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Impact of evolocumab on the pharmacodynamic profiles of clopidogrel in patients with atherosclerotic cardiovascular disease: a randomised, double-blind, placebo-controlled study

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KEYWORDS

- adjunctive pharmacotherapy
- biochemical markers
- clinical trials
- stable angina

Abstract

Background: The impact of intense low-density lipoprotein cholesterol (LDL-C) reduction using a pro-protein convertase subtilisin/kexin type 9 (PCSK9) inhibitor on profiles of platelet reactivity has yet to be explored.

Aims: Our aim was to investigate the effects of the PCSK9 inhibitor, evolocumab, on platelet reactivity in patients with atherosclerotic cardiovascular disease (ASCVD) on clopidogrel treatment.

Methods: This was a prospective, randomised, double-blind, placebo-controlled pharmacodynamic study in patients with ASCVD on clopidogrel treatment and with LDL-C levels ≥70 mg/dL despite a maximally tolerated statin dose. Patients were stratified according to levels of platelet reactivity using VerifyNow $P2Y_{12}$ reactivity units (PRU) into high platelet reactivity (HPR; PRU >208) or normal platelet reactivity (NPR; PRU >85 and ≤208). Each cohort was randomised to receive evolocumab 420 mg or placebo. The primary endpoint was the difference in PRU at 30 days.

Results: A total of 84 patients (HPR, n=37 [19 evolocumab vs 18 placebo]; NPR, n=47 [22 evolocumab vs 25 placebo]) were included. Evolocumab significantly reduced LDL-C compared to placebo at 14 (p<0.001) and 30 (p=0.001) days. At 14 days, PRU levels were significantly lower with evolocumab compared to placebo in the HPR (218.2±29.7 vs 246.6±35.2; p=0.017), but not in the NPR cohort (141.2±42.8 vs 148.2±41.7; p=0.578). At 30 days, there were no significant differences in PRU in the HPR (219.3±38.3 vs 240.9±51.8; p=0.161) or NPR (141.5±54.3 vs 158.6±40.8; p=0.229) cohorts.

Conclusions: Compared to placebo, evolocumab in adjunct to statin therapy did not significantly reduce platelet reactivity at 30 days in ASCVD patients on clopidogrel treatment despite intense LDL-C reduction. ClinicalTrials.gov: NCT03096288.

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Abbreviations

AA	arachidonic acid
ACS	acute coronary syndrome
ADP	adenosine diphosphate
ASCVD	atherosclerotic cardiovascular disease
CAD	coronary artery disease
CYP2C19	cytochrome P450 2C19
HDL-C	high-density lipoprotein cholesterol
HPR	high platelet reactivity
IQR	interquartile range
LDL-C	low-density lipoprotein cholesterol
LOF	loss-of-function
LTA	light transmission aggregometry
MA	maximum clot amplitude
MPA	maximum platelet aggregation
NPR	normal platelet reactivity
PAD	peripheral artery disease
PCSK9	proprotein convertase subtilisin/kexin type 9
PD	pharmacodynamics
PRU	$P2Y_{12}$ reactivity unit
TEG	thromboelastography coagulation analyser
TRAP	thrombin receptor-activating peptide

Introduction

Clopidogrel is the most broadly used oral platelet $P2Y_{12}$ receptor inhibitor in patients with atherosclerotic cardiovascular disease (ASCVD) manifestations[1]. Despite its established efficacy, pharmacodynamic (PD) studies have shown that approximately 30-40% of clopidogrel-treated patients have an impaired response and persist with high on-treatment platelet reactivity (HPR)[2,3]. Importantly, HPR is a marker of thrombotic risk, underscoring the need to optimise PD response profiles in clopidogrel-treated patients[2,3]. Lipid-lowering treatment with statins has been shown to reduce platelet reactivity among clopidogrel-treated patients[4-7]. Although the exact biological mechanism(s) involved in the modulation of platelet function induced by statin therapy remain elusive, there is accumulating evidence that circulating lipoproteins may affect platelet function[8,9]. In particular, low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein (VLDL), and especially oxidised LDL-C are atherogenic lipoproteins known to increase platelet activation[8,9]. Hence, reduction of these circulating lipoproteins may potentially modulate platelet function and PD response profiles to antiplatelet drugs.

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) plays an essential role in regulating cholesterol homeostasis by modulating LDL-C receptor degradation, thus reducing cellular uptake[10]. Evolocumab is a fully human monoclonal antibody that inhibits PCSK9, leading to a marked reduction in LDL-C and a lower incidence of cardiovascular events[10,11]. Whether such a reduction in cardiovascular events is due to solely LDL-C reduction or can also be attributed to other mechanisms, such as modulating thrombotic processes, is unknown. Of note, elevated circulating levels of PCSK9 have been associated with increased platelet reactivity[12]. However, the effects on platelet reactivity associated with intense LDL-C lowering induced by evolocumab remain unexplored. The aim of this study was to explore the impact of evolocumab on the PD effects of clopidogrel in patients with ASCVD.

Methods

STUDY DESIGN AND PARTICIPANTS

This was a prospective, randomised, double-blind, placebo-controlled PD study assessing the effects of evolocumab on platelet reactivity in patients with ASCVD treated with clopidogrel (ClinicalTrials.gov: NCT03096288). The study was performed at the University of Florida Health – Jacksonville (Jacksonville, FL, USA). Patients were screened for eligibility at the outpatient clinics of our healthcare system. Details on study inclusion and exclusion criteria are reported in the **Supplementary Appendix 1**. In brief, patients with ASCVD were screened and required to meet all of the following study entry criteria: a) ≥18 years old; b) treated with clopidogrel (75 mg once daily [od]), with or without low-dose aspirin (81 mg od), as per the standard of care, for at least 30 days; c) have PD profiles of clopidogrel response consistent with either normal (NPR) or high platelet reactivity according to consensus definitions[3]; d) fasting LDL-C ≥70 mg/dL or non-high-density lipoprotein cholesterol ≥100 mg/dL after ≥2 weeks of optimised stable lipid-lowering therapy with a maximally tolerated statin dose. Key exclusion criteria included treatment with any oral anticoagulant, treatment with any antiplatelet agent other than aspirin or clopidogrel in the prior 14 days, use of PCSK9 inhibitors in the prior 90 days or history of a serious hypersensitivity reaction to evolocumab. The study complied with the Declaration of Helsinki and was approved by the Western Institutional Review Board. All patients gave their written informed consent.

ASCVD was defined as a history of acute coronary syndromes (ACS), stable or unstable angina, coronary or other arterial revascularisation, stroke, transient ischaemic attack, or peripheral artery disease (PAD) presumed to be of atherosclerotic origin. HPR was defined as $P2Y_{12}$ reactivity units (PRU) >208 by VerifyNow $P2Y_{12}$ and NPR was defined as PRU between 85 and 208, in line with consensus definitions[3]. Patients meeting study entry criteria were stratified according to their baseline PRU into two cohorts: HPR and NPR.

After providing written informed consent, patients in each cohort were randomly assigned in a 1:1 fashion to receive a single subcutaneous administration of either evolocumab 420 mg or placebo (0.9% sodium chloride). Blood sampling for PD testing was conducted at 3 timepoints: baseline (before administration of randomised treatment), 14±2 days, and 30±2 days after randomised drug administration. At each timepoint, blood was collected before the morning dose of clopidogrel to measure trough levels of platelet reactivity (defined as 24±2 hrs after the last maintenance dose of clopidogrel). A flow diagram of the study design is illustrated in **Figure 1A**.

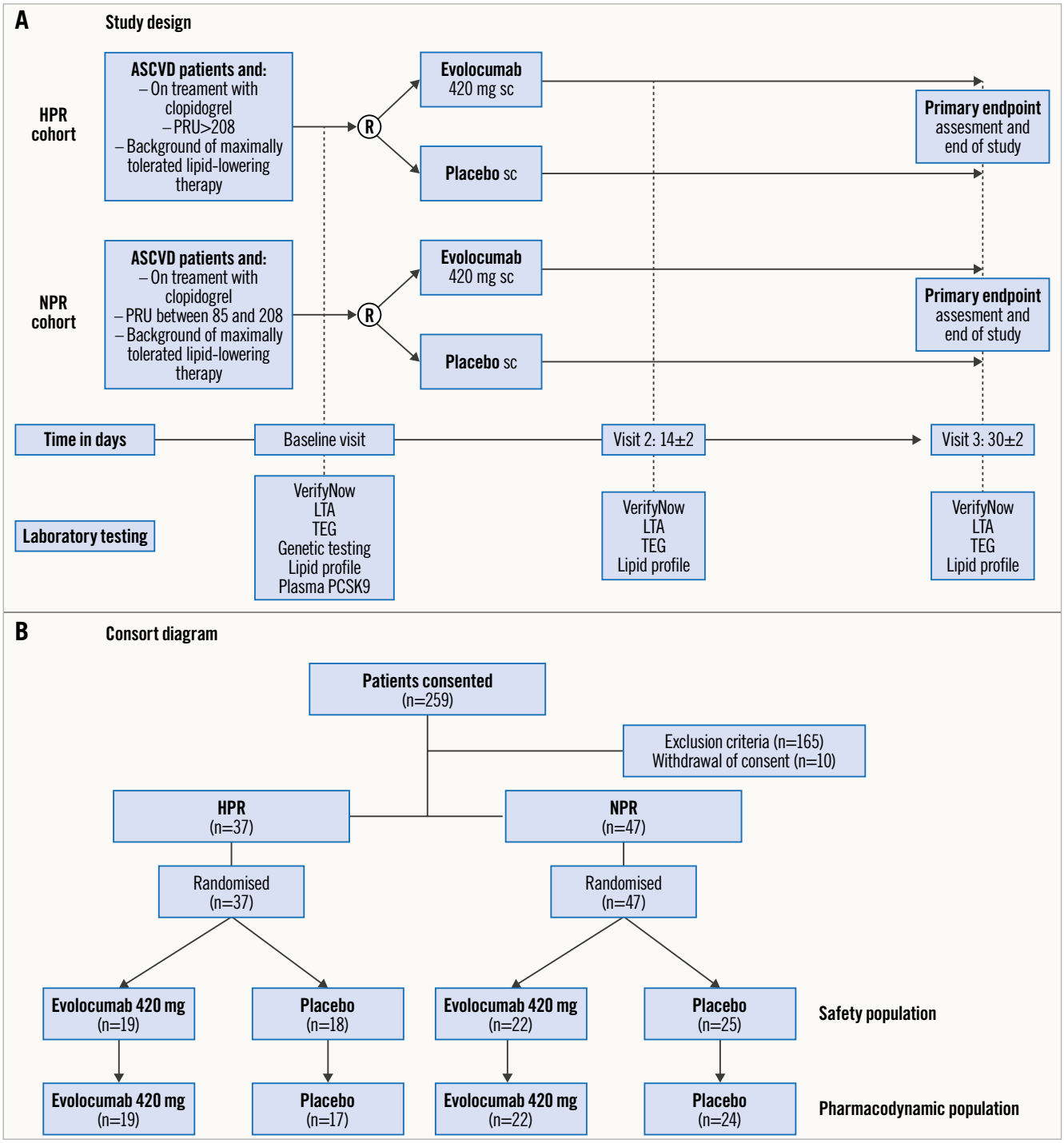


Figure 1. *Study design and consort diagram. A) Study design and (B) consort diagram. ASCVD: atherosclerotic cardiovascular disease; HPR: high platelet reactivity; LTA: light transmission aggregometry; NPR: normal platelet reactivity: PCSK9: proprotein convertase subtilisin/kexin type 9; PRU: $P2Y_{12}$ reactivity unit; R: randomisation; sc: subcutaneous; TEG: thromboelastography; S3: SAPIEN 3; TAV: transcatheter aortic valve; THV: transcatheter heart valve*

BLOOD SAMPLING AND LABORATORY ASSESSMENTS

Peripheral venous blood samples were drawn through a short venous catheter inserted into a forearm vein and collected in citrate, ethylenediaminetetraacetic acid, and serum tubes as appropriate for assessments. The first 2-4 mL of blood were discarded to avoid spontaneous platelet activation. PD assessments were conducted using three different assays: the VerifyNow PRU system (Werfen) with results reported in PRU, light transmission aggregometry (LTA; Chrono-Log) following arachidonic acid (AA; 1 mM), collagen (3µg/ml), adenosine diphosphate (ADP; 5 µM and 20 µM), and thrombin receptor-activating peptide (TRAP; 15 µM) stimuli with results reported as maximum platelet

aggregation (MPA%), and the thromboelastography coagulation analyser (TEG) 6s system (Haemonetics) using ADP and kaolin (thrombin pathway) as stimuli with results reported as maximum clot amplitude (MA, mm)[13-15]. At baseline, cytochrome P450 2C19 (CYP2C19) genotyping was performed with Spartan RX rapid genotyping (Spartan Bioscience) to determine the CYP2C19 (*1,*2,*3,*17) allele status[16]. Lipid profiles and PCSK9 levels (R & D Systems) were also assessed. A detailed description of laboratory assessments is provided in **Supplementary Appendix 2** and **Supplementary Appendix 3**.

STUDY OUTCOMES AND SAMPLE SIZE CALCULATION

The primary outcome was the reduction in platelet reactivity, as defined by VerifyNow PRU, between evolocumab and placebo at 30 days after randomisation in each cohort. The primary hypothesis of our study was that evolocumab would be associated with a reduction of 30 PRU in patients with HPR and NPR. This estimated treatment effect was arbitrarily chosen, given the absence of preliminary data in this setting; however, a reduction of 30 PRU approximates that previously observed with LDL-C lowering using statin therapy[4]. Statistical assumptions were derived for each cohort of patients based on the distribution of their PRU levels in an internal database analysis of a cohort of subjects on maintenance clopidogrel therapy (HPR: 247±29; NPR: 150±35). Based on these assumptions, a total of 34 and 46 patients were needed in the HPR and NPR cohorts, respectively, to detect a 30 PRU difference with 80% power and α of 0.05. Considering a possible dropout rate of 10%, up to a total of 90 patients were planned to be randomised. Secondary outcomes included comparing PD measures between evolocumab and placebo at each timepoint, assessed by VerifyNow PRU, LTA, TEG, HPR rates, and serum LDL-C levels.

STATISTICAL ANALYSIS PLAN

Categorical variables are expressed as frequencies and percentages. Continuous variables are presented as mean±standard deviation (SD) or median and interquartile range (IQR). A t-test was used to evaluate the primary outcome and all between-group comparisons of platelet reactivity and LDL-C levels. The Fisher's exact test or Pearson's chi-square were used for assessing comparisons of HPR and NPR rates between groups. Several exploratory analyses were performed to assess the effect of specific variables on the primary study outcome: 1) to assess the effect of the presence of CYP2C19 loss-of-function (LOF) alleles, we compared the PRU values of the poor or intermediate metaboliser versus others; 2) to assess the effect of baseline LDL-C levels, we compared the PRU values of patients with baseline LDL-C levels above median versus below median; 3) to assess the effect of baseline PCSK9 levels, we compared the PRU values between patients with baseline PCSK9 levels above median versus below median; 4) to assess the correlation between the change (Δ: baseline – follow-up) in PRU and LDL-C, we calculated the PRU and LDL-C at 30-day follow-up and compared them by means of Spearman's rank correlation coefficient.

A 2-tailed p-value of <0.05 was considered to indicate a statistically significant difference for all the above analyses. Our group performed statistical analyses using SPSS v28.0 software (IBM).

The safety population was composed of all patients who had received at least one dose of the study drug. The PD population included all patients with any PD data on the study drug without a major protocol deviation interfering the PD response.

Results

PATIENT POPULATION

Between October 2017 and October 2020, 259 subjects were identified and consented to participate in the study. Of these, 165 patients were screen failures and 10 patients withdrew. Thus, 84 patients (HPR, n=37; NPR, n=47) were randomised; all randomised patients were exposed to the study drug, representing the safety population; 82 patients (HPR, n=36; NPR, n=46) had valid PD data, representing the PD population. In the HPR cohort, 19 were randomised to evolocumab and 18 to placebo; in the NPR cohort, 22 to evolocumab and 25 to placebo. A consort diagram of the study population is illustrated in **Figure 1B**.

Baseline characteristics of the study population according to baseline platelet reactivity status (NPR or HPR) are summarised in **Table 1** and **Supplementary Table 1**. There were no significant differences between groups, except for a higher frequency of family history of coronary artery disease (CAD) and oral hypoglycaemic drugs in the placebo group in the HPR cohort and older age in the evolocumab group in the NPR cohort. There were no differences in baseline LDL-C levels between patients allocated to evolocumab versus placebo in the HPR (107.3±29.1 mg/dL vs 110.2±43.1 mg/dL; p=0.808) and NPR (91.2±30.5 mg/dL vs 99.6±31.3 mg/dL; p=0.365) cohorts **(Supplementary Table 1)**.

In the HPR cohort, 17 patients were carriers of CYP2C19 LOF alleles (all heterozygotes), without differences in frequency between the evolocumab group and placebo (57.9% vs 33.3%; p=0.169). In the NPR cohort, 10 patients were carriers of CYP2C19 LOF alleles (9 heterozygotes and 1 homozygote) without differences in frequency between the evolocumab group and placebo (18.2% vs 24.0%; p=0.583) **(Supplementary Table 2)**.

One ischaemic adverse event (non-ST-segment elevation myocardial infarction) occurred in the HPR cohort in a patient allocated to placebo. A complete list of adverse events is shown in **Supplementary Table 3**.

LIPID PROFILE FINDINGS

At 14 days, there was a significant reduction of the LDL-C levels with evolocumab compared to placebo in both the HPR (36.1±27.0 mg/dL vs 100.9±41.1 mg/dL; p<0.001) and NPR (18.0±14.6 mg/dL vs 91.2±32.2 mg/dL; p<0.001) cohorts. Reduced LDL-C levels persisted, albeit not as marked, at 30 days (HPR cohort: 45.9±15.9 mg/dL vs 103.8±42.6 mg/dL; p=0.001; NPR cohort: 30.9±12.0 mg/dL vs 95.0±34.3 mg/dL; p=0.001) **(Figure 2)**. Complete lipid profile results are provided in **Supplementary Table 4**.

Table 1. Baseline characteristics of the safety population.

		HPR (n=37)			NPR (n=47)		
		Evolocumab (n=19)	Placebo (n=18)	*p*-value	Evolocumab (n=22)	Placebo (n=25)	*p*-value
Age, yrs		63.4±8.1	62.5±8.1	0.794	63.3±8.2	58.3±8.1	0.040
Female gender		8 (42.1)	12 (66.7)	0.191	10 (45.5)	8 (32.0)	0.382
BMI, kg/m^2		32.8±8.6	32.0±7.0	0.747	30.3±5.6	31.2±6.1	0.561
Active smoking		6 (31.6)	5 (27.8)	0.451	8 (36.4)	7 (28.0)	0.280
Hypertension		17 (89.5)	17 (94.4)	0.521	20 (90.9)	23 (92.0)	0.645
Diabetes		7 (36.8)	11 (61.1)	0.194	13 (59.1)	14 (56.0)	0.533
Hyperlipidaemia		19 (100.0)	18 (100.0)	-	20 (90.9)	24 (96.0)	0.593
Family history of CAD		8 (42.1)	14 (77.8)	0.045	10 (45.5)	8 (32.0)	0.313
Peripheral artery disease		6 (31.6)	5 (27.8)	0.543	9 (40.9)	6 (24.0)	0.347
Stroke/TIA		4 (21.1)	7 (38.9)	0.295	5 (22.7)	3 (12.0)	0.446
Hepatic dysfunction		0	0	-	0	0	-
Prior MI		9 (47.4)	8 (44.4)	0.560	11 (50.0)	12 (48.0)	0.562
Prior PCI		12 (63.2)	12 (66.7)	0.548	14 (63.6)	15 (60.0)	0.502
Prior CABG		3 (15.8)	4 (22.2)	0.490	7 (31.8)	10 (40.0)	0.762
CHF		3 (15.8)	4 (22.2)	0.693	4 (18.2)	3 (12.0)	0.450
LVEF, %		55.0±12.2	51.9±11.3	0.720	52.1±9.5	50.6±9.3	0.603
Medications	Aspirin	16 (84.2)	13 (72.2)	0.447	18 (81.8)	22 (88.0)	0.690
	β-blocker	15 (78.9)	15 (83.3)	0.553	18 (81.8)	20 (80.0)	0.586
	ACEi/ARB	16 (84.2)	18 (100.0)	0.230	12 (54.5)	15 (60.0)	0.549
	High-dose statin	18 (94.7)	18 (100.0)	0.528	21 (95.4)	21 (84.0)	0.352
	Nitrates	7 (36.8)	7 (38.9)	0.583	6 (27.3)	9 (36.0)	0.279
	Proton pump inhibitor	4 (21.1)	3 (16.7)	0.532	6 (27.3)	13 (52.0)	0.136
	Calcium channel blocker	7 (36.8)	10 (55.6)	0.330	12 (54.5)	11 (44.0)	0.564
	Oral hypoglycaemic	3 (15.8)	9 (50.0)	0.038	5 (22.7)	12 (48.0)	0.127
	Insulin	5 (26.3)	8 (44.4)	0.336	6 (27.3)	6 (24.0)	0.963

Values are mean±SD or n (%). ACEi: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blockers; BMI: body mass index; CABG: coronary artery bypass graft; CAD: coronary artery disease; CHF: congestive heart failure; HPR: high platelet reactivity; LVEF: left ventricular ejection fraction; MI: myocardial infarction; NPR: normal platelet reactivity; PCI: percutaneous coronary intervention; SD: standard deviation; TIA: transient ischaemic attack

PHARMACODYNAMIC FINDINGS

There were no differences in baseline PRU levels between patients randomised to evolocumab versus placebo in the HPR (239.5±26.7 vs 248.5±37.5; p=0.419) and NPR (144.9±38.3 vs 148.8±29.7; p=0.704) cohorts. Baseline PD findings are summarised in **Supplementary Table 5**. PRU levels were significantly reduced in patients randomised to evolocumab compared to placebo at 14 days in the HPR (218.2±29.7 vs 246.6±35.2; p=0.017; mean difference: 28.4 [95% confidence interval {CI}: 5.4 to 51.4]), but not in the NPR (141.2±42.8 vs 148.2±41.7; p=0.578; mean difference: 7.0 [95% CI: −18.1 to 32.1]) cohort **(Figure 3, Supplementary Table 6)**. At 30 days, despite a numerical reduction, there were no significant differences in PRU levels, the primary endpoint of the study, between evolocumab and placebo in the HPR (219.3±38.3 vs 240.9±51.8 PRU; p=0.161; mean difference: 21.6 [95% CI: −9.0 to 52.3]) or NPR (141.5±54.3 vs 158.6±40.8 PRU; p=0.229; mean difference: 17.2 [95% CI: −11.7 to 46.0]) cohorts **(Figure 3, Table 2)**.

No significant differences in PD profiles between evolocumab and placebo were observed using other assays, including LTA with different agonists (AA, ADP, collagen and TRAP) and TEG, with the exception of MPA% with ADP 5 µM (evolocumab: 27.6±14.7 vs placebo: 37.9±18.9; p=0.046) and TEG ADP MA HPR rate (evolocumab: 63.6% vs placebo: 91.7%; p=0.021) in the NPR cohort at 30 days **(Figure 4, Table 2, Supplementary Table 6)**.

CORRELATION AND EXPLORATORY ANALYSES AT 30-DAY FOLLOW-UP

At baseline, in the HPR and NPR cohorts, there were moderate to high correlations between PRU and baseline levels of LDL-C (r_s=0.641; p=0.001 and r_s=0.410; p=0.005, respectively); there was no correlation with circulating PCSK9 levels in any cohort. At 14 and 30 days, there was a moderate correlation between PRU and LDL-C in the HPR cohort (r_s=0.453; p=0.008 and r_s=0.356; p=0.033, respectively), without a correlation in the NPR cohort (r_s=0.175; p=0.245 and r_s=0.245; p=0.101, respectively). In the

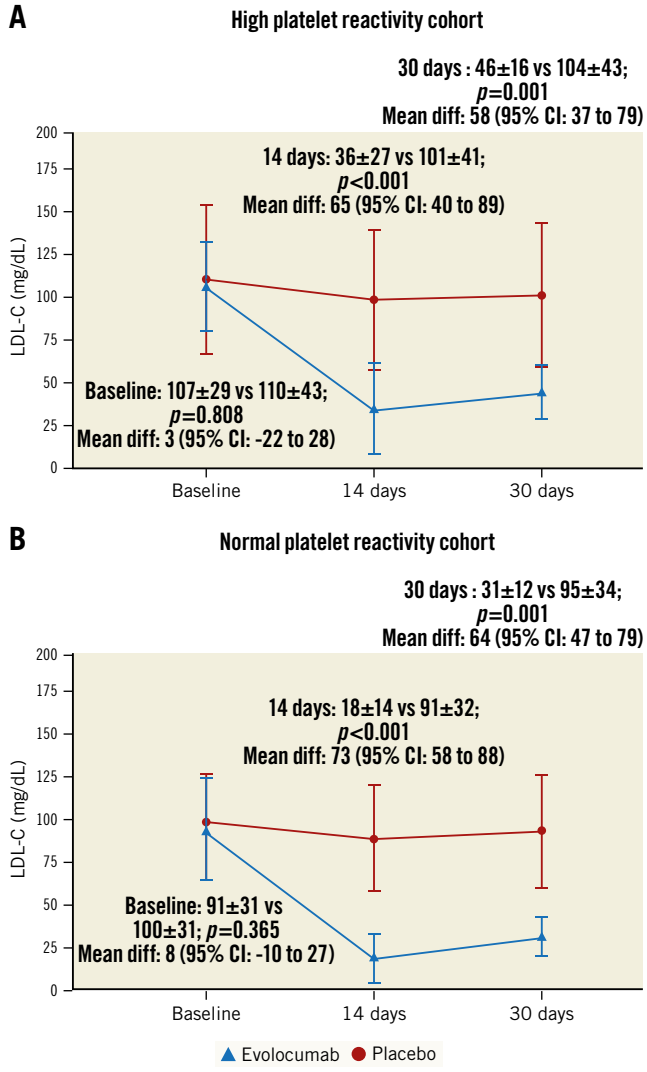


Figure 2. *LDL-C levels according to randomised drug and platelet reactivity status. Differences in LDL-C levels between evolocumab and placebo in the high platelet reactivity (HPR, n=36) and normal platelet reactivity cohorts (NPR, n=46) are reported as mean±SD and mean difference with 95% confidence intervals (95% CI). Dashed line represents ≥70 mg/dL. Values are expressed as mean. Error bars indicate SD. LDL-C: low-density lipoprotein cholesterol; SD: standard deviation*

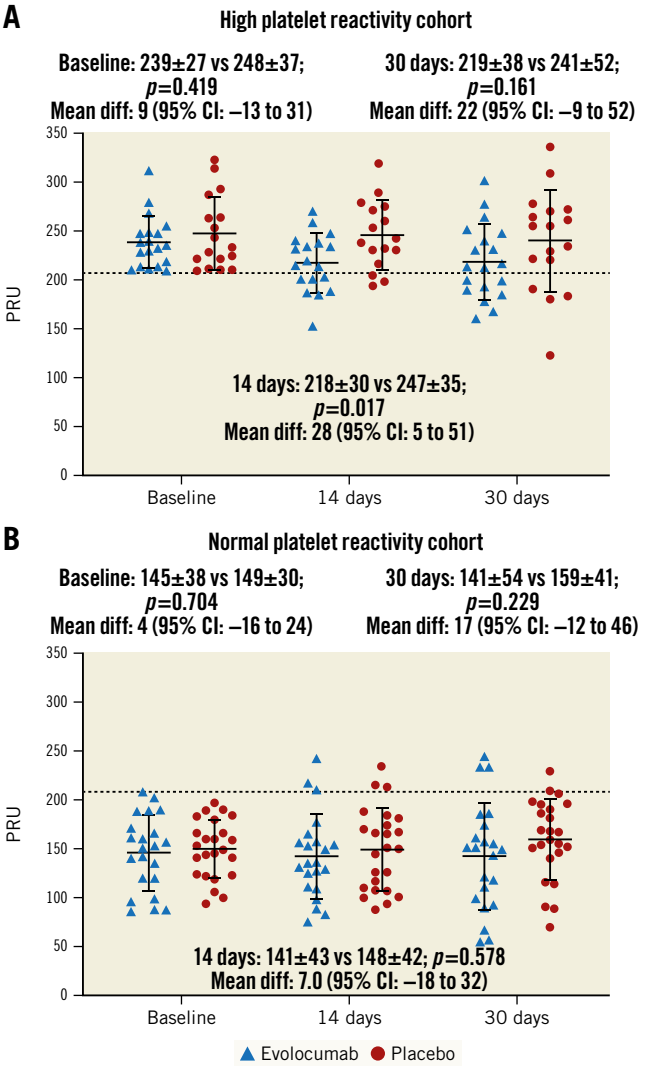


Figure 3. *Individual values of PRU according to randomised drug and platelet reactivity status. Differences in $P2Y_{12}$ reactivity units (PRU) between evolocumab and placebo in the high platelet reactivity (HPR, n=36) and normal platelet reactivity cohorts (NPR, n=46) are reported as mean±SD and mean difference with 95% confidence intervals (95% CI). Dashed line represents the predefined cut-off of HPR, >208 PRU. Solid lines with error bars indicate mean and SD. SD: standard deviation*

HPR cohort, at 14 days, there was a moderate correlation between ΔLDL-C and ΔPRU (r_s=0.361; p=0.039), but not at 30 days (r_s=0.104; p=0.545). In the NPR cohort, there were no correlations between ΔLDL-C and ΔPRU, neither at 14 days (r_s= −0.111; p=0.463) nor 30 days (r_s= −0.017; p=0.908).

At 30 days, among patients with baseline circulating levels of PCSK9 above the median, in the HPR cohort, there was a significant difference in PRU between evolocumab and placebo (209.7±33.3 vs 253.9±42.0; p=0.020), without a difference in the NPR cohort (162.3±55.1 vs 165.6±39.7; p=0.880). Among patients with baseline circulating levels of PCSK9 below the median, there were no differences in PRU between evolocumab and placebo in the HPR (227.8±44.8 vs 226.4±60.6; p=0.960) and NPR (130.5±46.2 vs 150.5±44.1; p=0.300) cohorts **(Figure 5, Supplementary Table 7, Supplementary Table 8).**

At 30 days, among carriers of a CYP2C19 LOF allele in the HPR cohort, there were significant differences in PRU levels between evolocumab compared to placebo (214.5±30.4 vs 261.8±45.8; p=0.027); there were no differences in the NPR cohort (176.8±51.5 vs 173.8±25.5; p=0.907). In non-carriers of a CYP2C19 LOF allele, there were no significant differences in PRU levels between evolocumab and placebo in the HPR cohort (225.3±52.4 vs 240.8±45.1; p=0.523) or NPR (127.1±51.5 vs 161.5±42.3; p=0.059) cohort **(Figure 5, Supplementary Table 9, Supplementary Table 10)**. Full

Table 2. Pharmacodynamic findings at 30-day follow-up.*

	HPR (n=36)			NPR (n=46)		
	Evolocumab (n=19)	Placebo (n=17)	*p*-value	Evolocumab (n=22)	Placebo (n=24)	*p*-value
VerifyNow PRU	219.3±38.3	240.9±51.8	0.161	141.5±54.3	158.6±40.8	0.229
HPR status	11 (57.9)	13 (76.5)	0.238	3 (13.6)	1 (4.2)	0.255
ADP 20 µL	61.2±13.0	61.4±12.2	0.963	42.7±17.3	53.7±20.5	0.057
HPR status	10 (52.6)	9 (52.9)	0.985	4 (19.0)	8 (33.3)	0.280
ADP 5 µL	43.5±14.3	48.4±14.5	0.311	27.6±14.7	37.9±18.9	0.046
HPR status	8 (42.1)	8 (47.1)	0.765	3 (14.3)	7 (29.2)	0.231
AA 1 mM†	2.0 (1.0-3.5)	34.0 (1.5-69.5)	0.260	1.0 (0.0-3.0)	2.0 (0.0-42.0)	0.308
TRAP 15 µM	73.9±8.8	75.1±10.4	0.707	66.4±17.6	72.1±10.4	0.204
Collagen 3 µg/mL†	53.8±24.2	65.3±14.3	0.127	36.9±29.9	44.5±30.5	0.446
TEG HKH MA	66.1±2.7	65.8±2.4	0.777	63.5±4.9	63.6±3.7	0.949
TEG ADP MA	63.3±11.3	62.8±4.1	0.838	51.2±15.5	56.9±9.4	0.145
HPR status	17 (89.5)	17 (100.0)	0.169	14 (63.6)	22 (91.7)	0.021

Values are mean±SD, median (interquartile range), or n (%). *Calculated in the pharmacodynamic population. †Calculated only in patients on concomitant aspirin treatment. AA: arachidonic acid; ADP: adenosine diphosphate; HKH: kaolin with heparinase; HPR: high platelet reactivity; MA: maximum amplitude; NPR: normal platelet reactivity; PRU: $P2Y_{12}$ reactivity units; SD: standard deviation; TEG: thromboelastography coagulation analyser; TRAP: thrombin receptor activator peptide

results of the exploratory analyses are reported in **Figure 5** and **Supplementary Table 7-Supplementary Table 13**.

Discussion

The present investigation aimed to assess the PD effects of evolocumab versus placebo on profiles of platelet reactivity in ASCVD patients treated with clopidogrel and a maximally tolerated dose of a statin. The key findings of this study can be summarised as follows: 1) compared to placebo, evolocumab was associated with a significant reduction in LDL-C levels at 14- and 30-day follow-up; 2) in patients with HPR, although at 14 days evolocumab was associated with a significant reduction in PRU, this was not confirmed at 30 days where the reduction did not reach statistical significance (primary endpoint); 3) in patients with NPR, evolocumab compared to placebo was not associated with a significant reduction in PRU at 14 and 30 days; 4) our overall PD findings were corroborated by a number of assays; and 5) exploratory analyses suggest that patients with HPR and elevated PCSK9 levels or who are carriers of CYP2C19 LOF alleles may experience an enhanced degree of reduction in platelet reactivity with evolocumab **(Central illustration)**.

Although clopidogrel therapy has been shown to be efficacious in different high-risk settings of patients with AVSCD, rates of ischaemic recurrences remain elevated despite this treatment regimen[2,3]. This can be, in part, attributed to the high interindividual variability in responses to clopidogrel[2,3]. Intuitively, the use of the more potent $P2Y_{12}$ receptor inhibitors, prasugrel and ticagrelor, represents a potential treatment option[17]. However, the risk of bleeding complications with their prolonged use has limited their broad uptake in clinical practice for long-term secondary prevention. The pleiotropic effects on thrombosis associated with lipid-lowering therapies, in particular statins, have been the subject of extensive research[8]. In statin-naïve patients with stable CAD and HPR, treatment with high-dose atorvastatin in addition to double-dose clopidogrel reduced platelet reactivity significantly more than double-dose clopidogrel alone[4]. To date, the exact biological mechanisms involved in statin-mediated modulation of platelet function are not fully understood, although likely attributed to both their lipid-lowering and non-lipid-related effects[18]. In fact, there is increasing evidence that circulating lipoproteins affect platelet function. In particular, LDL-C, VLDL, and especially oxidised LDL-C are atherogenic lipoproteins and increase platelet activation[9]. Also, familial hypercholesterolaemia is associated with increased platelet activity, such as hyperaggregability, and the application of a lipid-lowering drug resulted in a reduction of platelet reactivity[19]. It may therefore be argued that achieving intensified reduction of circulating lipoproteins may have an even greater impact on modulating platelet function than that observed with statins alone[20].

This is the first randomised controlled study assessing the role of intense LDL-C reduction with evolocumab on platelet function in ASCVD patients on clopidogrel treatment. In the HPR cohort, there was a significant reduction in PRU at 14 days which, however, was not sustained at 30 days, despite the numerical reduction. It may be argued that such findings could be attributed to the different magnitude of the LDL-C lowering effect over time. In fact, in our study evolocumab was associated with intense LDL-C reduction compared to placebo at 14- and 30-day follow-up, but this reduction was more pronounced at 14 than at 30 days. Consistent with our study, dose-finding studies of evolocumab also showed that 420 mg monthly was associated with an intense LDL-C level reduction (~70% reduction from baseline) at 2 weeks with a slight increase over 4 to 8 weeks. Instead, a dosing regimen of 140 mg every 2 weeks was associated with a less intense initial LDL-C level reduction

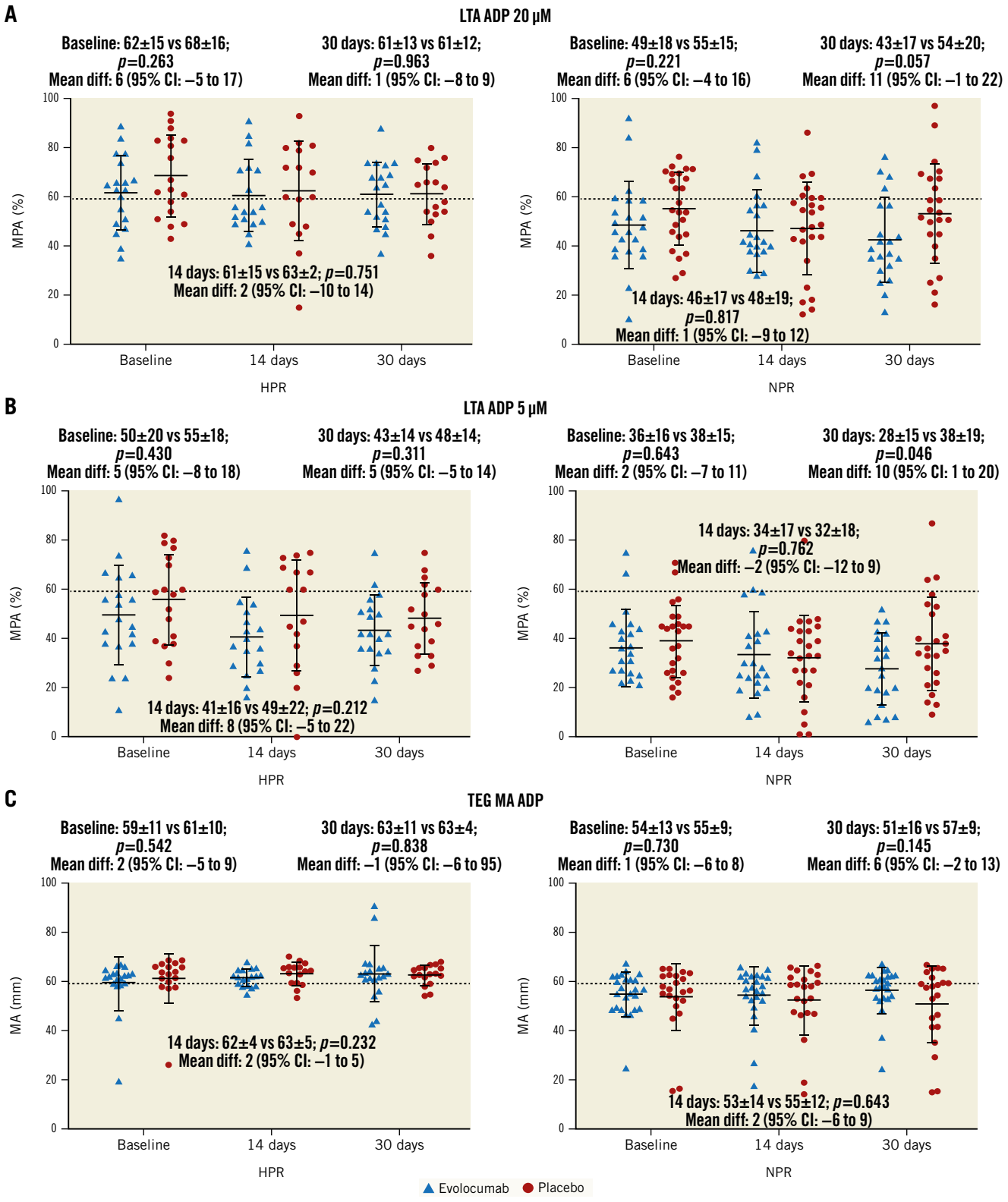


Figure 4. *Individual values of platelet reactivity measured by different assays according to randomised drug and platelet reactivity status. Differences in platelet reactivity tests between evolocumab and placebo in the high platelet reactivity (HPR, n=36) and normal platelet reactivity cohorts (NPR, n=46) are reported as mean±SD and mean difference with 95% confidence intervals (95% CI). A) Light transmission aggregometry (LTA) adenosine diphosphate (ADP) 20 µM is reported as maximum platelet aggregation percentage (MPA%). B) LTA ADP 5µM is reported as maximum platelet aggregation percentage (MPA%). C) Thromboelastography coagulation analyser (TEG) thrombin reported as maximum amplitude (MA) in mm. Dashed lines represent the predefined cut-off of HPR in the respective test. A and B: >59 MPA%; C: >47 mm. Solid lines with error bars indicate mean and SD. SD: standard deviation*

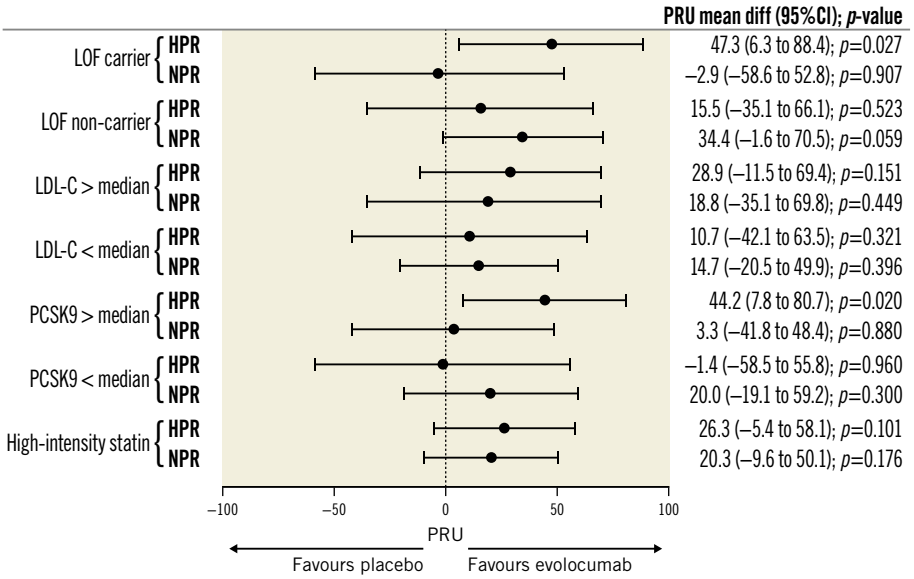


Figure 5. *Mean difference in PRU values according to HRP and NPR cohort and randomised drug in different exploratory analyses. Differences in PRU values between evolocumab and placebo are reported as mean±SD and mean difference with 95% confidence intervals (95% CI). >median denotes above the median of plasma levels and <median denotes below. High-intensity statin was defined as atorvastatin ≥40 mg or rosuvastatin ≥20 mg. HPR: high platelet reactivity; LDL-C: low-density lipoprotein cholesterol; LOF: loss-of-function allele; NPR: normal platelet reactivity; PCSK9: proprotein convertase subtilisin/kexin type 9; PRU: $P2Y_{12}$ reactivity units; SD: standard deviation*

(~55% reduction from baseline) but a more steady effect at 4 to 8 weeks[21]. These patterns have also been found in PD simulation models comparing different dosing regimens[22]. Our assumption is supported by findings from an *in vitro* study showing that atorvastatin dose-dependently inhibited platelet activation[22,23]. In addition, in the HPR cohort, at 14-day follow-up, there was a moderate correlation between ΔLDL-C and ΔPRU, but not at 30 days. Conversely, the reduction in PRU in the NPR cohort was very modest and non-significant. Overall, although our study suggests the potential for a PCSK9 inhibitor to modulate profiles of clopidogrel-induced platelet inhibition with an effect that depends on the magnitude of further LDL-C reduction, this was not fully supported by our investigation. Indeed, a treatment effect is more likely to occur among patients with HPR which is no longer observed or may be negligible if platelets are already adequately inhibited by clopidogrel.

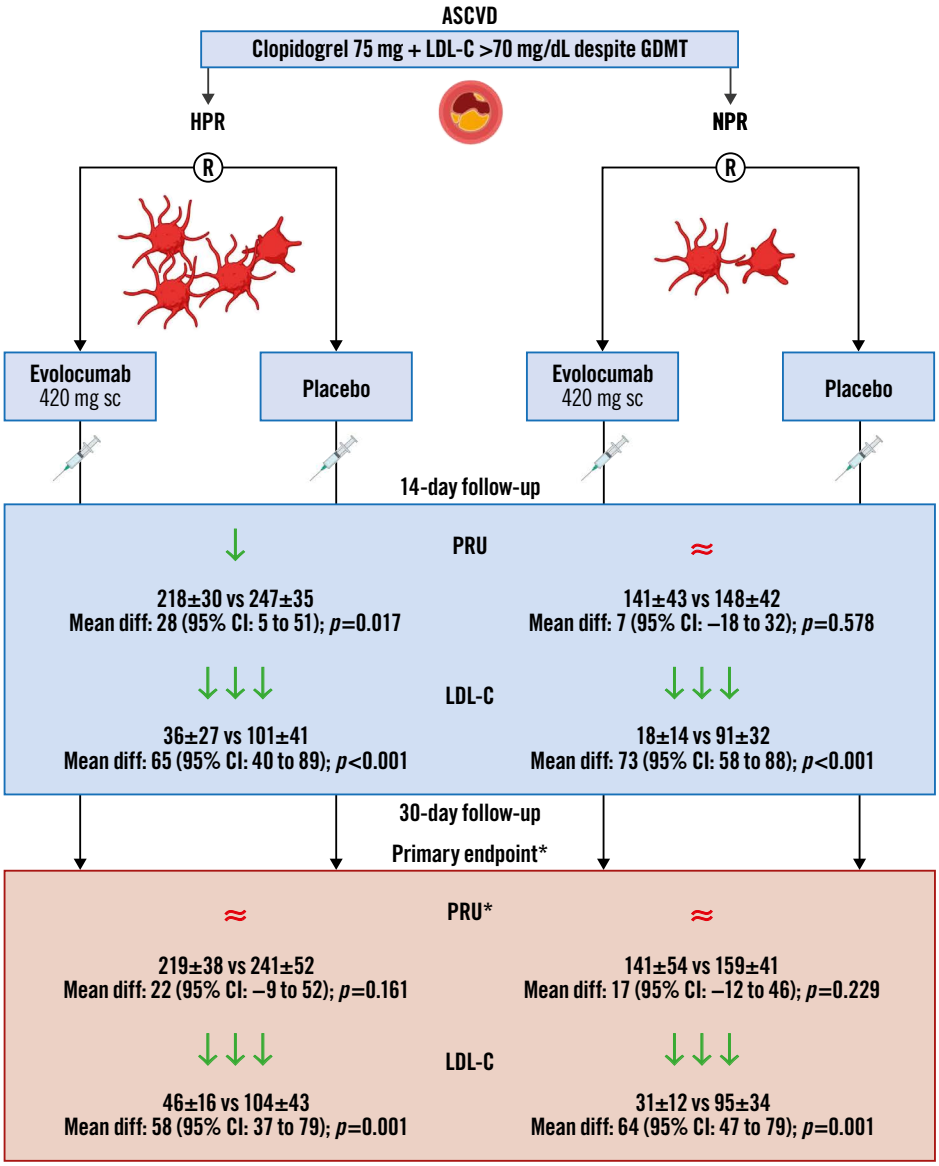
In line with prior investigations, we found a moderate correlation between LDL-C levels and PRU at baseline[8]. However, we did not find a correlation between PCSK9 levels and PRU. This could be related to the use of aspirin in most of our patients, which may modulate PCSK9 levels[24]. In fact, preclinical models have shown that circulating PCSK9 directly enhances platelet activation and *in vivo* thrombosis by binding to platelet CD36 and that this effect can be mitigated by the use of aspirin or PCSK9 inhibitors[24,25]. In a prospective observational study conducted in ACS patients undergoing percutaneous coronary intervention treated with more potent $P2Y_{12}$ inhibitors (i.e., prasugrel or ticagrelor), which are known to be associated with low platelet reactivity, elevated PCSK9 levels have been suggested to be associated with increased platelet reactivity and higher rates of ischaemic events[8]. However, in our study we enrolled patients with stable ACSVD while on clopidogrel, and we excluded those with low platelet reactivity. Overall, our data, rather than excluding the role of circulating PCSK9 in platelet reactivity in ASCVD, may underscore the gaps in knowledge of these complex biological pathways.

Exploratory analyses showed no differences in most PD outcomes assessed according to baseline LDL-C, baseline circulating PCSK9 levels, or concomitant treatment with high-dose statins. However, we found significant differences in PRU values in favour of evolocumab over placebo in the HPR cohort among carriers of CYP2C19 LOF alleles and patients with baseline levels of PCSK9 above the median. The reasons for these findings are unknown and may be attributed to play of chance. Nevertheless, these findings may be considered as hypothesis-generating for future research.

Limitations

Some limitations should be acknowledged. First, the PD nature of this study does not allow us to make any definitive conclusions on the clinical impact of our findings. Second, only one dosing regimen of evolocumab (420 mg subcutaneous monthly) was studied with a follow-up at 30 days. Third, our study excluded patients with low platelet reactivity and arbitrarily assumed a similar treatment effect in both the NPR and HPR cohorts. The rationale for this was that it would be unlikely for a treatment effect to be observed in these subjects, which is supported by our study findings that only the patients with HPR

CENTRAL ILLUSTRATION Impact of evolocumab on the pharmacodynamic profiles of clopidogrel in patients with atherosclerotic cardiovascular disease.



*The green arrow (↓) denotes a statistically significant reduction, and the red approximation symbol (≈) denotes no statistical difference. Values are reported as mean±SD and mean difference (95% confidence interval). ClinicalTrials.gov: NCT03096288. *The primary endpoint was the difference in PRU at 30 days. ASCVD: atherosclerotic cardiovascular disease; CI: confidence interval; LDL-C: low-density lipoprotein cholesterol; GDMT: guideline-directed medical therapy; PRU: $P2Y_{12}$ reactivity units; R: randomisation; sc: subcutaneous; SD: standard deviation*

were those with a PD efficacy signal. Furthermore, at the time of the trial design, there were no specific data on the treatment effects of lipid-lowering therapies in NPR patients with different profiles of clopidogrel response. Fourth, a type II error due to sample size cannot be ruled out. Whether a larger sample size would have resulted in significant differences in platelet reactivity between evolocumab and placebo is unknown and would warrant further investigation. Of note, in our study we found a mean difference in PRU of 28 in the HPR cohort and 10 in the NPR at 14 days, and one of 22 in the HPR and 17 in NPR at 30 days, respectively. Even though our study was powered to detect a difference of 30 PRU, an individual patient data meta-analysis involving over 3,000 patients reported that for every 10-unit increase in PRU value, there is a 4% increase in serious adverse cardiac events[26]. Moreover, our study was conducted among patients on a maximally tolerated dose of statins, which

have previously been shown to enhance clopidogrel-induced platelet inhibition[4]. Thus, it may be argued that evolocumab could have led to a greater magnitude of clopidogrel-induced platelet inhibition in stain-naïve patients. However, the effects of evolocumab on profiles of platelet reactivity among statin-naïve patients treated with clopidogrel remain unknown. Fifth, this trial was designed to assess the PD effects of evolocumab in stable ACSVD patients treated with clopidogrel. Therefore, the results cannot be extended to ACS patients or patients treated with other oral $P2Y_{12}$ inhibitors. However, ongoing randomised controlled trials are evaluating the role of PCSK9 inhibition in ACS patients[27,28]. Ultimately, despite randomisation, there were minor imbalances between the evolocumab and placebo groups in the HPR cohort (family history of CAD and oral hypoglycaemic drugs). However, as these imbalances were observed *post hoc* and are not correlated with the primary endpoint, no adjustments to the primary analysis were made[29].

Conclusions

In patients with ASCVD on clopidogrel treatment and with LDL-C levels above target despite a maximally tolerated statin dose, evolocumab was not associated with a significant reduction in platelet reactivity compared with placebo. Among patients with HPR, at 14 days, evolocumab was associated with a significant reduction in platelet reactivity, but this was not sustained at 30 days. Patients with NPR had non-significant reductions in platelet reactivity throughout the study. In both cohorts, evolocumab was associated with an intense reduction in LDL-C levels at 14 and 30 days.

Impact on daily practice

High on-treatment platelet reactivity while on clopidogrel is a marker of thrombotic risk. Optimising clopidogrel-induced antiplatelet effects is therefore an unmet clinical need. While potent $P2Y_{12}$ inhibitors can overcome HPR, the increased risk of bleeding limits their long-term use. LDL-C reduction using statins enhances clopidogrel-induced platelet inhibition. However, in this study, intense LDL-C reduction with PCSK9 inhibition with evolocumab did not significantly decrease platelet reactivity compared to a placebo among clopidogrel-treated patients on statin therapy. Nevertheless, among HPR patients, when LDL-C levels were the lowest and certain individual profiles were present, results suggested a potential treatment effect on platelet function with evolocumab. These observations support potential avenues of clinical investigation aimed at identifying patient cohorts in whom pleiotropic effects associated with evolocumab use may be observed.

Guest editor

This paper was guest edited by Franz-Josef Neumann, MD; *Department of Cardiology and Angiology II, University Heart Center Freiburg - Bad Krozingen, Bad Krozingen, Germany.*

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Conflict of interest statement

F. Franchi declares that he has received payment as an individual for consulting fees or honoraria from AstraZeneca, Bayer and Sanofi; and institutional payments for grants from PLx Pharma and The Scott R. MacKenzie Foundation. D.J. Angiolillo declares that he has received consulting fees or honoraria from Abbott, Amgen, AstraZeneca, Bayer, Biosensors, Boehringer Ingelheim, Bristol-Myers Squibb, Chiesi, Daiichi Sankyo, Eli Lilly, Haemonetics, Janssen, Merck, Novartis, PhaseBio, PLx Pharma, Pfizer, and Sanofi; he also declares that his institution has received research grants from Amgen, AstraZeneca, Bayer, Biosensors, CeloNova, CSL Behring, Daiichi Sankyo, Eisai, Eli Lilly, Gilead, Idorsia, Janssen, Matsutani Chemical Industry Co., Merck, Novartis, and the Scott R. MacKenzie Foundation. The other authors have no conflicts of interest to declare. The Guest Editor reports lecture fees paid to his institution from Amgen, Bayer Healthcare, Biotronik, Boehringer Ingelheim, Boston Scientific, Daiichi Sankyo, Edwards Lifesciences, Ferrer, Pfizer, and Novartis; consultancy fees paid to his institution from Boehringer Ingelheim; and grant support from Bayer Healthcare, Boston Scientific, Biotronik, Edwards Lifesciences, GlaxoSmithKline, Medtronic, and Pfizer.

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Supplementary data

Supplementary Appendix 1. Study selection criteria.

Supplementary Appendix 2. Laboratory testing.

Supplementary Appendix 3. Description of laboratory assays.

Supplementary Table 1. Baseline laboratory assessment and lipid profile.

Supplementary Table 2. CYP2C19 genetics according to platelet reactivity status and randomised drug.

Supplementary Table 3. Adverse events.

Supplementary Table 4. Lipid profile findings at 14 and 30 days.

Supplementary Table 5. Pharmacodynamic findings at baseline.

Supplementary Table 6. Pharmacodynamic findings at 14-day follow-up.

Supplementary Table 7. Pharmacodynamic and lipid profile findings at 30-day follow-up according to baseline circulating PCSK9 levels in the HPR cohort.

Supplementary Table 8. Pharmacodynamic and lipid profile findings at 30-day follow-up according to baseline circulating PCSK9 levels in the NPR cohort.

Supplementary Table 9. Pharmacodynamic and lipid profile findings at 30-day follow-up according to CYP2C19 LOF alleles in the HPR cohort.

Supplementary Table 10. Pharmacodynamic and lipid profile findings at 30-day follow-up according to CYP2C19 LOF alleles in the NPR cohort.

Supplementary Table 11. Pharmacodynamic and lipid profile findings at 30-day follow-up according to baseline LDL-C levels in the HPR cohort.

Supplementary Table 12. Pharmacodynamic and lipid profile findings at 30-day follow-up according to baseline LDL-C levels in the NPR cohort.

Supplementary Table 13. Pharmacodynamic and lipid profile findings at 30 days follow-up in patients treated with high-intensity dose statin at baseline.

The supplementary data are published online at: https://eurointervention.pcronline.com/doi/10.4244/EIJ-D-22-00719

Supplementary data

Supplementary Appendix 1. Study selection criteria.

Inclusion criteria:

1. Patients with ASCVD, defined as prior ACS, history of myocardial infarction, stable or unstable angina, coronary or other arterial revascularization, stroke, transient ischemic attack, or PAD presumed to be of atherosclerotic origin.
2. On therapy with clopidogrel (75mg od), with or without low-dose aspirin (81mg od), as per standard-of-care for at least 30 days.
3. HPR, defined as $P2Y_{12}$ reaction units (PRU) > 208 by VerifyNow $P2Y_{12}$, or normal platelet reactivity (NPR), defined as PRU between 85 and 208.
4. Fasting LDL-cholesterol ≥70 mg/dL or a non-high-density lipoprotein cholesterol (HDL-C) of ≥100 mg/dL after ≥2 weeks of optimized stable lipid-lowering therapy with maximally tolerated dose of statin, which would ideally include a high-intensity statin, but must be at least moderate intensity statin (i.e. atorvastatin 20 mg or equivalent, with or without ezetimibe. Maximal tolerated dose was defined based on patient clinical history (no statin re-challenge was performed).
5. Age ≥ 18 years old.

Exclusion criteria:

1. On treatment with any oral anticoagulant (vitamin K antagonists, dabigatran, rivaroxaban, apixaban, edoxaban).
2. On treatment with any antiplatelet agent other than aspirin and clopidogrel in the past 14 days.
3. Use of PCSK9 inhibitors in the past 90 days
4. Creatinine clearance <30 mL/minute.
5. Known severe hepatic impairment.
6. History of a serious hypersensitivity reaction to Evolocumab
7. Hemodynamic instability
8. Pregnant and breastfeeding women [women of childbearing age must use reliable birth control (i.e. oral contraceptives) while participating in the study].

Supplementary Appendix 2. Laboratory testing.

Peripheral venous blood samples were drawn through a short venous catheter inserted into a forearm vein and collected in citrate, EDTA, and serum tubes as appropriate for assessments. The first 2-4 mL of blood was discarded to avoid spontaneous platelet activation. Blood sampling was performed at 3 time points as indicated above in the study design section. Various PD assays were performed as described below:

1. VerifyNow $P2Y_{12}$
2. Light transmittance aggregometry (LTA)
3. Thrombelastograph Coagulation Analyzer TEG 6s Series system (CORA® system)

Supplementary Appendix 3. Description of laboratory assays.

VerifyNow (VN) PRU

The VerifyNow System is a turbidimetric based optical detection system which measures platelet induced aggregation as an increase in light transmittance (Werfen, MA, USA) and was utilized according to manufacturer's instructions, as previously described. The assay is based on microbead agglutination and uses specific reagents for the pathways of interest. The VN-PRU assay, by combining ADP+PGE1, measures changes in platelet function specific to $P2Y_{12}$ receptor inhibitors. The assay is based upon the ability of activated platelets to bind fibrinogen. Fibrinogen-coated microparticles aggregate in proportion to the number of GP IIb/IIIa receptors expressed. Microbead aggregation is more rapid and reproducible if platelets are activated. Therefore, the reagents are incorporated into the assay channel to induce platelet activation without fibrin formation. Light transmittance increases as activated platelets bind and aggregate fibrinogen-coated beads. The instrument measures this change in optical signal and reports results in $P2Y_{12}$ Reaction Units (PRU). HPR was defined as PRU>208 and NPR as PRU 85-208.

Light transmittance aggregometry (LTA)

Platelet aggregation was performed using LTA according to standard protocols. Blood was collected in citrated (3.2%) tubes. LTA was assessed using platelet rich plasma (PRP) by the turbidimetric method in a 2-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp., Havertown) as previously described. Platelet agonists included arachidonic acid (AA, 1 mM), collagen (3µg/ml), ADP (5 and 20 µM), and TRAP (15 µM). PRP was obtained as a supernatant after centrifugation of citrated blood at 1000 rpm for 10 minutes. The isolated PRP was kept at 37° C before use. Platelet poor plasma (PPP) was obtained by a second centrifugation of the blood fraction at 2800 rpm for 10 minutes. Light transmission was adjusted to 0% with the PRP and to 100% for the PPP for each measurement. Curves were recorded for 6 minutes and platelet aggregation was determined as the maximal percent change (MPA) in light transmittance from baseline using PPP as a reference. HPR was defined as MPA with 20 µM ADP > 59% and MPA with 5 µM ADP > 46%.

TEG 6s Series system (CORA® system)

The TEG 6s system (Haemonetics Corporation, Braintree, MA, USA) was used according to manufacture instructions. In brief, this is a new generation portable thrombelastography technology able to evaluate all phases of hemostasis, including time to clot formation, rate of clot formation, strength of clot and residual clot strength due to antiplatelet drugs, rate of clot lysis. Disposable assay cartridges contain all of the components necessary to allow the analyzer to prepare samples and perform hemostasis tests. The analyzer automatically draws the blood into the active area of the cartridge, meters the exact amount required for the test, and mixes it with the reagents spotted in the cartridge. The analyzer then monitors the harmonic motion of a pendant drop of blood in response to external vibration. As the sample transitions from a liquid state to a gel-like state during clotting, the modulus of elasticity and resonant frequency increase. The instrument measures these variations in resonant frequency during clotting and lysis. The results are displayed in a table and on a graphical tracing that reflects a hemostasis profile of clot formation. The resulting hemostasis profile is a measure of the time it takes for the first measurable clot to be formed, the kinetics of clot formation, the strength of the clot, and the breakdown of the clot, or fibrinolysis. In particular, the

PlateletMapping Cartridge are used to assess platelet function in patients who have received platelet inhibiting drugs. The PlateletMapping assay consists of a set of agonists, ADP and AA platelet agonists together with ActivatorF, which can measure the inhibition of platelet function. This assay specifically determines the MA (Maximum Amplitude, a measure of clot strength) and the reduction in MA due to antiplatelet therapy and reports it as a percentage of reduction in clot strength. The assay uses AA and ADP agonists to generate test results that reflect the inhibiting effects of antiplatelet agents such as TxA2 Inhibitors (e.g., aspirin) and ADP $P2Y_{12}$ inhibitors (e.g., clopidogrel). Since thrombin (present in blood samples) is the primary and most potent activator of platelets, its activity must be inhibited with heparin so that the platelet activating effects of ADP and AA can be measured. Since thrombin has been rendered inactive by heparin, activatorF is used to replace thrombin's role in the conversion of fibrinogen to fibrin and Factor XIII to Factor XIIIa. Thus, with this cross-linked fibrin network as the foundation, additional clot strength due to platelet-fibrin bonding related to ADP and AA platelet receptor activation can be measured. The HKH (Kaolin with Heparinase) reagent, a combination of kaolin and heparinase, generates test data for the uninhibited MA resulting from thrombin activation of the blood sample, while the heparinase neutralizes the effects of heparin. The HKH test also provides measures of R (Reaction time; the amount of time between the start of the test and the beginning of coagulation), K (the speed of formation of the clot from R time to a specific clot strength), Angle (the speed of clot strengthening), LY30 (Percent lysis 30 minutes after MA is finalized) and MA parameters; The activatorF test provides the contribution of fibrin to the overall strength of the clot. This test value is used in the calculation of aggregation/inhibition for MA ADP and MA AA. The AA and ADP test provide measures of MA, percent inhibition and percent aggregation. HPR was defined as MA ADP > 47 mm.

Genetic testing

Spartan RX rapid genotyping: Spartan RX (Spartan Bioscience Inc., Ontario, Canada) is the rapid genotyping system determining the CYP2C19 (*1,*2,*3,*17) allele status within 1 hour. This test consists of four separate steps intended to be done in less than 8 minutes: acquisition of a buccal swab; insertion of the swab into the cartridge; insertion of the reaction solution into the device; and analysis of CYP2C19 genotype triggered by a button on the device.

Lipid profile and PCSK9 levels

Blood for the evaluation of the effects on lipids was drawn at the same time points as described above for PD measurements. Cholesterol measures included a standard lipid profile and lipoprotein(a) [Lp(a)], which was measured by the central laboratory of our institution according to standard protocols. At baseline, blood samples were also collected for measurement of PCSK9 serum levels (R & D Systems, Minnepolis, USA), which were measured by ELISA as previously described.

Supplementary Table 1. Baseline laboratories assessment and lipid profile.[*]

	HPR (n=37)			NPR (n=47)		
	Evolocumab (n=19)	**Placebo (n=18)**	**Evolocumab (n=19)**	**Placebo (n=18)**	**Evolocumab (n=19)**	**Placebo (n=18)**
Hemoglobin, g/dL	12.4±1.4	12.2±1.6	0.620	14.1±1.7	14.0±1.3	0.817
Hematocrit, %	38.2±4.1	37.6±4.6	0.662	43.4±4.3	42.1±3.4	0.294
Platelets, x1,000/mm^3	250.8±73.2	229.2±47.5	0.307	219.5±60.7	257.0±74.0	0.068
Creatinine, mg/dL	1.1±0.3	1.0±0.3	0.476	1.0±0.3	1.0±0.2	0.831
eGFR, mL/min/1.73m^2	95.2±42.2	94.9±32.8	0.982	95.8±30.4	108.7±33.6	0.178
Total cholesterol, mg/dL	183.7±32.4	189.2±47.1	0.704	169.2±33.1	177.8±32.3	0.297
Triglycerides, mg/dL	113.2±53.1	105.8±60.2	0.698	122.7±55.7	146.3±87.9	0.289
HDL-C, mg/dL	50.6±13.5	54.6±19.0	0.473	47.7±13.6	48.2±15.8	0.904
LDL-C, mg/dL	107.3±29.1	110.2±43.1	0.808	91.2±30.5	99.6±31.3	0.365
Non-HDL-C, mg/dL	129.9±34.4	126.8±42.0	0.815	115.6±33.8	126.6±34.6	0.283
Lp(a), mg/dL	185.3±151.1	122.7±97.4	0.154	174.7±157.1	152.1±125.9	0.597
PCSK9, ng/mL	397.9±94.9	450.9±127.2	0.170	426.2±96.2	419.9±146.4	0.869

[*]Calculated in the safety population. Values are mean±SD. eGFR=estimated glomerular filtration rate; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; PCSK9=proprotein convertase subtilisin/kexin type 9.

Supplementary Table 2. CYP2C19 genetics according to platelet reactivity status and randomised drug.[*]

	HPR (n=37)			NPR (n=47)		
	Evolocumab (n=19)	**Placebo (n=18)**	**P-value**	**Evolocumab (n=22)**	**Placebo (n=25)**	**P-value**
CYP2C19 Genetics			0.418			0.318
*1/*1 no LOF	5 (26.3)	7 (38.9)		9 (40.9)	11 (44.0)	
*1/*17 no LOF	2 (10.5)	3 (16.7)		5 (22.7)	4 (16.0)	
*17/*17 no LOF	-	-		1 (4.5)	0	
*1/*2 heterozygous LOF	9 (47.4)	5 (27.8)		4 (18.2)	1 (4.0)	
*1/*3 heterozygous LOF	-	-		0	1 (4.0)	
*2/*17 heterozygous LOF	2 (10.5)	1 (5.6)		0	3 (12.0)	
*2/*2 homozygous LOF	-			0	1 (4.0)	
Inconclusive	-	-		2 (9.1)	1 (4.0)	
Positive system control error	1 (5.3)	0		-	-	
Not available	0	2 (11.1)		1 (4.5)	3 (12.0)	

[*]Calculated in the safety population. HRP=high platelet reactivity; NRP=normal platelet reactivity; LOF=loss-of-function.

Supplementary Table 3. Adverse events.

	Evolocumab (n=15)	**Placebo (n=3)**
Non-serious adverse events		
Nausea	1 (6,7)	
Headache	1 (6.7)	
Back pain	2 (13.3)	1 (33.3)
Non-cardiac chest pain	0	1 (33.3)
Nasal congestion	2 (13.3)	
Fatigue	2 (13.3)	
Cellulitis	1 (6.7)	
Fever	1 (6.7)	
Dyspepsia	1 (6.7)	
Chronic anemia	1 (6.7)	
Site injection bruising	1 (6,7)	
Left heat catheterization	1 (6.7)	0
Serious adverse events		
Gastrointestinal bleeding	1 (6.7)	0
Non-ST-segment elevation myocardial infarction	0	1 (3.3)

Values are expressed as frequencies and percentages.

Supplementary Table 4. Lipid profile findings at 14 and 30 days.[*]

	HPR (n=36)			NPR (n=46)		
	Evolocumab (n=19)	**Placebo (n=17)**	**p-value**	**Evolocumab (n=22)**	**Placebo (n=24)**	**p-value**
14 days follow-up						
Total Cholesterol, mg/dL	111.0±37.7	179.4±46.7	<0.001	85.2±26.0	169.9±32.6	<0.001
Triglycerides, mg/dL	127.7±146.8	114.7±52.6	0.728	87.4±36.6	154.9±63.8	0.001
HDL-C, mg/dL	52.7±14.6	55.6±18.4	0.617	50.2±16.1	47.7 ±14.4	0.578
LDL-C, mg/dL	36.1±27.0	100.9±41.1	<0.001	18.0±14.6	91.2±32.2	<0.001
Non-HDL, mg/dL	58.3±31.3	123.8±43.7	<0.001	34.9±13.2	122.2±33.1	<0.001
Lp(a), mg/dL	165.60±124.1	134.6±99.0±	0.424	134.9±119.3	143.1±131.4	0.828
30 days follow-up						
Total Cholesterol, mg/dL	117.2±22.8	180.9±49.0	0.001	98.5±22.1	172.5±41.6	0.001
Triglycerides, mg/dL	95.3±56.3	112.6±67.4	0.407	92.1±39.9	154.0±80.8	0.002
HDL-C, mg/dL	52.2±14.7	54.7±20.6	0.670	49.1±14.0	47.8±18.2	0.773
LDL-C, mg/dL	45.9±15.9	103.8±42.6	0.001	30.9±12.0	95.0 ±34.3	0.001
Non-HDL, mg/dL	65.0±20	127.4±48.3	0.001	49.3±12.6	124.7±40.3	0.001
Lp(a), mg/dL	173.6±132.8	125.3±99.7	0.223	156.5±144.4	149.9±124.4	0.872

Values are mean±SD [*]Calculated in the pharmacodynamic population. HDL-C=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; Lp(a)= Lipoprotein(a).

Supplementary Table 5. Pharmacodynamic findings at baseline.[*]

	HPR (n=36)			NPR (n=46)		
	Evolocumab (n=19)	**Placebo (n=17)**	**p-value**	**Evolocumab (n=22)**	**Placebo (n=24)**	**p-value**
Verify Now PRU	239.5±26.7	248.5±37.5	0.419	144.9±38.3	148.8±29.7	0.704
ADP 20µL	61.8±15.0	67.8±16.6	0.263	48.8±17.9	54.9±14.9	0.221
ADP 5µL	49.6±20.0	54.7±18.1	0.430	36.1±15.9	38.3±14.7	0.643
AA 1mM[†]	2.5 (2.0–60.0)	1.5 (0.0–35.0)	0.324	2.0 (0.0–3.0)	2.0 (2.0–5.0)	0.282
TRAP 15µM	77.9±9.1	79.4±7.1	0.579	70.0±10.5	73.5±10.3	0.250
Collagen 3µg/mL[†]	62.2±19.1	64.7±11.5	0.681	44.1±26.9	47.6±27.4	0.694
TEG HKH MA	65.6±2.8	65.7±2.9	0.954	63.7±4.5	62.9±4.2	0.550
TEG ADP MA	59.4±10.7	61.5±9.9	0.542	54.1±13.5	55.3±9.0	0.730

Values are mean±SD or median (interquartile range). [*]Calculated in the pharmacodynamic population. [†]Calculated only in patients on concomitant aspirin treatment. HRP=high platelet reactivity; NRP=normal platelet reactivity; PRU=platelet reactivity units; ADP= adenosine diphosphate; Max=maximum; AA= arachidonic acid; TRAP=thrombin receptor activator peptide; HKH=kaolin with heparinase; MA=maximum amplitude.

Supplementary Table 6. Pharmacodynamic findings at 14-day follow-up.[*]

	HPR (n=36)			NPR (n=46)		
	Evolocumab (n=19)	**Placebo (n=17)**	**p-value**	**Evolocumab (n=22)**	**Placebo (n=24)**	**p-value**
Verify Now PRU	218.2±29.7	246.6±35.2	0.017	141.2±42.8	148.2±41.7	0.578
HPR status (%)	11 (61.1)	13 (81.3)		3 (13.6)	3 (12.5)	0.909
ADP 20 µL	60.7±14.6	62.6±20.2	0.751	46.4±16.9	47.7±19.4	0.817
HPR status (%)	7 (38.9)	10 (62.5)	0.169	4 (18.2)	7 (29.2)	0.383
ADP 5 µL	40.8±16.1	49.4±22.4	0.212	33.5±17.5	32.0±17.9	0.762
HPR status (%)	6 (33.3)	9 (56.3)	0.179	4 (18.2)	4 (16.7)	0.892
AA 1mM[†]	2.0 (1.0–68.0)	3.0 (1.0–72.0)	0.919	1.0 (0.0–4.0)	2.0 (1.0–3.0)	0.530
TRAP 15 µM	73.2±18.7	78.9±14.9	0.330	68.9±11.0	72.4±11.2	0.286
Collagen 3µg/mL[†]	56.9±23.7	55.0±33.7	0.877	36.4±27.7	42.0±26.7	0.530
TEG HKH MA	66.1±2.7	65.8±2.4	0.945	63.5±4.9	63.6±3.7	0.974
TEG ADP MA	61.7±3.4	63.4±4.6	0.232	52.9±14.0	54.7±11.7	0.643
HPR status (%)	18 (100)	16 (100)	-	18 (81.8)	20 (83.3)	0.892

Values are mean±SD, median (interquartile range), or n (%). [*]Calculated in the pharmacodynamic population. [†]Calculated only in patients on concomitant aspirin treatment. HRP=high platelet reactivity; NRP=normal platelet reactivity; PRU=platelet reactivity units; ADP=adenosine diphosphate; Max=maximum; AA=arachidonic acid; TRAP=thrombin receptor activator peptide; HKH=kaolin with heparinase; MA=maximum amplitude.

Supplementary Table 7. Pharmacodynamic and lipid profile findings at 30-day follow-up according to baseline circulating PCSK9 levels in the HPR cohort.[*]

	Baseline PCSK9 above median (n=19)			Baseline PCSK9 below median (n=16)		
	Evolocumab (n=10)	Placebo (n=9)	p-value	Evolocumab (n=8)	Placebo (n=8)	p-value
Verify Now PRU	209.7±33.3	253.9±42.0	0.020	227.8±44.8	226.4±60.6	0.960
HPR status (%)	5 (50.0)	8 (88.9)	0.141	5 (62.5)	5 (62.5)	1.000
ADP 20 µL	57.6±10.6	62.4±11.0	0.343	64.1±15.9	60.1±14.1	0.599
HPR status (%)	3 (30.0)	5 (55.6)	0.370	6 (75.0)	4 (50.0)	0.608
ADP 5µL	40.8±8.9	53.2±13.6	0.029	44.5±19.0	43.0±14.4	0.861
HPR status (%)	3 (30.0)	6 (66.7)	0.179	4 (50.0)	2 (25.0)	0.608
AA 1mM[†]	1.0 (1.0-3.0)	67.5 (66.0–74.0)	0.066	2.5 (2.0–4.0)	2.0 (1.0–2.0)	0.394
TRAP 15µM	71.6±8.1	72.9±13.2	0.798	76.1±9.9	77.6±5.9	0.718
Collagen 3µg/mL[†]	52.3±24.8	69.7±11.8	0.137	55.5±27.8	65.6 ±13.1	0.684
TEG HKH MA	67.1±2.2	65.2±2.4	0.093	64.5±2.8	66.5±2.4	0.149
TEG ADP MA	59.6±9.2	63.8±2.6	0.199	64.6±9.3	61.6±5.2	0.441
HPR status (%)	8 (80.0)	9 (100.0)	0.474	8 (100.0)	8 (100.0)	-
Total Cholesterol, mg/dL	109.9±20.7	199.9±52.3	0.001	126.4±24.9	159.6±37.3	0.054
Triglycerides, mg/dL	98.3±71.5	106.2±78.4	0.410	88.0±37.3	119.8±57.0	0.209
HDL-C, mg/dL	49.2±11.1	63.8±24.7	0.109	55.6±19.3	44.5±7.1	0.161
LDL-C, mg/dL	41.1±10.4	115.0±49.0	0.001	53.1±20.2	91.3±32.5	0.014
Non-HDL, mg/dL	60.7 ±17.1	136.1±55.5	0.002	70.8±24.2	116.3±38.5	0.015
Lp(a), mg/dL	168.4±162.6	126.8±110.6	0.528	201.4±79.4	123.6±93.4	0.094

Values are mean±SD, median (interquartile range), or n (%). [*]Calculated in the pharmacodynamic population. [†]Calculated only in patients on concomitant aspirin treatment. PCSK9=proprotein convertase subtilisin/kexin type 9; PRU=platelet reactivity units; ADP=adenosine diphosphate; Max=maximum; AA=arachidonic acid; TRAP=thrombin receptor activator peptide; HKH=kaolin with heparinase; MA=maximum amplitude; HDL-C=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; Lp(a)=Lipoprotein(a).

Supplementary Table 8. Pharmacodynamic and lipid profile findings at 30-day follow-up according to baseline circulating PCSK9 levels in the NPR cohort.[*]

	Baseline PCSK9 above median (n=20)			Baseline PCSK9 below median (n=23)		
	Evolocumab (n=10)	**Placebo (n=10)**	**p-value**	**Evolocumab (n=11)**	**Placebo (n=12)**	**p-value**
Verify Now PRU	162.3±55.1	165.6±39.7	0.880	130.5±46.2	150.5±44.1	0.300
HPR status (%)	2 (20.0)	1 (10.0)	0.500	1 (9.1)	0	0.478
ADP 20 µL	47.9±16.3	57.3±18.1	0.238	39.7±17.5	51.7±21.3	0.170
HPR status (%)	3 (30.0)	4 (40.0)	0.500	1 (10.0)	3 (25.0)	0.594
ADP 5µL	32.6±15.2	43.4±21.3	0.208	23.5±13.8	34.1±16.1	0.118
HPR status (%)	3 (30.0)	4 (40.0)	0.500	0	2 (16.7)	0.481
AA 1mM[†]	1.0 (0.0-38.5)	2.0 (0.0–70.0)	0.955	0.5 (0.0–1.5)	1.5 (0.0–22.5)	0.270
TRAP 15µM	74.6±14.5	70.9±6.6	0.471	59.2±18.3	72.2±12.0	0.060
Collagen 3µg/mL[†]	50.0±33.8	50.9±24.8	0.957	27.9±21.6	41.9±32.6	0.300
TEG HKH MA	64.3±3.8	63.6±3.5	0.659	62.6±5.9	64.5±3.5	0.360
TEG ADP MA	50.9±11.6	55.8±12.7	0.337	51.3±18.2	58.0±4.5	0.207
HPR status (%)	8 (80.0)	10 (100.0)	0.474	6 (54.5)	11 (91.7)	0.069
Total Cholesterol, mg/dL	98.3±19.9	161.8±35.9	0.001	100.2±25.1	173.1±33.0	0.001
Triglycerides, mg/dL	75.1±23.3	147.6±58.0	0.002	109.2±47.0	140.1±50.5	0.145
HDL-C, mg/dL	51.0±12.4	47.7±16.4	0.618	47.9±16.4	49.2±21.4	0.877
LDL-C, mg/dL	32.3±10.0	84.5±34.1	0.001	30.4±14.2	96.0±24.3	0.001
Non-HDL, mg/dL	47.3 ±12.2	114.1±35.9	0.001	52.3±13.1	123.9±26.9	0.001
Lp(a), mg/dL	174.1 (56.0-219.0)	29.4 (10.7-203.1)	0.356	100.0 (8.9-282.0)	192.0 (64.8-219.8)	0.695

Values are mean±SD, median (interquartile range), or n (%). [*]Calculated in the pharmacodynamic population. [†]Calculated only in patients on concomitant aspirin treatment. PCSK9=proprotein convertase subtilisin/kexin type 9; PRU=platelet reactivity units; ADP=adenosine diphosphate; Max=maximum; AA=arachidonic acid; TRAP=thrombin receptor activator peptide; HKH=kaolin with heparinase; MA=maximum amplitude; HDL-C=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; Lp(a)=Lipoprotein(a).

Supplementary Table 9. Pharmacodynamic and lipid profile findings at 30-day follow-up according to CYP2C19 LOF alleles in the HPR cohort.[*]

	LOF (n=16)			No LOF (n=17)		
	Evolocumab (n=11)	**Placebo (n=5)**	**p-value**	**Evolocumab (n=7)**	**Placebo (n=10)**	**p-value**
Verify Now PRU	214.5±30.4	261.8±45.8	0.027	225.3±52.4	240.8±45.1	0.523
HPR status (%)	7 (63.3)	5 (100.0)	0.119	3 (42.9)	7 (70.0)	0.263
ADP 20 µL	63.0±10.6	65.6±13.8	0.685	61.7±14.8	59.5±12.5	0.743
HPR status (%)	7 (63.6)	4 (80.0)	0.513	3 (42.9)	4 (40.0)	0.906
ADP 5µL	45.9±10.2	52.8±17.5	0.331	43.7±17.1	47.2±13.1	0.641
HPR status (%)	5 (45.5)	2 (40.0)	0.838	3 (42.9)	5 (50.0)	0.772
AA 1mM[†]	1.5 (1.0-4.0)	72.0 (36.0–74.5)	0.036	3.0 (1.0–3.0)	2.0 (0.0–66.0)	0.755
TRAP 15µM	74.8±9.1	75.0±15.5	0.977	73.6±9.1	76.3±8.8	0.543
Collagen 3µg/mL[†]	57.9±24.2	70.3±14.8	0.367	50.6±25.7	65.6 ±13.1	0.211
TEG HKH MA	66.5±2.1	66.1±2.2	0.743	65.6±3.7	66.1±2.4	0.740
TEG ADP MA	66.1±13.0	62.2±3.5	0.523	59.2±8.2	63.4±3.5	0.167
HPR status (%)	10 (90.9)	5 (100.0)	0.486	6 (85.7)	10 (100.0)	0.218
Total Cholesterol, mg/dL	105.0±17.5	189.4±55.6	0.001	131.6±19.0	172.3±29.0	0.006
Triglycerides, mg/dL	81.5±27.2	129.0±105.1	0.168	123.0±82.0	115.2±48.6	0.808
HDL-C, mg/dL	49.3±8.6	56.8±32.5	0.471	52.9±19.8	54.0±16.3	0.898
LDL-C, mg/dL	39.5±14.5	106.8±37.1	0.001	54.0±15.0	95.4±28.0	0.003
Non-HDL, mg/dL	55.7 ±14.7	138.8±60.5	0.001	78.7±21.4	118.3±30.5	0.010
Lp(a), mg/dL	136.7±96.7	151.6±108.2	0.786	212.9±173.7	110.0±103.9	0.146

Values are mean±SD, median (interquartile range), or n (%). [*]Calculated in the pharmacodynamic population. [†]Calculated only in patients on concomitant aspirin treatment.
CYP2C19=Cytochrome P450 2C19; PRU=platelet reactivity units; ADP=adenosine diphosphate; Max=maximum; AA=arachidonic acid; TRAP=thrombin receptor activator peptide; HKH=kaolin with heparinase; MA=maximum amplitude; HDL-C=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; Lp(a)=Lipoprotein(a).

Supplementary Table 10. Pharmacodynamic and lipid profile findings at 30-day follow-up according to CYP2C19 LOF alleles in the NPR cohort.[*]

	LOF (n=10)			No LOF (n=29)		
	Evolocumab (n=4)	**Placebo (n=6)**	**p-value**	**Evolocumab (n=15)**	**Placebo (n=14)**	**p-value**
Verify Now PRU	176.8±51.5	173.8±25.5	0.907	127.1±51.5	161.5±42.3	0.059
HPR status (%)	1 (25.0)	0 (0)	0.400	1 (6.7)	7 (7.1)	0.741
ADP 20 µL	48.0±18.2	53.0±15.3	0.650	40.4±18.9	54.4±24.4	0.101
HPR status (%)	1 (25.0)	2 (33.3)	0.667	3 (21.4)	5 (35.7)	0.678
ADP 5µL	35.3±11.9	40.8±17.4	0.594	24.7±13.9	38.0±21.2	0.061
HPR status (%)	1 (25.0)	2 (33.3)	0.667	1 (7.4)	4 (28.6)	0.326
AA 1mM[†]	0 (0.0-3.0)	3.0 (0.0–42.0)	0.393	1.0 (0.0–22.0)	2.0 (0.5–70.5)	0.695
TRAP 15µM	65.3±11.4	74.7±10.9	0.226	69.0±19.7	69.4±9.3	0.952
Collagen 3µg/mL[†]	64.7±30.7	57.6±35.5	0.785	29.4±28.8	35.8 ±31.2	0.616
TEG HKH MA	62.9±2.8	62.9±3.1	0.997	63.6±5.7	64.1±3.7	0.757
TEG ADP MA	56.8±8.0	59.6±4.1	0.479	47.6±17.3	55.7±11.5	0.152
HPR status (%)	3 (75.0)	6 (100.0)	0.400	8 (53.3)	12 (85.7)	0.109
Total Cholesterol, mg/dL	82.8±10.2	184.0±34.6	0.001	102.2±22.5	172.5±48.3	0.001
Triglycerides, mg/dL	81.5±54.8	138.5±44.4	0.107	93.2±38.8	174.6±93.7	0.005
HDL-C, mg/dL	42.5±10.4	60.7±29.1	0.272	50.3±14.7	40.1±7.2	0.027
LDL-C, mg/dL	24.3±11.8	95.8±28.1	0.001	33.1±12.4	99.3±39.4	0.001
Non-HDL, mg/dL	40.3 ±7.0	123.3±31.2	0.001	51.9±13.2	132.4±46.5	0.001
Lp(a), mg/dL	154.4±74.9	131.9±97.8	0.697	173.6±163.0	156.3±144.2	0.768

Values are mean±SD, median (interquartile range), or n (%). [*]Calculated in the pharmacodynamic population. [†]Calculated only in patients on concomitant aspirin treatment.
CYP2C19=Cytochrome P450 2C19; PRU=platelet reactivity units; ADP=adenosine diphosphate; Max=maximum; AA=arachidonic acid; TRAP=thrombin receptor activator peptide; HKH=kaolin with heparinase; MA=maximum amplitude; HDL-C=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; Lp(a)=Lipoprotein(a).

Supplementary Table 11. Pharmacodynamic and lipid profile findings at 30-day follow-up according to baseline LDL-C levels in the HPR cohort.[*]

	Baseline LDL-C above the median (n=21)			Baseline LDL-C below the median (n=15)		
	Evolocumab (n=11)	**Placebo (n=10)**	**p-value**	**Evolocumab (n=8)**	**Placebo (n=7)**	**p-value**
Verify Now PRU	227.5±42.7	256.5±45.9	0.151	208.0±30.2	218.7±55.0	0.657
HPR status (%)	7 (63.6)	9 (90.0)	0.311	4 (50.0)	4 (57.1)	1.000
ADP 20 µL	62.0±13.5	66.7±9.6	0.374	60.0±13.2	53.7±11.9	0.354
HPR status (%)	6 (54.6)	8 (80.0)	0.361	4(50.0)	1 (14.3)	0.282
ADP 5µL	43.8±13.0	52.6±15.2	0.171	43.0±16.8	42.4±11.9	0.941
HPR status (%)	3 (27.3)	6 (60.0)	0.198	5 (62.5)	2 (28.6)	0.315
AA 1mM[†]	3.0 (1.0-4.0)	66.0 (2.0–70.5)	0.299	2.0 (1.0–2.0)	2.0 (0.0–66.0)	0.876
TRAP 15µM	73.7±8.2	78.9±7.6	0.151	74.1±10.2	69.7±12.0	0.454
Collagen 3µg/mL[†]	56.0±27.5	68.7±14.6	0.288	50.9±21.1	60.6 ±13.9	0.391
TEG HKH MA	66.5±2.2	66.4±2.0	0.972	65.5±3.4	64.9±2.9	0.732
TEG ADP MA	59.7±8.6	63.9±3.4	0.169	68.3±13.1	61.2±4.6	0.197
HPR status (%)	9 (81.8)	10 (100.0)	0.476	8 (100.0)	7 (100.0)	-
Total Cholesterol, mg/dL	118.3±24.8	204.9±48.9	0.001	115.6±21.4	146.7±22.4	0.017
Triglycerides, mg/dL	100.8±65.6	126.6±76.0	0.415	87.6±43.3	92.6±51.4	0.843
HDL-C, mg/dL	53.2±15.4	54.2±23.9	0.908	50.8±14.8	55.4±16.7	0.575
LDL-C, mg/dL	45.0±15.0	125.5±43.2	0.001	47.3±18.0	72.9±11.7	0.007
Non-HDL, mg/dL	65.1 ±18.7	155.6±47.4	0.001	64.9±23.0	91.3±11.8	0.017
Lp(a), mg/dL	181.8±146.0	161.3±106.7	0.720	162.3±121.1	73.9±64.7	0.108

Values are mean±SD, median (interquartile range), or n (%). [*]Calculated in the pharmacodynamic population. [†]Calculated only in patients on concomitant aspirin treatment. LDL-C=low-density lipoprotein cholesterol; PRU=platelet reactivity units; ADP=adenosine diphosphate; Max=maximum; AA=arachidonic acid; TRAP=thrombin receptor activator peptide; HKH=kaolin with heparinase; MA=maximum amplitude; HDL-C=high-density lipoprotein cholesterol; Lp(a)=Lipoprotein(a).

Supplementary Table 12. Pharmacodynamic and lipid profile findings at 30-day follow-up according to baseline LDL-C levels in the NPR cohort.[*]

	Baseline LDL-C above the median (n=21)			Baseline LDL-C below the median (n=25)		
	Evolocumab (n=9)	**Placebo (n=12)**	**p-value**	**Evolocumab (n=13)**	**Placebo (n=12)**	**p-value**
Verify Now PRU	144.0±62.8	162.8±49.1	0.449	139.7±50.3	154.4±32.0	0.396
HPR status (%)	2 (22.2)	1 (8.3)	0.553	1 (7.7)	0	0.520
ADP 20 µL	45.0±18.7	58.2±20.9	0.168	41.2±17.0	49.3±19.9	0.289
HPR status (%)	2 (22.2)	7 (58.3)	0.197	2 (15.4)	1 (8.3)	0.531
ADP 5µL	29.9±17.2	43.8±22.9	0.162	26.2±13.4	32.1±12.1	0.266
HPR status (%)	3 (33.3)	6 (50.0)	0.670	0	1 (8.3)	0.480
AA 1mM[†]	2.0 (0.0-3.0)	2.0 (1.0–71.0)	0.875	1.0 (0.0–2.0)	2.0 (0.0–42.0)	0.401
TRAP 15µM	63.4±17.0	76.5±11.8	0.055	68.3±18.4	67.8±6.9	0.922
Collagen 3µg/mL[†]	23.0 (5.0-65.0)-	61.5 (58.0-75.0)	0.492	40.0 (3.0-61.0)	40.0 (12.0-58.0)	1.000
TEG HKH MA	63.3±3.2	63.1±2.9	0.891	63.7±5.9	64.1±4.5	0.847
TEG ADP MA	50.9±11.6	55.8±12.7	0.384	51.3±18.2	58.0±4.5	0.230
HPR status (%)	5 (55.6)	10 (83.3)	0.331	9 (69.2)	12 (100.0)	0.096
Total Cholesterol, mg/dL	99.8±20.4	189.2±45.9	0.001	97.5±23.9	155.8±30.0	0.001
Triglycerides, mg/dL	85.1±32.1	168.9±101.6	0.028	96.9±45.1	139.0±53.4	0.044
HDL-C, mg/dL	47.7±9.5	46.7±11.2	0.831	50.2±16.8	48.8±23.8	0.873
LDL-C, mg/dL	35.1±12.5	110.9±41.1	0.001	27.9±11.2	79.2±15.0	0.001
Non-HDL, mg/dL	52.1 ±15.6	142.5±49.4	0.001	47.4±10.3	106.9±16.2	0.001
Lp(a), mg/dL	128.4 (15.7-223.5)	203.1 (178.0-231.0)	0.492	157.0 (56.0-268.2)	42.0 (15.7-191.0)	0.208

Values are mean±SD, median (interquartile range), or n (%). [*]Calculated in the pharmacodynamic population. [†]Calculated only in patients on concomitant aspirin treatment. LDL-C=low-density lipoprotein cholesterol; PRU=platelet reactivity units; ADP=adenosine diphosphate; Max=maximum; AA=arachidonic acid; TRAP=thrombin receptor activator peptide; HKH=kaolin with heparinase; MA=maximum amplitude; HDL-C=high-density lipoprotein cholesterol; Lp(a)=Lipoprotein(a).

Supplementary Table 13. Pharmacodynamic and lipid profile findings at 30-day follow-up in patients treated with high-intensity dose statin at baseline.[*]

	HPR (n=34)			NPR (n=41)		
	Evolocumab (n=18)	**Placebo (n=16)**	**p-value**	**Evolocumab (n=21)**	**Placebo (n=20)**	**p-value**
Verify Now PRU	217.7±38.7	244.1±51.9	0.108	137.1±51.7	157.5±42.5	0.176
HPR status (%)	10 (55.6)	13 (81.3)	0.152	2 (9.5)	1 (5.0)	0.519
ADP 20µL	60.5±13.1	62.9±10.6	0.553	41.0±15.9	56.2±20.1	0.011
HPR status (%)	9 (50.0)	9 (56.3)	0.744	3 (15.0)	7 (35.0)	0.273
ADP 5µL	42.4±13.9	49.8±13.8	0.136	27.0±14.8	40.3±18.6	0.017
HPR status (%)	7 (38.9)	8 (50.0)	0.730	3 (15.0)	6 (30.0)	0.451
AA 1mM[†]	2.0 (1.0-4.0)	34.0 (1.5-69.5)	0.256	1.0 (0.0-2.5)	2.0 (0.0-70.0)	0.161
TRAP 15µM	73.6±9.0	75.2±10.7	0.644	65.9±17.9	72.5±11.2	0.171
Collagen 3µg/mL[†]	53.6±25.1	65.3±14.3	0.140	35.4±30.2	48.2±29.6	0.217
TEG HKH MA	65.9±2.7	65.7±2.5	0.824	63.8±4.7	63.3±3.9	0.688
TEG ADP MA	61.8±9.3	62.8±4.2	0.690	51.9±15.5	57.5±6.5	0.144
HPR status (%)	16 (88.9)	16 (100)	0.487	14 (66.7)	19 (95.0)	0.045
Total Cholesterol, mg/dL	117.2±23.5	183.6±49.4	0.001	96.0±19.4	170.2±42.3	0.001
Triglycerides, mg/dL	93.7±57.5	107.5±66.1	0.524	92.5±40.8	144.9±84.0	0.018
HDL-C, mg/dL	52.1±15.2	55.8±20.8	0.563	47.7±12.6	47.6±18.5	0.982
LDL-C, mg/dL	46.4±16.2	106.4±42.6	0.001	29.8±11.2	94.9±34.9	0.001
Non-HDL, mg/dL	65.2±20.6	129.2±49.5	0.001	48.3±12.0	122.6±40.8	0.001
Lp(a), mg/dL	183.1±129.9	130.3±100.8	0.192	164.1±143.7	131.4±109.1	0.426

Values are mean±SD, median (interquartile range), or n (%). [*]Calculated in the pharmacodynamic population. [†]Calculated only in patients on concomitant aspirin treatment. High-intensity statin was defined as atorvastatin ≥40 mg or rosuvastatin ≥20mg. PRU=platelet reactivity units; ADP=adenosine diphosphate; Max=maximum; AA=arachidonic acid; TRAP=thrombin receptor activator peptide; HKH=kaolin with heparinase; MA=maximum amplitude; HDL-C=high-density lipoprotein cholesterol; LDL-C=low-density lipoprotein cholesterol; Lp(a)=Lipoprotein(a).