# A prospective, randomized clinical trial of antiretroviral therapies on carotid wall thickness

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**Objective:** This article compares the effects of initiating three contemporary antiretroviral therapy (ART) regimens on progression of carotid artery intima–media thickness (IMT) over 3 years.

**Design:** Randomized clinical trial.

**Setting:** Multicenter (26 institutions).

**Patients:** ART-naive HIV-infected individuals ($n = 328$) without known cardiovascular disease or diabetes mellitus.

**Intervention:** Random assignment to tenofovir/emtricitabine along with atazanavir/ritonavir (ATV/r), darunavir/ritonavir (DRV/r), or raltegravir (RAL).

**Main outcome measures:** Right-sided carotid IMT was evaluated by B-mode ultrasonography before ART initiation, and then after 48, 96, and 144 weeks. Comparisons of yearly rates of change in carotid IMT used mixed-effects linear regression models that permitted not only evaluation of the effects of ART on carotid IMT progression but also how ART-associated changes in traditional risk factors, bilirubin, and markers of HIV infection were associated carotid IMT progression.

**Results:** HIV-1 RNA suppression rates were high in all arms (>85%) over 144 weeks. Modest increases in triglycerides and non-high-density lipoprotein cholesterol levels were observed in the protease inhibitor-containing arms compared with decreases with RAL. In contrast, carotid IMT progressed more slowly on ATV/r [8.2, 95% confidence interval (5.6, 10.8) μm/year] than DRV/r [12.9 (10.3, 15.5) μm/year, $P = 0.013$]; changes with RAL were intermediate [10.7 (9.2, 12.2) μm/year, $P = 0.15$ vs. ATV/r; $P = 0.31$ vs. DRV/r]. Bilirubin and non-high-density lipoprotein cholesterol levels appeared to influence carotid IMT progression rates.

**Conclusion:** In ART-naive HIV-infected individuals at low cardiovascular disease risk, carotid IMT progressed more slowly in participants initiating ATV/r than those initiating DRV/r, with intermediate changes associated with RAL. This effect may be due, in part, to hyperbilirubinemia. 

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## Introduction

The relative contributions of antiretroviral therapy (ART), traditional cardiovascular disease (CVD) risk factors, inflammation, and immune activation on arterial injury and atherosclerosis progression in HIV-infected individuals are not known [1]. Previous observational studies, including studies of carotid intima–media thickness (IMT), have suggested that patients with HIV infection are at increased risk for CVD [1,2]. Some studies suggest that certain ART, including certain protease inhibitors and abacavir, may cause increased CVD risk in patients with HIV infection [3,4]. However, differences in the prevalence of CVD risk factors among individuals with HIV infection, which may partially be related to ART, and the effects of persistent inflammation and immune activation may also contribute to increased CVD risk among individuals with HIV [5,6].

Newer ART regimens have less adverse metabolic effects than older protease inhibitor-based regimens. For example, both boosted darunavir (darunavir/ritonavir, DRV/r) and boosted atazanavir (atazanavir/ritonavir, ATV/r) are associated with less adverse lipid profiles, less insulin resistance, and less lipodystrophy than older protease inhibitor regimens [7–9]. Similarly, raltegravir (RAL), an integrase inhibitor, has relatively neutral metabolic effects [10,11]. These three modern ART regimens were directly compared for virologic, tolerability and metabolic outcomes in AIDS Clinical Trials Group study A5257 (ClinicalTrials.gov: NCT00851799 and NCT00811954), a prospective, randomized trial [12]. Previous comparisons of the arterial effects of ART have been limited by inclusion of individuals who received prior ART. The present study – a predefined, prospective substudy of A5257 – offered the unique opportunity to isolate the effects of three modern ART regimens from the persistent effects of previous ART.

The primary objective of this study was to compare the effects of three contemporary ART regimens on carotid IMT progression, a validated marker of carotid wall injury and future CVD risk [13–15]. Carotid IMT is a common surrogate endpoint in clinical trials of CVD risk reduction such as those using lipid-lowering and antihypertensive therapies [13–15]. Considering its antioxidant effects and the increased incidence of hyperbilirubinemia among individuals who take atazanavir, we also evaluated (*post hoc*) the effects of on-treatment bilirubin levels on carotid IMT progression.

## Materials and methods

### Study design

Study A5260s, a substudy of AIDS Clinical Trial Group Study A5257, is a prospective, longitudinal evaluation of ART-naïve, HIV-infected individuals without known CVD, diabetes mellitus, or use of lipid-lowering medications. Eligible A5257 participants were randomized equally to one of the three regimens of tenofovir/emtricitabine along with ATV/r, DRV/r, or RAL; randomization was stratified by screening HIV RNA level (>100 000 or ≤100 000 copies/ml) and Framingham 10-year Coronary Heart Disease Risk Score (<6% or ≥6% risk). Substudy visits were scheduled at baseline, 4, 24, 48, 96, and 144 weeks after treatment initiation. Final substudy visits could occur at any time between 112 and 144 weeks after treatment initiation. Study A5260s as well as its parent study, A5257, was approved by the Institutional Review Boards of all 26 participating sites and all participants provided written, informed consent (see Appendix A, http://links.lww.com/QAD/A722). Current use of statins, fish oil (>2 g/day), fibric acid derivatives, or niacin (>1000 mg/day) was exclusionary at enrolment. Detailed inclusion and exclusion criteria for the study were reported previously in our baseline, cross-sectional analysis [16].

### Carotid artery ultrasonography

B-mode images of the distal right common carotid artery (CCA) and the right carotid artery bifurcation were acquired with a high-resolution linear array ultrasound transducer with simultaneous electrographic tracings before ART initiation, and then after 48, 96, and 112–144 weeks [16]. The right CCA was imaged in cross-section and the transducer moved laterally into the jugular vein so they were stacked with the vein displayed on top of the artery. The transducer was rotated 90°, maintaining the jugular vein stacked above the CCA and bifurcation while obtaining a longitudinal view of both the vessels, emphasizing the far wall. Images were sent electronically to the University of Southern California Atherosclerosis Research Unit Core Imaging and Reading Center for quality control and interpretation by a single experienced technician. Carotid artery 'lesions' were defined as focal regions of IMT of at least 1.5 mm. The CCA and bifurcation were measured with a proprietary automated edge detection program. Paired ultrasound and phantom scans were obtained at baseline, but within group, coefficient of variants was paired at baseline CCA. The coefficients of variation for common carotid IMT scans and paired baseline carotid bifurcation IMT scans were 1.09 and 1.27%, respectively [16].

### Brachial artery reactivity assessments

Brachial artery flow-mediated dilation (FMD) was measured by ultrasound after induction of reactive hyperemia by lower arm occlusion, prior to ART initiation, and then after 4, 24, and 48 weeks, as described previously [16]. These scans were measured at the University of Wisconsin Atherosclerosis Imaging Research Program Core Laboratory.

### Laboratory and biomarker assessments

Blood samples were obtained from participants who were required to fast for at least 8 h and sent to core laboratories

for analyses, as described previously [16]. Lipoproteins as well as GlycA and GlycB were quantified by nuclear magnetic resonance spectroscopy at LipoScience (Raleigh, North Carolina, USA). Inflammatory biomarkers were measured at the University of Vermont Laboratory for Clinical Biochemistry on plasma stored at −70°C. These measures included high-sensitivity C-reactive protein (hsCRP) by nephelometry (Siemens BNII Nephelometer; Siemens Healthcare, Indianapolis, Indiana, USA), interleukin-6, and D-dimer. Plasma markers of monocyte and immune activation (soluble CD14 and CD163) were determined using ELISA (R&D Systems, Minneapolis, Minnesota, USA).

Peripheral blood mononuclear cells were isolated and cryopreserved within 30 h of blood collection. All specimens were stored in liquid nitrogen at −70°C. Immunophenotyping was performed on cryopreserved peripheral blood mononuclear cells using multicolor flow cytometry. The fluorochrome-conjugated antibodies used were anti-CD3 PE-Cy7 (clone SK7), anti-CD8 APC (clone RPA-T8), anti-HLADR FITC (clone L243), anti-CD38 PE (clone HB7), anti-CD14 APC (clone M5E2), anti-CD16 PE-Cy7 (clone 3G8) (BD Biosciences, San Jose, California, USA, and Invitrogen, Carlsbad, California, USA). Samples were acquired on an LSR-II flow cytometer Fluorescence Minus One and controls were prepared on controls for each run. Monocytes were gated as DUMP (CD2, CD3, CD19, CD20, and CD56) negative; HLADR-positive cells and proinflammatory monocytes were characterized and expressed as proportional percentages ($CD14^+$, $CD16^+$) as previously described. T-cell activation ($CD4^+CD38^+DR^+$ and $CD8^+CD38^+DR^+$) was measured by flow cytometry. Data were compensated and analyzed in FlowJo software, version 9.3.3 (Treestar, Ashland, Oregon, USA).

## Sample size considerations and statistical analysis

The primary hypothesis was that progression of right CCA IMT would be faster over 144 weeks in participants initiating a ritonavir-boosted protease inhibitor-containing regimen compared with a RAL-containing regimen. The target sample size of 110 participants/group provided 80% power to detect a difference in the rate of CCA IMT progression between the pooled-protease inhibitor regimens and RAL of 1.3 μm/year. In the event of a significant difference between ATV/r and DRV/r, the study was designed to examine all pairwise treatment group comparisons.

Primary treatment group comparisons used an intention-to-treat approach; supportive analyses were restricted to on-treatment and successfully treated populations (Fig. 1). All analyses of IMT outcomes used mixed-effects linear regression model with random intercept and slope and unstructured covariance matrix on the random effect and adjusted for stratification factors. The treatment group effect of interest was the difference in the rate of change (i.e. the slope) of IMT over time (i.e. the interaction between the treatment group and time). Given randomization, the primary analysis included week 0 IMT in the outcome vector; supportive analyses included only on-treatment outcomes and adjusted for baseline IMT measure. Similar treatment group comparisons for the endothelial function week 24 change from baseline were conducted using linear regression with adjustment for week 0 baseline brachial artery diameter.

Associations between CCA IMT progression and traditional CVD risk factors, HIV-related measures, and other biomarkers were examined in analyses restricted to the successfully treated cohort to minimize confounding due to lack of control of HIV-1 viremia

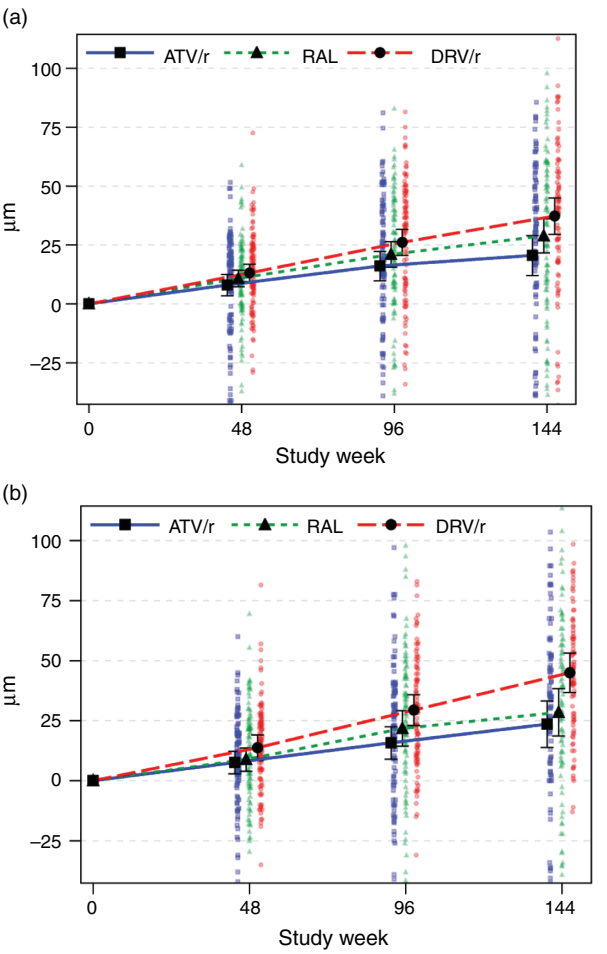


**Fig. 1. (a) Change in common carotid artery intima–media thickness progression by study week (intention to treat). (b) Change in carotid artery bifurcation intima–media thickness progression by study week (intention to treat).** Point estimates and error bars give mean and 95% confidence intervals, respectively. ATV/r, atazanavir/ritonavir; DRV/r, darunavir/ritonavir; RAL, raltegravir.

(Fig. 1). Baseline and early on-treatment (week 24 or 48) biomarker effects on both the level and rate of CCA IMT progression were included as continuous outcomes; time-updated proximal effects were also examined. To explore the potential of treatment-mediated pathways, analyses were also performed with adjustment for the treatment group. Attenuation of the estimated treatment group differences would be expected if treatment group differences were mediated by given biomarker changes.

To explore the potential that ATV/r-associated hyperbilirubinemia mediated the effects of this regimen on CCA IMT progression, we examined how adjustment for bilirubin elevations impacted the estimated treatment group differences. Specifically, early on-treatment bilirubin levels were parameterized as binary cut points of 0.6, 0.8, and 1.0 mg/dl and on a continuous scale [17].

Inference for all treatment comparisons was assessed against a type I error of 2.5% with 97.5% confidence intervals; all other inference was performed at a 5% type 1 error rate. Analyses were performed using SAS Version 9.2 (Cary, North Carolina, USA).

## Results

### Participant characteristics

The 334 participants in A5260s were similar to those enrolled in A5257 except for a smaller proportion of females and blacks and slightly higher $CD4^+$ cell count (Supplemental Table S1, http://links.lww.com/QAD/A722). Baseline participant characteristics for the 328 participants in the analysis population are in Table 1; no notable treatment group imbalances were seen. The Framingham coronary heart disease risk was estimated to be at least 6%/10 years in 12% of participants; 13% had metabolic syndrome and 9% had carotid lesions at baseline (6% ATV/r, 8% RAL, 12% DRV/r). Eighty-four percent completed study follow-up, and two participants died. Final CCA IMT scans occurred more than 112 weeks

**Table 1. Baseline characteristics by treatment group.**

| Characteristics[a] | Treatment group | | | |
|---|---|---|---|---|
| | Overall ($N=328$) | ATV/r ($N=109$) | RAL ($N=106$) | DRV/r ($N=113$) |
| Sex | | | | |
| Men | 294 (90%) | 99 (91%) | 94 (89%) | 101 (89%) |
| Women | 34 (10%) | 10 (9%) | 12 (11%) | 12 (11%) |
| Age (years) | 36 (28, 45) | 37 (31, 45) | 36 (27, 44) | 35 (27, 46) |
| Race/ethnicity | | | | |
| Non-Hispanic white | 144 (44%) | 53 (49%) | 43 (41%) | 48 (42%) |
| Non-Hispanic black | 105 (32%) | 34 (31%) | 34 (32%) | 37 (33%) |
| Hispanic | 65 (20%) | 20 (18%) | 20 (19%) | 25 (22%) |
| Asian/other/more than one race | 13 (4%) | 2 (2%) | 8 (8%) | 3 (3%) |
| 10-year risk of hard coronary artery disease[b] (%) | | | | |
| Low (<6%) | 289 (88%) | 96 (88%) | 96 (91%) | 97 (86%) |
| Medium/high (≥6%) | 39 (12%) | 13 (12%) | 10 (9%) | 16 (14%) |
| HIV-1 RNA ($log_{10}$ copies/ml) | 4.5 (4.0, 5.1) | 4.6 (4.0, 5.1) | 4.5 (4.1, 5.1) | 4.5 (4.0, 4.9) |
| $CD4^+$ cell count (cells/μl) | 349 (203, 455) | 350 (211, 461) | 343 (185, 445) | 355 (207, 461) |
| SBP (mmHg) | 117 (108, 125) | 117 (107, 124) | 117 (107, 125) | 117 (108, 125) |
| DBP (mmHg) | 74 (68, 80) | 74 (68, 80) | 74 (68, 80) | 76 (68, 80) |
| Fasting total cholesterol (mg/dl) | 152 (133, 177) | 153 (134, 178) | 150 (135, 178) | 150 (131, 172) |
| Fasting triglycerides (mg/dl) | 107 (78, 151) | 114 (86, 160) | 105 (76, 150) | 98 (74, 133) |
| Fasting HDL cholesterol (mg/dl) | 37 (31, 45) | 36 (32, 43) | 39 (32, 45) | 37 (30, 47) |
| Fasting non-HDL cholesterol (mg/dl) | 112 (94, 136) | 114 (96, 142) | 111 (97, 136) | 111 (89, 131) |
| Calculated fasting low-density lipoprotein cholesterol (mg/dl) | 89 (73, 109) | 91 (69, 109) | 89 (74, 110) | 88 (71, 107) |
| BMI ($kg/m^2$) | 25 (22, 28) | 26 (23, 29) | 24 (22, 28) | 24 (22, 27) |
| Waist circumference (cm) | 88 (81, 98) | 92 (81, 100) | 89 (82, 96) | 86 (81, 95) |
| Metabolic syndrome | 44 (13%) | 16 (15%) | 14 (13%) | 14 (12%) |
| Current smoker | 124 (38%) | 44 (40%) | 39 (37%) | 41 (36%) |
| Total bilirubin (mg/dl) | 0.50 (0.40, 0.60) | 0.50 (0.40, 0.60) | 0.45 (0.30, 0.60) | 0.40 (0.40, 0.60) |
| Brachial artery diameter (mm) | 0.43 (0.39, 0.46) | 0.43 (0.39, 0.46) | 0.43 (0.39, 0.46) | 0.43 (0.39, 0.46) |
| Brachial artery flow-mediated dilation (%) | 4.5 (3.0, 6.3) | 4.4 (3.2, 6.3) | 4.3 (2.6, 5.9) | 4.5 (3.1, 6.6) |
| Common carotid artery IMT (μm) | 0.65 (0.59, 0.70) | 0.66 (0.59, 0.72) | 0.64 (0.58, 0.69) | 0.64 (0.59, 0.70) |
| Carotid artery bifurcation IMT (μm) | 0.74 (0.65, 0.82) | 0.74 (0.65, 0.81) | 0.72 (0.64, 0.83) | 0.74 (0.66, 0.83) |
| Evidence of carotid lesions | 28 (9%) | 7 (6%) | 8 (8%) | 13 (12%) |

ATV/r, atazanavir/ritonavir; DRV/r, darunavir/ritonavir; HDL, high-density lipoprotein; IMT, intima–media thickness; RAL, raltegravir. All lipid panel measures are from samples that were centrally tested.
[a]Medians (first and third quartiles) or number (%).
[b]Myocardial infarction or death from coronary heart disease.

after starting treatment; 88% ($N=288$) had a baseline and at least 2 postentry scans.

HIV-1 RNA suppression rates were high in all arms throughout the study (>85% through 144 weeks). The virologic, immunologic, and metabolic responses to these regimens and their side-effects in the parent study have been reported elsewhere [12]. As in the parent study, modest and sustained increases in non-high-density lipoprotein (HDL) cholesterol and triglycerides levels were observed with initiation of ATV/r and DRV/r that were maintained over time, in contrast with decreases in these parameters observed with RAL [12]. There were trends toward increasing use of lipid medications in all groups over the duration of the study with similar numbers of participants on statins (two to five per group) at the final visit.

## Effects of treatment on carotid intima–media thickness progression

Significant differences between treatment groups were noted in the annual rate of change of the right CCA and bifurcation IMT values (Table 2, Fig. 1). Patients initiating ATV/r [8.2 (5.6, 10.8) μm/year] progressed more slowly than those initiating DRV/r [12.9 (10.3, 15.5) μm/year, $P=0.013$]. There was intermediate progression for those initiating RAL [10.7 (9.2, 12.2) μm/year; $P=0.15$ vs. ATV/r; $P=0.31$ vs. DRV/r]. The ATV/r benefit was more pronounced in the on-treatment and successfully treated cohorts [6.6 (3.2, 10.0) μm/year with ATV/r compared with 12.8 (9.7, 15.9) μm/year with DRV/r, $P=0.007$, and 10.2 (8.3, 12.2) μm/year with RAL, $P=0.044$). Similar findings were seen in the carotid bifurcation (Table 2, Fig. 1b).

Associations of longitudinal CCA IMT progression with baseline and early on-treatment risk factors, plasma biomarkers (hsCRP, interleukin-6, D-dimer, sCD14, sCD163, GlycA, and GlycB), and measures of cellular immune activation (%$CD4^+$: $CD38^+HLADR^+$ T cells, %$CD8^+$: $CD38^+HLADR^+$ T cells, and %$CD14^+CD16^+$ monocytes) are shown in Table 3. With the exception of weak evidence of associations suggesting faster CCA IMT progression for higher levels of baseline Homeostasis Model Assessment of Insulin Resistance ($P=0.06$) and hsCRP ($P=0.06$), no associations between CCA IMT progression and baseline risk factors or biomarkers were apparent ($P>0.19$). There was an association between faster CCA IMT progression and higher on-treatment (week 24) levels of non-HDL cholesterol ($P=0.011$). This association did not change upon adjustment for treatment group and estimated treatment effects were unchanged suggesting that the treatment group differences were not mediated through the non-HDL cholesterol pathway. No other associations between CCA IMT progression and early on-treatment or time-updated (proximal) levels of biomarkers were apparent ($P>0.12$, Table 3).

## Effects of treatment on carotid artery lesions and brachial artery reactivity

New carotid lesions were uncommon. Among the 28 patients with evidence of lesions at baseline, 22 patients (79%) were continually observed with evidence of lesions at every study week thereafter. At the final visit, lesions were apparent in similar proportions among 28 patients (10% in the ATV/r, 15% in the DRV/r, and 7% in the RAL groups). Rates of carotid IMT progression did not differ after 48 ($P=0.97$) and 96 weeks ($P=0.97$) based on the presence or absence of carotid lesions.

FMD did not change significantly within any treatment arm ($P>0.35$). No significant differences in FMD between arms were noted at any study week ($P>0.28$). More than half of all patients in all treatment arms had changes in FMD within 2% at each study week, 61% at

**Table 2. Annualized rates of change in common carotid artery and carotid artery bifurcation intima–media thickness by treatment group and treatment group comparisons.**

| | CCA IMT (μm/year)[a,c] | | CCA IMT (μm/year)[a,d] | | CCA IMT (μm/year)[b,d] | | Carotid bifurcation IMT (μm/year)[a,c] | |
|---|---|---|---|---|---|---|---|---|
| Estimated progression rates by treatment group (95% confidence intervals)[c] | | | | | | | | |
| | | *P* | | *P* | | *P* | | *P* |
| ATV/r | 8.2 (5.6, 10.8) | **<0.001** | 7.9 (5.2, 10.6) | **<0.001** | 6.6 (3.2, 10.0) | **<0.001** | 8.7 (5.6, 11.8) | **<0.001** |
| DRV/r | 12.9 (10.3, 15.5) | **<0.001** | 12.6 (9.8, 15.3) | **<0.001** | 12.8 (9.7, 15.9) | **<0.001** | 14.7 (11.6,17.8) | **<0.001** |
| RAL | 10.7 (9.2, 12.2) | **<0.001** | 10.4 (8.7, 12.1) | **<0.001** | 10.2 (8.3, 12.2) | **<0.001** | 11.5 (9.7, 13.3) | **<0.001** |
| Estimated difference (first minus second arm) in progression rates (97.5% confidence intervals)[c] | | | | | | | | |
| | | *P* | | *P* | | *P* | | *P* |
| ATV/r vs. DRV/r | −4.7 (−8.9, −0.4) | **0.013** | −6.0 (−11.0, −1.0) | **0.007** | −4.7 (−9.0, −0.5) | **0.013** | −6.2 (−11.3, −1.1) | **0.007** |
| ATV/r vs. RAL | −2.8 (−7.0, 1.5) | 0.15 | −2.3 (−7.4, 2.7) | 0.30 | −2.7 (−7.0, 1.5) | 0.15 | −4.6 (−9.7, 0.5) | 0.044 |
| DRV/r vs. RAL | 1.9 (−2.4, 6.2) | 0.31 | 3.7 (−1.4, 8.7) | 0.11 | 2.0 (−2.3, 6.3) | 0.30 | 1.6 (−3.3, 6.5) | 0.46 |

ATV/r, atazanavir/ritonavir; CCA, common carotid artery; DRV/r, darunavir/ritonavir; IMT, intima–media thickness; RAL, raltegravir. Values in bold are statistically significant.
[a]Intention to treat.
[b]Successfully treated cohort.
[c]Analyses adjusted for screening HIV-1 RNA level and Framingham risk scores.
[d]Analyses adjusted for screening HIV-1 RNA level and Framingham risk scores and baseline CCA IMT (μm).

**Table 3. Longitudinal common carotid artery intima–media thickness associations with baseline and week 24 or 48 biomarker in successfully treated population.**

| | Additional annualized rate of IMT (μm/year) per unit change in biomarker (95% confidence interval) | | | |
|---|---|---|---|---|
| | Baseline biomarker[a] | | Week 24 or 48 biomarker[b] | |
| | | *P* | | *P* |
| Homeostasis model assessment of insulin resistance ($log_{10}$) | 5.27 (−0.29, 10.83) | 0.06 | 1.56 (−3.63, 6.74) | 0.55 |
| Non-high-density lipoprotein cholesterol (per 30 mg/dl) | 1.24 (−0.62, 3.09) | 0.19 | 2.22 (0.52, 3.93) | 0.011 |
| D-dimer ($log_{10}$ μg/ml) | 1.97 (−2.10, 6.04) | 0.34 | 1.08 (−2.92, 5.08) | 0.60 |
| High sensitivity C-reactive protein ($log_{10}$ μg/ml) | 3.93 (−0.18, 8.04) | 0.06 | −0.96 (−4.29, 2.37) | 0.57 |
| Interleukin-6 ($log_{10}$ pg/ml) | 2.54 (−4.29, 9.37) | 0.46 | −2.27 (−6.53, 1.98) | 0.29 |
| Soluble CD14 ($log_{10}$ ng/ml) | 3.03 (−14.75, 20.81) | 0.74 | 4.37 (−14.39, 23.12) | 0.65 |
| Soluble CD163 ($log_{10}$ ng/ml) | 4.93 (−3.36, 13.22) | 0.24 | 0.36 (−10.92, 11.64) | 0.95 |
| GlycA (μmol/l) | −0.00 (−0.03, 0.02) | 0.94 | −0.01 (−0.03, 0.02) | 0.67 |
| GlycB (μmol/l) | 0.03 (−0.04, 0.11) | 0.37 | 0.03 (−0.03, 0.09) | 0.41 |
| %$CD4^+$: $CD38^+HLADR^+$ T cells | −0.07 (−0.20, 0.06) | 0.28 | −0.15 (−0.34, 0.05) | 0.15 |
| %$CD8^+$: $CD38^+HLADR^+$ T cells | −0.05 (−0.18, 0.08) | 0.45 | −0.12 (−0.28, 0.03) | 0.12 |
| %$CD14^+CD16^+$ monocytes | −0.05 (−0.25, 0.15) | 0.63 | 0.02 (−0.14, 0.17) | 0.83 |

IMT, intima–media thickness.
[a]Analyses adjusted for screening HIV-1 RNA level and Framingham risk scores, baseline common carotid artery intima–media thickness (μm), and baseline biomarker.
[b]Analyses adjusted for screening HIV-1 RNA level and Framingham risk scores and baseline common carotid artery intima–media (μm).

week 4, 56% at week 24, and 59% at week 48. Changes in FMD after 4, 24, and 48 weeks were not associated with 48 and 96-week changes in CCA IMT. Brachial artery diameters remained stable over time in all treatment groups with median changes not exceeding 0.01 mm.

### Effects of bilirubin elevations on carotid intima–media thickness progression

Bilirubin elevations are common during treatment with atazanavir. We hypothesized that the antioxidant effect of asymptomatic increases in unconjugated bilirubin in the ATV/r may contribute to slower progression of carotid IMT [18]. As expected, total bilirubin levels increased rapidly and remained elevated among individuals assigned to ATV/r (Supplemental Figure S2, http://links.lww.com/QAD/A722). In general, higher levels of on-treatment bilirubin were associated with slower progression of CCA IMT (Table 4). However, of all the analyses performed, the only effects that reached statistical significance were the weeks 4 and 24 bilirubin levels using a binary cut point of 0.6 mg/dl ($P = 0.021$ and 0.027, respectively). Similar trends were seen for the higher cut points of 0.8 and 1.0 mg/dl; however, these results did not achieve statistical significance ($P > 0.12$). Upon adjustment for weeks 4 and 24 bilirubin levels using the 0.6 mg/dl cut point, the treatment group differences in the rate of CCA IMT change between ATV/r and DRV/r as well as between ATV/r and RAL were attenuated with a loss of the previously described statistical significance for the DRV/r arm comparisons; this attenuation was not seen at the higher thresholds (Table 4 and Supplemental Table S2, http://links.lww.com/QAD/A722).

**Table 4. Longitudinal common carotid artery intima–media thickness associations with weeks 4 and 24 total bilirubin levels in successfully treated population.**

| | Difference in annual rates of change (μm/year) with 95% confidence interval | |
|---|---|---|
| Bilirubin level at week 4[a] | | *P* |
| Continuous (mg/dl) | −0.79 (−2.77, 1.19) | 0.43 |
| > vs. ≤0.6 | −4.49 (−8.30, −0.67) | 0.021 |
| > vs. ≤0.8 | −2.37 (−6.47, 1.72) | 0.25 |
| > vs. ≤1.0 | −3.35 (−7.68, 0.98) | 0.13 |
| Bilirubin level at week 24[a] | | *P* |
| Continuous (mg/dl) | −1.13 (−2.95, 0.69) | 0.22 |
| > vs. ≤0.6 | −4.21 (−7.95, −0.47) | 0.027 |
| > vs. ≤0.8 | −2.61 (−6.67, 1.46) | 0.21 |
| > vs. ≤1.0 | −3.38 (−7.65, 0.88) | 0.12 |

CCA, common carotid artery.
[a]Analyses adjusted for screening HIV-1 RNA level and Framingham risk scores and baseline CCA intima–media thickness (μm).

## Discussion

In this prospective, randomized clinical trial of 328 HIV-infected individuals beginning their first ART regimen, we found that after 3 years, both CCA and carotid bifurcation IMT progressed more slowly in participants initiating ATV/r than those initiating DRV/r, with intermediate changes associated with RAL, despite more salutary lipid effects with RAL. These differences were more pronounced in participants who remained successfully treated on their randomized regimen for the study duration. The mean differences in carotid IMT progression between the ATV/r and DRV/r are clinically relevant and on the order of one-half to one-third the effect of statin therapy in patients with high cholesterol. Our data also suggest that low-level hyperbilirubinemia may have affected CCA IMT progression and mediated the ATV/r effect.

It is unclear whether differences in ART regimens or their metabolic effects influence CVD risk, in large part because few randomized clinical trials assessing ART have had a significant number of adverse CVD events due to the young age of the participants and short duration of follow-up [1]. Thus, evaluating the effects of ART on surrogate endpoints that predict adverse CVD events is necessary. This was the first prospective randomized clinical trial to evaluate the effects of contemporary ART on carotid IMT, a proven surrogate of CVD risk in clinical trials in individuals without HIV infection [13–15]. Patients were followed for up to 144 weeks with high levels of drug compliance and data availability, and although a greater number of participants discontinued ATV/r for tolerability than DRV/r and RAL, the findings of our intention-to-treat analysis were strengthened in the setting of successful treatment.

We chose carotid IMT progression as a primary endpoint for this study because it is a well established surrogate for CVD risk and for evaluating the arterial and CVD risk effects of preventive therapies such as lipid-lowering and antihypertensive medications [13–15]. Although a recent meta-regression suggested that carotid IMT progression is not a strong marker of CVD risk in observational studies [19], in clinical trials, a recent meta-analysis demonstrated that for each 10 μm/year slower rate of carotid IMT, there was an 18% lower odds for myocardial infarction [14]. Statin therapy was also associated with a 12 μm/year lower rate of carotid IMT progression and a 52% reduction in CVD events [13]. These findings strongly support use of change in carotid IMT as a surrogate endpoint of changes in CVD risk in treatment intervention studies.

Our finding of a benefit of ATV/r over DRV/r was unexpected. In fact, A5260s was originally designed with the assumption of no difference in carotid IMT progression between ATV/r and DRV/r and that these arms would be pooled for comparison with participants assigned to receive RAL. On assessment for baseline and on-treatment risk factors and biomarkers associated with carotid IMT progression, statistically significant associations were detected only with early (week 24) non-HDL cholesterol levels and total blood bilirubin higher than 0.6 mg/dl. Furthermore, the adjustment for on-treatment differences impacted the estimated treatment group difference only for bilirubin levels. Although the bilirubin analyses were exploratory, *post hoc*, and limited by almost nonoverlapping bilirubin distributions between the ATV/r compared with the DRV/r and RAL groups, it is plausible that the ATV/r benefit was mediated by bilirubin. Bilirubin is an antioxidant that improves endothelial function and retards progression of atherosclerosis [20,21]. In a recent report from the National Health and Nutrition Examination Survey, total bilirubin levels were associated with total mortality in the United States and values above 0.6 as well as values below 0.4 mg/dl were significantly associated with mortality in models fully adjusted for, among other factors, CVD, diabetes mellitus, cancer, use of certain medications, kidney function, and liver function as well as smoking, alcohol, and BMI [17]. Owing to the fact that atherosclerosis involves arterial oxidative stress, bilirubin has been considered a possible target for as an antiatherosclerotic agent. Further supporting this observation is our on-treatment analysis that biases the study population toward individuals with long-term ATV/r and thus bilirubin exposure; it suggested an even greater ATV/r benefit.

In this study, starting ART was not associated with improvements in FMD after 4, 24, and 48 weeks. A previous randomized clinical trial of ART-naive individuals randomized to receive three class-sparing older ART regimens demonstrated significant endothelial dysfunction that improved after 4 and 24 weeks in all ART arms with successful treatment [22]. There were notable differences between participants in A5152s and our study with the appearance that our participants were healthier. Compared with those in A5152s, our participants, prior to ART initiation, had higher FMD (4.5 vs. 3.7%). Indeed, the FMD in our study was similar to that observed after 4 weeks of ART in A5152s. Our participants also had higher average measures of HDL cholesterol (39 vs. 31 mg/dl) and $CD4^+$ cell count (349 vs. 245 cells/μl), and lower HIV RNA levels (4.5 vs. 4.8 $\log_{10}$ copies/ml). Of note, a more recent cross-sectional study also demonstrated that neither use of ATV/r nor total bilirubin levels were associated with improved FMD in ART-treated and suppressed individuals [23].

## Limitations

As there was no ART-experienced control group, the generalizability of our findings to ART-experienced patients is uncertain. As there were no HIV-infected but untreated patients, regression to the mean for the FMD results cannot be excluded. The participants in the study on average were young, at low CVD risk, and only 10% were females, so generalizability to older patients, especially women, may be limited. Nevertheless, this is the largest report with the longest follow-up describing, to date, the effects of modern ART on CVD risk among treatment-naïve HIV-infected individuals. This study is relatively small and underpowered to detect modest risk factor associations with carotid IMT progression as well as the effects of elevated bilirubin levels among individuals receiving ATV/r. The large number of sites may also have introduced variability into the measures of carotid IMT and FMD and may have obscured some relationships; however, markers of variability were excellent. As few individuals in the RAL and DRV/r groups had extreme bilirubin elevations and because treatment group and

extreme bilirubin elevations were collinear, these associations require cautious interpretation. These caveats, as well as the absence of a large distribution of bilirubin levels within the ATV/r group precluded our ability to definitively establish whether bilirubin elevations contributed to the effect of atazanavir on progression of carotid IMT.

### Conclusion

Among treatment-naive individuals, carotid IMT progresses more slowly on ATV/r than DRV/r, with intermediate changes in individuals receiving RAL, despite its more salutary lipid effects. Low-level hyperbilirubinemia seems to affect carotid IMT progression and may mediate some of the effect of ATV/r on IMT progression.

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### Conflicts of interest

J.H.S. is Director of an ultrasound lab that receives research funding from Gilead. He receives royalties from the Wisconsin Alumni Research Foundation for intellectual property related to carotid ultrasound and vascular age (technology not used in this study). T.T.B. has served as a consultant for Bristol-Myers Squibb, GlaxoSmithKline, Merck, Abbott, Gilead, ViiV Healthcare and has received research funding from Merck and GlaxoSmithKline. G.A.M. has served as a consultant, speaker, and has received research funding from Bristol-Myers Squibb, GlaxoSmithKline, Gilead, Merck, and Tibotec. She also chaired a Data and Safety Monitoring Board for a Pfizer-funded study. The other authors have no conflicts of interest.

*Clinical trial registration information: ClinicalTrials.gov identifier: NCT00851799; http://clinicaltrials.gov/show/NCT00851799.*

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