Viral Resistance with Topical RT-Microbicides

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Overview

• What antiretrovirals (ARV) are being considered as candidate microbicides?
• How do they work?
• What is ARV resistance and how does it evolve?
• Could ARV resistance occur in microbicide trials?
• How might ARV resistance impact the design of MTN microbicide trials?
  – Phase 1/2
  – Phase 2B/3
## Approved Antiretroviral Drugs

<table>
<thead>
<tr>
<th>NRTI</th>
<th>NNRTI</th>
<th>PI</th>
<th>FI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zidovudine</td>
<td>Nevirapine</td>
<td>Ritonavir</td>
<td>Enfurvitide (Maraviroc)</td>
</tr>
<tr>
<td>Didanosine</td>
<td>Delavirdine</td>
<td>Indinavir</td>
<td></td>
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<tr>
<td>Zalcitabine</td>
<td>Efavirenz</td>
<td>Nelfinavir</td>
<td></td>
</tr>
<tr>
<td>Stavudine</td>
<td></td>
<td>Saquinavir</td>
<td></td>
</tr>
<tr>
<td>Lamivudine</td>
<td></td>
<td>Amprenavir</td>
<td></td>
</tr>
<tr>
<td>Abacavir</td>
<td></td>
<td>Lopinavir/r</td>
<td></td>
</tr>
<tr>
<td>Tenofovir</td>
<td></td>
<td>Fosamprenavir/r</td>
<td></td>
</tr>
<tr>
<td>Emtricitabine</td>
<td></td>
<td>Tipranavir/r</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Darunavir/r</td>
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</table>
## Microbicide Pipeline

<table>
<thead>
<tr>
<th></th>
<th>Pre-Clinical</th>
<th>Safety</th>
<th>Efficacy</th>
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</thead>
<tbody>
<tr>
<td><strong>Entry Inhibitors</strong></td>
<td>Cyanovirin BMS806 Plant lectins New Polyanions</td>
<td>VivaGel CAP Polystyrene sulfate</td>
<td>Pro2000 Carraguard Buffergel</td>
</tr>
<tr>
<td><strong>NRTI</strong></td>
<td></td>
<td>PMPA</td>
<td></td>
</tr>
<tr>
<td><strong>NNRTI</strong></td>
<td>DABO MIV-150</td>
<td>UC-781 TMC-120</td>
<td></td>
</tr>
<tr>
<td><strong>Membrane active</strong></td>
<td></td>
<td>SLS</td>
<td></td>
</tr>
<tr>
<td><strong>Unclassified</strong></td>
<td>Bacteria</td>
<td>Praneem</td>
<td></td>
</tr>
<tr>
<td><strong>Combination</strong></td>
<td>PC-815* Truvada NRTI/NNRTI NRTI/P NNRTI/P</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: PC-815 is a combination of NRTI/P and NNRTI.*
## RT-Inhibitor Microbicides

<table>
<thead>
<tr>
<th>Microbicide</th>
<th>Phase</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMPA (Tenofovir)</td>
<td>II</td>
<td>CONRAD/IPM</td>
</tr>
<tr>
<td>UC-781</td>
<td>I</td>
<td>CONRAD</td>
</tr>
<tr>
<td>TMC-120</td>
<td>I</td>
<td>IPM/Tibotec</td>
</tr>
<tr>
<td>PC-815 (MIV-150 + Carraguard)</td>
<td>Pre-clinical</td>
<td>Population Council</td>
</tr>
</tbody>
</table>
Adapted from Shattock and Moore, Nat Rev Microbiol, 2003
Mechanism of Action
Nucleoside and Nucleotide RTIs (NRTI)

- **Zidovudine (AZT)**
- **Stavudine (d4T)**
- **Zalcitabine (ddC)**
- **Lamivudine (3TC)**
- **Tenofovir (TDF)**
- **Didanosine (ddI)**
- **Abacavir (ABC)**
- **Emtricitabine (FTC)**
NRTI – Mechanism of Action

AZT (Zidovudine)

Intracellular metabolism

AZT-TP
NRTI – Mechanism of Action

AZT (Zidovudine)

Intracellular metabolism

AZT-TP

Incorporation by HIV RT

Competition!

Nascent DNA

Viral RNA/DNA
NRTI – Mechanism of Action

AZT (Zidovudine)

Intracellular metabolism

AZT-TP

Incorporation by HIV RT

dTTP

Competition!

Nascent DNA

Viral RNA/DNA

Incorporation by HIV RT

Chain-termination

Intracellular metabolism

Viral RNA/DNA

Incorporation by HIV RT

Chain-termination

Viral RNA/DNA

Incorporation by HIV RT

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Viral RNA/DNA

Incorporation by HIV RT

Chain-termination

Viral RNA/DNA
DNA Chain Termination by Nucleoside Analogues

Viral RNA

DNA

Viral RT

AZT
Nonnucleoside RTIs (NNRTIs)

Nevirapine (Viramune)

Efavirenz (Sustiva)

Delavirdine (Recriptor)
NNRTI Mechanism of Action

HIV-1 Drug Resistance

- High viral replication (~$10^{11}$ virions/day)
  - Error prone RT ($3 \times 10^{-5}$/bp/cycle)
- All single & many double mutants likely pre-exist
  - Rapidly selected by monotherapy or dual therapy with drugs for which 1-2 mutations confer resistance
- Multiple mutations are selected and accumulate with continued viral replication during therapy
  - Resistance/cross-resistance to multiple drugs
HIV-1 Drug Resistance

- Recombination between resistant variants
  - Speeds accumulation of mutations on the same genome
- HIV-1 target flexibility
  - Preserved function despite many substitutions
  - e.g., >25% of 99 amino acids in PR can vary
Fitness vs. Drug Resistance

- Drug-resistant variants are less fit than wildtype when drug is absent
  - Leads to decay of resistant variants when drug is removed
- Drug-resistant variants are more fit than wildtype when drug is present
  - Fitness advantage leads to emergence of the resistant variant
- Example
  - K65R: 3-10 fold resistance
  - 50% fitness of wildtype when drug is absent
Mechanism of NRTI Resistance

- Discrimination
- Excision
Discrimination

Resistance mutations enable HIV-1 RT to preferentially incorporate the natural dNTP substrate over the NRTI-TP

Examples: K65R, L74V, K70E, M184V
Discrimination

Excision

Resistance mutations facilitate excision or removal of the chain-terminating NRTI-MP from the 3’-terminus of the primer

**Examples:** Thymidine analogue mutations (TAMS)
Excision

Mechanism of NNRTI Resistance

Resistance mutations, such as K103N and Y181C, affect the association and dissociation constants of the NNRTI-RT binding interaction.
NNRTI Resistance

# RT Resistance

## Resistance

<table>
<thead>
<tr>
<th>Resistance</th>
<th>High-Level</th>
<th>Intermediate</th>
<th>Low-level</th>
<th>Contributes</th>
<th>None</th>
</tr>
</thead>
</table>

### Mutations

- **M184V**

- **3TC FTC**

- **AZT D4T TDF ABC DDI 3TC FTC**

- **http://hivdb.stanford.edu/cgi-bin/NRTIResiNote.cgi**
Limited Inherent Potency of the Regimen
  - Single/dual drug therapy
Suboptimal Drug Exposure
  - Incomplete Adherence
  - Unfavorable PK (or antagonism)
  - Resistant virus (de novo or transmitted)

Incomplete Inhibition of Viral Replication

Selection of Pre-existing Mutants
Evolution of New Mutants

Reduction in Drug Susceptibility

Limit Current/Future Treatment Options
Antiretroviral Resistance in the Clinic
Definitions are Important

• **Genotypic resistance**
  – Assay sensitivity
  – Single mutations
  – Multiple mutations
  – Use of Virtual Phenotype™

• **Phenotypic resistance**
  – Fold change in sensitivity (> 2.5, 5, or 10)

• **Virological response to ART**
  – Proportion with VL < 50, 400 copies per mL
  – Time to undetectable VL
  – Time to failure
Appearance of 3TC-Resistant Mutations in Treated Patients

Schuurman et al, JID 1995; 171:1411

Wild type at codon 184

RNA Copies/ml

Weeks after start of 3TC
Resistance Associated with Mother-to-Child Transmission Prevention Studies
Nevirapine Resistance

Eshleman S et al. AIDS Res Hum Retrovirol 2004
Consequences of NVP Resistance

Jourdain et al. NEJM 2004
ART Resistance in Treatment Naive Patients
# Prevalence of Resistant Virus in Treatment Naive Patients

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Prevalence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIEDRP (USA: 1995 - 2000)</td>
<td>377</td>
<td>12.4%</td>
<td>Little SJ et al. NEJM 2002</td>
</tr>
<tr>
<td>CPCRA (USA: 1999 - 2001)</td>
<td>491</td>
<td>11.6%</td>
<td>Novak RM et al. CID 2005</td>
</tr>
</tbody>
</table>
Is Primary Resistance to ARV Increasing?

Little SJ et al. NEJM 2002
Response* to ART in Subjects with Primary Resistance

Little SJ et al. NEJM 2002

Pillay D et al. AIDS 2006

*Proportion with HIV viral load > 500 copies mL plasma
RT-Microbicide Resistance Scenarios
Individuals with Chronic or Acute HIV Infection
Chronic HIV-1 infection Exposed to RT Microbicides

- Local selection of resistant variants is likely with a single drug
  - Potential for systemic dissemination
  - Potential for horizontal or vertical transmission
  - May persist for certain drugs – NNRTI

- Systemic selection will depend on drug exposure
  - If low exposure likely to be a minor resistant population and not detected by standard genotype methods

- Impact on response to subsequent therapy unclear
Pre-Existing Mutant at 0.01%

Limit of Detection for Std Genotype
Monotherapy Selects Mutant

Detected by Standard Genotype
Response to Treatment

% Mutant

Time (Months)

Re-selection of "Low Frequency" Mutant
Acute HIV-1 infection with Oral or Topical ARV

• For NRTI PrEP, SIV/macaque studies show that initial breakthrough infection is wild type! (unprotected cells)
  – Resistant virus will be selected with continued PrEP but not if PrEP is stopped in time
  – Should revert to wild type with PrEP discontinuation unless transmitted virus was drug-resistant (no wildtype)

• Breakthrough infection of topical PrEP is likely to be wild type with systemic dissemination related to systemic exposure
  – Risk of horizontal or vertical transmission of resistant virus if PrEP is continued
Modeling RT Microbicide Resistance

• Phase III placebo controlled study
• 10,000 women followed for 12 months
• Monte Carlo Simulation (N = 10,000)
• Model parameters
  – Clinical efficacy (0-90%)
  – High absorption (50 – 90%)
  – Low absorption (1-3%)

Wilson et al. CROI 2007 Abstract 999
Incidence of Resistance

Wilson et al. CROI 2007 Abstract 999
Frequency of HIV Testing

Wilson et al. CROI 2007 Abstract 999
What do We Know From HPTN-050?
<table>
<thead>
<tr>
<th>Group</th>
<th>Category</th>
<th>PMPA</th>
<th>Dose</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Sexually abstinent HIV-negative</td>
<td>0.3%</td>
<td>QD</td>
<td>12</td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td>1.0%</td>
<td>QD</td>
<td>12</td>
</tr>
<tr>
<td>A3</td>
<td></td>
<td>0.3%</td>
<td>BID</td>
<td>12</td>
</tr>
<tr>
<td>A4</td>
<td></td>
<td>1.0%</td>
<td>BID</td>
<td>12</td>
</tr>
<tr>
<td>B</td>
<td>Sexually active HIV-negative</td>
<td>1.0%</td>
<td>BID</td>
<td>12</td>
</tr>
<tr>
<td>C</td>
<td>Sexually abstinent HIV-positive</td>
<td>1.0%</td>
<td>BID</td>
<td>12</td>
</tr>
<tr>
<td>D</td>
<td>Sexually active HIV-positive</td>
<td>1.0%</td>
<td>BID</td>
<td>12</td>
</tr>
</tbody>
</table>
HPTN-050 PK Data

Oral 300 mg TDF single dose 24 hours post-dose (C24)

Oral 300 mg single dose C24*

LLOQ
HPTN-050 Virology

- HIV was detected in the plasma of 13/24 HIV+ women at Day 0 and 12/24 at Day 14, but in CVL of only 2 women at Day 0 and none at Day 14.
  - No new resistance mutations evolved in plasma or CVL after 14 days of TFV gel use.
  - No pt. had high level TFV mutations e.g K65R
Unanswered Questions

- What is the relationship between systemic absorption and the development of resistance?
- Will microbicide formulation or route of delivery alter risk of resistance?
- Could resistance occur during seroconversion?
- What about superinfection or viral recombination?
Trial Design Issues

• Which patients should be studied?
  – Seroconverters
  – Chronically infected

• What assay should be used to assess viral resistance?

• What samples should be evaluated?
  – Plasma
  – Cervicovaginal or rectal secretions
  – Tissue

• What duration of study?
Implications for MTN Trials (1)

• Phase 1/2 studies in HIV positive participants
  – Avoid inadvertent exposure of those with chronic HIV-1 infection to topical or oral ARV PrEP
    • Resistance selection is very likely
    • Subsequent transmission is possible
    • Could affect subsequent treatment response
Implications for MTN Trials (2)

- Detect acute HIV-1 infection on PrEP trials ASAP (HPTN-035, MTN-003)
  - Avoid selection of ARV-resistant virus
  - Could be transmitted
  - Could affect subsequent treatment response
- Possible need to increase frequency of HIV testing
- Study subsequent response to therapy carefully (MTN-015)
Summary

• RT microbicide resistance is likely in participants with chronic HIV infection who should not be enrolled in Phase 1/2 studies
• Phase 2B studies using RT microbicides should identify seroconverters ASAP and stop therapy
• Long term follow-up of these seroconverters is very important
Acknowledgements

John Mellors MD