**Introduction**

Many biological risk factors for HIV acquisition, including mucosal epithelial disruption, the availability of CCR5+/CD4+ target cells, sexually transmitted infections (STI), disturbances in the vaginal microbiota, such as bacterial vaginosis (BV), are well characterized and accepted. However, the association between innate immune markers and HIV acquisition remains unclear.

Understanding the association between female genital innate immunity and HIV acquisition is important for:

- the design of HIV prevention technologies
- understanding clinical trial results
- understanding the potential impact prevention technologies on the HIV epidemic

**Methods**

Case-cohort design to analyze the association between female genital tract (FGT) immune markers and HIV acquisition.

- **Cases**: women in Kenya and South Africa who became HIV infected during follow-up (60 weeks) in a clinical trial of Truvada (TDF/FTC) for HIV prevention (FEM-PrEP)

- **Sub-cohort**: 15% random selection independent of case status from women HIV RNA and DNA PCR negative at enrollment

- **FGT immune markers**: FGT macrophage inflammatory protein (MIP)-1α, MIP-1β, interleukin (IL)-6, IL-8, IL-10, IL-1β, interferon gamma induced-protein-10, regulated on activation normal T cell expressed & secreted (RANTES), granulocyte macrophage colony-stimulating factor (GMCSF) and secretory leukocyte peptidase inhibitor (SLPI)

- **Recent sexual exposure**: prostate-specific antigen (PSA) qualitative testing used to control for recent semen exposure

- **Statistical analysis**: Cox models with pseudo likelihood and robust variance estimation

- **Age, injectable contraceptive use, reproductive tract infection PSA and TDF/FTC were controlled.**

- **Exploratory analyses evaluated influence of exposure coding.**

- **SAS® software version 9.3 (SAS Institute, Cary, NC).**

**Study population**

- Over half of women were ≤24 years old.
- High baseline STI/BV prevalence (14% had ≥1 STI (NG, CT and/or T. vaginalis); 45% had BV).
- 100% baseline use of effective contraceptive method (protocol requirement) (66% injectables, 26% oral contraceptives, 8% sterilized/IUD/implant).
- 98% continued use of effective contraception throughout follow-up.
- TDF/FTC use low (50% of follow-up visit intervals among sub-cohort women in the active arm was classified as no/low TDF/FTC use, 26% some use and 24% good/excellent use).

**Bivariable analysis**

- Younger age, TDF/FTC concentrations consistent with some and good/excellent (vs. no/low) use and higher IL-1α concentrations were significantly associated with case status.

**Multivariable analysis**

- No immune markers significantly associated (p>0.05) with HIV acquisition when modeled as continuous exposure variables.
- IL-1β associated with HIV acquisition among women PSA positive at baseline.
- MIP-β, IL-8 and RANTES significantly associated with HIV acquisition among women PSA positive at baseline in placebo arm.

**Sensitivity analyses**

- Conducted to explore influence of variable coding on effect estimates.
- Variations of exposure variable coding had varying influence – both in estimate directionality (Figure 2) and statistical significance (Figure 3) - on primary effect estimates.

**Conclusions**

- Nested case-cohort study design allowed assessment of innate immune markers and HIV acquisition in vivo among a large sample of women.
- Increased HIV acquisition among women in placebo arm with higher concentrations of RANTES, IL-1β, MIP-1β and IL-8 biologically plausible.
- RANTES both protective and inflammatory → binds CCR5 HIV co-receptor and target cell chemotactant.
- RANTES elevation correlated with increased HIV-1 targets cells in genital mucosa and HIV acquisition.
- IL-1β activates the NF-κβ pathway → activation of pro-inflammatory cytokines.
- IL-1β elevation proposed as mechanism of increased HIV risk among women with BV.
- MIP-1β and IL-8 chemotactant of CCR5+ CD4+ T cells and other HIV susceptible cell types.
- MIP-1β and IL-8 elevation associated with increased HIV acquisition among South African women.
- Consistent restriction of significant findings to women PSA positive at baseline may be explained by PSA positivity as an indicator of future HIV exposure and acquisition risk.
- Influence of exposure coding on point estimates and statistical significance is important.
- Coding schemes generally not reported in published literature.
- Potential inconsistency in coding across studies limits comparability.
- Establishment of a minimal set of standard exposure classification and/or reporting criteria would be useful for advancing our understanding of female innate immunity and HIV acquisition.
- Analysis may be underpowered; FGT specimens only available for 164 women from the selected cohort (i.e., 8% person-sample from original cohort).
- Genital specimens only systematically collected at baseline in the parent trial which may have led to exposure misclassification.