

BRIEF COMMUNICATION

# Evolocumab, a PCSK9-Monoclonal Antibody, Rapidly Reverses Coronary Artery Endothelial Dysfunction in People Living With HIV and People With Dyslipidemia

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**BACKGROUND:** PCSK9 (proprotein convertase subtilisin/kexin type 9) is well recognized for its important role in cholesterol metabolism. Elevated levels are associated with increased cardiovascular risk and inhibition with PCSK9 antibodies (PCSK9i) lowers cardiovascular events in patients with coronary artery disease. PCSK9 levels are also elevated in people living with HIV (PLWH) and those with dyslipidemia. Because increased PCSK9 in PLWH is associated with impaired coronary endothelial function, a barometer of coronary vascular health, we tested the hypothesis that PCSK9i improves impaired coronary endothelial function in dyslipidemia without coronary artery disease and in PLWH with nearly optimal/above goal low-density lipoprotein cholesterol levels.

**METHODS AND RESULTS:** We performed a single-center study in 19 PLWH and 11 with dyslipidemia to evaluate the effects of the PCSK9i evolocumab on coronary endothelial function using cine 3T MRI to noninvasively measure coronary endothelial function, assessed as the changes in coronary cross-sectional area and coronary blood flow from rest to that during isometric handgrip exercise, a known endothelial-dependent vasodilator. Before evolocumab, there was a decrease or no coronary vasodilation and no increase in coronary blood flow (the normal responses) to isometric handgrip exercise in either group. Following 6 weeks of evolocumab, 480 mg q4 weeks, the % cross-sectional area changes from rest to isometric handgrip exercise were  $+5.6\pm 5.5\%$  and  $+4.5\pm 3.1\%$  in the PLWH and dyslipidemia groups, respectively, both  $P<0.01$  versus baseline. Improved cross-sectional area was paralleled by a significant coronary blood flow improvement in both groups.

**CONCLUSIONS:** To our knowledge, these data represent the first evidence that PCSK9 inhibition improves coronary artery health in PLWH and people with dyslipidemia.

**REGISTRATION:** URL: <https://www.clinicaltrials.gov>; Unique identifier: NCT03500302.

**Key Words:** endothelial function ■ HIV ■ inflammation ■ magnetic resonance imaging ■ proprotein convertase subtilisin/kexin type 9

**A**bnormal coronary endothelial function (CEF) is considered a “barometer” of vascular health both because it is a driver of the development, progression, and clinical manifestations of atherosclerotic cardiovascular disease (ASCVD) and importantly because impaired CEF improves in response to preventive

interventions like statins and angiotensin-converting enzyme inhibitors.<sup>1,2</sup> Normal CEF is primarily mediated by endothelial cell nitric oxide release,<sup>3</sup> which can be assessed by measuring changes in coronary cross sectional area and coronary blood flow in response to endothelial-dependent stimuli, such as isometric

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This work was presented in part as an oral abstract at the European Society of Cardiology Congress, September 2, 2019, in Paris, France.

For Sources of Funding and Disclosures, see page 7.

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## Nonstandard Abbreviations and Acronyms

<b>CEF</b>	coronary endothelial function
<b>PCSK9</b>	proprotein convertase subtilisin/kexin type 9
<b>PLWH</b>	people living with HIV

handgrip exercise (IHE). Conventional CEF measures required invasive cardiac catheterization, which precluded studies in stable, low-risk populations. Recently established noninvasive coronary magnetic resonance imaging (MRI) methods, combined with IHE, can quantify nitric oxide-mediated CEF.<sup>4</sup> Thus, noninvasive MRI is an appealing tool to quantify the effectiveness of medical interventions designed to improve CEF and thereby enhance coronary vascular health and ultimately decrease the risk of future ASCVD events.

Dyslipidemia and HIV infection are associated with accelerated ASCVD. Both are pro-inflammatory states and inflammation is now understood to be an important nontraditional risk factor involved in all stages of atherosclerosis.<sup>5</sup> It was recently recognized that PCSK9 (proprotein convertase subtilisin/kexin type 9), a protease produced in the liver, adversely modulates the expression of low-density lipoprotein (LDL) receptors, which bind and remove LDL cholesterol (LDL-C). Beyond its role in cholesterol homeostasis, PCSK9 is prevalent in human atherosclerotic plaque and in isolated vascular, smooth muscle, and endothelial cells treated with inflammatory stimuli.<sup>6</sup> PCSK9 serum levels are also elevated in people living with HIV (PLWH)<sup>7</sup> and those with dyslipidemia<sup>8</sup> and in a large 15-year prospective cohort study was associated with incident cardiovascular events independent of established vascular risk factors.<sup>9</sup> However, it is important to note that the independent association of serum PCSK9 with ASCVD risk is not supported in some studies.<sup>10–12</sup> Furthermore, we showed that elevated PCSK9 serum levels in PLWH are associated with coronary artery vascular endothelial dysfunction.<sup>7</sup> Recently, the development of monoclonal antibodies to PCSK9, which rapidly and effectively reduce LDL levels represented a clinical breakthrough in the treatment of familial hypercholesterolemia and coronary artery disease patients who are statin intolerant or who do not experience sufficient lipid lowering on statins alone.<sup>13</sup> Because PCSK9 inhibitors are effective and generally well tolerated, they represent an appealing treatment strategy in PLWH in whom concurrent liver disease and frequent drug interactions can frequently preclude statin use. Furthermore, a recent study demonstrated a local, anti-inflammatory effect on the vasculature, suggesting an additional mechanism responsible for their benefit.<sup>14</sup>

PCSK9 inhibitors therefore may offer a practical, clinically relevant, but as yet untested means to improve endothelial function and vascular health. Using our noninvasive reproducible MRI methods to evaluate nitric-oxide mediated CEF, we tested the hypothesis that the PCSK9 monoclonal antibody evolocumab improves abnormal CEF in PLWH and, in another group, those with dyslipidemia, both pro-inflammatory states.

## MATERIALS AND METHODS

The data and analytical methods that support the findings of this study are available from the corresponding author on reasonable request.

This was a single-center pilot clinical trial conducted at the Johns Hopkins Hospital in 19 PLWH and 11 patients with dyslipidemia without HIV. The goal was to assess whether the PCSK9 inhibitor evolocumab improved CEF in these two groups. Participants were stable asymptomatic outpatients with no recent (<3 months) change in medications with the following lipids at the screening visit: fasting LDL-C >70 mg/dL; fasting triglycerides <500 mg/dL as well as abnormal CEF measured on screening MRI at baseline, defined as <5% increase in coronary cross-sectional area (CSA) from rest to that during IHE, an endothelial-dependent stressor. In the PLWH group, participants were taking stable antiretroviral therapy, that is, no change in the prior 3 months with clinically controlled HIV viral load (plasma HIV RNA concentration ≤100 copies/mL). None of the participants had known coronary artery disease.

Biomarker and CEF studies were obtained before and at 1 week and 6 -week following initiation of evolocumab 420 mg every 4 weeks in the PLWH group and before and at 6 weeks after initiation in the dyslipidemia group. The protocol was approved by the institutional review board at the Johns Hopkins University School of Medicine and complies with the Declaration of Helsinki. All participants provided written informed consent after the study was explained to them and the trial was registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (NCT03500302).

## MRI Study Protocol and Image Analysis

Participants underwent a baseline MRI study of CEF in the fasting state (>8 hours) before the administration of any prescribed vasoactive medications at rest and during continuous IHE using MRI methodology as previously reported.<sup>7</sup> Images were taken perpendicular to a proximal or midcoronary arterial segment that was straight over a distance of ≈20 mm. Alternating anatomical images were acquired to measure CSA, and phase contrast (velocity-encoded) images were

acquired to quantify coronary blood flow velocity used to calculate coronary blood flow (CBF) as previously described<sup>4</sup> to evaluate the CEF end points of interest: %CSA change and %CBF change from rest to that during IHE. Images were collected at baseline and during 4 to 7 minutes of continuous IHE using an MRI-compatible dynamometer (Stoelting, Wood Dale, IL) at 30% of maximum grip strength (Figure 1A through 1F).<sup>4</sup> Both per-segment and per-patient analyses were performed. MRI interpretation was performed by two independent readers (Y.A., A.H.) who were blinded to clinical and laboratory information at the time of analysis.

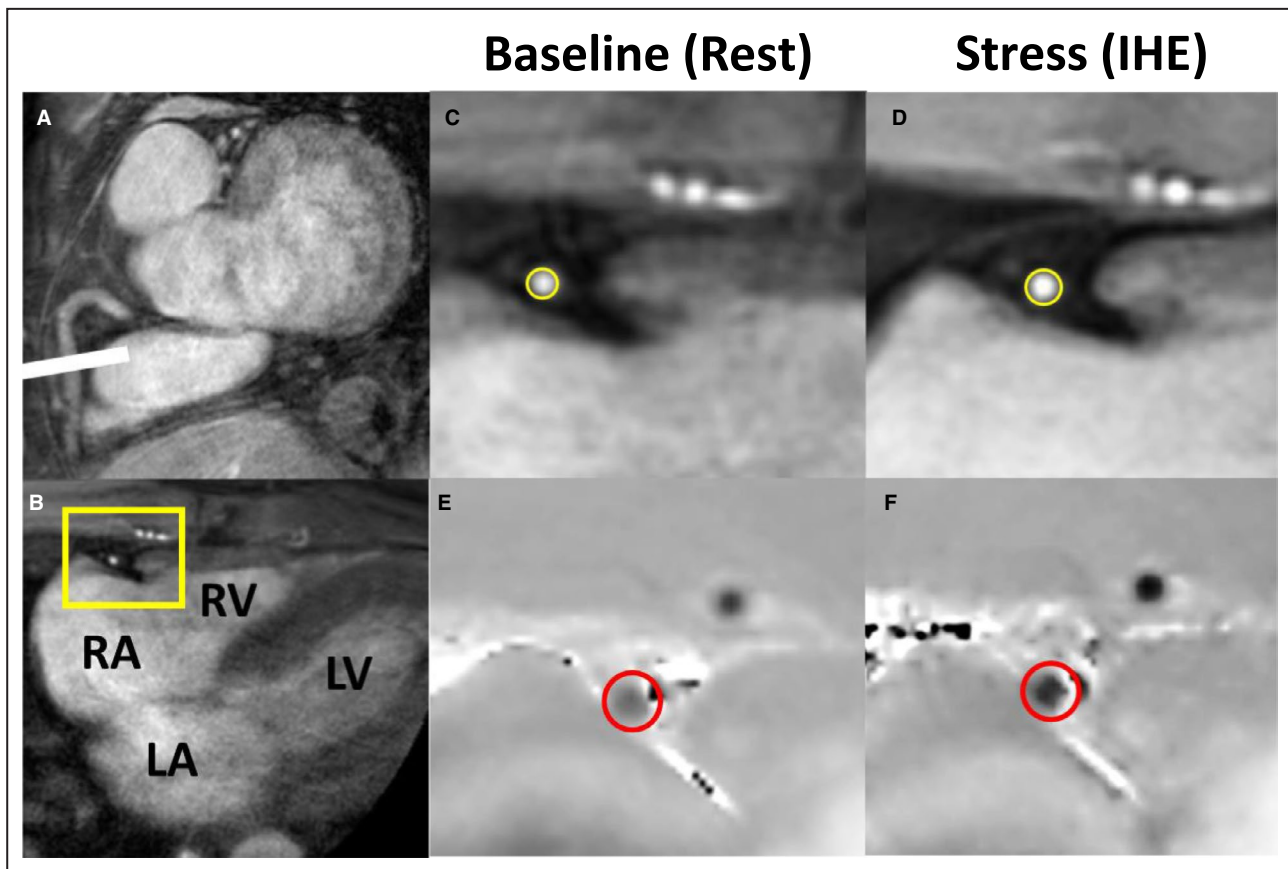
### Laboratory Measurements

Venous blood samples were collected on the day of the MRI study after an overnight fast to determine the concentrations of high-sensitivity C-reactive protein,

interleukin-6, interferon-gamma, tumor necrosis factor-alpha and, in the HIV group, soluble CD163. Serum samples were analyzed for the aforementioned markers using commercially available ELISA kits (quantikine ELISA; Bio-Techne Corporation, Minneapolis, MN). A fasting lipid panel was also obtained and analyzed in the Johns Hopkins Hospital clinical laboratory on the day of the study.

### Statistical Analysis

Statistical analysis was performed with Prism 8.00 (GraphPad Software, La Jolla, CA). The data were tested for normality using the Shapiro-Wilk test. Parametric (Student *t* test) and nonparametric (Wilcoxon signed rank test for paired data and Wilcoxon rank sum test for nonpaired data) tests were used when appropriate for normally distributed and skewed data, respectively, to compare the percentage changes (from rest



**Figure 1.** Representative magnetic resonance imaging (MRI) of the right coronary artery (RCA) in an HIV-infected individual. In this MRI scan, a scout scan of the RCA is shown (A) together with the location for cross-sectional imaging (white line). B, A view of the RCA cross-section is shown (yellow box) perpendicular to image (A). The yellow box in (B) is magnified to show a cross-sectional image of the RCA (yellow circles) at rest (C) and during isometric handgrip stress (IHE) (D) in a study participant with HIV whose coronary endothelial function (CEF) improved following initiation of PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitor therapy, with an increase in coronary area with IHE. Magnified coronary flow velocity image of the RCA in the same subject is shown at rest (E) and during IHE (F) (red circles). The signal phase is proportional to flow velocity with the darker pixels in the velocity phase contrast images during IHE indicating higher velocity in the stress image compared with that at rest. LA indicates left atrium; LV, left ventricle; RA, right atrium; and RV, right ventricle.

to those during IHE) in CSA and CBF obtained at baseline, that is, before initiation of evolocumab, and the follow-up time points. Normally distributed data were summarized using means and SDs, whereas nonnormally distributed data were summarized using medians and interquartile ranges. Statistical significance was defined as a 2-tailed  $P \leq 0.05$ .

## RESULTS

### Study Population

Baseline characteristics are presented in Table 1. Eleven patients with dyslipidemia without HIV who were scheduled to receive evolocumab for clinical indications and 19 PLWH on stable highly active antiretroviral therapy and with undetectable HIV RNA were studied separately before (baseline) and after (1 and 6 weeks in PLWH and 6 weeks in patients with dyslipidemia) evolocumab initiation.

### CEF Results

Before evolocumab, CEF was impaired in both groups as compared with CEF in healthy individuals.<sup>7</sup> IHE did not induce the normal coronary vasodilation or increase in CBF in either group; mean stress-induced CSA changes were  $-2.3 \pm 5.9\%$  in PLWH ( $P=0.27$  rest versus IHE) and  $-1.2 \pm 3.9\%$  in the dyslipidemia group ( $P=0.46$  rest versus IHE). Notably, CEF trended higher after only 1 week of evolocumab in the PLWH group (%CSA change from rest to IHE:  $-2.3 \pm 5.9\%$  at baseline pre-PCSK9 to  $+1.6 \pm 6.8\%$  at 1 week follow-up,  $P=0.07$ ), with a statistically significant improvement at 6 weeks (with %CSA changes from rest to IHE:  $-2.3 \pm 5.9\%$  at baseline pre-PCSK9i (PCSK9 inhibitor) to  $+5.6 \pm 5.5\%$ ,  $P=0.0012$  versus pre-PCSK9i) in PLWH (Figure 2A). CEF was also significantly higher after 6 weeks in the dyslipidemia group with % CSA change from rest to IHE from  $-1.2 \pm 3.9\%$  pre-PCSK9i to  $+4.5 \pm 3.1\%$  at 6 weeks ( $P < 0.0001$  versus pre-PCSK9i Figure 2B). We also observed improved mean stress-induced coronary blood flow at the 6-week time point in both groups (PLWH baseline mean CBF % change with IHE:  $+5.2 \pm 11.3\%$  increased to  $+15.3 \pm 10.5\%$  at 6 weeks,  $P=0.02$ ; dyslipidemia baseline mean CBF % change:  $+9.9 \pm 6.6\%$  increased to  $+21.7 \pm 13.6\%$  at 6 weeks,  $P=0.02$ , Figure 2C and 2D).

### LDL-Cholesterol and Inflammatory Biomarkers Results

The %LDL-C reduction with evolocumab was significant at 1 week in the PLWH group ( $-52.5 \pm 15.8\%$ ,  $P=0.003$  versus pre PCSK9i) and at 6 weeks in the PLWH and dyslipidemia groups,  $-69.2 \pm 12.5\%$  ( $P=0.001$  versus pre PCSK9i) and  $-59.9 \pm 18.4\%$

**Table 1. Cohort Characteristics**

Characteristics	PLWH (n=19)	Dyslipidemia Patients (n=11)
Age, y	52±9	59±10
Male sex, n (%)	15 (79)	9 (82)
Black, n (%)	14 (74)	2 (18)
BMI	26±5	28±3
PCI/CABG, n (%)	0	0
Hypertension, n (%)	5 (26)	3 (27)
Diabetes mellitus, n (%)	0	1 (9)
Smoker, n (%)	3 (16)	2 (18)
ACE-inhibitor, n (%)	2 (11)	2 (18)
Statin, n (%)	4 (22)	7 (64)
High intensity, n (%)	1 (5)	5 (45)
Moderate intensity, n (%)	3 (16)	2 (18)
Non-statin LDL-C lowering, n (%)	0	10 (91)
Beta-blocker, n (%)	1 (5)	3 (27)
ASA, n (%)	5 (26)	5 (45)
Total cholesterol, mg/dL	189±48	231±85
LDL-C, mg/dL	118±44	150±66
HDL-C, mg/dL	48±11	51±15
Triglycerides, mg/dL	108±48	164±76
Non-HDL-C, mg/dL	141±47	180±76
ASCVD 10 y risk, %	8.6±3.7	15.2±6.8
ASCVD ≥7.5%, n (%)	11 (58)	9 (82)
HAART, n (%)	19 (100)	N/A
NRTI use, n (%)	19 (100)	N/A
NNRTI use, n (%)	1 (5)	N/A
PI use, n (%)	0	N/A
Current abacavir use, n (%)	8 (42)	N/A
CD4+ T-cell count, cells/μL	707±350	N/A
Viral load <20 copies/mL, n (%)*	17 (89)	N/A

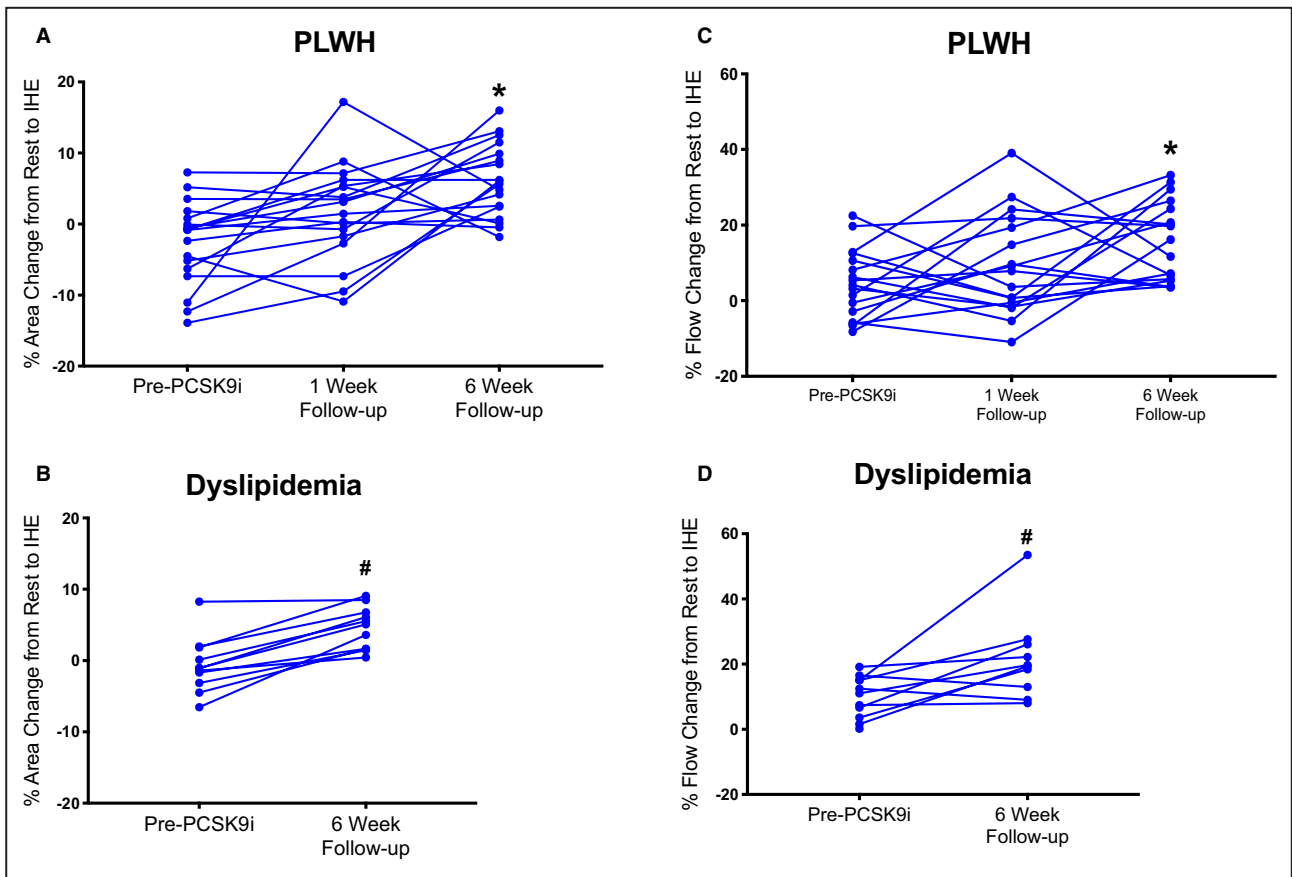
Categorical variables shown as count (%), and continuous variables are shown as mean (SD) or median [Q1, Q3] if data were skewed. ACE indicates angiotensin-converting enzyme; ASA, aspirin; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; CABG, coronary artery bypass grafting; CD4, cluster of differentiation 4; HAART, highly active antiretroviral therapy; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PCI, percutaneous coronary intervention; PI, protease inhibitor; and PLWH, people living with HIV.

\*Two patients had viral load >20 copies/mL (23 and 27 copies/mL).

( $P=0.002$  versus pre PCSK9i), respectively. A significant relationship was not detected between the LDL-C reduction and CEF improvement in either group.

Inflammatory biomarkers, including high-sensitivity C-reactive protein, interleukin-6, interferon-gamma, tumor necrosis factor-alpha, and soluble CD163 were unchanged in both groups over 6 weeks.

The baseline and follow-up biological parameters, including coronary endothelial function (CSA and CBF), lipids (LDL-C and non- high-density lipoprotein cholesterol) results, as well as those for the inflammatory



**Figure 2. Magnetic resonance imaging (MRI) assessment of coronary endothelial function in people living with HIV (PLWH) and people with dyslipidemia.**

Percentage changes from baseline in coronary artery cross-sectional area (CSA) during isometric handgrip stress (IHE) are shown for PLWH (A) and people with dyslipidemia (B). \* $P=0.0012$  and # $P<0.0001$  vs respective pre-PCSK9i (proprotein convertase subtilisin/kexin type 9 inhibitor) baseline. Percentage changes from baseline in coronary blood flow (CBF) during IHE are shown for PLWH (C) and people with dyslipidemia (D). \*# $P=0.02$  vs respective pre-PCSK9i-baseline. MRIs were analyzed blinded to group and timepoint. HIV+ N=19; Dyslipidemia N=11.

biomarkers (hsCRP, interleukin-6, interferon-gamma, tumor necrosis factor-alpha, and soluble CD163) are presented in Table 2.

## DISCUSSION

Arterial endothelial cell function is a critical determinant of the development and progression of vascular disease and an independent predictor of cardiovascular events.<sup>2</sup> In vivo endothelial cell function can be assessed by the changes in vascular dimension and flow in response to endothelial-dependent stresses.<sup>4</sup> In addition, interventions that are associated with decreased cardiovascular risk, including statins, angiotensin-converting enzyme inhibition, and exercise also improve the vascular response to endothelial-dependent stresses, further suggesting that this response can be considered a “barometer” of vascular health.<sup>1</sup> Prior studies assessing the benefit of such interventions, however, required repeated invasive

coronary catheterization measures and therefore limited the opportunity to study coronary endothelial cell function in stable, otherwise healthy subjects. Our group developed and validated a noninvasive MRI methodology to assess CEF by measuring changes in coronary dimension and flow in response to IHE, an endothelial-dependent stressor.<sup>3,4</sup> The results were shown to be reproducible on the same day and over 8 weeks, longer than the time studied in this report, and the responses to IHE were nearly completely blocked with L-NMMA, indicating that the MRI responses to IHE primarily reflect nitric oxide-mediated CEF.<sup>3</sup> We recently reported that CEF assessed using this methodology was significantly decreased in PLWH<sup>15</sup> and that impaired CEF was associated with elevated levels of PCSK9.<sup>7</sup>

We now report that PCSK9 inhibition with evolcumab significantly improves abnormal coronary endothelial function after only 6 weeks in PLWH with a mean near optimal/above goal LDL-C and in PLWH

**Table 2. Biological Parameters of the Study Groups at Baseline and Follow-Up**

	Baseline	1-wk Follow-Up	6-wk Follow-Up	P Value [Baseline vs 6-wk]
<b>PLWH (n=19)</b>				
Coronary endothelial function				
Stress-induced %CSA changes	-2.3±5.9	+1.6±6.8	+5.6±5.5	<0.01
Stress-induced %CBF changes	+5.2±11.3	+11.1±15.0	+15.3±10.5	0.02
Lipids				
LDL-C, mg/dL	118±44	64±39	37±19	<0.01
Non-HDL-C, mg/dL	141±47	77±36	54±20	<0.01
Inflammatory biomarkers				
hsCRP, mg/L	1.81 [1.04, 5.57]	2.46 [0.76, 4.59]	3.08 [0.99, 6.10]	1.00
IL-6, pg/mL	0.72 [0.58, 1.20]	0.77 [0.61, 1.14]	0.80 [0.62, 1.31]	0.58
IFN-gamma, pg/mL	8.50 [4.86, 10.21]	9.32 [5.29, 11.45]	7.10 [5.20, 10.53]	0.73
TNF-alpha, pg/mL	2.22 [1.53, 2.59]	1.98 [1.64, 2.50]	1.98 [1.43, 2.26]	0.82
Soluble CD163, ng/mL	422±116	492±186	479±200	0.30
<b>Dyslipidemia patients (n=11)</b>				
Coronary endothelial function				
Stress-induced %CSA changes	-1.2±3.9	N/A	+4.5±3.1	<0.01
Stress-induced %CBF changes	+9.9±6.6	N/A	+21.7±13.6	0.02
Lipids				
LDL-C, mg/dL	150±66	N/A	63±42	<0.01
Non-HDL-C, mg/dL	180±76	N/A	87±47	<0.01
Inflammatory biomarkers				
hsCRP, mg/L	1.65 [1.29, 6.91]	N/A	1.70 [1.47, 3.10]	0.63
IL-6, pg/mL	1.11 [0.52, 3.10]	N/A	0.65 [0.31, 4.21]	0.56
IFN-gamma, pg/mL	18.04 [9.10, 48.41]	N/A	14.48 [8.91, 33.68]	0.85
TNF-alpha, pg/mL	1.85 [1.59, 4.33]	N/A	1.87 [1.56, 2.98]	0.77
Soluble CD163, ng/mL	542±233	N/A	555±218	0.67

Continuous variables are shown as mean (SD) or median [Q1, Q3] if data were skewed. CBF indicates coronary blood flow; CD163, cluster of differentiation 163; CSA, cross-sectional area; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; IFN-gamma, interferon gamma; IL-6, interleukin 6; LDL-C, low-density lipoprotein cholesterol; PLWH, people living with HIV; and TNF-alpha, tumor necrosis factor alpha.

with dyslipidemia. Moreover, the effect of PCSK9 inhibition on coronary endothelial function was rapid, with a trend toward improvement after only 1 week, followed by a significant improvement in CEF after 6 weeks in both groups. To our knowledge, these data represent the first evidence that PCSK9 inhibition improves coronary artery endothelial dysfunction, an important driver of ASCVD.

Although inhibition with PCSK9 led to a dramatic improvement in CEF, there was no significant change in serum inflammatory markers over the course of the study, similar to findings in prior studies.<sup>16</sup> A previous study suggested that the ASCVD protective effects of PCSK9 inhibition may be related to a local anti-inflammatory effect on the arterial wall rather than to changes in systemic inflammatory biomarkers.<sup>14</sup> In addition, PCSK9 serum levels are associated with ASCVD risk independent of LDL-C concentration in a prospective cohort study examining incident cardiovascular events over a 15-year follow-up period.<sup>9</sup> However, other studies

reported no or only a weak association between PCSK9 serum levels and ASCVD risk.<sup>10-12</sup> A direct regulatory impact of PCSK9 on lectin-type oxidized LDL receptor-1, a major pro-inflammatory modulator, has been reported in isolated vascular endothelial- and smooth muscle cells.<sup>17</sup> In patients treated for dyslipidemia, peripheral endothelial function is improved in those on a statin but not in those on ezetimibe despite comparable LDL-C levels, suggesting pleotropic statin effects. We extend these findings in this study, demonstrating for the first time that PCSK9 inhibition rapidly improves in vivo CEF in people with dyslipidemia and in PLWH, including some with near optimal/above goal baseline LDL-C. We propose a novel axis for the development and progression of ASCVD in people with these pro-inflammatory diseases whereby pro-inflammatory states trigger PCSK9 expression, leading to adverse effects on nitric oxide-mediated CEF. Inhibiting PCSK9 may therefore break the link between the pro-inflammatory stimuli and endothelial injury.

In PLWH, PCSK9 inhibition may offer an attractive therapeutic option as dyslipidemia is common and statins are often underprescribed. In a recent report from the National Ambulatory Medical Care Survey/National Hospital Ambulatory Medical Care Survey only 23.6% of HIV patients with known diabetes mellitus, cardiovascular disease, or dyslipidemia were prescribed statins.<sup>18</sup> PLWH have profound endothelial dysfunction<sup>15</sup> and elevated PCSK9 levels,<sup>7</sup> and a recently published study showed that PCSK9 inhibition with evolocumab was safe and significantly reduced atherogenic lipoprotein levels in dyslipidemic PLWH on maximally tolerated statin therapy.<sup>19</sup> Moreover, the side effect profile of PCSK9 inhibitors is favorable with virtually no drug interactions, important considerations for PLWH who are on antiretroviral therapy and frequently have concomitant liver disease. However, it is important to note that the PLWH group in this study would not be eligible for evolocumab therapy using current guidelines. Taken together, PCSK9 inhibition may represent an important new treatment strategy for improving vascular function in PLWH.

**Limitations:** Our pilot clinical study was limited by the relatively small sample size; however, the sample size was adequate to detect a significant change in CEF after 6 weeks. In addition, statins also improve vascular health and as not all of the participants were on high dose statins, it is possible that if they were, the impact of PCSK9 inhibition may have been less marked. Lastly, one limitation was that we did not have a placebo control group, as this was beyond the scope of funding for this study.

In conclusion, short-term evolocumab significantly and rapidly improves CEF, a barometer of vascular health and driver of ASCVD, in PLWH with a mean near optimal/above goal LDL-C and in people with dyslipidemia, both pro-inflammatory states associated with increased ASCVD risk.

## ARTICLE INFORMATION

Received February 14, 2020; accepted June 8, 2020.

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### Acknowledgments

The investigators gratefully acknowledge the participants who donated their time and energies toward this study and the advancement of scientific knowledge. The investigators also thank their research coordinators, Frances A. Kirkland and Shannon Kelley. Amgen provided the evolocumab for this study. Amgen had no role in the design of the study, the collection, management or interpretation of the data, or the statistical analysis. Amgen reviewed the first submitted version of the manuscript but was not involved in the writing or approval of the manuscript or the decision to submit the manuscript for publication.

## Sources of Funding

This work was supported by the National Heart, Lung, and Blood Institute (R01HL147660, R01HL125059, and R01HL120905), the American Heart Association (19CDA34760040, 17GRNT33670943), the Johns Hopkins Center for AIDS Research (P30AI094189), the Johns Hopkins Magic That Matters Grant, the Johns Hopkins Ciccarone Center for the Prevention of Heart Disease, and the Clarence Doodeman Endowment of Johns Hopkins Cardiology.

## Disclosures

Dr Leucker reports a research grant from Amgen. Dr Brown serves as a consultant to Gilead Sciences, Viiv Healthcare, Merck, and Theratechnologies. The remaining authors have no disclosures to report.

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