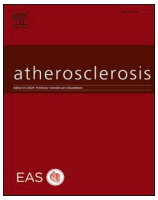
Atherosclerosis 392 (2024) 117504

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis



Effects of alirocumab on endothelial function and coronary atherosclerosis in myocardial infarction: A PACMAN-AMI randomized clinical trial substudy

Emrush Rexhaj [a,1], Sarah Bär [a,1], Rodrigo Soria [a], Yasushi Ueki [a], Jonas D. Häner [a], Tatsuhiko Otsuka [a], Raminta Kavaliauskaite [a], George CM. Siontis [a], Stefan Stortecky [a], Hiroki Shibutani [a], David Spirk [b,c], Thomas Engstrøm [d], Irene Lang [e], Laura Morf [a], Maria Ambühl [a], Stephan Windecker [a], Sylvain Losdat [f], Konstantinos C. Koskinas [a], Lorenz Räber [a,*], on behalf of the PACMAN-AMI Investigators

[a] Department of Cardiology, Bern University Hospital Inselspital, Freiburgstrasse 18, 3010, Bern, Switzerland
[b] Institute of Pharmacology, Bern University Hospital and University of Bern, Freiburgstrasse 18, 3010, Bern, Switzerland
[c] Sanofi, Suurstofi 2, 6343, Risch-Rotkreuz, Switzerland
[d] Department of Cardiology, Rigshospitalet, Copenhagen University Hospital, Blegdamsvej 9, 20100, Copenhagen, Denmark
[e] Department of Cardiology, Medical University of Vienna, Währinger Gürtel 18-20, 1090, Vienna, Austria
[f] CTU Bern, University of Bern, Mittelstrasse 43, 3012, Bern, Switzerland

ARTICLE INFO

Keywords:
Endothelial function
Flow-mediated dilation
PCSK9 inhibitor
Alirocumab
Coronary atherosclerosis
Intracoronary imaging

ABSTRACT

Background and aims: The effects of protein convertase subtilisin/kexin type 9 (PCSK9) inhibitors on endothelial function as assessed by flow-mediated dilation (FMD) in patients with acute myocardial infarction (AMI) are unknown. Therefore, we aimed to investigate the effects of the PCSK9 inhibitor alirocumab added to high-intensity statin on FMD, and its association with coronary atherosclerosis in non-infarct related arteries using intracoronary intravascular ultrasound (IVUS), near-infrared spectroscopy (NIRS), and optical coherence tomography (OCT).

Methods: This was a pre-specified substudy among patients recruited at Bern University Hospital, Switzerland, for the randomized-controlled, double-blind, PACMAN-AMI trial, which compared the effects of biweekly alirocumab 150 mg *vs.* placebo added to rosuvastatin. Brachial artery FMD was measured at 4 and 52 weeks, and intracoronary imaging at baseline and 52 weeks.

Results: 139/173 patients completed the substudy. There was no difference in FMD at 52 weeks in the alirocumab (n = 68, 5.44 ± 2.24%) *versus* placebo (n = 71, 5.45 ± 2.19%) group (difference = −0.21%, 95% CI −0.77 to 0.35, $p = 0.47$). FMD improved throughout 52 weeks in both groups similarly ($p < 0.001$). There was a significant association between 4 weeks FMD and baseline plaque burden (IVUS) (n = 139, slope = −1.00, $p = 0.006$), but not with lipid pool (NIRS) (n = 139, slope = −7.36, $p = 0.32$), or fibrous cap thickness (OCT) (n = 81, slope = −1.57, $p = 0.62$).

Conclusions: Among patients with AMI, the addition of alirocumab did not result in further improvement of FMD as compared to 52 weeks secondary preventative medical therapy including high-intensity statin therapy. FMD was significantly associated with coronary plaque burden at baseline, but not with lipid pool or fibrous cap thickness.

* Corresponding author.
E-mail address: lorenz.raeber@insel.ch (L. Räber).
[1] The first two authors contributed equally to the study.

https://doi.org/10.1016/j.atherosclerosis.2024.117504
Received 1 December 2023; Received in revised form 22 February 2024; Accepted 27 February 2024
Available online 6 March 2024

1. Introduction

The vascular endothelium plays a key role in the pathogenesis of atherosclerosis and its impairment precedes the development of structural atherosclerotic alterations and later manifest cardiovascular disease [1,2]. Flow-mediated dilation (FMD) allows to evaluate the systemic vascular endothelial-dependent function [2–4] and is considered the "gold standard" for non-invasive evaluation of endothelial function [3,4]. The prognostic value of FMD for cardiovascular outcomes has been shown in several meta-analyses with a pooled relative cardiovascular risk reduction by 8–13% per 1% increase in FMD [5–9].

Statins, besides low-density lipoprotein cholesterol (LDL-C) lowering, have pleiotropic effects [10], which include anti-inflammatory properties and positive effects on endothelial function. As such, FMD has been shown to improve under statin therapy in several studies on subjects at increased risk for [11,12], and manifest atherosclerotic disease [13,14], related to higher bioavailability of nitric-oxide [15].

PCSK9 inhibitors lower LDL-C by upregulation of hepatic LDL-C receptors and do improve cardiovascular outcomes [16,17]. The body of evidence on potential pleiotropic effects of PCSK9, especially involvement in inflammatory and oxidative processes, is increasing [18,19]. Some small studies observed an improvement of FMD after short-term treatment with PCSK9 inhibitors in patients with chronic coronary syndromes (CCS) [20–22], diabetes mellitus [23], and familial hypercholesterolemia [24]. However, the effect of PCSK9 inhibition on FMD among acute myocardial infarction (AMI) patients has never been investigated. Further, the association between brachial artery FMD and the extent of coronary atherosclerosis according to intracoronary imaging and regression/progression of atherosclerosis is unknown. Therefore, as a pre-specified substudy of the randomized-controlled PACMAN-AMI (PCSK9 antibody AliroCuMab on coronary Atherosclerosis in patieNts with Acute Myocardial Infarction) trial [25], we aimed to assess the effect of the PCSK9 inhibitor alirocumab added to high-intensity statin therapy with rosuvastatin on FMD, as well as the association between FMD and the extent and stability of coronary atherosclerosis in multimodality intracoronary imaging and regression/progression of atherosclerosis in AMI patients throughout 1 year.

2. Patients and methods

2.1. Study design PACMAN-AMI main trial

The design and primary results of the PACMAN-AMI trial have been published previously [25,26]. Briefly, PACMAN-AMI was an investigator-initiated, randomized, double-blind, placebo-controlled, European multi-center study to evaluate the effect of intensive lipid lowering therapy with alirocumab added to high-intensity statin therapy with rosuvastatin on coronary atherosclerosis by multi-modality intracoronary IVUS, NIRS, and OCT in patients with AMI undergoing percutaneous coronary intervention (PCI). Detailed inclusion and exclusion criteria have been reported elsewhere [25]. In brief, patients were eligible if 1) they had two non-infarct related arteries (non-IRA) suitable for intracoronary imaging with non-obstructive atherosclerotic disease (visual estimate >20 to <50% angiographic diameter stenosis), 2) LDL-C level ≥125 mg/dL if patients were stain-naïve or had not been on a stable (≥4 weeks) statin regimen at the time of screening; or LCL-C ≥70 mg/dL if patients were on an unchanged statin treatment for ≥4 weeks prior to study enrolment. The study was conducted at 9 academic centres in: Switzerland (5), Austria (1), Denmark (1) and the Netherlands (2). A total of 300 patients were randomized in a 1:1 ratio to either alirocumab subcutaneous 150 mg biweekly or matching placebo between May 09, 2017 and October 07, 2020. Web-based randomization was performed using randomly varying block sizes of 2, 4, or 6 patients, stratified by study site, use of stable (≥4 weeks) statin treatment at presentation, and type of AMI (ST-elevation myocardial infarction (STEMI) *vs.* non-ST-elevation myocardial infarction). Analysis was per intention-to-treat. All patients underwent serial coronary angiography and intracoronary imaging at baseline and 52 weeks follow-up. Written informed consent was obtained from each patient, the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by the local ethics committee on research on humans.

2.2. PACMAN-AMI pre-specified FMD substudy

This was a pre-specified substudy of the PACMAN-AMI trial [26] among patients enrolled at Bern University Hospital (n = 173). FMD was measured at 4 and 52 weeks after AMI. The 4 weeks timepoint for FMD baseline measurement was chosen, since in the setting of AMI (especially STEMI), postponing angiography and PCI for FMD measurement would not have been feasible, and an FMD measurement directly after PCI could have been affected by the acute (inflammatory) effects of MI [27,28], reperfusion, PCI [29], and initiated medication (e.g. antihypertensive therapy, betablockers) [3,4] and would not have represented a valid baseline measurement. At 4 weeks, the acute phase of myocardial infarction was passed and co-medication administered in steady-state doses, therefore representing an appropriate setting for baseline assessment of FMD. Follow-up FMD measurement was performed at 52 weeks before repeating coronary angiography and intracoronary imaging.

2.3. FMD measurement

FMD is defined as the 1-min hyperemia-induced brachial artery percentage change of maximum diameter from baseline diameter (i.e. $\text{FMD}\ (\%) = \frac{(\textit{Maximum Diameter} - \textit{Baseline Diameter})}{\textit{Baseline Diameter}} * 100$) (Supplemental Fig. 1) [3,4]. FMD is the most common non-invasive method used to assess systemic endothelial-dependent vascular function. Although the technique is not easy to be performed, it is well standardized, and since many years an established procedure at our institution [30,31]. FMD is affected by the classical cardiovascular risk factors [32], correlates with coronary artery endothelial function [33–35], and is an independent predictor of cardiovascular outcomes [36], also in patients with already established coronary heart disease [37]. Moreover, its easy use in different clinical trials that assessed the effect of treatment or life style modification on endothelial function [38] makes it the gold method adapted to the present study.

FMD was assessed in all patients by a highly experienced analyst (ER) blinded to treatment allocation and according to current recommendations [3,4]. The increase of the brachial artery diameter evoked by reactive hyperemia was determined using high-resolution ultrasound (Esaote MyLab30 Gold, Esaote SpA, Italy) and automatic wall tracking software (Cardiovascular Suite, Quipu, Pisa, Italy). The brachial artery was identified above the antecubital fossa with a high-resolution ultrasound device and a high frequency (7–10 MHz) linear array probe. The ultrasound probe was fixed in a stereotactic clamp with micrometer movement capabilities and Doppler flow was recorded continuously

throughout the assessment. After 1 min of baseline measurements, a pressure cuff placed around the forearm was inflated to 250 mmHg for 5 min. After deflation of the cuff, the hyperemia-induced changes of brachial artery diameter and flow were continuously measured. Endothelium-independent dilation of the brachial artery was assessed by measuring the increase of the brachial artery diameter evoked by 50 μg oral glyceryl-trinitrate.

2.4. Endpoints

The pre-specified primary endpoint was the absolute difference in FMD between the placebo and alirocumab group at 52 weeks. Secondary endpoints were the absolute difference in FMD between groups at 4 weeks and the difference of absolute change in FMD from week 4 to week 52 between groups. Pre-specified intracoronary imaging endpoints were the association between FMD at 4 weeks and IVUS-derived percent atheroma volume (PAV) (%), NIRS-derived maximum lipid core burden index 4 mm (maxLCBI4mm), and OCT-derived minimum fibrous cap thickness (minFCT) (μm) at baseline and the association between FMD at 52 weeks and change in PAV, maxLCBI4mm, and minFCT. As exploratory endpoints, we assessed the association between FMD at 4 weeks and baseline LDL-C as well as during the study period.

2.5. Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) or median [interquartile range (IQR)] and categorical variables are presented as counts with percentages. We hypothesized a clinically relevant absolute difference in FMD between the placebo and alirocumab group at 52 weeks of 1.5% and a standard deviation of 1.5. For a power of 90% at a two-sided alpha of 0.05, the required sample size was 62 (i.e. 31 patients per arm). Considering a drop-out rate of 25%, we aimed to enroll at least 78 patients. Patient characteristics, medication, and laboratory values were compared using Student t-tests, Fisher's exact tests or Wilcoxon-Mann-Withney tests, as appropriate. Changes in FMD over time were compared between groups using repeated linear models. The association FMD and between intracoronary imaging findings (i.e. PAV, maxLCBI4mm, minFCT) as well as LDL-C were assessed using linear regressions including FMD, trial arm and the interaction between FMD and trial arm as explanatory variables. All analyses were conducted in R version 4.2.0. Significance tests were two-tailed with a significance level set to 0.05.

3. Results

3.1. Patient population

Of 173 enrolled patients at Bern University Hospital, 139 (80%) (n = 68 alirocumab, n = 71 placebo) underwent serial FMD measurement. Reasons for exclusion of patients were enrollment before ethical approval of the FMD substudy, withdrawn consent for FMD measurement, logistical problems with FMD measurement, or study restrictions due to the COVID-19 pandemic (Fig. 1). Mean age was 58.1 years, 16% were female, 10% had diabetes mellitus, 42% were active smokers, and baseline LDL-C level was 155.9 mg/dl (Table 1). Characteristics of patients included in *vs.* excluded from the PACMAN-AMI FMD substudy at Bern University Hospital were similar (Supplemental Table 1). Medication at FMD baseline (i.e. 4 weeks) is shown in Table 1, and medication at trial enrollment (timepoint 0) and 52 weeks is shown in Supplemental Table 2. LDL-C at FMD baseline (i.e. 4 weeks) was 24.5 ± 15.9 in the alirocumab and 77.7 ± 26.1 mg/dl in the placebo group ($p < 0.001$), and at 52 weeks it was 25.1 ± 27.5 mg/dl and 76.3 ± 27.3 mg/dl, respectively ($p < 0.001$). Complete lipid profiles at trial enrollment (timepoint 0), 4 weeks, and 52 weeks are provided in Supplemental Table 3. Blood pressure and heart rate were similar between groups at the time point of FMD measurement at 4 and 52 weeks (Table 2).

3.2. Primary and secondary FMD endpoints

At 52 weeks, FMD was 5.44 ± 2.24% in the alirocumab and 5.45 ± 2.19% in the placebo group (between-group difference of −0.21%, 95% CI −0.77 to 0.35, $p = 0.47$) (Fig. 3, Table 2). FMD at 4 weeks was 4.52 ± 1.87% in the alirocumab group and 4.32 ± 1.62% in the placebo group (between-group difference = 0.20%, 95% CI -0.47 to 0.87, $p = 0.56$). Compared to 4 weeks, 52 weeks FMD was significantly improved in both groups (alirocumab: from 4.52 ± 1.87% to 5.44 ± 2.24%, $p < 0.001$; placebo: from 4.32 ± 1.62% to 5.45 ± 2.19%, $p < 0.001$) (Supplemental Fig. 2, Table 2). Endothelium-independent vasodilation was similar in both groups (alirocumab: 11.66 ± 3.77% *vs.* placebo: 12.21 ± 3.18%, $p = 0.36$). Baseline brachial artery diameter and ischemia-induced increase in blood flow were similar in both groups (Table 2).

3.3. FMD vs. intracoronary imaging endpoints at baseline

PAV as assessed by IVUS at baseline (timepoint 0) showed a significant association with FMD at 4 weeks (slope = −1.00, 95% CI -1.70 to −0.30), $p = 0.006$), There was trend towards a significant association between changes in PAV and FMD throughout 52 weeks (slope = −0.19, 95% CI -0.40 to 0.02, $p = 0.08$) (Fig. 2A–B, Fig. 3, Supplemental Table 4). Also, if assessed categorically, using 3 concepts of comparison groups, i.e. 1) PAV regressors *vs.* non-regressors, 2) PAV change above *vs.* below the median of the population, or 3) triple regressors *vs.* non-triple regressors (i.e. concomitant regression in PAV and maxLCBI4mm and increase in minFCT [39]), there were no significant differences in the change in FMD (Supplemental Table 5). For maxLCBI4mm in NIRS (Fig. 2C–D) and minFCT in OCT (Fig. 2E–F) with FMD, no significant associations between baseline values or changes over time were observed (Supplemental Table 4).

3.4. FMD vs. LDL-C

There were no signifcant associations between baseline LDL-C and FMD at 4 weeks (slope = −0.65, 95% CI-3.83 to 2.53, $p = 0.69$), or changes in LDL-C and FMD throughout 52 weeks (slope = 1.23, 95% CI -2.55 to 5.01, $p = 0.52$) (Supplemental Fig. 3, Supplemental Table 4).

4. Discussion

PACMAN-AMI FMD represents the largest pre-specified study to assess the effects of intensive lipid-lowering therapy with the PCSK9 inhibitor alirocumab added to high-intensity statin therapy with rosuvastatin on FMD. To our knowledge, it is the first trial conducted in an AMI population and the first study to investigate the association between FMD and the extent and stability of coronary atherosclerosis as assessed with multi-modality intracoronary imaging in two non-infarct related coronary arteries. The salient findings of our study can be summarized as follows: 1) In an AMI population, 52 weeks of secondary preventative medical therapy led to a significant improvement in endothelial function as assessed by FMD. However, the addition of a PCSK9 inhibitor to high-intensity statin therapy did not result in further beneficial effects on FMD. 2) There was a significant association between coronary plaque burden as assessed by intracoronary IVUS and

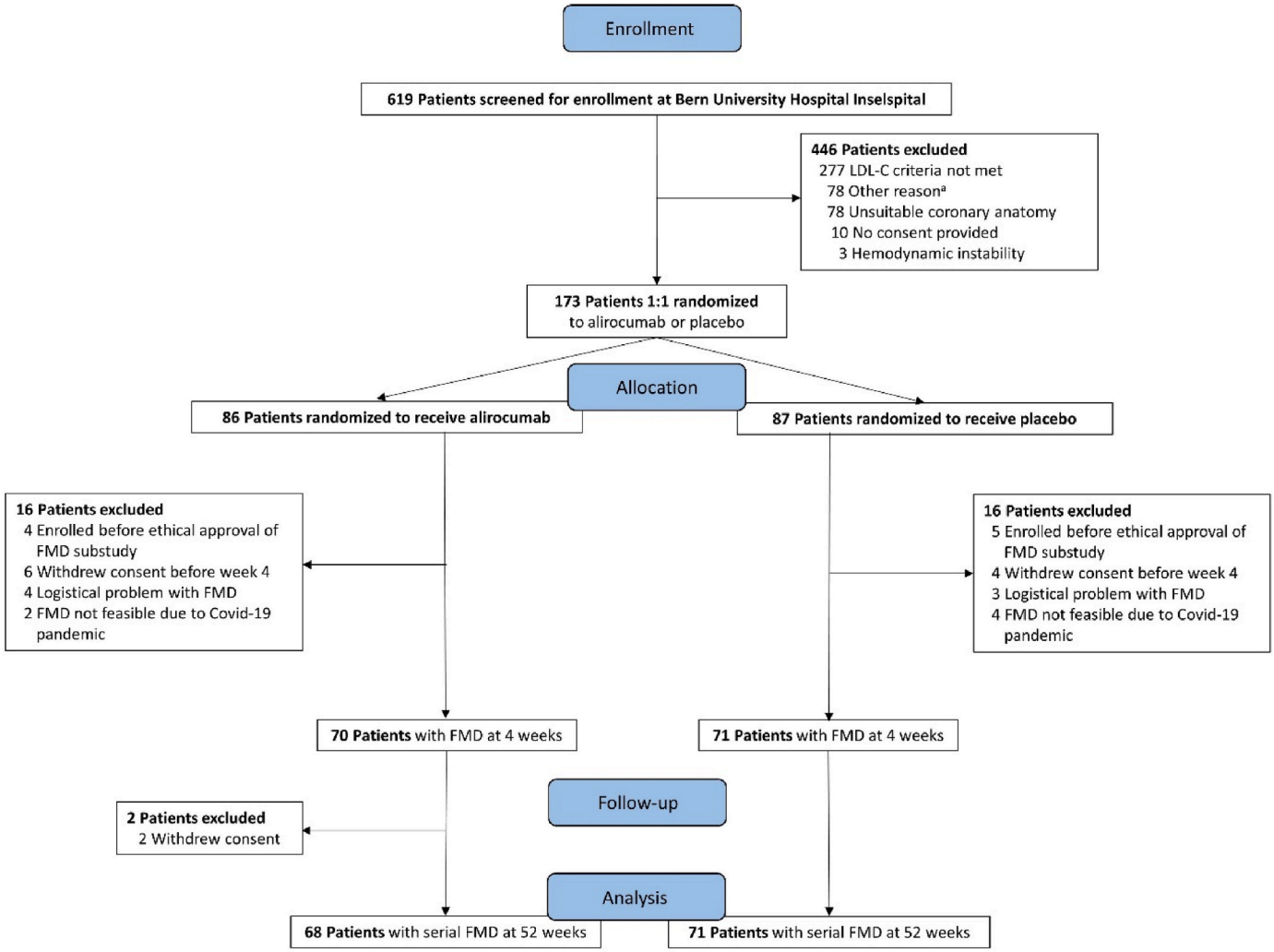


Fig. 1. CONSORT flow diagram.
FMD = flow-mediated dilation. [a]Specific reasons for other exclusions were not documented. See eTable in Supplement 4 in the main publication [26] for additional inclusion and exclusion criteria.

brachial artery FMD at baseline, and a trend towards an association between changes in plaque burden and FMD throughout 52 weeks (Fig. 3). 3) No significant associations between FMD and coronary lipid pool as assessed by NIRS or fibrous cap thickness as assessed by OCT at baseline and the respective changes over 52 weeks were observed.

4.1. Potential pleiotropic effects of PCSK9

Inflammation is a major driver of atherosclerotic plaque formation, progression, vulnerability and its associated cardiovascular events [40]. Reducing inflammation by the interleukin 1 inhibitor canakinumab was shown to successfully reduce cardiovascular events post myocardial infarction, however at the cost of increased incidence of fatal infection or sepsis [41]. Therefore, agents mitigating inflammatory processes at the arterial wall are an interesting and promising treatment target in cardiovascular diseases. Chronic vascular inflammation induces endothelial dysfunction [42], and, since evidence from in-vitro, murine and limited human studies on potential LDL-C-independent, pleiotropic effects of PCSK9 in inflammatory and oxidative processes is increasing [18,19], we investigated the effects of PCSK9 inhibitors on endothelial function as assessed by FMD. We did not observe any improvement of FMD in the alirocumab as compared to the placebo treatment arm. However, opposite to statins, the clinical role of PCSK9 inhibitors in the pathogenesis of atherosclerosis, beyond its key role lipid-metabolism, still remains to be determined. For example, PCSK9 inhibitors have no effect on the inflammatory marker high-sensitivity C-reactive protein (hs-CRP) [43]. Consistent with this previous observation, hs-CRP values were similar in both groups at 52 weeks in the present study.

4.2. Prior evidence on the effects of PCSK9 inhibition on FMD

In line with previous observational evidence on patients with CCS [21,22] and familial hypercholesterolemia [24], we observed an improvement in FMD throughout 52 weeks of secondary prevention medical therapy including high-intensity rosuvastatin in all patients. However, the beneficial effect was not related to PCSK9 inhibitor treatment, since rosuvastatin alone showed similar improvement of FMD in the control group. Consistent with our findings, other small observational studies showed that alirocumab treatment, added to maximal tolerated lipid-lowering therapy, did not improve FMD in patients with established atherosclerotic cardiovascular disease and elevated LDL-cholesterol [44,45]. This contrasts the findings of a small RCT conducted by Sposito et al. [23], where greater improvement in FMD after 4 months treatment with the PCSK9 inhibitor evolocumab added to background sodium-glucose transporter-2 therapy with empagliflozin (n = 55) as compared to empagliflozin alone (n = 55) among patients with diabetes mellitus type 2 was observed [23]. In another RCT by Rehberger et al. [20], patients at least 6 months after a

Table 1
Patient baseline characteristics.

Clinical characteristics	N	Alircoumab (N = 68)	N	Placebo (N = 71)	*p*-value
Age (years)	68	57.5 ± 10.1	71	58.7 ± 8.4	0.45
Sex (female)	68	8 (12%)	71	14 (20%)	0.25
Type of AMI, n (%)					
NSTEMI	68	36 (53%)	71	32 (45%)	0.40
STEMI		32 (47%)		39 (55%)	
Statin/LDL-C status at baseline, n (%)					
On statin for at least 4 weeks prior to PCI and LDL-C ≥70 mg/dl		9 (13%)		9 (13%)	
No statin and LDL-C ≥125 mg/dl	68	59 (87%)	71	62 (87%)	1.00
BMI (kg/m^2)	68	27.4 ± 4.2	71	27.9 ± 4.2	0.49
Systolic blood pressure (mmHg)	68	123.2 ± 21.8	71	126.5 ± 21.4	0.36
Diastolic blood pressure (mmHg)	68	73.9 ± 13.0	71	73.1 ± 14.2	0.71
Heart rate (/min)	68	81.9 ± 14.8	71	78.8 ± 12.6	0.19
Family history of CAD or CVD, n (%)	68	18 (27%)	71	23 (32%)	0.46
Peripheral arterial disease, n (%)	68	1 (2%)	71	0 (0.0%)	0.49
Diabetes mellitus, n (%)	6	6 (9%)	8	8 (11%)	0.78
Diabetes type, n (%)					
Type 1	6	1 (2%)	8	0 (0%)	0.43
Type 2		5 (7%)		8 (11%)	
Arterial hypertension, n (%)	68	29 (43%)	71	32 (45%)	0.86
Hypercholesterolemia, n (%)	68	62 (91%)	71	65 (92%)	1.00
Active smoker, n (%)	68	34 (50%)	71	25 (35%)	0.09
History of smoking, n (%)	68	44 (65%)	71	44 (62%)	0.86
Previous myocardial infarction, n (%)	68	0 (0.0%)	71	0 (0.0%)	–
Previous PCI, n (%)	68	0 (0.0%)	71	0 (0.0%)	–
Premature[a] CAD, cerebral or peripheral vascular disease, n (%)	68	20 (29%)	71	16 (23%)	0.44
History of stroke, n (%)	68	0 (0%)	71	1 (1%)	1.00
History of TIA, n (%)	68	1 (2%)	71	1 (1%)	1.00
Laboratory values at trial enrollment (Time point 0)					
Total cholesterol (mg/dl)	68	207.4 ± 30.1	71	208.0 ± 32.7	0.92
LDL-C (mg/dl)	68	156.2 ± 28.4	71	155.5 ± 36.0	0.89
HDL-C (mg/dl)	68	41.1 ± 9.9	71	42.4 ± 9.6	0.42
Lipoprotein (a) (mg/dl)	68	9.1 [38.3]	71	10.9 [45.4]	0.40
hs-CRP (mg/l)	68	2.1 [3.2]	71	2.7 [5.6]	0.78
Peak CK (U/L)	67	831.0 ± 1283.7	71	618.4 ± 855.8	0.25
Peak hs-Troponine ng/ml	68	1.94 ± 4.36	71	1.15 ± 2.25	0.18
Hematocrit (L/L)	68	0.4 ± 0.0	70	0.4 ± 0.1	0.18
Hemoglobin (g/dl)	68	14.5 ± 1.3	71	14.1 ± 1.3	0.13
White blood cell count (/μl)	68	10′300 ± 3′900	71	10′500 ± 3′200	0.72
HbA1c (%)	68	5.8 ± 1.0	71	5.7 ± 0.8	0.48
Creatinine (mg/dl)	68	0.86 ± 0.17	71	0.87 ± 0.17	0.58
Medication at 4 weeks	N	Alirocumab (N = 94)	N	Placebo (N = 99)	*p*-value
Statin, n (%)	68	68 (100%)	71	71 (100%)	–
Ezetimibe, n (%)	68	0 (0%)	71	0 (0%)	–
Aspirin, n (%)	68	67 (99%)	71	71 (100%)	0.49
Clopidogrel, n (%)	68	5 (7%)	71	1 (1%)	0.11
Ticagrelor, n (%)	68	55 (81%)	71	66 (93%)	0.044
Prasugrel, n (%)	68	8 (12%)	71	4 (6%)	0.24
Novel oral anticoagulant, n (%)	68	4 (6%)	71	1 (1%)	0.20
ACE inhibitor, n (%)	68	55 (81%)	71	53 (75%)	0.42
ATII antagonist, n (%)	68	8 (12%)	71	14 (20%)	0.25
Betablocker, n (%)	68	65 (96%)	71	63 (89%)	0.21
Calcium antagonist, n (%)	68	3 (4%)	71	7 (10%)	0.33
Oral antidiabetics, n (%)	68	3 (4%)	71	7 (10%)	0.33
Insulin, n (%)	68	2 (3%)	71	1 (1%)	0.61
Lipid profile at 4 weeks					
Total cholesterol (mg/dl)	68	85.8 ± 20.6	71	144.0 ± 28.6	<0.001
LDL-C (mg/dL)	68	24.5 ± 15.9	71	77.7 ± 26.1	<0.001
HDL-C (mg/dl)	68	49.1 ± 10.8	71	47.7 ± 10.6	0.44
non-HDL-C (mg/dl)	68	37.2 ± 17.4	71	96.6 ± 27.6	<0.001
Lipoprotein (a) (mg/dl)	68	5.4 [32.8]	71	13.2 [80.3]	0.006
hs-CRP (mg/l)	68	1.5 [2.2]	71	1.3 [2.0]	0.82
Apolipoprotein A1 (mg/dl)	68	134.9 ± 20.0	71	133.8 ± 20.7	0.74
Apolipoprotein B (mg/dl)	68	34.0 ± 13.1	71	75.2 ± 18.5	<0.001

Values are mean ± standard deviation (SD), number (%) or median [interquartile range]. *p*-values are from Student t-tests, Fisher's exact tests, or Wilcoxon-Mann-Withney tests. [a]<55 years in males and <60 years in females (first degree relatives). ACE = angiotensin converting enzyme, AMI = acute myocardial infarction, ATII = angiotensin II receptor antagonist, BMI = body mass index, CAD = coronary artery disease, CK = creatine kinase, CRP = C-reactive protein, CVD = cardiovascular disease, HDL-C = high-density lipoprotein cholesterol, hs = high sensitivity, LDL-C = low-density lipoprotein cholesterol, NSTEMI = non-ST-elevation myocardial infarction, PCI = percutaneous coronary intervention, STEMI = ST-elevation myocardial infarction, TIA = transitory ischemic attack.

Table 2
Primary and secondary FMD endpoints.

	Alirocumab				Placebo				Between-arm difference in change	
	N	4 weeks	52 weeks	Change (95% CI)	*p*-value	N	4 weeks	52 weeks	Change (95% CI)	*p*-value	Difference (95% CI)	*p*-value
FMD (%)	68	4.52 ± 1.87	5.44 ± 2.24	0.92 (0.52–1.32)	<0.001	71	4.32 ± 1.62	5.45 ± 2.19	1.13 (0.74–1.52)	<0.001	0.92 0.52 to 1.32)	0.47
BL diameter, mm	68	3.84 ± 0.62	3.86 ± 0.84	0.01 (−0.10 to 0.13)	0.79	71	3.89 ± 0.53	3.88 ± 0.55	−0.01 (−0.12 to 0.10)	0.86	0.02 (−0.13 to 0.18)	0.76
Flow (%)	67	885.0 ± 391.8	771.0 ± 339.9	−114.0 (−220.1 to −8.0)	0.04	70	761.1 ± 255.7	788.0 ± 363.6	26.9 (−76.9 to 130.6)	0.61	140.9 (−289.3 to 7.4)	0.06
SBP (mmHg)	67	129.3 ± 17.0	128.8 ± 17.4	−0.5 (−4.6 to 3.7)	0.82	70	126.8 ± 16.2	130.8 ± 15.9	4.1 (−0.0 to 8.1)	0.05	−4.5 (−10.3 to 1.3)	0.13
DBP (mmHg)	67	77.8 ± 9.6	78.3 ± 9.9	0.4 (−1.9 to 2.8)	0.72	70	79.8 ± 9.6	80.3 ± 11.7	0.5 (−1.8 to 2.8)	0.68	−0.1 (−3.3 to 3.2)	0.97
HR (bpm)	68	65 ± 11	65 ± 11	0 (−3 to 2)	0.77	70	66 ± 10	65 ± 11	−2 (−4 to 1)	0.16	1 (−2 to 5)	0.43
LDL-C (mg/dl)	68	156.2 ± 28.4	25.1 ± 27.5	−131.2 (−140.0 to −122.3)	<0.001	71	155.5 ± 36.0	76.3 ± 27.3	−79.2 (−87.9 to −70.6)	<0.001	51.9 (−64.3 to −39.6)	<0.001

Values are mean ± standard deviation or marginal difference (95%). *p*-values are from repeated models. Flow refers to ischemia-induced increase in blood flow. BL = baseline, CI = confidence interval, DBP = diastolic blood pressure, FMD = flow-mediated dilation, HR = heart rate, LDL-C = low-density lipoprotein cholesterol, SBP = systolic blood pressure.

myocardial infarction, <55 years old with high Lp(a) levels were randomized to receive placebo (n = 31), evolocumab (n = 34), or alirocumab (n = 35) for 6 months. Significant improvement in FMD was observed at 6 months for evolocumab, but not for alirocumab or placebo treated patients.

These differences to our study finding may be explained by several factors. Firstly, there was large variation in baseline FMD (Sposito et al., 2.6% [23], Rehberger et al., 11.0% [20], PACMAN-AMI, 4.4%) possibly representing patient populations with various degrees of baseline endothelial dysfunction. Secondly, in the other studies, 100% of patients underwent a 2 [20] or 4 months [23] run-in phase with uptitration of statins, after which patients remained on a stable statin regimen throughout the study period. Importantly, more than half of patients received low or moderate intensity statin therapy including simvastatin or smaller doses of rosuvastatin [23]. Conversely, in PACMAN-AMI, only high-intensity rosuvastatin 20 mg was used and 87% of the patients were statin-naïve prior to study enrollment, i.e. a high-intensity statin was initiated at the same time as the PCSK9 inhibitor and the combined effect of initiated high-intensity rosuvastatin and alirocumab *vs.* rosuvastatin was assessed at 52 weeks, whereas the others measured the effects of PCSK9 inhibitors added to an established, mostly non-high-intensity statin regimen. Thus, it appears possible, that in PACMAN-AMI, rosuvastatin initiation at trial enrollment induced its established effects on FMD [14], and may have attenuated a potential added benefit of alirocumab on FMD.

Interestingly, in the study by Rehberger et al. [44] and our trial, there was no significant improvement in FMD related to alirocumab, opposite to the studies on evolocumab [21–24], which consistently reported improvement in FMD. However, since alirocumab and evolocumab are both fully human monoclonal antibodies that share the same mechanism of action and a similar pharmacodynamic/kinetic properties [46,47], and demonstrated consistent effects on clinical outcomes [16,17] and coronary atherosclerosis as assessed with intracoronary imaging [26,48,49], it seems rather unlikely that the discrepancies in the effects on FMD are related to the type of PCSK9 inhibitor. They may rather be explained by differences in patient population, study design, timepoint of FMD assessment, co-medication, and treatment duration.

Further research by adequately designed and powered studies on the effects of PCSK9 inhibitors on endothelial function in different patient populations is warranted. The INTENSITY-HIGH (NCT03355027) study [50] is currently investigating the effects of PCSK9 inhibition on vascular inflammation and FMD in stable cardiovascular disease.

4.3. Association between FMD and coronary atherosclerosis

PACMAN-AMI FMD represents the first pre-specified, prospective study to investigate the association between FMD and the extent and stability of coronary atherosclerosis with the use of multi-modality intracoronary imaging using IVUS, OCT and NIRS in an AMI population. Our findings indicate an association between 4 weeks brachial artery FMD and non-IRA coronary plaque burden before treatment initiation, but not between FMD and the extent of lipid pools or minimal fibrous cap thickness. Throughout 52 weeks of secondary preventative medical therapy, FMD similarly improved in both the alirocumab and placebo group, whereas more pronounced coronary plaque regression and stabilization had been observed with alirocumab added to rosuvastatin as compared to placebo [26]. Accordingly, changes in FMD and intracoronary imaging parameters from baseline to 52 weeks were not significantly associated – but a trend towards an association between changes in PAV and FMD was observed. These findings are novel and lack comparable prior evidence [51,52].

4.4. Association between FMD and LDL-C

There have been inconclusive results with respect to the association between LDL-C and endothelial function in patients on PCSK9 inhibitors

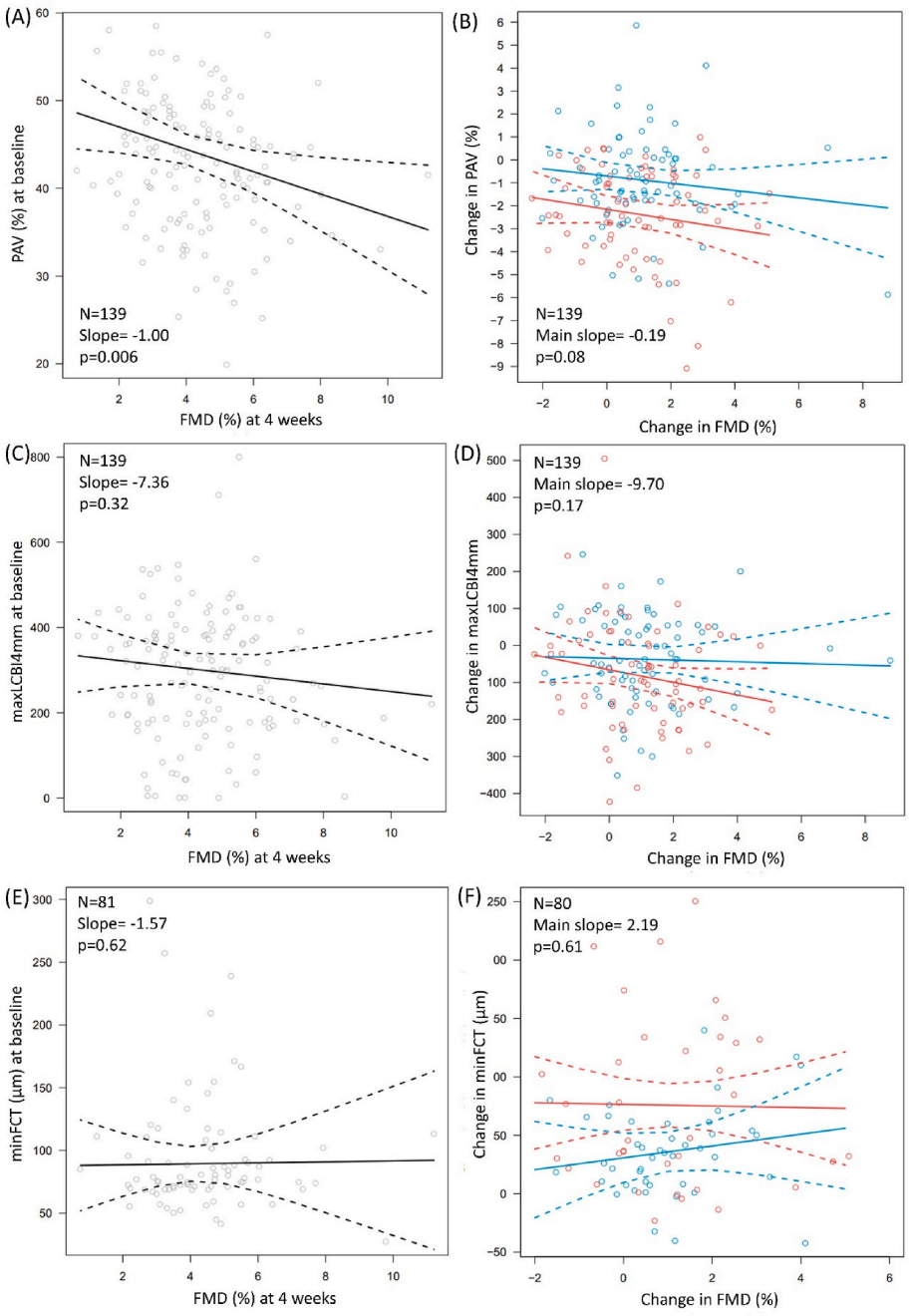


Fig. 2. Association between FMD and intracoronary imaging endpoints.
(A) Percent atheroma volume (PAV) at baseline *vs.* flow-mediated dilation (FMD) at 4 weeks. (B) Change in PAV from baseline to 52 weeks *vs.* change in FMD from 4 to 52 weeks. (C) Maximum lipid core burden index 4 mm (maxLCBI4mm) at baseline *vs.* FMD at 4 weeks. (D) Change in maxLCBI4mm from baseline to 52 weeks *vs.* change in FMD from 4 to 52 weeks. (E) Minimum fibrous cap thickness (minFCT) at baseline *vs.* FMD at 4 weeks. (F) Change in minFCT from baseline to 52 weeks *vs.* change in FMD from 4 to 52 weeks. For A, C, E: Lines depict the slope across both arms (solid) and associated 95% confidence intervals (dashed). For B, D, F: Lines depict the slope per arm (solid) and associated 95% confidence intervals (dashed). *p*-values are from the main slope. Red: alirocumab, Blue: placebo. PAV, maxLCBI4mm, and minFCT were averaged across vessels for each patient.

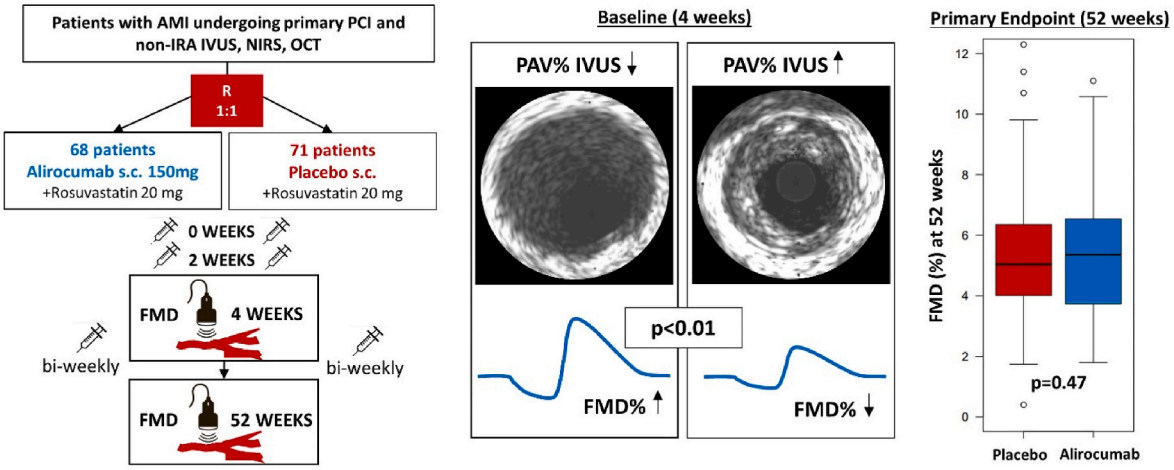


Fig. 3. Graphical abstract.
AMI = acute myocardial infarction, FMD = flow-mediated dilation, i.c. = intracoronary, IVUS = intravascular ultrasound, non-IRA = non-infarct related artery, NSTEMI = non-ST-elevation myocardial infarction, PAV = percent atheroma volume, STEMI = ST-segment elevation infarction.

[21,22,24] and statins alone [13,15]. While some studies reported significant linear association between LDL-C reduction and FMD improvement on PCSK9 inhibitor treatment (r = 0.39–0.69) [21,24], others found no association between absolute values or changes in FMD and LDL-C [22]. In our study, we did not find a significant association between baseline LDL-C and FMD (slope = −0.41, $p = 0.80$), or changes in LDL-C and FMD throughout 52 weeks (slope = −0.70, $p = 0.50$).

4.5. Potential clinical implications of the PACMAN-AMI FMD substudy

The results of our trial suggest that improvement in endothelial function can be achieved throughout 1 year after AMI by guideline-based secondary preventative medical therapy including a high-intensity statin. However, we did not observe a direct additional benefit on FMD by adding the PCSK9 inhibitor alirocumab to rosuvastatin. In line with these findings, no overall improvement in coronary hemodynamics with alirocumab *vs.* placebo in non-obstructive non-IRA as assessed by Quantitative Flow Ratio has been observed in another substudy of the PACMAN-AMI trial [53]. On the contrary, with respect to morphological changes on the coronary artery level, PCSK9 inhibition on top of statin therapy induces a more pronounced regression in plaque burden [26,48,49] and angiographic stenosis [53], along with plaque stabilization as compared to placebo [26,49]. Further functional and morphological effects of PCSK9 inhibitors in the evolution of atherosclerosis should be subject to future studies.

4.6. Limitations

The results of this study must be discussed in the light of several limitations. 1) The study population for this substudy was monocentric and consists of 80% of totally enrolled patients at Bern University Hospital Inselspital. However, characteristics of in- *vs.* excluded patients enrolled at Bern University Hospital were similar. 2) FMD was not measured before initiation of the study drugs, as FMD measurement before primary PCI would not have been feasible and potentially confounded by the acute effects of MI [27], reperfusion, PCI [29], and initiated medication [3,4] and therefore, was not a valid baseline measurement. At 4 weeks, the acute phase of AMI was passed and co-medication administered in steady-state doses, therefore representing an appropriate setting for the baseline assessment of FMD. However, this timepoint already represents the between-group effects of two doses of alirocumab. The primary endpoint, however, was independent of the 4 weeks timepoint. 3) We did not obtain biomarkers of endothelial function such as nitric-oxide.

4.7. Conclusions

In patients with AMI, improvement in endothelial function as assessed by brachial artery FMD was achieved throughout 1 year with guideline-based secondary preventative medical therapy including a high-intensity statin. However, the addition of the PCSK9 inhibitor alirocumab, did not result in further improvement of endothelial function. FMD was significantly inversely related to non-IRA intracoronary plaque burden before treatment initiation, but not to lipid pool or fibrous cap thickness.

Trial registration

ClinicalTrials.gov:NCT03067844.

Financial support

This study was conducted in an independent academic setting and funded by the Bern University Hospital.

Data availability

The data underlying this article will be shared upon reasonable request from the corresponding author.

CRediT authorship contribution statement

Emrush Rexhaj: Conceptualization, Methodology, Validation, Investigation, Resources, Supervision, Writing – review & editing. **Sarah Bär:** Investigation, Resources, Data curation, Writing – original draft, Visualization, Project administration. **Rodrigo Soria:** Investigation, Resources, Writing – review & editing. **Yasushi Ueki:** Investigation, Resources, Data curation, Validation, Writing – review & editing. **Jonas D. Häner:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Tatsuhiko Otsuka:** Investigation, Resources, Data curation, Writing – review & editing. **Raminta Kavaliauskaite:** Investigation, Resources, Data curation, Writing – review & editing, Project administration. **George CM. Siontis:** Investigation, Writing – review & editing. **Stefan Stortecky:** Investigation, Writing – review & editing. **Hiroki Shibutani:** Investigation, Data curation, Writing – review & editing. **David Spirk:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Funding acquisition. **Thomas Engstrøm:** Investigation, Writing – review & editing. **Irene Lang:** Investigation, Writing – review & editing. **Laura Morf:** Resources,

Project administration, Writing – review & editing. **Maria Ambühl:** Resources, Project administration, Writing – review & editing. **Stephan Windecker:** Investigation, Resources, Writing – review & editing. **Sylvain Losdat:** Methodology, Validation, Formal analysis, Data curation, Visualization, Writing – review & editing. **Konstantinos C. Koskinas:** Conceptualization, Methodology, Validation, Investigation, Supervision, Funding acquisition, Writing – review & editing. **Lorenz Räber:** Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Visualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Rexhaj reports research grants from the Swiss National Science Foundation, Innosuisse-Suisse Innovation Agency, Swiss Heart Foundation, Mach-Gaensslen Stiftung Schweiz; speaker fees to institution from Servier; consulting fees to institution from Boehringer Ingelheim, outside the submitted work. Dr. Bär reports research grants to the institution from Medis Medical Imaging Systems, Abbott, Bangerter-Rhyner Stiftung; and personal research grants from the Swiss National Science Foundation, and the University of Turku, Finland, outside the submitted work. Prof. Ueki reports grants from Astellas Pharma, personal fees from Abbott Vascular, Amgen, Bayer, Daiichi Sankyo, Kowa, NIPRO, and Novartis, outside the submitted work. Dr. Siontis reports personal fees from Abbott Vascular, outside the submitted work. Prof. Stortecky reports research grants to the institution from Edwards Lifesciences, Medtronic, Abbott Vascular, and Boston Scientific and speaker fees from Boston Scientific, outside the submitted work. Prof. Spirk reports personal fees from Sanofi-Aventis (Suisse), outside the submitted work. Prof. Engstrøm reports speaker fees from Abbott, outside the submitted work. Prof. Lang reports grants and personal fees from Janssen, and AOPOrphan; personal fees from MSD; and grants from Neutrolis, outside the submitted work.

Prof. Windecker reports research, travel or educational grants to the institution from Abbott, Abiomed, Amgen, Astra Zeneca, Bayer, Biotronik, Boehringer Ingelheim, Boston Scientific, Bristol-Myers Squibb, Cardinal Health, CardioValve, Corflow Therapeutics, CSL Behring, Daiichi Sankyo, Edwards Lifesciences, Guerbet, InfraRedx, Janssen-Cilag, Johnson & Johnson, Medicure, Medtronic, Merck Sharp & Dohm, Miracor Medical, Novartis, Novo Nordisk, Organon, OrPha Suisse, Pfizer, Polares, Regeneron, Sanofi-Aventis, Servier, Sinomed, Terumo, Vifor, V-Wave. Prof. Windecker serves as advisory board member and/or member of the steering/executive group of trials funded by Abbott, Abiomed, Amgen, Astra Zeneca, Bayer, Boston Scientific, Biotronik, Bristol-Myers Squibb, Edwards Lifesciences, Janssen, MedAlliance, Medtronic, Novartis, Polares, Recardio, Sinomed, Terumo, V-Wave and Xeltis with payments to the institution but no personal payments. He is also member of the steering/executive committee group of several investigator-initiated trials that receive funding by industry without impact on his personal remuneration. Dr. Koskinas reports grants from Sanofi, Regeneron, and Infraredx during the conduct of the PACMAN-AMI main trial and personal fees from Amgen and Daiichi Sankyo, outside the submitted work. Dr. Losdat is employed by CTU Bern, University of Bern, which has a staff policy of not accepting honoraria or consultancy fees. However, CTU Bern is involved in design, conduct, or analysis of clinical studies funded by not-for-profit and for-profit organizations. In particular, pharmaceutical and medical device companies provide direct funding to some of these studies. For an up-to-date list of CTU Bern's conflicts of interest: http://www.ctu.unibe.ch/research/declaration_of_interest/index_eng.html. Prof. Räber reports research grants to the institution from Abbott Vascular, Biotronik, Boston Scientific, Heartflow, Sanofi, Regeneron, Medis Medical Imaging Systems, Bangerter-Rhyner Stiftung, Swiss National Science foundation, Swiss Heart Foundation; speaker or consultation fees by Abbott Vascular, Amgen, AstraZeneca, Canon, Medtronic, Novo Nordisk, Occlutech, Sanofi, Vifor, outside the submitted work. All other authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2024.117504.

References

[1] R.A. Brown, E. Shantsila, C. Varma, G.Y.H. Lip, Current Understanding of Atherogenesis, Am. J. Med. 130 (2017) 268–282, https://doi.org/10.1016/j.amjmed.2016.10.022.

[2] J.E. Deanfield, J.P. Halcox, T.J. Rabelink, Endothelial function and dysfunction: testing and clinical relevance, Circulation 115 (2007) 1285–1295, https://doi.org/10.1161/CIRCULATIONAHA.106.652859.

[3] D.H.J. Thijssen, R.M. Bruno, A.C.C.M. van Mil, S.M. Holder, F. Faita, A. Greyling, P. L. Zock, S. Taddei, J.E. Deanfield, T. Luscher, D.J. Green, L. Ghiadoni, Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans, Eur. Heart J. 40 (2019) 2534–2547, https://doi.org/10.1093/eurheartj/ehz350.

[4] M.C. Corretti, T.J. Anderson, E.J. Benjamin, D. Celermajer, F. Charbonneau, M. A. Creager, J. Deanfield, H. Drexler, M. Gerhard-Herman, D. Herrington, P. Vallance, J. Vita, R. Vogel, Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery, J. Am. Coll. Cardiol. 39 (2002) 257–265, https://doi.org/10.1016/S0735-1097(01)01746-6.

[5] Y. Inaba, J.A. Chen, S.R. Bergmann, Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis, Int J Cardiovasc Imaging 26 (2010) 631–640, https://doi.org/10.1007/s10554-010-9616-1.

[6] D.J. Green, H. Jones, D. Thijssen, N.T. Cable, G. Atkinson, Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? Hypertension 57 (2011) 363–369, https://doi.org/10.1161/HYPERTENSIONAHA.110.167015.

[7] R.T. Ras, M.T. Streppel, R. Draijer, P.L. Zock, Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis, Int. J. Cardiol. 168 (2013) 344–351, https://doi.org/10.1016/j.ijcard.2012.09.047.

[8] Y. Xu, R.C. Arora, B.M. Hiebert, B. Lerner, A. Szwajcer, K. McDonald, C. Rigatto, P. Komenda, M.M. Sood, N. Tangri, Non-invasive endothelial function testing and the risk of adverse outcomes: a systematic review and meta-analysis, Eur Heart J Cardiovasc Imaging 15 (2014) 736–746, https://doi.org/10.1093/ehjci/jet256.

[9] Y. Matsuzawa, T. Kwon, R.J. Lennon, L.O. Lerman, A. Lerman, Prognostic value of flow-mediated vasodilation in brachial artery and Fingertip artery for cardiovascular events: a systematic review and meta-analysis, J. Am. Heart Assoc. 4 (2015) e002270, https://doi.org/10.1161/JAHA.115.002270.

[10] A. Oesterle, U. Laufs, J.K. Liao, Pleiotropic effects of statins on the cardiovascular system, Circ. Res. 120 (2017) 229–243, https://doi.org/10.1161/CIRCRESAHA.116.308537.

[11] E. Taneva, K. Borucki, L. Wiens, R. Makarova, C. Schmidt-Lucke, C. Luley, S. Westphal, Early effects on endothelial function of atorvastatin 40 mg twice daily and its withdrawal, Am. J. Cardiol. 97 (2006) 1002–1006, https://doi.org/10.1016/j.amjcard.2005.10.032.

[12] F.C. Lario, M.H. Miname, J.M. Tsutsui, R.D. Santos, I. Kowatsch, J.C.N. Sbano, J.A. F. Ramires, R. Kalil Filho, W. Mathias, Atorvastatin treatment improves myocardial and peripheral blood flow in familial hypercholesterolemia subjects without evidence of coronary atherosclerosis, Echocardiography 30 (2013) 64–71, https://doi.org/10.1111/j.1540-8175.2012.01810.x.

[13] J. Dupuis, J.C. Tardif, P. Cernacek, P. Théroux, Cholesterol reduction rapidly improves endothelial function after acute coronary syndromes. The RECIFE (reduction of cholesterol in ischemia and function of the endothelium) trial, Circulation 99 (1999) 3227–3233, https://doi.org/10.1161/01.cir.99.25.3227.

[14] R. Rawlings, A. Nohria, P.-Y. Liu, J. Donnelly, M.A. Creager, P. Ganz, A. Selwyn, J. K. Liao, Comparison of effects of rosuvastatin (10 mg) versus atorvastatin (40 mg) on rho kinase activity in caucasian men with a previous atherosclerotic event, Am. J. Cardiol. 103 (2009) 437–441, https://doi.org/10.1016/j.amjcard.2008.10.008.

[15] S. John, M. Schlaich, M. Langenfeld, H. Weihprecht, G. Schmitz, G. Weidinger, R. E. Schmieder, Increased bioavailability of nitric oxide after lipid-lowering therapy in hypercholesterolemic patients: a randomized, placebo-controlled, double-blind study, Circulation 98 (1998) 211–216, https://doi.org/10.1161/01.CIR.98.3.211.

[16] M.S. Sabatine, R.P. Giugliano, A.C. Keech, N. Honarpour, S.D. Wiviott, S. A. Murphy, J.F. Kuder, H. Wang, T. Liu, S.M. Wasserman, P.S. Sever, T.R. Pedersen, FOURIER steering committee and investigators, evolocumab and clinical outcomes in patients with cardiovascular disease, N. Engl. J. Med. 376 (2017) 1713–1722, https://doi.org/10.1056/NEJMoa1615664.

[17] G.G. Schwartz, P.G. Steg, M. Szarek, D.L. Bhatt, V.A. Bittner, R. Diaz, J. M. Edelberg, S.G. Goodman, C. Hanotin, R.A. Harrington, J.W. Jukema, G. Lecorps, K.W. Mahaffey, A. Moryusef, R. Pordy, K. Quintero, M.T. Roe, W.J. Sasiela, J.-F. Tamby, P. Tricoci, H.D. White, A.M. Zeiher, ODYSSEY OUTCOMES committees and investigators, alirocumab and cardiovascular outcomes after acute coronary syndrome, N. Engl. J. Med. 379 (2018) 2097–2107, https://doi.org/10.1056/NEJMoa1801174.

[18] E. Punch, J. Klein, P. Diaba-Nuhoho, H. Morawietz, M. Garelnabi, Effects of PCSK9 targeting: alleviating oxidation, inflammation, and atherosclerosis, J. Am. Heart Assoc. 11 (2022) e023328, https://doi.org/10.1161/JAHA.121.023328.
[19] E. Yurtseven, D. Ural, K. Baysal, L. Tokgözoğlu, An update on the role of PCSK9 in atherosclerosis, J. Atherosclerosis Thromb. 27 (2020) 909–918, https://doi.org/10.5551/jat.55400.
[20] A. Rehberger Likozar, M. Šebeštjen, Smoking and diabetes attenuate beneficial effects of PSCK9 inhibitors on arterial wall properties in patients with very high lipoprotein (a) levels, Atherosclerosis Plus 50 (2022) 1–9, https://doi.org/10.1016/j.athplu.2022.07.001.
[21] G. Maulucci, F. Cipriani, D. Russo, G. Casavecchia, C. Di Staso, L. Di Martino, A. Ruggiero, M. Di Biase, N.D. Brunetti, Improved endothelial function after short-term therapy with evolocumab, J Clin Lipidol 12 (2018) 669–673, https://doi.org/10.1016/j.jacl.2018.02.004.
[22] J. Schremmer, L. Busch, S. Baasen, Y. Heinen, R. Sansone, C. Heiss, M. Kelm, M. Stern, Chronic PCSK9 inhibitor therapy leads to sustained improvements in endothelial function, arterial stiffness, and microvascular function, Microvasc. Res. 148 (2023) 104513, https://doi.org/10.1016/j.mvr.2023.104513.
[23] A.C. Sposito, I. Breder, J. Barreto, J. Breder, I. Bonilha, M. Lima, A. Oliveira, V. Wolf, B. Luchiari, H.R. do Carmo, D. Munhoz, D. Oliveira, O.R. Coelho-Filho, O. R. Coelho, J.R. Matos-Souza, F.A. Moura, L.S.F. de Carvalho, W. Nadruz, T. Quinaglia, S.T. Kimura-Medorima, EXCEED-BHS3 Group, Evolocumab on top of empagliflozin improves endothelial function of individuals with diabetes: randomized active-controlled trial, Cardiovasc. Diabetol. 21 (2022) 147, https://doi.org/10.1186/s12933-022-01584-8.
[24] A. Di Minno, M. Gentile, G. Iannuzzo, I. Calcaterra, M. Tripaldella, B. Porro, V. Cavalca, M.D. Di Taranto, E. Tremoli, G. Fortunato, P.O.F. Rubba, M.N.D. Di Minno, Endothelial function improvement in patients with familial hypercholesterolemia receiving PCSK-9 inhibitors on top of maximally tolerated lipid lowering therapy, Thromb. Res. 194 (2020) 229–236, https://doi.org/10.1016/j.thromres.2020.07.049.
[25] C. Zanchin, K.C. Koskinas, Y. Ueki, S. Losdat, J.D. Häner, S. Bär, T. Otsuka, A. Inderkum, M.R.J. Jensen, J. Lonborg, G. Fahrni, A.S. Ondracek, J. Daemen, R.-J. van Geuns, J.F. Iglesias, C.M. Matter, D. Spirk, P. Juni, F. Mach, D. Heg, T. Engstrom, I. Lang, S. Windecker, L. Räber, Effects of the PCSK9 antibody alirocumab on coronary atherosclerosis in patients with acute myocardial infarction: a serial, multivessel, intravascular ultrasound, near-infrared spectroscopy and optical coherence tomography imaging study–Rationale and design of the PACMAN-AMI trial, Am. Heart J. 238 (2021) 33–44, https://doi.org/10.1016/j.ahj.2021.04.006.
[26] L. Räber, Y. Ueki, T. Otsuka, S. Losdat, J.D. Häner, J. Lonborg, G. Fahrni, J. F. Iglesias, R.-J. van Geuns, A.S. Ondracek, M.D. Radu Juul Jensen, C. Zanchin, S. Stortecky, D. Spirk, G.C.M. Siontis, L. Saleh, C.M. Matter, J. Daemen, F. Mach, D. Heg, S. Windecker, T. Engstrøm, I.M. Lang, K.C. Koskinas, PACMAN-AMI collaborators, effect of alirocumab added to high-intensity statin therapy on coronary atherosclerosis in patients with acute myocardial infarction: the PACMAN-AMI randomized clinical trial, JAMA 327 (2022) 1771–1781, https://doi.org/10.1001/jama.2022.5218.
[27] I.M. Seropian, C. Sonnino, B.W. Van Tassell, L.M. Biasucci, A. Abbate, Inflammatory markers in ST-elevation acute myocardial infarction, Eur Heart J Acute Cardiovasc Care 5 (2016) 382–395, https://doi.org/10.1177/2048872615568965.
[28] L.S.F. Carvalho, N. Panzoldo, S.N. Santos, R. Modolo, B. Almeida, J.C. Quinaglia E Silva, W. Nadruz, E.C. de Faria, A.C. Sposito, Brasilia Heart Study Group, HDL levels and oxidizability during myocardial infarction are associated with reduced endothelial-mediated vasodilation and nitric oxide bioavailability, Atherosclerosis 237 (2014) 840–846, https://doi.org/10.1016/j.atherosclerosis.2014.10.103.
[29] J.R. Spiro, J.E. Digby, G. Ghimire, M. Mason, A.G. Mitchell, C. Ilsley, A. Donald, M. C.D. Dalby, R.K. Kharbanda, Brachial artery low-flow-mediated constriction is increased early after coronary intervention and reduces during recovery after acute coronary syndrome: characterization of a recently described index of vascular function, Eur. Heart J. 32 (2011) 856–866, https://doi.org/10.1093/eurheartj/ehq401.
[30] U. Scherrer, S.F. Rimoldi, E. Rexhaj, T. Stuber, H. Duplain, S. Garcin, S.F. de Marchi, P. Nicod, M. Germond, Y. Allemann, C. Sartori, Systemic and pulmonary vascular dysfunction in children conceived by assisted reproductive technologies, Circulation 125 (2012) 1890–1896, https://doi.org/10.1161/CIRCULATIONAHA.111.071183.
[31] T.A. Meister, S.F. Rimoldi, R. Soria, R. von Arx, F.H. Messerli, C. Sartori, U. Scherrer, E. Rexhaj, Association of assisted reproductive technologies with arterial hypertension during adolescence, J. Am. Coll. Cardiol. 72 (2018) 1267–1274, https://doi.org/10.1016/j.jacc.2018.06.060.
[32] D.S. Celermajer, K.E. Sorensen, V.M. Gooch, D.J. Spiegelhalter, O.I. Miller, I. D. Sullivan, J.K. Lloyd, J.E. Deanfield, Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis, Lancet 340 (1992) 1111–1115, https://doi.org/10.1016/0140-6736(92)93147-F.
[33] T.J. Anderson, A. Uehata, M.D. Gerhard, I.T. Meredith, S. Knab, D. Delagrange, E. H. Lieberman, P. Ganz, M.A. Creager, A.C. Yeung, A.P. Selwyn, Close relation of endothelial function in the human coronary and peripheral circulations, J. Am. Coll. Cardiol. 26 (1995) 1235–1241, https://doi.org/10.1016/0735-1097(95)00327-4.
[34] B. Takase, A. Uehata, T. Akima, T. Nagai, T. Nishioka, A. Hamabe, K. Satomura, F. Ohsuzu, A. Kurita, Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease, Am. J. Cardiol. 82 (1998) 1535–1539, https://doi.org/10.1016/S0002-9149(98)00702-4.
[35] J.D. Barth, Which tools are in your cardiac workshop? Carotid ultrasound, endothelial function, and magnetic resonance imaging, Am. J. Cardiol. 87 (2001) 8–14, https://doi.org/10.1016/S0002-9149(01)01419-9.
[36] J. Yeboah, A.R. Folsom, G.L. Burke, C. Johnson, J.F. Polak, W. Post, J.A. Lima, J. R. Crouse, D.M. Herrington, Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study, Circulation 120 (2009) 502–509, https://doi.org/10.1161/CIRCULATIONAHA.109.864801.
[37] Y. Kitta, J. Obata, T. Nakamura, M. Hirano, Y. Kodama, D. Fujioka, Y. Saito, K. Kawabata, K. Sano, T. Kobayashi, T. Yano, K. Nakamura, K. Kugiyama, Persistent impairment of endothelial vasomotor function has a negative impact on outcome in patients with coronary artery disease, J. Am. Coll. Cardiol. 53 (2009) 323–330, https://doi.org/10.1016/j.jacc.2008.08.074.
[38] M. Charakida, S. Masi, T.F. Lüscher, J.J.P. Kastelein, J.E. Deanfield, Assessment of atherosclerosis: the role of flow-mediated dilatation, Eur. Heart J. 31 (2010) 2854–2861, https://doi.org/10.1093/eurheartj/ehq340.
[39] F.G. Biccirè, J. Häner, S. Losdat, Y. Ueki, H. Shibutani, T. Otsuka, R. Kakizaki, T. M. Hofbauer, R.-J. van Geuns, S. Stortecky, G.C. Siontis, S. Bär, J. Lønborg, D. Heg, C. Kaiser, D. Spirk, J. Daemen, J. Iglesias, S. Windecker, T. Engstrøm, I. Lang, K. C. Koskinas, L. Räber, Concomitant coronary atheroma regression and stabilization in response to lipid-lowering therapy, J. Am. Coll. Cardiol. (23) (2023) S0735–S1097, https://doi.org/10.1016/j.jacc.2023.08.019, 06468–9.
[40] N. Ruparelia, J.T. Chai, E.A. Fisher, R.P. Choudhury, Inflammatory processes in cardiovascular disease: a route to targeted therapies, Nat. Rev. Cardiol. 14 (2017) 133–144, https://doi.org/10.1038/nrcardio.2016.185.
[41] P.M. Ridker, B.M. Everett, T. Thuren, J.G. MacFadyen, W.H. Chang, C. Ballantyne, F. Fonseca, J. Nicolau, W. Koenig, S.D. Anker, J.J.P. Kastelein, J.H. Cornel, P. Pais, D. Pella, J. Genest, R. Cifkova, A. Lorenzatti, T. Forster, Z. Kobalava, L. Vida-Simiti, M. Flather, H. Shimokawa, H. Ogawa, M. Dellborg, P.R.F. Rossi, R.P.T. Troquay, P. Libby, R.J. Glynn, Antiinflammatory therapy with canakinumab for atherosclerotic disease, N. Engl. J. Med. 377 (2017) 1119–1131, https://doi.org/10.1056/NEJMoa1707914.
[42] P. Vallance, J. Collier, K. Bhagat, Infection, inflammation, and infarction: does acute endothelial dysfunction provide a link? Lancet 349 (1997) 1391–1392, https://doi.org/10.1016/S0140-6736(96)09424-X.
[43] Y.-X. Cao, S. Li, H.-H. Liu, J.-J. Li, Impact of PCSK9 monoclonal antibodies on circulating hs-CRP levels: a systematic review and meta-analysis of randomised controlled trials, BMJ Open 8 (2018) e022348, https://doi.org/10.1136/bmjopen-2018-022348.
[44] T. Metzner, D.R. Leitner, G. Dimsity, F. Gunzer, P. Opriessnig, K. Mellitzer, A. Beck, H. Sourij, T. Stojakovic, H. Deutschmann, W. März, U. Landmesser, M. Brodmann, G. Reishofer, H. Scharnagl, H. Toplak, G. Silbernagel, Short-term treatment with alirocumab, flow-dependent dilatation of the brachial artery and use of magnetic resonance diffusion tensor imaging to evaluate vascular structure: an exploratory pilot study, Biomedicines 10 (2022) 152, https://doi.org/10.3390/biomedicines10010152.
[45] J. Cacciottolo Paul, Kostapanos Michalis, Hubsch Annette, Vamvaka Evangelia, Kaloyirou Fotini, Helmy Joanna, H. Sancho Elena, Pavey Holly, Maki-Petaja Kaisa, Ian Wilkinson, Cheriyan Joseph, The effects of alirocumab versus ezetimibe on top of statins on vascular inflammation and function, J. Am. Coll. Cardiol. 77 (2021) 159, https://doi.org/10.1016/S0735-1097(21)01518-7, 159.
[46] E.A. Stein, S. Mellis, G.D. Yancopoulos, N. Stahl, D. Logan, W.B. Smith, E. Lisbon, M. Gutierrez, C. Webb, R. Wu, Y. Du, T. Kranz, E. Gasparino, G.D. Swergold, Effect of a monoclonal antibody to PCSK9 on LDL cholesterol, N. Engl. J. Med. 366 (2012) 1108–1118, https://doi.org/10.1056/NEJMoa1105803.
[47] C.S. Dias, A.J. Shaywitz, S.M. Wasserman, B.P. Smith, B. Gao, D.S. Stolman, C. P. Crispino, K.V. Smirnakis, M.G. Emery, A. Colbert, J.P. Gibbs, M.W. Retter, B. P. Cooke, S.T. Uy, M. Matson, E.A. Stein, Effects of AMG 145 on low-density lipoprotein cholesterol levels, J. Am. Coll. Cardiol. 60 (2012) 1888–1898, https://doi.org/10.1016/j.jacc.2012.08.986.
[48] S.J. Nicholls, R. Puri, T. Anderson, C.M. Ballantyne, L. Cho, J.J.P. Kastelein, W. Koenig, R. Somaratne, H. Kassahun, J. Yang, S.M. Wasserman, R. Scott, I. Ungi, J. Podolec, A.O. Ophuis, J.H. Cornel, M. Borgman, D.M. Brennan, S.E. Nissen, Effect of evolocumab on progression of coronary disease in statin-treated patients: the GLAGOV randomized clinical trial, JAMA 316 (2016) 2373–2384, https://doi.org/10.1001/jama.2016.16951.
[49] S.J. Nicholls, Y. Kataoka, S.E. Nissen, F. Prati, S. Windecker, R. Puri, T. Hucko, D. Aradi, J.-P.R. Herrman, R.S. Hermanides, B. Wang, H. Wang, J. Butters, G. Di Giovanni, S. Jones, G. Pompili, P.J. Psaltis, Effect of evolocumab on coronary plaque phenotype and burden in statin-treated patients following myocardial infarction, JACC Cardiovasc Imaging 15 (2022) 1308–1321, https://doi.org/10.1016/j.jcmg.2022.03.002.
[50] P.J. Cacciottolo, M.S. Kostapanos, E. Hernan Sancho, H. Pavey, F. Kaloyirou, E. Vamvaka, J. Helmy, A. Hubsch, C.M. McEniery, I.B. Wilkinson, J. Cheriyan, Investigating the lowest threshold of vascular benefits from LDL cholesterol lowering with a PCSK9 mAb inhibitor (alirocumab) in patients with stable cardiovascular disease (INTENSITY-HIGH): protocol and study rationale for a randomised, open label, parallel group, mechanistic study, BMJ Open 11 (2021) e037457, https://doi.org/10.1136/bmjopen-2020-037457.
[51] T. Sawada, T. Emoto, Y. Motoji, M. Hashimoto, H. Kageyama, D. Terashita, T. Mizoguchi, T. Mizuguchi, M. Iwasaki, K. Taira, H. Okamoto, Y. Matsuo, S. Kim, A. Takarada, M. Yokoyama, Possible association between non-invasive parameter of flow-mediated dilatation in brachial artery and whole coronary plaque

vulnerability in patients with coronary artery disease, Int. J. Cardiol. 166 (2013) 613–620, https://doi.org/10.1016/j.ijcard.2011.11.101.

[52] T. Nemoto, Y. Minami, M. Yamaoka-Tojo, T. Sato, Y. Muramatsu, R. Kakizaki, K. Fujiyoshi, T. Hashimoto, K. Meguro, T. Shimohama, T. Tojo, J. Ako, Impaired flow-mediated dilation and severity and vulnerability of culprit plaque in patients with coronary artery disease, Int. Heart J. 60 (2019) 539–545, https://doi.org/10.1536/ihj.18-531.

[53] S. Bär, R. Kavaliauskaite, T. Otsuka, Y. Ueki, J.D. Häner, G.C.M. Siontis, S. Stortecky, H. Shibutani, F. Temperli, C. Kaiser, J. Iglesias, R. Jan van Geuns, J. Daemen, D. Spirk, T. Engstrøm, I. Lang, S. Windecker, K.C. Koskinas, S. Losdat, L. Räber, Impact of alirocumab on plaque regression and haemodynamics of non-culprit arteries in patients with acute myocardial infarction: a prespecified substudy of the PACMAN-AMI trial, EuroIntervention (2023), https://doi.org/10.4244/EIJ-D-23-00201. EIJ-D-23-00201.