Outline

1. Immune Activation and HIV Susceptibility
2. Sex work and HIV risk
3. HESN and Immune Quiescence
4. Inducing Immune Quiescence

Inflammation and HIV Susceptibility

- Activated CD4+ T cell is many times more susceptible to HIV infection and produces more virus
- STIs, which cause genital inflammation, are known to be cofactors in HIV acquisition
- GML-treated monkeys reduced inflammation and lowered susceptibility to SIV infection (QS Li et al. Nature, 2009)
- STEP HIV vaccine trial – high baseline IFN-γ ELISpot correlated with HIV infection, not HIV-specific responses (Huang et al PLoS One 2014)

Caprisa 004 - Genital Inflammation and HIV Risk

- Figure 1: Unsupervised hierarchical clustering was used to visualize the variation in cytokine concentrations in individual women and to cluster women according to the similarity of their cytokine expression profiles (using ClustVis Direct Explorer). Women who later became human immunodeficiency virus (HIV) positive were significantly more likely to have lower IFN-γ concentrations than those who remained seronegative.

2) Conceptual Model of Sex Work and HIV Risk: Role of HIV Exposure and Immune Activation

Mucosal Immune activation

Phases of sex work
- Casual Sex
- Transactional Sex
- Self ID as FSW
- Experienced FSW

Study cohorts
- Transitions Cohort
- MARPS Cohort

HIV Exposure

Impact of sex work on cellular activation

If ....

Immune Activation = ↑ HIV risk

....then does

Immune Quiescence = ↓ HIV risk?

3) Nairobi Sex worker cohort:
T cell Immune Quiescence
Nairobi Sex Worker Study
Pumwani cohort
- Est. in 1985, open cohort > 4000 women enrolled
- Average 4 clients/day
- Most are HIV+ at entry, those not seroconvert within 2 yrs
- ~110 uninfected despite up to 500 unprotected exposures
- Exposure or co-factor determinants not different
- HIV resistance defined as:
  - No evidence of HIV infection
  - Still active in sex work
  - Followed in cohort for >7 years

HIV resistance defined as:
1. No evidence of HIV infection
2. Still active in sex work
3. Followed in cohort for >7 years

Immune Quiescence in HESN
- Lower overall gene expression, CD4+ T cells and whole blood
- Lower gene expression in HIV and T cell receptor pathways
- Lower resting PBMC cytokine production
- Lower level of cellular activation on T cell
- Higher T regs in the blood
- Lower level of FGT chemokines/cytokines
- Lower level of CD4+ CCR5+ T cells, lower anti-proteases at FGT
- Normal Antigen recall function – not immune suppression
- OVERALL, T cells seem to be resting or quiescent
- Termed this phenotype T cell Immune Quiescence

IQ in other HESN cohort
- Amsterdam MSM Cohort,
  - Lower % of activated (HLA-DR, CD38) and proliferating (Ki67) CD4+
    in HESN. (Koning et al J Immunol 2005; 175; 6117-6122)
- Discordant couples, Central African Republic
  - Lower levels of HIV-1 infection prior to PHA stimulation. (Begaud et al Retrovirology 2005; June 22; 3:35)
- HESN Men, Ugandan
  - Lower levels of CD4+ T cells activation (HLA-DR and CCR5) and reduced susceptibility to in vitro HIV-1 infection prior to PHA stimulation. (Kuebler et al JID 2015)
- Discordant couples, Colombia
- MSM, USA
  - HESN associated with low CD8+ CD38+DR+ T cell activation status (Kuebler et al, JID, 2015)

Caprisa 004 - Genital Inflammation and HIV Risk
- Unsupervised hierarchical clustering was used to visualize the variation in cytokine concentrations in individual women and to cluster women
  - According to the similarity of their cytokine expression profiles using Cluster/Dendrograms. Women who later became human immunodeficiency virus
4) Immune Quiescence: Can it be induced?

Hypothesis and objectives

**Hypothesis:**
The Immune Quiescence phenotype observed in HESN can be induced

**Objectives:**
- Feasibility of conducting a clinical trial phase I in low-risk women in Nairobi, Kenya (Baba Dogo and Pumwani clinics)
- Determining if drugs can induce T cell immune quiescence
- If oral administration decrease the number of HIV target cells at the female genital

Choice of Anti-inflammatory drugs

- **Safe**
  - FDA and Health Canada approved
  - Proven long-term track record
  - Minimal side-effects
  - Significantly speeds the development time lines
- **Accessible**
  - Must be accessible to areas most affected by HIV
- **Affordable**
  - Generic versions available
  - Low-cost

Drugs chosen

- **Hydroxychloroquine - HCQ**
  - nonsteroidal anti-inflammatory drug (NSAID)
  - Used to treat
    - Malaria
    - Arthritis – inflammatory condition
    - Lupus – autoimmune condition
  - Daily usage for prevention
    - Daily-use dose 200 mg
    - Rheumatoid arthritis – inflammatory condition
    - Lupus – autoimmune condition
  - CIHR funding - Fowke

- **Acetylsalicylic acid (ASA)**
  - nonsteroidal anti-inflammatory drug (NSAID)
  - Used to treat
    - Headache, fever, pain
  - Daily usage for prevention
    - Daily-use dose 81 mg
    - Arthritis – inflammatory condition
    - Stroke
    - Heart disease
    - Dementia
  - Grand Challenge Canada Funding – Lajoie

Inducing Immune Quiescence - Design

Participants: 40 Low Risk – HCQ, 37 Low Risk - Aspirin

- Month 1: Phase I: Assessment of the baseline immune activation
- Month 2 and 3: Phase II: Assessment of immune activation during hydroxychloroquine or acetylsalicylic acid
- Follow-up visit

- Samples collected (blood, cytobrush/scaper, cervico vaginal lavage), questionnaire
- Sampling was performed 5-7 days post-menses

No drug-associated adverse events
Sample Analysis

**Blood, CMC**
- Cytokines, Chemokines
- Drug levels
- Cell phenotype

**Flow Cytometry Panels**
- T cell activation blood panel:
  - CD3 PeCy5
  - CD4 FITC
  - CD8v500
  - CCR5 V450
  - HLA-DR APC-h7
  - CD161 APC
  - CD95 PE
  - CD69 PeCy7
  - CCR7 PeCF594
  - CD45RA Alexa 700
- CMCs panel
  - CD3 PeCy5
  - CD4 FITC
  - CD8v500
  - CCR5 V450
  - HLA-DR APC-h7
  - CD161 APC
  - CD95 PE
  - CD69 PeCy7
  - Live/Dead ECD

**Visit 1 (wk -2) vs Visit 3 (wk 6) for HCQ and ASA - No change**

**Blood**
- CD4/CD8
- CD69
- CD95
- HLA-DR
- CCR7
- CD45Ra

**CMC**
- CD4/CD8
- CD69
- CD95
- HLA-DR

**HCQ arm: Blood compartment**

**ASA arm: Blood compartment**

**HCQ arm: FGT compartment**

**ASA arm: FGT compartment**

Reduction but did not reach significance

Visit 1
Visit 2
Visit 3

CD8+ CD161+MFI
CD4+CCR5+MFI

Anova p=0.008

CD8+ CD161++MFI
CD4+CCR5+

Relative proportion of CD4+CCR5+

p=0.01
Conclusion

- HCQ and ASA decrease the expression of CCR5 on CD4+ T cells at the systemic compartment

- Daily oral administration of ASA for 6 wks reduces the level HIV target cells at the female genital tract

Next steps....

- Measuring Cytokines/Chemokines expression
- Measuring drug levels (blood and FGT)
- Finalizing data analysis

- Next phase of the study in high-risk population
  - Longer duration on drug regimen
  - Larger sample size
  - Mechanism of action

What does this mean?

- Reducing inflammation may help reduce HIV risk.
- Women commented the drugs were non-stigmatizing.
- Can be used with other approaches, eg microbicide or vaccine.
- As adherence is a major concern
  - would an intravaginal ring be more effective?
- Possible intervention for women transitioning to sex work???

What does this mean?

- Reducing inflammation may help reduce HIV risk.
- Women commented the drugs were non-stigmatizing.
- Can be used with other approaches, eg microbicide or vaccine.
- As adherence is a major concern
  - would an intravaginal ring be more effective?
- Possible intervention for women transitioning to sex work???

Thanks

<table>
<thead>
<tr>
<th>Collaborators</th>
<th>The Funders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frank Plummer</td>
<td>MHRC</td>
</tr>
<tr>
<td>Blake Ball</td>
<td>CHIR</td>
</tr>
<tr>
<td>Julie Lajoie</td>
<td>BM Gates Foundation</td>
</tr>
<tr>
<td>Ma Luo</td>
<td>Grand Challenges Canada</td>
</tr>
<tr>
<td>Joshua Kimani</td>
<td></td>
</tr>
<tr>
<td>Julius Oyugi</td>
<td></td>
</tr>
<tr>
<td>Elijah Songok</td>
<td></td>
</tr>
<tr>
<td>Majengo Clinic staff</td>
<td></td>
</tr>
<tr>
<td>MCH Clinic staff</td>
<td></td>
</tr>
</tbody>
</table>

Winnipeg and Nairobi Research Teams
Laboratory of Viral Immunology

Nairobi Team:
- Lucy Mwangi
- Ken Oduor
- Dominic Ouma

Women of the Nairobi cohorts