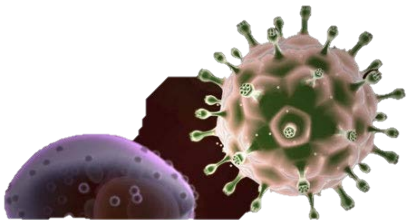


Integrated HIV-1 DNA load during stably suppressive ART is associated with the frequency of CD8 cells expressing HLA-DR/DP/DQ

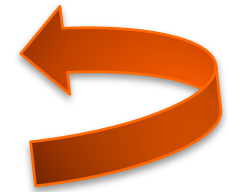
Ruggiero A¹, De Spiegelaere W², Cozzi-Lepri A³, Kiselina M², Pollakis G¹, Beloukas A¹, Mackie N⁴, Vandekerckhove L², Phillips A³, Geretti AM¹;
ERAS Study Group

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During long-term suppressive ART

- Stable integrated HIV-1 DNA load in CD4 T-cells
- Persistent detection of HIV-1 RNA in plasma
- Immune dysfunction
- Chronic inflammation
- Gut damage and bacterial translocation



Study Aim

To investigate factors associated with integrated HIV-1 DNA load in patients receiving first-line NNRTI-based ART with consistent VL <50 cps



Study Population

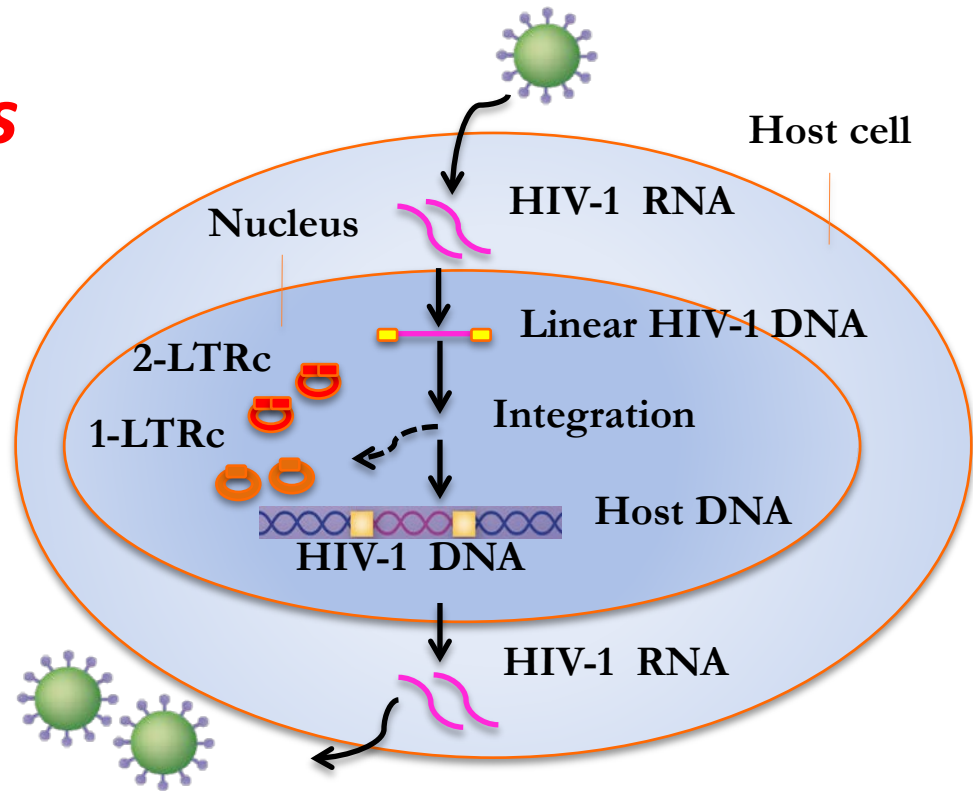
- ✓ 50 patients on first-line ART
- ✓ 2 NRTIs + EFV or NVP
- ✓ VL <50 cps within 6 months of starting ART
- ✓ Continuous VL suppression <50 cps for up to 14 yrs
- ✓ No blips, no treatment interruption
- ✓ No change of initial NNRTI
- ✓ Change of initial NRTI allowed (e.g., for toxicity)



Measured parameters

Virological

- Residual plasma HIV-1 RNA (rVL)*
- Total, integrated, and 2-LTRc DNA in PBMC



Immunological

- CD4 + CD26, CD38, or CD69 on fresh PBMC
- CD8 + CD38, or HLA-DR/DP/DQ on fresh PBMC
- sCD14 in plasma

***50% & 95% detection thresholds 1 & 3 HIV-1 RNA cps/ml**

Characteristics

| | All | Suppressive ART (yrs) | | P |
|---|---------------|-----------------------|---------------|--------|
| | | <6.4 | >6.4 | |
| Total no | 50 | 25 | 25 | - |
| Male gender n (%) | 40 (80) | 22 (88) | 18 (72) | 0.28 |
| Age yrs* | 46 (40-53) | 44 (36-49) | 48 (43-55) | 0.04 |
| Nadir CD4 count* | 206 (110-265) | 246 (160-292) | 176 (97-213) | 0.007 |
| Current CD4 count* | 572 (478-734) | 552 (442-606) | 640 (502-794) | 0.05 |
| Pre-ART VL log₁₀ cps* | 5.0 (4.7-5.5) | 4.8 (4.5-5.3) | 5.1 (4.9-5.6) | 0.09 |
| EFV n (%) | 40 (80) | 22 (44) | 18 (36) | 0.29 |
| Changed NRTI n (%) | 25 (50) | 4 (16) | 21 (84) | <0.001 |

*Median (IQR)



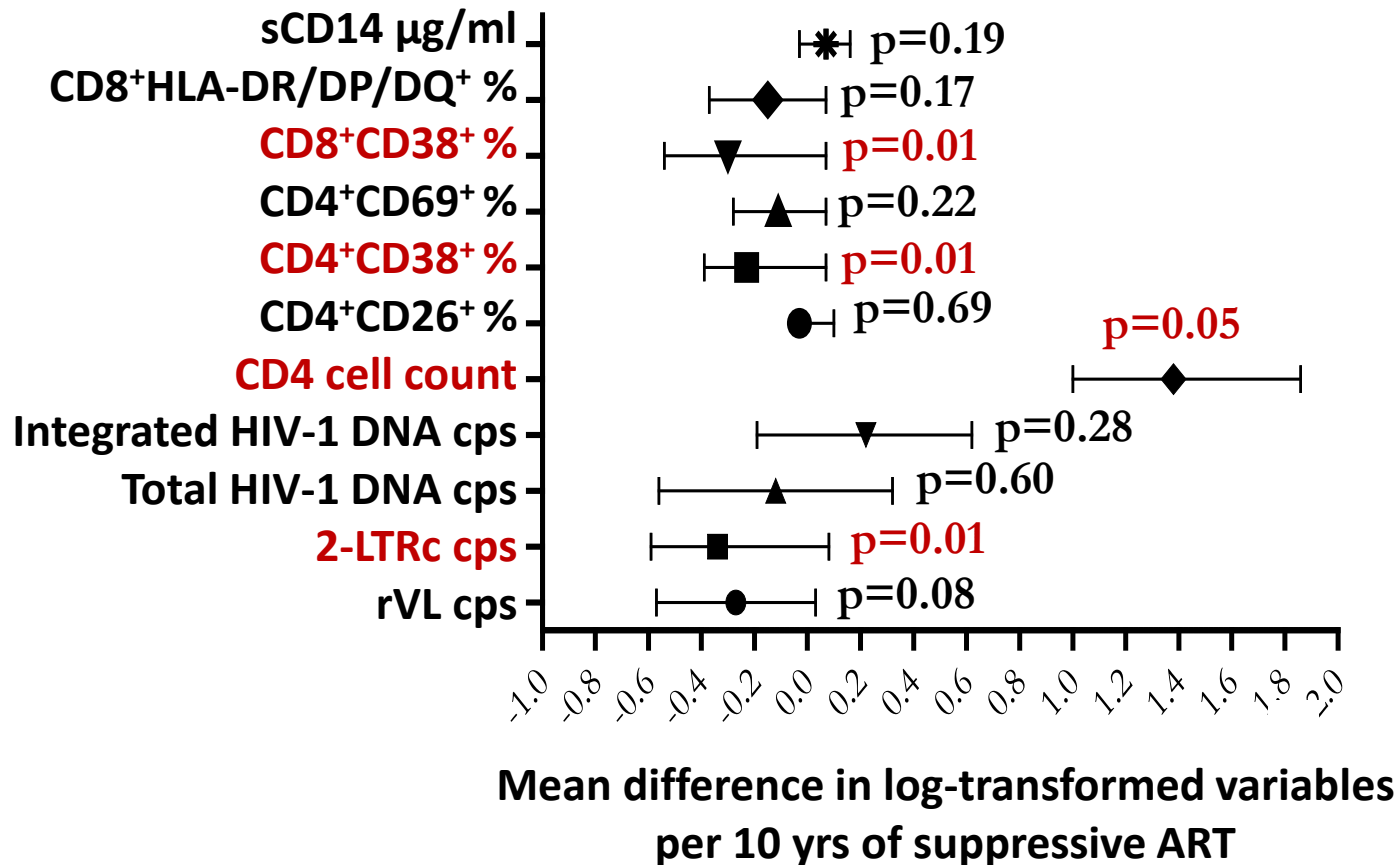
Characteristics

| | All | Suppressive ART (yrs) | | P |
|---|---------------|-----------------------|---------------|------|
| | | <6.4 | >6.4 | |
| Total no | 50 | 25 | 25 | - |
| rVL+ n (%) | 29 (58) | 15 (60) | 14 (56) | 1.00 |
| rVL cps* | 2 (2-4) | 2 (2-7) | 2 (2-4) | 0.33 |
| 2-LTRc+ n (%) | 16 (32) | 10 (40) | 6 (25) | 0.36 |
| 2-LTRc cps/10⁶ PBMC* | 3 (3-7) | 3 (3-7) | 3 (3-3) | 0.28 |
| Total HIV-1 DNA log₁₀ cps/10⁶ PBMC* | 2.6 (2.3-2.9) | 2.6 (2.4-2.9) | 2.5 (2.2-2.7) | 0.36 |
| Integrated HIV-1 DNA log₁₀ cps/10⁶ PBMC* | 1.9 (1.7-2.2) | 1.9 (1.7-2.1) | 1.8 (1.7-2.4) | 0.91 |

*Median (IQR)



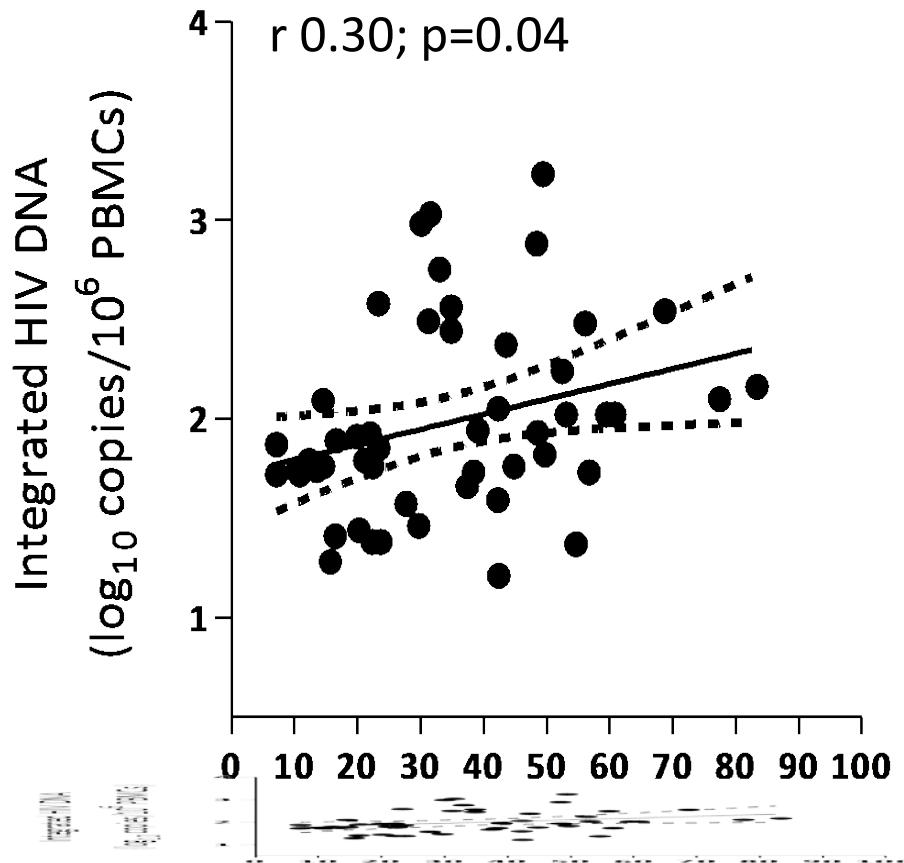
Effect of duration of suppressive ART on measured parameters



Subjects with highest and lowest Integrated HIV-1 DNA load

| Characteristic | Integrated HIV-1 DNA quartile | | P |
|--|-------------------------------|----------------------|-------------|
| | Lowest (n=13) | Highest (n=13) | |
| Nadir CD4 count* | 180 (80-240) | 208 (118-260) | 0.41 |
| Changed NRTI backbone, n (%) | 8 (61) | 5 (38) | 0.43 |
| Pre-ART VL log ₁₀ cps* | 4.9 (4.6-5.5) | 5.1 (5.0-5.5) | 0.26 |
| rVL cps* | 2 (2-3) | 2 (2-5) | 0.89 |
| 2-LTRc cps* | 3 (3-7) | 3 (3-7) | 0.65 |
| CD4 count | 721 (552-758) | 513 (482-640) | 0.25 |
| CD4 ⁺ CD38 ⁺ % | 23 (17-34) | 20 (16-24) | 0.28 |
| CD8 ⁺ CD38 ⁺ % | 4 (3-7) | 3 (2-7) | 0.43 |
| CD8⁺HLA-DR/DP/DQ⁺ % | 24 (16-37) | 35 (32-49) | 0.01 |
| sCD14 µg/ml | 1.8 (1.6-2.3) | 2.4 (2.1-2.7) | 0.04 |

Integrated HIV-1 DNA load positively correlated with frequency of CD8⁺HLA-DR/DP/DQ⁺ cells



No correlation with

- rVL
- 2-LTRc
- CD4⁺CD38⁺
- CD8⁺CD38⁺ %



Integrated HIV-1 DNA load positively correlated with frequency of CD8⁺HLA-DR/DP/DQ⁺ cells

| Factor | Univariate | | Multivariable | |
|--|--------------------------|-------------|--------------------------|-------------|
| | Mean difference (95% CI) | P | Mean difference (95% CI) | P |
| VL <50 cps (10 yrs →) | 0.22 (-0.19, 0.62) | 0.28 | 0.23 (-0.20, 0.66) | 0.30 |
| Pre-ART VL (log ₁₀ cps ↑) | 0.10 (-0.08, 0.28) | 0.27 | 0.13 (-0.05, 0.30) | 0.15 |
| rVL (log ₁₀ cps ↑) | 0.20 (-0.17, 0.57) | 0.28 | 0.27 (-0.08, 0.62) | 0.13 |
| CD4 (100 cells ↑) | 0.03 (-0.02, 0.08) | 0.25 | 0.02 (-0.03, 0.07) | 0.38 |
| CD8⁺HLA-DR/DP/DQ⁺ (50↑) | 0.38 (0.02, 0.74) | 0.04 | 0.51 (0.15, 0.86) | 0.01 |
| sCD14 (log ₁₀ μg/ml ↑) | 0.97 (-0.14, 2.08) | 0.09 | 0.90 (-0.16, 1.97) | 0.10 |

Summary

- ❖ Integrated HIV-1 DNA load and % CD8⁺HLA-DR/DP/DQ⁺ cells stable during long-term suppressive ART and positively associated
- ❖ Higher integrated HIV-1 DNA load associated with higher sCD14 levels in unadjusted analyses
- ❖ Integrated HIV-1 DNA load not correlated with measures of recent/ongoing HIV-1 replication (rVL, 2-LTRc, % CD4⁺CD38⁺ and CD8⁺ CD38⁺ cells)



CD8⁺ CD38⁻ HLA-DR⁺ cells

- ❖ May have regulatory and effector functions
- ❖ Preferentially generated in response to low antigenic stimulation
- ❖ May play a key role in controlling HIV and other infections
- ❖ In the context of ART, expansion may be triggered by low-level HIV expression (with or without virus production), other persistent pathogens, or microbial translocation from the gut



Three main hypothesis

1. Low-level HIV production stimulates CD8⁺HLA-DR/DP/DQ⁺ cells and continuously replenishes the integrated reservoir
2. CD8⁺HLA-DR/DP/DQ⁺ cells directly stimulate activation and proliferation of HIV-infected cells, causing expansion of the reservoir (+/- virus production)
3. A stimulant / multiple stimulants act simultaneously on CD8⁺HLA-DR/DP/DQ⁺ cells and the reservoir, causing an indirect association between the two parameters



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