State of the Art in Microbicide Research

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MTN Regional Meeting
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Vaginal/Cervical Tissues and Natural Barriers to HIV Infection

- Vaginal transmission of HIV during intercourse can occur via vaginal or cervical epithelium.
- Women having an intact epithelium without inflammatory changes and a *Lactobacillus*-predominant microflora have a relatively low risk of infection.
- Damage to epithelium allows greater access to submucosal T cells, macrophages and dendritic cells.
- Microbicides should NOT disrupt innate barriers to infection while decreasing the viral load present in the vagina.
What is a Topical Microbicide?

A substance that can be applied topically to prevent transmission of sexually transmitted infections including HIV.
A Tale of Two N-9 Trials

- Cameroon — 1995 - 1998
- UNAIDS — 1996 - 2001
# N-9 Film – Effect on HIV Acquisition

<table>
<thead>
<tr>
<th></th>
<th>No. of Women</th>
<th>No. of HIV Infections</th>
<th>HIV Incidence 100 p-y</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo Film</td>
<td>575</td>
<td>46</td>
<td>6.6</td>
<td>1.0</td>
</tr>
<tr>
<td>N-9 Film</td>
<td>595</td>
<td>48</td>
<td>6.7</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.68-1.52)</td>
</tr>
</tbody>
</table>

Source: Roddy, NEJM 1998
### UNAIDS N-9 Trial - Results Among Women with Regular FU

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<thead>
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<th></th>
<th>No. of HIV infections</th>
<th>HIV incidence 100 p-y</th>
<th>RR (95% CI)</th>
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</thead>
<tbody>
<tr>
<td>Replens®</td>
<td>33</td>
<td>9.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Advantage S®</td>
<td>53</td>
<td>16.4</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Ref: VanDamme, Lancet 2002: 360:971-7
N-9 Impact on Genital Inflammation

- Classical safety studies required by the FDA did not detect significant safety signals for N-9 during phase 1 and 2 studies except when women used N-9 multiple times daily (Hillier, AIDS, 2005; 39:1-8)

- 3 daily applications of N-9 (150 mg) induced an increase in IL-1, TNF and IL-8, and a decrease in SLPI (Fichorova, J Infect Dis 2001;184:418)
The Change in Microbicide Research Following N-9

- Early concept was that commercially available products could be used to prevent HIV- using approved products for new indications (phase 4 studies)
- In 2000-2002 it became clear that **new** products had to be developed for use as microbicides
- Drug development model: regulatory construct based on phase 1, 2, 3 studies under guidance from FDA
What was the Outcome of the Shift in Microbicide Paradigm?

- Clinical trial sites had to be prepared to conduct studies at standards acceptable to US FDA, rather than epidemiologic standards.
- Need for rapid growth in infrastructure at the sites in the developing world: labs, pharmacies, training in regulatory trial conduct.
- New drug product development required up-front investment in formulation and scale-up.
- HUGE increase in costs for safety testing, clinical trials, drug manufacture and scale-up.
Top Priorities After N-9

- Engage FDA in defining a regulatory pathway for the evaluation and approval of microbicide products
- Develop clinical trial site infrastructure for regulatory trials
- Get the most promising products into the field to evaluate their effectiveness in large scale trials as soon as possible
Microbicides for Prevention of HIV-1 Transmission

- Reduce trauma to mucosa
- Maintain vaginal pH
- Reduce epithelial inflammation

What Intervention to Evaluate?

- No clear way yet to identify “best in class” which should move forward
- Real disagreement in the field regarding preference of broad spectrum vs. HIV-specific approach for microbicides
- Important to test different classes of microbicides having different mechanisms of action
Non-trial Issues of Relevance To Topical Microbicides

- Lack of a well-established correlation between in vitro, animal models, and clinical testing
- Insufficient knowledge on vaginal transmission of HIV and other STD pathogens
- Lack of optimal formulations
- Insufficient knowledge on cervico-vaginal and intercourse physiology
- Insufficient knowledge on of impact of contraceptive hormones, gel products and genital microbes on innate immune defenses in the vagina
<table>
<thead>
<tr>
<th>Sponsors</th>
<th>Product</th>
<th>No. Women Seen</th>
<th>No. Women to Enroll</th>
<th>Complete (Est)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIAID</td>
<td>BufferGel, PRO 2000</td>
<td>9000</td>
<td>3100</td>
<td>2008</td>
</tr>
<tr>
<td>Pop Council</td>
<td>Carraguard</td>
<td>12,540</td>
<td>6300</td>
<td>2007</td>
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<tr>
<td>FHI/USAID</td>
<td>Savvy</td>
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<tr>
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<tr>
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<td>PRO 2000</td>
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Carraguard, Cellulose sulfate and PRO 2000 are sulfated or sulfonated polysaccharides
Increasing Concerns Regarding Safety of Broad Spectrum Microbicides in 2007

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>OUTCOME</th>
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<tbody>
<tr>
<td>N-9 Gel</td>
<td>Trial stopped, evidence of harm</td>
</tr>
<tr>
<td>N-9 Film</td>
<td>Trial completed, no evidence of harm or benefit</td>
</tr>
<tr>
<td>Savvy (C31G)</td>
<td>Trial stopped due to futility, no evidence of harm</td>
</tr>
<tr>
<td>Cellulose Sulfate</td>
<td></td>
</tr>
<tr>
<td>CONRAD</td>
<td>Trial stopped, trend toward evidence of harm</td>
</tr>
<tr>
<td>FHI</td>
<td>Trial stopped, no harm</td>
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## Effectiveness Trials 2007

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Carraguard, Cellulose sulfate and PRO 2000 are sulfated or sulfonated polysaccharides.
Focusing on Future Success: The Pros and Cons

- The failure of N-9, cellulose sulfate and Savvy to have any impact on HIV transmission have made some people question whether any microbicide can ever work for prevention of HIV.
- The INCREASED risk of HIV in some studies has raised questions about whether any product can be safe enough.
- The microbicides having the greatest potency against HIV (ART’s) have not yet entered testing.
- Products used independent of coitus may not have the same impact on innate immunity.
- Products used daily will not require adherence to gel usage just prior to intercourse.
Why Would Topical Microbicides Increase Risk of HIV?

- Increase in local inflammation and recruitment of target cells.
- Disturbances of innate defense factors in the reproductive tract
  » Antiviral activity
  » Antimicrobial peptides
  » SLPI
  » Normal flora
- Exfoliation/disruption of epithelium

MTN IS EVALUATING THIS VERY CAREFULLY
### How Do We Discriminate Between Infection-Related vs. Microbicide-Related Changes in Innate Immune Function of the Reproductive Tract?

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<tr>
<th>Breaks in the Genital Epithelium: Genital Ulcer Disease</th>
<th>vs. Chemical Epithelial Disruption</th>
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<td><strong>Cervical Inflammation:</strong> GC, Ct, Trich</td>
<td>vs. Product-related inflammation</td>
</tr>
<tr>
<td><strong>Vaginal inflammation and symptoms:</strong> (BV, yeast, Trich) Irritation and Itching</td>
<td>vs. Vulvar/vaginal irritation due to product use</td>
</tr>
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</table>
Why Assess Vaginal Microflora?

1. Because altered flora is associated with acquisition of HIV
2. Because altered flora is linked with changes in cytokines, chemokines, SLPI and other surrogates of safety
### Longitudinal Studies Evaluating Vaginal Flora and HIV Acquisition in Women

<table>
<thead>
<tr>
<th>Author</th>
<th>No Women</th>
<th>Adjusted Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taha, AIDS, 1999</td>
<td>1196 Pregnant women in Malawi</td>
<td>Amsel criteria for BV One 1.5 Two 2.4 Three 3.7 P=0.04</td>
</tr>
<tr>
<td>Martin, JID, 1999</td>
<td>Sex workers in Mombasa, Kenya</td>
<td>Absence of vaginal lactobacilli 2.0 (95% CI 1.2-3.5)</td>
</tr>
<tr>
<td>Myer, JID, 2005</td>
<td>Non-pregnant women in Cape Town, S. Africa</td>
<td>Nugent &gt;7 OR 2.0 (95% CI; 1.1-3.6)</td>
</tr>
</tbody>
</table>
Vaginal Microbicides: Detecting Toxicities That Increase Pathogen Transmission

- Mouse HSV-2 Vaginal transmission model which
  - Directly tests for toxic effects that increase susceptibility to HSV-2
  - Provides assessment of anti HIV effect
  - Identifies toxic effects that correlate with HSV-2 susceptibility

- Evaluation of N-9, BZK, SDS, components of Savvy, BufferGel, HEC placebo

Ref: Cone et al, BMC Infect Dis 2006; 6:90
20 microliters of product applied to vagina of progesterone-treated mice

Antiviral activity assessed at 4 hour intervals for up to 24 hours compared to PBS treated controls

Factors assessed
  » Susceptibility to HSV
  » Number of cell layers
  » Cellular debris
  » Number of receptors
  » Supplementary cytokines

Ref: Cone et al, BMC Infect Dis 2006; 6:90
Vaginal HSV-2 Susceptibility and Toxic Effects vs. Time After a Single Dose of 2% N9
Vaginal HSV-2 Susceptibility 12 Hours After a Single Application of Candidate Microbicides

Ref: Cone et al
Vaginal HSV-2 Susceptibility and Toxic Effects vs. Time After a Single Dose of 2% N9

- N-9, Savvy components, BZK and SDS all enhanced HSV-2 susceptibility at 12 hours in mouse model
- Buffer Gel and HEC placebo did not impact HSV-2 susceptibility
- Important targets for safety studies: inflammatory cytokines, exfoliation of columnar epithelium

Ref: Cone et al, BMC Infect Dis 2006; 6:90
Assessing Anti-Viral Activity of Cervicovaginal Fluid Following Microbicide Use

- Assessment of vaginal fluid for anti-HIV and anti-HSV activity prior to and after use of topical microbicide
- HeLa cells or human macrophages inoculated with CVL spiked with replication defective HIV
- Human cervical cells inoculated with CVL and challenged with HSV-2

Assessing Anti-Viral Activity of Cervicovaginal Fluid Following Microbicide Use

- **Study population:**
  - 10 HIV+ women received 2g 0.5% PRO 2000 gel
  - 1 HIV+ women received 2g placebo
- Women with concurrent genital infection excluded
- Cervicovaginal lavage samples obtained 48 hours before and 1 hour after PRO2000 exposure
- Levels of IL-1B, IL-8 and SLP I in CVL measured at each visit.

Assessing Anti-Viral Activity of Cervicovaginal Fluid Following Microbicide Use

- Anti HIV activity –
  » 4.0 ± 1.3 log reduction (PRO 2000)
  » 0.2 ± 0.4 log reduction (Placebo)
- Anti HSV activity:
  » 3.0 ± .2 log reduction (PRO 2000)
  » 0.6 ± .2 log reduction (Placebo)
- Marked reduction in IL-1B, IL-8 and SLPI following product exposure, similar for PRO & Placebo

Assessing Impact of PRO2000 on Immune Factors in the Vagina

- 24 healthy nonpregnant women using PRO 2000 DAILY for 14 days
- PRO2000 elicits a decline in immune mediators in the vagina (Beta defensins, immunoglobulins, and IL-1 Ra, a range of cytokines) during product use
- No decline in intrinsic antiviral activity
- Will the temporary decline in some mediators enhance risk?

Is There Anything We Have Learned that Make us Worry About BufferGel and PRO2000?

- Best models available today show that BufferGel and PRO 2000 do NOT disrupt innate immune function in the vagina
- PRO 2000 has good activity against both HSV and HIV in cervicovaginal fluid
- Our blinded safety data to date do not show worrisome trends
The recent failures in the microbicide field should not discourage us from continuing to press forward with our current studies.

Newer ART based microbicides having high potency against HIV and used independent of coitus have enormous potential.
## Product Pipeline in 2007*

<table>
<thead>
<tr>
<th>Membrane Disruption</th>
<th>Defense Enhancers</th>
<th>Entry Inhibitors</th>
<th>Replication Inhibitors</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Clinical</td>
<td></td>
<td>PSC-Rantes Cyanovirin</td>
<td>MIV-150 TMC-125</td>
<td>Aptamers</td>
</tr>
<tr>
<td>Phase 1</td>
<td>Acidform™ Lactobacillus crispatus</td>
<td>VivaGel™ Invisible condom™</td>
<td>UC781 TMC 120 ring PMPA TMC120 gel</td>
<td></td>
</tr>
<tr>
<td>Phase 2/2B</td>
<td>Lactobacillus</td>
<td></td>
<td></td>
<td>Praneem Polyherbal</td>
</tr>
<tr>
<td>Phase 3</td>
<td>BufferGel™</td>
<td>Carraguard PRO 2000 (0.5%, 2%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Active planning, ongoing, or recently completed studies
I have my microbicide

I just took my V to a whole new level.