Estrogen blocks HIV re-emergence from latency and points to gender-specific differences in HIV reservoirs

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Gender-specific differences affecting reactivation of latent HIV
Scully, Deeks, Chomont, Gandhi, Bacchetti, Johnston & Karn

- Aim 1: To determine if there are differences in HIV-1 reservoir size, potential for reactivation and residual inflammation between fully suppressed men and women.

- Aim 2: To address how to appropriately include women in studies of cure and eradication strategies

- Enrollment is focused on women of reproductive age who are not taking any exogenous hormone therapy including oral contraceptives.

- Leukapacks from 10 matched men and women selected from larger study of 100 patients
Induction of HIV transcription in clones of latently infected T-cells is strictly dependent upon NF-κB

A. Fluorescent Microscopy

- TNF-α

+ TNF-α

DAPI/Actin  d2EGFP

B. Flow cytometry

% of Max

Unstimulated  TNF-α

d2EGFP

10^0  10^1  10^2  10^3
ESR1 is a central mediator of HIV latency in Jurkat T-cells.
Primary cell models: Reporter and polarization conditions

A

HIV-1 Reporter Virus

CD8a-d2EGFP-IRES-Nef

Rev

Env

Tat

Vpu

Rev

LTR

Tat (H13L)

5’

Δgag

3’

B

Polarization and Infection

Activation/Polarization of Naive CD4+ T-cells

Infection of Effector Cells

Isolate CD8a+ Cells

Quiescent Cells

HIV Reactivation

Day 0

3

6 7

14

21

25

Polarization Cytokines

CD3/CD28 Beads

High IL-2 + IL-23

Low IL-2 + IL-23

C

Effector Cells

Quiescent Cells

Cyclin D3

Ki67

0 50 100 150 200

0 20 40 60 80

45.08% 17.65%

30.67% 6.57%

0.00% 98.36%

0.00% 1.62%

D

Unstimulated

TCR-stimulation

Unstimulated

Stimulated 0.71%

Unstimulated

Stimulated 84.83%

Unstimulated

95.64%

Unstimulated

12.27%
Blocking of ESR-1 and its upstream modulator SRC3 re-activates latent HIV-1 provirus in primary T-cells.
ESR-1 antagonists re-activate latent HIV-1 provirus while ESR-1 agonists inhibit HIV-1 transcription
Envelop mRNA Detection by Ion Torrent Sequencing (EDITS) assay for patient cells

A. Primer sets for the detection of singly spliced env mRNA

- **env mRNA**
- **Fwd**
- **ssREV**
- **n-ssFwd**
- **n-ssRev**

B. RNA induction assay

C. RNA induction by different LRAs
EDITS assays demonstrate that estradiol is a potent inhibitor of proviral reactivation.
Estradiol inhibition of proviral reactivation is gender-specific
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Conclusions

• ESR antagonists are well established drugs and can be considered as components of clinical studies aimed at inducing proviral reactivation.

• Estradiol at peak menstrual cycle levels is a potent inhibitor of viral reactivation suggesting important differences between men and women for viral replication and reservoir sizes.

• The design of regimens for proviral reactivation needs to account for estrogen, and perhaps other hormones, as confounding factors affecting potency.
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