Barriers to Cure HIV in Women

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17th Residential Course on Clinical Pharmacology of Antiretrovirals,

January 19-21, 2022

Overview

- History of inclusion/exclusion of women
- Epidemiology of HIV in Women
- Sex-differences in Natural History
- Sex-differences in Immune Response
- HIV Latency and Transcriptional Activity
- Final Considerations and Clinical Relevance

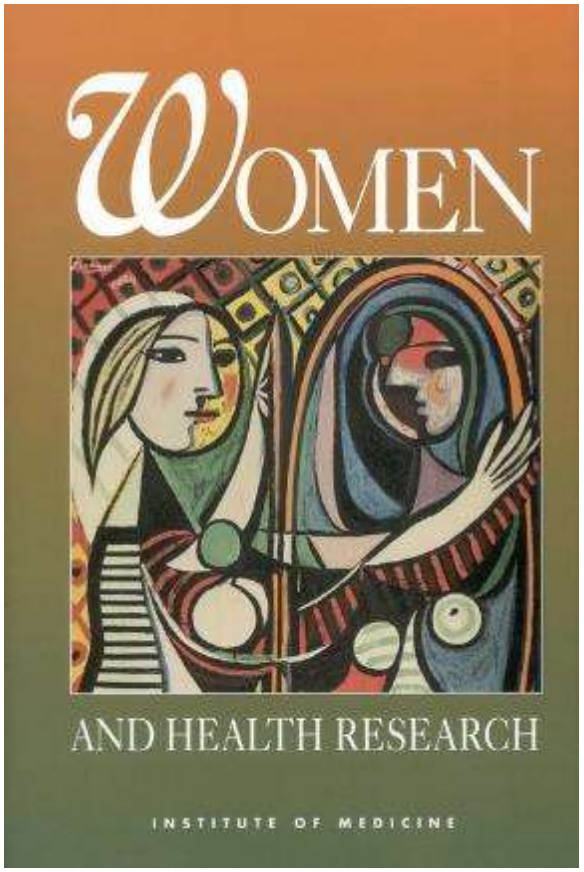
History of Inclusion/Exclusion

- Clinical research during the 1960's and 1970's
- Default: Women of reproductive age excluded
  - Reproductive toxicity
  - Variability in menstrual cycle
  - Concerns about women's autonomy
  - Sample size too large
- FDA 1977 guidelines: *"excluding women with childbearing potential from participating in phase 1 and early phase 2 clinical studies until reproductive toxicity studies were conducted and some evidence of effectiveness had become available."*
- **Most research conducted in males and extrapolated to women**

Slide adapted from Liza Dawson

Policy Updates

- October 1992 Committee to determine Ethical and Legal Issues Relating to the Inclusion of Women in Clinical Studies.
- NIH Revitalization Act of 1993.
  - Women and minorities must be included in clinical studies unless scientific rationale for exclusion.
- Institute of Medicine report 1994: Women and Health Research.
- **Increasing advocacy for more research for and with women.**



Slide adapted from Liza Dawson

Epidemiology of HIV

- Women account for >**50%** of people with HIV
  - In sub-Saharan Africa >60% of PWH are women
  - Young women are disproportionally affected →leading cause of death

- Most cure-related research and clinical trials
  - take place in countries where MSM represent the greatest proportion of the epidemic
  - **Clinical research focus on cisgender MSM**

Women and Men are Different

- Disease pathogenesis has sex specificity
  - Autoimmunity
  - Cancer
  - Infectious diseases
  - Vaccine responses

Klein and Flanagan, *Nat Rev Immunol* 16, 626-638. 2016,
Markle and Fish, *Trends Immunol* 35, 97-104, 2014.
Edgren, G., et al., Eur. J. Epidemiol.27, 187–196, 2012.
Klein et al., *Lancet Infect Dis* 10, 338-349, 2010.

	Pathogen load	Prevalence or Incidence	Severity
	Intensity		
Viruses			
HIV			
Influenza virus (avian H7N9)	N.D.		
Influenza virus (2009 H1N1)	N.D.		
MERS-CoV	N.D.		
Hepatitis B Virus			
Bacteria			
Mycobacterium tuberculosis	N.D.		
Legionella pneumophila	N.D.		
Campylobacter jejuni	N.D.		
Leptospira spp.	N.D.		
Parasites			
Plasmodium falciparum			
Toxoplasma gondii	N.D.		
Schistosoma mansoni			
Entamoeba histolytica	N.D.		
Fungi			
Paracoccidioides brasilliensis			
Aspergillosis fumigatus	N.D.		
Crptococcus neoformans	N.D.		

= **Male Bias**

= **No Observed Bias**

= **Female Bias**

N.D. = **Not Determined**

Vom Steeg and Klein, PLOS Pathogens, 2016

Natural History of Viral Load

The New England Journal of Medicine

INITIAL PLASMA HIV-1 RNA LEVELS AND PROGRESSION TO AIDS IN WOMEN AND MEN

TIMOTHY R. STERLING, M.D., DAVID VLAHOV, PH.D., JACQUIE ASTEMBORSKI, M.H.S., DONALD R. HOOVER, PH.D., M.P.H., JOSEPH B. MARGOLICK, M.D., PH.D., AND THOMAS C. QUINN, M.D.

TABLE 2. INITIAL PLASMA HIV-1 RNA LEVELS AND CD4+ LYMPHOCYTE COUNTS AFTER SEROCONVERSION.

VARIABLE	MEN	WOMEN	P VALUE*
Median plasma HIV-1 RNA level (copies/ml)	50,766	15,103	<0.001
Median CD4+ lymphocyte count (per mm^3)	659	672	0.48

*P values were determined with the Wilcoxon rank-sum test.

Farzadegan et al. **Lancet** 1998
Sterling et al. **JID** 1999
Prins et al. **AIDS** 1999
Sterling et al. **NEJM** 2001
Toulami et al. **AIDS** 2004
Gandhi et al. **CID** 2002
Meditz et al. **JID** 2011

Natural History of HIV –Disease Progression

Although the initial level of HIV RNA was lower in women than in men, the rates of progression to AIDS were similar. Treatment guidelines that are based on the viral load, rather than the $CD4^+$ lymphocyte count, will lead to differences in eligibility for ART according to sex (here: 74% versus 37%).

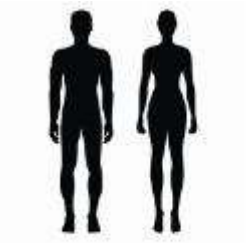
Sex based differences in HIV pathogenesis

- 13 studies evaluating differences in viral load
- Total participants assessed: 6702 men and 3894 women
- 7 of 9 cross sectional studies showed lower viral loads in women (0.13 to 0.35 $\log_{10}$ lower, approx. 2-fold lower)
- 4 longitudinal studies showed lower viral loads in women (.033-0.78 $\log_{10}$ lower, 2-6-fold reduction)
- Differences likely attenuate with disease progression, are not protective against immunologic decline

Gandhi et al, CID, 2002.

Possible Sex Differences

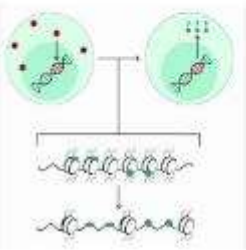
- Socioeconomic factors
- Less symptoms during Primary Infection
  - Less likely to be diagnosed and treated early
- More side effects from ART
  - More likely to interrupt treatment



Anatomic Differences:
-Acquisition sites: female genital tract versus rectal mucosa
-Hormonal modulation of risk at the female genital tract
-Drug penetration to mucosal sites

Genetic differences:
-Gene dosage effects of X chromosome encoded genes/ incomplete X inactivation
-Regulatory function of X-encoded microRNAs
-Estrogen responsive elements in promoters of multiple immune active genes

Immune cell phenotypes:
-Higher interferon alpha production from plasmacytoid dendritic cells from women
-Sex differences in the efficacy of vaccines
-Hormone modulation of immune cell function



Latency maintenance:
-Estrogen blockade of HIV transcriptional activation
-Sex specific epigenetic modifications in immune cells

Microbiome:
-Female genital tract and rectal mucosa with distinct microbiome compositions that determine local inflammation and acquisition risk
-Direct effects of the vaginal microbiome on local antiretroviral drug levels
-Sex hormone modulation of the gut microbiota that contributes to systemic inflammation

Scully, 2018

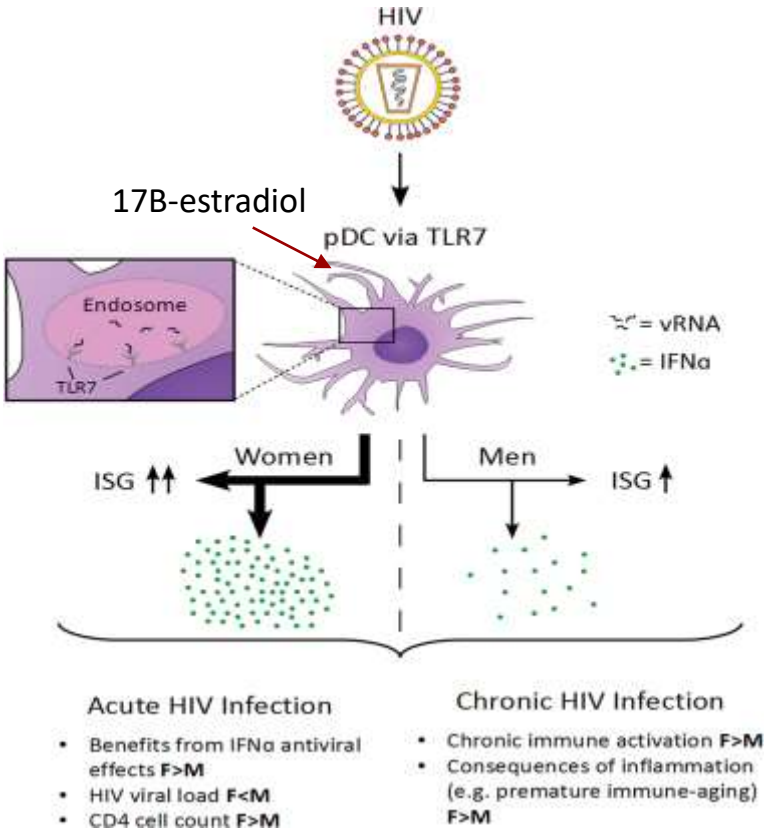
Immune Response

- Women have
  - More autoimmune diseases
  - More robust response to vaccines (increased antibody titers and Interferon response)

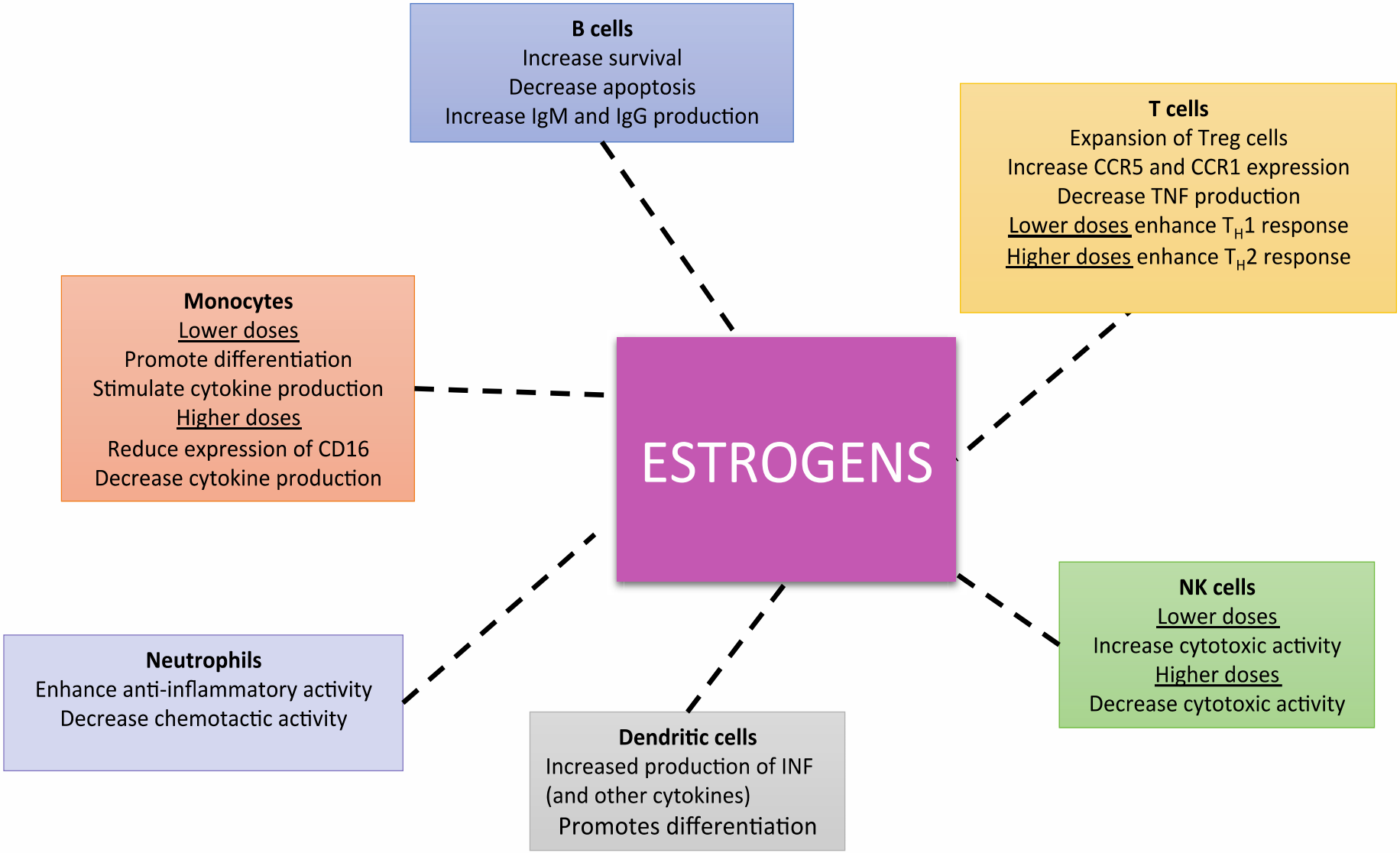
Potential Mechanisms of Sex Differences in HIV Infection

IFN responses appear to play a significant role, and are modulated by sex hormones

"IFN-α and ISG induction might explain some of the differences in the clinical manifestations of HIV infection between women and men, including the <u>better control</u> of HIV viremia by women during primary infection (as a consequence of the anti–HIV-1 effect of IFN-α) and the higher level of immune activation and <u>accelerated disease progression</u> in women during chronic infection, after adjustment for the level of viral replication"



Addo et al. **JID** 2014



Gianella et al, 2016, JIAS, Barriers to a cure for HIV in women

Progestin

- Receptors on various immune cells
- During pregnancy and luteal phase
  - → Immune suppressive
- During secretory phase
  - → Increase uterine immune cells

What about HIV Reservoirs?

- Impact **of hormones** on HIV persistence
  - Menstrual cycle, menopause, pregnancy, lactation
  - Hormonal contraceptive
- HIV Reservoir **size and distribution** in tissues
  - Anatomic (Adipose tissue, CNS, genital tract)
  - Metabolic
  - Pharmacokinetic

Sex Differences in HIV DNA Reservoir

- Fourati et al. J. **Antimicrob. Chemother.** 2014 and Cuzin et al. **AIDS** 2015:
  - Odds ratio of 2.3 and 1.5, respectively, for female sex association with low (<100 copies/million PBMCs) HIV DNA

- Scully et al. **JID** 2019:
  - No evident sex difference in total HIV DNA in CD4 T cells, (26 women and 26 men)

- Prodger et al. **JCI Insight 2020**
  - No evident sex difference in total HIV DNA in CD4 T cells, (n=57 women, n=33 men)

- Gandhi et al. **JID** 2020:
  - No evident sex difference for intact (IPDA) proviral levels or decay rates (n = 12 women, n = 32 men)

- Kirk et al. **JID** 2020
  - Female sex associated with lower intact (IPDA) proviral levels (n = 25 women, n = 66 men)

- Falcinelli et al **JID** 2020
  - No difference in the frequency of persistent HIV in resting CD4 T cells (qVOA, IPDA, total HIV DNA), (n=22 women, n=39 men)

- Gianella et al. **OFID** 2020
  - Premenopausal status was independently associated with lower HIV DNA compared with postmenopause (P<0.01)

Sex Differences in Inducible HIV Reservoir and HIV Transcription

- Prodger et al. **CID** 2017
  - Trend toward female sex association with lower replication competent provirus (QVOA) frequencies in Uganda but not US cohort

- Prodger et al. **JCI Insight** 2020
  - Lower replication competent provirus (QVOA) frequencies in women from Uganda cohort

- Scully et al. **JID** 2019
  - No significant differences in inducible HIV RNA levels in CD4 T cells from women vs. men (EDITS and TILDA)
  - Lower cellular cellular HIV RNA expression in women

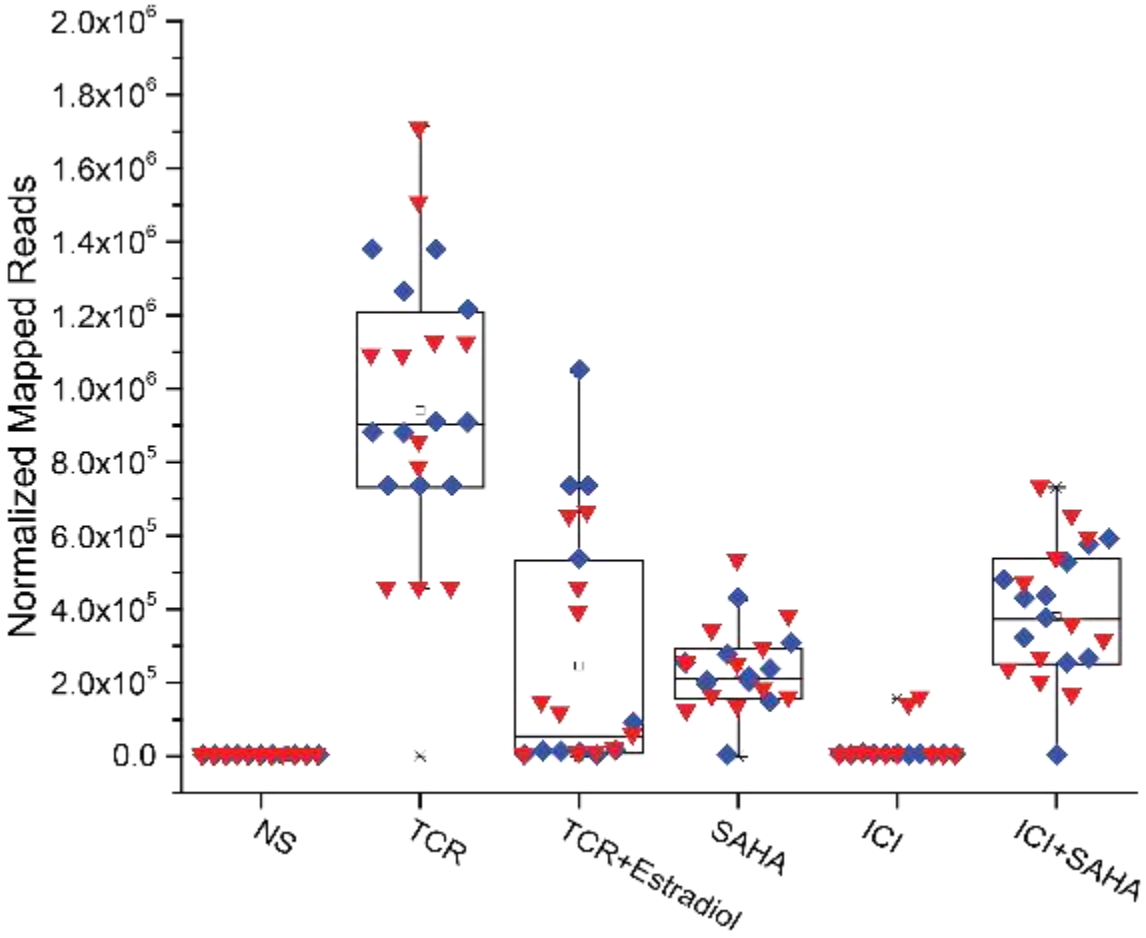
- Gianella et al. **OFID** 2020
  - Lower cellular HIV RNA expression in women

Estrogen receptor-1 is a key regulator of HIV-1 latency that imparts gender-specific restrictions on the latent reservoir

Biswajit Das[a,1], Curtis Dobrowolski[a,1], Benjamin Luttge[a], Saba Valadkhan[a], Nicolas Chomont[b,c], Rowena Johnston[d], Peter Bacchetti[e], Rebecca Hoh[f], Monica Gandhi[f], Steven G. Deeks[f], Eileen Scully[g], and Jonathan Karn[a,2]

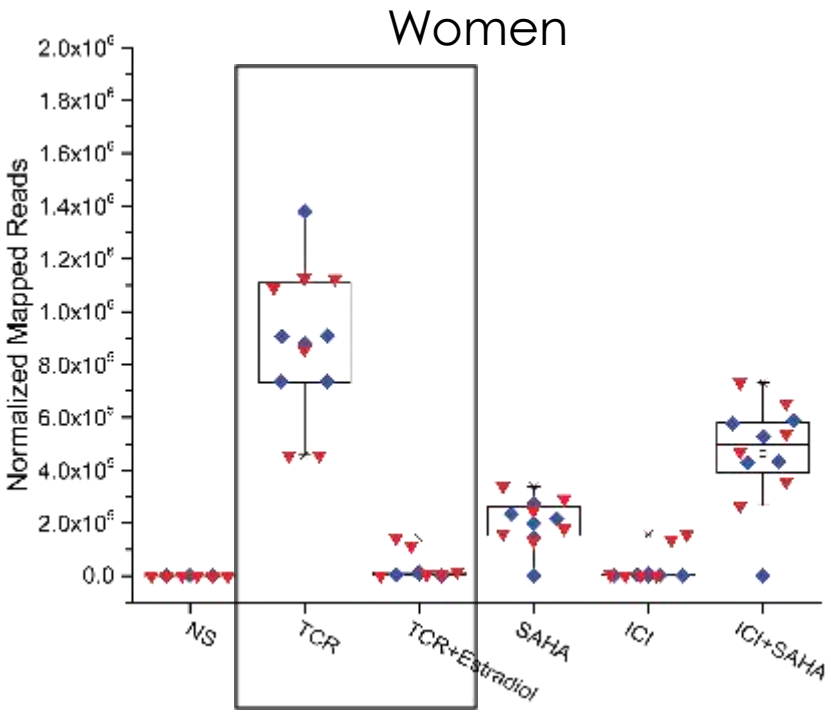
→ Estrogen identified in an shRNA screen as a potent inhibitor of latency reversal in cell lines and latency models

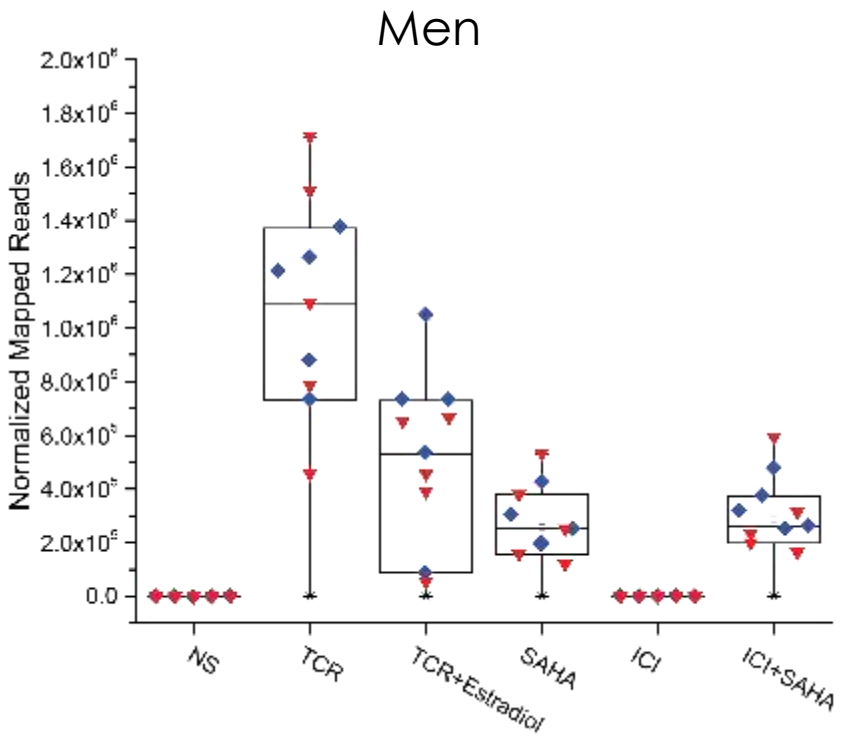
Direct hormonal regulation of latency reversal



ICI=Fulvestrant (Estrogen receptor antagonist)

Estrogen Effect is Sex Specific



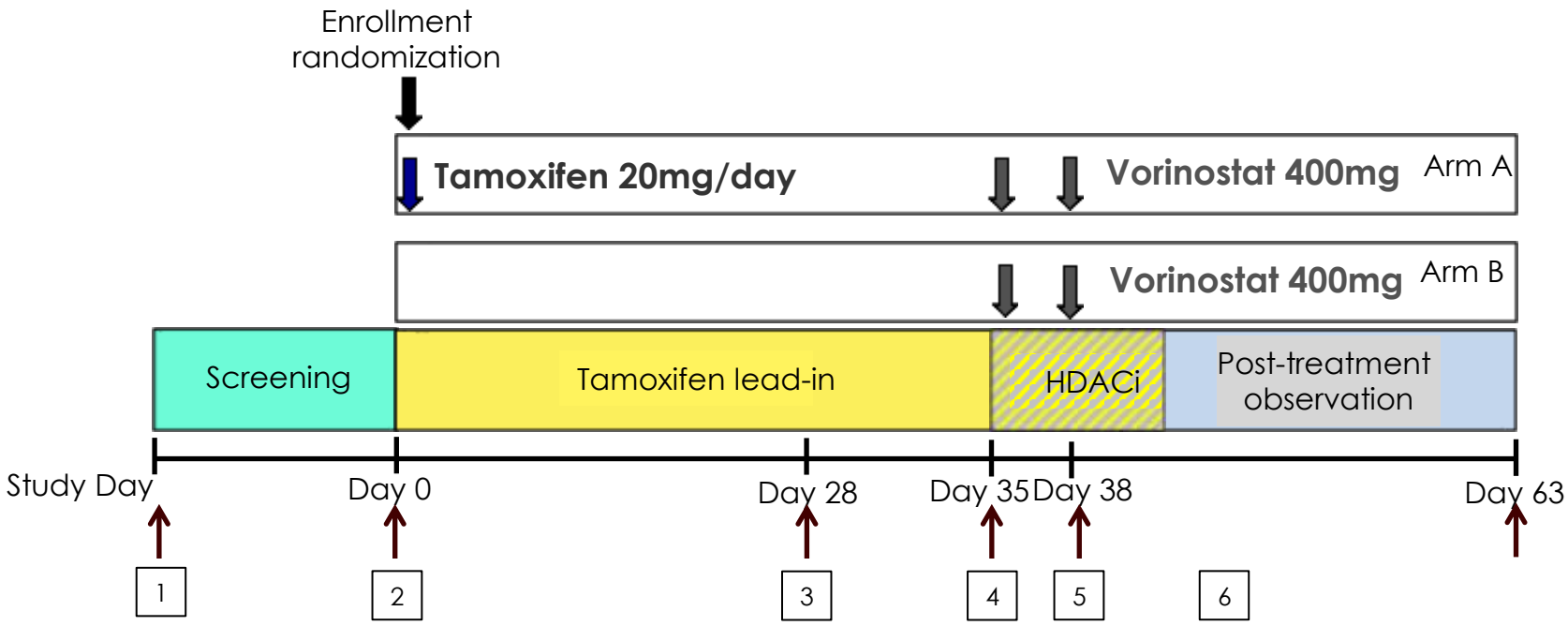


Summary

- Important differences between men and women in HIV persistence.
- **Estradiol at peak menstrual cycle levels is a potent inhibitor of viral reactivation.**
- Possibly interfering with “Kick and kill”.

Clinical translation: ACTG 5366 (Chair: Dr. Eileen Scully)

- Selective estrogen receptor antagonist (SERM) in combination with an HDAC inhibitor may enhance virus reactivation



Results (unpublished)

- Tamoxifen did not enhance Vorinostat effect on HIV transcription (in postmenopausal women)
- No safety issues
- Women can be recruited to cure-related studies.
  - The 31 participants were recruited quickly, and without difficulty, from study sites across the United States.
  - They had a median age of 57, were 58% black and had been on ART for a median of 7.5 years.

Sex differences in HIV Persistence and Reservoir Size during Aging

Gianella et al, CID, 2021

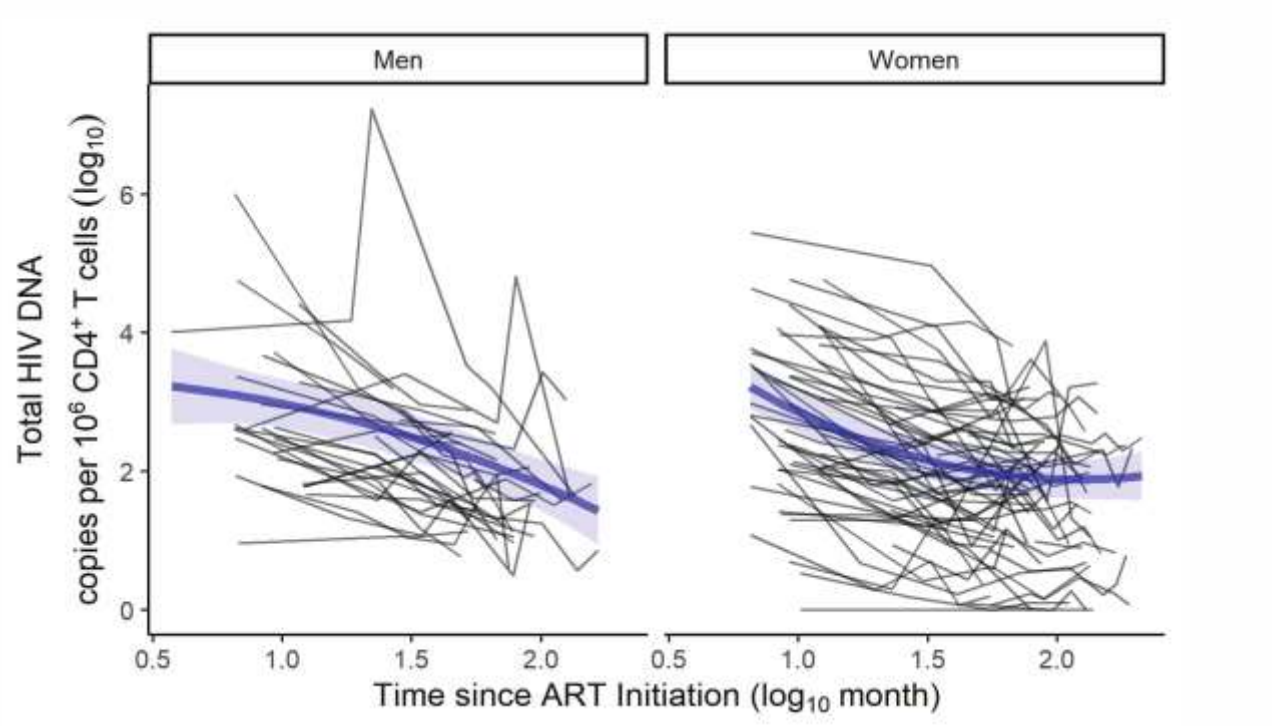
AIDS Clinical Trials Group Longitudinal Linked Randomized Trials (ALLRT): Longitudinal blood samples (N=409) from virally suppressed cisgender women (N=59) and men (N=31).

Chicago Women's Interagency HIV Study (WIHS): Longitudinal blood samples (N=49) from virally suppressed cisgender women (N=15) across 3 reproductive stages (pre-, peri- and post-menopause).

Authors: S.A. Rawlings, M. Nakazawa, A. Chaillon, M. Strain, L. Layman, E. Scully, B. Scott, C. Pacis, K. M. Weber, A. Landay, C. Anderson, J. Karn

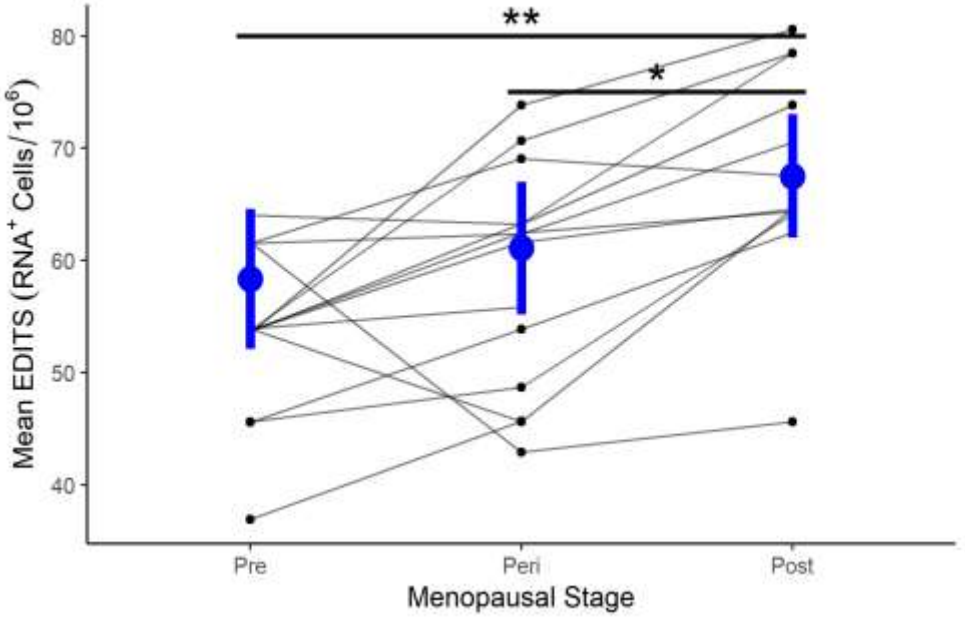
Results

- Overall, we observed a significant decline of total HIV DNA over time in men and women ($p<0.01$).
- The rate of change significantly differed between sexes ($p<0.01$)
  - women have significantly slower rate of decline as compared to men which becomes more pronounced with age.



Results

- The levels of inducible HIV RNA increased across reproductive stages in women (p<0.001).



- We did not observe a difference in the dynamic of cell-associated HIV RNA measures in the absence of ex vivo stimulation between sexes (p=N.S.).

Conclusions

- Sex specific HIV reservoir dynamic.
  - While total HIV DNA (including intact and defective genomes) declines more slowly in women than in men, the inducible reservoir (enriched in replication competent virus) increases in women after menopause.
  - The divergent behavior of the reservoirs in both sexes is an important parameter to be considered in cure trials.

One Cure May Not Fit All

- Eradication strategies need to account for estrogen (and perhaps other hormones).

- Estrogen antagonists are well established
  - E.g. Tamoxifen
  - Components of future clinical studies

Women in Research

- 12,946 participants across 125 studies
- Median of 11% were women (range: 0- 89%)
- 32 studies reported **no** women enrolled

TABLE 2. PARTICIPANTS PER INTERVENTION TYPE

Intervention type	*Number of participants*	*Median number of participants*	*Median number of women (number of studies missing data)*	*Median number of nonwhite (number of studies missing data)*
Early treatment	3,285	34	5 (5)	8 (23)
Cell therapy	245	8	0 (5)	4 (9)
Immune modulation	7,516	30	5 (10)	7 (30)
Treatment intensification	394	13	1 (2)	13 (14)
Reactivation	113	11	3 (1)	n/a (6)
Treatment interruption	4,566	40	7 (9)	14 (36)
Therapeutic vaccine	2,381	36	3 (8)	5 (24)

Rowena Johnston, 2015, ARHR

Early Phase Clinical Trials

- Potentially **high or unknown risk** but lack of clinical benefit.
  - Arguments regarding need for fair access to direct benefits of research may be weaker.
- Many early phase studies do not lead to successful products.
  - Arguments about needing women in these trials to support the evidence base for women's health care might be weaker
- Some review boards argue that women of reproductive potential should be excluded from early phase research and included only later when safety profile is better known.
- **FDA**: Considers well suppressed women as healthy volunteers. Safety barrier high. Contraceptive requirements very strict, often preventing women from joining studies even if they are willing

Slide adapted from Liza Dawson

Preclinical Research

- Most commonly performed with male animals
  - Costs and preservation of breeding stocks
  - Less standard conditions (hormonal)

- This bias may obscure **key sex differences** that could guide clinical studies

Importance of Sex Balance

- Failure to enroll women in every step of research
  - Critical knowledge gaps
  - Missed opportunity to learn about sex-differences on basic biological responses.

Challenges to Enroll Women

- Competing priorities
- Collect sex-specific samples is a challenge
- Need samples throughout menstrual cycle
- Established recruitment practices

Conclusions

- Sex-based differences affect the natural history and immune pathogenesis of HIV infection
  - **Likely also affect HIV reservoirs**
- Targeted enrolment of women in clinical trials and careful sex-based analysis
  - Design sex-specific approaches to HIV eradication
- Consider cis- and trans-gender women
  - Role of exogenous and endogenous sex hormones

Recommendations

1. Sex-based analyses are imperative at any stage of clinical research in order to understand the biological differences in HIV reservoir dynamics.

2. Clinical trial protocols can be written to include sex-based comparisons so that necessary information can be collected as trials are conducted

3. Targeted enrolment of women is a necessity in all phases of clinical research (match the demographics)

4. Publications must always report # of women enrolled, even when it's zero.

5. Engage community and other women.

Transgender Women

- Same considerations for transgender women
- Exclusion of women based on gender identity problematic
- Transgender women may be affected differently
  - Role of exogenous sex hormones
- HIV interventions need to be designed to be safe, culturally and socially appropriate and effective for all affected populations.

Acknowledgments

- Eileen Scully
- Jon Karn
- Karine Dube
- Katy Godfrey
- Liz Barr
- Liza Dawson