Sensitive Next-Generation Sequencing of HIV-1 in ASPIRE

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Dapivirine Intravaginal Ring (DPV IVR)

- Safe and effective to prevent HIV-1 infection in women
- Unlike tenofovir and FTC, DPV never used therapeutically
- Part of NNRTI class of drugs (same as NVP and EFV)
Risk of Resistance from DPV IVR Unknown

- Using DPV IVR during undetected acute infection
- Continuing DPV IVR after breakthrough infection
- Failure of DPV IVR to protect against partner with NNRTI resistant virus

SELECTED RESISTANCE

TRANSMITTED RESISTANCE
Look Carefully for Imbalance between Arms

Resistance in Dapivirine Arm

Resistance in Placebo Arm
Outline

• Methods used to detect HIV drug resistance

• Resistance objective in ASPIRE

• Preliminary Results
How HIV Drug Resistance is Measured

Sanger “Standard” Sequencing
Consensus of all HIV quasispecies in sample
≥20% detected

Next-Generation Sequencing (NGS)
Each virion individually sequenced
Thousands of sequences per sample
≥1% detected

Sample of HIV virions from plasma
Standard Sequencing Region

HIV Target Sequence

Protease
Codons 1 - 99

Full Length Reverse Transcriptase
Codons 1 - 560

Forward primer

Reverse primer
NGS Sequencing Region

Protease
Codons 1 - 99

Full Length Reverse Transcriptase
Codons 1 - 560

212 152 150

Mutations important for NNRTI resistance covered in this region

HIV Target Sequence
Principles of Sensitive NGS Assay

UMI = Unique Molecular Identifier
Unique tag for each HIV genome

HIV TARGET SEQUENCE

UMI

MiSeq Element
Needed for machine to read the sample

PTID ID
Allows multiplexing of samples

PTID ID

MiSeq Element
Principles of Sensitive NGS cont.

Thousands of sequences are generated per sample.

Sensitivity of resistance detection can be determined individually for each sample and depends on HIV recovery from sample.
# Standard Genotyping vs NGS

<table>
<thead>
<tr>
<th>Standard Genotyping</th>
<th>NGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene Region includes protease and full-length RT</td>
<td>Targeted gene region include part of RT important for NNRTI resistance</td>
</tr>
<tr>
<td>Long &amp; shallow sequence read length</td>
<td>Short &amp; deep sequence read length</td>
</tr>
<tr>
<td>One sequence per sample</td>
<td>Thousands of sequences per sample with each virus genome individually tagged</td>
</tr>
<tr>
<td>Detect mutations at 20% or greater</td>
<td>Detect mutations at 1% or greater</td>
</tr>
</tbody>
</table>
Objective

- To evaluate seroconverters in MTN-020 (ASPIRE) for evidence of HIV drug resistance associated with DPV ring use using standard genotyping and NGS.
HIV Diagnosis in ASPIRE

2 Rapid Tests

Western blot

HIV Diagnosis and Confirmation

One or two positive

- Product discontinuation
- Plasma collection for resistance testing
Plasma collected and stored after 1st positive rapid test. If confirmed positive...

- HIV RNA PCR
  - Performed at site
  - Results used for participant care

- Standard Genotype
  - Performed at Virology Core
  - Results returned to sites

- NGS
  - Performed at Virology Core
  - Research only – not for clinical care
## Methods

### Standard Genotyping

All seroconverters from both arms tested

### NGS

<table>
<thead>
<tr>
<th>PHASE I</th>
<th>DPV ARM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;95 pg/ml plasma DPV</td>
</tr>
<tr>
<td></td>
<td>residual drug levels of &lt;23 per 5 mg</td>
</tr>
<tr>
<td></td>
<td>at any follow up visit</td>
</tr>
</tbody>
</table>

**PLB ARM:** 1:1 random match

| PHASE II | Remaining specimens both arms |

Sample stored after first positive rapid tested
Results
Standard Genotyping

168 ASPIRE Seroconverters

165 (98%) Successfully Sequenced

69 DPV Ring Arm
96 Placebo Ring Arm

3 HIV RNA <200 c/mL
## DPV-Associated NNRTI Mutations: Standard

**Frequency among participants who acquired HIV-1 infection after enrollment while on study product**

<table>
<thead>
<tr>
<th>Mutation*</th>
<th>PLB Ring N = 96</th>
<th>DPV Ring N = 68</th>
</tr>
</thead>
<tbody>
<tr>
<td>L100I</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K103N</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>E138K</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Y181C</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*All differences were not significant between arms, p > 0.05*

*Based on in vitro selection and cross-resistance data from Schader SM et al. AAC 2012 and Fletcher P et al. AAC 2009*
Other NNRTI Mutations: Standard

Among participants who acquired HIV-1 after enrollment while on study product

<table>
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<tr>
<th>Mutation</th>
<th>PLB Ring (N = 96)</th>
<th>DPV Ring (N = 68)</th>
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</thead>
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<tr>
<td>V90I</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>K101E</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>K103S</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>V106M</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>V108I</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>E138A</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>E138G</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>V179D</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>V179T</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>H221Y</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

All differences were not significant between arms, p > 0.05
Full-length Analysis of RT

- No novel amino acid changes across all of RT were associated with seroconversion in the DPV arm
Standard Genotyping

NO DIFFERENCE

DPV RING ARM
8 of 69 (11.6%) with NNRTI mutations

PLB RING ARM
10 of 96 (10.4%) with NNRTI mutations
168 ASPIRE Seroconverters

123 Successfully Sequenced

62 DPV Ring Arm

61 Placebo Ring Arm

3 HIV RNA <200 c/mL
37 Not Selected for Testing*
5 Failed/Re-testing

*32 Placebo + 5 DPV ring non-adherent defined by low plasma drug levels or high residual ring levels
**DPV-Associated NNRTI Mutations: NGS**

Frequency among participants who acquired HIV-1 infection after enrollment while on study product

<table>
<thead>
<tr>
<th>Mutation</th>
<th>PLB Ring N = 61</th>
<th>DPV Ring N = 62</th>
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<tr>
<td>L100I</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K103N</td>
<td>0*</td>
<td>2</td>
</tr>
<tr>
<td>E138K</td>
<td>0</td>
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<tr>
<td>Y181C</td>
<td>0</td>
<td>0</td>
</tr>
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*1 PTID with K103N identified by standard genotyping not yet sequenced by NGS

K103N at 100% for both PTIDs same as standard genotype

No new low frequency DPV-associated mutations detected.
### Other NNRTI Mutations: NGS

Among participants who acquired HIV-1 after enrollment while on study product

<table>
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<tbody>
<tr>
<td>V90I</td>
<td>No difference</td>
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<tr>
<td>K101E</td>
<td>No difference</td>
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<td>K103S</td>
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<td>No difference</td>
<td></td>
</tr>
<tr>
<td>V108I</td>
<td>No difference</td>
<td></td>
</tr>
<tr>
<td>E138A</td>
<td><strong>Note</strong>*</td>
<td></td>
</tr>
<tr>
<td>E138G</td>
<td>No difference</td>
<td></td>
</tr>
<tr>
<td>V179D</td>
<td>No difference</td>
<td></td>
</tr>
<tr>
<td>V179T</td>
<td>No difference</td>
<td></td>
</tr>
</tbody>
</table>

*1 new E138A at 9% frequency detected in 1 PTID from PLB Ring Arm
Any Amino Acid Differences in RT? (NGS)

1. Evaluate full gene region amino acids 80 – 212 in RT
2. Compare number of low-frequency mutants (1 - 20% frequency) at every position
3. Chi-square test to compare placebo vs dapivirine arm
Other Amino Acid Differences: NGS

No significant differences between arms
NGS

DPV RING ARM
0 of 62 (0%) with low-frequency NNRTI mutations

PLB RING ARM
1 of 61 (1.6%) with low-frequency NNRTI mutations
Summary

• NNRTI mutation frequency did not differ by arm (p > 0.05)

• DPV-associated mutations E138K, L100I or Y181C were not detected in ASPIRE by standard or sensitive sequencing.

• The polymorphism E138A was the most common mutation amongst seroconverters but its frequency did not differ by arm.
Conclusion

• DPV-associated resistance mutations were not detected in ASPIRE by standard or sensitive resistance analysis.

• The frequency of NNRTI mutations in seroconverters from ASPIRE did not differ by arm indicating that the NNRTI resistance was likely transmitted and not selected by DPV ring use.
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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)

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- **Malawi**: Blantyre site (Malawi College of Medicine-John Hopkins University Research Project): Bonus Makanani, Taha E. Taha
- **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
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