Pioneering systems biology in clinical microbicide trials – MTN-007 and beyond

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The ever interesting story of tenofovir gel

I. Immunological effects of tenofovir gel

II. Potential longer-term effects and how to study them

III. Hypothetical ramifications beyond the microbicide field
MTN-007 study design

4 study arms
N=15/arm

2% Nonoxynol 9 (N9) gel
1% Tenofovir gel
Placebo gel
No treatment

V1: Baseline
Min of 7 days

V2: Single gel application
30-45 minutes

V3: 7 once daily gel applications

V4: Colon Bx
Rectum Bx

MTN-007 rectal biopsies yielded excellent RNA quality and quantity

n=381
## Number of genes changing expression after 7 days of treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Up</th>
<th>Down</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonoxynol-9</td>
<td>60</td>
<td>56</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>137</td>
<td>505</td>
</tr>
<tr>
<td>HEC</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>No treatment</td>
<td>17</td>
<td>6</td>
</tr>
</tbody>
</table>

Consensus data across 8 study subjects
Tenofovir inhibits the anti-inflammatory arm of gut immunity

Confirmation by immunohistology

Rectal biopsies in MTN-007

IL-10

CD7

0

VII

IL-10

p = 0.0168

CD7

p = 0.0006

Positive cells / mm²

Positive cells / mm²
Do the immunological effects of tenofovir gel also occur in the genital tract?

- Will be investigated in MTN-014, a vaginal-rectal cross-over study of 1% tenofovir gel
- Was investigated *in vitro* with primary vaginal epithelial cells established from four healthy women
IL-10 in vaginal epithelial cells

The immunological effects of tenofovir are complex

- Tenofovir is an immune modulator rather than stimulator in the mucosa ("anti-anti-inflammatory")
- The consequences of this property on HIV susceptibility remain unclear, but ongoing studies in CAPRISA 004 subjects suggest that in the presence of inflammation they may become clinically relevant (Jo-Ann Passmore)
II. Potential longer-term effects and how to study them
Tenofovir increases epithelial cell proliferation ...
... and generally enhances cell viability

![Graph showing z-scores and log10(p-values) for epithelial cell death, leukocyte migration induction, and tumor cell viability.]

Proteomics: Adam Burgener
Tenofovir causes mitochondrial dysfunction

Mitochondrial ATP6 gene suppression

**Tenofovir**

- mRNA copies / 1000 HBB copies

**Nonoxynol 9**

- p = 0.0002
- p = 0.4911
AZT, another NRTI, was highly carcinogenic in topical rodent studies

### Incidence of Vaginal Histopathologic Findings in Mice Given Zidovudine Intravaginally

<table>
<thead>
<tr>
<th>Finding</th>
<th>EC</th>
<th>VC</th>
<th>5</th>
<th>20</th>
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<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>13</td>
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<tr>
<td>Epithelial dysplasia</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

*(n = 50 / arm)*

*Note. EC, environmental control; VC, vehicle control.*

Ayers KM et al.  
Nonclinical toxicology studies with zidovudine: genetic toxicity tests and carcinogenicity bioassays in mice and rats.  
Thus, carcinogenicity assessment of topical microbicides could be important, but is difficult.

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Systems and Carcinogenic Impact Assessment of Topical Microbicides on the Human Mucosa

- Systems studies: MTN-014 and MTN-017
- Carcinogenicity screening models
Carcinogenicity screening of microbicides

**HISTORIC STANDARD MOUSE CARCINOGENICITY STUDY**
(excessively expensive)

- AZT in saline (n=50)
- 0.9% saline (n=50)

Direct daily vaginal exposure ≤730 days

26% vaginal carcinomas in AZT arm (0% in placebo)

**PROPOSED MOUSE CARCINOGENICITY STUDY**

- Tenofovir gel (n=30)
- AZT gel (n=30)
- Dapivirine gel (n=30)
- Placebo gel (n=30)

Direct daily vaginal gel ≤120 days

Tumor formation

**PROPOSED HUMAN CELL CARCINOGENICITY STUDY**

- Cervix
- Rectum
  - (n=3 each)

Tenofavirov
AZT
Dapivirine
No Treatment
HPV E6/E7*
HPV E6/E7+Tenofovir*
HPV E6/E7+AZT*
HPV E6/E7+Dapivirine*

Cell culture 60 days

Mutagenicity
(Gene expression microarrays)

E6/E7+ cervical cells only

**Xenografts (n=126):**
10 mice/cell line + 6 positive controls

Tumor formation

*E6/E7 only with cervical cells

NSG

≤180 days

NSG
III. Hypothetical ramifications beyond the microbicide field
Hypothesized effects of NRTIs on HIV latency

NRTI

Integrated virus transcription inhibitor (IVTI)
- Lack of viral gene expression
  - Latently infected cells escape immune system
  - Decelerated decay rate of latently infected cells

Anti-anti-inflammatory
- Persistent immune activation
  - Latently infected cells persist and expand

Increased cell survival and proliferation
- Escape of natural cell death


There is as yet no clinical evidence whatsoever for NRTI carcinogenicity or latency-prolonging effects.
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Study Participants & Clinic Staff
MTN-007 Investigators

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