

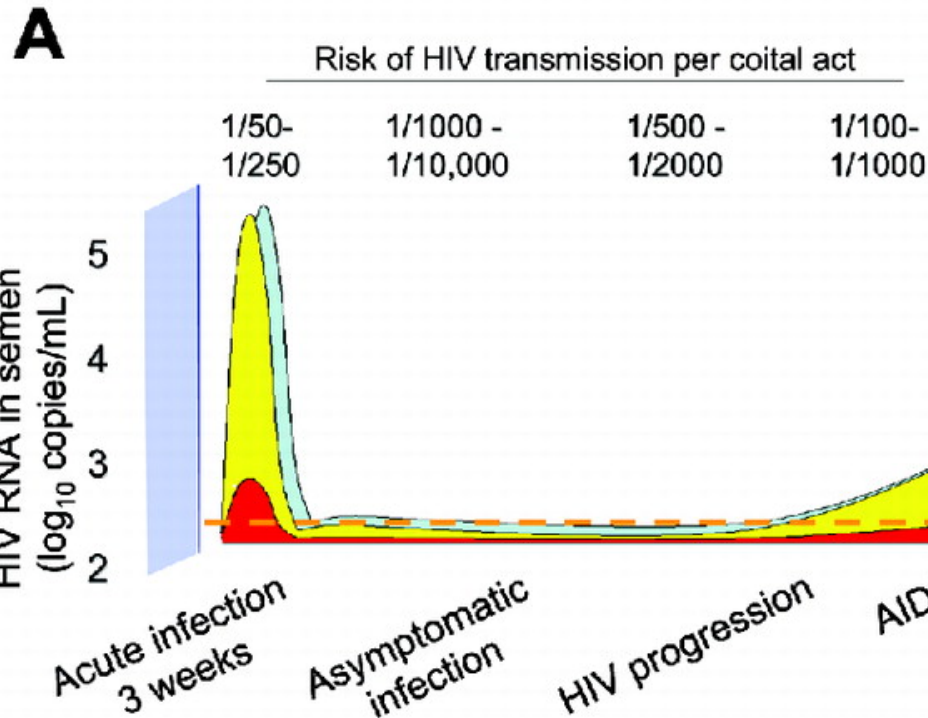
Development of Clinical Biomarkers Predictive of Microbicide Efficacy



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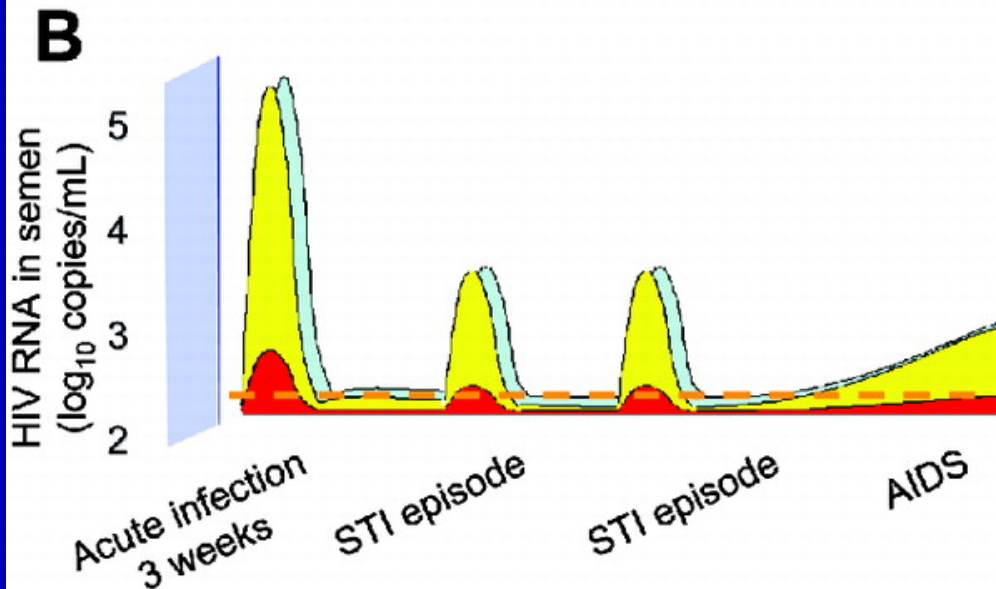
Gaps in Pre-clinical and Clinical Assessment of Efficacy

- No gold standard
- No *in vitro* or animal model recapitulates human transmission
- Optimal assays not defined
 - Reliance on cell lines, laboratory isolates- are they predictive?
 - How much *in vitro* activity is needed for protection *in vivo*?
 - Need to test multiple clades representing R5/X4 viruses
 - Impact of cervicovaginal secretions & semen on activity
 - Formulation effects
 - Physiology: hormones, contractions, trauma



Prediction of the efficiency of HIV transmission according to HIV burden in the genital tract.

A: Probability of male-to-female HIV transmission per coital act, as a function of disease stage in the index case. *Yellow*, Expected distribution of viral burden in semen among men over time; *red*, theoretical effect of a biological intervention designed to reduce viral excretion; *dashed line*, a potential threshold for HIV transmission. **B:** Determinants of high HIV transmission probability: acute infection, sexually transmitted infection (STI), and AIDS. *The Journal of Infectious Diseases* 2005;191:1391-1393



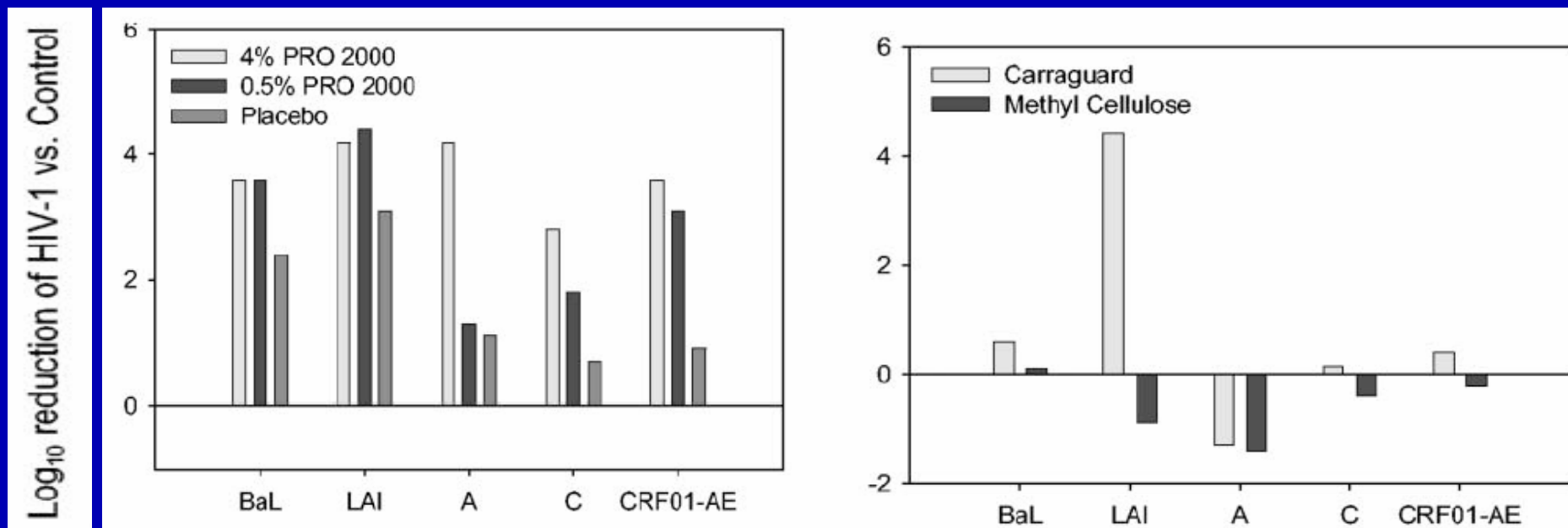
Viral Shedding in the Genital Tract

- The female & male genital tracts represent distinct compartments for HIV-1 replication/evolution.
- Plasma viral load may fail as a marker of infectivity of genital secretions.
- HIV-1 transmission intervention strategies need to incorporate prevention, diagnosis & rx of prevalent STIs
- Gap in knowledge regarding potential impact of topical microbicides on locally replicating virus
 - Would microbicides reduce viral load in women who are HIV-infected?
 - Could microbicides be of value in preventing perinatal HSV transmission?
 - Would microbicide use select for resistant viruses?

Gaps in Pre-clinical Efficacy Testing

- How much anti-HIV activity in vitro using which virus, which cells, and which assays will predict efficacy in vivo?
- How much anti-HSV activity will predict activity in vivo?
- Which HIV clades and how many different isolates should be tested in vitro?

Prevention of PBMC Infection by Different Clades



Primary isolates: A (Central Africa & Asia), C (Central & South Africa), and CRF01-AE (Asia)
 Virus cultured with PBMCs in the presence or absence of product and placebo;
 Infection was measured by the release of p24gag in the supernatants
 from day 4 or 7 of culture.

Need to Test Microbicides in Presence of Genital Tract Secretions

- Most preclinical studies conducted with virus introduced in buffer or media
- Previously demonstrated that polyanionic microbicides retain anti-HIV & anti-HSV activity over broad pH range & in presence of cervicovaginal secretions (CVL)
- What about seminal proteins?

Impact of Seminal Fluid on Candidate Drugs

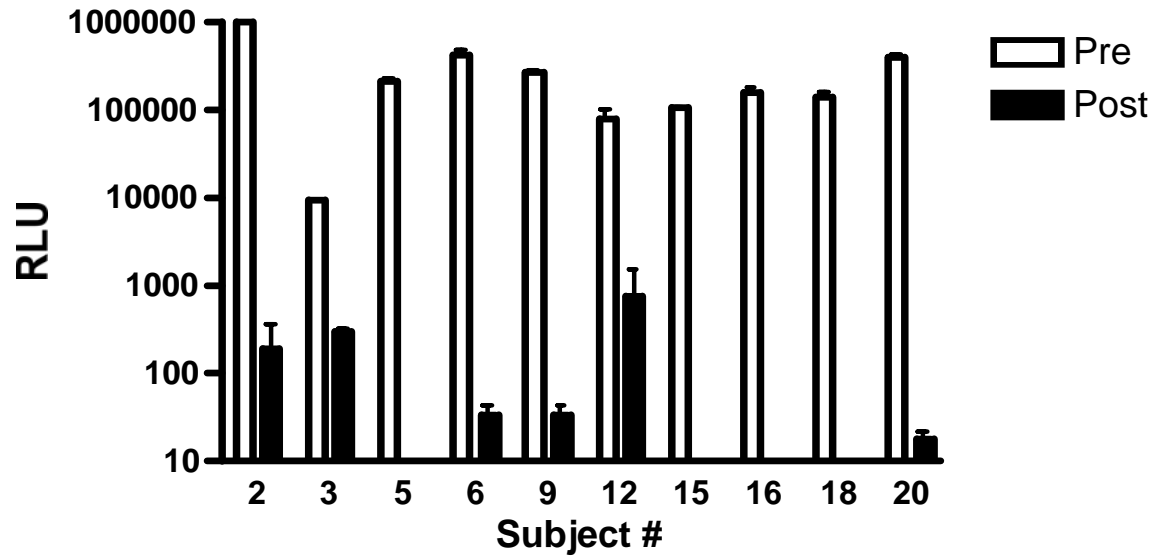
- Semen interferes with polyanionic microbicides
- Interference translates to murine HSV model
- Maps to high molecular weight fraction
 - Fibronectin-1 & Lactoferrin
 - Mechanism studies suggest that seminal proteins bind to the HSV envelope (and, to a lesser extent, epithelial cell) and compete with binding of polyanionic microbicides thus interfering with anti-viral activity
 - Mechanism studies with HIV in progress
- Confirmation of protein identities & mechanisms should promote the development of strategies to overcome this interference

Development of Surrogate Marker of Microbicide Activity Following Vaginal Application in Vivo

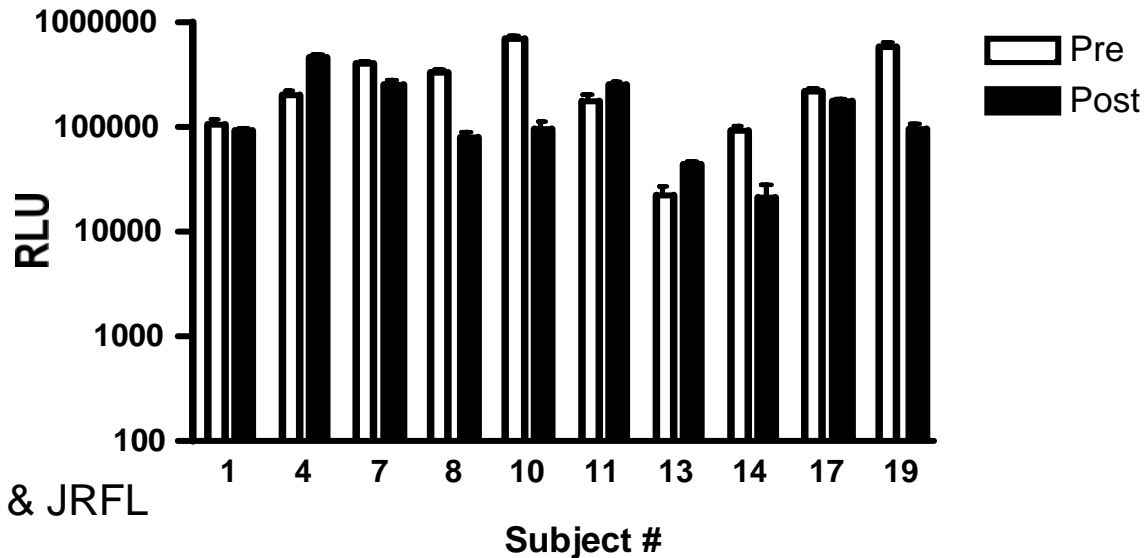
- There is no data demonstrating anti-HIV or anti-HSV activity of a microbicide following vaginal application
- To determine the extent of anti-HIV & anti-HSV activity in cervical fluid obtained 1 h after gel application using a spiking strategy
 - 0.5% PRO 2000 vs. matched Placebo gel
- Enrolled 20 HIV-infected women

Anti-HIV Activity of CVL Pre & Post Gel

PRO 2000

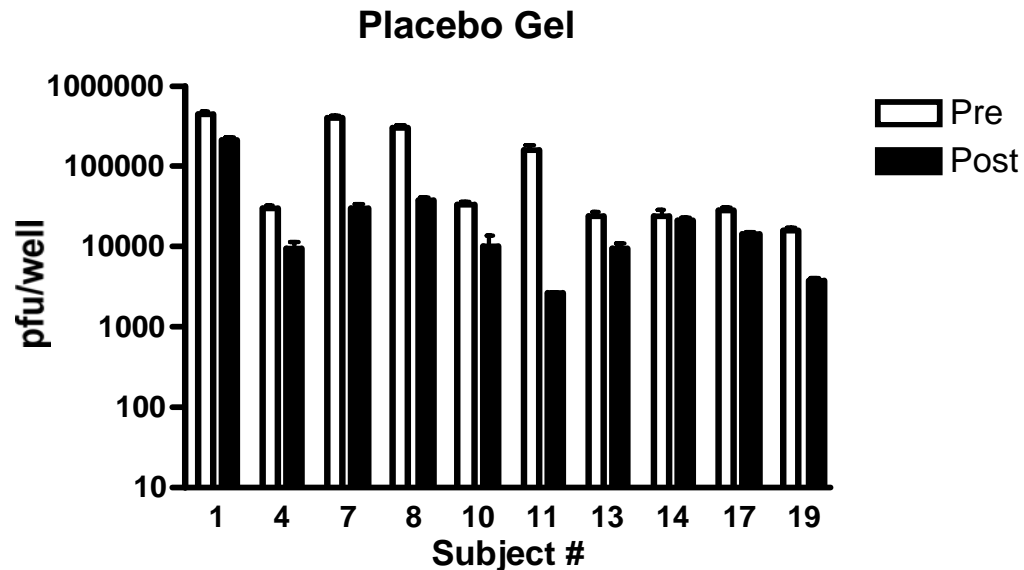
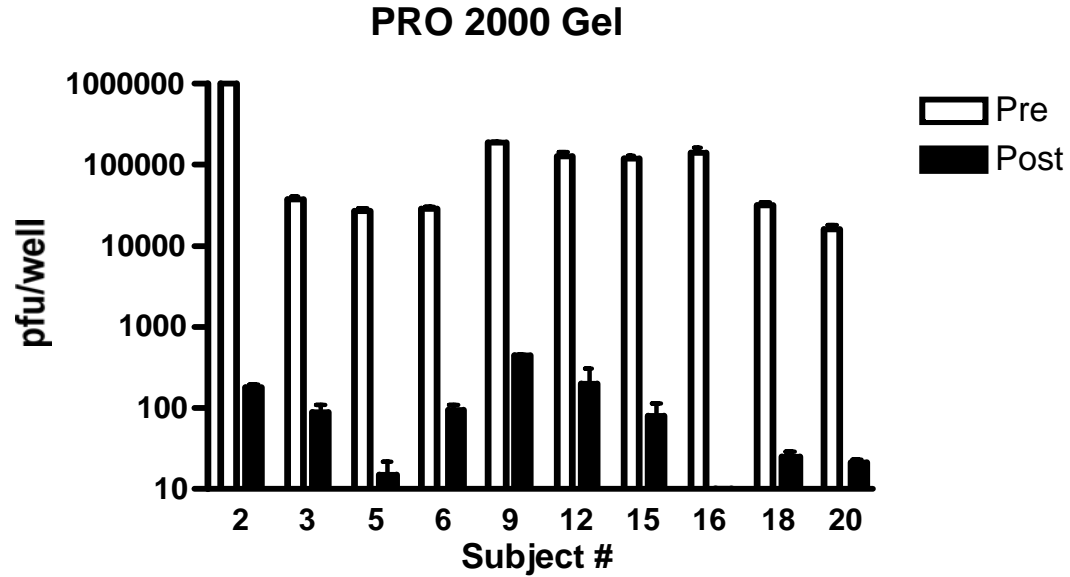


Placebo

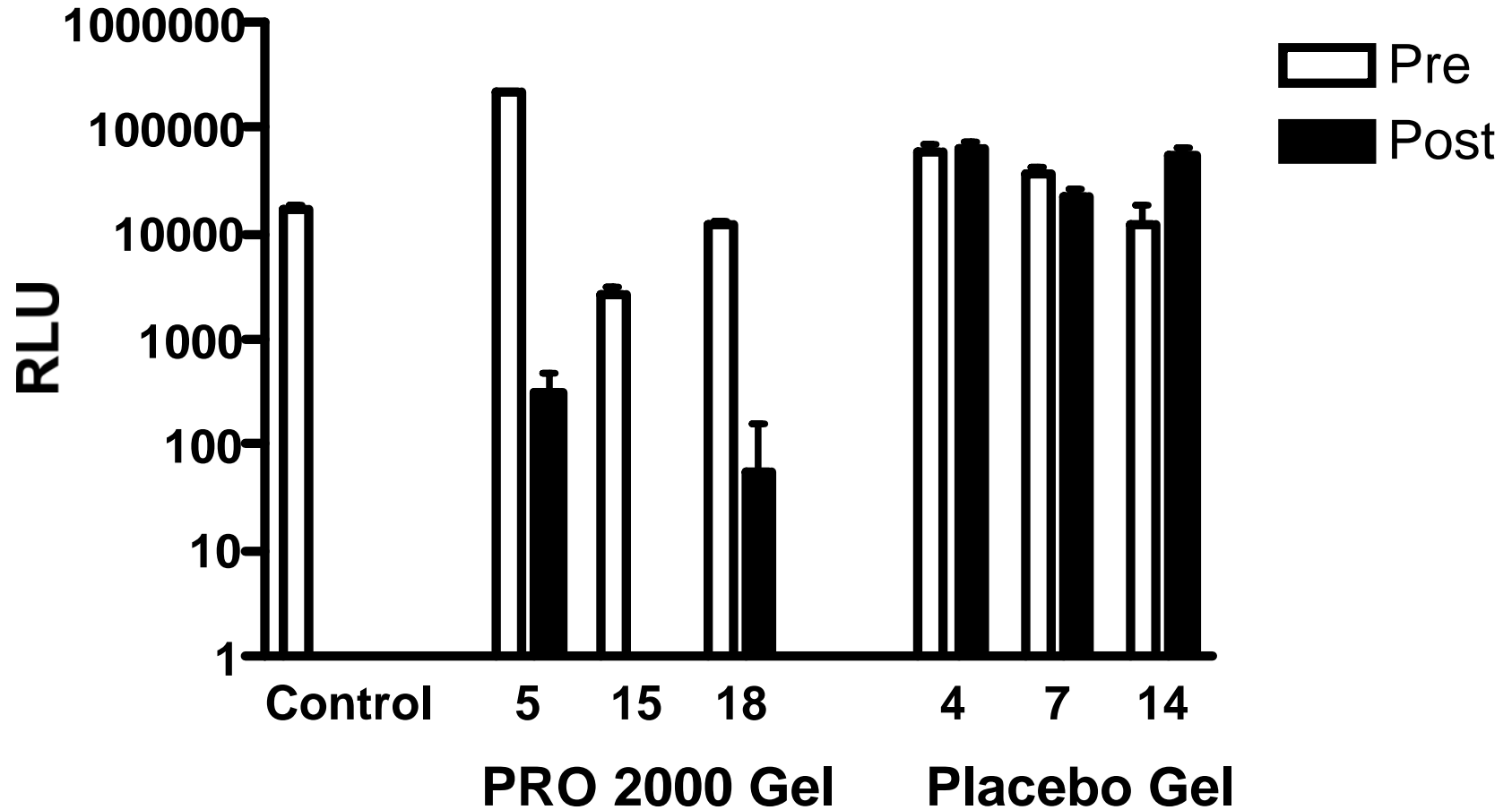


HeLaCD4-CCR5 cells & JRFL

CVL Obtained Post PRO 2000 Inhibits HSV Infection



CVL Post-Application Inhibits HIV Infection of Human Macrophages



Conclusions: Single Dose Trial

- CVL obtained 1 hour post-application of 0.5% PRO 2000 Gel significantly inhibits HIV infection by R5 virus
 - CCR5-expressing HeLa cells & macrophages
- CVL obtained post-application inhibits HSV-2 infection
- Concentration of PRO 2000 in CVL ~ 100-300 $\mu\text{g/ml}$
- Placebo Gel had little or no anti-viral activity
- No acute inflammatory response observed

Implications

- 0.5% PRO 2000 is sufficiently bioavailable and retains substantial anti-HIV and anti-HSV activity 1 hour after intravaginal application
- These data may help interpret results obtained in ongoing Phase IIb/III trials
- This strategy may provide surrogate marker to predict efficacy of candidate microbicides prior to initiating large scale studies
 - Studies with additional drugs in progress
- Expand model to post-coital activity

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