Open Forum Infectious Diseases

MAJOR ARTICLE

A Randomized Placebo Controlled Trial of Aspirin Effects on Immune Activation in Chronically Human Immunodeficiency Virus-Infected Adults on Virologically Suppressive Antiretroviral Therapy

Meagan P. O'Brien,[1] Peter W. Hunt,[2] Douglas W. Kitch,[3] Karin Klingman,[4] James H. Stein,[5] Nicholas T. Funderburg,[6] Jeffrey S. Berger,[7] Pablo Tebas,[8] Brian Clagett,[9] Daniela Moisi,[9] Netanya S. Utay,[10] Fran Aweeka,[2] and Judith A. Aberg[1]

[1]Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York; [2]Department of Medicine, University of California San Francisco; [3]Harvard T.H. Chan School of Public Health, Boston, Massachussetts; [4]HIV Research Branch, Therapeutics Research Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; [5]Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison; [6]School of Health and Rehabilitation Sciences, Ohio State University, Columbus; [7]Department of Medicine, NYU School of Medicine, New York, New York; [8]Department of Medicine, Hospital of the University of Pennsylvania, Philadelphia; [9]Case Western Reserve University, Cleveland, Ohio; [10]Department of Medicine, University of Texas Medical Branch at Galveston

Background. Immune activation persists despite suppressive antiretroviral therapy (ART) in human immunodeficiency virus (HIV) infection and predicts non-Acquired Immune Deficiency Syndrome (AIDS) comorbidities including cardiovascular disease. Activated platelets play a key role in atherothrombosis and inflammation, and platelets are hyperactivated in chronic HIV infection. Aspirin is a potent inhibitor of platelet activation through the cyclooxygenase-1 (COX-1) pathway. We hypothesized that platelet activation contributes to immune activation and that aspirin would reduce immune activation and improve endothelial function in ART-suppressed HIV-infected individuals.

Methods. In this prospective, double-blind, randomized, placebo-controlled 3-arm trial of 121 HIV-infected participants on suppressive ART for >48 weeks, we evaluated the effects of 12 weeks of daily aspirin 100 mg, aspirin 300 mg, or placebo on soluble and cellular immune activation markers, flow-mediated dilation (FMD) of the brachial artery, and serum thromboxane B_2, a direct readout of platelet COX-1 inhibition.

Results. The 300-mg and 100-mg aspirin arms did not differ from placebo in effects on soluble CD14, interleukin (IL)-6, soluble CD163, D-dimer, T-cell or monocyte activation, or the other immunologic endpoints measured. Endothelial function, as measured by FMD, also was not significantly changed when comparing the 300-mg and 100-mg aspirin arms to placebo.

Conclusions. Aspirin treatment for 12 weeks does not have a major impact on soluble CD14, IL-6, soluble CD163, D-dimer, T-cell or monocyte activation, or FMD, suggesting that inhibition of COX-1-mediated platelet activation does not significantly improve HIV-related immune activation and endothelial dysfunction. Although future studies are needed to further identify the causes and consequences of platelet activation in ART-treated HIV infection, interventions other than COX-1 inhibition will need to be explored to directly reduce immune activation in treated HIV infection.

Keywords. aspirin; CD14; HIV; platelets.

Advances in antiretroviral therapy (ART) have increased survival of the human immunodeficiency virus (HIV)-infected population; however, life expectancy remains shorter, and the incidence of many morbidities including cardiovascular disease (CVD), remains higher than the general population, particularly among those who initiate ART at more advanced disease stages [1–3]. Although ART toxicities and traditional risk factors may contribute to increased risk, elevated markers of innate immune activation, inflammation, and thrombosis (eg, soluble [s]CD14, interleukin [IL]-6, D-dimer) independently predict morbidity and mortality and CVD [4–7].

Platelets are key mediators in hemostasis, thrombosis, and atherogenesis, but they may also contribute to inflammation [8]. Platelets express Toll-like receptors (TLRs) [9] and can become activated in response to proinflammatory cytokines or bacterial components [10–12], both of which are elevated in HIV infection [13, 14]. Increased platelet reactivity is associated with an increased risk of cardiovascular events [15–18], thus, antiplatelet therapy is recommended in those at high risk for or with known CVD.

Because platelets are activated in HIV infection and may contribute to immune activation [19–23], we hypothesized that blocking platelet activation with aspirin, an antiplatelet drug that irreversibly inhibits the cyclooxygenase-1 (COX-1)

Received 10 October 2016; editorial decision 22 December 2016; accepted 29 December 2016.
Correspondence: M. P. O'Brien, MD, One Gustave L. Levy Place, Box 1090, New York, NY 10029 (meagan.o'brien@mssm.edu).

Open Forum Infectious Diseases®

DOI: 10.1093/ofid/ofw278

pathway, might decrease immune activation, coagulation, and improve surrogate markers of CVD. A previous uncontrolled, open-label study showed that 1 week of low-dose aspirin reduced immune activation (eg, sCD14 and T-cell activation) in ART-treated HIV-infected patients [23]. The goals of this trial were to evaluate the longer-term immune effects of aspirin and its effects on endothelial function.

METHODS

Study Design

The AIDS Clinical Trials Group (ACTG) A5331 study was a double-blind, randomized, placebo-controlled 3-arm trial in ART-suppressed HIV-infected individuals who were not currently taking daily aspirin or nonsteroidal anti-inflammatory drugs. Participants were randomized to receive aspirin 100 mg, aspirin 300 mg, or placebo for 12 weeks, and were followed for 4 weeks after treatment was stopped. Aspirin and placebo tablets were supplied by Bayer Pharma AG, Germany. A permuted block randomization algorithm with the 3 treatments randomized in a 1:1:1 ratio, block size = 6, was generated by the ACTG Data Management Center (DMC), to determine the order of treatments. Unique sets of Study Identification Numbers (SIDs) were allocated to study sites. The ACTG DMC mailed out a prescription list to the site pharmacist (unblinded) containing a mapping of SID numbers to their corresponding treatment regimen. Participants were randomized to a treatment based on the permuted blocks, which were dynamically generated by the randomization system. Once the treatment for a given participant was determined, the Subject Enrollment System assigned an SID number corresponding to this treatment regimen. The site pharmacist was provided the SID number and used the prescription list to determine the appropriate treatment to dispense. Participants, care providers, and study team members were blinded. For the immunological studies, we collected blood samples 6 times: at pre-entry (1 day to 1 week before entry), entry (up to 3 days after randomization and on/before the first dose of study drug), at 2 weeks of study drug, at 11 weeks of study drug, at 12 weeks of study drug, and at 16 weeks, 4 weeks after study drug was stopped. We collected blood twice, at pre-entry/entry and week 11 or 12 to enable us to average 2 measures at each timepoint and thereby minimize intrasubject variability in soluble markers of inflammation [24]. We evaluated the effects of aspirin on endothelial function by measuring flow-mediated diameter (FMD) of the brachial artery at entry, before the first dose of study drug, and after 12 weeks of treatment, while still receiving treatment. Participants underwent clinical evaluations, detailed bleeding questionnaires, safety laboratory testing, and blood sample collection and storage at these visits. Serum specimens, ethylenediaminetetraacetic acid-treated specimens, and peripheral blood mononuclear cells (PBMCs) were cryopreserved for batched assays. The primary endpoint of the study was the change over 12 weeks in sCD14, chosen because of its low intrasubject variability compared with other soluble markers (eg, IL-6) and significance as a predictor of morbidity and mortality.

Study Participants

We enrolled participants ≥18 years of age, with chronic HIV infection on suppressive ART (HIV ribonucleic acid [RNA] below the quantification limit for at least 48 weeks, transiently detectable blips <500 copies/mL were allowed if flanked by undetectable values). Major exclusion criteria included a history of gastrointestinal or central nervous system bleeding, recent severe illness, liver or kidney disease, uncontrolled diabetes, pregnancy or breast-feeding, and use of immunosuppressive medications within 24 weeks of study entry. The study was conducted at 15 clinical research sites of the ACTG network. Institutional review boards at each site approved the study. All participants provided written informed consent. The trial was registered using Clinical Trials Registration NCT02155985.

Evaluations

Laboratory Evaluations

We measured plasma sCD14, D-dimer, IL-6, and sCD163 at pre-entry, entry, 2 weeks, 11 weeks, 12 weeks, and 16 weeks. We measured plasma kynurenine/tryptophan (KT) ratio, inflammatory monocyte subsets, and cellular markers of T-cell activation and exhaustion at entry and 12 weeks [25]. Serum thromboxane (TXB_2) is a direct measure of the capacity of platelets to synthesize TXA_2, and we used it as a specific measure of the pharmacological effect of aspirin on platelets and as a surrogate marker of adherence to the intervention. We measured serum TXB_2 levels at entry, 2 weeks, 12 weeks, and 16 weeks (4 weeks after stopping study drug). We measured urine 11-dehydrothromboxane ($11dhTxB_2$) (corrected for creatinine clearance) at entry and 12 weeks as a secondary measure of aspirin effectiveness. Urine $11dhTxB_2$ is a measure of overall platelet activation and a nonspecific measure of total-body TXB_2, because up to 30% of TXB_2 excreted in the urine is derived from extraplatelet sources [26]. All blood specimens were drawn and processed at the local sites. The PBMCs, plasma, and serum were shipped to Case Western Reserve University School of Medicine (Cleveland, OH) for central analysis. Peripheral blood mononuclear cells were thawed and stained with Live/Dead Fixable Yellow (Invitrogen, Carlsbad, CA) and divided into 2 aliquots for staining of T cells and monocytes. Cells were then stained with CD3 PerCP (BioLegend, San Diego, CA), CD4 AF700, CD8 APC-H7, HLA-DR PE, CD38 APC, and PD-1 BV421 (BD Biosciences, San Jose, CA) and with CD14 Pacific Blue, CD16 PE, and CD69 PE-Cy7 (BD Biosciences, San Jose, CA) and acquired on an LSRII flow cytometer (BD Biosciences, San Jose, CA), using FacsDiva software (BD Biosciences, San Jose, CA). Frequencies of activated ($CD38^+HLA\text{-}DR^+$) and exhausted (PD1+) CD4 and CD8 T cells and monocyte subsets (CD14/CD16/CD69) were determined by gating based on isotype controls using FacsDiva software. Levels of plasma sCD14, IL-6,

sCD163, serum TXB_2 (R&D Systems, Inc., Minneapolis, MN), D-dimer (Diagnostica Stago, Asnières sur Seine, France), and urine $11dhTxB_2$ (Corgenix, Broomfield, CO) were measured by enzyme-linked immunosorbent assay. Urine creatinine (random) was measured using colorimetric analysis (Affiliated Medical Services, Wichita, KS). Plasma kynurenine and tryptophan levels were measured by high-performance liquid chromatography (University of California, San Francisco, CA), and the KT ratio was calculated, as previously described [27].

Endothelial Function

Endothelial function was assessed by FMD of the brachial artery, a well established measure of endothelium-dependent vasodilation, as previously described [28, 29]. Participants were required to be fasting and to not use any tobacco-containing products for 8 hours before the study. Each study was recorded digitally and sent to the University of Wisconsin (Madison, WI) core ultrasound laboratory where data analysis was performed.

Statistical Analysis

The primary endpoint was the change in sCD14 levels from baseline (average of pre-entry/entry) to week 12 (average of week 11/week 12). Based on prior studies, a 0.07 $\log_{10}$ sCD14 between-arm difference would be associated with a 25% decreased odds of a nonacquired immune deficiency syndrome event or nonaccidental death [6], suggesting it would be a clinically significant reduction, and this guided the effect size for the study, which required 40 participants per study arm. Secondary endpoints included (1) changes from baseline to week 12 in soluble markers of inflammation and coagulation (average of pre-entry/entry compared with average of week 11/week 12) and (2) changes from entry to week 12 in T-cell markers of activation and exhaustion, monocyte subsets, plasma KT ratio, serum TXB_2 levels, urine $11dhTxB_2$ to urine creatinine ratio, and FMD.

To examine the biologic effects of aspirin, we used as-treated analyses, limiting the analysis to participants on treatment for the duration of the study and who had sCD14 data from baseline and week 12. Mean changes (log transformed when appropriate) within treatment arms and mean differences between arms were estimated using a single regression model. If data were log transformed, the exponent of model estimates were used to estimate geometric mean fold changes within arms and the percent difference in geometric mean fold changes between arms. To assess whether the treatment effects varied across baseline subgroups, regression models consisting of the treatment arm main effect, the baseline covariate of interest, and their interaction were used for each baseline covariate individually. We explored interactions by baseline factors (age, sex, smoking status, ART regimen containing 2 nucleoside reverse-transcriptase inhibitors + integrase strand transfer inhibitor, sCD14, sCD163, IL-6, D-dimer, serum TXB_2, urine TXB_2, plasma KT ratio, T-cell activation and exhaustion, monocyte subset percentage, and FMD). Relationships among variables were assessed with Spearman correlations. All statistical tests were 2-sided with a nominal alpha level of 0.05 with no adjustment for multiple testing.

RESULTS

Characteristics of Participants

A total of 170 participants were screened and 121 participants were enrolled at 15 US sites between August 2014 and March 2015. Reasons for screen failure are provided in Figure 1. Five participants did not complete the study treatment due to one of the following: protocol-defined toxicity of blood in stools

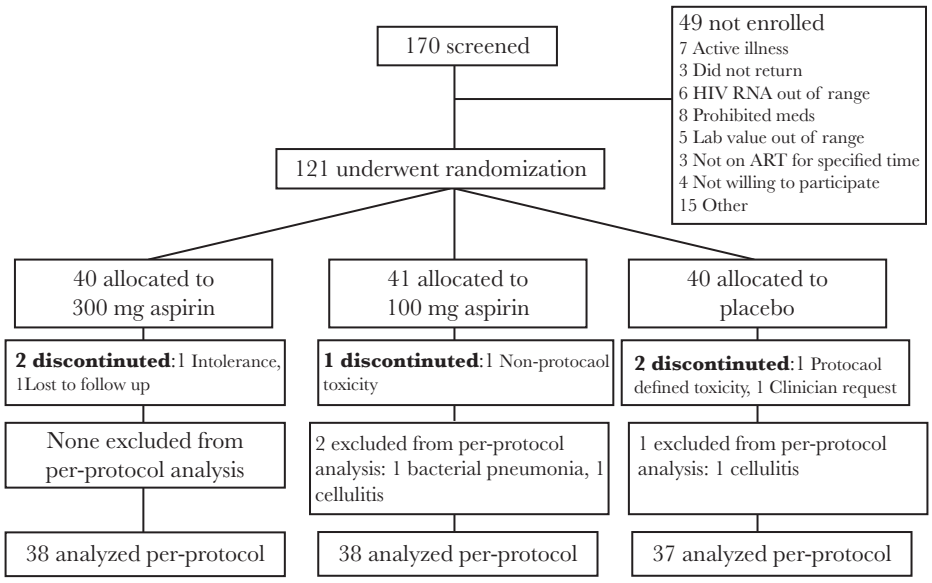

Figure 1. Study flow chart of screening, enrollment, study completion, and analysis. Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; RNA, ribonucleic acid.

Table 1. Characteristics of Participants at Time of Enrollment Into the Study[a]

		Treatment (N)			
Characteristics		300 mg (N = 40)	100 mg (N = 41)	Placebo (N = 40)	Total (N = 121)
Age (median)		48	50	49	49
Q1, Q3		(38, 54)	(46, 56)	(42, 57)	(42, 55)
Sex	Male	32 (80)	31 (76)	35 (88)	98 (81)
	Female	8 (20)	10 (24)	5 (13)	23 (19)
Race/ Ethnicity	White Non-Hispanic	21 (53)	18 (44)	21 (53)	60 (50)
	Black Non-Hispanic	11 (28)	19 (46)	11 (28)	41 (34)
	Hispanic	7 (18)	4 (10)	8 (20)	19 (16)
	Asian/Pacific Islander	1 (3)	0 (0)	0 (0)	1 (1)
CD4 (median)		573	629	642	599
Q1, Q3		436, 684	481, 856	436, 747	456, 777
Smoking	Never	18 (46)	25 (61)	25 (64)	68 (57)
	Previously	11 (28)	7 (17)	6 (15)	24 (20)
	Currently	10 (26)	9 (22)	8 (21)	27 (23)
Statin use		7 (19)	6 (14)	6 (16)	19 (16)
ART	ABC	2 (5)	1 (2)	4 (10)	7 (6)
	INSTI + 2 NRTI	14 (35)	8 (20)	12 (30)	34 (28)
	NNRTI + 2 NRTI	12(30)	23(56)	14 (35)	49 (40)
	PI + 2 NRTI	12(30)	12(29)	16 (40)	40 (33)

Abbreviations: ABC, abacavir; ART, antiretroviral therapy; INSTI, integrase strand transfer inhibitor; N, number; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

[a]Data are presented as No. (%) or median (interquartile range: Q1, Q3).

Table 2. Soluble Markers and Platelet Markers at Baseline[a]

		Treatment (N)			
Markers		300 mg (N = 38)	100 mg (N = 38)	Placebo (N = 36)	Total (N = 112)
sCD14 (ng/mL)	Mean (SD)	1933 (781)	2021 (611)	1864 (458)	1941 (631)
	Min, Max	828, 4136	889, 3264	1195, 3088	828, 4136
	Median	1652	2043	1851	1852
	Q1, Q3	1402, 2169	1544, 2459	1538, 2108	1465, 2280
sCD163 (ng/mL)	Mean (SD)	565 (276)	648 (369)	692 (470)	634 (379)
	Min, Max	251, 1462	198, 1918	277, 2823	198, 2823
	Median	470	571	584	551
	Q1, Q3	356, 738	387, 794	418, 693	391, 731
IL6 (pg/mL)	Mean (SD)	1.53 (1.16)	1.61 (1.62)	2.28 (4.57)	1.80 (2.83)
	Min, Max	0.39, 5.16	0.22, 9.36	0.22, 28.30	0.22, 28.30
	Median	1.13	1.15	1.30	1.19
	Q1, Q3	0.82, 1.73	0.85, 1.85	0.82, 2.16	0.83, 1.85
D-dimer (ng/mL)	Mean (SD)	158 (75)	165 (83)	170 (96)	164 (84)
	Min, Max	54, 403	49, 434	51, 406	49, 434
	Median	147	137	145	145
	Q1, Q3	108, 175	109, 208	107, 199	109, 192
Serum KT ratio (%)	Mean (SD)	38.5 (9.2)	40.4 (13.0)	39.0 (9.2)	39.3 (10.6)
	Min, Max	22.1, 57.6	21.1, 73.5	24.5, 62.6	21.1, 73.5
	Median	37.1	36.8	37.7	37.3
	Q1, Q3	31.4, 45.6	32.3, 49.0	31.1, 45.9	31.4, 46.4
Serum TXB_2 (ng/mL)	Mean (SD)	31.6 (34.5)	31.4 (21.6)	30.9 (23.4)	31.3 (26.9)
	Min, Max	1.9, 144.0	1.7, 81.3	7.0, 130.8	1.7, 144.0
	Median	19.1	30.4	27.0	25.4
	Q1, Q3	12.4, 36.2	13.9, 41.2	13.2, 38.9	12.6, 38.8
Urine 11dhTXB_2/creatinine ratio (pg/mg)	Mean (SD)	4552 (3271)	4637 (2869)	5488 (4421)	4855 (3513)
	Min, Max	822, 13 852	429, 11 114	1016, 19 284	429, 19 284
	Median	3496	3,545	4789	3829
	Q1, Q3	1984, 6289	2527, 6452	1917, 8308	2122, 6683

Abbreviations: 11dhTXB_2, 11-dehydrothromboxane; IL, interleukin; KT, kynurenine/tryptophan; Max, maximum; Min, minimum; Q, quartile; s, soluble; SD, standard deviation; TXB_2, thromboxane.

[a]Data are presented as mean with SD and median (interquartile range: Q1, Q3).

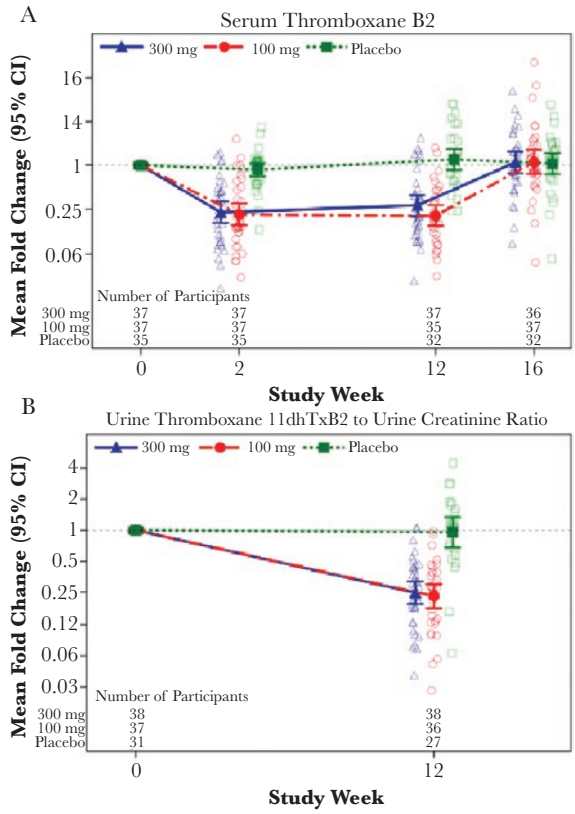

Figure 2. Effects of 12 weeks of aspirin 300 mg, aspirin 100 mg, and placebo with 4-week washout on serum thromboxane (TXB_2) and urinary TXB_2 11-dehydrothromboxane to urine creatinine ratio, shown as mean-fold change (95% confidence interval [CI]) from baseline.

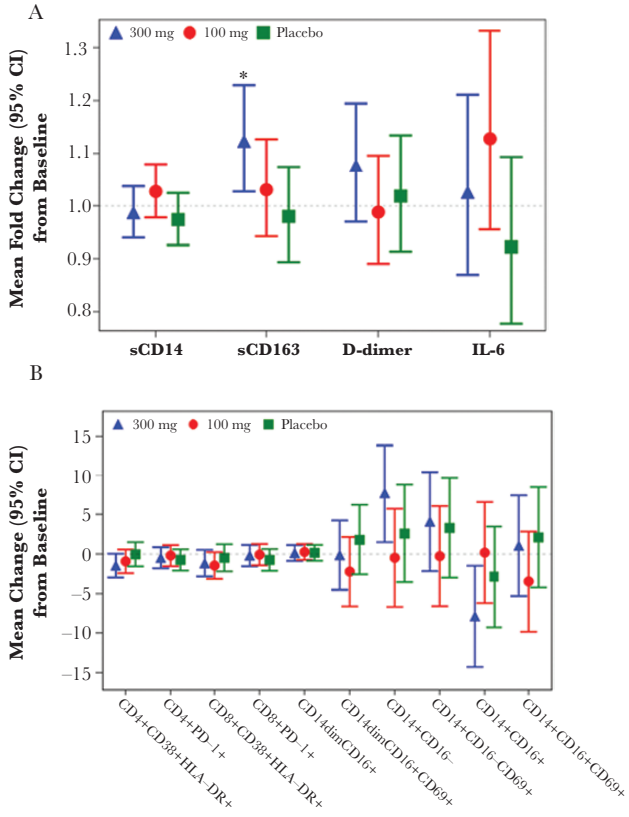

Figure 3. (A) Effects of 12 weeks of aspirin 300 mg, aspirin 100 mg, and placebo on soluble markers soluble (s)CD14, sCD163, D-dimer, interleukin (IL)-6; *, sCD163, increased with 300-mg daily aspirin (14.7%; 95% confidence interval [CI], 0.8–30.4; $P = .037$) compared with placebo. (B) Effects of 12 weeks of aspirin 300 mg, aspirin 100 mg, and placebo on flow cytometry markers of T-cell activation ($CD4^+CD38^+HLA\text{-}DR^+$, $CD8^+CD38^+HLA\text{-}DR^+$), T-cell exhaustion ($CD4^+PD1^+$, $CD8^+PD1^+$), and inflammatory monocyte subsets ($CD14^{dim}CD16^+CD69^+$, $CD14^{dim}CD16^-CD69^+$, $CD14^+CD16^+$, $CD14^+CD16^+CD69^+$), shown as mean-fold change (95% CI) from baseline.

(placebo), nonprotocol-defined low-grade toxicity of constipation/bloating (100 mg), clinician request not in best interest of patient (placebo), intolerance (300 mg), and lost to follow up (300 mg). Three additional participants were not included in the per-protocol analysis because they had serious bacterial infections (predefined exclusion, because of the known inflammatory effects) during the study period. Two participants in the aspirin 100 mg group were excluded: 1 had bacterial pneumonia during week 8 of the study, and 1 had finger cellulitis diagnosed during week 13 of the study. One study participant in the placebo group had foot cellulitis during week 12 of the study (Figure 1). Baseline characteristics are summarized in Table 1: 98 (81%) of the participants were male, 60 (50%) were non-White, 27 (23%) were current smokers, 19 (16%) were on statins, 7 (6%) were on an abacavir-containing ART regimen, and 34 (28%) were on an integrase inhibitor-containing regimen. Self-reported adherence to study drug was high with approximately 90% of study participants reporting 100% adherence. Aspirin was safe and well tolerated. There were no study discontinuations due to serious adverse events. Plasma HIV RNA levels did not change during treatment and remained below the limits of quantification.

Impact of Aspirin on Thromboxane Levels

Baseline serum TXB_2 and urine11dhTxB_2 (corrected for creatinine clearance) are summarized in Table 2. Serum TXB_2 log_{10}-transformed data were used for analysis. Serum TXB_2 levels did not change significantly in the placebo group and were reduced at 2 weeks and 12 weeks in the 300-mg group and 100-mg group and returned to baseline 4 weeks after stopping study drug, as summarized in Figure 2A. The estimated geometric mean fold change was 0.28 (95% confidence interval [CI], .20–.38) in the 300-mg arm, 0.20 (95% CI, .15–0.28) in the 100-mg arm, and 1.21 (95% CI, .86–1.69) in the placebo arm. Compared with placebo, the 300-mg arm had a mean 76.7% (95% CI, 63.1–85.3) greater reduction ($P < .001$) and the 100-mg arm had a mean 83.1% (95% CI, 73.0–89.4) greater reduction ($P < .001$). The levels of urine 11dhTxB_2 (corrected for creatinine clearance) paralleled serum TXB_2 changes as shown in Figure 2B. The estimated geometric mean fold change was 0.25 (95% CI, .19–.32) in the 300-mg arm, 0.23 (95% CI, .18–.30) in the 100 mg arm, and 0.96 (95% CI, 0.71–1.30) in the placebo arm. Compared with placebo, the 300-mg arm had a 74.2% (95% CI,

Table 3. Cellular Markers at Baseline[a]

Cellular Markers		Treatment (N)			
		300 mg (N = 38)	100 mg (N = 38)	Placebo (N = 36)	Total (N = 112)
$CD4^+CD38^+HLA\text{-}DR^+$ (%)	Mean (SD)	12.9 (7.2)	11.4 (5.8)	11.7 (5.9)	12.0 (6.3)
	Min, Max	3.3, 28.1	2.7, 28.3	3.4, 26.1	2.7, 28.3
	Median	12.6	10.3	10.7	10.5
	Q1, Q3	6.7, 17.6	6.4, 16.4	6.8, 13.9	6.7, 16.2
$CD4^+$ $PD\text{-}1^+$ (%)	Mean (SD)	32.2 (9.7)	32.7 (10.6)	36.3 (15.0)	33.7 (12.0)
	Min, Max	9.7, 49.9	13.8, 59.8	15.6, 71.0	9.7, 71.0
	Median	33.5	30.3	33.1	32.4
	Q1, Q3	26.4, 38.7	23.8, 41.5	24.5, 43.2	25.4, 40.4
$CD8^+CD38^+$ $HLA\text{-}DR^+$ (%)	Mean (SD)	12.6 (10.0)	10.3 (5.8)	11.0 (5.1)	11.3 (7.3)
	Min, Max	2.5, 40.9	2.7, 25.7	3.0, 23.3	2.5, 40.9
	Median	8.6	9.2	11.3	9.5
	Q1, Q3	6, 18	6.1, 12.7	6.6, 13.7	6.5, 13.8
$CD8^+$ $PD\text{-}1^+$ (%)	Mean (SD)	26.0 (10.6)	29.3 (10.2)	30.2 (12.7)	28.5 (11.2)
	Min, Max	8.8, 46.4	9.1, 52.7	13.4, 56.7	8.8, 56.7
	Median	24.1	30.2	29.3	27.9
	Q1, Q3	17.2, 33.4	19.5, 37.5	20.9, 37.8	19.5, 35.2
$CD14^{dim}CD16^+$ (%)	Mean (SD)	5.7 (3.4)	7.8 (5.2)	6.5 (4.3)	6.7 (4.4)
	Min, Max	0.9, 15.3	1.1, 23.1	0.9, 17.9	0.9, 23.1
	Median	4.8	5.8	6.1	5.7
	Q1, Q3	3.5, 7.3	4.2, 11.7	3.2, 8.0	3.7, 8.1
$CD14^{dim}CD16^+CD69^+$ (%)	Mean (SD)	26.6 (17.9)	25.1 (16.8)	28.9 (17.2)	26.9 (17.2)
	Min, Max	1.8, 72.0	3.3, 63.8	2.4, 63.3	1.8, 72.0
	Median	23.9	24	28.6	24.9
	Q1, Q3	11.2, 39.5	8.2, 38.9	14.7, 38.9	12.4, 38.9
$CD14^+CD16^-$ (%)	Mean (SD)	65.7 (20.8)	67.5 (18.0)	72.1 (16.4)	68.4 (18.5)
	Min, Max	12.4, 91.0	20.0, 89.0	4.3, 94.6	4.3, 94.6
	Median	73.5	72.2	74.1	73.5
	Q1, Q3	50.2, 81.9	55.5, 79.6	64.6, 81.0	60.0, 80.3
$CD14^+CD16^-CD69^+$ (%)	Mean (SD)	50.2 (19.3)	52.2 (22.0)	58.0 (24.3)	53.5 (22.0)
	Min, Max	4.5, 88.4	8.8, 84.0	6.3, 91.6	4.5, 91.6
	Median	51.4	55.6	63.9	54.6
	Q1, Q3	38.8, 65.2	35.9, 69.1	41.1, 80.6	39.5, 69.3
$CD14^+CD16^+$ (%)	Mean (SD)	29.6 (21.7)	25.5 (18.7)	22.1 (17.4)	25.7 (19.4)
	Min, Max	4.7, 85.5	6.3, 77.4	3.3, 94.7	3.3, 94.7
	Median	20.3	17.5	21.0	20.1
	Q1, Q3	13.2, 47.2	10.4, 40.5	9.2, 27.0	11.5, 32.6
$CD14^+CD16^+CD69^+$ (%)	Mean (SD)	55.3 (21.6)	53.7 (24.0)	59.1 (23.7)	56.0 (23.0)
	Min, Max	6, 94	9.3, 86.4	11.0, 90.8	6, 94
	Median	58.6	59.1	58	58.6
	Q1, Q3	41.2, 70.8	30.2, 72.8	42.4, 80.9	41.9, 75.2

Abbreviations: Q, quartile; SD, standard deviation.

[a]Data are presented as percent with mean (SD) and median (interquartile range; Q1, Q3).

61.5–82.7) greater reduction ($P < .001$) and the 100-mg arm had a 76.1% (95% CI, 64.2–84.0) greater reduction ($P < .001$), without evidence for a dose-response effect.

Impact of Aspirin on Soluble Markers

Baseline soluble markers are summarized in Table 2. As shown in Figure 3A, the levels of sCD14 (our primary endpoint) did not change significantly during 12 weeks of aspirin treatment compared with placebo, regardless of aspirin dose tested. The estimated geometric mean fold change was 0.99 (95% CI, .94–1.04) in the 300-mg arm, 1.03 (95% CI, .98–1.08) in the 100-mg arm, and 0.97 (95% CI, .93–1.02) in the placebo arm. Compared with placebo, the 300-mg arm had a 1.4% (95% CI, −5.5 to 8.8) greater increase ($P = .70$) and the 100-mg arm had a 5.5% (95% CI, −1.7 to 13.3) greater increase ($P = .14$). There was also no evidence for a difference in week 12 sCD14 changes between pooled aspirin arms and placebo ($P = .28$).

As shown in Figure 3A, plasma IL-6 and D-dimer levels did not change significantly between baseline and 12 weeks

of aspirin treatment compared with placebo. The estimated geometric mean fold change in IL-6 at 12 weeks was 1.03 (95% CI, .87–1.21) in the 300-mg arm, 1.13 (95% CI, .96–1.33) in the 100-mg arm, and 0.92 (95% CI, .78–1.09) in the placebo arm, without significant differences between groups. The estimated geometric mean fold change in D-dimer at 12 weeks was 1.08 (95% CI, .97–1.19) in the 300-mg arm, 0.99 (95% CI, .89–1.10) in the 100-mg arm, and 1.02 (95% CI, .91–1.13) in the placebo arm, also without significant differences between groups. As shown in Figure 3A, the estimated geometric mean fold change in sCD163 between baseline and 12 weeks was 1.12 (95% CI, 1.03–1.23) in the 300-mg arm, 1.03 (95% CI, .94–1.13) in the 100-mg arm, and 0.98 (95% CI, .89–1.07) in the placebo arm. Soluble CD163 significantly increased with 300 mg daily aspirin (14.7%; 95% CI, .8–30.4; $P = .037$) compared with placebo, whereas there was no significant change with 100 mg daily aspirin (5.2%; 95% CI, −7.5 to 19.6; $P = .44$) compared with placebo.

Impact of Aspirin on Cellular Markers

Baseline cellular markers are summarized in Table 3. Because platelet activation induces a proinflammatory phenotype in circulating classical monocytes, with increased expression of CD16 [30], we tested whether aspirin might affect the frequency of circulating monocyte subsets. The frequencies of classical ($CD14^+CD16^-$), intermediate ($CD14^+CD16^+$), and nonclassical ($CD14^{dim}CD16^+$) monocyte subsets did not change significantly during 12 weeks of aspirin treatment compared with placebo. We also found no evidence for a change in plasma KT ratio, T-cell activation, and exhaustion (Figure 3B, Table 4).

Impact of Aspirin on Flow-Mediated Dilation

Flow-mediated dilation measurements in the 300-mg and 100-mg aspirin arms did not change from baseline to week 12 compared with placebo. At week 12, the mean change was −0.32% (95% CI, −1.17 to .54) in the 300-mg arm, −0.98% (95% CI, −1.85 to −0.11) in the 100-mg arm, and −0.20% (95% CI, −1.08 to .69) in the placebo arm. Compared with placebo, neither the 300-mg arm difference, −0.12% (95% CI, −1.34 to 1.11; $P = .85$), nor the 100-mg arm difference, −0.79 (95% CI, −2.03 to .45; $P = .21$), were statistically significant (Figure 4). See Table 5 for a complete listing of FMD parameters between baseline and 12 weeks of study drug, including relative FMD (%), average diameter of brachial artery (mm), brachial artery flow (cubic centimeter [cc]/min), and average diameter of reactive hyperemia (cc/min).

Assessment of Interactions

Interactions of study drug effects with current smoking, sex, age, ART regimen, and baseline marker tertile were assessed, combining aspirin arms to improve power. These analyses were mostly unrevealing, but they did appear to show less of an aspirin-mediated increase in sCD163 among current nonsmokers ($P = .036$) and women ($P = .031$) and greater reductions in D-dimer among current smokers ($P = .027$). Compared with placebo at 12 weeks, the mean percentage change of D-dimer in current smokers taking aspirin (300-mg and 100-mg arms pooled) was −25.1% (95% CI, −44.3 to .8) and in nonsmokers taking aspirin (pooled arms) it was 8.9% (95% CI, −5.8 to 25.8) ($P = .027$). These differences were modest and have to be considered with caution in the setting of multiple hypothesis testing. Spearman correlations were also performed within the 2 aspirin groups for all variables. Baseline serum TXB_2 correlated with urine 11-dhTxB_2 (0.30; $P = .014$), as did the change in serum TXB_2 with urine 11-dhTxB_2 in response to aspirin therapy (0.41; $P = .001$). No other significant associations were found.

DISCUSSION

In this randomized, double-blind, placebo-controlled trial, 12 weeks of aspirin treatment, at either 100 or 300 mg daily, failed to reduce plasma levels of sCD14 in virologically suppressed HIV-infected participants on ART. There were also no consistent differences between the 300-mg or 100-mg aspirin arms

Table 4. Effects of 12 Weeks of Aspirin on Markers of Immune Activation, Exhaustion, and KT Ratio[a]

	Mean Change Difference			
Marker	300 mg	*P* Values	100 mg	*P* Values
$\%CD14^+CD16^-$	5.09 (−3.66 to 13.84)	.25	−3.10 (−11.84 to 5.65)	.48
$\%CD14^+CD16^-CD69^+$	4.16 (−2.18 to 10.49)	.85	−3.59 (−12.55 to 5.37)	.43
$\%CD14^+CD16^+$	−4.96 (−14.01 to 4.05)	.28	3.07 (−5.96 to 12.10)	.50
$\%CD14^+CD16^+CD69^+$	−1.05 (−10.04 to 7.94)	.82	−5.50 (−14.59 to 3.40)	.22
$\%CD14^{dim}CD16^+$	−0.02 (−1.45 to 1.41)	.96	0.12 (−1.31 to 1.54)	.87
$\%CD14^{dim}CD16^+CD69^+$	−1.94 (−8.17 to 4.29)	.54	−4.04 (−10.27 to 2.19)	.20
$\%CD4^+CD38^+HLADR^+$	−1.42 (−2.98 to 0.05)	.19	−0.91 (−3.06 to 1.25)	.41
$\%CD4^+PD1^+$	0.28 (−1.63 to 2.20)	.77	0.56 (−1.36 to 2.47)	.56
$\%CD8^+CD38^+HLADR^+$	−0.67 (−3.09 to 1.75)	.58	−0.99 (−3.41 to 1.43)	.42
$\%CD8^+PD1^+$	0.56 (−1.38 to 2.50)	.57	0.67 (−1.27 to 2.61)	.50
KT ratio	1.71 (−1.52 to 4.94)	.30	−1.34 (−4.61 to 1.93)	.42

Abbreviations: CI, confidence interval; KT, kynurenine/tryptophan.

[a]Data are presented as mean change difference (95% CI) vs placebo.

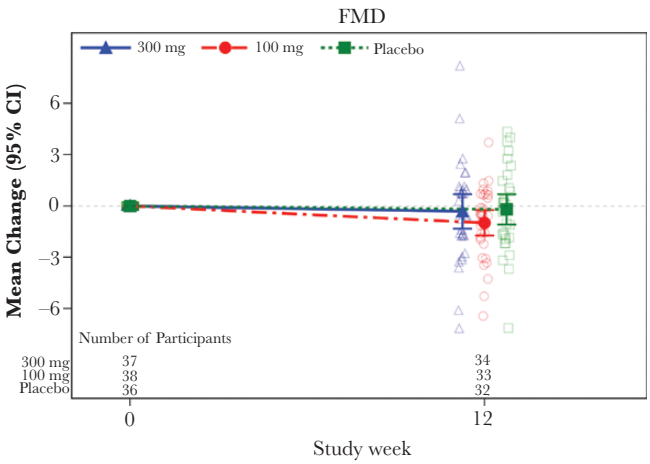

Figure 4. Effects of 12 weeks of aspirin 300 mg, aspirin 100 mg, and placebo on flow-mediated dilation, shown as mean change (95% confidence interval [CI]). Abbreviation: FMD, flow-mediated dilation.

versus placebo for FMD, D-dimer, or any of the other immunologic endpoints.

These results differ from the previous open-label study showing that 1 week of 81 mg daily aspirin reduced sCD14 in ART-treated HIV-infected participants. Reasons for these disparate results are unclear. A transient reduction in immune activation seems unlikely because there was also no evidence of a reduction in sCD14 in the current trial at week 2. The baseline sCD14 was higher in the previous uncontrolled trial; therefore, we hypothesized that aspirin effects on sCD14 might only be apparent in persons with a higher baseline sCD14, but we did not find any evidence for interaction by baseline level of sCD14 in the current study. The previous uncontrolled trial also had a greater prevalence of tobacco smokers (56%) than our current trial (23%). Nevertheless, we failed to identify consistent interactions between smoking and changes in immunologic endpoints by treatment arm in the current trial, with the exception of possibly greater aspirin-mediated reductions in D-dimer among smokers. Likewise, we did not see an appreciable change in IL-6, D-dimer, the proportional representation of monocyte subsets, or T-cell immune activation overall. There was an increase in sCD163 in the aspirin 300-mg arm, suggesting potentially "increased" monocyte activation with high-dose aspirin. Because we did not see an increase in sCD14 or any other monocyte subsets in the aspirin 300-mg arm, it is likely that this difference was spurious. Although we cannot exclude other potential cardiovascular benefits of aspirin in this setting (ie, on thrombosis or atherosclerosis), endothelial function as assessed by FMD failed to improve after 12 weeks of either dose of aspirin in our current trial. Thus, although we cannot fully explain the different inferences from the prior uncontrolled trial of aspirin, our current placebo-controlled trial suggests that aspirin—at the common clinical doses assessed—is unlikely to have a clinically significant effect on immune activation or endothelial function in most ART-suppressed individuals.

It has been suggested that inflammation modifies platelet function, leading to increased platelet reactivity and reduced

Table 5. FMD Parameters[a]

		Treatment (N)			
FMD Parameters		300 mg (N = 38)	100 mg (N = 38)	Placebo (N = 36)	Total (N = 112)
Relative FMD (%) week 0	Median	4.55	4.84	3.69	4.26
	Q1, Q3	3.24, 5.76	2.94, 6.56	2.47, 5.32	2.63, 6.22
Relative FMD (%) week 12	Median	3.03	3.73	3.94	3.73
	Q1, Q3	2.23, 5.26	2.48, 4.76	1.88, 5.26	2.23, 5.18
Average diameter of brachial artery (mm) week 0	Median	0.43	0.43	0.45	0.44
	Q1, Q3	0.40, 0.48	0.39, 0.49	0.40, 0.47	0.40, 0.48
Average diameter of brachial artery (mm) week 12	Median	0.43	0.44	0.44	0.44
	Q1, Q3	0.40, 0.48	0.40, 0.50	0.41, 0.47	0.40, 0.49
Brachial artery flow (cc/min)	Median	133.13	165.36	130.33	141.84
week 0	Q1, Q3	106.94, 224.32	87.72, 227.36	95.61, 193.32	98.00, 224.32
Brachial artery flow (cc/min)	Median	146.07	151.64	147.85	147.25
week 12	Q1, Q3	108.43, 245.52	78.38, 259.84	97.01, 201.38	94.10, 241.37
Average diameter of reactive hyperemia (mm)	Median	0.45	0.45	0.46	0.46
90 sec, week 0	Q1, Q3	0.42, 0.50	0.41, 0.51	0.43, 0.49	0.42, 0.50
Average diameter of reactive hyperemia (mm)	Median	0.45	0.45	0.46	0.46
90 sec, week 12	Q1, Q3	0.41, 0.50	0.43, 0.51	0.44, 0.50	0.43, 0.50
Relative hyperemia brachial flow (cc/min) week 0	Median	864.00	840.64	840.84	848.56
	Q1, Q3	691.67, 1157.86	752.07, 1062.10	642.98, 1154.69	694.90, 1139.49
Relative hyperemia brachial flow (cc/min) week 12	Median	761.07	932.95	855.67	857.82
	Q1, Q3	638.30, 1078.83	758.95, 1102.68	726.25, 1082.85	673.63, 1090.50

Abbreviations: cc, cubic centimeter; FMD, flow-mediated dilation; Q, quartile.

[a]Data are presented as median (interquartile range: Q1, Q3).

antiplatelet drug efficacy in persons with known CVD [16, 31–33]. Several studies have shown that platelets are activated in HIV infection [19–23], and a previously reported study demonstrated reduced aspirin drug efficacy in HIV-infected persons compared with healthy controls, with less of a reduction in urine 11dhTxB$_2$ and less inhibition of platelet aggregation by aspirin [23]. Although the 300 mg and 100 mg aspirin arms in our study achieved a mean reduction of 70.1% and 83.1%, respectively, in serum TXB$_2$ levels (both $P < .001$ vs placebo), both groups had weaker aspirin-mediated reductions than are typically observed in historical HIV-uninfected, where a typical response to low-dose aspirin therapy is to reduce serum TXB$_2$ by >95% or to below a level of 5 ng/mL [34–36]. Analyzing further, only 25% achieved a reduction in serum TXB$_2$ >90% and 49% achieved a reduction <5 ng/mL in the 300-mg arm and only 24% achieved a reduction in serum TXB$_2$ >90% and 42% achieved a reduction <5 ng/mL in the 100-mg arm at 12 weeks.

Similarly to ART-treated HIV-infected persons, persons with diabetes mellitus type 2 have increased platelet activation and reduced antiplatelet drug efficacy. Although platelet activation and impaired antiplatelet response in diabetes is associated with poor glycemic control, inflammation, and increased platelet turnover [37, 38], the cause of platelet activation and impaired aspirin response in HIV infection is incompletely understood. We found a weak association between baseline serum TXB$_2$ and baseline sCD14 (rho = 0.21, $P = .081$), suggesting HIV-related bacterial translocation as a potential contributor to platelet activation, via platelet-expressed TLR4 [39, 40], but we found no association between baseline serum TXB$_2$ and baseline IL-6, sCD163, or D-dimer. We also found no association between the change in serum TXB$_2$ in response to 12 weeks of aspirin therapy and baseline sCD14, IL-6, sCD163, or D-dimer.

CONCLUSIONS

Future studies are needed to identify the causes and consequences of platelet activation and reduced antiplatelet drug efficacy in ART-treated HIV infection. These studies may have important clinical implications for the prevention and treatment of CVD in patients with HIV infection. Until further data are available, the use of aspirin for the primary and secondary prevention of cardiovascular events in HIV-infected patients should be individualized and prescribed according to the current guidelines for the general population [41, 42].

Acknowledgments

We are grateful for the commitment of the study participants and the study staff at the AIDS Clinical Trials Group (ACTG) sites. The following persons assisted in conducting A5331: Linda Boone, Laurie S. Meyers, Lynette Purdue, Karen Cavanagh, David L. Shugarts, Michael Dorosh, Adam Manzella, and Elmar Detering (Bayer AG).

Disclaimer. Bayer AG played no role in the design of the study, in data collection or analysis, or in manuscript preparation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Financial support. Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number UM1 AI068634 (Hughes), UM1 AI068636 (Kuritzkes), UM1 AI106701 (Coombs) and UM1 AI069532 (Aberg). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Potential conflicts of interest. Aspirin and placebo tablets were supplied by Bayer Pharma AG, Germany. P. W. H. reports personal fees from Merck, personal fees from Gilead, personal fees from Viiv, all for consulting activities outside the submitted work. J. H. S. reports other from Wisconsin Alumni Research Foundation, other from UptoDate, outside the submitted work. J. A. A. reports grants from National Institute of Allergy and Infectious Diseases, during the conduct of the study; grants from Bristol Myers Squibb, grants from Gilead, other from Janssen, other from Merck, other from Viiv, outside the submitted work. N. T. F. reports grants from NIH National Heart, Lung, and Blood Institute, other from Gilead, outside the submitted work. D. W. K. reports other from NIH, during the conduct of the study. N. S. U. reports grants and personal fees from Tobira Therapeutics, Inc, personal fees from Gilead Sciences, Inc, outside the submitted work. P. T. reports personal fees from Merck, personal fees from Glaxo, outside the submitted work. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Freiberg MS, Chang CC, Kuller LH, et al. HIV infection and the risk of acute myocardial infarction. JAMA Intern Med **2013**; 173:614–22.
2. Legarth RA, Ahlström MG, Kronborg G, et al. Long-term mortality in HIV-infected individuals 50 years or older: a nationwide, population-based cohort study. J Acquir Immune Defic Syndr **2016**; 71:213–8.
3. Rasmussen LD, May MT, Kronborg G, et al. Time trends for risk of severe age-related diseases in individuals with and without HIV infection in Denmark: a nationwide population-based cohort study. Lancet HIV **2015**; 2:e288–98.
4. Longenecker CT, Jiang Y, Orringer CE, et al. Soluble CD14 is independently associated with coronary calcification and extent of subclinical vascular disease in treated HIV infection. AIDS **2014**; 28:969–77.
5. Sandler NG, Wand H, Roque A, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis **2011**; 203:780–90.
6. Tenorio AR, Zheng Y, Bosch RJ, et al. Soluble markers of inflammation and coagulation, but not T-cell activation, are predictors of non-AIDS-defining morbid events during suppressive antiretroviral treatment. J Infect Dis **2014**; 210:1248–59.
7. Hunt PW, Sinclair E, Rodriguez B, et al. Gut epithelial barrier dysfunction and innate immune activation predict mortality in treated HIV infection. J Infect Dis **2014**; 210:1228–38.
8. Linden MD, Jackson DE. Platelets: pleiotropic roles in atherogenesis and atherothrombosis. Int J Biochem Cell Biol **2010**; 42:1762–6.
9. Shiraki R, Inoue N, Kawasaki S, et al. Expression of Toll-like receptors on human platelets. Thromb Res **2004**; 113:379–85.
10. Jayachandran M, Brunn GJ, Karnicki K, et al. In vivo effects of lipopolysaccharide and TLR4 on platelet production and activity: implications for thrombotic risk. J Appl Physiol (1985) **2007**; 102:429–33.
11. von Hundelshausen P, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. Circ Res **2007**; 100:27–40.
12. Garraud O, Cognasse F. Platelet Toll-like receptor expression: the link between "danger" ligands and inflammation. Inflamm Allergy Drug Targets **2010**; 9:322–33.
13. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med **2006**; 12:1365–71.
14. Vanpouille C, Introini A, Morris SR, et al. Distinct cytokine/chemokine network in semen and blood characterize different stages of HIV infection. AIDS **2016**; 30:193–201.
15. Pastori D, Pignatelli P, Farcomeni A, et al. Urinary 11-dehydro-thromboxane B2 is associated with cardiovascular events and mortality in patients with atrial fibrillation. Am Heart J **2015**; 170:490–7.e1.
16. Eikelboom JW, Hankey GJ, Thom J, et al. Incomplete inhibition of thromboxane biosynthesis by acetylsalicylic acid: determinants and effect on cardiovascular risk. Circulation **2008**; 118:1705–12.
17. Faraday N, Becker DM, Yanek LR, et al. Relation between atherosclerosis risk factors and aspirin resistance in a primary prevention population. Am J Cardiol **2006**; 98:774–9.

18. Ferro D, Basili S, Roccaforte S, et al. Determinants of enhanced thromboxane biosynthesis in patients with systemic lupus erythematosus. Arthritis Rheum **1999**; 42:2689–97.
19. Damien P, Cognasse F, Lucht F, et al. Highly active antiretroviral therapy alters inflammation linked to platelet cytokines in HIV-1-infected patients. J Infect Dis **2013**; 208:868–70.
20. Mayne E, Funderburg NT, Sieg SF, et al. Increased platelet and microparticle activation in HIV infection: upregulation of P-selectin and tissue factor expression. J Acquir Immune Defic Syndr **2012**; 59:340–6.
21. Singh MV, Davidson DC, Jackson JW, et al. Characterization of platelet-monocyte complexes in HIV-1-infected individuals: possible role in HIV-associated neuroinflammation. J Immunol **2014**; 192:4674–84.
22. Davidson DC, Jackson JW, Maggirwar SB. Targeting platelet-derived soluble CD40 ligand: a new treatment strategy for HIV-associated neuroinflammation? J Neuroinflammation **2013**; 10:144.
23. O'Brien M, Montenont E, Hu L, et al. Aspirin attenuates platelet activation and immune activation in HIV-1-infected subjects on antiretroviral therapy: a pilot study. J Acquir Immune Defic Syndr **2013**; 63:280–8.
24. Hunt PW, Shulman NS, Hayes TL, et al. The immunologic effects of maraviroc intensification in treated HIV-infected individuals with incomplete CD4+ T-cell recovery: a randomized trial. Blood **2013**; 121:4635–46.
25. Schroecksnadel K, Winkler C, Wirleitner B, et al. Aspirin down-regulates tryptophan degradation in stimulated human peripheral blood mononuclear cells in vitro. Clin Exp Immunol **2005**; 140:41–5.
26. Dobaczewski M, Nocun M, Zavodnik I, et al. Targeting the urine and plasma determinants of thromboxane A2 metabolism in detection of aspirin effectiveness. Blood Coagul Fibrinolysis **2008**; 19:421–8.
27. Favre D, Mold J, Hunt PW, et al. Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of TH17 to regulatory T cells in HIV disease. Sci Transl Med **2010**; 2:32ra6.
28. Torriani FJ, Komarow L, Parker RA, et al. Endothelial function in human immunodeficiency virus-infected antiretroviral-naive subjects before and after starting potent antiretroviral therapy: The ACTG (AIDS Clinical Trials Group) Study 5152s. J Am Coll Cardiol **2008**; 52:569–76.
29. Stein JH, Brown TT, Ribaudo HJ, et al. Ultrasonographic measures of cardiovascular disease risk in antiretroviral treatment-naive individuals with HIV infection. AIDS **2013**; 27:929–37.
30. Passacquale G, Vamadevan P, Pereira L, et al. Monocyte-platelet interaction induces a pro-inflammatory phenotype in circulating monocytes. PLoS One **2011**; 6:e25595.
31. Larsen SB, Grove EL, Kristensen SD, Hvas AM. Reduced antiplatelet effect of aspirin is associated with low-grade inflammation in patients with coronary artery disease. Thromb Haemost **2013**; 109:920–9.
32. Bernlochner I, Steinhubl S, Braun S, et al. Association between inflammatory biomarkers and platelet aggregation in patients under chronic clopidogrel treatment. Thromb Haemost **2010**; 104:1193–200.
33. Ge H, Zhou Y, Liu X, et al. Relationship between plasma inflammatory markers and platelet aggregation in patients with clopidogrel resistance after angioplasty. Angiology **2012**; 63:62–6.
34. Patrono C, García Rodríguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. N Engl J Med **2005**; 353:2373–83.
35. Patrono C, Rocca B. Drug insight: aspirin resistance–fact or fashion? Nat Clin Pract Cardiovasc Med **2007**; 4:42–50.
36. Reilly IA, FitzGerald GA. Inhibition of thromboxane formation in vivo and ex vivo: implications for therapy with platelet inhibitory drugs. Blood **1987**; 69:180–6.
37. Neergaard-Petersen S, Hvas AM, Grove EL, et al. The influence of haemoglobin A1c levels on platelet aggregation and platelet turnover in patients with coronary artery disease treated with aspirin. PLoS One **2015**; 10:e0132629.
38. Geisler T, Mueller K, Aichele S, et al. Impact of inflammatory state and metabolic control on responsiveness to dual antiplatelet therapy in type 2 diabetics after PCI: prognostic relevance of residual platelet aggregability in diabetics undergoing coronary interventions. Clin Res Cardiol **2010**; 99:743–52.
39. Berthet J, Damien P, Hamzeh-Cognasse H, et al. Human platelets can discriminate between various bacterial LPS isoforms via TLR4 signaling and differential cytokine secretion. Clin Immunol **2012**; 145:189–200.
40. Zhang G, Han J, Welch EJ, et al. Lipopolysaccharide stimulates platelet secretion and potentiates platelet aggregation via TLR4/MyD88 and the cGMP-dependent protein kinase pathway. J Immunol **2009**; 182:7997–8004.
41. Bibbins-Domingo K; U.S. Preventive Services Task Force. Aspirin use for the primary prevention of cardiovascular disease and colorectal cancer: U.S. preventive services task force recommendation statement. Ann Intern Med **2016**; 164:836–45.
42. Smith SC Jr, Benjamin EJ, Bonow RO, et al. AHA/ACCF secondary prevention and risk reduction therapy for patients with coronary and other atherosclerotic vascular disease: 2011 update: a guideline from the American Heart Association and American College of Cardiology Foundation endorsed by the World Heart Federation and the Preventive Cardiovascular Nurses Association. J Am Coll Cardiol **2011**; 58:2432–46.

APPENDIX

We acknowledge the following personnel and AIDS Clinical Trials Unit grants:

Cornelius N Van Dam MD and Kim Epperson - Greensboro CRS (Site 3203) Grant U01 AI069423; Dr Paul Sax MD and Cheryl Keenan RN BC - Brigham and Women's Hospital (Site 107) Grant UM1AI068636-10; Teri Flynn ANP-BC and Amy Sbrolla RN BSN - Massachusetts General Hospital (Site 101) Grant 2UM1AI069412-09; Carl J Fichtenbaum MD and Michelle Saemann RN - Case CTU, Cincinnati CRS (Site 2401) Grant AI-69501; Roberto C Arduino MD and Martine M Diez - HART CRS (Site 31473) Grants 2 UM1 AI069503-10 and 5 UM1 AI068636-10; Beverly E Sha MD and Janice M Fritsche APRN - Rush University Medical Center CRS (Site 2702) Grant U01 AI069471; Dr Kara Chew MD and Sana Majid - UCLA Care Center (CRS 601) Grant AI069424; Eric S Daar and Ruben Lopez – Harbor UCLA CRS(Site 603) Grant A1 069424 and UCLA CTSI Grant UL1TR000124; Kelly Walsh and Constance Benson MD - UCSD AntiViral Research Center CRS (Site 701) Grant AI069432; Kristen Allen BSN RN CCRP and Jane Baum BSN RN CCRP - Case CRS (Site 2501) Grant AI69501; Annie Luetkemeyer MD and Joann Volinski RN - UCSF AIDS CRS (Site 801) Grant 5UM1AI069496; Jonathan Oakes BA and David Currin RN - Chapel Hill CRS (Site 3201) Grants UM1 AI069423, CTSA: UL1TR001111, CFAR: P30 AI050410; Brenda Jackson RN and Joan Gottesman RN - Vanderbilt Therapeutics CRS (Site 3652) Grants UM1AI069439 and TR000445; Nina Lambert and Sherrie Wolfe - Northwestern University CRS (Site 2701) Grants 2UM1 AI 069471 and UL1TR001422; Graham Ray and Cathi Basler - University of Colorado Hospital CRS (Site 6101) Grants 2UM1AI069432 and UL1 TR001082