The E138A Mutation Amplifies Resistance to Dapivirine in Combination with NNRTI Mutations

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on behalf of the MTN-020/ASPIRE Study Team

THE INTERNATIONAL WORKSHOP ON HIV DRUG RESISTANCE AND TREATMENT STRATEGIES
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Dapivirine Intravaginal Ring

- Dapivirine (DPV) is a diarylpyrimididine (DAPY) non-nucleoside RT inhibitor (NNRTI)

- A monthly intravaginal matrix ring (IVR) containing 25 mg of DPV reduced the risk of HIV-1 infection among African women.

<table>
<thead>
<tr>
<th>Study</th>
<th>HIV-1 incidence reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
</tr>
<tr>
<td>MTN020 (ASPIRE)</td>
<td>27%</td>
</tr>
<tr>
<td>IPM027 (RING)</td>
<td>31%</td>
</tr>
</tbody>
</table>

(Baeten J et al., NEJM 2016; Nel A et al., NEJM 2016; Brown E, AIDS 2016)
E138A was the most prevalent NNRTI mutation in the ASPIRE trial

Standard population genotype testing of all seroconverters
(n=168)

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Standard population genotype testing of all seroconverters (n=168)

Resistance mutations (n=24)

RTI mutations (n=18)  PI mutations (n=6)

(Baeten J et al., NEJM 2016)
E138A was the most prevalent NNRTI mutation in the ASPIRE trial.

- Standard population genotype testing of all seroconverters (n=168)
- Resistance mutations (n=24)
  - RTI mutations (n=18)
    - E138A (n=8)
  - PI mutations (n=6)
    - Other mutations (n=10)

(Baeten J et al., NEJM 2016)
E138A in HIV-1 Reverse Transcriptase

- Occurs naturally in 5% of treatment-naïve HIV-1-subtype C-infected individuals

- Selected by DAPY-class NNRTIs and causes 3-fold resistance to ETR and RPV
Questions

• Is E138A selected by DPV IVR ring?

• Does E138A confer resistance to DPV in subtype C virus from seroconverters?

• Could E138A reduce protective efficacy of the DPV IVR?
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• Does E138A confer resistance to DPV in subtype C virus from seroconverters?

• Could E138A reduce protective efficacy of the DPV IVR?
Similar frequency of E138A by arm in the ASPIRE trial

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Placebo Ring (N = 96)</th>
<th>DPV Ring (N = 68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V90I</td>
<td>1 (1%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>K101E</td>
<td>1 (1%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>K103N</td>
<td>2 (2%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>K103S</td>
<td>0 (0%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>V106M</td>
<td>0 (0%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>V108I</td>
<td>0 (0%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td><strong>E138A</strong></td>
<td><strong>5 (5%)</strong></td>
<td><strong>3 (4%)</strong></td>
</tr>
<tr>
<td>E138G</td>
<td>0 (0%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>V179D</td>
<td>2 (2%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>V179I/T</td>
<td>0 (0%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>H221Y</td>
<td>1 (1%)</td>
<td>1 (1.5%)</td>
</tr>
</tbody>
</table>

All differences were not significant, p > 0.05

(Baeten J et al., NEJM 2016)
Questions

• Is E138A selected by DPV IVR ring?

• Does E138A confer resistance to DPV in subtype C virus from seroconverters?

• Could E138A reduce protective efficacy of the DPV IVR?
Phenotyping participant samples containing E138A HIV

- Participant full-length HIV RT was cloned into xxLAI

- Transfected 293T cells to propagate recombinant viruses

- Assessed drug susceptibility to DPV in TZM-bl single round assay
E138A was associated with decreased susceptibility to DPV in some backgrounds but not others

<table>
<thead>
<tr>
<th>Arm</th>
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<tbody>
<tr>
<td>DPV</td>
<td>No DRM composite</td>
</tr>
<tr>
<td>DPV</td>
<td>E138A, V179I/T</td>
</tr>
<tr>
<td>DPV</td>
<td>V108I/V, E138A</td>
</tr>
<tr>
<td>DPV</td>
<td>E138A, V179D</td>
</tr>
<tr>
<td>Placebo</td>
<td>No DRM composite</td>
</tr>
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(Penrose, et al. CROI 2017)
E138A was associated with decreased susceptibility to DPV in some backgrounds but not others

<table>
<thead>
<tr>
<th>Arm</th>
<th>Genotype</th>
<th>Mean IC₅₀ ±SD (nM)</th>
<th>Fold-Change compared to wild type</th>
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<tbody>
<tr>
<td>DPV</td>
<td>No DRM composite</td>
<td>0.7 ± 0.2</td>
<td>-</td>
</tr>
<tr>
<td>DPV</td>
<td>E138A, V179I/T</td>
<td>4.0 ± 0.8</td>
<td>5.7</td>
</tr>
<tr>
<td>DPV</td>
<td>V108I/V, E138A</td>
<td>1.4 ± 0.3</td>
<td>2.0</td>
</tr>
<tr>
<td>DPV</td>
<td>E138A, V179D</td>
<td>0.4 ± 0.1</td>
<td>0.57</td>
</tr>
<tr>
<td>Placebo</td>
<td>No DRM composite</td>
<td>1.0 ± 0.3</td>
<td>-</td>
</tr>
<tr>
<td>Placebo</td>
<td>K101E, E138A</td>
<td>4.6 ± 1.4</td>
<td>4.6</td>
</tr>
<tr>
<td>Placebo</td>
<td>E138A</td>
<td>4.2 ± 0.9</td>
<td>4.2</td>
</tr>
<tr>
<td>Placebo</td>
<td>E138A</td>
<td>3.0 ± 0.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Placebo</td>
<td>E138A</td>
<td>1.2 ± 0.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Placebo</td>
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<td>1.4 ± 0.3</td>
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(Penrose, et al. CROI 2017) In bold: P < 0.01
Further analysis

• To explore the genetic basis for reductions in DPV susceptibility from E138A alone and in combination with other NNRTI mutations
E138A was associated with decreased susceptibility to DPV in some backgrounds but not others

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<tr>
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<td>1.4</td>
</tr>
</tbody>
</table>

(Penrose, et al. CROI 2017)
Methods: Systematic reversion of NNRTI DRM to wild-type

- **Bulk Clone**
  - Clone full length participant HIV RT into xxLAI

- **Single Colony Isolate**
  - Isolate single genome from bacterial culture

- **Reversion**
  - Systematically revert NNRTI DRMs to WT codon

- **Wild-type**
  - Complete reversion of all NNRTI DRM
E138A alone causes low level resistance to DPV

Arm: PLB ring

Bulk Clone

- **E138A**
  - $4.2 \pm 0.9 \text{nM}$
  - $4.2 \text{FC}$

Single Colony Isolate

- **E138A**
  - $4.6 \pm 1.7 \text{nM}$
  - $4.6 \text{FC}$

Reversion

- **Wild type**
  - $0.87 \pm 0.06 \text{nM}$
  - $0.87 \text{FC}$

$IC_{50}$ DPV$\pm$STDEV nM
E138A responsible for low level DPV resistance when combined with V108I

**Arm: DPV ring**

- **Bulk Clone**
  - V108I, E138A
    - IC₅₀: 1.4 ± 0.3 nM
    - 2.0 FC

- **Single Colony Isolate**
  - V108I, E138A
    - IC₅₀: 2.6 ± 0.9 nM
    - 3.7 FC

- **Reversion**
  - V108I
    - IC₅₀: 0.52 ± 0.0 nM
    - 0.7 FC
  - E138A
    - IC₅₀: 1.7 ± 0.5 nM
    - 2.4 FC

- **Wild type**
  - IC₅₀: 0.75 ± 0.1 nM
  - 1.1 FC
K101E and E138A have synergistic effect in increasing resistance to DPV

Arm: PLB ring

**Bulk Clone**
- K101E, E138A
  - 4.6 ± 1.6nM
  - 4.6FC

**Single Colony Isolate**
- K101E, E138A
  - 12 ± 1.4nM
  - 12FC

**Reversion**
- K101E
  - 3.5 ± 0.6nM
  - 3.5FC
- E138A
  - 4.1 ± 1.1nM
  - 4.1FC
- Wild type
  - 1.0 ± 0.1nM
  - 1.0FC

IC₅₀ DPV±STDEV nM
E138A and V179I/T have a synergistic effect in increasing resistance to DPV

<table>
<thead>
<tr>
<th>Arm: DPV ring</th>
<th>Bulk Clone</th>
<th>Single Colony</th>
<th>Isolate</th>
<th>Reversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>E138A + V179I/T</td>
<td>4.0 ± 0.8nM</td>
<td>12 ± 4nM</td>
<td>0.67 ± 0.1nM</td>
<td>0.75 ± 0.1nM</td>
</tr>
<tr>
<td></td>
<td>5.7FC</td>
<td>17FC</td>
<td>1FC</td>
<td>1.1FC</td>
</tr>
</tbody>
</table>

IC_{50} DPV±STDEV nM
E138A enhances DPV resistance in combination with some NNRTI DRMs in ASPIRE samples
Questions

• Is E138A selected by DPV IVR ring?

• Does E138A confer resistance to DPV in subtype C virus from seroconverters?

• Could E138A reduce protective efficacy of the DPV IVR?
In-vitro DPV levels far exceed resistance conferred by E138A

‡Mean cervical tissue (600 ng/mL) and cervical vaginal fluid (5,700 ng/mL) DPV concentrations found after 28 days (D28) of DPV ring use in the Phase 1 MTN-013 study
Findings

• Is E138A selected by DPV IVR ring?
  – No significant difference in E138A prevalence between arms

• Does E138A confer resistance to DPV in subtype C virus from seroconverters?
  – Low-level DPV resistance conferred by E138A in some seroconverters but not others

• Could the presence of E138A reduce protective efficacy of the DPV IVR?
  – In-vitro DPV levels far exceed resistance conferred by E138A
Additional findings

• In two seroconverters, E138A with another NNRTI DRM (V179I, V179T, K101E) had a synergistic effect in elevating DPV resistance.
  – These findings expand our understanding of resistance profiles for DPV
  – Resistance monitoring in seroconverters using investigational antiretroviral products for HIV prevention is critical.
MTN-020/ASPIRE Study Team

• MTN-020/ASPIRE leadership: Jared M. Baeten (protocol chair), Thesla Palanee-Phillips (protocol co-chair), Elizabeth R. Brown (protocol statistician), Katie Schwartz (FHI 360 senior clinical research manager), Lydia E. Soto-Torres (DAIDS medical officer)

• Study sites:
  – Malawi: Lilongwe site (University of North Carolina Project): Francis Martinson
  – South Africa: Cape Town site (University of Cape Town): Linda-Gail Bekker
  – South Africa: Durban eThekwini site (Centre for AIDS Programme of Research in South Africa): Gonasagrie Nair
  – South Africa: Johannesburg site (Wits Reproductive Health and HIV Institute): Thesla Palanee-Phillips
  – Uganda: Kampala site (Makerere University-Johns Hopkins University Research Collaboration): Flavia Matovu Kiweewe, Clemensia Nakabiito
  – Zimbabwe: Chitungwiza-Seke South, Chitungwiza-Zengeza, Harare-Spilvha sites (University of Zimbabwe-University of California San Francisco Collaborative Research Program): Nyaradzo M. Mgodi, Felix Mhlanga, Zvavahera M. Chirenje


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• Microbicides Trials Network Statistical and Data Management Center (Fred Hutchinson Cancer Research Center): Elizabeth R. Brown, Jennifer Berthiaume, Marla Husnik, Karen Patterson, Barbra A. Richardson, Daniel W. Szydlo


• International Partnership for Microbicides: Zeda Rosenberg, Annalene Nel

• MTN-020/ASPIRE participants and their communities; MTN-020 Community Working Group; MTN-020 Study Monitoring Committee; DAIDS MNDSMB

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