

## Short Communication: The Effect of Rosuvastatin on Vascular Disease Differs by Smoking Status in Treated HIV Infection

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### Abstract

Smoking is an important contributor to cardiovascular disease risk and is highly prevalent in the HIV population. In the Stopping Atherosclerosis and Treating Unhealthy Bone with Rosuvastatin in HIV trial (SATURN-HIV), a 96-week, randomized placebo-controlled study testing the effect of rosuvastatin on subclinical vascular disease and immune activation in HIV-infected adults, rosuvastatin improved immune activation and arrested common carotid artery intima media thickness (CCA IMT) progression. In this exploratory analysis, ANOVA was used to test for effect modification by smoking. One-hundred forty-seven adults were included (72 in rosuvastatin group; 75 in placebo group). Groups were similar at baseline. Overall, mean  $\pm$  SD age was  $45.4 \pm 9.9$  years, 115 (78%) were men and 100 (68%) were African American. Ninety-three (63%) were current smokers (mean  $\pm$  SD  $0.6 \pm 0.44$  packs/day) and another 24 (16%) were smokers in the past. There were statistically significant randomization group by smoking status interactions for 0–24 ( $p = .01$ ) and 0–48 ( $p < .01$ ) week changes in proportion of activated CD4<sup>+</sup> T cells and for 0–48 ( $p < .01$ ) and 0–96 (trend only;  $p = .06$ ) week changes in CCA IMT. No effect modification by smoking was detected for changes in markers of inflammation or monocyte activation. The beneficial effect of rosuvastatin on CCA IMT was not apparent in smokers although T cell activation improved to a greater degree in this subgroup.

**Keywords:** smoking, inflammation, subclinical vascular disease, HIV, antiretroviral therapy

### Introduction

PEOPLE LIVING WITH HIV are at increased risk of cardiovascular disease (CVD) events.<sup>1</sup> We and others have shown that 3-hydroxy-3 methylglutaryl coenzyme A reductase inhibitors or statins improve subclinical vascular disease in HIV-infected adults on antiretroviral therapy (ART).<sup>2,3</sup> Further, in the Stopping Atherosclerosis and Treating Unhealthy Bone with Rosuvastatin in HIV (SATURN-HIV) trial, rosuvastatin improved markers of inflammation including interferon  $\gamma$  inducible protein-10 (IP-10) and lipoprotein-associated phospholipase A2 (Lp-PLA2), immune activation including soluble CD14 (sCD14), proportion of tissue factor expressing patrolling monocytes and proportion of activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells<sup>4–6</sup> and oxidized low density lipoprotein (oxLDL).<sup>7</sup>

Smoking is highly prevalent in people living with HIV<sup>8</sup> and has long been recognized as an important contributor to CVD.<sup>9</sup> Understanding the effect of smoking on CVD risk reduction interventions such as statins is relevant to HIV care providers because it will help to prioritize which patients

should be targeted for the intervention. The aim of this exploratory analysis was to assess whether smoking status modifies the effect of rosuvastatin on select outcome measures that differed between groups over the SATURN-HIV study.

The SATURN-HIV study is a 96-week, single site, randomized, placebo-controlled trial to evaluate the effect of rosuvastatin on immune activation and subclinical vascular disease in HIV-infected adults on ART. The primary results of this trial have been published<sup>2</sup> and full eligibility criteria may be found on clinicaltrials.gov (NCT01218802). In brief, participants were  $\geq 18$  years old with HIV-1 infection on stable ART for at least 3 months with HIV-1 RNA  $< 1,000$  copies/mL and fasting LDL  $\leq 130$  mg/dl. Additional entry criteria included proportion of CD8<sup>+</sup> T cells that express CD38 and human leukocyte antigen (HLA)-DR  $\geq 19\%$  or high sensitivity C-reactive protein (hsCRP)  $\geq 2$  mg/liter. Known coronary artery disease or diabetes or inflammatory conditions were exclusionary. Randomization was to rosuvastatin 10 mg daily or matching placebo. In this exploratory, *post hoc* analysis, we assessed whether smoking status

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modified the effect of rosuvastatin on changes in markers of subclinical vascular disease, immune activation, and inflammation that differed between groups over the study. The study was approved by the University Hospitals Cleveland Medical Center Institutional Review Board.

### Study Evaluations

All participants underwent carotid intima media thickness (IMT) measurement by high resolution ultrasound at 0, 48, and 96 weeks as previously described.<sup>10</sup> Additionally, a 12-h fasting blood draw was performed at 0, 24, 48, and 96 weeks. Samples were cryopreserved at  $-80^{\circ}\text{C}$  until analyzed in batches. Cellular markers of immune activation were phenotyped from peripheral blood mononuclear cells by flow cytometry as previously described.<sup>4</sup>  $\text{CD4}^{+}$  and  $\text{CD8}^{+}$  T cells expressing CD38 and HLA-DR (activated) were quantified as a percentage of the  $\text{CD4}^{+}$  and  $\text{CD8}^{+}$  lymphocyte population, respectively. Monocyte subsets including  $\text{CD14}^{+}\text{CD16}^{+}$  (inflammatory) and  $\text{CD14}^{\text{dim}}\text{CD16}^{+}$  (patrolling) were quantified as a percentage of the monocyte population. Monocyte subset expression of tissue factor was also quantified. Soluble markers of monocyte activation (sCD14), systemic and vascular inflammation (IP-10 and Lp-PLA2), and oxLDL were measured by ELISA (sCD14 and IP-10; R&D Systems, Minneapolis, MN; Lp-PLA2; diaDexus, Inc., CA; oxLDL; Mercodia, Uppsala, Sweden).

### Statistical Analysis

ANOVA was used to model each outcome. Outcomes evaluated were those that differed statistically between groups in the SATURN-HIV study, that is, absolute changes in log-transformed common carotid artery (CCA) IMT, IP-10, Lp-PLA2, sCD14, proportion of tissue factor expressing patrolling monocytes, proportion of activated  $\text{CD4}^{+}$  and  $\text{CD8}^{+}$  T cells and oxLDL over 48 and 96 weeks. Independent variables included in each model were baseline value for the outcome measure, group, smoking status, and a group by smoking status interaction. All statistical tests were two-sided and considered statistically significant if  $p < .05$  in this hypothesis-generating study. Analyses were performed using SAS v. 9.4 (The SAS Institute, Cary, NC).

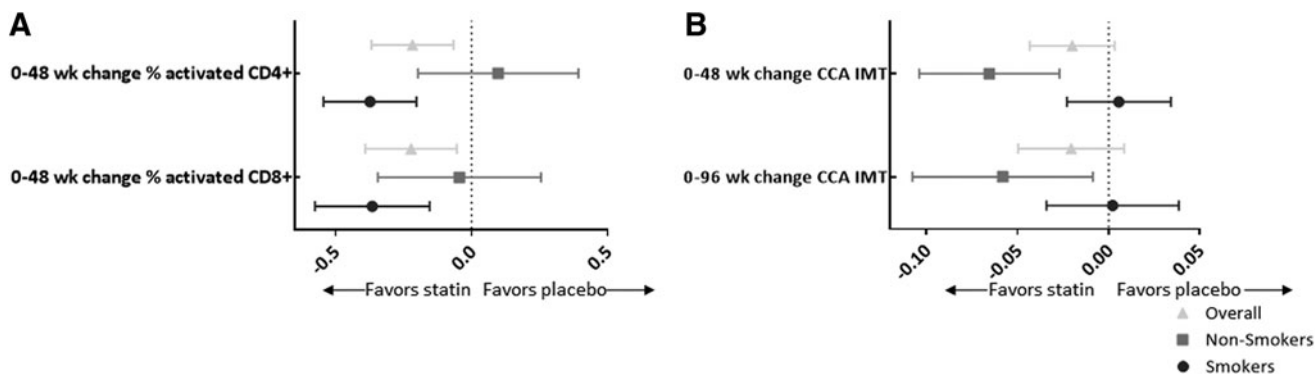
### Results

One hundred forty-seven adults were randomized (72 to rosuvastatin and 75 to placebo). The randomization groups were similar at baseline. Overall, mean  $\pm$  SD age was  $45.4 \pm 9.9$  years, 78% were men and 68% were African American. Sixty-three percent were current smokers (mean of  $0.6 \pm 0.44$  packs/day) and another 16% were past smokers. Mean known duration of HIV infection was  $12 \pm 7$  years and current and nadir  $\text{CD4}^{+}$  T cell counts were  $640 \pm 300$  and  $200 \pm 146$  cell/mm<sup>3</sup>, respectively. Most (78%) had HIV-1 RNA  $< 50$  copies/ml. At baseline, current smokers were similar to nonsmokers based on each of these factors as well.

There were statistically significant randomization group by smoking status interaction terms for 0–24 ( $p = .01$ ) and 0–48 ( $p < .01$ ) week changes in proportion of activated  $\text{CD4}^{+}$  T cells and a trend toward significance for 0–48 week change in activated  $\text{CD8}^{+}$  T cells ( $p = .07$ ). In addition, the interaction was significant for 0–48 week change in CCA IMT ( $p < .01$ ) and trended toward significance for 0–96 week change in CCA IMT ( $p = .06$ ). The stratum-specific estimates reveal that the improvement in  $\text{CD4}^{+}$  and  $\text{CD8}^{+}$  T cell activation with rosuvastatin is statistically significant for the smoking group only. In contrast, the beneficial effect of rosuvastatin on CCA IMT was significant for the nonsmoking group only. For smokers, rosuvastatin did improve T cell activation; however, the beneficial effect of rosuvastatin on CCA IMT was not apparent in this group (Fig. 1). No effect modification by smoking was detected for changes in other markers tested.

### Discussion

In this analysis, we have shown that the beneficial effects of rosuvastatin on T cell activation and subclinical vascular disease differ between HIV-infected smokers and nonsmokers. Interestingly, the improvement in T cell activation with rosuvastatin was only apparent in HIV-infected smokers; whereas, the beneficial effect of rosuvastatin on CCA IMT was not present in this group. At a first glance, the effect of smoking status on CCA IMT and T cell activation may seem contradictory; however, we have previously shown that the changes in T cell activation did not predict changes in CCA IMT<sup>2</sup> and so these effects are likely independent of one another.



**FIG. 1.** (A) The symbols represent the mean difference in 0–48 week change in log-transformed proportion of activated  $\text{CD8}^{+}$  and  $\text{CD4}^{+}$  T cells between rosuvastatin and placebo groups. The error bars show the 95% confidence interval around the means. (B) The symbols represent the mean difference in 0–48 and 0–96 week change in log-transformed common carotid artery intima media thickness between rosuvastatin and placebo groups. The error bars again show the 95% confidence interval around the means. For both figures, a mean difference less than 0 favors the rosuvastatin group.

Although the mechanisms linking smoking to CVD are not fully known, a major contributor is smoking-induced oxidative stress that leads to endothelial dysfunction, lipid peroxidation, and platelet activation.<sup>11–13</sup> Further, in the Women's Health Study, smoking was associated with higher levels of hsCRP, interleukin-6, soluble intercellular adhesion molecule type 1, and E-selectin—four out of the five inflammatory markers tested.<sup>14</sup> In HIV, smokers had higher CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation and exhaustion, measures of monocyte activation (sCD14), and microbial translocation (bacterial lipopolysaccharide).<sup>15</sup> Atherosclerosis is known to be an inflammatory disease. T cells, macrophages, and mast cells dominate early atherosclerotic lesions and cytokines accelerate progression of the lesions, which may ultimately result in plaque rupture.<sup>16</sup> In HIV-related CVD, there have been many links to systemic<sup>17</sup> and vascular inflammation,<sup>18</sup> and monocyte activation.<sup>19,20</sup> Given this, it stands to reason that smoking may increase the risk of CVD in HIV even more than in the general population, which has been suggested by Freiberg *et al.* who showed a significant interaction between HIV and smoking status in risk of prevalent CVD.<sup>21</sup>

Whether smoking modifies the effect of statins on immune activation and CVD risk in the general population is largely unknown. However, in the Jupiter Trial, the hazard ratio for the combined primary endpoint of myocardial infarction, stroke, revascularization, unstable angina, or death from CVD was similar for smokers and nonsmokers and the interaction term was not statistically significant ( $p = .63$ ).<sup>22</sup> Even though this is counter to what we have shown with CCA IMT, it is possible that with the heightened immune activation that characterizes chronic HIV infection, the additional effect of smoking may be too much to correct with statins.

Because SATURN-HIV was not designed to test effect modification, we may not have detected all relevant interactions due to limited power. As only participants with fasting LDL  $\leq 130$  mg/dl and heightened hsCRP or evidence of increased T cell activation were included in this study, results may not be generalizable to all HIV-infected individuals. Last, smoking status was not a stratification variable during randomization. As such, while baseline factors were similar between randomization groups and by smoking status, all factors may not have been evenly distributed across interaction subgroups.

In conclusion, current smoking modifies the effect of rosvastatin on CCA IMT and T cell activation. The beneficial effect of rosvastatin on CCA IMT was not apparent in smokers even though T cell activation appears to improve more in this group. Targeting HIV-infected smokers with low LDL for primary CVD prevention utilizing rosvastatin may not be appropriate unless smoking cessation is achieved; however, further studies are needed to confirm these findings.

### Acknowledgments

This study was supported by the National Institutes of Health (grant numbers K23HL116209 to C.O.H., HD070490 and NR012642 to G.A.M.). Study drugs were provided by AstraZeneca. Technical assistance was provided by the Center for AIDS Research, Case Western Reserve University (P30 AI36219). Results were presented at the Conference on Retroviruses and Opportunistic Infections in Boston, Massachusetts, February 22–25, 2016.

### Disclosure Statement

C.O.H. has served on a medical advisory board for Gilead Sciences. G.A.M. has received research grants from BMS, Gilead Sciences, Merck, and GSK and has served as a consultant to BMS, Viiv/GSK, ICON, and Gilead.

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