HIV Prevention and the Vaginal Microbiome

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Efficacy of Oral and Vaginal TFV-Based PrEP is Variable; Especially in Women

<table>
<thead>
<tr>
<th>Study (location)</th>
<th>Population</th>
<th>Design</th>
<th>Relative reduction in HIV incidence in intention-to-treat analysis</th>
<th>PrEP detection in blood samples from non-seroconverters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partners PrEP Study</td>
<td>4747 heterosexual men and women with HIV infected partners (serodiscordant couples)</td>
<td>1:1:1 randomization to daily oral TDF, FTC/TDF, or placebo</td>
<td>TDF: 67% (95% CI 44-81%, p&lt;0.0001) FTC/TDF: 75% (95% CI 56-87%, p&lt;0.0001)</td>
<td>Detection of tenofovir in blood associated with 82-90% HIV protection.</td>
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<tr>
<td>TDFZ Study (Botswana)</td>
<td>1219 heterosexual men and women</td>
<td>1:1 randomization to daily oral FTC/TDF or placebo</td>
<td>FTC/TDF: 63% (95% CI 22-63%, p&lt;0.01)</td>
<td>73%</td>
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<tr>
<td>IPrEx (Brazil, Ecuador, Peru, South Africa, Thailand, US)</td>
<td>2499 MSM and transgender women</td>
<td>1:1 randomization to daily oral FTC/TDF or placebo</td>
<td>FTC/TDF: 44% (95% CI 13-63%, p=0.006)</td>
<td>Detection of tenofovir associated with 92% HIV protection, high adherence with &gt;95% protection.</td>
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<tr>
<td>CAPRISA 004 (South Africa)</td>
<td>989 woman</td>
<td>1:1 randomization to intercourse-associated use of tenofovir vaginal gel or placebo</td>
<td>Tenofovir gel: 39% (95% CI 8-60%, p&lt;0.02)</td>
<td>Detection of high concentrations of tenofovir (&gt;1000 ng/mL) in cervicovaginal fluid associated with 74% reduced HIV risk.</td>
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<tr>
<td>FEM-PrEP (Kenya, South Africa, Tanzania)</td>
<td>2120 women</td>
<td>1:1 randomization to daily oral FTC/TDF or placebo</td>
<td>FTC/TDF: No HIV protection</td>
<td>35-38% at a single visit, 26% at two consecutive visits</td>
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<td>VOICE (South Africa, Uganda, Zimbabwe)</td>
<td>5029 women</td>
<td>1:1:1:1 randomization to daily oral TDF, FTC/TDF, oral placebo, tenofovir vaginal gel, or gel placebo</td>
<td>TDF: No HIV protection FTC/TDF: No HIV protection Tenofovir gel: No HIV protection</td>
<td>≤30% of samples had tenofovir detected. ≤50% of women in each of the active arms never had tenofovir detected, at any time during their follow-up</td>
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</table>
# Variable and Modest Efficacy of Dapivirine Ring

<table>
<thead>
<tr>
<th></th>
<th>IPM 027</th>
<th>MTN 020</th>
<th>HOPE</th>
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<tbody>
<tr>
<td><strong>Enrollment</strong></td>
<td>1959 women 18-45 years 1300 active arm</td>
<td>2629 women 18-45 years 1325 active arm</td>
<td>1456 women from MTN020 Open-label</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td>31% [1-51] effective</td>
<td>27% [1-46]</td>
<td>39% estimated protection (35 infections)</td>
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<tr>
<td><strong>HIV incidence</strong></td>
<td>4.1% active; 6.1% placebo</td>
<td>3.3% active; 4.5% placebo</td>
<td>2.7% [1.9-3.8] vs 4.4 [3.2-5.8]</td>
</tr>
</tbody>
</table>
Factors that may impact PrEP efficacy

Behavior
Adherence

STI/HIV Risk

Microbiota
- Semen
- Hormones
- STI
  "Inflammatory state"

Drug
PK/PD

STI
HIV
Risk

"Inflammatory state"
# Mechanisms of drug transport into and out of target cells

<table>
<thead>
<tr>
<th>Drug</th>
<th>Tenofovir (TFV)</th>
<th>TFV disoproxil fumarate (TDF)</th>
<th>TFV alafenamide (TAF)</th>
<th>Dapivirine (DPV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structure</strong></td>
<td><img src="image1" alt="TFV structure" /></td>
<td><img src="image2" alt="TDF structure" /></td>
<td><img src="image3" alt="TAF structure" /></td>
<td><img src="image4" alt="DPV structure" /></td>
</tr>
<tr>
<td><strong>IC$_{50}$ ($\mu$M)</strong></td>
<td>2.5</td>
<td>0.1</td>
<td>0.05</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Transport mechanism</strong></td>
<td>Endocytosis</td>
<td>Passive diffusion</td>
<td>Passive diffusion</td>
<td>Passive diffusion ?</td>
</tr>
<tr>
<td><strong>Energy dependency</strong></td>
<td>ATP-dependent non-saturable</td>
<td>ATP-independent saturable Carboxylesterase-1 rate-limiting</td>
<td>ATP-independent saturable Cathepsin A rate-limiting</td>
<td>Unknown uptake/efflux mechanisms Metabolized by CYP4503A4/5</td>
</tr>
<tr>
<td><strong>Saturability</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Enzymes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Source</strong></td>
<td>Taneva et al., AAC, 2015</td>
<td>Taneva et al., AAC, 2015</td>
<td>Taneva et al., (in prep)</td>
<td>To et al., Biochem Pharmacol, 2015</td>
</tr>
</tbody>
</table>

TFV entry into FGT epithelial and immune cells by endocytosis reflects limited expression of organic anion transports (Taneva, AAC 2015)
Complexities of Drug PK
Mechanism of intracellular activation of TAF

Drugs released into complex environment that may modulate drug PK and Efficacy
Hypothesis: Vaginal Microbiota Differ Across Populations and Impact PK/PD by the following mechanisms:

- Modulation of vaginal pH
- Bacteria can bind/absorb drugs and alter drug availability
- Bacteria release products that may degrade drugs or interfere with drug uptake
Hypothesis: Vaginal Microbiota Differ Across Populations and Modulate PK
Bronx and Thika Women Treated for BV

Serebrenik, Sultan, Wang, Hunte, Srinivasan, Keller, Herold, in review
pH Modulates Intracellular (T cell) drug levels

Taneva et al, JCI Insight, 2018, Jul 12;3(13).
bacteria in liquid culture

bacterial suspension at OD=4 for uptake assay

radiolabeled drugs added for 2 h

2X washes

cell-associated radioactivity

extracellular radioactivity

L. crispatus actively transports and phosphorylates TFV
Dapivirine is “Sticky”
Adheres to Live or Heat-Killed Bacteria
Translates into decreased antiviral activity

Similar effects on drug bioavailability with fresh vaginal swabs
**Bacteria release products that may degrade drugs or interfere with drug uptake**

- **Jurkat**
- **CD4+ T**
- **VK2**
- **HaCAT**
- **TZM-bl**

**Cell-associated TFV (%)**

- **G. vaginalis (DNF01662)**
- **A. vaginae (DNF00720)**

**Cell-associated drug (% of control)**

- **TFV**
- **TDF**
G. vaginalis and other bacteria secrete adenine which blocks TFV Endocytosis; A. vaginae consumes adenine
Adenine (and bacterial culture supernatants) shift the TFV Antiviral Dose Response Curve

Adenine levels

- Broth: 66 μM
- G. vag sups: 246 μM
- A vag sups: 9.5 μM
- L. crispatus: 281 μM
Putting it all together

Microbiome modulates drug PK/PD through multiple mechanisms

• Tenofovir transport (endocytosis) is reduced at higher pH
• Bacteria bind indiscriminantly to dapvirine
• TFV is actively transported and phosphorylated by bacteria (*L. crispatus*) which may then serve as a drug reservoir
• *G. vaginalis* (and others) release adenine which inhibits endocytosis thus reducing TFV bioavailability
• Strong **adherence** and **optimized drug release** overcome the effects of microbiome (and semen) on drug PK/D
Implications and future directions

- Preclinical and early clinical studies should assess impact of microbiota, pH and mucosal molecules on drug PK/PD within the genital tract
  - Ongoing studies suggest little effect of vaginal microbiota on rilpivirine (NNRTI) and dolutegravir (integrase inhibitor) PK
- The impact of vaginal microbiota on systemically delivered HIV PrEP not well studied
  - May be more limited as the site of protection is likely peripheral blood/lymph nodes and not within the female genital tract
- Microbiome and mucosal immune environment also modulate risk of HIV acquisition/transmission
  - Higher rates of HIV acquisition/transmission with BV
- Protection is a balance between viral inoculum and drug PK/PD
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