Disrupting HIV Maturation and Infectivity with Mercaptobenzamide Molecules

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Mercaptobenzamide: 247
Snapshot of HIV/AIDS today

• 37 people million infected
  - 25.5 million in Sub-Saharan Africa
  - 5.1 million in India / Southeast Asia
  - 1.6 million Eastern Europe / Central Asia
  - 2.1 million Latin America and Caribbean
  - 1.1 million in U.S.A. and North America
  - 0.9 million Western / Central Europe

• 20 Million people receiving antiretroviral therapy

• 30 drugs to treat HIV, used in combination, not a cure

• Cost: $20,000 to $36,000 per person per year for a lifetime (32 years)

• Countries where 10% or more new infections are resistant
  - Argentina, Guatemala, Namibia, Nicaragua, Uganda, Zimbabwe

54% of people infected with HIV are aware of their infection.

Antiviral Activity

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>TC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEM-SS (HIV-1(IIIB))</td>
<td>0.6</td>
<td>&gt;100</td>
</tr>
<tr>
<td>PBMC (HIV-1 Clade B)</td>
<td>5.74</td>
<td>&gt;100</td>
</tr>
<tr>
<td>monocyte-macrophage</td>
<td>1.97</td>
<td>&gt;100</td>
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</table>

- Remarkably non-toxic!
- Similar low micromolar activity against HIV Clades: A, B, C, D, E, F, G, O
- Antiviral activity is the same against HIV viruses with resistance to known drugs.
- No activity against HIV Reverse Transcriptase, Integrase, Protease, Viral Entry
- In combination with 23 FDA-approved HIV Drugs, synergistic or additive effects
  - No synergistic toxicity
- After several attempts, not able to generate 247-resistant HIV strains
- Not expensive to synthesize - $15 / gram
- 247 is a microbicide that can prevent SIV infection

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Application as Microbicide

Protection for Uninfected Women and Men who have sex with HIV-Infected Men

- Topical prevention
- Prevent HIV transmission during sexual intercourse
- CAPRISA Clinical Trial – July, 2010
- 1% tenofovir gel used by women in South Africa
- HIV transmission lowered 39%
- ASPIRE Clinical Trial – dapivirine in contraceptive ring
- February, 2016 – monthly usage reduces infections by 30%

Tenofovir
Nucleotide Reverse Transcriptase Inhibitor (NRTI)

Gel: Apply just before intercourse

Dapivirine
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)

Ring Formulation:
Slow release over 1 month (only for women)
Microbicide study in SIV-Infected Rhesus Macaques:

- Microbicide: 0.8% 247 in Hydroxyethylcellulose (HEC) Gel
- Animals challenged weekly, for total of 15 challenges
  
  (12 animals in microbicide group, 10 in control group)
- Intravaginal challenge 3 hours after application of microbicide
- Animals will be followed for 40 weeks after completion of challenges
**247** does not cause inflammation in SIV-Infected Rhesus Macaques:

Histology of vaginal biopsies collected day 7 post 1% SAMT vaginal gel administration

Ron Veazey, Tulane University School of Medicine

**247** can be formulated into rings for slow release:

Slow release of **247** measured over 30 days

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Patrick Kiser, Northwestern University

Marc Baum, John Moss
Oak Crest Institute of Science
HIV Viral Replication Cycle

**Entry**
- gp41
- gp120
- CD4
- CCR5

**Transcription**
- Viral RNA
- Integration

**Reverse Transcription**
- Viral RNA

**Assembly**
- Viral RNA
- New Viral RNA

**Translation to polyproteins**
- Gag

**Host Genome**
- Nucleocapsid Protein (NC)

**Maturation**
- HIV Protease

**Budding**
Drug Development:
- Entry
- Reverse Transcription (RT)
- Integration
- Maturation (Protease Inhibitors)
HIV Viral Replication Cycle

Drug Development:
- Entry
- Reverse Transcription (RT)
- Integration
- Maturation (Protease Inhibitors)

247 mechanism: Inactivation of Gag

Host Genome
Nucleus

Viral DNA
Viral RNA
New Viral RNA
Reverse Transcription
Integration
Translation to polyproteins
Assembly
Budding
Maturation
HIV Protease

Entry
CD4
CCR5

gp41
gp120

= Nucleocapsid Protein (NC)
Immature HIV Treated Cells

Electron Microscopy

Mature HIV Untreated Cells

HIV Infected Cell Treated with 247

Budded Immature HIV

Budded Mature HIV

HIV Infected Cell

David Ott (NCI)
247 specifically acetylates lysines and cysteines in HIV Gag

247 does NOT acetylate every lysine in HIV Gag.
In HIV-infected H9 Cells:

Treat with 247

Examining isolated virions:
Gag aggregation and processing defects

Not Infected

Infected

Radiolabel modifies Gag


David Ott (NCI)

Lisa Jenkins (NCI)
Conclusions:

- **247** reacts with HIV Gag by acetylation of cysteines and lysines
- **247** acetylates Gag at several cysteines and lysines, both *in vitro* and in cells
- There is a unique pattern of HIV Gag acetylation that disrupts viral maturation
- Not able to generate HIV resistance to **247**
- **247** is an effective microbicide against SIV *in vivo*, rhesus macaques
- **247** does not cause irritation
- **247** can be formulated into different delivery devices
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