# HIV e infiammazione

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**IL SOTTOSCRITTO LEONARDO CALZA**

***IN QUALITÀ DI RELATORE DELL'EVENTO IN CORSO, AI SENSI DELL'ART. 3.3 SUL CONFLITTO DI INTERESSI, PAG. 17 DEL REG. APPLICATIVO DELL'ACCORDO STATO-REGIONI DEL 5/11/09, PER CONTO DEL PROVIDER*** **DICHIARA CHE NEGLI ULTIMI DUE ANNI HA AVUTO I SEGUENTI RAPPORTI ANCHE DI FINANZIAMENTO CON SOGGETTI PORTATORI DI INTERESSI COMMERCIALI IN CAMPO SANITARIO:**

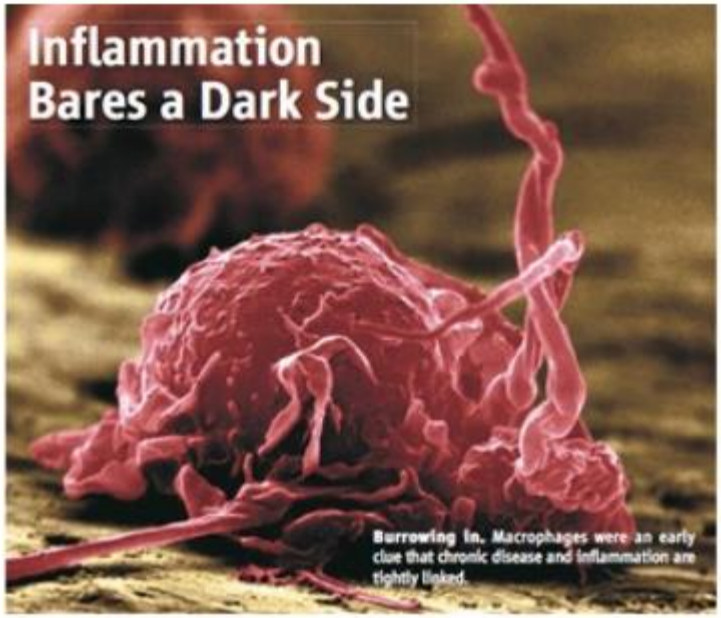
-JANSSEN

-VIIV

-MSD

-GILEAD


# Chronic inflammation is also harmful in non-HIV-infected adults




"Over the past decade, it has become widely accepted that inflammation is a driving force behind chronic diseases that will kill nearly all of us . . ."

*Courtesy of Peter Reiss*

Science , 2010

# Inflammatory and Coagulation Biomarkers and Mortality in Patients with HIV Infection

Lewis H. Kuller[1], Russell Tracy[2], Waldo Belloso[3], Stephane De Wit[4], Fraser Drummond[5], H. Clifford Lane[6], Bruno Ledergerber[7], Jens Lundgren[8], Jacqueline Neuhaus[9], Daniel Nixon[10], Nicholas I. Paton[11], James D. Neaton[9*], for the INSIGHT SMART Study Group

- INSIGHT SMART randomized, open-label trial
- 5472 HIV-positive patients with CD4+ cell count >350/mmc
- Drug Conservation (DC) versus Virologic Suppression (VS) groups

**Table 5.** Risk of Death Associated with Latest Level of Each Biomarker

| Biomarker | OR[a] | 95% CI | *p*-Value |
|---|---|---|---|
| hsCRP (µg/ml) | 2.4 | 1.4–4.2 | 0.003 |
| Amyloid A (mg/l) | 1.4 | 1.0–1.9 | 0.09 |
| Amyloid P (µg/ml) | 0.7 | 0.4–1.0 | 0.04 |
| IL-6 (pg/ml) | 2.0 | 1.2–3.1 | 0.006 |
| D-dimer (µg/ml) | 2.2 | 1.1–4.1 | 0.02 |
| F1.2 (pmol/l) | 1.1 | 0.7–1.7 | 0.77 |

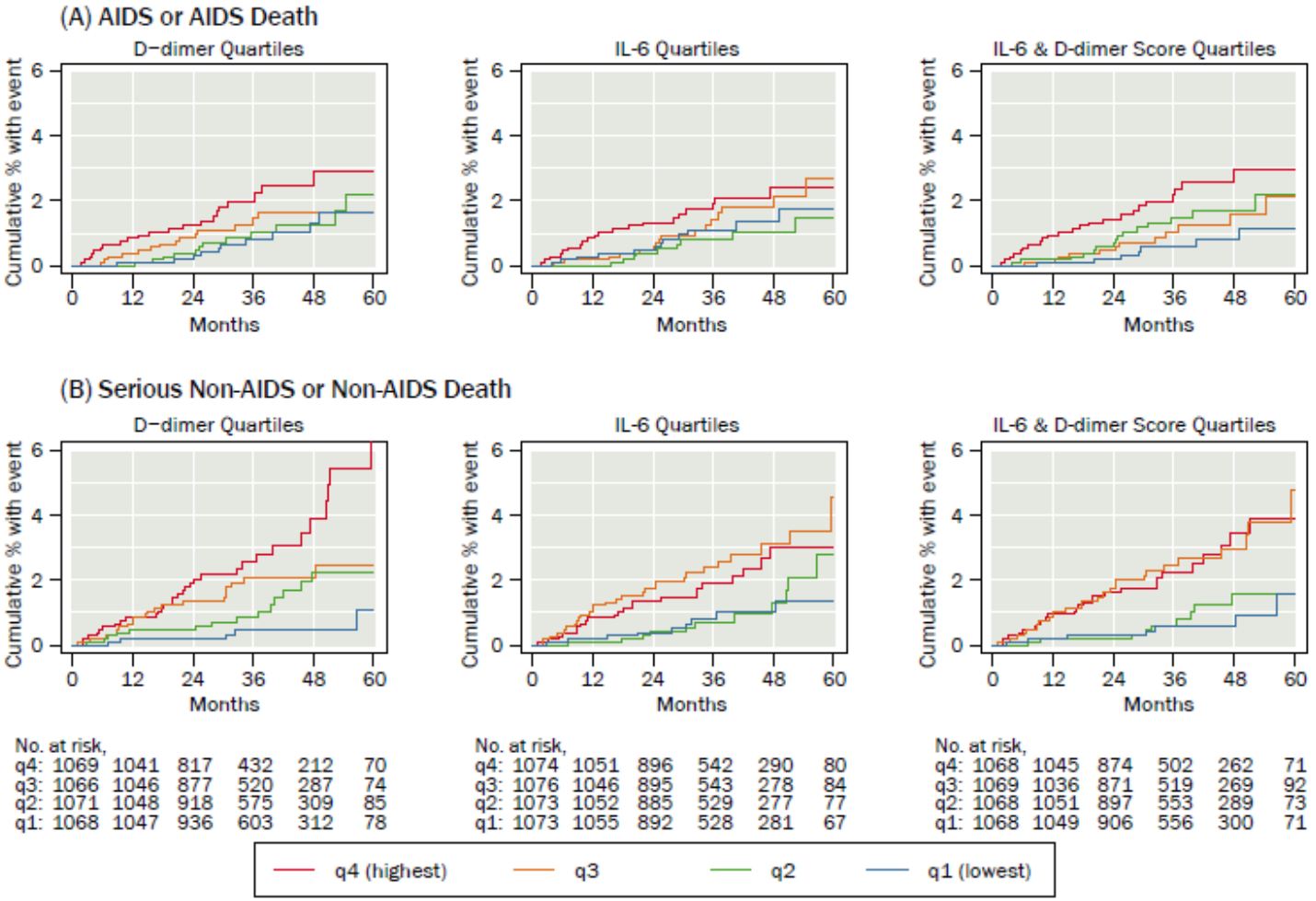
*(PLoS Med 2008)*

# Inflammatory Biomarkers Predict Risk for All-Cause Mortality among Treated Adults

| | | T cells | Innate | | | | Microbes | Coagulation | | Fibrosis |
|---|---|---|---|---|---|---|---|---|---|---|
| Cohort | Design | T cell acti-vation | CRP | IL-6 | K/T IDO | Cysta-atin C | sCD-14 | D-dimer | Fibrin-ogen | Hyalu-ronic Acid |
| SMART ESPRIT | Case-control | | ✓ | ✓ | | | ✓ | ✓ | | ✓ |
| FRAM | Cohort | | ✓ | | | ✓ | | | ✓ | |
| SOCA/ SCOPE | Cohort | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | | |
| UARTO | Cohort | ✓ | | | ✓ | | | | | |
| VACS | Cohort | | | | | | ✓ | ✓ | | |
| FIRST (Pre-ART) | Case-control | | ✓ | ✓ | | | | ✓ | | ✓ |
| Pfidisas (Pre-ART) | Case-control | | ✓ | ✓ | | | | ✓ | | |

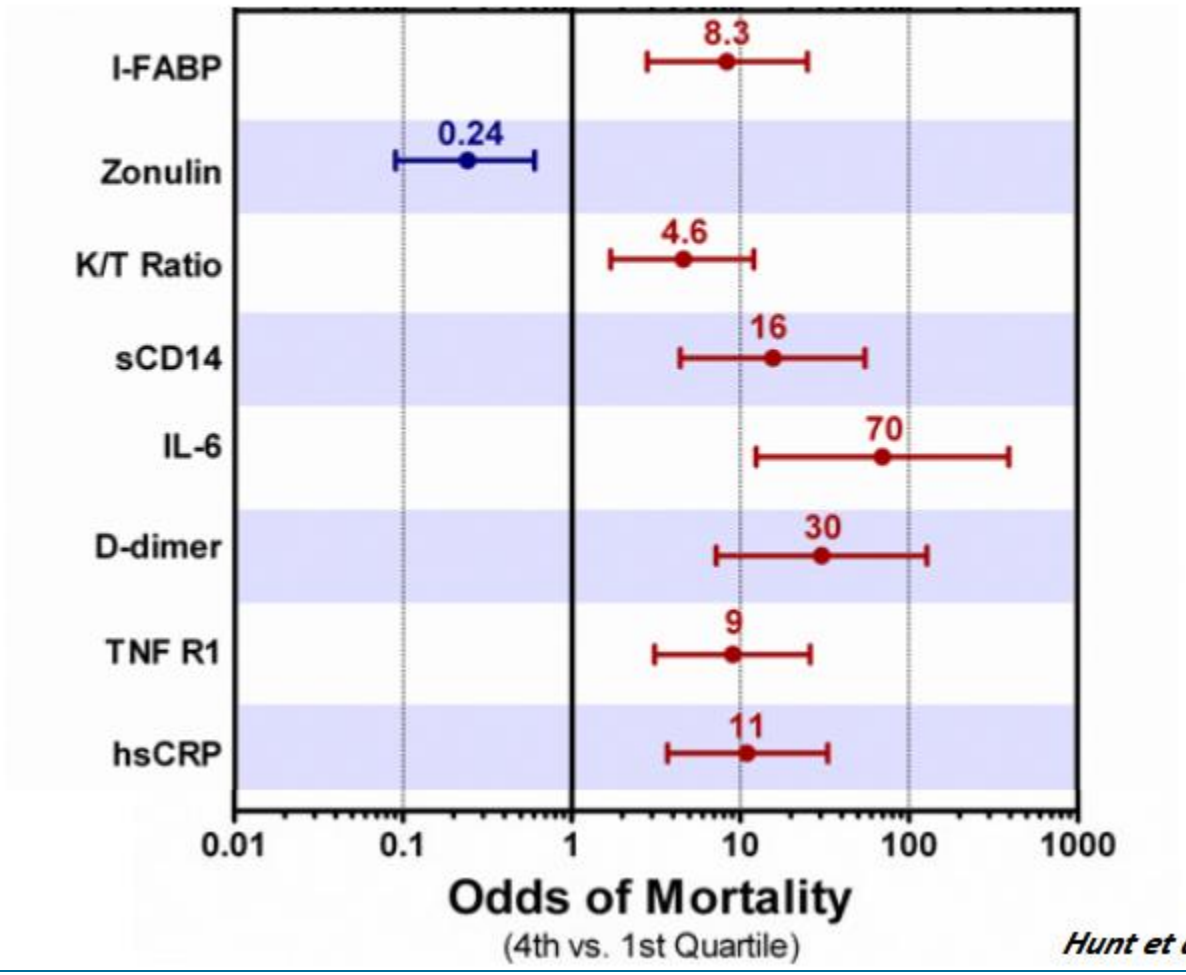
# Association of Inflammation and Coagulation with Clinical Risk in the START Trial

**FIGURE 3: Kaplan-Meier estimates for the cumulative percent of participants experiencing (A) AIDS or AIDS-death, and (B) Serious non-AIDS or non-AIDS death, by quartiles of D-dimer, IL-6 and the IL-6 & D-dimer score at baseline.**




*(Baker J et al., CROI 2017, Abstract 623)*

# Effect on inflammation in predicting mortality higher in HIV disease than the general population (SOCA/SCOPE)

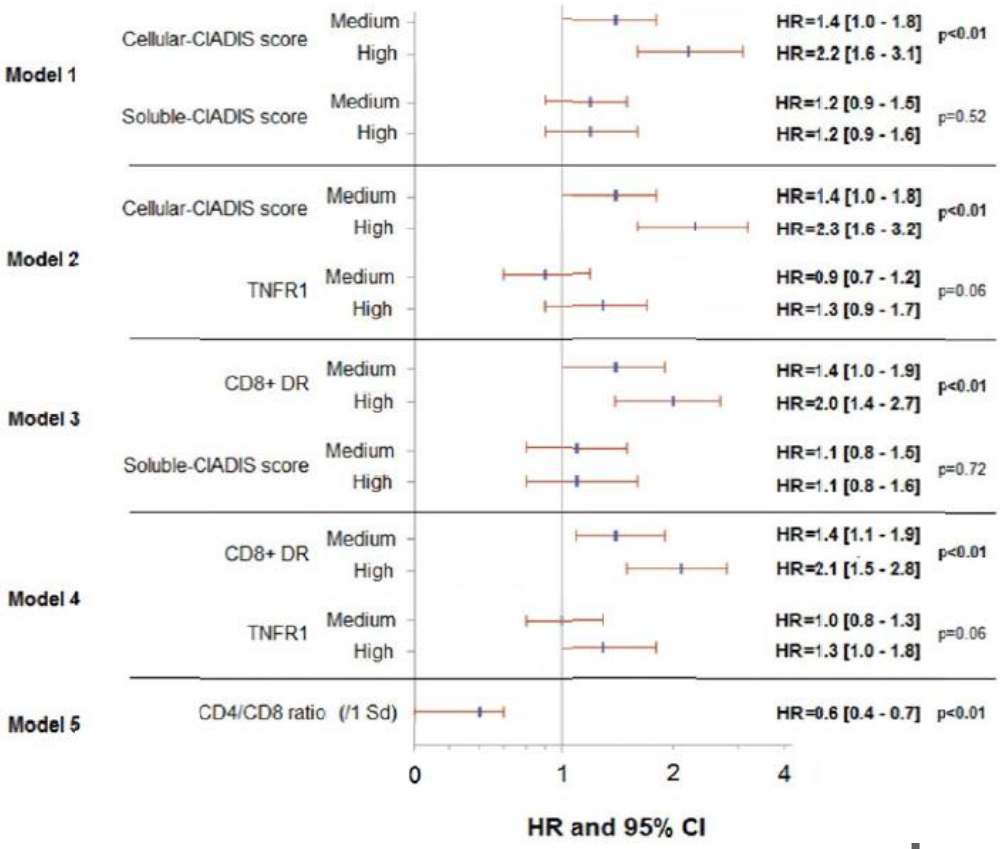



*Hunt et al CROI 12*

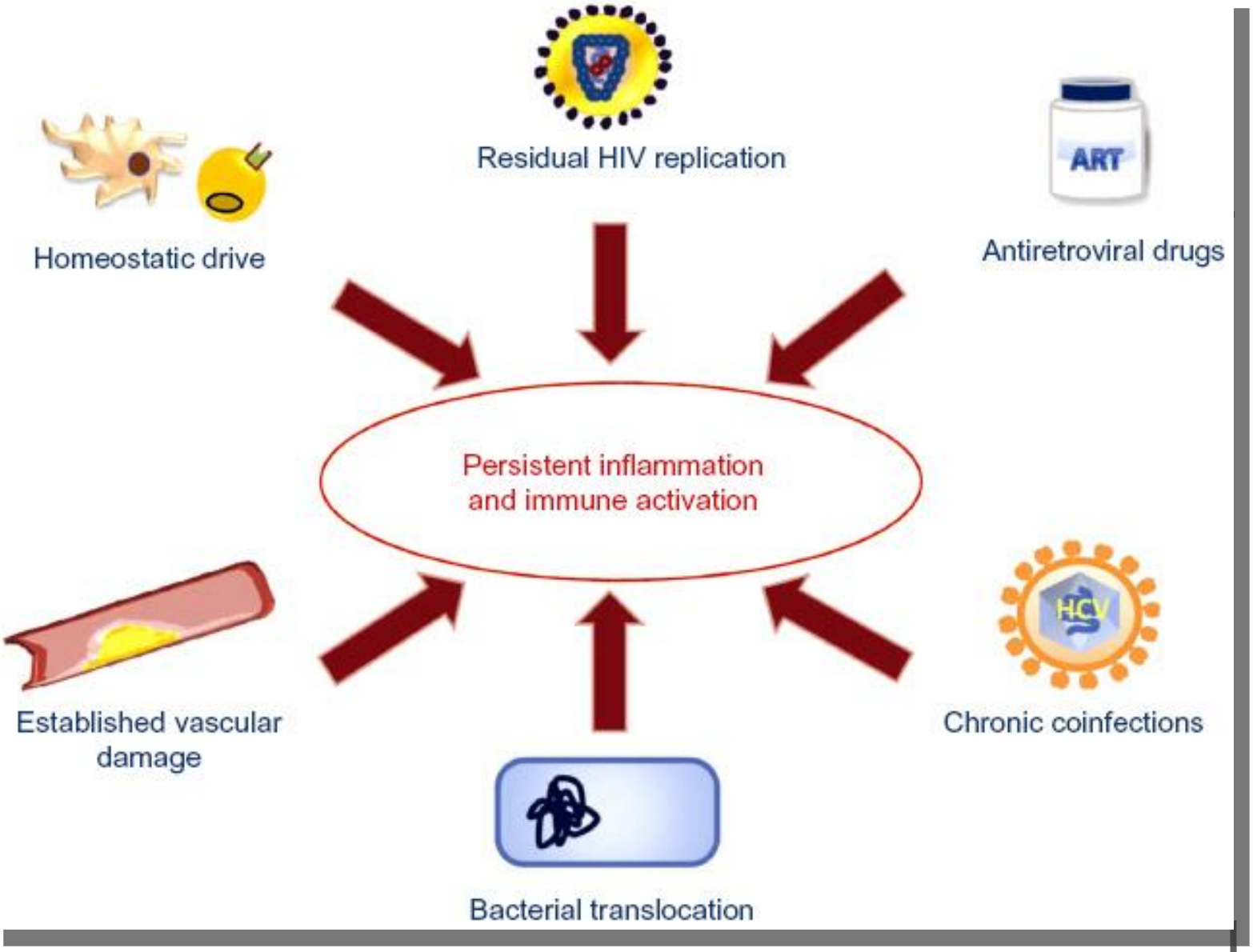
# Multimorbidity, age-related comorbidities and mortality: association of activation, senescence and inflammation markers in HIV adults.

- CIADIS (Chronic Immune Activation and Senescence) Study
- 828 HIV+ patients with undetectable viral load

**Figure 2. Hazard ratios (HR) with 95% confidence intervals estimated by the Cox proportional hazards models* with delayed entry for the association between CIADIS scores and the risk of the first age-related comorbidities over a 3-year follow-up (n=828).**




*(Duffau P et al., AIDS 2018)*



# Inflammatory and Coagulation Biomarkers and Mortality in Patients with HIV Infection

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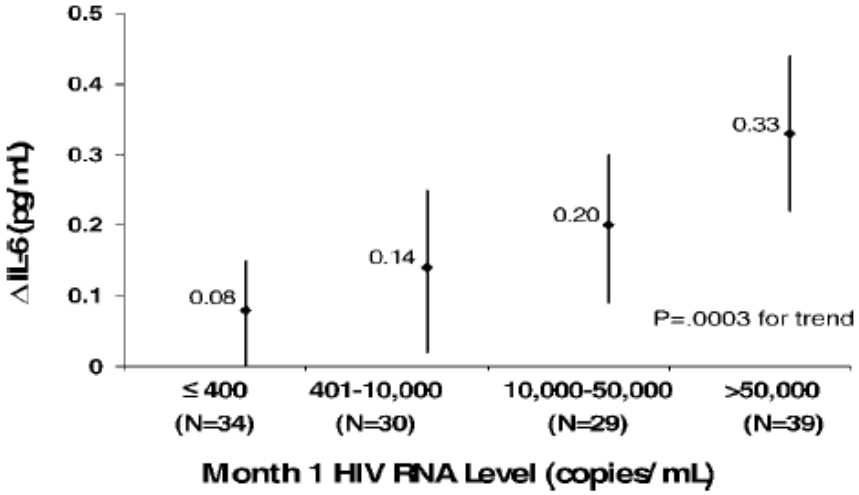
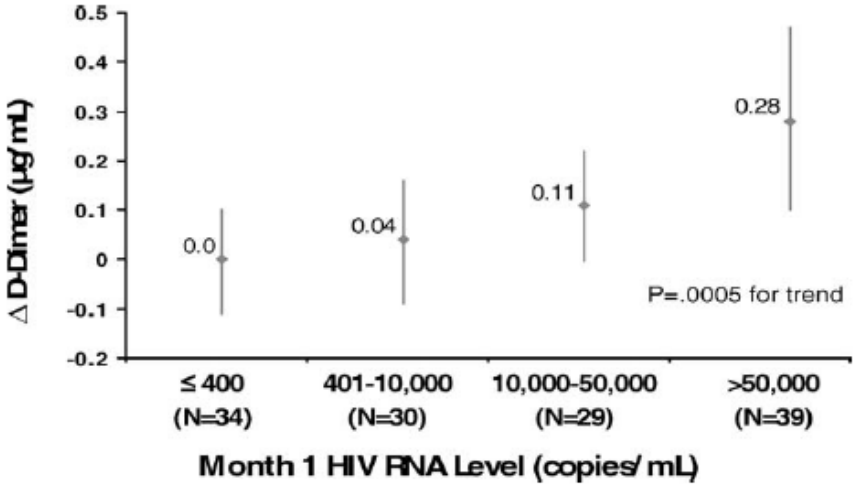



*(PLoS Med 2008)*

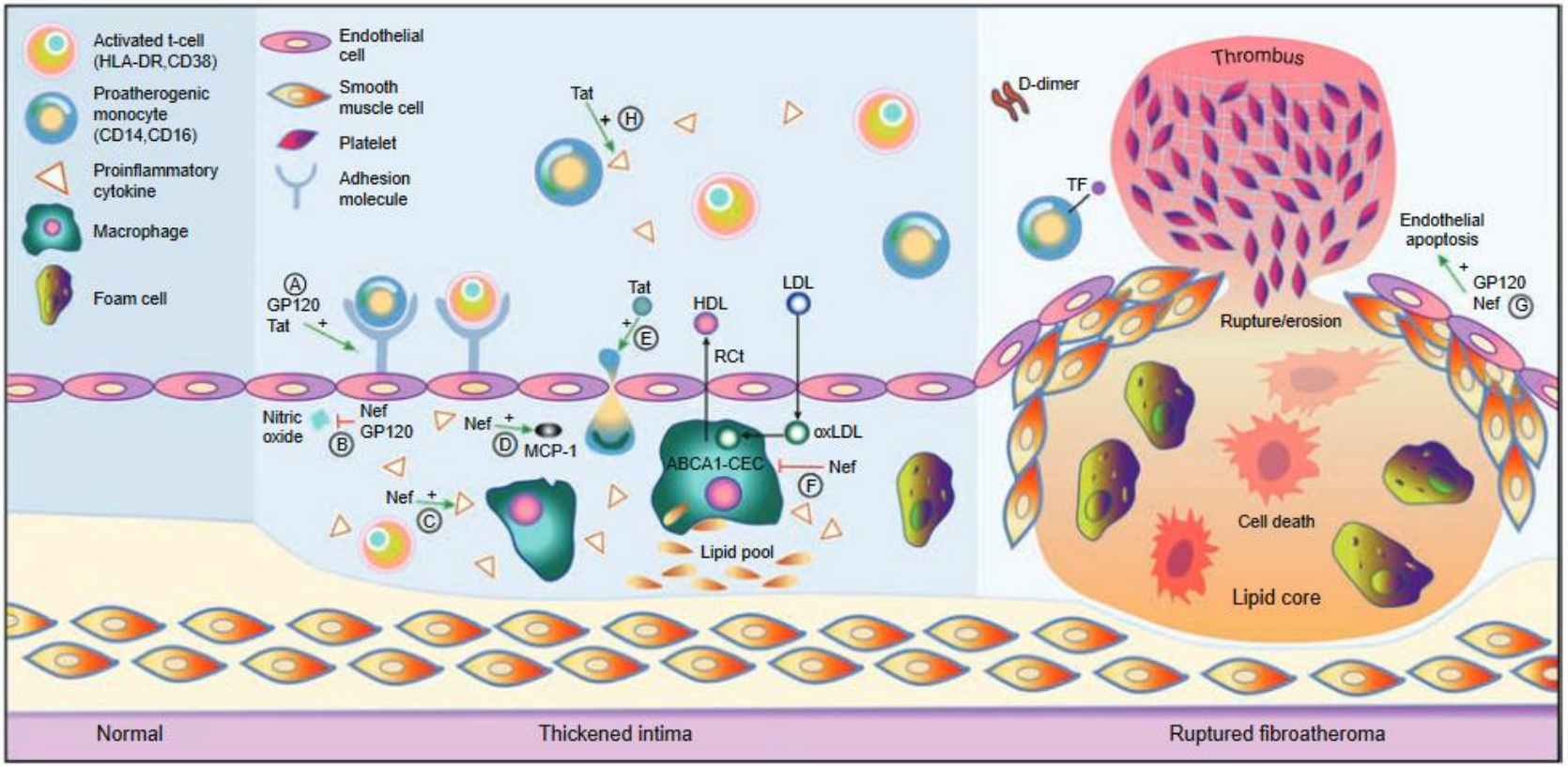
# Residual Viraemia in HIV-1-Infected Patients with Plasma Viral Load ≤20 copies/ml is Associated with Increased Blood Levels of Soluble Immune Activation Markers

- Observational, prospective study
- 32 HIV-positive patients on cART with HIV RNA <20 copies/mL
- 24-month follow-up

**Table 2** The effect of individual time-points with detectable residual viraemia (RV) on concomitant virologic and immunologic parameters in 32 HIV-1-infected patients with HIV-1 RNA ≤20 copies/ml in the 24 months study-period. RV was assessed by either transcription-mediated amplification (TMA) technique or reverse transcriptase (RT-) polymerase chain reaction (PCR) technique.

| | TMA-RV[a] | | PCR-RV[b] | |
|---|---|---|---|---|
| | Mean (95% CI)[c] | *P*-value | Mean (95% CI)[c] | *P*-value |
| Proviral-HIV-1-DNA[d] | 0.99 (0.67–1.46) | 0.949 | 1.11 (0.76–1.63) | 0.596 |
| Absolute changes in soluble immune activation markers[e] | | | | |
| sTNFrII (ng/ml) | 0.234 (0.027–0.441) | 0.030 | 0.254 (0.073–0.434) | 0.007 |
| $\beta_2$-microglobulin (nmol/l) | 22 (5–39) | 0.016 | 20 (4–35) | 0.016 |
| IgA ($\mu$mol/l) | 0.67 (−0.27–1.61) | 0.169 | 0.82 (0.07–1.58) | 0.035 |
| IgG ($\mu$mol/l) | 3.11 (−0.66–6.88) | 0.111 | −0.10 (−5.85–5.65) | 0.973 |
| IgM ($\mu$mol/l) | −0.01 (−0.17–0.15) | 0.922 | −0.21 (−1.40–0.98) | 0.736 |

*(Ostrowski SR et al., Scand J Immunol 2008)*




*(Nou E et al., AIDS 2016)*

# Association of Residual Plasma Viremia and Intima-Media Thickness in Antiretroviral-Treated Patients with Controlled Human Immunodeficiency Virus Infection

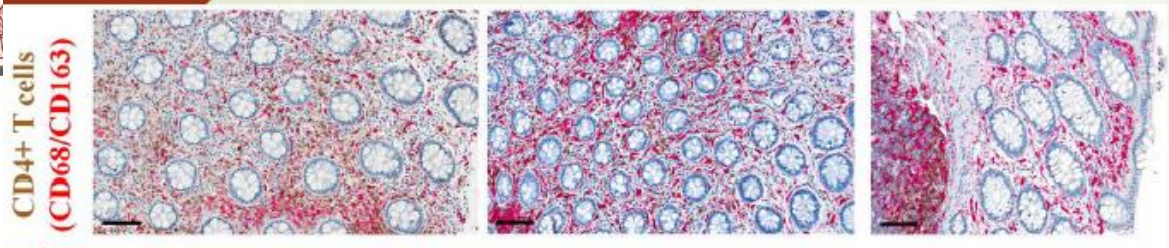
- Cross-sectional study
- 47 male HIV-positive patients
- >4 years of cART with HIV RNA <20 copies/mL

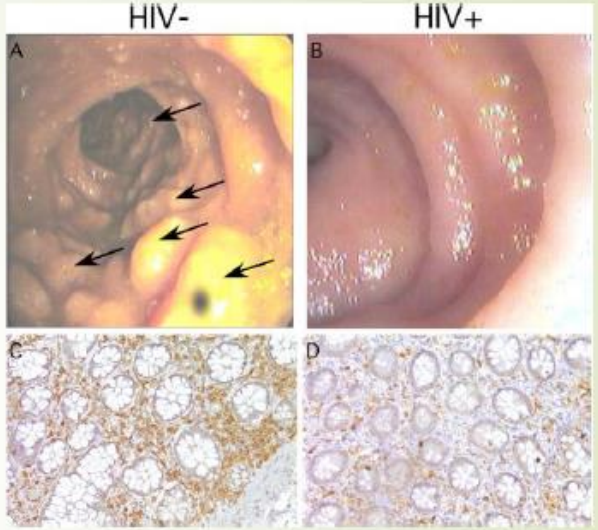
**Table 3.** Total and common carotid artery intima media thicknesses between viremia groups.

| | Total c-IMT | | | | cca-IMT | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariate | | Multivariable | | Univariate | | Multivariable | |
| | Δ* (95% CI) | *p* | Δ* (95% CI) | *p* | Δ* (95% CI) | *p* | Δ* (95% CI) | *p* |
| D- vs UD-viremia groups | 0.045 (0.002, 0.089) | 0.04 | 0.021 (−0.012, 0.055) | 0.2 | 0.074 (0.026, 0.123) | 0.003 | 0.059 (0.022, 0.095) | 0.002 |

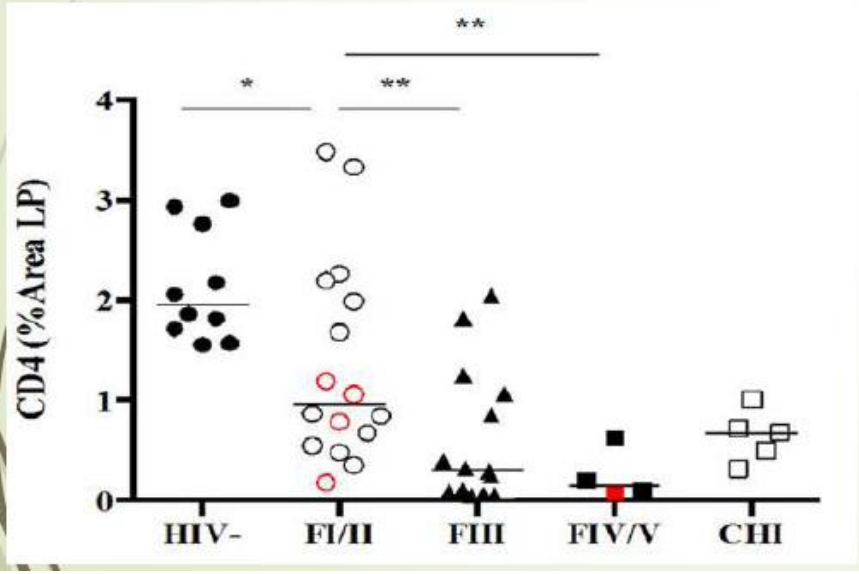
*(Boyd A et al., PLoS One 2014)*

# Earliest depletion of gut-associated CD4+



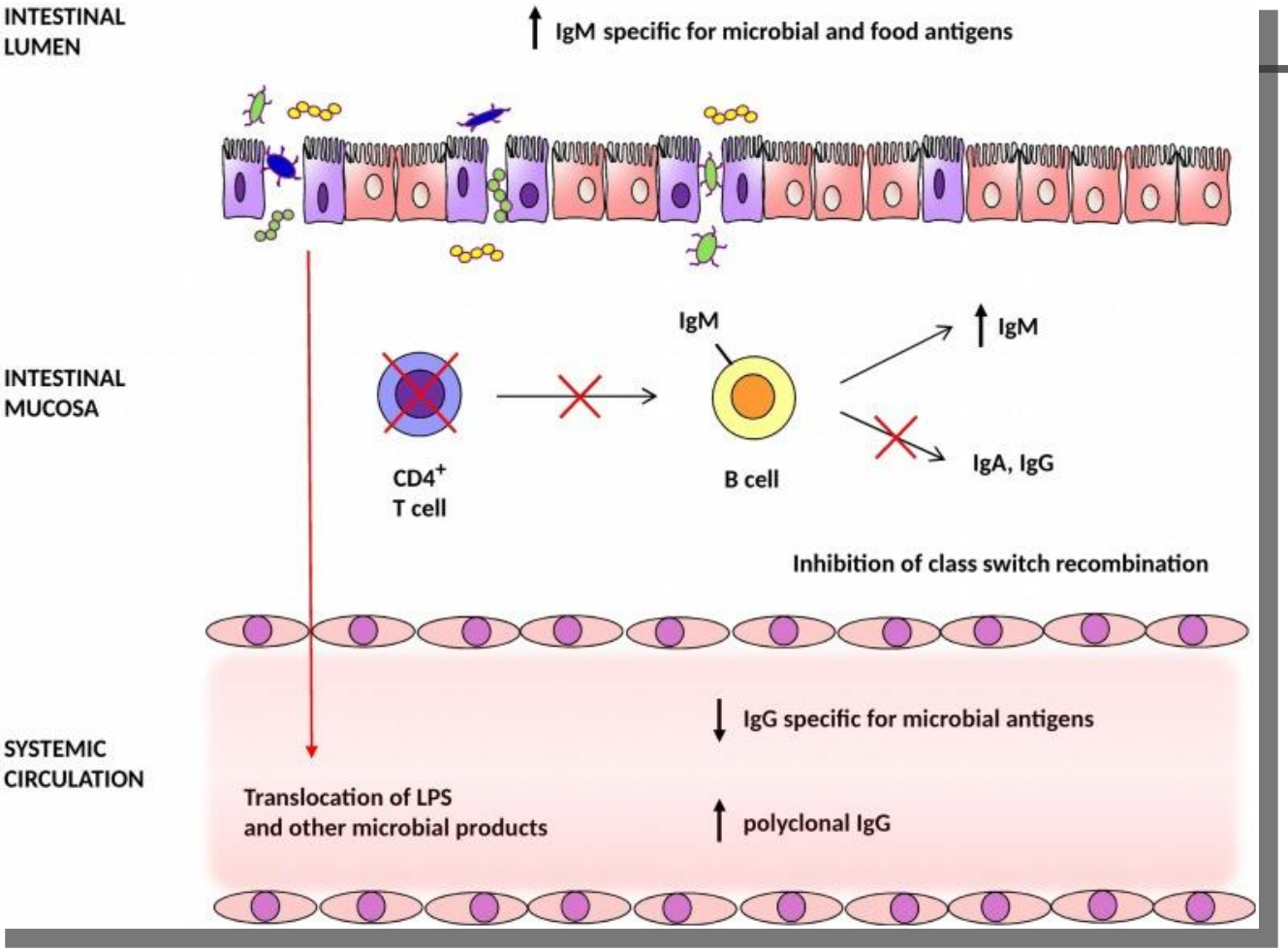

Brenchley et al. Nat Med 2006




Shuetz et al. Plos Path 2014

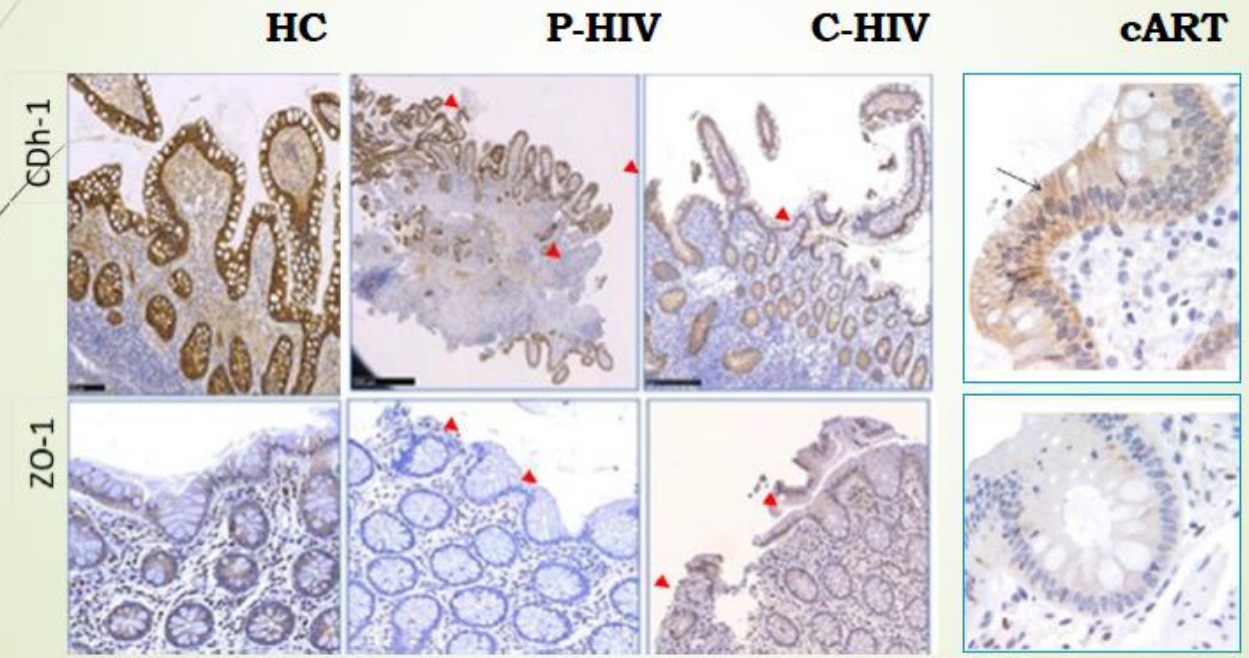
RESEARCH ARTICLE

# Dysregulation of Systemic and Mucosal Humoral Responses to Microbial and Food Antigens as a Factor Contributing to Microbial Translocation and Chronic Inflammation in HIV-1 Infection



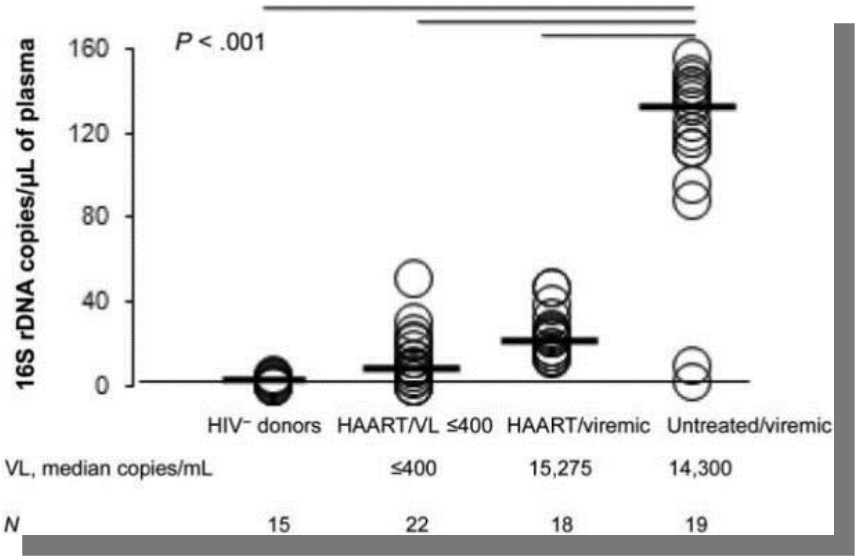

*(Hel Z et al., PLoS Pathog 2017)*

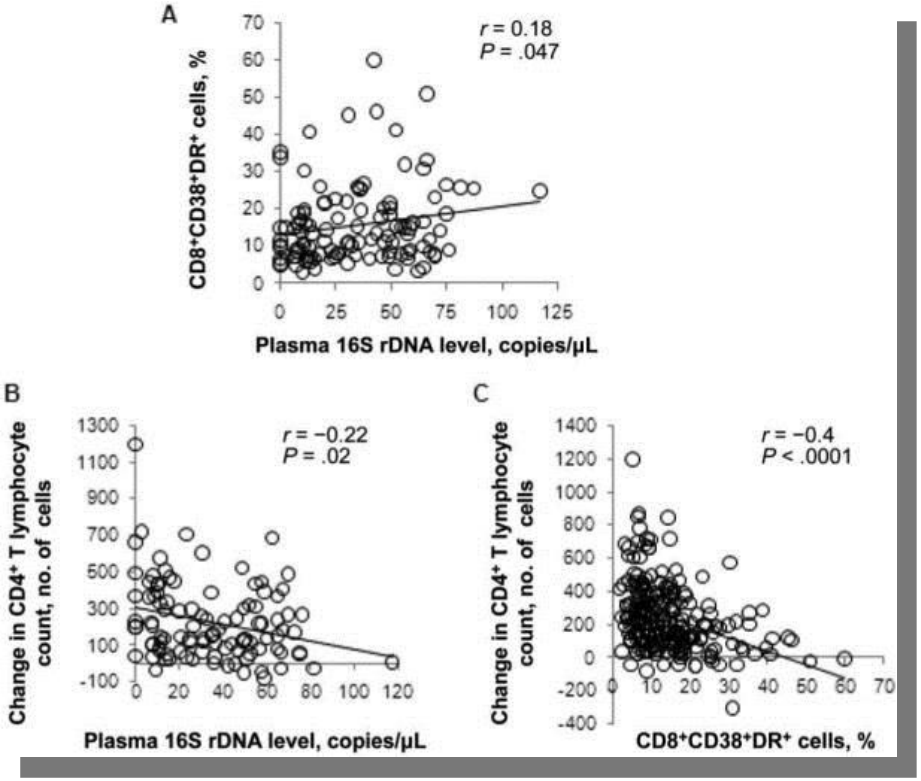
# Early depletion of gut tight junctions that is not reverted by cART




Cannizzo et al. CROI 2018

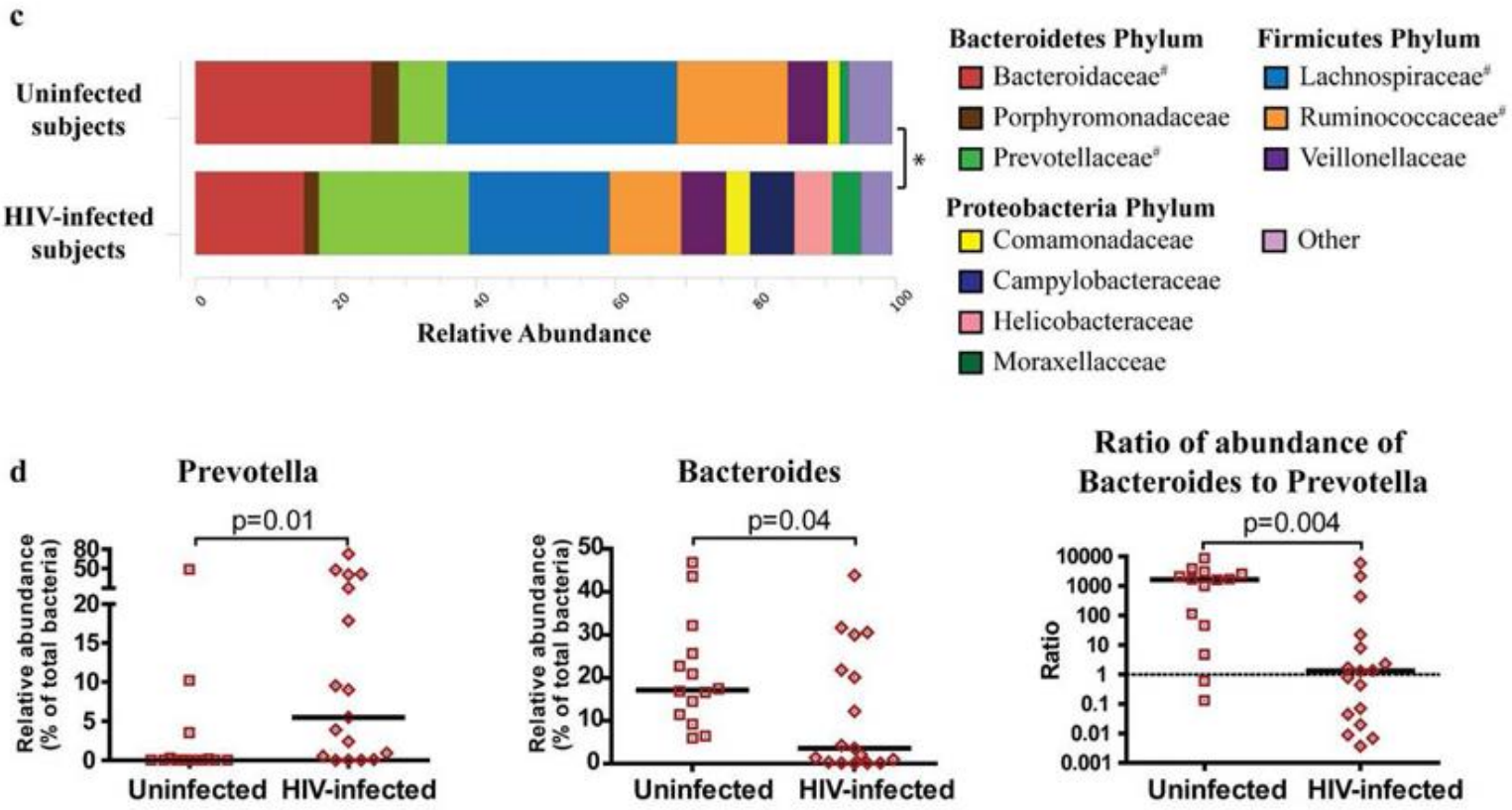
# Plasma Levels of Bacterial DNA Correlate with Immune Activation and the Magnitude of Immune Restoration in Persons with Antiretroviral-Treated HIV Infection




*(Jiang W et al., J Infect Dis 2009)*

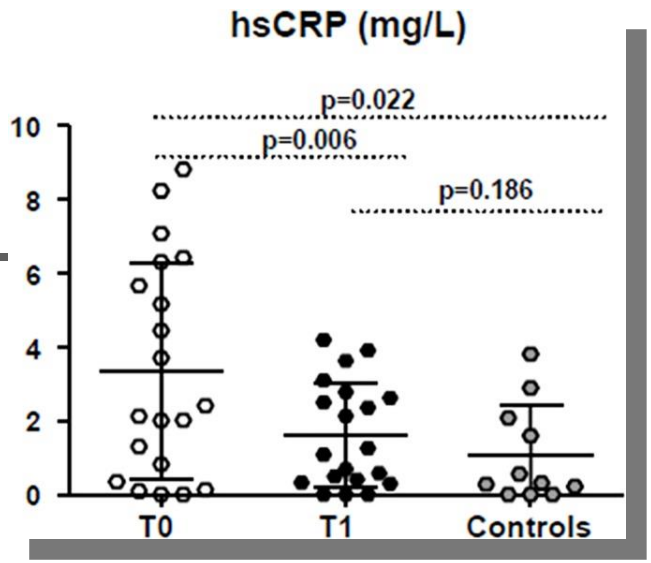
# An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia




*(Dillon SM et al., Mucosal Immunol 2014)*

# Probiotics Reduce Inflammation in Antiretroviral Treated, HIV-Infected Individuals: Results of the "Probio-HIV" Clinical Trial

- Observational study
- 20 HIV-positive patients on suppressive cART supplemented with probiotics vs 11 HIV-negative controls
- 48-week follow-up



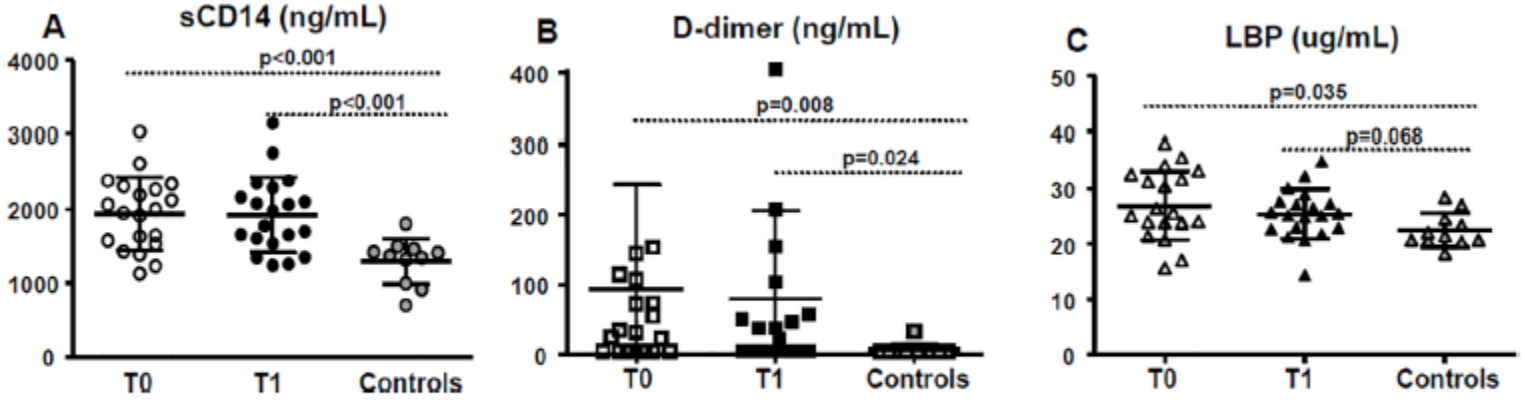
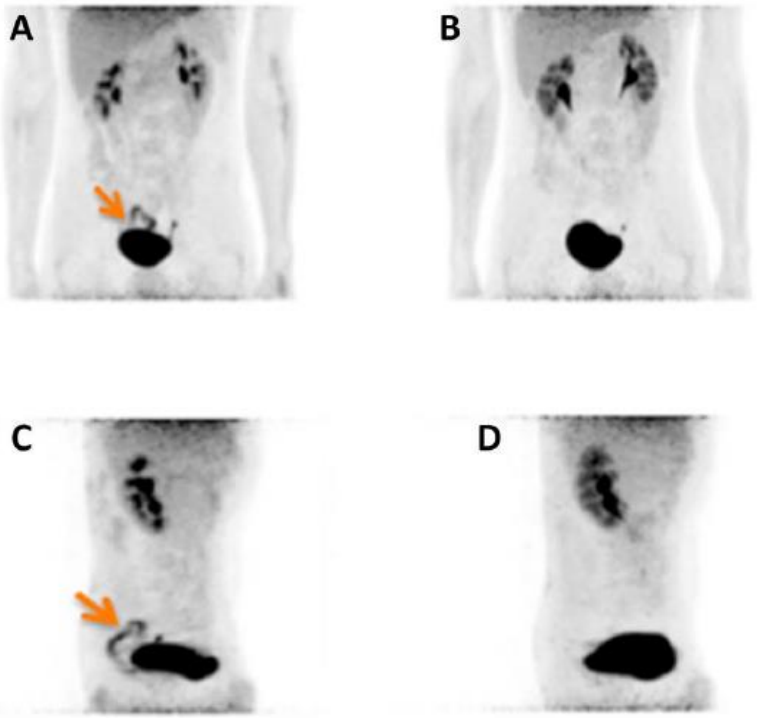



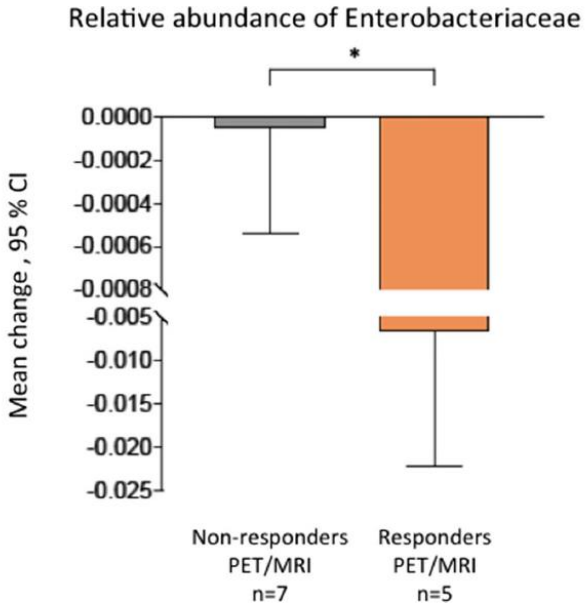

**Fig 3. Plasma levels of sCD14, d-dimer and LBP.** Scatter plots of sCD14 (A), d-dimer (B) and Lipopolysaccharide Binding Protein before (T0) and after probiotics' intake (T1) compared with controls. Horizontal bars in the scatter plot represent mean value with SD. considered statistically significant.

*(d'Ettorre G et al., PLoS One 2015)*

# Effect of *Lactobacillus rhamnosus GG* Supplementation on Intestinal Inflammation Assessed by PET/MRI Scans and Gut Microbiota Composition in HIV-Infected Individuals

- Prospective study
- 15 cART-naive and 30 cART treated patients
- LGG treatment for 8 weeks
- 18F-FDG PET/MRI evaluation




*(Arnbjerg CJ et al., J Acquir Immune Defic Syndr 2018)*

# Inflammatory and Coagulation Biomarkers and Mortality in Patients with HIV Infection

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- INSIGHT SMART randomized, open-label trial
- 5472 HIV-positive patients with CD4+ cell count >350/mmc
- Drug Conservation (DC) versus Virologic Suppression (VS) groups

**Table 7.** Biomarker, CD4+ Cell Count Change, and HIV-RNA Level Change 1 mo after Randomization

| Biomarker | DC Group, Mean (SD) or % | VS Group, Mean (SD) or % | Average Difference (SE)[a] | *p*-Value[a] |
|---|---|---|---|---|
| hsCRP (µg/ml) ($log_{10}$) | 0.02 (0.41) | 0.00 (0.41) | 0.02 (0.03) | 0.63 |
| Amyloid A (mg/l) ($log_{10}$) | 0.03 (0.38) | 0.01 (0.38) | 0.00 (0.03) | 0.88 |
| Amyloid P (µg/ml) ($log_{10}$) | −0.02 (0.20) | 0.01 (0.20) | −0.03 (0.01) | 0.05 |
| IL-6 (pg/ml) ($log_{10}$) | 0.12 (0.30) | 0.01 (0.34) | 0.08 (0.02) | 0.0005 |
| D-dimer (µg/ml) ($log_{10}$) | 0.09 (0.31) | −0.03 (0.31) | 0.11 (0.02) | <0.0001 |
| F1.2 (pmol/l) ($log_{10}$) | −0.04 (0.34) | −0.03 (0.27) | −0.02 (0.02) | 0.44 |
| CD4+ cell count (cells/$mm^3$) | −104 (180) | 18 (188) | −130 (16) | <0.0001 |
| Change in HIV-RNA (copies/ml) ($log_{10}$) | 1.17 (1.37) | −0.31 (0.97) | 1.46 (0.08) | <0.0001 |
| HIV-RNA ≤400 copies/ml at 1 mo (%) | 16.7 | 67.1 | −50.4 | <0.0001 |

*(PLoS Med 2008)*

# Association of Suboptimal Antiretroviral Therapy Adherence With Inflammation in Virologically Suppressed Individuals Enrolled in the SMART Study

**Table 2. Distribution of Biomarker Concentrations in Participants With Available Baseline Data According to Adherence Category and Fold Difference in Available Baseline Inflammatory and Coagulopathy Biomarker Plasma Concentrations in Suboptimally Adherent, Virologically Suppressed PLWH on ART Enrolled in SMART**

| | | | | | Unadjusted Analysis | | | Adjusted Analysis[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 100% Adherence | | Suboptimal Adherence | | | | | | | |
| Biomarker | No. | Mean (SD) | No. | Mean (SD) | Fold Higher Level Compared With 100% Adherence[b] | 95% CI | *P* Value | Fold Higher Level Compared With 100% Adherence[b] | 95% CI | *P* Value |
| IL-6, pg/mL | 2372 | 2.60 (6.93) | 391 | 2.93 (4.53) | 1.16 | 1.06–1.26 | .0005 | 1.09 | 1.01–1.18 | .02 |
| D-dimer, μg/mL | 2382 | 0.30 (0.59) | 394 | 0.38 (1.06) | 1.17 | 1.05–1.29 | .002 | 1.11 | 1.01–1.22 | .03 |
| hsCRP, μg/mL | 2397 | 3.72 (7.08) | 396 | 4.27 (7.51) | 1.07 | 0.94–1.23 | .31 | 1.04 | 0.91–1.17 | .58 |

Abbreviations: ART, antiretroviral therapy; CI, confidence interval; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; PLWH, people living with HIV.

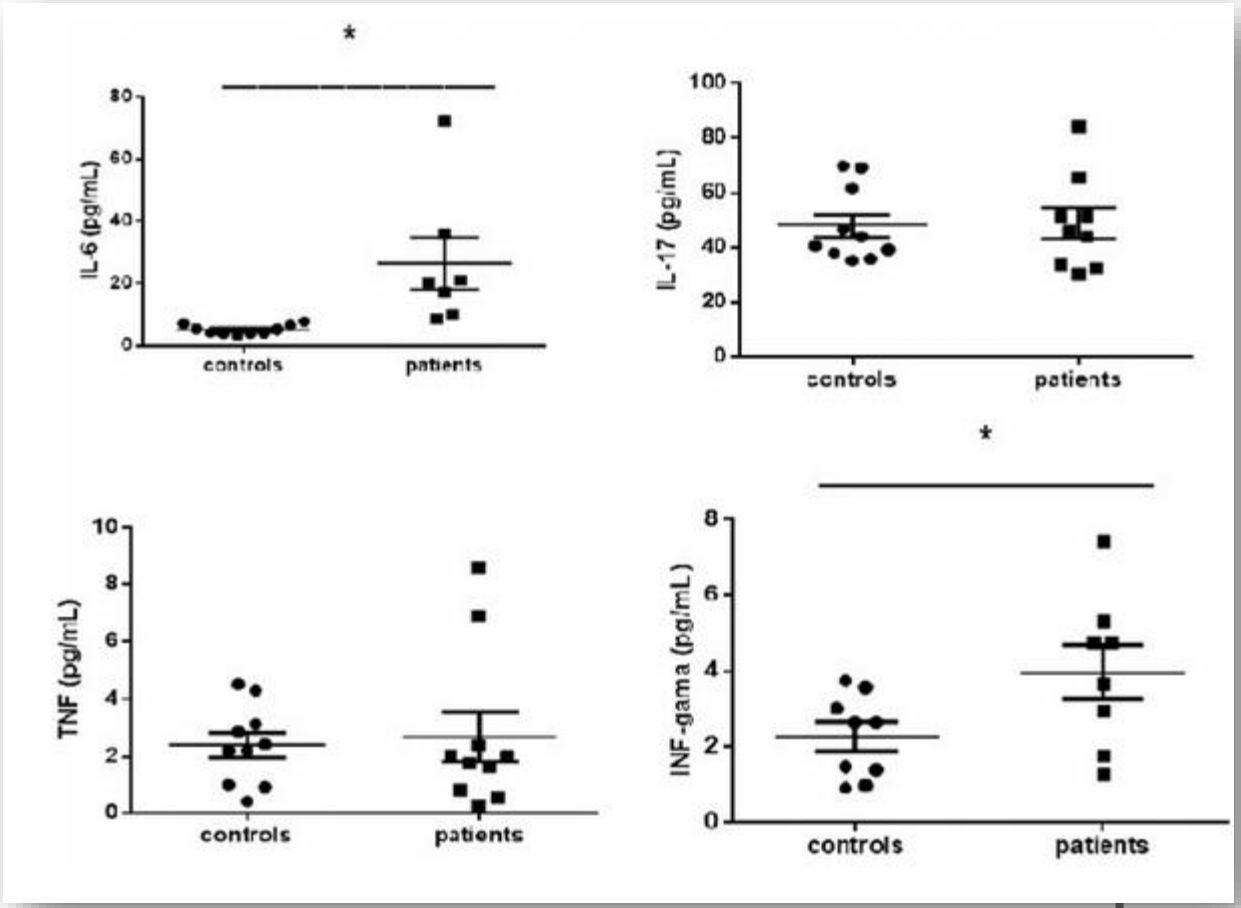
[a]Models were adjusted for covariates including age, race, gender, body mass index, time on ART, HIV exposure group, baseline viral load, baseline and nadir $CD4^+$ T-cells, hepatitis B or C co-infection, smoking, and regimen type.

[b]100% adherence defined as no report of any missed doses for any drug in the preceding 7-day period.

*(Castillo-Mancilla JR et al., Open Forum Infect Dis 2017)*

# Changes in inflammatory/cardiac markers of HIV positive patients

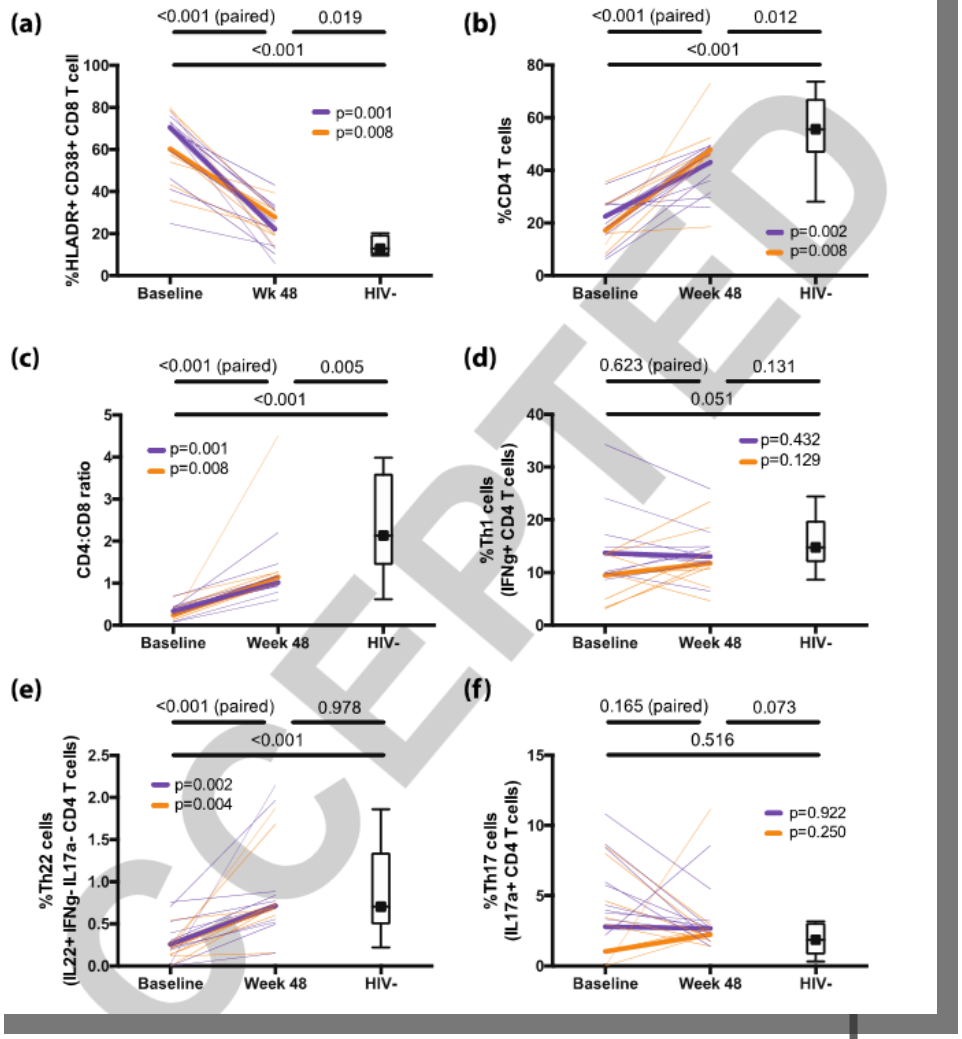
- Cross-sectional study
- 10 HIV-positive patients with undetectable HIV RNA
- 10 HIV-negative controls




*(Rezer JFP et al., Microb Pathog 2018)*

# Impact of intensified antiretroviral therapy during early HIV infection on gut immunology and inflammatory blood biomarkers

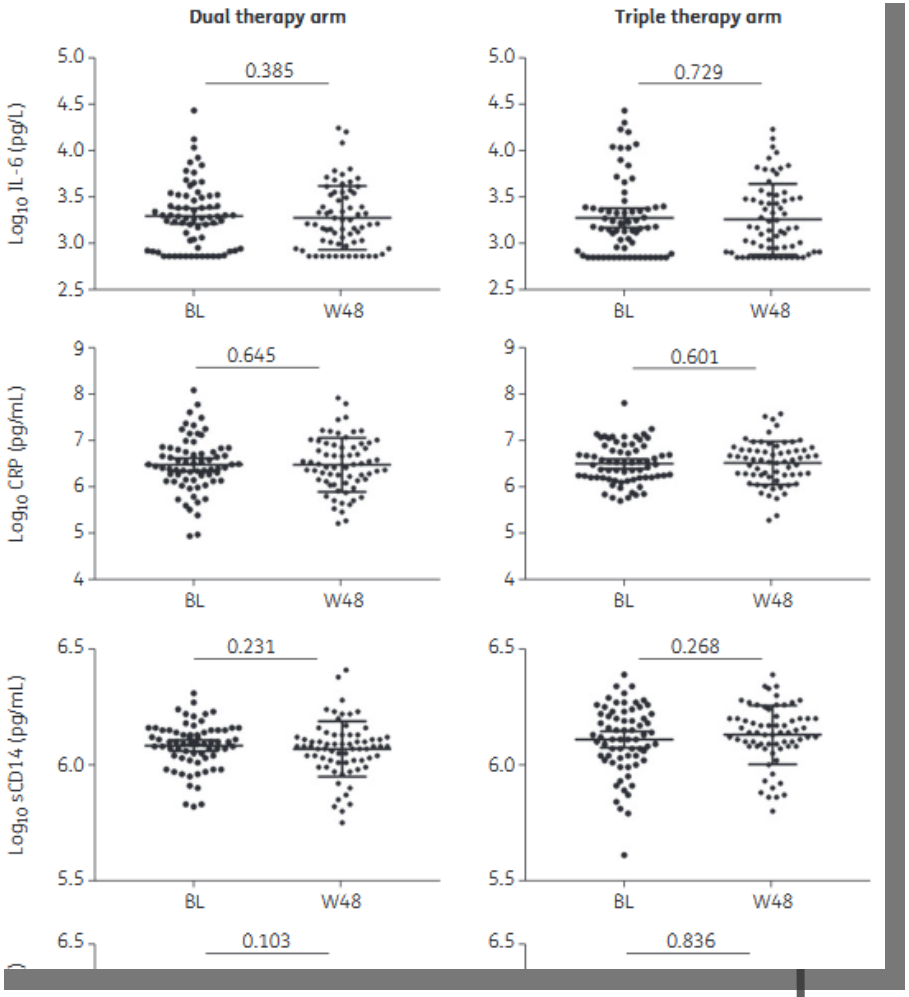
- Randomized, double-blind trial
- 22 ART-naive patients with early HIV infection
- TDF/FTC + LPV/r versus TDF/FTC + LPV/r + RAL + MVC
- 48-week follow-up




*(Kim CJ et al., AIDS 2017)*

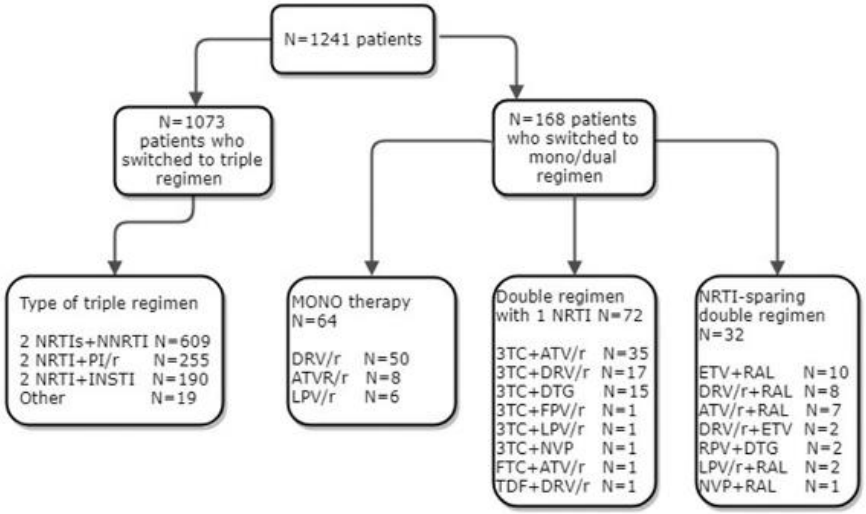
# Systemic inflammation markers after simplification to atazanavir/ ritonavir plus lamivudine in virologically suppressed HIV-1-infected patients: ATLAS-M substudy

- ATLAS-M substudy
- Randomized, open-label trial
- 139 HIV-positive patients
- 48-week follow-up




*(Belmonti S et al., J Antimicrob Chemother 2018)*

# Switching to dual/monotherapy determines an increase in CD8+ in HIV-infected individuals: an observational cohort study




- ICONA Cohort Study
- 1241 HIV-positive patients
- 24-month follow-up

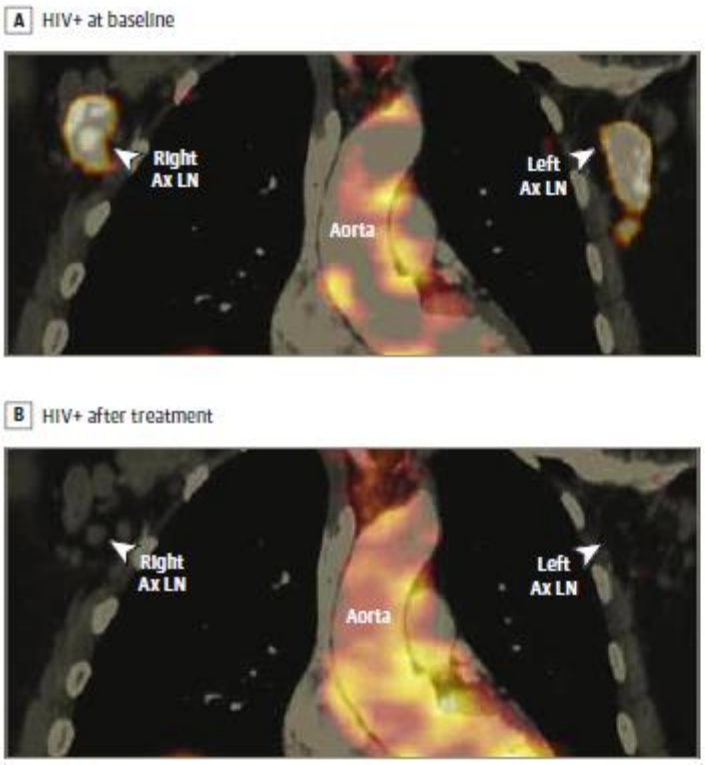
| | Triple (*N* – 622) | Dual (*N* – 57) | *p* value | Mono (*N* – 45) | *p* value |
|---|---|---|---|---|---|
| CD4/CD8 ratio | | | | | |
| At switch, mean (SD) | 0.76 (0.45) | 0.80 (0.37) | 0.167 | 0.90 (0.48) | 0.025 |
| 24 months after switch, mean (SD) | 0.93 (0.71) | 0.88 (0.43) | 0.824 | 1.02 (0.50) | 0.105 |
| Change, mean (SD) | +0.17 (0.58) | +0.08 (0.19) | 0.024 | +0.12 (0.22) | 0.786 |
| CD8 cell count | | | | | |
| At switch, mean (SD) | 912 (458) | 882 (471) | 0.396 | 812 (363) | 0.173 |
| 24 months after switch, mean (SD) | 867 (451) | 911 (411) | 0.337 | 913 (575) | 0.870 |
| Change, mean (SD) | −45 (401) | +28 (256) | 0.017 | +101 (404) | 0.012 |
| CD4 cell count | | | | | |
| At switch, mean (SD) | 588 (285) | 614 (269) | 0.402 | 654 (274) | 0.040 |
| 24 months after switch, mean (SD) | 683 (294) | 703 (272) | 0.285 | 795 (332) | 0.006 |
| Change, mean (SD) | +95 (181) | +89 (175) | 0.943 | +141 (229) | 0.205 |

*(Mussini C et al., BMC Med 2018)*

# Effects of Antiretroviral Therapy on Immune Function and Arterial Inflammation in Treatment-Naive Patients With Human Immunodeficiency Virus Infection

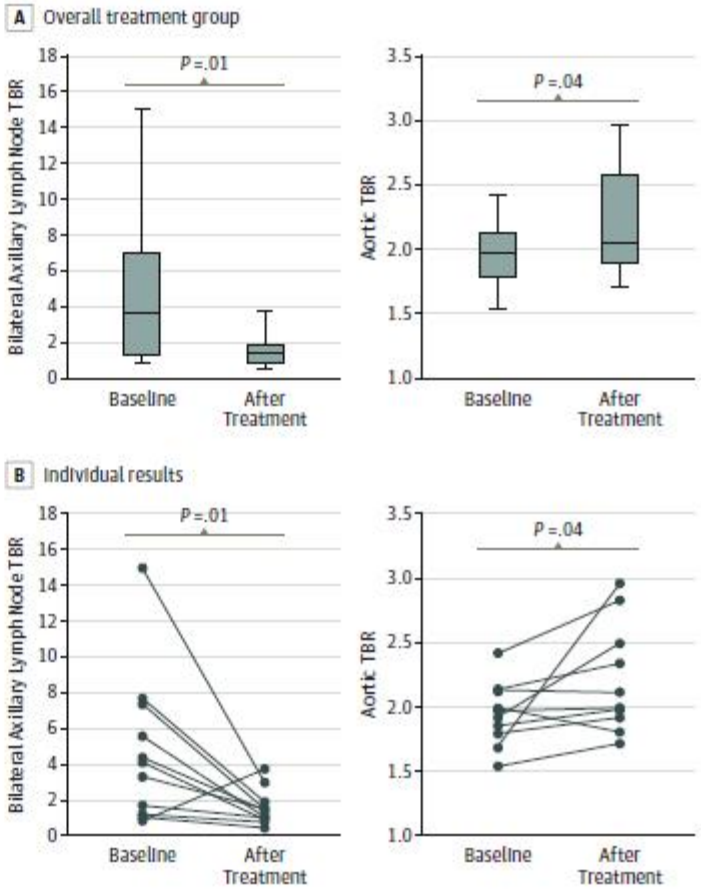
- Observational study
- 12 ARV-naive HIV+ patients starting TDF/F/EVG/Cobi
- PET evaluation at baseline and after 6 months

Figure 1. Representative Fludeoxyglucose F 18 Positron Emission Tomographic Imaging of the Aorta and Axillary Lymph Nodes (Ax LNs)




Increased aortic target-background ratio shown in a treatment-naive patient with human immunodeficiency virus infection before (A) and after (B) antiretroviral therapy using elvitegravir, cobicistat, emtricitabine, and tenofovir disoproxil fumarate. In contrast, lymph node target-background ratio decreased with combined ART in the same individual.

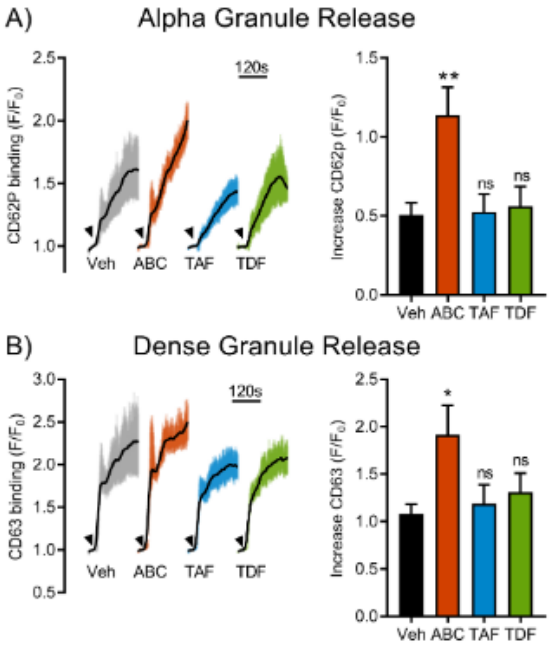
Figure 2. Effects of Combination Antiretroviral Therapy (ART) on Fludeoxyglucose F 18 Positron Emission Tomography ([18F]-FDG-PET) Parameters




*(Zanni MV et al., JAMA Cardiol 2016)*

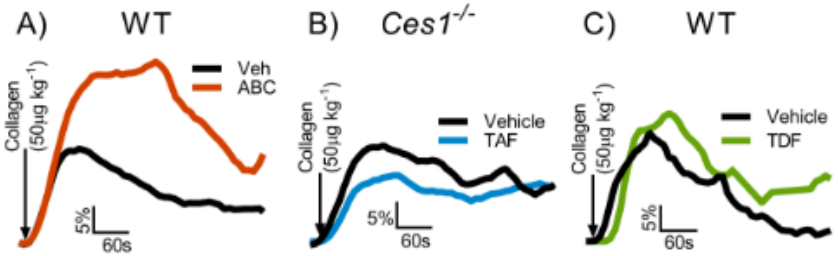
# Comparative Impact of Antiretrovirals on Human Platelet Activation

## Abacavir increases platelet granule release




Collagen-evoked (10µg/mL) platelet alpha (A) and dense (B) granule release were monitored in real-time by flow cytometry. Treatment with $C_{max}$ concentrations of ABC, but not TAF or TDF, enhanced platelet granule release. Granule release is a critical step in platelet activation (n=7)

## Abacavir enhances platelet aggregation *in vivo*

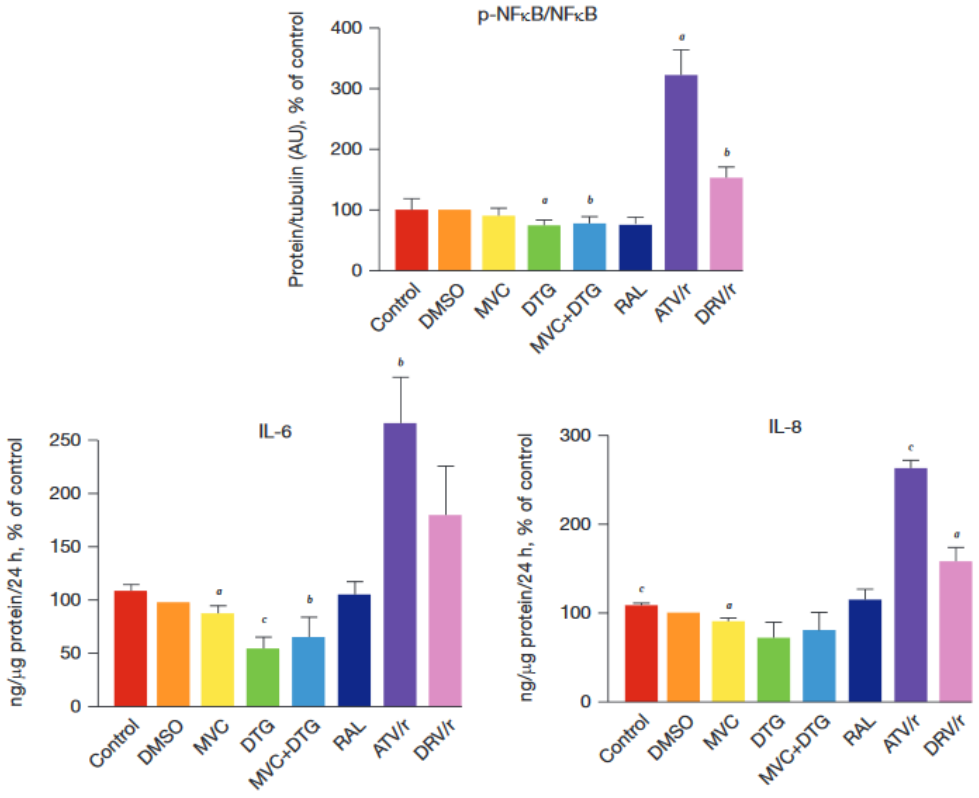



Representative collagen-induced *in vivo* aggregation responses in the presence

*(Taylor KA et al., Abstract 673, CROI 2018)*

# Impact of CCR5, integrase and protease inhibitors on human endothelial cell function, stress, inflammation and senescence

**Figure 2.** Effect of antiretrovirals on inflammation in adult donors' cells




*(Alfonso P et al., Antivir Ther 2017)*

# Changes in intestinal microbiota in HIV-1-infected subjects following cART initiation: influence of CD4+ T cell count

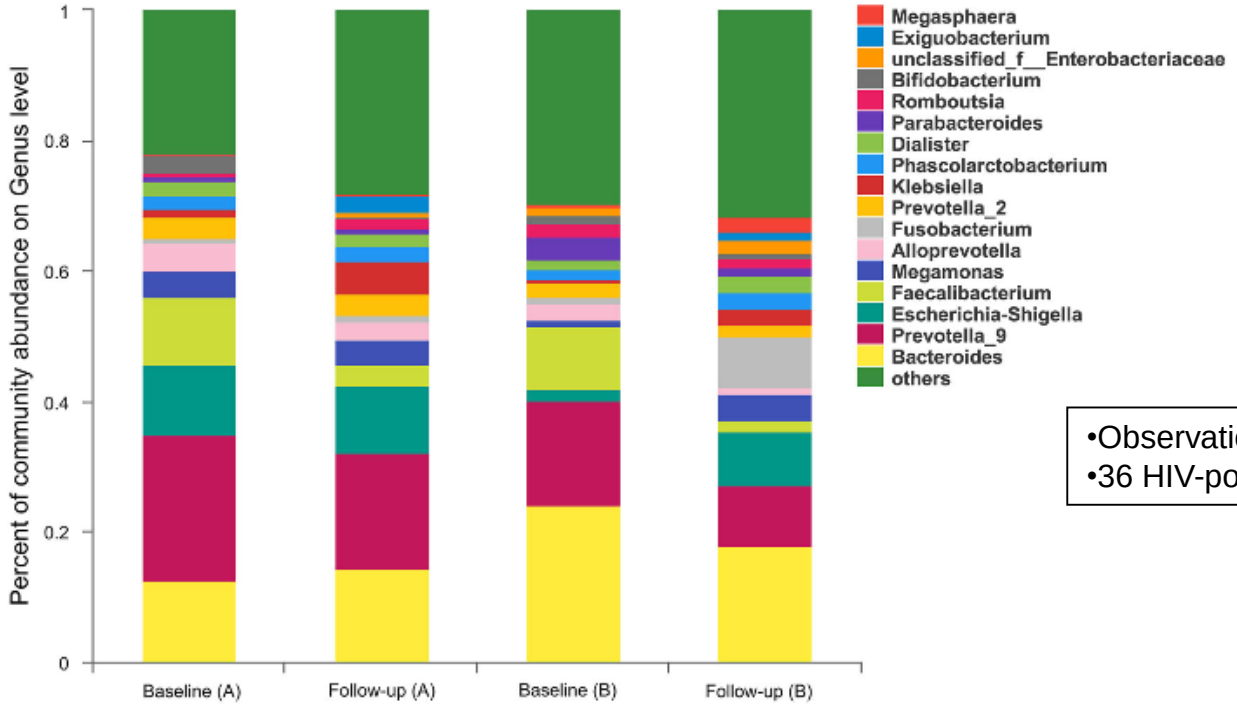



**Fig. 4 The grouped microbial composition at the genus level at baseline and follow-up. a** HISs with baseline $CD4^+$ T cell counts <300/mm$^3$; **b** HISs with baseline $CD4^+$ T cell counts >300/mm$^3$

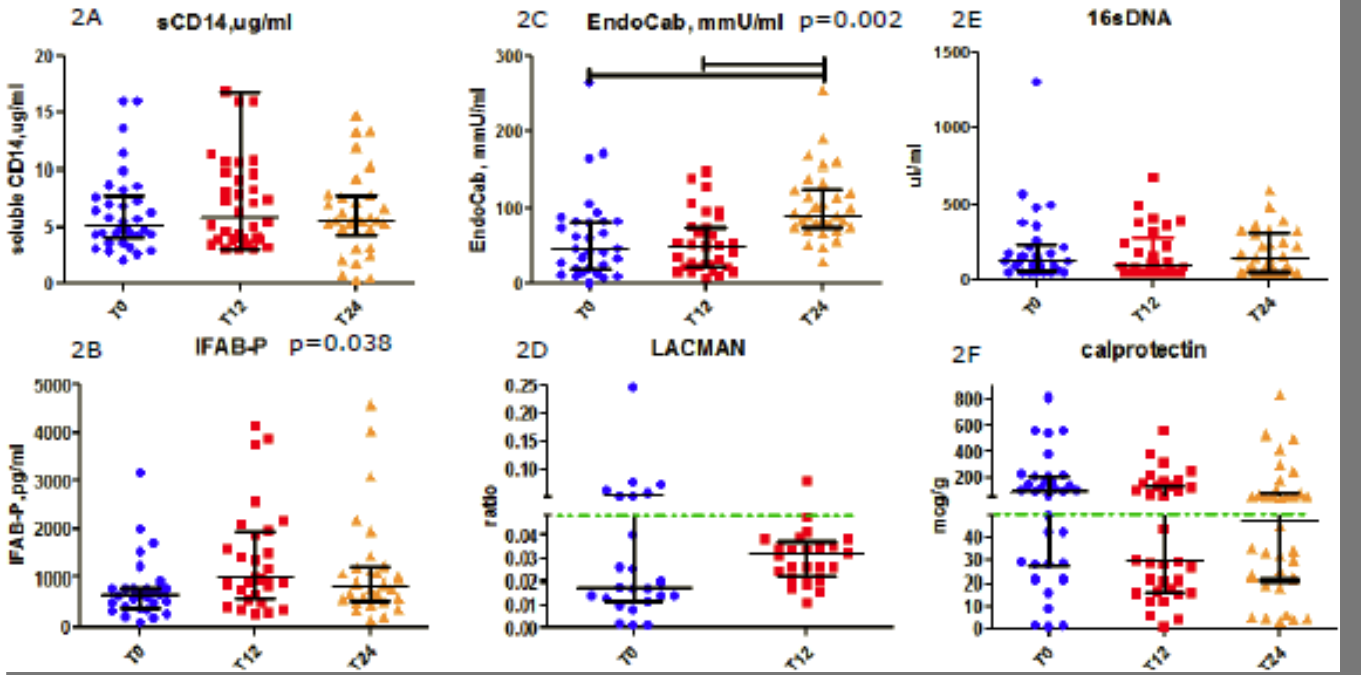
- Observational study
- 36 HIV-positive cART-naive patients

*(Ji Y et al., Emerg Microbes Infect 2018)*

# Two-year cART does not restore peripheral blood and intestinal HIV-related dysbiosis

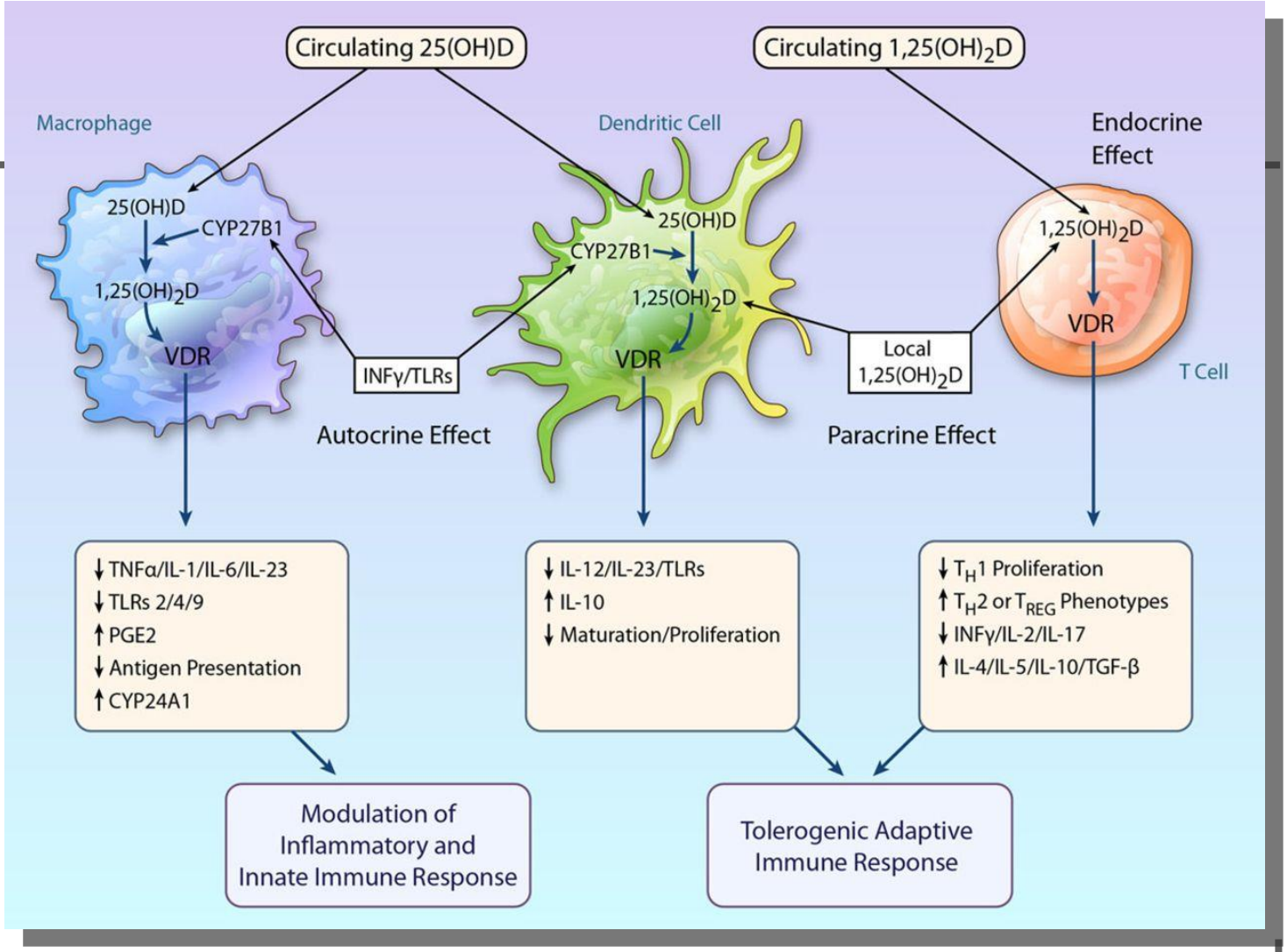
**Figure 2. Microbial Traslocation, gut barrier permeability/damage and inflammation parameters in the course of cART**

No modifications of plasma sCD14 (2A), 16S-rDNA (2E) and LPS (not shown) were measured, albeit an increase of EndoCab (2C). A rise in IFAB-P (2B) and no variations of E-cadherin (not shown), LAC/MAN ratio (2D) and calprotectin (2F) were also observed. Of note however, pathologic LAC/MAN ratios and calprotectin (green dotted line shows reference values) decreased on cART.

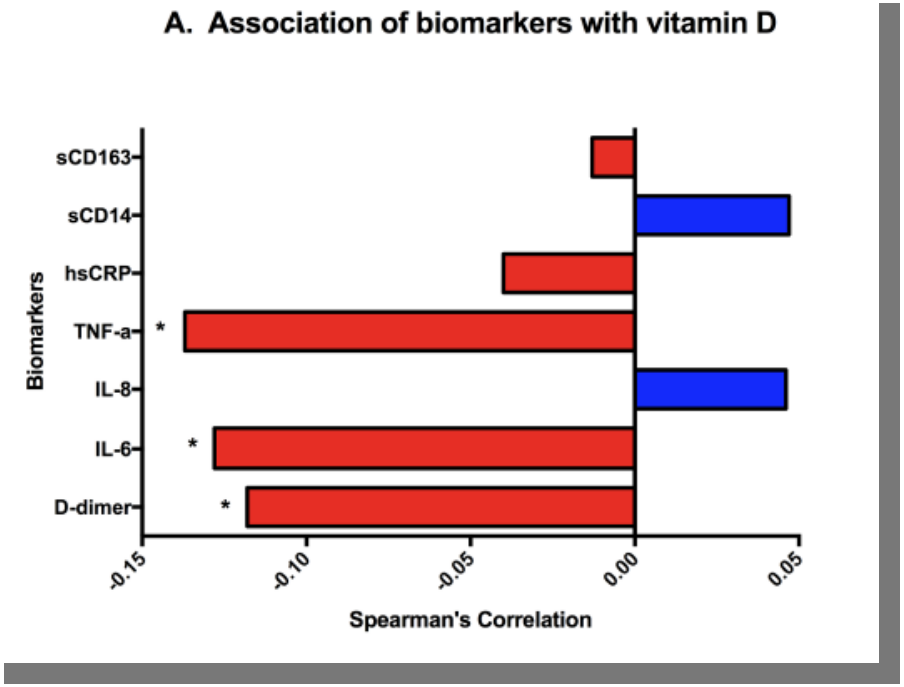



*(Tincati C et al., CROI 2017, Abstract 215)*

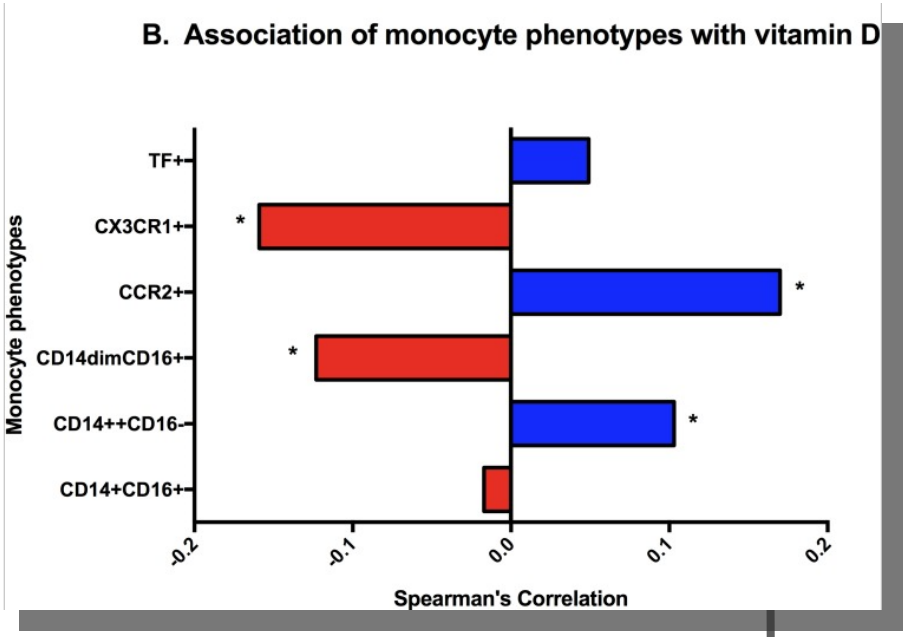
# Vitamin D and inflammation



# Vitamin D deficiency is associated with IL-6 levels and monocyte activation in HIV-infected persons



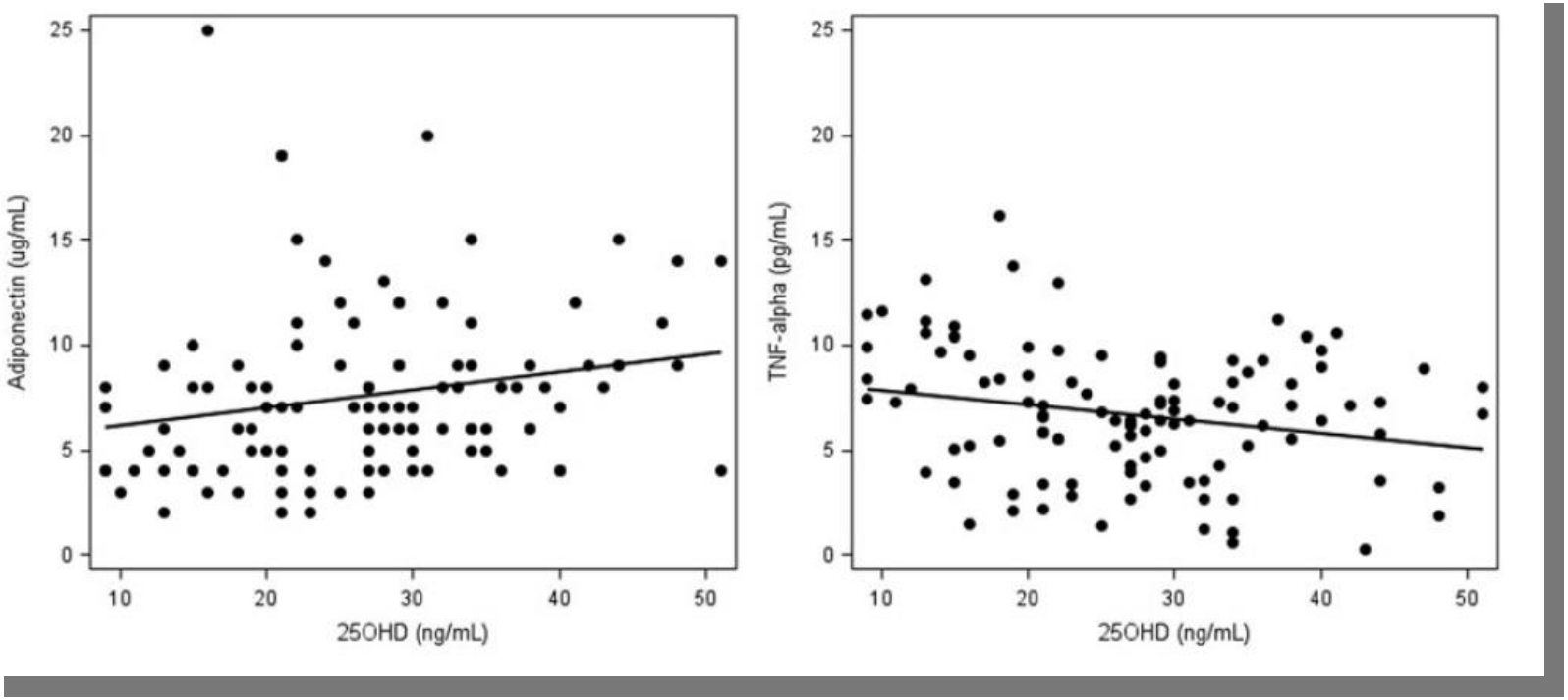

- Cross-sectional study
- 663 HIV-infected patients




*(Manion M et al., PLoS One 2017)*

# Vitamin D Levels and Markers of Inflammation and Metabolism in HIV-Infected Individuals on Suppressive Antiretroviral Therapy

- Observational, prospective study
- 106 HIV-positive patients on stable cART
- HIV RNA <200 copies/mL

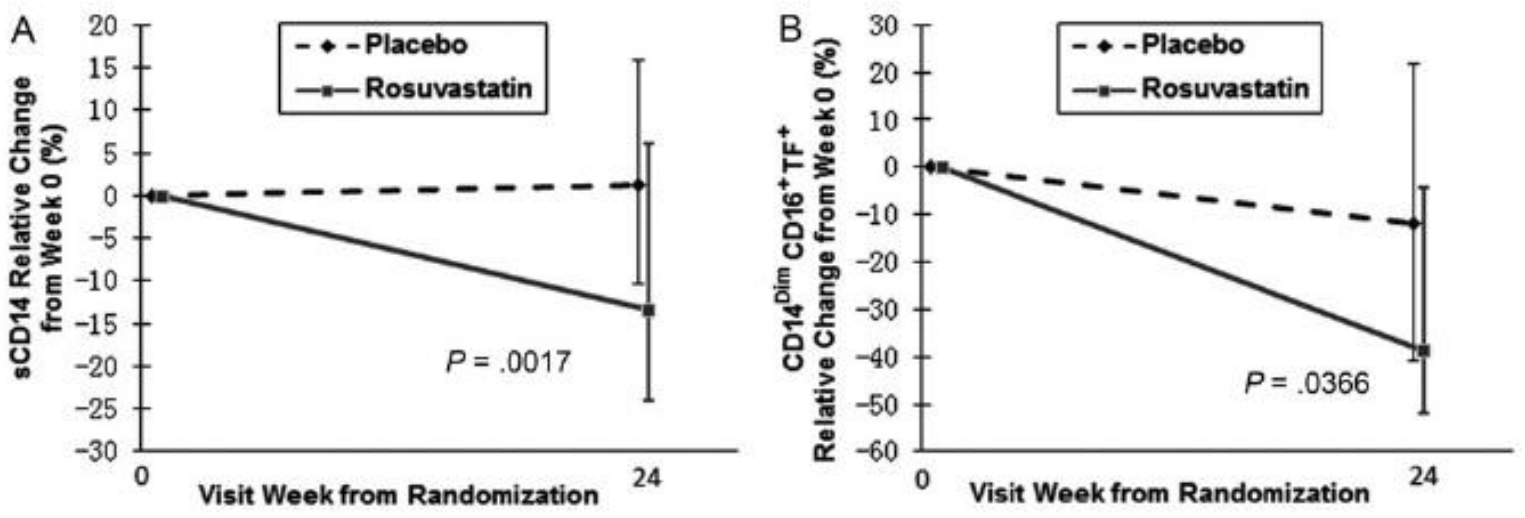



*(Hoffman RM et al., AIDS Res Hum Retroviruses 2016)*

***Anti-inflammatory therapy?***

# Rosuvastatin Treatment Reduces Markers of Monocyte Activation in HIV-Infected Subjects on Antiretroviral Therapy

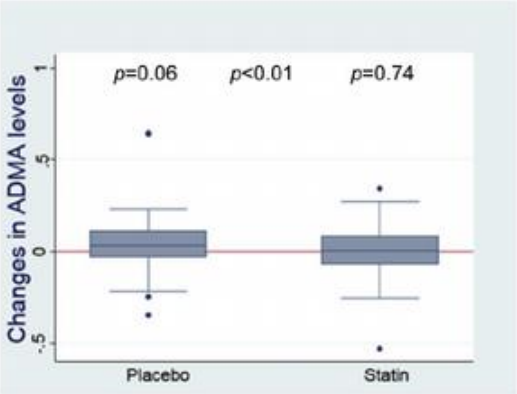
- SATURN randomized, double-blinded trial
- 147 HIV+ patients
- Stable cART (>12 weeks)
- LDL cholesterol <130 mg/dL
- hsCRP >2 mg/L and/or CD8+ cell activation

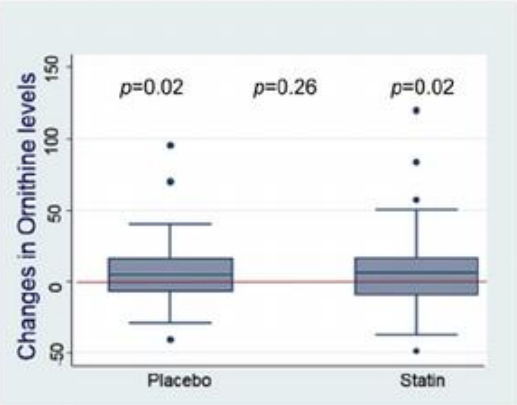


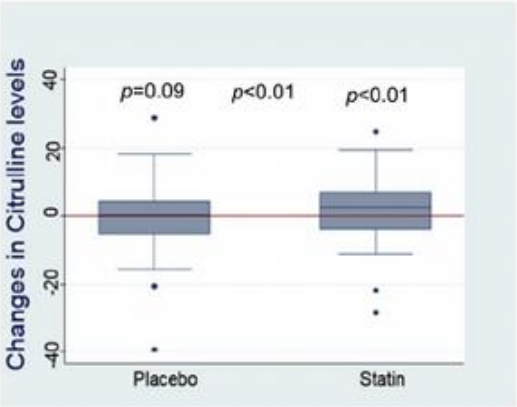

*(Fundferburg NT et al., Clin Infect Dis 2014)*

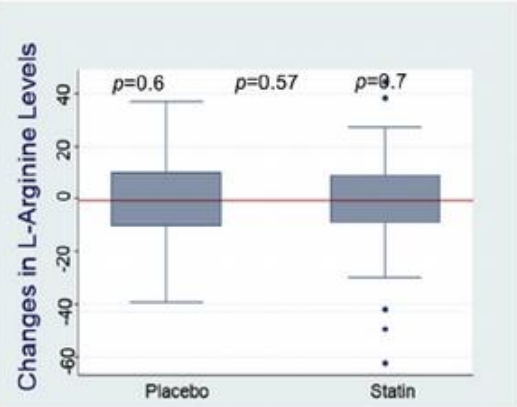
# Effect of statin on arginine metabolites in treated HIV-infection

- SATURN randomized, double-blind, controlled trial
- 147 HIV+ patients on suppressive cART with LDL <130 mg/dL
- Rosuvastatin (10 mg daily) or placebo for 12 months



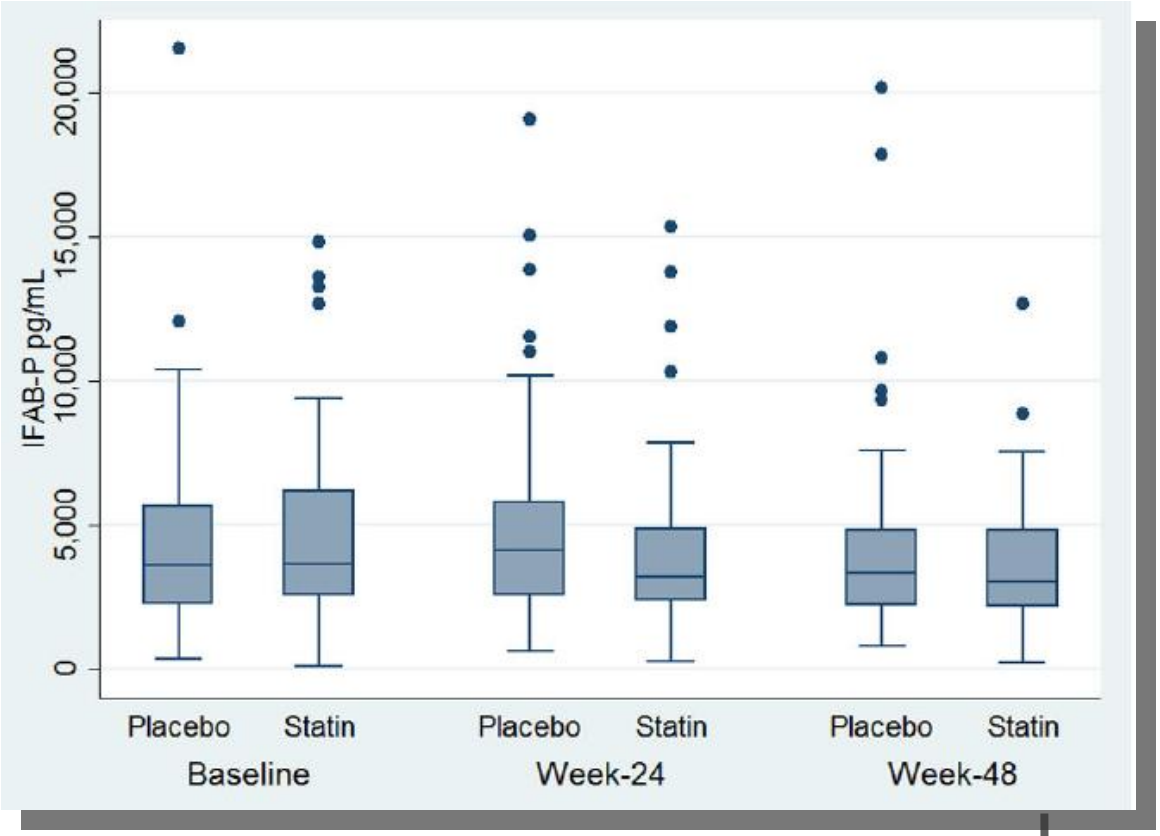




*(Diraj al-Fargo S et al., Atherosclerosis 2017)*

# Rosuvastatin Decreases Intestinal Fatty Acid Binding Protein (I-FABP), but Does Not Alter Zonulin or Lipopolysaccharide Binding Protein (LBP) Levels, in HIV-Infected Subjects on Antiretroviral Therapy

- SATURN randomized, double-blind, controlled trial
- 147 HIV+ patients on suppressive cART with LDL <130 mg/dL
- Rosuvastatin (10 mg daily) or placebo for 12 months




*(Funderburg NT et al., Pathog Immun 2016)*

# Effects of statin therapy on coronary artery plaque volume and high-risk plaque morphology in HIV-infected patients with subclinical atherosclerosis: a randomised, double-blind, placebo-controlled trial

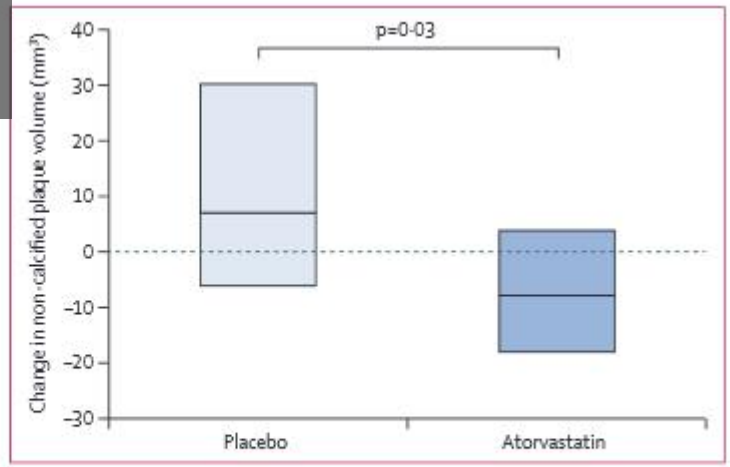



*Figure 2:* **Comparison of the 1 year change in non-calcified plaque volume in study participants**

See Online for appendix

Median and IQR.

- Randomised, controlled, double-blind study
- 40 HIV+ patients with subclinical atherosclerosis and normal LDL-c
- Atorvastatin vs placebo
- 1-year follow-up

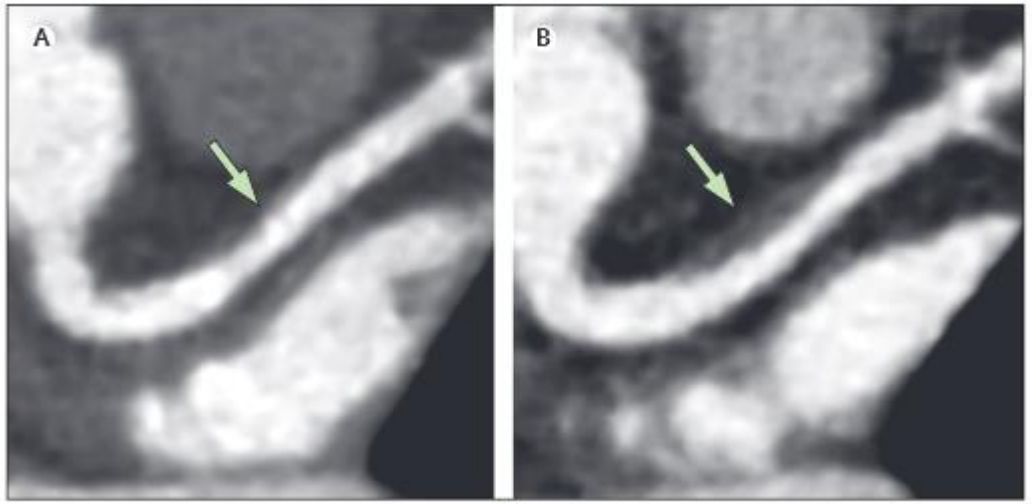
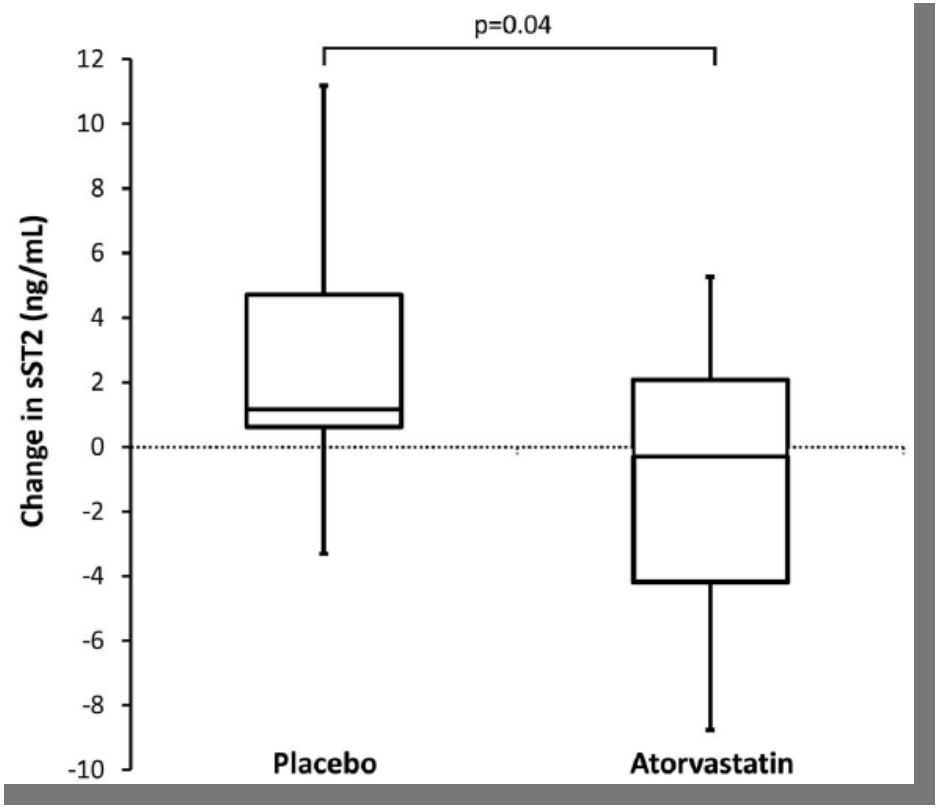



*Figure 3:* **Increasing non-calcified plaque in proximal left anterior descending (LAD) coronary artery in patient on placebo**

*(Lo J et al., Lancet HIV 2015)*

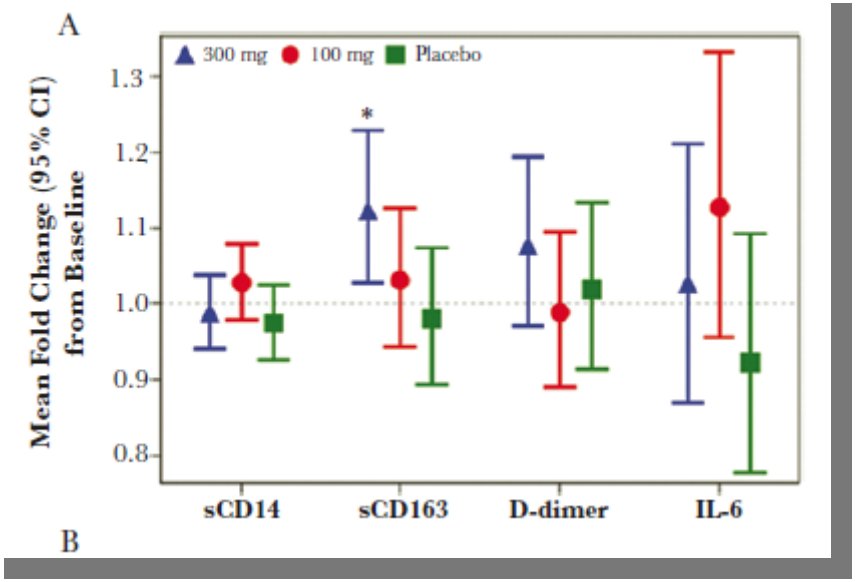
# Statin Effects on Myocardial Fibrosis Markers in People Living With HIV

- Double-blind, randomized, placebo--controlled trial
- 40 HIV-positive patients on suppressive cART
- Atorvastatin 40 mg daily vs placebo
- 12-month follow-up

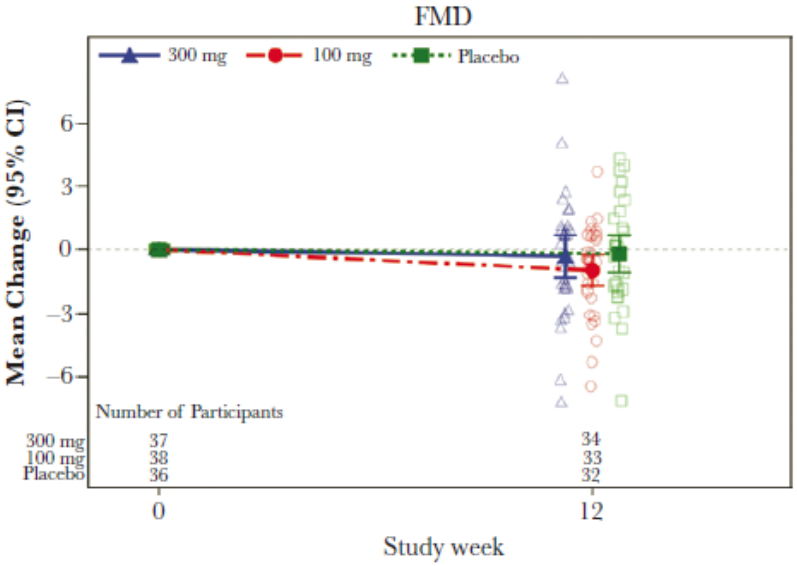



*(deFilippi C et al., J Acquir Immune Defic Syndr 2018)*

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

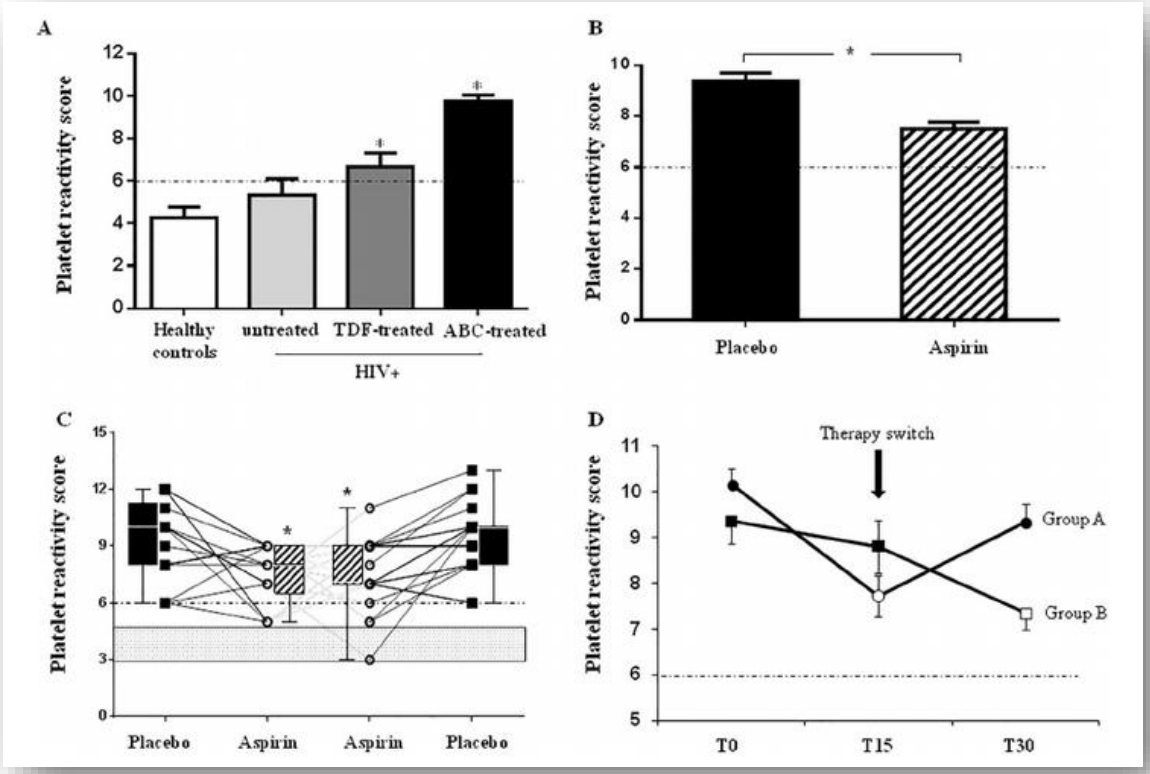



- Randomized, double-blind, controlled trial
- 121 HIV-infected patients on suppressive cART for >48 weeks
- 12-week treatment with aspirin 100 mg qd, 300 mg qd or placebo
- Evaluation of soluble markers and brachial artery flow-mediated dilation (FMD)




*(O'Brien MP et al., Open Forum Infect Dis 2017)*

# Effect of aspirin treatment on abacavir-associated platelet hyperreactivity in HIV-infected patients




- Randomized, placebo-controlled, cross-over trial
- 40 HIV-infected patients with ABC-associated platelet hyperreactivity
- Aspirin 100 mg daily vs placebo for 15 days with subsequent cross-over

*(Falcinelli E et al., Int J Cardiol 2018)*

# Inflammation in HIV infection: conclusions

- Unique, higher than expected and stable over time
- Multiple pathological mechanisms
- Crucial role of intestinal wall abnormalities with altered microbiome and bacterial translocation
- Multiple effects of the combination antiretroviral therapy
- Inability to fully suppress the inflammation
- Anti-inflammatory and pleiotropic effects of statins could be useful
- Novel therapeutic approaches targeting inflammation should be evaluated