Using soluble immune mediators to study the mucosal milieu

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What to sample?

• Genital tract secretions
  – Easily obtained
  – Swabs have no dilution, but minimal volume
  – Cervicovaginal lavage (CVL) will dilute, but larger volume

• Genital tract tissue
  – More invasive
  – Variability in immune cells
  – Reproducibility/sample bias
  – Vaginal vs cervical tissue
MTN-001

• Phase 2 Adherence and Pharmacokinetics Study of Oral and Vaginal Preparations of Tenofovir

• Participants randomized to regimen sequence:
  – 6 wks daily oral TDF
  – 6 wks daily vaginal TFV gel
  – Both

• CVL collected at baseline & each 6 week period

• US sites: Pittsburgh, Bronx NY, Cleveland, Birmingham

• African sites: Bothas Hill and Unkomass SA; Makerere Uganda

Hendrix, C.W., et al. PLOS ONE 2013
Hypotheses & Objectives

• **Primary:**
  – Antiviral activity of CVL will correlate with drug levels
  – CVL antiviral activity will be greater following vaginal gel used compared to oral PrEP

• **Secondary:**
  – Baseline activity may correlate with concentrations of antimicrobial peptides, cytokines, chemokines and/or flora
  – Mucosal environment at baseline may differ between U.S. and African women

• **Implications**
  – Will activity and drug levels of CVL correlate with clinical trial outcomes?
  – Can CVL antiviral activity provide biomarker of potential PrEP efficacy?
• 49/55 participants had >90% inhibitory activity after vaginal gel compared to 1/53 on oral.
• “Risk” of having anti-HIV activity of ≥90% was 47.2 times higher on gel than no product.

Herold, BC et al., J AIDS 2014
Correlation of PK/PD

- HIV inhibitory activity correlated significantly with [TFV]; Spearman rho=0.74; but NOT with other immune mediators
- For each $1\log_{10}$ ↑ in [TFV], the likelihood of HIV activity ≥90% ↑ 1.50X (95% CI: 1.29, 1.74, p<0.001)

Herold, BC et al., J AIDS 2014
Were there PK/PD differences between U.S. and African women?

Herold, BC et al., *J AIDS* 2014
Summary

• Women have variable levels of innate anti-HIV activity (baseline)
• Oral TFV did not increase anti-HIV activity beyond baseline
• Topical TFV did increase anti-HIV activity
  – Higher TFV levels significantly correlated to better anti-HIV activity
• No significant difference in anti-HIV activity between U.S. and African women when topical gel was used
Hypotheses & Objectives

• **Primary:**
  – Antiviral activity of CVL will correlate with drug levels
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• **Secondary:**
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• **Implications**
  – Will activity and drug levels of CVL correlate with clinical trial outcomes?
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Baseline CVL testing

• Total protein (BCA)
• Cytokines/chemokines (luminex): IL-1β, IL-6, IP-10, IL-8, MIP-1α, MIP-3α
• Innate factors (ELISA): SLPI, Lf, MPO, HβD1, HβD2, HβD3, HNP1-3, MPO
• Antimicrobial activity
  – Anti-HIV
  – Anti-HSV
  – Anti-E. coli

MTN BSWG unpublished data 2014
Significant (p < .001) differences found between U.S. and African women

MTN BSWG unpublished data 2014
Soluble mediators

Significant ($p = .001$) differences found between U.S. and African women

MTN BSWG unpublished data 2014
Differences in soluble mucosal immunity between U.S. and African women

<table>
<thead>
<tr>
<th>Variable</th>
<th>U.S. ♀ % (n) or mean (SD) n=73</th>
<th>African ♀ % (n) or mean (SD) n=73</th>
<th>Univariable p-value</th>
<th>Multivariable p-value (controlling for log₁₀ total ptn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log₁₀ total ptn</td>
<td>2.76 (0.31)</td>
<td>2.28 (0.35)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Log₁₀ MPO</td>
<td>2.72 (0.93)</td>
<td>2.38 (0.82)</td>
<td>0.02</td>
<td>0.3</td>
</tr>
<tr>
<td>Log₁₀ SLPI</td>
<td>5.37 (0.40)</td>
<td>4.90 (0.50)</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Log₁₀ Lactoferrin</td>
<td>3.04 (0.52)</td>
<td>2.56 (0.60)</td>
<td>&lt;0.001</td>
<td>0.1</td>
</tr>
<tr>
<td>Log₁₀ HβD-1</td>
<td>3.45 (0.39)</td>
<td>2.92 (0.50)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log₁₀ HβD-2</td>
<td>3.27 (0.53)</td>
<td>3.02 (0.84)</td>
<td>0.04</td>
<td>0.9</td>
</tr>
<tr>
<td>Log₁₀ HβD-3</td>
<td>3.12 (0.45)</td>
<td>2.72 (0.51)</td>
<td>&lt;0.001</td>
<td>0.1</td>
</tr>
<tr>
<td>Log₁₀ HNP1-3</td>
<td>4.54 (0.65)</td>
<td>4.37 (0.67)</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Log₁₀ IL-1β</td>
<td>0.94 (0.88)</td>
<td>0.54 (0.78)</td>
<td>0.005</td>
<td>0.2</td>
</tr>
<tr>
<td>Log₁₀ IL-6</td>
<td>0.94 (0.71)</td>
<td>0.51 (0.67)</td>
<td>&lt;0.001</td>
<td>0.07</td>
</tr>
<tr>
<td>Log₁₀ IP-10</td>
<td>2.24 (0.83)</td>
<td>1.84 (0.77)</td>
<td>0.004</td>
<td>0.4</td>
</tr>
<tr>
<td>MIP-1α &gt; LL</td>
<td>51% (37)</td>
<td>53% (39)</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Log₁₀ IL-8</td>
<td>2.93 (0.53)</td>
<td>2.56 (0.56)</td>
<td>&lt;0.001</td>
<td>0.9</td>
</tr>
<tr>
<td>Log₁₀ MIP-3α</td>
<td>1.71 (0.78)</td>
<td>1.33 (0.82)</td>
<td>0.006</td>
<td>0.6</td>
</tr>
<tr>
<td>E. coli activity %</td>
<td>31.74 (35.73)</td>
<td>16.81 (22.73)</td>
<td>0.003</td>
<td>0.8</td>
</tr>
</tbody>
</table>

MTN BSWG unpublished data 2014
Differences in soluble mucosal immunity between U.S. white and black women

<table>
<thead>
<tr>
<th>Variable</th>
<th>U.S. White ♀ % (n) or mean (SD) n=35</th>
<th>U.S. Black ♀ % (n) or mean (SD) n=35</th>
<th>Univariable p-value</th>
<th>Multivariable p-value (controlling for log₁₀ total ptn)</th>
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<td>Log₁₀ total ptn</td>
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<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Log₁₀ MPO</td>
<td>2.99 (0.78)</td>
<td>2.45 (1.02)</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Log₁₀ SLPI</td>
<td>5.39 (0.39)</td>
<td>5.36 (0.42)</td>
<td>0.3</td>
<td>0.5</td>
</tr>
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<td>Log₁₀ Lactoferrin</td>
<td>3.03 (0.52)</td>
<td>3.05 (0.53)</td>
<td>0.9</td>
<td>0.6</td>
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<tr>
<td>Log₁₀ HβD-1</td>
<td>3.51 (0.40)</td>
<td>3.37 (0.38)</td>
<td>0.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Log₁₀ HβD-2</td>
<td>3.28 (0.42)</td>
<td>3.32 (0.42)</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Log₁₀ HβD-3</td>
<td>3.04 (0.40)</td>
<td>3.21 (0.44)</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Log₁₀ HNP1-3</td>
<td>4.62 (0.63)</td>
<td>4.45 (0.69)</td>
<td>0.3</td>
<td>0.09</td>
</tr>
<tr>
<td>Log₁₀ IL-1β</td>
<td>0.84 (0.91)</td>
<td>1.04 (0.90)</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Log₁₀ IL-6</td>
<td>1.02 (0.81)</td>
<td>0.86 (0.63)</td>
<td>0.4</td>
<td>0.08</td>
</tr>
<tr>
<td>Log₁₀ IP-10</td>
<td>2.19 (0.61)</td>
<td>2.23 (1.00)</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>MIP-1α &gt; LL</td>
<td>59% (20)</td>
<td>43% (15)</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Log₁₀ IL-8</td>
<td>2.93 (0.50)</td>
<td>2.94 (0.58)</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Log₁₀ MIP-3α</td>
<td>1.80 (0.82)</td>
<td>1.62 (0.76)</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>E. coli activity %</td>
<td>32.46 (37.77)</td>
<td>32.20 (33.06)</td>
<td>0.3</td>
<td>0.2</td>
</tr>
</tbody>
</table>

MTN BSWG unpublished data 2014
Summary

• U.S. women had significantly higher levels of all variables except HNP1-3 (trend) and MIP-1α as compared to African women.

• In multivariable regression models controlling for differences in $\log_{10}$ total protein only SLPI and HβD-1 remained significantly different (IL-6 trend) between U.S. and African women.

• Differences between U.S. white and black women were modest.

• U.S. white women had significantly higher levels of MPO and HβD-1 (IL-6 trend) controlling for differences in $\log_{10}$ total protein compared to U.S. black women.
Conclusions

• Measurable PD activity in mucosal fluids correlated to topical drug levels (not oral PrEP) irrespective of nationality

• U.S. women have a higher “set point” of soluble innate immune factors than African women
  – Consistent with findings from Cohen et al. AIDS 2010

• “Normal ranges” of the soluble mediators need to be determined based on location of study participants
Acknowledgements

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