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HIV Superinfection and the CAPRISA 004 Trial

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Background:

- The CAPRISA 004 was a double-blind randomized clinical trial of 1% tenofovir (n=445 women) or placebo vaginal gel (n=444 women) applied before sex and another within 12 hours after sex based in South African medical clinics (1).
- Sexually-active, non-pregnant HIV-uninfected women were enrolled and followed for 30 months.
- The tenofovir vaginal gel reduced HIV acquisition by an estimated 39% overall, and by 54% in women with high gel adherence.
- The HIV incidence in the tenofovir arm was found to be 5.6 per 100 women-years (38 out of 680.6 women-years) compared with 9.1 per 100 women-years (60 out of 660.7 women-years) in the placebo gel arm (incidence rate ratio = 0.61; P = 0.017).
- Superinfection is defined when an individual is initially infected with an HIV-1 variant(s) and is subsequently infected with a new distinctly different variant.
- A recent study from Rakai Health Sciences in Uganda found similar rate of HIV incidence (Incidence=1.15/100py) and the rate of HIV superinfection within their population (SI=1.44/100py)(2).
- A study of high-risk female bar workers in Kenya found the rate of SI to be half of the rate of primary HIV infection(3).

Hypothesis:

The rate of HIV superinfection among seroconverters from the CAPRISA 004 trial was similar to the primary HIV incidence rate in the trial.

Methods:

- 96 women seroconverted during the CAPRISA 004 trial, the first time point and last time point before ART initiation were selected for analysis.
- Viral RNA was extracted using the QIAmp Viral RNA Minikit.
- Each sample was separately amplified for the p24 and gp41 regions using a RT-PCR reaction.
- This first round product was used in a nested PCR reaction to amplify a specific 350-440 base pair region with unique primer barcodes designed for the 454 NGS platform.
- Barcoded products were sent to the NIAID Core Facility in Hamilton, Montana for purification and pyrosequencing.

Definition of Superinfection Used For Analysis:

•New phylogenetically distinct viral strain in second time point sample (either intra- or inter- subtype) that has a ample genetic distance from the first sample

- ***p24 ≥0.59% per year or gp41 ≥0.98% per year***

Amplification Results	Placebo Arm	Tenofovir Arm
Total Number of Seroconverters	61	35
Number of Successful Amplifications	47	29
Failure Rate	23.00%	17.10%

Results:

- 76 women were screened for superinfection (47 both regions, 12 gp41 & 17 p24 only).
- All women were infected with subtype C except for one woman with subtype A.
- Two women were found to be superinfected (2.6%) (Figure 1)
- Viral load increased by >0.5 log during the window when superinfection occurred in both women (Figure 2).

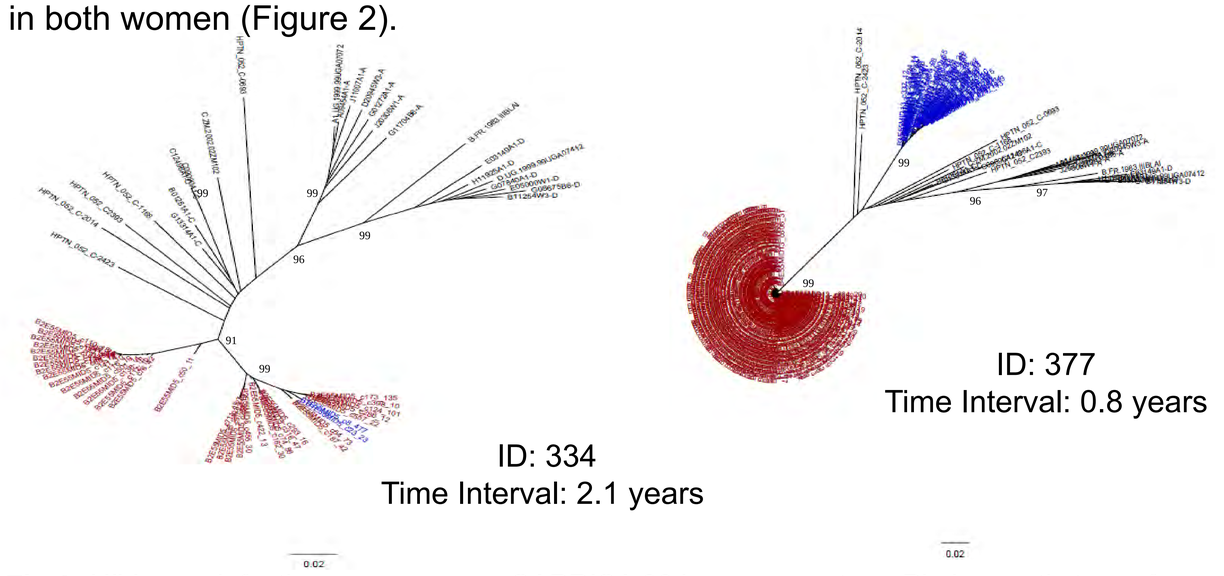

Fig 1: HIV superinfection cases among CAPRISA 004 seroconverters. Phylogenetic tree of consensus *gp41* viral sequences (10 reads) of a seroconversion (Blue) and a follow-up sample (Red), Distance is indicated for the tree by the scale at bottom of the tree, and samples are grouped with a selection of subtype reference sequences (black).

ID	Baseline Subtype	Baseline Reads	Baseline Con_Seq	SI Subtype	Follow-up Reads	Follow-up Con_Seq	Cons_Seq SI Strain	Baseline VL	Follow-up VL
334	C	1,287	2	C	5,139	33	45%	6,850	27,962
377	C	8,211	49	C	20,450	221	100%	245,000	337,496

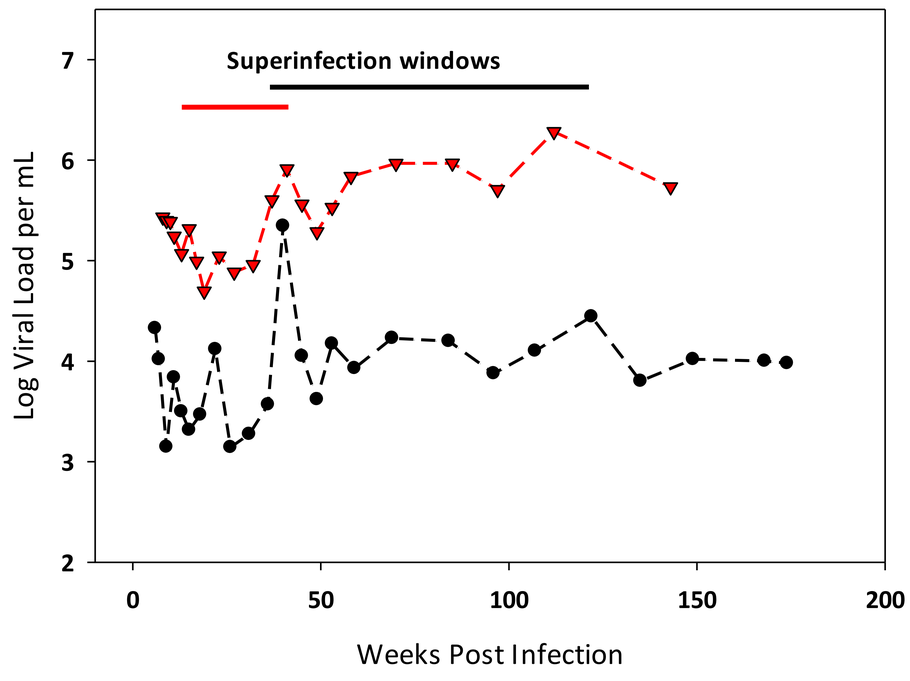

FIG 2: Viral loads from HIV SI cases. Viral load measurements for the two women (placebo arm, red; tenofovir arm, black) who became superinfected are shown, with the time frame in which SI occurred shown above.

Conclusions:

- Rate of superinfection within this population was found to be **1.5/100py** (CI=0.7-7.1), which was significantly less than primary HIV incidence in the total CAPRISA 004 trial (IRR=0.20; p=0.003)
- These date differ than previous findings in the study in Rakai, Uganda, but agree with the findings from Kenya.
- A possible factor influencing these difference in findings is that CAPRISA 004 participants and Kenyan bar workers had very frequent follow-up visits (monthly) as opposed to annual visits in the Rakai cohort.

References:

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