Poster #743

Rosuvastatin Lowers Cystatin C in HIV-infected Subjects on Antiretroviral Therapy: SATURN-HIV

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BACKGROUND

In chronic treated HIV-infection, both creatinine and cystatin C based estimates of glomerular filtration rate ($eGFR_{cr}$ and $eGFR_{cys}$) are less accurate than combined estimates ($eGFR_{cr\text{-}cys}$) when compared to measured GFR; however, $eGFR_{cys}$ is a better predictor of mortality and cardiovascular events. This may be explained by non-GFR determinants of plasma cystatin C levels, particularly inflammation. Statins may improve cystatin C levels by decreasing inflammation. ***Therefore, the objectives of this study were:***

1. To assess the effect of rosuvastatin 10mg daily on plasma cystatin C concentrations over 24 weeks.
2. To explore the relationships between baseline and 0-24 week changes in cystatin C with measures of cardiovascular risk and biomarkers of inflammation and immune activation.

METHODS

Design: Randomized, double-blind, placebo controlled trial

Subjects:

- The **S**topping **A**therosclerosis and **T**reating **U**nhealthy bone with **R**osuvastati**N** in HIV (SATURN-HIV) trial randomized participants to 10mg daily or matching placebo. This is a pre-specified 0-24 week analysis of cystatin C changes.
- All were ≥18 years of age, on stable ART with LDL-cholesterol (LDL-C) ≤130mg/dL and had either heightened T-cell activation (CD8+CD38+HLA-DR+ ≥19%) or increased inflammation (high sensitivity C-reactive protein (hsCRP ≥2mg/L) at baseline.

Kidney Function

- **Plasma cystatin C** concentration was measured by particle enhanced immunonepholometric assay on a BNII nephelometer at the University of Vermont Laboratory for Clinical Biochemistry Research.
- **Glomerular filtration rate ($eGFR_{cr}$)** was estimated using the creatinine-based 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Measures of Cardiovascular Risk:

- **Mean-max common carotid artery intima-media thickness (CCA-IMT):** the maximum IMT of the distal 1cm of the CCA far wall was measured using semi-automated edge detection software at 3 separate angles bilaterally (anterior, lateral, and posterior; 6 total measurements). The mean of the 6 maximum measurements was used for analyses.
- **Carotid distensibility** was calculated using the same formula [(2*(Ds-Dd)/Dd)/PP] used in the Women's Interagency Health Study (WIHS) and the Multicenter AIDS Cohort Study (MACS). Ds, systolic diameter; Dd, diastolic diameter; PP, pulse pressure.
- **Flow-mediated dilation (FMD) and hyperemic velocity (VTI) of the brachial artery was** measured by ultrasound with semi-automated edge detection software pre- and post- reactive hyperemia using the forearm occlusion method and 5 minute occlusion time.
- **Coronary artery calcium (CAC) score and epicardial fat:** Total CAC score (Agatston method) and epicardial fat volume were quantified from a non-contrast 64-slice multi-detector CT scan of the chest.

Inflammation and Immune Activation:

- **CD4+ and CD8+ T-cell activation (CD38 and HLA-DR expression)**
- **Inflammatory (CD14+CD16+) and patrolling ($CD14^{dim}CD16$+) monocytes.**
- **Plasma markers of monocyte activation: Soluble CD163 (sCD163) and CD14 (sCD14),**
- **Plasma biomarkers of inflammation and endothelial activation**

Statistics:

- Within- and between-group comparisons were made using t-tests or Wilcoxon tests as appropriate. ANCOVA was used to adjust means for clinically relevant variables. Univariable followed by multivariable linear regression was used to explore relationships between baseline cystatin C and markers of inflammation and immune activation. All non-Normally distributed variables were log-transformed for regression analyses. Spearman correlation analysis was used to examine associations between baseline cystatin C and measures of cardiovascular risk as well as 0-24 week changes in cystatin C and 0-24 week changes in inflammation/immune activation markers. All participants were analyzed as part of the group they were randomized to. Missing data in the longitudinal analyses were not imputed.

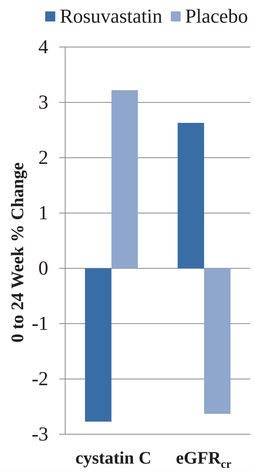
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RESULTS

Table 1: The baseline characteristics of 147 study subjects were balanced between groups. Eleven subjects (5 rosuvastatin and 6 placebo) discontinued the study before week 24.

Table 1	Rosuvastatin n=72	Placebo N=75
Demographics		
Age (years)	45 (41, 51)	47 (39, 53)
Male sex	81%	76%
African American race	69%	67%
HIV Parameters		
HIV duration (years)	11 (6, 17)	12 (6, 19)
Current CD4+ count (cells/µl)	608 (440, 848)	627 (398, 853)
Nadir CD4+ count (cells/µl)	173 (84, 312)	190 (89, 281)
Undetectable viral load (<50 copies/ml)	78%	77%
ART duration (years)	5.2 (3.1, 9.9)	5.9 (3.3, 9.6)
Current Protease Inhibitor Use	50%	48%
Current TDF Use	89%	88%
Metabolic and Cardiovascular Risk Factors		
Body Mass Index (kg/m^2)	27 (23, 30)	27 (23, 30)
Active Hepatitis B	4%	5%
Active Hepatitis C	7%	9%
Systolic Blood Pressure (mmHg)	122 (112, 136)	120 (110, 132)
Anti-hypertensive Medication Use	28%	24%
HDL Cholesterol (mg/dL)	47 (38, 58)	46 (37, 57)
LDL Cholesterol (mg/dL)	96 (76, 107)	97 (77, 121)
HOMA-IR ≥2.5	31%	41%
Smoking		
Current	60%	67%
Past	15%	16%
Never	25%	17%
Framingham Risk Score (% 10-year risk)	3 (1, 7)	4 (1, 7)
Kidney Function		
$eGFR_{cr}$ (CKD-EPI)	99 (85, 117)	100 (87, 118)
Cystatin C (mg/L)	0.84 (0.75, 0.99)	0.83 (0.71, 0.95)
Urine ACR >30	17%	17%

Figure: After 24 weeks, statin use slowed mean $eGFR_{cr}$ decline and decreased cystatin C. Similar results were seen for % change. Adjustment for protease inhibitor use and baseline $eGFR_{cr}$ did not change the cystatin C results; however, after adjustment for 0-24 week change in $eGFR_{cr}$, 0-24 week change in cystatin C was no longer significant between groups; p=0.099.



Cystatin C

	Absolute Change (mg/dL)	Within Group P-value	Between Group P-value	Percent Change	Within Group P-value	Between Group P-value
Rosuvastatin 10mg	-0.034 (0.130)	**0.035**	**0.008**	-2.77 (15.7)	**0.040**	**0.013**
Placebo	+0.023 (0.139)	0.198		3.22 (17.3)	0.227	

$eGFR_{cr}$

	Absolute Change ($ml/min/1.73m^2$)	Within Group P-value	Between Group P-value	Percent Change	Within Group P-value	Between Group P-value
Rosuvastatin 10mg	+1.61 (12.8)	0.307	**0.033**	+2.63 (14.2)	0.297	**0.023**
Placebo	-3.08 (12.7)	**0.047**		-2.63 (13.4)	0.109	

Table 2: Baseline cystatin C was associated with several markers of inflammation and immune activation and with current PI use, but not tenofovir. Soluble intercellular adhesion molecule-1 (sICAM-1) and tumor necrosis factor-α receptors I and II (TNF-RI/ II) were associated with cystatin C in a multivariable model independent of $eGFR_{cr}$. Only variables with p<0.25 in univariable analyses are shown (except for tenofovir use). Cystatin C and all biomarkers of inflammation were log-transformed prior to analyses. All variance inflation factors <1.5.

Table 2	Univariable Analysis		Multivariable Analysis	
	Estimate	p-value	Estimate	p-value
Kidney function				
$eGFR_{cr}$ (per 10 ml/min/$1.73m^3$)	**-0.06**	**<0.0001**	**-0.022**	**0.005**
Demographics and traditional kidney risk factors				
Age (per decade)	**0.077**	**<0.0001**		
Caucasian race (vs. all other)	**0.089**	**0.028**		
Male sex (vs. female)	0.069	0.123		
SBP (per 10mmHg)	0.016	0.152	**0.021**	**0.018**
DBP (per 10mmHg)	0.022	0.245		
HIV-specific factors and co-infections				
Nadir CD4 (per 100 cells/m^3)	**-0.02**	**0.042**		
HIV Duration (per year)	**0.006**	**0.024**		
ART Duration (per year)	0.006	0.093		
Current PI use (vs. no PI use)	**0.079**	**0.034**	**0.072**	**0.009**
Hepatitis B co-infection	0.132	0.129		
Hepatitis C co-infection	0.079	0.246		
Markers of inflammation and immune activation				
CD14+ CD16+ monocytes	0.034	0.210		
CD14dimCD16+ monocytes				
Soluble CD163	**0.174**	**<0.0001**		
Soluble CD14				
CD4+ DR+ 38+	**0.091**	**0.031**		
CD8+ DR+ 38+	0.052	0.13		
IL-6	**0.120**	**<0.0001**		
sICAM-1	0.049	0.156	**-0.058**	**0.041**
sVCAM-1	**0.216**	**<0.0001**		
sTNF-RI	**0.306**	**<0.0001**	**0.299**	**<0.0001**
sTNF-RII	**0.246**	**<0.0001**	**0.272**	**<0.0001**

Table 3: Higher cystatin C concentration was associated with worse vascular structure and function and higher volume of epicardial adipose tissue, an ectopic visceral fat depot. After adjustment for age, sex, and race, higher cystatin C was independently associated with higher CCA-IMT (p=0.029) and higher epicardial adipose tissue volume (p=0.02; logistic regression model not shown).

Table 3	Spearman r	p-value
Brachial FMD (%)	0.037	0.66
Brachial hyperemic VTI (cm)	-0.130	0.12
Mean-max CCA-IMT (µm)	**0.339**	**<0.0001**
Carotid distensibility (10^{-6}*$Newtons^{-1}$*m^2)	**-0.193**	**0.02**
Coronary calcium score	**0.170**	**0.04**
Epicardial adipose tissue (cm^3)	**0.314**	**0.0001**

Table 4: Changes in cystatin C concentration were correlated with changes in sTNF-RII receptors in both arms of the study; whereas significant correlations with changes in CD4+ T-cell activation and sICAM-1 were only observed in the statin arm. Within the statin arm, changes in cystatin C did not correlate with changes in LDL cholesterol (r = -0.127, p=0.31).

Table 4	Rosuvastatin		Placebo	
	Spearman r	p-value	Spearman r	p-value
CD14+CD16+ monocytes	-0.137	0.283	0.140	0.257
CD14dimCD16+ monocytes	0.075	0.559	0.037	0.764
Soluble CD163	-0.003	0.978	-0.064	0.607
Soluble CD14	0.118	0.348	-0.018	0.885
CD4+CD38+HLA-DR+ T-cells	**0.281**	**0.025**	0.022	0.862
CD8+CD38+HLA-DR+ T-cells	0.159	0.210	0.049	0.691
IL-6	0.010	0.933	0.058	0.634
sICAM-1	**0.460**	**<0.0001**	0.186	0.126
sVCAM-1	0.180	0.144	0.109	0.375
sTNF-RI	0.184	0.136	0.111	0.362
sTNF-RII	**0.372**	**0.002**	**0.314**	**0.009**

CONCLUSIONS

- In this study, rosuvastatin 10mg daily reduced plasma cystatin C and slowed kidney function decline over 24 weeks. These findings suggest that HIV-infected subjects with elevated markers of inflammation despite effective ART are a sub-group of patients that may gain a protective renal benefit from statins.
- Plasma cystatin C was associated with inflammation and endothelial activation at baseline independent of $eGFR_{cr}$, and changes in cystatin C correlated with changes in inflammation, endothelial activation, and T-cell activation (but not LDL change) suggesting that some of the statin effect on plasma cystatin C levels may be related to changes in systemic inflammation and immune activation.
- Cystatin C is a potent predictor of cardiovascular events and mortality in chronic HIV infection and was independently associated with CVD risk markers in this study. Further studies are needed (see poster #797) to determine whether this cystatin C-CVD relationship may be explained by non-GFR factors such as inflammation.