Monitoring for Drug Resistance by Genotyping

Urvi M Parikh, PhD
MTN Virology Core Lab
Outline

- What is Drug Resistance?
- Genotyping Algorithm
- Standard vs Sensitive Resistance Testing
- Sequencing Protocols
  - ViroSeq
  - Allele-specific PCR
  - Single Genome Sequencing
- Interpreting the Data
What is drug resistance?

- High error rate of HIV causes misincorporations, resulting in changes in genome

- Some changes enable HIV to replicate in presence of antiviral compounds, thus reducing drug effectiveness
MTN Study Drugs

- MTN-001 – tenofovir
- MTN-002 – tenofovir
- MTN-003 – tenofovir, TDF, TDF/FTC
- MTN-004 – SPL7013 (VivaGel™)
- MTN-005 – non-medicated intravaginal ring
- MTN-015 – “seroconverter”
  - HPTN-035 – BufferGel, PRO2000/5 Gel
Mutations of Interest

Tenofovir

- K65R (3%)
- K70E (0.24%)
- L74V (rare)
- Q151M (rare)
- T69SS (rare)
- A62V and S68G
  - Compensatory
  - Replication capacity

FTC

- M184V

Virus with K65R causes resistance to FTC

M184V makes the virus MORE SUSCEPTIBLE to Tenofovir
Microbicide Resistance Unlikely

- **BufferGel**
  - Carbopol974P
  - Maintains acidic pH of vagina
  - Virus inactivated at pH 4 – 5.8

- **Pro2000/5**
  - Inhibits virus entry into cells
  - Non-specific mechanism

From Weber PLOS Med 2005
Genotyping Algorithm

Plasma Samples from VOICE

- ViroSeq Standard Genotyping
  - Resistance mutations detected
    - Report mutations
    - Subset of samples
    - Identify new mutations or linkages between known mutations
  - NO Resistance mutations detected
    - Subset of samples
    - Single Genome Sequencing (unknown changes)
- NO Resistance mutations detected
  - Report that participant does not have drug resistance
Why Sensitive Testing?

- Standard sequencing can miss mutations that are present at <25% of the population
- Minority variants can be associated with treatment failure (Johnson PLOS Med 2008)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Baseline Minority Mutations</th>
<th>Bulk Genotype Mutations at Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>M184V</td>
<td>Unk</td>
</tr>
<tr>
<td>25</td>
<td>M184V</td>
<td>M184V</td>
</tr>
<tr>
<td>31</td>
<td>K103N</td>
<td>K103N, M184V</td>
</tr>
<tr>
<td>41</td>
<td>K103N, M184V</td>
<td>K103N, M184V</td>
</tr>
<tr>
<td>44</td>
<td>K103N</td>
<td>K103N</td>
</tr>
<tr>
<td>63</td>
<td>K103N</td>
<td>Unk</td>
</tr>
<tr>
<td>67</td>
<td>Y181C</td>
<td>WT</td>
</tr>
</tbody>
</table>

All patients were reported as having wild type infection by standard sequencing.
To cDNA

Plasma virus

To cDNA

PCR Bulk cDNA

Detections the “majority” or “population” variant

Misses bases present at <25%

N = G and A

N = G and T

Reported T

Actually T and G

STANDARD SEQUENCE
(Population)
Protocol: ViroSeq Coverage

- **protease**
  - codons 1-99
  - Forward primer

- **reverse transcriptase**
  - codons 1-335
  - Reverse primer

1.8 kb RT PCR product

7 overlapping sequence primers:
- A
- B
- C
- D
- F
- G
- H
ViroSeq™ HIV-1 Antiretroviral Drug Resistance Report

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Drug</th>
<th>Evidence of Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRTI</td>
<td>EPIVIR® (lamivudine, 3TC)</td>
<td>Resistance**</td>
</tr>
<tr>
<td></td>
<td>EMTRIVA® (emtricitabine, FTC)</td>
<td>Resistance**</td>
</tr>
<tr>
<td></td>
<td>RETROVIR® (zidovudine, AZT)</td>
<td>Resistance**</td>
</tr>
<tr>
<td></td>
<td>VIDEX® (didanosine, ddI)</td>
<td>Resistance***</td>
</tr>
<tr>
<td></td>
<td>ZERIT® (stavudine, d4T)</td>
<td>Resistance***</td>
</tr>
<tr>
<td></td>
<td>ZIAGEN® (abacavir, ABC)</td>
<td>Resistance**</td>
</tr>
<tr>
<td></td>
<td>VIREAD® (tenofovir, TDF)</td>
<td>Resistance**</td>
</tr>
<tr>
<td>NNRTI</td>
<td>RESCRIPTOR® (delavirdine, DLV)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>SUSTIVA® (efavirenz, EFV)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>VIRAMUNE® (nevirapine, NVP)</td>
<td>None</td>
</tr>
<tr>
<td>PI⁺</td>
<td>AGENERASE® (amprenavir, APV)</td>
<td>Resistance*</td>
</tr>
<tr>
<td></td>
<td>LEXIVA® (ritonavir, POS)</td>
<td>Resistance*</td>
</tr>
<tr>
<td></td>
<td>CRIXIVAN® (indinavir, IDV)</td>
<td>Resistance***</td>
</tr>
<tr>
<td></td>
<td>FORTOVASE®/ INVIRASE® (saquinavir, SQV)</td>
<td>Resistance*</td>
</tr>
<tr>
<td></td>
<td>KALETRA® (lopinavir + ritonavir, LPV)</td>
<td>Resistance***</td>
</tr>
<tr>
<td></td>
<td>NORVIR® (ritonavir, RTV)</td>
<td>Resistance***</td>
</tr>
<tr>
<td></td>
<td>VIRACEPT® (neviravir, NFV)</td>
<td>Resistance***</td>
</tr>
<tr>
<td></td>
<td>REYATAZ® (atazanavir, ATV)</td>
<td>Resistance***</td>
</tr>
<tr>
<td></td>
<td>APTIVUS® (tipranavir, TPV)</td>
<td>Resistance***</td>
</tr>
</tbody>
</table>

Drug Class   | Drug Resistance Mutations Identified
Allele-Specific PCR (ASPCR)

1. **Plasma virus** to cDNA
2. 1st round PCR
3. Amplify target region
4. Quantify with CYBR green
5. Dilute DNA to 10⁷ copies/reaction
6. Amplify and Quantify with CYBR green

**Discriminatory Primer**
- Detects % Mutant

**Total Primer**
- Detects ALL
Allele-specific PCR (ASPCR)

Round 1 – Amplify pol region

HIV-1 pol

PCR Product – Dilute to $10^7$ copies

Detection Limit: 0.1% mutant
(Halvas, J Clin Micro 2006)
Single Genome Sequencing (SGS)

Plasma virus → To cDNA → Dilute to 30% positive → cDNA 1 copy/rxn → Sequence Positives

Sensitivity depends on number of genomes sequenced

20 sequences detects mutations present at 10%

Ref: Palmer et al., J Clin Micro 2005
Single Genome Sequencing (SGS)

<table>
<thead>
<tr>
<th>Certainty of Detection</th>
<th>1%</th>
<th>5%</th>
<th>10%</th>
<th>25%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>90%</td>
<td>230</td>
<td>45</td>
<td>22</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>95%</td>
<td>298</td>
<td>59</td>
<td>29</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>99%</td>
<td>459</td>
<td>90</td>
<td>44</td>
<td>16</td>
<td>7</td>
</tr>
</tbody>
</table>

# Sequences needed to detect mutation present at

Table from L. Halvás
## What is the difference?

<table>
<thead>
<tr>
<th>Method</th>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIROSEQ</td>
<td>CLINICAL (USA FDA-</td>
<td>Population genotype – major mutations</td>
</tr>
<tr>
<td></td>
<td>approved)</td>
<td></td>
</tr>
<tr>
<td>ASPCR</td>
<td>RESEARCH ONLY</td>
<td>% of a specific mutant</td>
</tr>
<tr>
<td>SGS</td>
<td>RESEARCH ONLY</td>
<td>All mutations, major and minor polymorphisms</td>
</tr>
</tbody>
</table>
## How will we use the data?

<table>
<thead>
<tr>
<th>Method</th>
<th>What we learn</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIROSEQ</td>
<td>If patient has virus with resistance mutations, can help decide what therapy to put her on</td>
</tr>
<tr>
<td>ASPCR</td>
<td>Gives an idea if patient has “undetected” resistance, and to what extent</td>
</tr>
<tr>
<td>SGS</td>
<td>Gives a picture of the diversity of virus in the patient to help better understand how resistance occurred</td>
</tr>
</tbody>
</table>
Finally, remember...

- If the microbicide **PROTECTS** against HIV, **drug resistance is not** an issue!!!
- Drug resistance is a concern if:
  - A positive person uses a microbicide
  - The microbicide does not protect and the participant becomes infected
- **MONITORING** for drug resistance can assure us that resistance is not occurring, or help identify the correct drugs for treatment
Questions?