL-C) and the change in FFR, the change in lipid core burden index (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 coronary flow reserve and index of microvascular resistance 5. Change in inflammatory phenotype of peripheral blood mononuclear cells and monocytes
Safety clinical endpoints	Composite of patient-oriented composite endpoints (POCE): All-cause death Any stroke Any myocardial infarction Any revascularization (not mentioned: revascularization of study vessel at planned follow-up) Unplanned ischemia driven percutaneous coronary intervention (PCI) of target lesion Any unplanned ischemia driven PCI
Safety endpoints	Adverse events, serious adverse events

Abbreviations: FFR, fractional flow reserve; IRA, infarct-related artery; max$LCBI_{4mm}$, maximum lipid core burden index within a 4 mm segment; NIRS, near-infrared spectroscopy; IVUS, intravascular ultrasound; PAV, percent atheroma volume; TAV, total atheroma volume; PB, plaque burden; MLA, minimum lumen area; LDL-C, low-density lipoprotein cholesterol; LCBI, lipid core burden index; POCE, composite of patient-oriented composite endpoints; PCI, percutaneous coronary intervention.

Supplementary Table 3. Medication at discharge.

	Evolocumab (n = 74)	**Placebo (n = 75)[a]**
Aspirin – no. (%)	74 (100.0%)	73 (97.3%)
ADPRI (ticagrelor/clopidogrel/prasugrel) – no. (%)	74 (100.0%)	75 (100.0%)
DAPT – no. (%)	74 (100.0%)	73 (97.3%)
Statin use – no. (%)	74 (100.0%)	74 (98.7%)
- High-intensity statin therapy – no. (%)	73 (98.6%)	68 (90.7%)
o Atorvastatin 40 mg – no. (%)	57 (77.0%)	47 (63.5%)
o Atorvastatin 80 mg – no. (%)	9 (12.2%)	12 (16.2%)
o Rosuvastatin 20 mg – no. (%)	5 (6.8%)	9 (12.2%)
o Rosuvastatin 40 mg – no. (%)	2 (2.7%)	0 (0.0%)
Other lipid-lowering drugs		
- Ezetimibe – no. (%)	6 (8.1%)	6 (8.0%)
- Fibrates – no. (%)	1 (1.4%)	0 (0.0%)
ACE inhibitor – no. (%)	56 (75.7%)	60 (80.0%)
ARB – no. (%)	6 (8.1%)	8 (10.7%)
Beta-blocker – no. (%)	64 (86.5%)	70 (93.3%)

Abbreviations: ADPRI, adenosine diphosphate receptor inhibitors; DAPT, dual antiplatelet therapy; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker.

[a] One patient, randomized to placebo, dropped out of the study before discharge.

Supplementary Table 4. Medication at follow-up.

	Evolocumab (n = 72)[a]	**Placebo (n = 73)[a]**
Aspirin – no. (%)	69 (95.8%)	68 (93.2%)
ADPRI (ticagrelor/clopidogrel/prasugrel) – no. (%)	71 (98.6%)	73 (100.0%)
DAPT – no. (%)	68 (94.4%)	68 (93.2%)
Statin use – no. (%)	70 (97.2%)	70 (95.9%)
- High-intensity statin therapy – no. (%)	70 (97.2%)	66 (90.4%)
o Atorvastatin 40 mg – no. (%)	55 (78.6%)	43 (61.4%)
o Atorvastatin 80 mg – no. (%)	8 (11.4%)	12 (17.1%)
o Rosuvastatin 20 mg – no. (%)	5 (7.1%)	11 (15.7%)
o Rosuvastatin 40 mg – no. (%)	2 (2.9%)	0 (0.0%)
Other lipid-lowering drugs		
- Ezetimibe – no. (%)	5 (6.9%)	6 (8.2%)
- Fibrates – no. (%)	1 (1.4%)	0 (0.0%)
ACE inhibitor – no. (%)	46 (63.9%)	53 (72.6%)
ARB – no. (%)	8 (11.1%)	11 (15.1%)
Beta-blocker – no. (%)	57 (79.2%)	64 (87.7%)

Abbreviations: ADPRI, adenosine diphosphate receptor inhibitors; DAPT, dual antiplatelet therapy; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker.

[a] 143 underwent coronary angiography at follow-up. However, 145 completed clinical follow-up of the trial.

Supplementary Table 5. Baseline characteristics of all patients and of patients who underwent baseline intravascular ultrasound (IVUS) imaging.

	All patients (n = 150)	IVUS at baseline (n = 95)
Randomized to		
- Evolocumab – no. (%)	74 (49.3%)	48 (50.5%)
- Placebo – no. (%)	76 (50.7%)	47 (49.5%)
Demographics		
- Age – years (±SD)	64.2 (8.5)	65.1 (8.3)
- Sex, male – no. (%)	123 (82.0%)	78 (82.1%)
- Sex, female – no. (%)	27 (18.0%)	17 (17.9%)
BMI – kg/m^2 (±SD)	27.4 (4.0)	27.5 (4.0)
Cardiovascular risk factors		
- Hypertension – no. (%)	59 (39.3%)	38 (40.0%)
- Dyslipidemia – no. (%)	63 (42.0%)	38 (40.0%)
- Family history of premature CAD – no. (%)	57 (38.0%)	37 (38.9%)
- Smoking history – no. (%)	110 (73.3%)	70 (73.7%)
o Current Smoker – no. (%)	45 (30.0%)	25 (26.3%
- Diabetes mellitus – no. (%)	15 (10.0%)	7 (7.4%)
o Insulin-treated diabetes mellitus – no. (%)	4 (2.7%)	1 (1.1%)
Medical history		
- Stroke or TIA – no. (%)	7 (4.7%)	5 (5.3%)
- Peripheral artery disease (PAD) – no. (%)	3 (2.0%)	1 (1.1%)
- Prior myocardial infarction – no. (%)	20 (13.3%)	13 (13.7%)
- Prior PCI – no. (%)	24 (16.0%)	16 (16.8%)
- Premature CVD (CAD/stroke/TIA/PAD) – no. (%)	8 (5.3%)	7 (7.4%)
Baseline lipid-lowering therapy		
- Any statins – no. (%)	41 (27.3%)	26 (27.4%)
o High-intensity statin therapy[a] – no. (%)	15 (10.0%)	8 (8.4%)
- Ezetimibe – no. (%)	5 (3.3%)	1 (1.1%)
Other cardiac medications		
- Aspirin – no. (%)	33 (22.0%)	21 (22.1%)
- ADPRI (ticagrelor/clopidogrel/prasugrel) – no. (%)	8 (5.3%)	7 (7.4%)
- DAPT – no. (%)	4 (2.7%)	4 (4.2%)
- ACE inhibitor – no. (%)	18 (12.0%)	12 (12.6%)
- ARB – no. (%)	13 (8.7%)	8 (8.4%)
- Beta-blocker – no. (%)	27 (18.0%)	18 (18.9%)
Type of ACS		
- STEMI – no. (%)	53 (35.3%)	29 (30.5%)
- NSTEMI – no. (%)	90 (60.0%)	61 (64.2%)
- UAP – no. (%)	7 (4.7%)	5 (5.3)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CAD, coronary artery disease; TIA, transient ischemic attack; PAD,

peripheral artery disease; PCI, percutaneous coronary intervention; CVD, cardiovascular disease; ADPRI, adenosine diphosphate receptor inhibitors; DAPT, dual antiplatelet therapy; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; ACS, acute coronary syndrome; STEMI, ST-elevation myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction, UAP unstable angina pectoris.

[a] Atorvastatin ≥ 40 mg, rosuvastatin ≥ 20 mg or simvastatin ≥ 80mg.

Supplementary Table 6. Change in lipid levels of all patients from baseline to 12-week follow-up.

Measurement	Evolocumab (n = 66)	Placebo (n = 68)	p-value
Triglycerides, mmol/L			
Baseline – mean (SD)	1.5 (0.8)	1.8 (1.5)	
Follow-up – mean (SD)	1.0 (0.5)	1.3 (0.8)	
Adjusted mean change (95% CI)	-0.5 (-0.7 to -0.3)	-0.3 (-0.5 to -0.1)	
Adjusted mean difference in change (95% CI) compared to placebo	-0.2 (-0.4 to -0.0)		0.03
Total cholesterol, mmol/L			
Baseline – mean (SD)	5.3 (1.2)	5.4 (1.2)	
Follow-up – mean (SD)	2.3 (0.6)	3.6 (0.9)	
Adjusted mean change (95% CI)	-3.0 (-3.2 to -2.7)	-1.7 (-2.0 to -1.4)	
Adjusted mean difference in change (95% CI) compared to placebo	-1.3 (-1.5 to -1.0)		< 0.001
non-HDL-C, mmol/L			
Baseline – mean (SD)	4.1 (1.2)	4.2 (1.3)	
Follow-up – mean (SD)	1.1 (0.5)	2.4 (0.8)	
Adjusted mean change (95% CI)	-3.0 (-3.3 to -2.7)	-1.7 (-2.0 to -1.4)	
Adjusted mean difference in change (95% CI) compared to placebo	-1.3 (-1.5 to -1.0)		< 0.001
LDL-C, mmol/L			
Baseline – mean (SD)	3.4 (1.1)	3.4 (1.1)	
Follow-up – mean (SD)	0.6 (0.5)	1.8 (0.7)	
Adjusted mean change (95% CI)	-2.7 (-2.9 to -2.5)	-1.5 (-1.7 to -1.3)	
Adjusted mean difference in change (95% CI) compared to placebo	-1.2 (-1.4 to -1.0)		< 0.001
HDL-C, mmol/L			
Baseline – mean (SD)	1.2 (0.3)	1.2 (0.4)	
Follow-up – mean (SD)	1.2 (0.2)	1.2 (0.3)	
Adjusted mean change (95% CI)	0.0 (-0.0 to 0.1)	-0.0 (-0.1 to 0.0)	
Adjusted mean difference in change (95% CI) compared to placebo	0.1 (-0.0 to 0.1)		0.06

Abbreviations: non-HDL-C, non-high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

For patients treated with evolocumab, one serial measurement for triglycerides and LDL-C was missing (n = 65), while two serial measurements for non-HDL-C were missing (n = 64). For patients treated with placebo, one serial measurement was missing for triglycerides, non-HDL-C and LDL-C (n = 67).

CU conversion factors: To convert total cholesterol, LDL-C, HDL-C, and non-HDL-C values to mg/dL, divide by 0.0259; to convert triglycerides values to mg/dL, divide by 0.0113.

Supplementary Table 7. Overall changes in FFR, maxLCBI$_{4mm}$, and atheroma volume parameters.

Intracoronary physiology	**Overall (n = 142)**
Fractional flow reserve	
Baseline – mean (SD)	0.78 (0.05)
Follow-up – mean (SD)	0.78 (0.07)
Mean change (95% CI)	0.00 (-0.01 to 0.01)
Near-infrared spectroscopy parameters	**Overall (n = 85)**
MaxLCBI$_{4mm}$	
Baseline – mean (SD)	358.5 (175.5)
Follow-up – mean (SD)	321.3 (170.5)
Mean change (95% CI)	-37.3 (-63.2 to -11.3)
LCBI$_{total}$[a]	
Baseline – mean (SD)	87.6 (60.8)
Follow-up – mean (SD)	72.3 (51.5)
Mean change (95% CI)	-15.3 (-24.7 to -5.9)
Intravascular ultrasound parameters	**Overall (n = 86)**
Percent atheroma volume, %	
Baseline – mean (SD)	47.6 (7.2)
Follow-up – mean (SD)	47.2 (6.8)
Mean change (95% CI)	-0.5 (-1.1 to 0.2)
Normalized total atheroma volume, mm^3	
Baseline – mean (SD)	376.5 (129.1)
Follow-up – mean (SD)	367.5 (121.7)
Mean change (95% CI)	-9.0 (-18.1 to 0.1)
Maximum plaque burden, %	
Baseline – mean (SD)	70.8 (7.0)
Follow-up – mean (SD)	70.0 (6.9)
Mean change (95% CI)	-0.8 (-1.6 to 0.0)
Minimum lumen area, mm^2	
Baseline – mean (SD)	3.7 (0.9)
Follow-up – mean (SD)	3.6 (1.0)
Mean change (95% CI)	-0.1 (-0.2 to 0.1)

Abbreviations: maxLCBI$_{4mm}$, maximum lipid core burden index within any 4 mm segment; LCBI$_{total}$, lipid core burden index over the total vessel.

[a] Serial LCBI$_{total}$ values were missing for four patients (n = 81).

Trial sponsor: Radboudumc

Radboudumc (sponsor) manages the collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, with ultimate authority over these activities.

Data availability statement

The detailed study protocol and statistical analysis plan are provided in the supplementary appendices. Anonymised participant source data are available from the corresponding author upon reasonable request.

Author statement

F.M., J.L., and R.G. designed the study and drafted this paper. R.O., C.B., A.I., M.M., J.C., D.W., P.S., V.P., D.H., T.C., C.C., P.D., L.N., A.D., M.W., and N.R. were responsible for execution of the study and data acquisition. H.R., R.B., and A.C., contributed to data analysis. All authors reviewed the work critically and gave final approval of this work to be published.

Functional ***I***mprovement of non-infarc***T*** rela***T***ed coronary artery stenosis by ***E***xtensive LDL-C ***R***eduction with a PCSK9 Antibody

FITTER V8.0

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PROTOCOL TITLE: **F**unctional **I**mprovement of non-infarc**T** rela**T**ed coronary artery stenosis by **E**xtensive LDL-C **R**eduction with a PCSK9 Antibody

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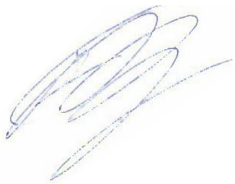
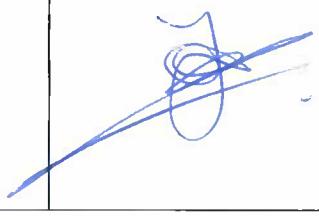
Coordinating Investigator: Drs. F.B. Mensink Cardiology Radboud University Medical Center		31-10-23
Coordinating Investigator: Drs. J. Los Cardiology Radboud University Medical Center		31-10-23

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
ACS	**Acute Coronary Syndrome**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CABG	**Coronary Artery Bypass Graft**
CAD	**Coronary Artery Disease**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
ESC	**European Society of Cardiology**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
FFR	**Fractional Flow Reserve**
FH	**Familial Hypercholesterolemia**
GCP	**Good Clinical Practice**
HIST	**High Intensity Statin Therapy**
HoFH	**Homozygous Familial Hypercholesterolemia**
IB	**Investigator's Brochure**
IC	**Informed Consent**
ICA	**Invastive Coronary Imaging**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
IMR	**Index of Microcirculatory Resistance**
IRA	**Infarct Related Artery**
IVUS	**Intra Vascular Ultra Sound**
LCBI	**Lipid Core Burden Index**
LDL-C	**Low Density Lipoprotein Cholesterol**
MACE	**Major adverse cardiac events**

MaxLCBI$_{4mm}$	**Lipid core burden index at the 4mm maximal segment**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
MI	**Myocardial Infarction**
MVD	**Multi Vessel Disease**
NIRS	**Near InfraRed Spectroscopy**
PB	**Plaque Burden**
PCI	**Percutaneous Coronary Intervention**
PBMC	**Peripheral Blood Mononuclear Cell**
PCSK9	**Proprotein convertase subtilisin/kexin type 9**
PV	**Plaque Volume**
RCT	**Randomized Controlled Trial**
(S)AE	**(Serious) Adverse Event**
SD	**Standard Deviation**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organization or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidizing party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: In a large number of patients presenting with acute coronary syndrome (ACS) multivessel disease is identified. Optimal treatment approach for bystander lesions in non-infarct related arteries (non-IRAs) has not been well established. Some RCTs favor preventive complete revascularization over deferred PCI. Background medical treatment wasn't optimal in these studies, however, which could have caused bias. Revascularization of lesions in the non-IRA can be guided by fractional flow reserve (FFR). In addition, the Near-InfraRed Spectroscopy (NIRS) derived lipid core burden index (LCBI) has been identified as independent risk factor for future coronary events. Potent lipid lowering therapy might improve FFR and LCBI. We want to investigate if optimizing LDL-C lowering therapy after an ACS has an effect on functional impairment of a non-IRA lesion and could thus prevent mechanical intervention (PCI or CABG).
Objective: To evaluate the effect of maximal LDL-C reduction by Evolocumab on top of high intensity background lipid-lowering therapy on FFR and max$LCBI_{4mm}$ of non-IRA lesions, in patients presenting with MVD-ACS. Secondly, to evaluate the effect of maximal LDL-C reduction on plaque characteristics of non-IRA lesions and to correlate baseline LDL-C, lipid core burden, and plaque characteristics with changes in FFR. Thirdly, to evaluate the change in microvascular function. Finally, to investigate the relation between LDL-C reduction and change in pro-inflammatory monocyte phenotypes.
Study design: This is a multi-center, randomized, double blind, placebo controlled clinical trial.
Study population: Patients presenting with MVD-ACS will be included in this study. Patients must be 18 years or older. We aim to include at least 150 patients to achieve adequate power for this study.
Intervention: The patients will be randomized 1:1 to (A), one group will receive 140mg Evolocumab every two weeks (Q2W) for 12 weeks, using personal injectors; (B) the other group will receive placebo. All participants will receive high intensity statin therapy (HIST) as background therapy (Atorvastatin 40mg or equivalent).
Main study parameters/endpoints: The primary study endpoints are the change in FFR from baseline to follow-up in non-IRA lesions, and the change in Max$LCBI_{4mm}$ from baseline to follow-up in the non-IRA. The secondary invasive imaging study endpoints are the change from baseline to follow-up in the non-IRA of: percent atheroma volume, normalized total atheroma, volume, maximum plaque burden (PB) and minimum luminal area. Exploratory endpoints include correlations between on-treatment LDL-C, Max$LCBI_{4mm}$, plaque characteristics, and FFR. Main endpoint for the immunological parameters is the comparison of monocyte phenotype between the groups at t=12 weeks post-ACS.
Nature and extent of the burden and risks associated with participation, benefit and group relatedness: After inclusion, all patients have to undergo staged FFR +/- NIRS, meaning they will undergo invasive strategy for a second time, and if needed additional stenting of significant lesions, with the associated periprocedural risks (e.g. death, stroke, MI, vascular complications), however these risks are quite small (<2% major complications) .

1. INTRODUCTION AND RATIONALE

In a large number of patients presenting with acute coronary syndrome (ACS) multivessel disease is identified [1]. 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) [2-5]. 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 [6]. 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 [7]. 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 shown to induce regression of coronary atherosclerotic plaque volume (PV) in patients with coronary artery disease (CAD) [8]. 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 [9-11]. 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 (MACE) [12, 13]. 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 [12, 13]. A recent study demonstrated the effect on plaque composition in 52 weeks [14]. 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. [15] The change in plaque volume might be closely related to FFR changes.

Furthermore, 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 [16, 17]. Hypercholesterolemia also activates the innate immune system bone marrow progenitors resulting in long-term activation of the innate immune system [18]. In patients with familial hypercholesterolemia (FH), PCSK9 treatment reduced monocyte CCR2 expression and ex-vivo migratory capacity [19]. 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.

Evolocumab was the first PCSK9-inhibitor approved for clinical use in 2015 for lowering of LDL-C as an adjunct to diet in patients with familial hypercholesterolemia (FH), primary hypercholesterolemia and in patients with homozygous familial hypercholesterolemia (HoFH). Evolocumab has been evaluated in several large scale studies as GLAGOV (N = 968) [8] and FOURIER (N= 27564) [15] on surrogate and clinical endpoints with important benefits and established safety and tolerability.

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, and change in microvascular circulation. We will 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. OBJECTIVES

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 Hypotheses:

1A. Addition of PCSK-9 inhibitors to treatment post-ACS will lead to significant reduction of functional impairment of a non-IRA lesion in patients with MVD-ACS.
1B. Addition of PCSK-9 inhibitors to treatment post-ACS will lead to significant reduction of lipid core burden of a non-IRA lesion in patients with MVD-ACS.
2A. Addition of PCSK-9 inhibitors to treatment post-ACS will lead to significant plaque reduction in a non-IRA lesion in patients with MVD-ACS.
2B. Change in functional impairment will be more pronounced in patients with higher baseline lipid core burden
2C: Addition of PCSK-9 inhibitors to treatment post-ACS will lead to significant amelioration of the microvascular circulation in patients with MVD-ACS.
2D. LDL-C lowering attenuates the pro-inflammatory myeloid cell reprogramming by ACS.

3. STUDY DESIGN

3.1 Type of study

The study is designed as a multi-center, randomized, placebo controlled clinical trial. The principal trial center will be the Radboudumc.

3.2 Summary of the study design

Patients with ACS will be screened for the study. Informed consent will be obtained. The culprit lesion in the IRA will be treated with a Drug Eluting Stent (DES). Bystander non-IRA lesions will be investigated with FFR. Patients with FFR values between 0.67 and 0.85 will be eligible for this study. Participants will be randomized and treated for 12 weeks with either Evolocumab or placebo. Both groups will be treated with high intensity statin therapy as background therapy. FFR at 12 weeks will be the endpoint. Patients with FFR values ≤0.80 at follow up will be treated with PCI in the same setting. In study sites with Near-infrared spectroscopy (NIRS) availability, at the baseline and follow up IVUS-NIRS will be performed during coronary angiography. IMR measurements will also be performed at baseline and follow-up, when possible.

In a subgroup of patients included in the Radboudumc, the inflammatory phenotype of peripheral blood mononuclear cells (PBMCs) and Percoll-isolated monocytes will be explored using flow cytometry and cytokine production capacity and foam cell formation [20]. In addition, we will explore the underlying metabolic, epigenetic, and transcriptomic mechanisms. The immunological side study will investigate the relation between LDL-C reduction and reduction of pro-inflammatory monocyte phenotypes.

3.3 Treatment

Evolocumab (140mg) will be administered every two weeks (Q2W) on day 1 through week 12 with personal injectors, containing 1 mL deliverable volume of 140 mg/mL Evolocumab or an identical volume of placebo. Evolocumab will be administered Q2W in accordance with the instructions in the Summary of Product Characteristics (SmPC). In total, six 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. Evolocumab and placebo will be manufactured and packaged by Amgen Europe B.V. and distributed using Amgen clinical investigational product distributions procedures. Detailed information on the nonclinical effects of Evolocumab and its clinical effects in this patient population is provided in the Investigator's Brochure (IB). The EU SmPC and IB provide detailed product information for investigators in the Netherlands.

Atorvastatin or other high-intensity statins will be used as background therapy in both groups with a dosage of ≥ 40 mg daily (QD) or equivalent (80mg simvastatin or 20mg rosuvastatin). If not receiving atorvastatin ≥ 40 mg or equivalent, the investigator must attest that higher dose statin therapy has been considered but is not appropriate for this subject (e.g., dose not tolerated or other significant concern).

3.4 Duration of the study

The follow-up time will be 3 months. The inclusion time is estimated to be 24 months, the study should be completed in 30 months. The study will be held at three or more study sites, in the Netherlands only.

4. STUDY POPULATION

4.1 Population (base)

The research population will be recruited from the general patient population presenting with ACS. Patients treated with PCI and one or more additional non-IRA stenoses with FFR 0.67 – 0.85 will be eligible for this study.

4.2 Inclusion criteria

In order to be eligible for this study, a subject must meet all the following criteria:
- ACS with PCI of infarct related artery
- Multivessel Disease (MVD)
- FFR of non-IRA lesion: 0.67 - 0.85
- Age ≥ 18 years at screening

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Refusal or inability to provide informed consent
- Prior coronary artery bypass graft
- Known left ventricular ejection fraction (LVEF) < 30%
- Untreated functional left main stem stenosis (FFR ≤ 0.80)
- Contra-indication for antithrombotic therapy according to ESC guidelines
- Non-IRA stenosis not amenable for PCI treatment (operator's decision)
- Complicated IRA treatment, with one or more of the following:
    - Extravasation
    - Permanent no re-flow after IRA treatment (TIMI flow 0-1)
    - Inability to implant a stent
- Known severe cardiac valve dysfunction that will require surgery in the follow-up period.
- Severe kidney disease defined as an eGFR < 30 ml/min.
- Known severe liver disease defined as Child-Pugh score of 10-15.
- Female subject is pregnant, breastfeeding or planning to become pregnant or planning to breastfeed during treatment and for an additional 15 weeks after the last dose of investigational product. Females of childbearing potential should only be included in the study after a confirmed menstrual period and a negative highly sensitive serum pregnancy test.
- Female subjects of childbearing potential unwilling to use 1 acceptable method of effective contraception during treatment and for an additional 15 weeks after the last dose of investigational product.
- Female subject who has not used an acceptable method(s) of birth control for at least 1 month prior to screening, unless the female subject is sterilized or postmenopausal.

4.4 Sample size calculation

4.4.1 Powered primary physiological endpoint

This study is powered to detect a difference in the primary endpoint (FFR of lesions in non-IRA).
In a previous trial [11], 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 [21]. 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

4.4.2 Powered primary invasive imaging endpoint

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 [15] 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.

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

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

5. TREATMENT OF SUBJECTS

5.1 INVESTIGATIONAL PRODUCT

5.1.1 Name and description of investigational products

Investigational products in this study include Evolocumab (140mg/ml) and matched placebo. Detailed information regarding the storage, preparation, destruction, and administration of Evolocumab can be found in the Pharmacy Instruction Guide (PIG). These will be provided separately from this protocol.
The medical device(s) used in this study include(s): Prefilled AI/pen.
For detailed information about the pharmacological properties of Evolocumab we refer to the Investigator's Brochure or SmPC, which will also be provided separately from this protocol.

5.1.2 Production and storage

Evolocumab will be produced and delivered to the sponsor (Radboudumc) by Amgen. An overview on the tasks delegated by the sponsor to Amgen is presented in the Investigator Initiated Research Agreement between sponsor and Amgen, "Study Drug Exhibit".
Evolocumab and placebo should be kept in the refrigerator between +2 °C and +8 °C. It should be kept in the original box for protection from light. If removed from the refrigerator, Evolocumab or placebo can be kept in the original box at room temperature (25 °C), but has to be administered in one month time.
The AI/Pens should be inspected for investigational product quality,
expiry, and damage before using. Damaged, expired, or degraded product should not be used and any issues with the AI/Pens should be reported to Amgen.

5.1.3 Route of administration

Evolocumab is given subcutaneously and can be injected in the belly, thigh or upper arm. Injection sites should be alternated between administered dosages, and injections should not be given in areas where the skin is painful, erythematous or when a hematoma is present. Evolocumab can be administered by the participant him/herself, after having received adequate training by an expert who is trained in administering the product.
Evolocumab antibodies will be given as a sterile solution of exactly 1.0ml. This solution will contain 140mg Evolocumab (deliverable volume 140mg/ml). This solution will be administered using a disposable, prefilled, single use auto-injector/pen for fixed-dose subcutaneous injection.

Placebo will consist of the same sterile solution with a deliverable volume of 1.0ml, without addition of the Evolocumab antibodies. The personal autoinjector is the same as the one used for administration of Evolocumab.

5.1.4 Dosage and dosage adjustments

The recommended dosage for Evolocumab is 140mg every two weeks or 420mg once a month, the two dosages are clinically equivalent (with atherosclerotic vascular disease in adults as indication).
Dosage adjustment is not needed in participants with mild to intermediately impaired renal function. Dosage adjustment is not needed in participants with mild liver function impairment. No dosage adjustment is needed for elderly patients (≥65y).

5.1.5 Schedule

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. The first dose will be given clinically during hospital stay. Participants will receive injection training by certified healthcare providers. First dosing must be observed by a healthcare provider. The remaining 5 doses of Evolocumab or placebo will be administered at home by the participant or a designee. Subjects who do not wish to self-inject at home can return to the clinic for their injections.
The participant (or designee) must have demonstrated competency at administration of subcutaneous injections before self-administration is permitted.
In total, 6 doses of IMP (Evolocumab or Placebo) will be administered. The last dose will be given in week 10. This is 2 weeks before the follow-up procedure. The follow-up procedure will be in week 12.

5.1.6 Contra-indications and precautions

Evolocumab not indicated for use in patients with severely decreased renal function (eGFR <30 ml/min/1,73m2).
In patients with intermediate or severe liver function impairment, total Evolocumab exposure is decreased, which could lead to less LDL-C lowering.
No data is available on the effect of Evolocumab on fertility and pregnancy.

5.1.7 Allergic reactions and adverse reactions

The needle protector of the AI/pen consists of dry natural rubber (latex derivative), which could lead to allergic reaction.
Angioedema, rash, and urticaria have occurred. Common adverse reactions in clinical trials (> 5% of patients treated with Evolocumab and occurring more frequently than placebo): nasopharyngitis, upper respiratory tract infection, influenza, back pain, and injection site reactions.

6. METHODS

6.1 Study endpoints

6.1.1 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).

6.1.2 Secondary endpoints

Secondary invasive imaging (IVUS) endpoints are:
1. The change in percent atheroma volume (PAV, mm^3)
2. The change in normalized total atheroma volume (TAV, mm^3)
3. The change in maximum plaque burden (%)
4. The change in minimum luminal area (MLA, mm^2)

6.1.3 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.

6.2 Randomization, blinding and treatment allocation

Patients who present themselves with ACS will be screened for this study after hospitalization. Written informed consent will be obtained (see chapter 9.2 Ethical considerations). Patients will undergo invasive coronary angiography (ICA) and the culprit lesion in the IRA will be treated with PCI, conform local and international guidelines. FFR on non-IRA lesions is performed and if FFR is in the earlier mentioned range (FFR 0.67 – 0.85) patients will be eligible for this study. Patients are randomized after the initial procedure into two groups (Evolocumab or placebo). The randomization will take place in 1:1 fashion. Both the patient and the researcher will be blinded for received treatment.

6.3 Study procedures

6.3.1 Percutaneous intervention (PCI), Fractional Flow Reserve (FFR) and Index of Microcirculatory Resistance

ICA and PCI of the culprit lesion will be performed according to standard procedures and practice, either a transradial or a transfemoral approach is used. Before ICA, ACS medication and antithrombotic therapy is given conform the ESC guidelines. Iodized contrast is giving during the procedure. ICA imaging will be performed with a biplane or monoplane cardiovascular X-ray system in at least two orthogonal directions. If a non-IRA lesion is present, FFR measurements across the stenosis of the non-IRA lesion will be performed. If possible, IMR will be calculated using distal coronary flow measurements. More information about the exact FFR/IMR procedure can be found in the appendix. When the FFR is above 0.85, patients will be treated conservatively according to current guidelines and excluded from this study. When the FFR < 0.67, the coronary lesion will be stented, and patients will be excluded from the study. When the FFR value is between 0.67 and 0.85, patients are included in the study. Patients will be randomized to the Evolocumab or placebo group and are treated with LDL-C lowering therapy for a total period of 12 weeks. Two weeks after the last dose of Evolocumab or placebo FFR will be re-assessed and the lesion stented when FFR ≤ 0.80. If the follow-up FFR >0.80, conservative treatment will be implemented.
Patients who need an invasive coronary procedure after at least 8 weeks of treatment should have their FFR re-assessed at this procedure before any intervention in the study vessel. Patients with invasive procedures 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.
Images of ICA, FFR/IMR/NIRS and follow up ICA and FFR/IMR/NIRS will be sent to the sponsor.

6.3.2 Near-infrared spectroscopy (NIRS)

In centers capable of NIRS the U.S. Food and Drug Administration–approved NIRS-IVUS system will be used in this study. The combined IVUS-NIRS 3.2-F rapid exchange catheter (InfraReDx, Burlington, Massachusetts), will be positioned distal to a side branch in the target lesion. 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 mm^2 of vessel wall from a depth of approximately 1 mm in the direction from the luminal surface toward the adventitia. 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, with a maximum imaging length of 12 cm. 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. Core laboratory personnel are blinded to all other patient and outcome data. The fraction of yellow pixels obtained from the chemogram, an image map derived from the NIRS measurements, is multiplied by 1000 to compute the LCBI. NIRS data will be analyzed off-line by an independent core lab blinded to all other patient and outcome data.

Raw data of all FFR/IMR/NIRS-measurements will be obtained and sent to the sponsor. These include equalization, resting Pd/Pa measurement, hyperemic Pd/Pa measurement (FFR), hyperemic distal coronary flow measurements (IMR) and a drift check.

6.3.3 Monocyte phenotype analysis

Blood will be drawn after revascularization for baseline measurement of monocyte phenotype. Monocyte phenotype will be determined using a FACS panel and cytokine production capacity of peripheral blood mononuclear cells after 24 hours of incubation with

LPS/Pam3Cys and cholesterol crystals (IL-1b). At 4 and 12 weeks measurements will be repeated (in the morning after overnight fast) with additional MACS isolation of monocytes for RNA and ChiP-sequencing. More information about the immunological study can be found in the appendix.

6.3.4 Gathering of data

Demographics, clinical history and other clinical data at baseline and follow-up will be documented as outlined in the CRF.

6.3.5 Blood sampling

Peripheral blood samples will be taken according to local standard care protocol from patients who present with ACS in the Emergency Care (EC). This will be done either within 24 hours prior to PCI, or immediately after PCI (CBC, blood chemistry, lipids, CK, CK-MB, hs-Troponin). Secondly, during or directly after ICA, after a patient has given written consent, 50ml of blood will be drawn from the arterial sheath for monocyte phenotype analysis. Additionally, 10,5ml of blood will be drawn for the serum pregnancy test (see chapter 6.3.6) and inflammatory markers (hsCRP, IL-1β and IL-6).
During a follow-up visit at 1 week post-procedure lipids and inflammatory markers (hsCRP, IL-1β and IL-6) will again be measured and another 10ml of blood will be drawn.
During a follow-up visit at 4 weeks post-procedure a total of 60ml of blood will be drawn (lipids, hsCRP, IL1β-IL-6 and monocyte phenotyping)
During the last follow-up visit at 12 weeks post-procedure 53ml of blood will be drawn for lipids and monocyte phenotyping.

A total blood volume of 183,5ml (3 times 50ml for monocyte phenotyping, 3,5ml for pregnancy testing, 3 times 7ml for inflammatory markers, 3 times 3ml for lipids) will be drawn from a participant during this study.
Only participants included in the Radboudumc will have blood drawn for monocyte phenotyping and for measurement of IL-1β and Il-6. Participants in other centers will have blood drawn only for the pregnancy test, lipids and for hsCRP measurements. A total blood volume of 16,5ml will be drawn from these participants during the study.

Importantly, any LDL-C measurements during the study (except LDL-C at screening and follow-up after 12 weeks) will be blinded for the researchers.

Blood samples for monocyte phenotyping will be stored at the Radboudumc laboratory of experimental internal medicine (Laboratorium Experimentele Interne Geneeskunde). Samples will only be used for the research purposes mentioned in this study.
All samples that are kept in the Radboudumc laboratory are coupled with a unique numerical code. Patient information isn't linked to the sample directly, and a special key is needed to access the codes. The storage site is secured against unauthorized access according to GCP guidelines.
The patient material will only be used for analyses described in this protocol. We do not expect that the samples will yield any information of importance to participants' health. No genetic testing will be performed on patient material. If there are accidental findings of importance, investigators will act according to the document 'nevenbevindingen' in the 'intergraal kwaliteit system (IKS)' of the Radboudumc, and inform patients as instructed.

6.3.6 Pregnancy testing

Patients will have to undergo a serum pregnancy test at screening. Blood will be drawn for the arterial sheath during the procedure. If pregnant, patients are excluded from this study. Male patients or post-menopausal females are exempt from this rule.

6.3.7 EKG
EKGs will be acquired directly after presentation at the Emergency Care (EC) or directly before ICA. A copy of an EKG at presentation will be sent to the sponsor.

6.3.8 Follow-up
Radboudumc:
At 1 week, 4 weeks and 12 weeks there will be a hospital visit. At 6 weeks and 8 weeks there will be a telephonic contact.

Other sites:
At 1 week and at 12 weeks there will be a hospital visit. At 4 weeks, 6 weeks and 8 weeks there will be a telephonic contact.

6.4 Withdrawal of individual subjects
Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The intention-to-treat principle will be used when a patient is not treated according to the allocated treatment strategy. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

6.4.1 Specific criteria for withdrawal
Patients who are unable to make an independent decision regarding participation will be withdrawn from the study.

6.5 Replacement of individual subjects after withdrawal.
Because the power calculation used already accounts for possible dropout, participants who withdraw after randomization will not be replaced with new participants.

6.6 Follow up of subjects withdrawn from treatment
Patients who withdraw from this study will receive normal standard care post-ACS.

6.7 Premature termination of the study
The principal investigator will notify the accredited METC within 15 days if the study is terminated for any reason.
The investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

7. SAFETY REPORTING

7.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

7.2 Aes, SAEs and SUSARs

7.2.1 Adverse Events (Aes)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.
In light of the recent Coronavirus (COVID-19) pandemic, a question is added to the CRF whether an event is possibly related to an infection with SARS-CoV-2, as this could influence our results.
AEs will be recorded from time of signature of informed consent till 30 days after participant received the last dose of study medication (IMP).

7.2.2 Serious adverse events (SAEs)

A serious adverse event is an AE that fulfils one or more of the following criteria:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalization or prolongation of existing inpatients' hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- or any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.
The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

The sponsor will report the SAEs through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

SAEs will be recorded from time of signature of informed consent till 30 days after participant received the last dose of study medication (IMP).

7.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

- the event must be serious (see chapter 8.2.2);
- there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
- the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in: Summary of Product Characteristics (SPC) for an authorized medicinal product; Investigator's Brochure for an unauthorized medicinal product.

The sponsor will report expedited the following SUSARs through the web portal ToetsingOnline to the METC and inform the manufacturer simultaneously:
SUSARs that have arisen in the clinical trial that was assessed by the METC;
SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

Additionally all SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC and the manufacturer simultaneously. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.
The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

7.3 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

7.4 End of study safety report

Within one calendar year after the end of the study the sponsor will generate an unblinded SAE listing for the manufacturer to reconcile with previous submitted yearly SAE listings.

7.5 Pregnancy

Details of all pregnancies and/or lactation in female subjects and, if indicated, female partners of male subjects will be collected after the start of study treatment and until 15 weeks after the last dose of investigational product.
If a pregnancy is reported, the investigator is to inform Amgen within 10 days of learning of the pregnancy and/or lactation. Any female subject who becomes pregnant while participating will discontinue study treatment.
Amgen Global Patient Safety will follow-up with the investigator regarding additional information that may be requested.
Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered serious adverse events.

8. STATISTICAL ANALYSIS

8.1 General

A detailed description of the entire statistical analysis can be found in the statistical analysis plan (SAP).

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.

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

Analysis of the primary outcome will be done using an ANCOVA model as primary analysis using the FAS and IVUS-NIRS population. ANCOVA model will include group and randomisation stratification factor per 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.

For continuous secondary outcome parameters the approach applied for primary parameter will be followed.
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 necessary.

Multiplicity correction for primary endpoints and secondary endpoints will be done to maintain the overall familywise error rate at 0.05.

8.2 Interim analysis

No interim analysis is planned for this study.

8.3 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).

8.4 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 need 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 can 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.

8.5 Statistical analytical issues

8.5.1 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.

8.5.2 Adjustments for covariates

For covariates used in the ANCOVA model, please see chapter 8.1. No further adjustment for covariates are intended for the primary and secondary endpoints.

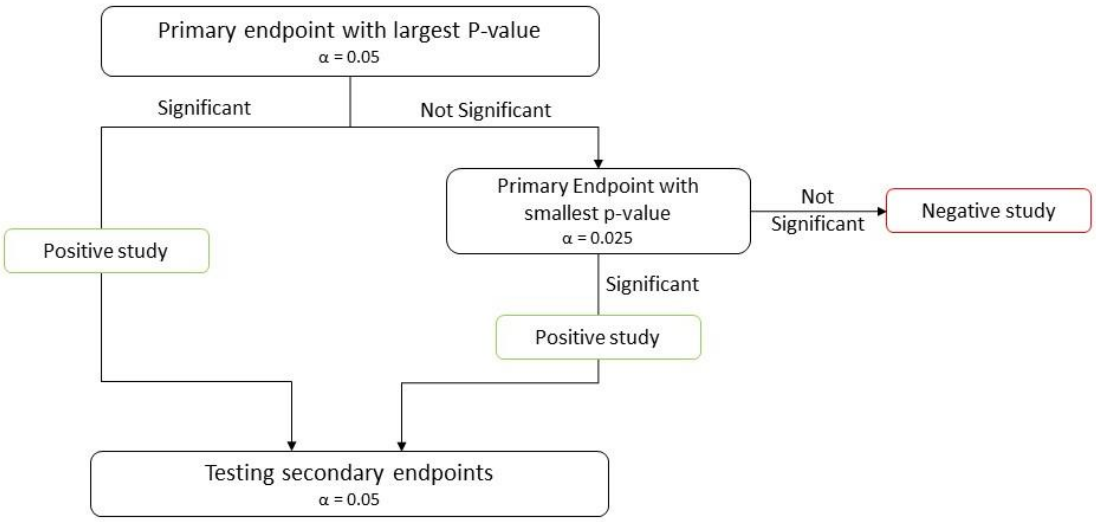
8.5.3 Multicenter studies

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

8.5.4 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 hypothesis 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 hypothesis 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 H_0 for the secondary endpoints. For example, endpoint 2b. will only be tested if endpoint 2a. has a p-value <0.05 etc.

8.5.5 Examination of subgroups

Prespecified stratified analyses of the primary and secondary endpoints will be performed according to the following characteristics: age, gender, diabetes mellitus, ACS type, vessel localization, FFR at baseline, statin use at baseline, LDL-level at baseline, LDL reduction throughout study period, max$LCBI_{4mm}$ 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 max$LCBI_{4mm}$ at baseline
5. Change in max$LCBI_{4mm}$ will be higher in patients with high LDL-C at baseline
6. Change in max$LCBI_{4mm}$ will be higher in statin naive patients

9. ETHICAL CONSIDERATIONS

9.1 Regulation statement

This study is conducted in full accordance with the principles of the "Declaration of Helsinki" (as amended in Tokyo, Venice, and Johannesburg), with ICH-GCP, and with the laws and regulations of the Netherlands.

9.2 Recruitment and consent

It is the responsibility of the investigators to obtain written informed consent. The information is intended to give each participant a thorough understanding of the purpose and the nature of the trial, the cooperation required, anticipated benefits, and potential hazards of the study. The investigator also explains that the patient is completely free to refuse or to withdraw from the trial and that if he does so he receives standard treatment with the same degree of care. A consent form (in the native language) will be made available. The consent form must be signed and dated by both the investigator and the patient. The fact that informed consent was obtained is recorded in the case report form.

9.2.1 Written informed consent

Patients who are hospitalized for ACS are screened if they are eligible for this study. Patients who do not have to undergo ICA immediately will be informed about the study before ICA occurs. These patients will receive ICA within 72 hours of hospitalization. Participants will receive a subject information sheet and informed consent form, preferably within 1 hour after arrival. Patients will then have the opportunity to ask questions about the study and are given sufficient time to decide on participation.
These patients have to sign a written consent in which they agree to participate in the study. Patients are only eligible for this study if they have at least one non-IRA lesion. If the FFR is not 0.67-0.85, no further measurements should be performed. Patients who do not meet the inclusion criteria are noted as screen failures.
If the patient meets the inclusion criteria, they will be randomized in a 1:1 fashion to treatment group or placebo. Patients who refuse to give written informed consent are excluded from the study.

9.2.2 Oral consent for NIRS measurement

If there is adequate time before the index procedure, the patient is informed of the entire FITTER study and full written informed consent is given prior to the index procedure. However, patients who have to undergo ICA immediately or who do not have sufficient time before ICA cannot give the written informed consent before the procedure. In these cases oral consent is asked directly before or during the index procedure for necessary data acquisition (e.g. IVUS-NIRS). This NIRS measurement will only be performed when all other inclusion criteria and no exclusion criteria are met. Full written consent for the entire study will be obtained after the index procedure and before randomization and the start of medication. Patients who refuse to give written informed consent are excluded from the study and will receive the standard medical care.

9.2.3 Dialogue and companionship

The dialogue regarding study participation will take place in the Emergency Care, in the Coronary Care Unit or the Cardiology ward. The staff is experienced in obtaining informed consent in this environment, also during acute circumstances. Enrollment is omitted if the patient feels the need for a companion to decide on participation, as a companion is almost never present before coronary angiography.

9.3 Benefits and risks assessment, group relatedness

Staged PCI for patients with multivessel disease presenting with ACS has been the preferred strategy for many years where ad-hoc multivessel PCI in STEMI was considered potentially harmful (Class III STEMI recommendation, ESC guidelines). Recent studies demonstrated the safety of ad-hoc multivessel PCI in selected STEMI patients, changing the recommendation to IIA. Increased risk of unscheduled PCI was observed when bystander lesions were treated conservatively with clinical follow-up without staged procedures.
FFR of the non-IRA can thus detect patients with a significant functional impairment of flow (FFR <0.67). In this group immediate PCI would be a better option than deferred treatment. Therefore, the proposed schedule minimizes the risks of cardiac events related to the non-IRA lesions during the study period.
On the other hand, lesions with less flow impairment will be treated with medication first, possibly ameliorating flow and avoiding additional PCI of the lesion in a later stage. The risks of FFR and NIRS-IVUS during staged invasive procedures are known to be low.

9.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO. The Radboud University Medical Center (RUMC) has a liability insurance concluded at the ‘Onderlinge Waarborgmaatschappij Centramed’ b.a., Postbus 7374, 2701 AJ Zoetermeer.
The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.
1. € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 5.000.000,-- (i.e. five million Euro) for death or injury for all subjects who participate in the Research;
3. € 7.500.000,-- (i.e. seven and a half million Euro) for the total damage incurred by the organization for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.
4. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

10.1 Handling and storage of data and documents

The investigator will maintain a study file, which he will use to file the IB, protocol, drug accountability records, correspondence with the IEC, the sponsor/investigator, and other study-related documents.
The sponsor/investigator will archive and retain all documents pertaining to the study for at least 15 years after the last approval.
The data generated will be encoded and a separate patient identification log will be created. The key to the code will only be available to the principal investigator and delegated investigators. The acquired encoded data imputed in the eCRF will be accessible with passwords to the researchers involved. Personal data will comply to the Dutch Personal Data Protection Act.

10.2 Monitoring and Quality Assurance

During the study, the study sites will be monitored to review study progress, Investigator and patient compliance with clinical protocol requirements and any emergent problems. The monitor visit will include: patient informed consent, patient recruitment and follow-up, SAE documentation and reporting, AE documentation, IMP allocation, IMP accountability and quality of the data. Details will be specified in a monitoring plan.

10.3 Amendments

A ‘substantial amendment’ is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree.
the safety or physical or mental integrity of the subjects of the trial;
the scientific value of the trial;
the conduct or management of the trial; or
the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

10.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

10.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC, the competent authority and the manufacturer simultaneous of the end of the study within a period of 90 days. The end of the study is defined as the last patient’s last visit.

The sponsor will notify the METC and the manufacturer immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC,the competent authority and the manufacturer within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC, the Competent Authority and the manufacturer simultaneously.

10.6 Public disclosure and publication policy

The investigators are committed to the publication and widespread dissemination of the results of the study and they will therefore considered for publication or presentation at (scientific) symposia and congresses.

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12. APPENDICES

12.1 Appendix 1: Flowcharts

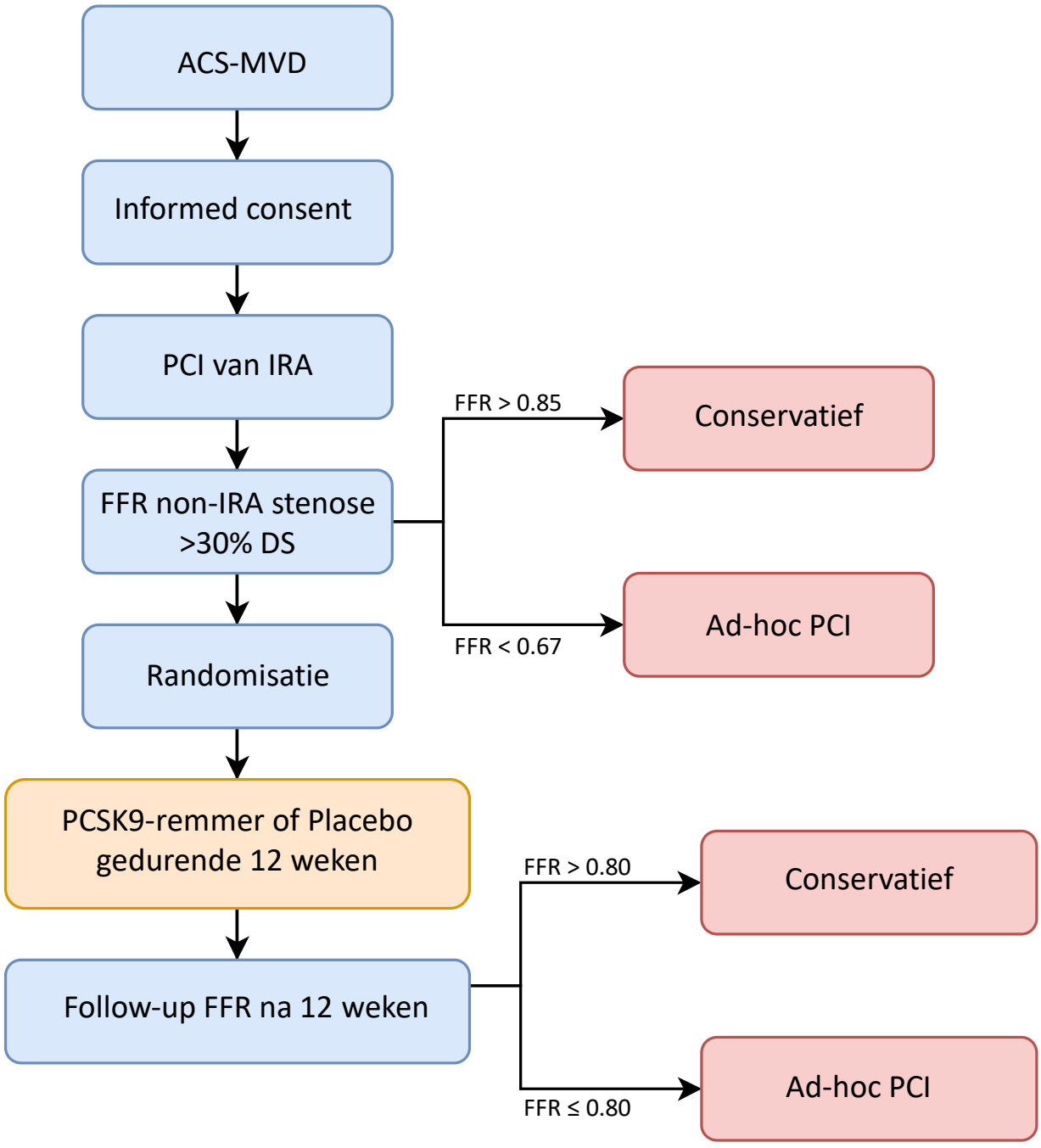


Figure 1. Flow-chart of the FITTER trial; ACS-MVD= acute coronary syndrome with multivessel disease, IRA= infarct related artery, non-IRA = non-infarct related artery, DS = diameter stenosis. FFR = fractional flow reserve., PCI = percutaneous coronary intervention.

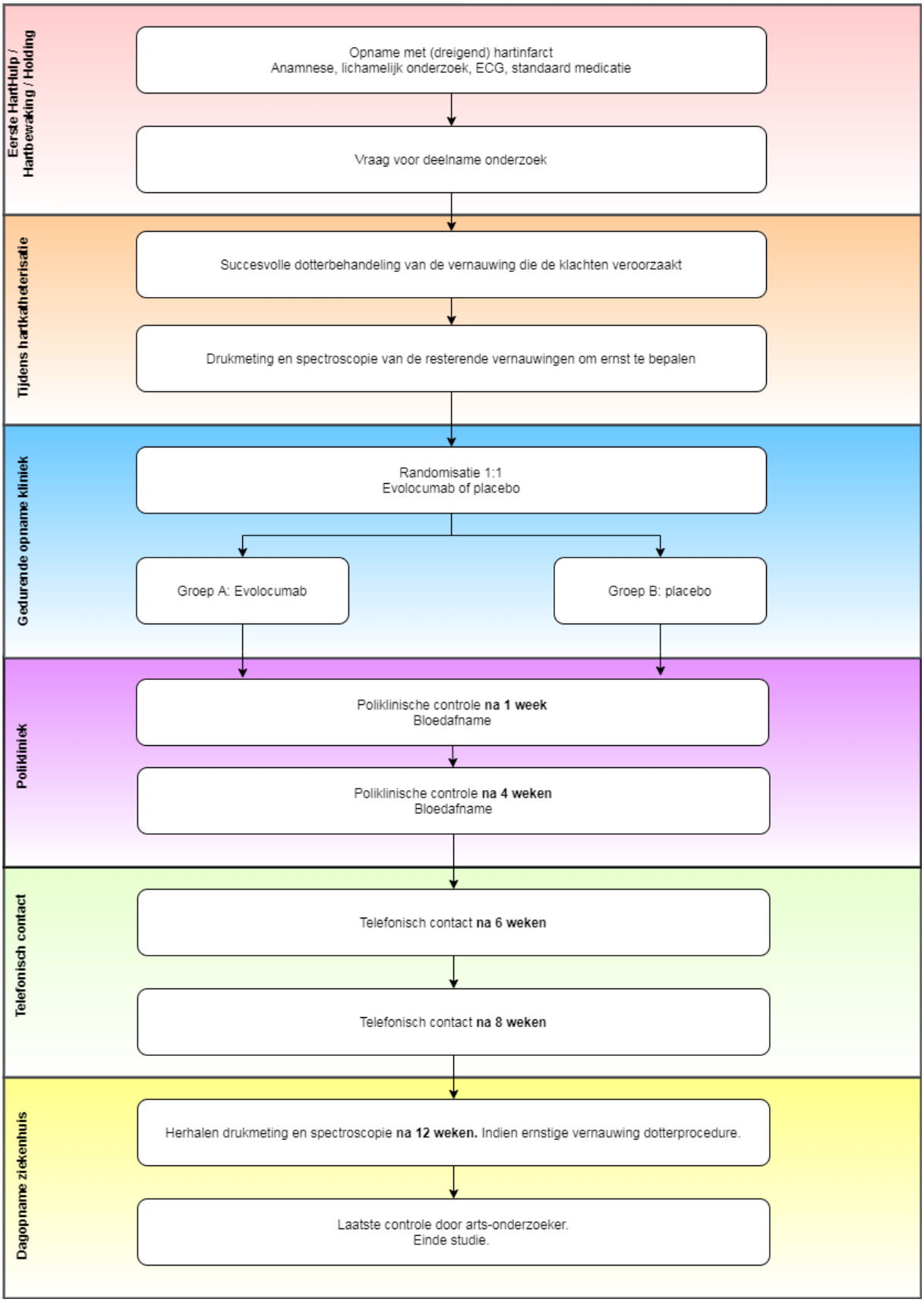


Figure 2. Flowchart of basic study protocol (in Dutch).

12.2 Appendix 2: Statin intensity table

Table. Classification of Statin Therapies

Statin	High-Intensity	Moderate-Intensity	Low-Intensity
	Lowers LDL >50%	Lowers LDL 30% to 49%	Lowers LDL <30%
Atorvastatin	40 mg – 80 mg	10 mg – 20 mg	
Rosuvastatin	20 mg – 40 mg	5 mg – 10 mg	
Lovastatin		40 mg	20 mg
Simvastatin		20 mg – 40 mg	10 mg
Pravastatin		40 mg – 80 mg	10 mg – 20 mg
Fluvastatin (XL)		80 mg	
Fluvastatin		40 mg (twice daily)	20 mg – 40 mg
Pitavastatin		2 mg – 4 mg	1 mg

LDL=low-density lipoprotein.
Source: *Circulation*. 2013;129(25 suppl 2):S1-S45.

12.3 Appendix 3: Event table

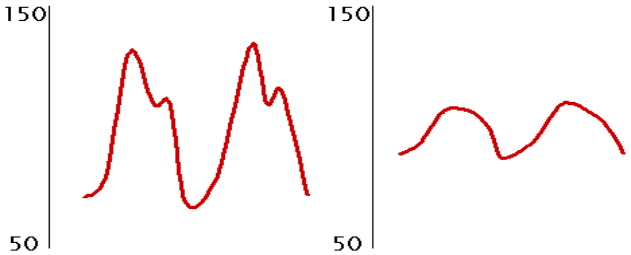
	First presentation	Procedure	Post-PCI/ Discharge	Double-blind Treatment Period and Follow-up						
Visit number	Day 0	Day 0	Day 1	Day 7 (±1d) week 1	Day 14 (±1d) week 2	Day 28 (±1d) week 4	Day 42 (±1d) week 6	Day 56 (±1d) week 8	Day 70 (±1d) week 10	Day 84 – 91 Week 12
Mode of visit	In hospital	In hospital	In hospital	Visit	Home	Phone call / Visit	Phone call	Phone call	Home	Clinical visit
Patient information and informed consent	X	X								
Review of inclusion/exclusion criteria	X	X								
Demographic characteristics	X									
Medical History	X									
Physical examination	X			X		X[2]				X
Randomization			X							
CBC, blood chemistry, CK, CK-MB, hs-Troponin	X[1,3]		X[1]							
Lipids (3ml)	X[1]			X		X[2]				X
Monocyte phenotyping (50ml)		X[2]				X[2]				X[2]
hsCRP (3,5ml)		X		X		X[2]				
IL-1β, IL-6 (3,5ml)		X[2]		X[2]		X[2]				
Serum pregnancy test (3,5ml)		X								
12-lead ECG	X		X							X
Injection training and dispensing diary			X							
Medication regimen			X		X	X	X	X	X	
Adverse event monitoring	X	X	X	X		X	X	X		X
Angiography + FFR / IMR		X								X
NIRS		X[4]								X[4]

[1]Standard of care
[2]Radboudumc only
[3]CK, CK-MB, and Troponin are drawn prior to PCI
[4]Selected centers only

12.4 Appendix 4: SOP: FFR/IMR measurement

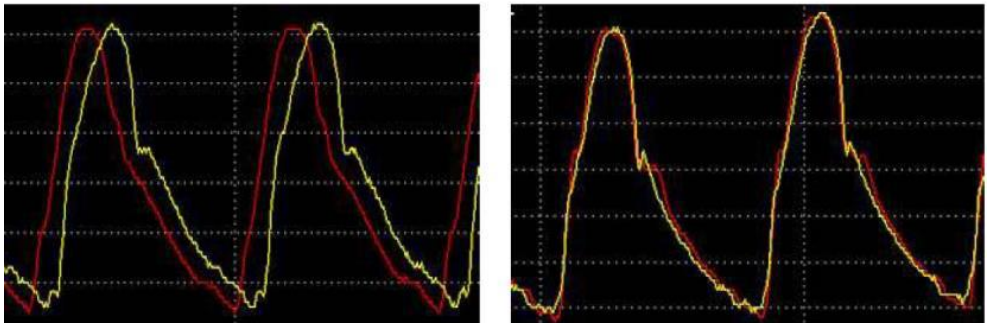
Fractional flow reserve (FFR) should be performed according to the following standard operating protocol.

1. Ensure that the height of the aortic pressure transducer is on the midline of the patient and further make sure to keep the aortic pressure transducer on the midline throughout the entire FFR measurement.
2. Ensure that 200 – 300 mcg IC nitroglycerine is administered before proceeding.
3. Flush catheter with saline.
4. Ensure that the guide catheter is coaxial with vessel and the aortic pressure waveform is not damped. If aortic pressure trace appears damped, ideally disengage the guide catheter to ensure an optimum aortic pressure trace.



Normal aortic waveform with a clearly visible dicrotic notch(left) and damped aortic waveform without a dicrotic notch (right)

5. Wait 15 seconds for the saline (or contrast) to lose hyperemic effect and normalize the distal pressure waveform to the aortic waveform.
6. Ensure that Pd/Pa is 1.00. If the Pd/Pa ratio is not 1.00, normalize again. Also ensure that the aortic and distal pressure waveform have complete temporal alignment.



Waveforms with incorrect (left) and correct (right) temporal alignment

7. Position the wire and pressure sensor at least 3 vessel diameters distal to the lesion to be evaluated. Make sure the wire position is documented
8. Before administering a hyperemic agent, record the Pd/Pa ratio for at least 15 heartbeats. Before starting the recording, please wait for any contrast, saline or other compounds administered intracoronary to lose their temporary hyperemic effect (this typically takes around 15 seconds).
9. Administer hyperemic agent. IC bolus injection or IV adenosine administration are both accepted in this study and the choice is left to the discretion of the operator.
    - For IC adenosine administration, 150 mcg is advised. After administering the adenosine, flush the catheter with ~5 milliliter of saline and start the recording. Record FFR and ensure that a 2 beat Pd/Pa average is used for calculation of FFR.

- For IV adenosine administration, 140 mcg/kg/min adenosine administered through a central or peripheral vein is advised. Wait for stable hyperemia to occur, either for up to 3 minutes in duration or as determined by the physician. Record FFR and ensure that a 3 beat Pd/Pa average is used for calculation of FFR.

10. Record the hyperemic Pd/Pa (FFR) ratio for at least 15 heartbeats. Make sure the recording is uninterrupted for the entire duration, no injection of contrast, or saline, or disruption to the aortic pressure transducer should be made during this recording phase. The operator is free to repeat the FFR measurement until a satisfactory FFR measurement has been obtained.
11. Once FFR measurements are complete, pull the guide wire back to the guide catheter and assess for pressure sensor drift of the Pd/Pa recording. Record and store this for at least 5 heartbeats. If the average Pd/Pa at the guiding catheter is between 0.98 and 1.02 (1.00 ± 0.02), the procedure is finished. If this is not the case, repeat the procedure from step 6 onwards.

Index of Microcirculatory Resistance should be calculated according to the following standard operating protocol [22]:

1. Place the pressure wire in the mid-distal segment of the coronary artery.
2. It is recommended to use a three-way valve system for saline injection.
3. Flush the guide catheter of all contrast and air bubbles, and ensure that it is engaged in the coronary ostium.
4. Ensure the aortic pressure (Pa, RED) is recorded.
5. 3 ml bolus injections of room temperature saline (x 3) (a temperature decline of at least 2 °C should typically be obtained; repeat the injections for an outlying transit time to ensure all three curves are similar).
6. Switch on IV adenosine (140 ug/kg/min) and wait for two minutes (confirm clinical response to adenosine).
7. Flush the guide catheter of saline that may have warmed in the guide catheter in the patient.
8. 3ml bolus injections of room temperature of saline (x 3) during hyperemia.
9. The apparent IMR is calculated by multiplying the distal coronary pressure by the mean transit time of a 3 ml bolus of saline at room temperature during coronary hyperemia induced by intravenous adenosine.
10. 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)].
11. 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.

12.5 Appendix 5: Protocol immunological study

Background
In contrast to the conventional immunological dogma, our group and others have recently described that innate immune cells, including monocytes and macrophages, can build a long-term immunological memory after brief exposure to micro-organisms or microbial products. This innate immune memory has been termed trained immunity and is mediated by profound metabolic and epigenetic reprogramming [23]. In the context of recurrent infections, this mechanism can provide powerful protection against reinfection [24]. We have recently established that trained immunity can also be induced by endogenous atherogenic substances, including oxidized low-density lipoprotein and lipoprotein (a) particles [20, 25]. Therefore, we have hypothesized that trained immunity contributes to the pathophysiology of atherosclerosis [26].

Indeed, in patients with dyslipidemia or in patients with established coronary atherosclerosis, circulating monocytes have a trained immune phenotype, characterized by an augmented cytokine production capacity, metabolic reprogramming towards increased glycolysis, and enrichment of activating histone modifications [27, 28].

Recent murine studies have revealed that trained immunity also occurs at the level of myeloid progenitors in the bone marrow, and that this mechanisms explains the long-lasting persistence of trained monocytes in the circulation [18, 29, 30]. In atherosclerosis-prone *LDLR*-/- mice, a Western type diet induced pro-inflammatory reprogramming of hematopoietic stem and progenitors cells, which persists for weeks also after switching back to chow diet [18]. Accumulation of intracellular cholesterol and mevalonate are key components of this reprogramming [31].

Importantly, also a myocardial infarction itself can induce a long-term activation of the innate immune system: 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 [16]. Therefore, we now propose that in the first weeks after an ACS occurred, there is an optimal time window for preventing atherosclerosis progression by powerful lowering of plasma cholesterol. In patients with familial hypercholesterolemia (FH), PCSK9 treatment reduced monocyte CCR2 expression and ex-vivo migratory capacity [19].

Hypothesis
We therefore hypothesize that rapid initiation of powerful cholesterol lowering with PCSK9 inhibitors immediately after a myocardial infarction, prevents the long-term pro-inflammatory reprogramming of circulating myeloid cells.

Objective
To test this hypothesis, in a subgroup of patients included in the Radboudumc, the inflammatory phenotype of peripheral blood mononuclear cells (PBMCs) and Percoll-isolated monocytes will be explored using flow cytometry and cytokine production capacity and foam cell formation [20]. In addition, we will explore the underlying metabolic, epigenetic, and transcriptomic mechanisms. The immunological side study will investigate the relation between LDL-C reduction and reduction of pro-inflammatory monocyte phenotypes.

Methods:
Blood will be drawn immediately after revascularization for baseline measurement of monocyte phenotype. At 4 and 12 weeks measurements will be repeated (in the morning after overnight fast) with additional MACS isolation of monocytes for RNA and ChiP-sequencing.

Detailed description of methods:

Population:
Number of patients is 40, 20 treated with Evolocumab and 20 treated with placebo.

Measurements:
- Extensive flow cytometric characterization of circulating myeloid cells (FACS as described previously [32].
- Sysmex (monocyte number)
- Cytokine and chemokine production capacity of PBMC's after 24 h incubation with LPS/Pam3Cys and cholesterol crystals (IL-1b).
- Seahorse measurements will be done to measure the glycolysis and oxidative phosphorylation.
- CD14+ MACS isolation of monocytes will be performed for transcriptomic and epigenetic analysis (with chromatin immunoprecipitation).
- To assess the effect of rapid lipid lowering on circulating inflammatory markers, we will perform proteomics analysis (platform Inflammation and Cardiovascular 2 of OLINK).

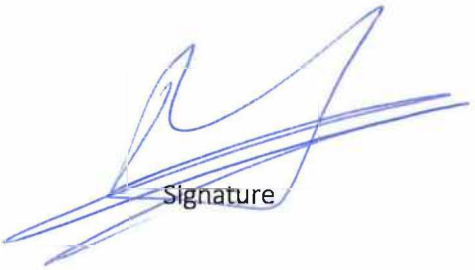
Statistical analysis plan

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.

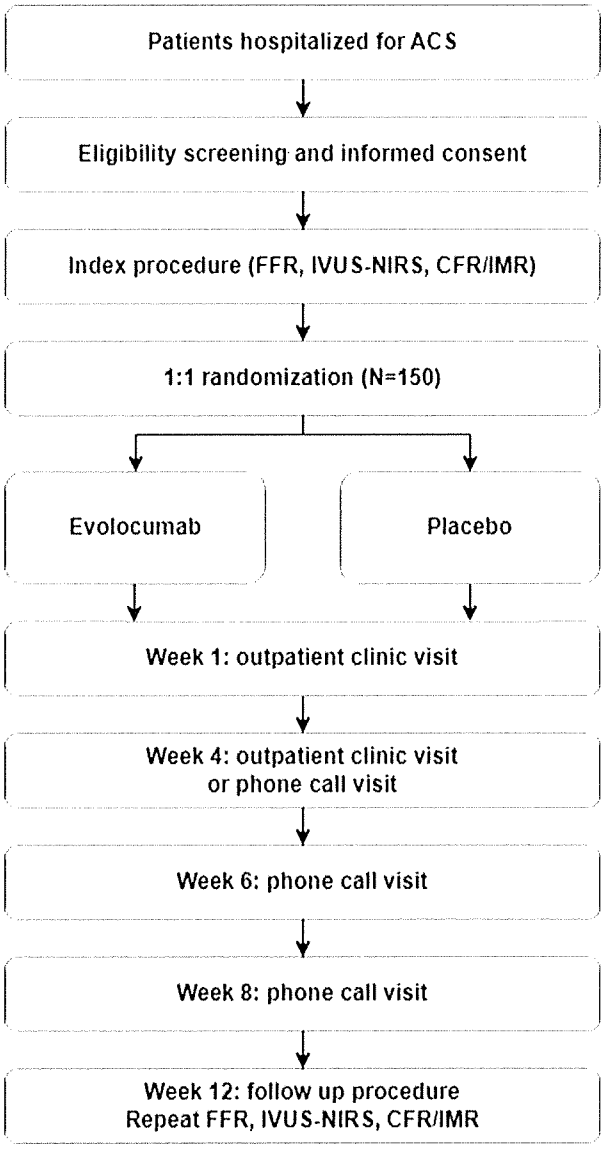
After all queries have been resolved and after the data has been locked, the trial statistician will produce all tables with the unblinded information, correctly assigning the patients to treatment evolocumab or placebo.

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.

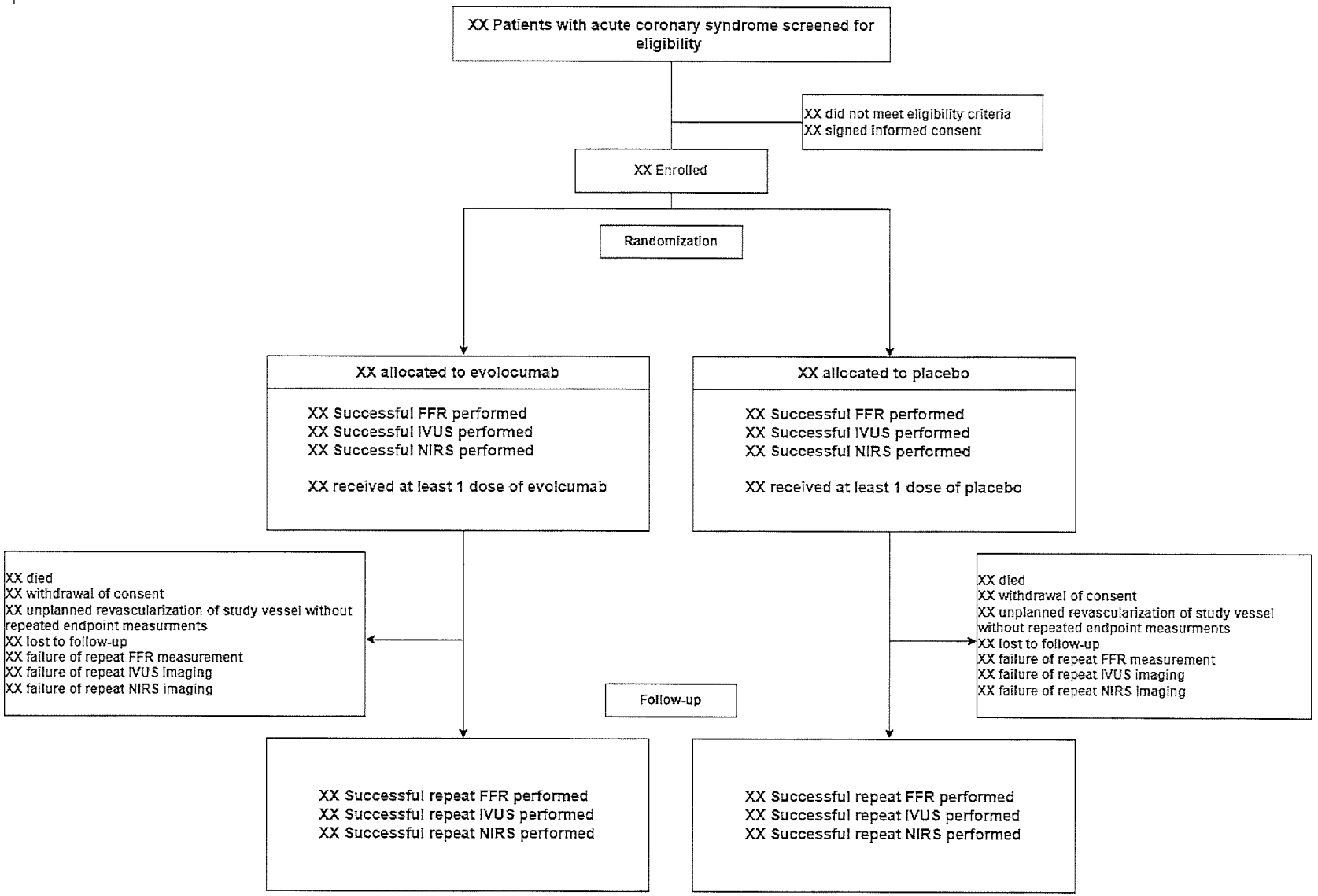
Deaths are reported in the Adverse Events with date of death, confirmed in follow-up visit as patient died item, and end of study.

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

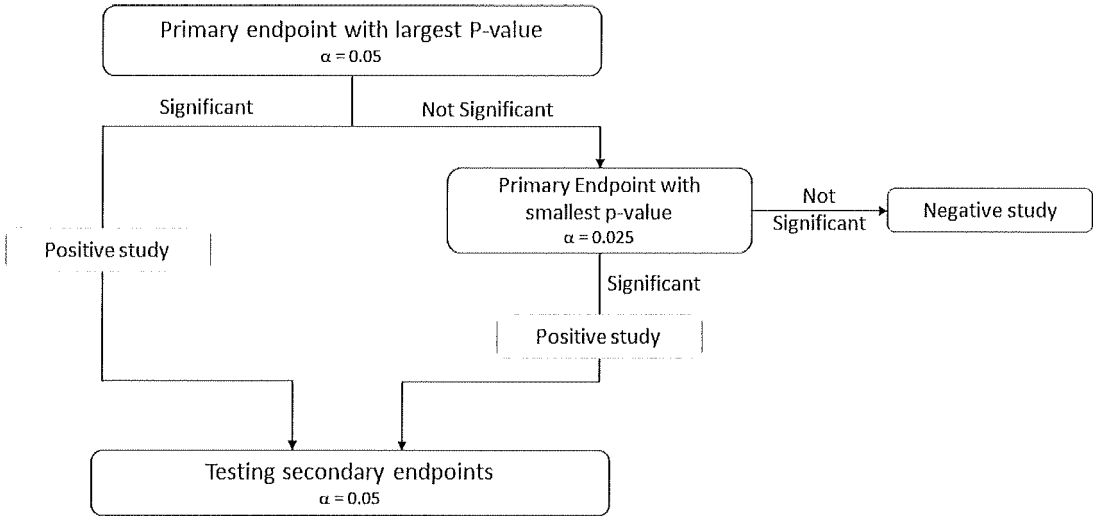
For our lesion-based analysis, multiple separate lesions within the same vessel or patient can be included. A random effect for lesion in the ANCOVA will be used to account for the correlation among the lesions within patient.

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