

In vitro Modeling of the Therapeutic Impact of Statins and ApoA-I mimetics on Atherogenesis in Chronic Treated HIV (018)

In vitro Modeling of the Impact of TLR4-LOX-1 cellular signalling in Atherogenesis in Chronic Treated HIV (P29)

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21st International Workshop on
Co-morbidities and Adverse
Drug Reactions in HIV

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Drug Reactions in HIV Basel, Switzerland, 5–6 November 2019

Disclosures

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Background (1)

Dissecting pathogenesis of atherosclerotic CVD in chronic treated HIV

Observational Human studies:

Clinical databases
Surrogate measures of atherosclerosis
Plasma: biomarkers
PBMCs: Immunological studies



Animal models

Mechanistic human studies

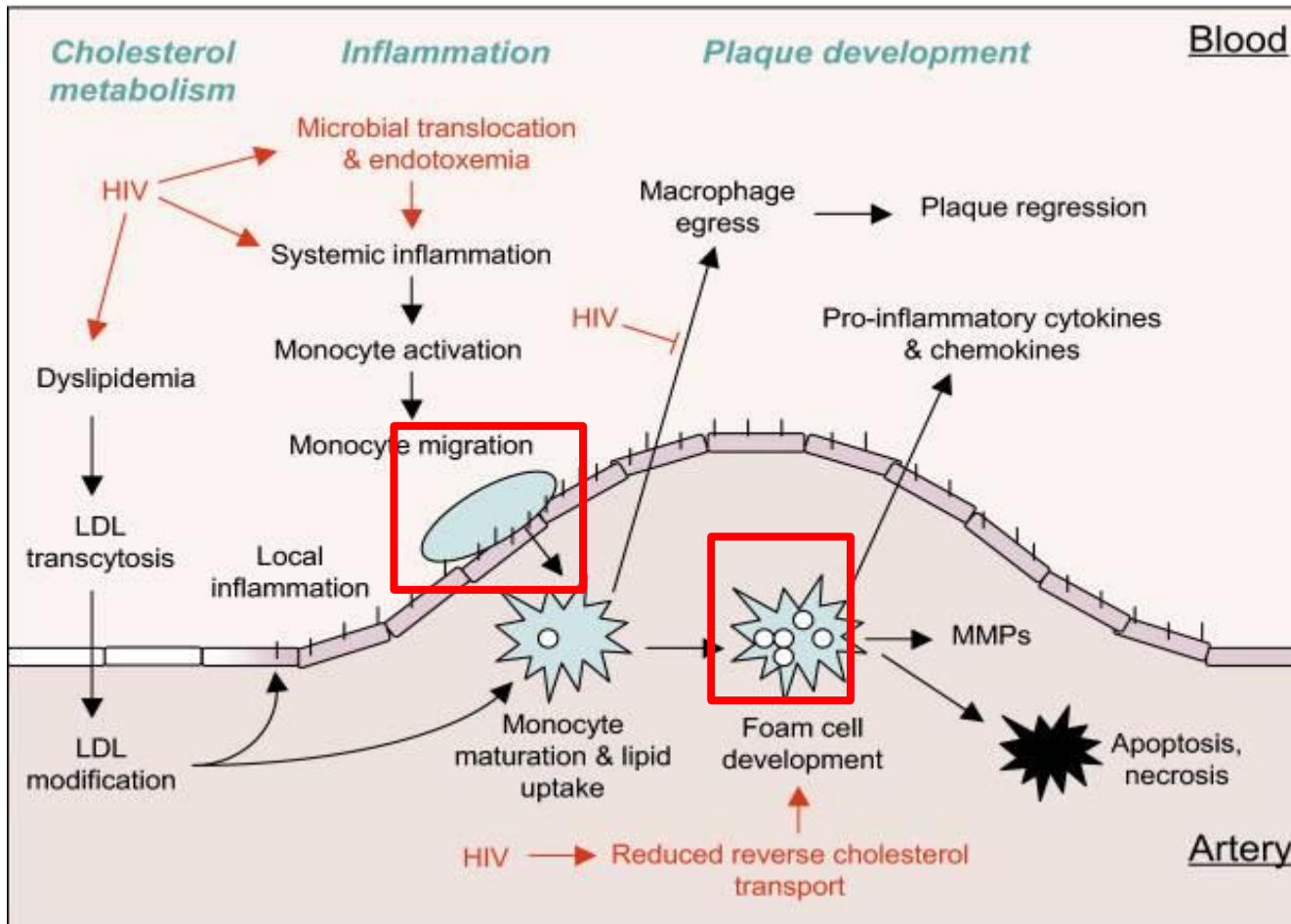
Clinical trials and determination of molecular signatures of disease in response to specific intervention

Mechanistic human studies

Ex vivo models of atherosclerosis

Background (2)

The macrophage: the intersection between HIV infection and atherosclerosis.



sCD163

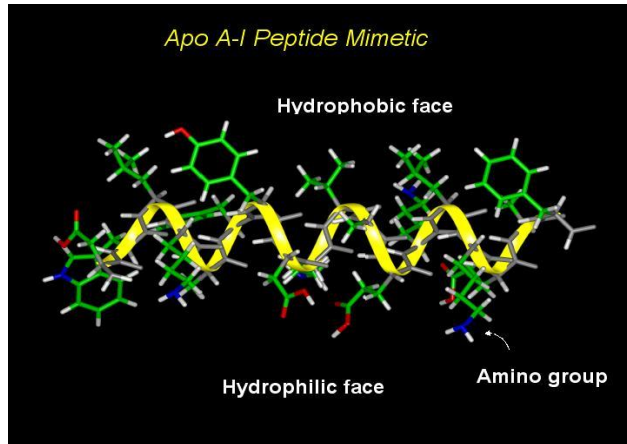
sCD14

CD14+CD16+

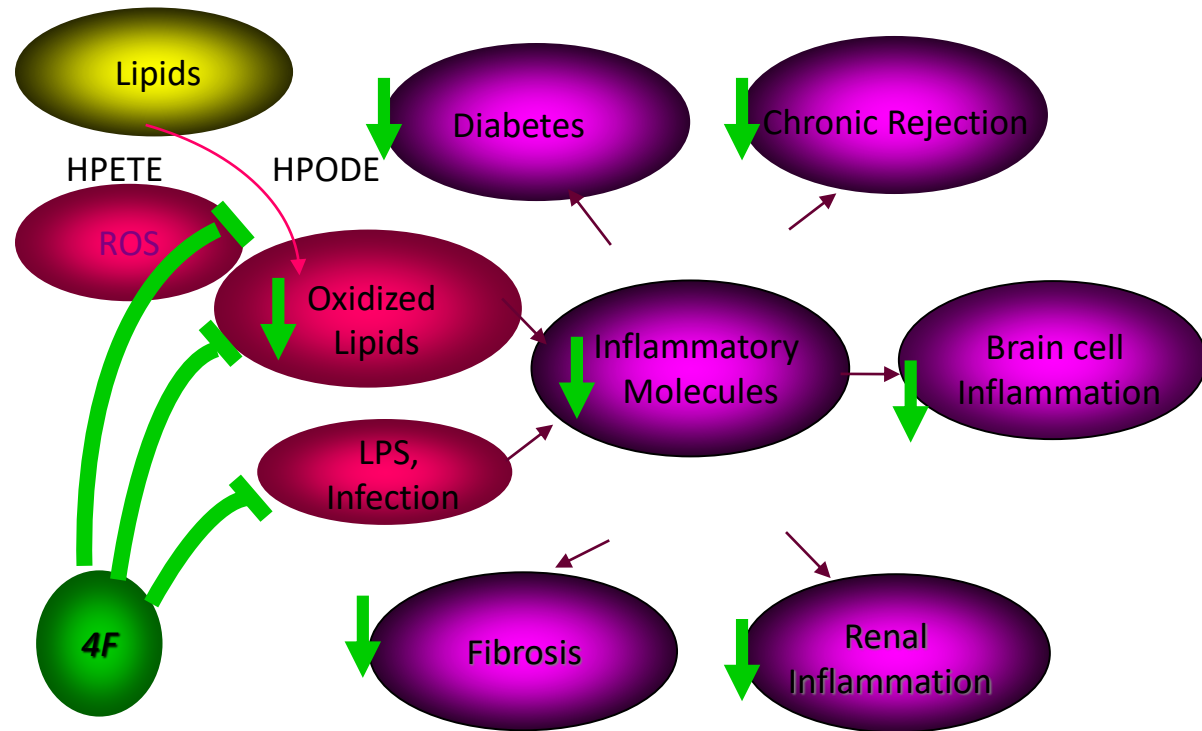
Background (3) ApoA-1 mimetic peptides.

4F Ac- D-W- F-K-A-F-Y-D-K-V-F-E-K-L-K-E-A-F-NH₂

6F Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-NH₂



4F affinity for oxidized lipids is 100 000x that of apoA-I!

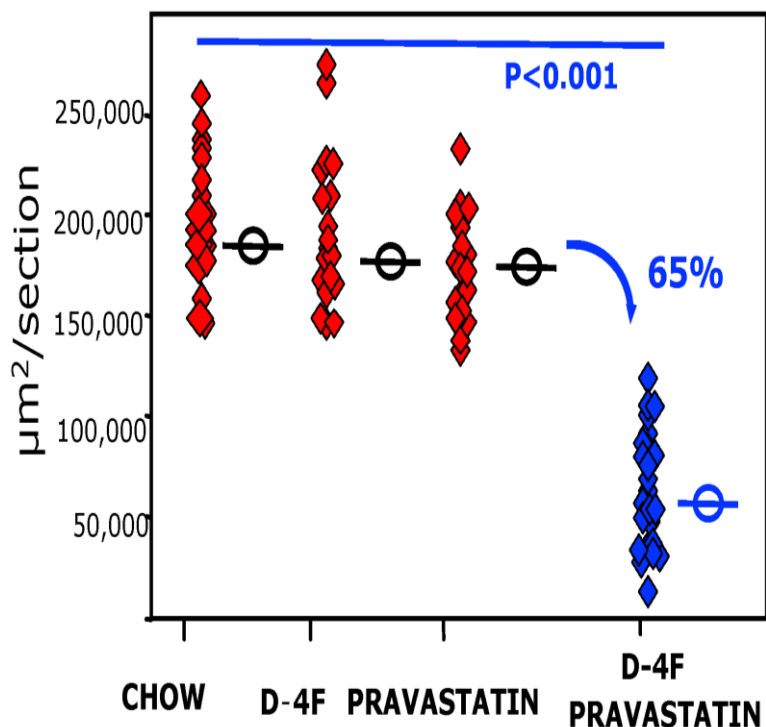


Background (4) Statins synergize with apoA-I mimetics in vivo to attenuate atherosclerotic CVD

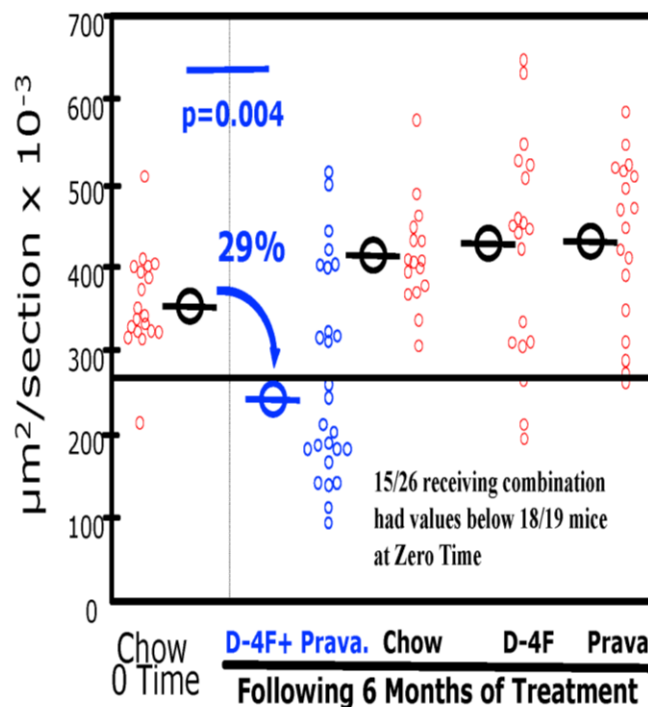
D-4F and Statins Synergize to Render HDL Antiinflammatory in Mice and Monkeys and Cause Lesion Regression in Old Apolipoprotein E-Null Mice

Mohamad Navab, G.M. Anantharamaiah, Susan Hama, Greg Hough, Srinivasa T. Reddy, Joy S. Frank, David W. Garber, Shaila Handattu, Alan M. Fogelman

PREVENTION



REVERSAL

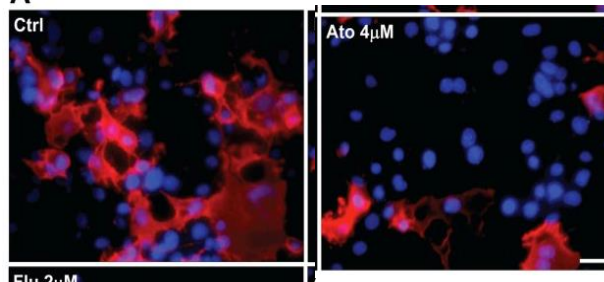
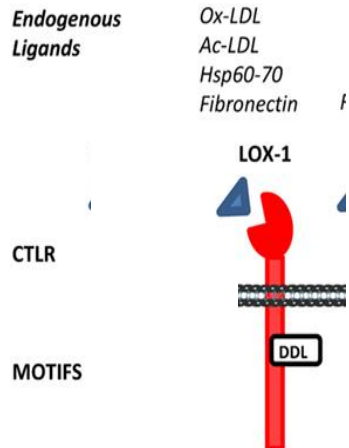


Background (5) TLR4 and LOX-1 are therapeutic targets for statins and also have key roles in atherogenesis

Cell Cycle 14:10, 1583-1595; May 15, 2015; © 2015 Taylor & Francis Group, LLC

Molecular mechanism of statin-mediated LOX-1 inhibition

Silvia Biocca^{1,*}, Federico Iacovelli², Sara Matarazzo¹, Giulia Vindigni¹, Francesco Oteri³, Alessandro Desideri², and Mattia Falconi^{2,*}



[Pharmacogenet Genomics](#). 2008

Sep;18(9):803-13.

Statins inhibit toll-like receptor 4-mediated lipopolysaccharide signaling and cytokine expression.

[Hodgkinson CP](#)¹, [Ye S](#).

[Mol Neurobiol](#). 2015 Apr;51(2):623-32.

Lectin-like ox-LDL receptor-1 (LOX-1)-Toll-like receptor 4 (TLR4) interaction and autophagy in CATH.a differentiated cells exposed to angiotensin II.

[Ding Z](#)¹, [Liu S](#), [Wang X](#), [Khaidakov M](#), [Dai Y](#), [Deng X](#), [Fan Y](#), [Xiang D](#), [Mehta JL](#).

PMID: [25950192](#), [24902807](#)

Experimental Design

Healthy donors
(18-40 yo)

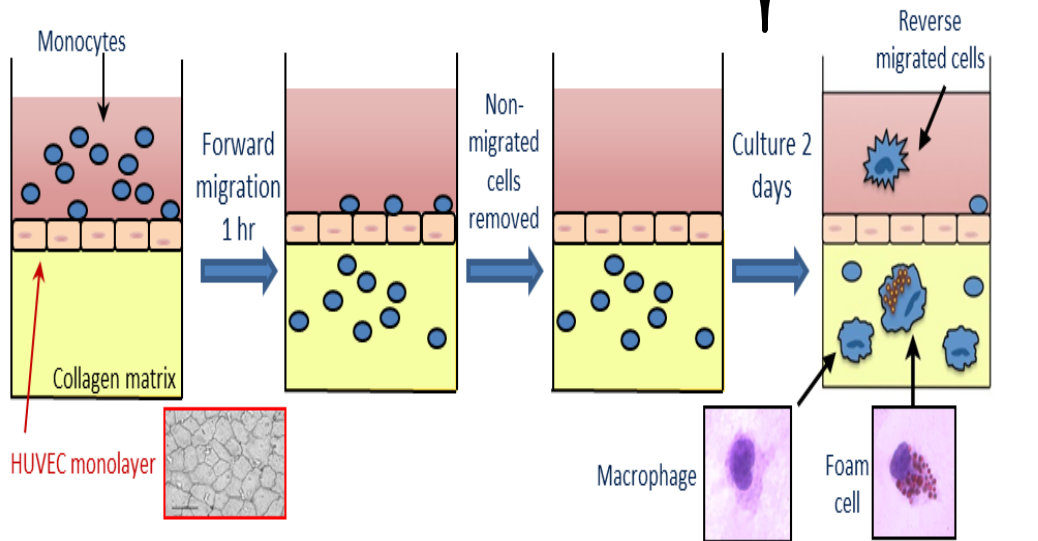
PBMC

Pooled Plasma
(HIV-)

Pooled Plasma
(HIV+)

HIV+ participants

- 40-60 years old
- 10 males
- Suppressed viremia (<50 copies/ml)
- All on identical ART: elvitegravir / cobicistat / emtricitabine / tenofovir alafenamide



Atorvastatin 50 μ M (1X) and
500 μ M (10X)

4F 10 μ g/ml (1X) and 100
 μ g/ml (10X)

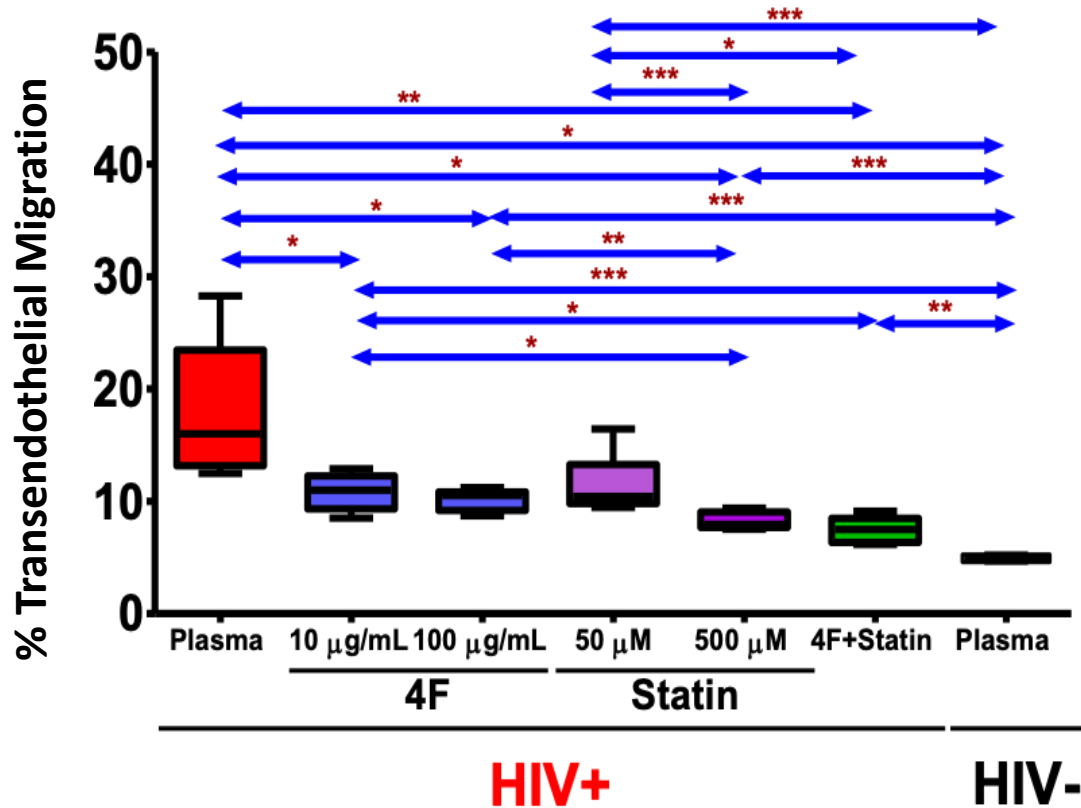
Transendothelial migration (TEM)

- % of cells that migrated through endothelium

Flow cytometry

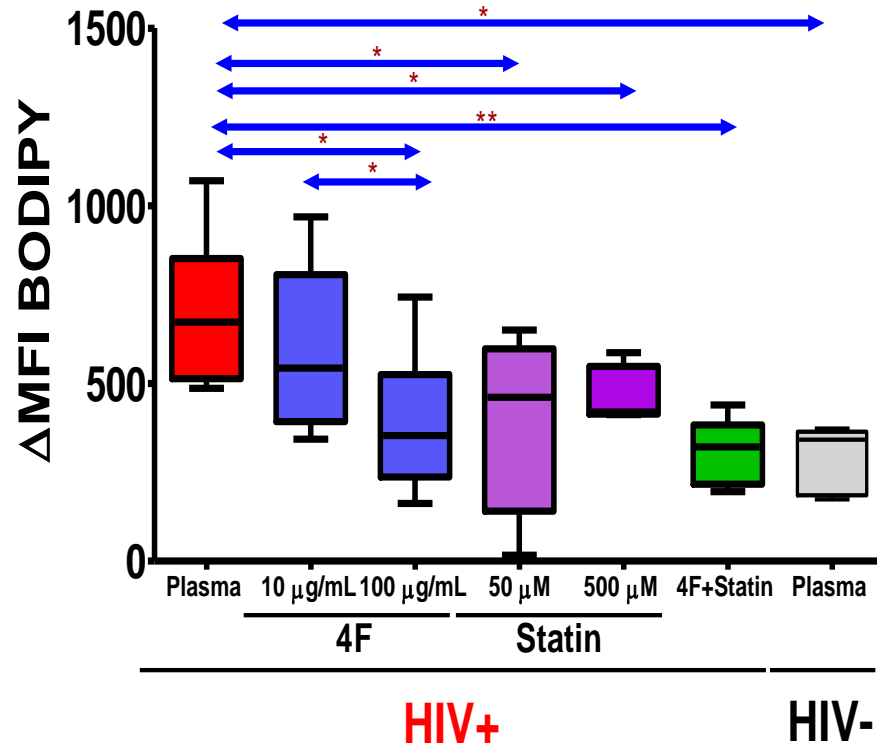
- **MDFCF** (Δ MFI BODIPY of CD33+ macrophages inside the gel: fluorescence intensity of BODIPY compared to negative staining control)
- Cellular levels of TLR4 and LOX-1 in CD33+ myeloid cells.

Results (1) Atorvastatin and 4F ApoA-I mimetic peptide attenuate proatherogenic properties (monocyte chemotaxis) of HIV plasma



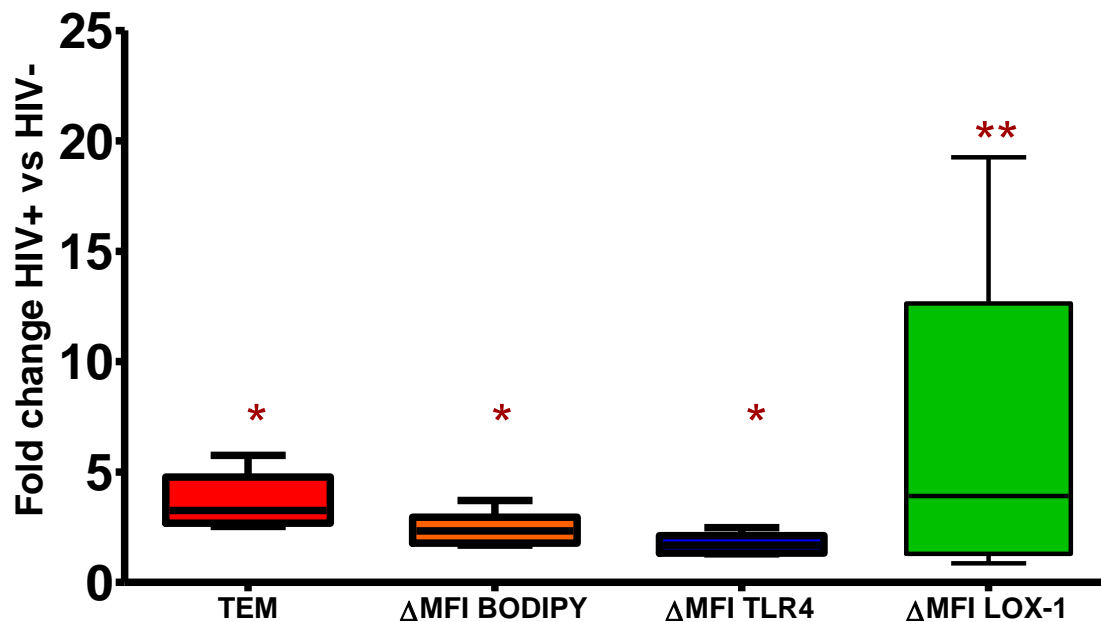
- When media-containing HIV+ compared to HIV- plasma was added to HUVECs, a significantly increased proportion of monocytes underwent transendothelial migration (TEM)(median migrated cells 16 vs. 4.9%, respectively)
- In the presence of HIV plasma, both atorvastatin (at both 50 and 500 µM) and 4F (at both 10 and 100 µg/ml) attenuated TEM of M/M (p<0.05 for all comparisons).
- **The combined treatment with 4F (10 µg/ml) and atorvastatin (50 µM) reduced TEM compared to 50 µM atorvastatin and 10 µg/ml 4F alone (p<0.05).**

Results (2) Atorvastatin and 4F ApoA-I mimetic peptide attenuate proatherogenic properties (Monocyte derived foam cell formation) of HIV plasma



- When media-containing HIV+ compared to HIV- plasma was added to HUVECs, a significantly increased proportion of CD33+ macrophages inside the collagen gel had increased lipid content per cell (median Δ MFI BODIPY 673 vs. 342, respectively)($P < 0.05$)
- In the presence of HIV-plasma, both atorvastatin (at both 50 and 500 μ M) and 4F (at both 10 and 100 μ g/ml) attenuated MDFCF ($p < 0.05$ for all comparisons).
- **There was a trend that the combined treatment with 4F (10 μ g/ml) and atorvastatin (50 μ M) reduced MDFCF compared to 10 μ g/ml 4F alone ($p = 0.08$).**

Results (3) The Pattern Recognition Receptors TLR4 and LOX-1 are upregulated in monocytes from healthy donors during ex vivo atherogenesis induced by HIV plasma



Compared to HIV-plasma, HIV+ plasma induced

- a mean 3.6-fold increase in TEM,
- a mean 2.4-fold in MDSCF,
- a mean 1.75 fold increase in TLR4
- a mean 8.3-fold increase in LOX-1 in CD33+ macrophages inside the collagen gel (p<0.05 for all paired comparisons)

Conclusions

- HIV-plasma from patients on potent ART with no clinical CVD directly induces key mechanisms of early atherogenesis (TEM and MDFCF) in parallel to increases in membrane protein levels of TLR4 and LOX-1 in macrophages within the collagen gel (ex vivo model of arterial wall).
- Both atorvastatin and 4F attenuated the *ex vivo* proatherogenic effects of HIV-plasma.
- Atorvastatin and 4F had additive antiatherogenic ex vivo effects (TEM, MDFCF).
- The combination of statins and oral apoA-I mimetics can be a novel therapeutic strategy for atherosclerosis in chronic treated HIV and needs to be further validated *in vivo*.
- The role of the TLR4-LOX-1 axis in atherosclerosis in chronic treated HIV needs to be further studied *in vivo*.

Acknowledgements



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CALIFORNIA HIV/AIDS
RESEARCH PROGRAM



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on Aging



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and Blood Institute



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University of California Los Angeles