Basics of HIV Resistance and Testing

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Virco

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Overview

- Review basic concepts on Resistance
- Determine how resistance tests work and are interpreted
- When should resistance tests be used?
- What is the relevance of the Resistance phenomenon and what do we know about resistance to new classes?
Overview

- Review basic concepts on Resistance
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What is Resistance?

- The reduced susceptibility of a patient’s viral isolate to suppression by an antiviral drug

- A change that improves viral replication in the presence of an inhibitor

- The point at which an ARV agent can no longer effectively inhibit viral reproduction
HIV-1: Replicative Cycle

1. Attachment
2. Entry
3. Reverse transcription
4. Integration
5. Transcription and translation
6. Assembly and budding

Host cell
Viral DNA
Proviral DNA
Hstl DNA
Nucleus
HIV
RNA
Wild Type Virus

- Non-mutant, drug-susceptible virus
- No previous effect from medication
- Reference virus
Quaisi-Species

- Viral isolates are composed of various groups of virus
  - Wild Type (Sensitive)
  - Mutated (Resistant)
  - Mutated (Non-resistant)

- Acquired resistance

- Selective pressure from current medication
Selective Drug Pressure

- Drug pressure drives selective forces for genetic changes in the viral genome
- **Mutations** arising under ART allow virus to escape from the inhibitory effect of the drug
- Mutations that develop are associated with ARV agents being administered
- “Minority variants” — <20% of population
Selective Drug Pressure

- Ongoing replication under selective pressure...

- Increasing amount of resistant mutations are able to develop

- No longer minority variants
Selective Drug Pressure

- Ongoing replication under selective pressure...
- Increasing amount of resistant mutations are able to develop
- What happens if ARVs are discontinued?
Selective Drug Pressure

- In absence of ARV pressure, resistant clones are “overgrown” with Wild Type virus and fade to “undetectable”
- Resistance is a genetic characteristic, so it is “archived” and can be re-expressed rapidly
- Absence of resistance on RT does NOT guarantee susceptibility!
- Clinical history is critical
Overview

- Review basic concepts on Resistance

- Determine how resistance tests work and are interpreted

- When should resistance tests be used?

- What is the relevance of the Resistance phenomenon and what do we know about resistance to new classes?
Types of Resistance Tests and a Few Examples

- **Genotype**
  - TruGENE® (Siemens)

- **Phenotype**
  - Antivirogram® (Virco)
  - PhenoSense™ (Monogram)

- **Calculated Phenotype:**
  - Virco® TYPE HIV-1 (Virco)
HIV Resistance Testing Assays

RESISTANCE

- The (in)ability of HIV to replicate in the presence of Antiretroviral Drugs
- Caused by changes in relevant parts of the virus genome (mutations)

Genotyping Assay
- Indirect measure of the viral susceptibility to Antiretroviral Drugs
- Based on sequence (mutations) of relevant parts of the viral genome
- Requires interpretation of sequence information
- QUALITATIVE

Calculated Phenotyping Assay
- Correlative database, frequently updated with GT and PT
- PT fold change of virus is calculated from mutations in GT, with interpretation supported by clinical outcomes data base
- QUANTITATIVE

Conventional Phenotyping Assay
- Direct measure of the ability of the virus to grow in the presence of antiretrovirals
- Compared to laboratory reference strain
- QUANTITATIVE
GENOTYPE
Genotypic assays step by step

1. PATIENT
2. PLASMA (> 200 µl)
3. Viral Gag/PR/RT gene isolation
4. total RNA
5. extraction
6. cDNA
7. RT
8. PCR
9. Gag/PR/RT GENES (amplicon)

Interpretation:
- Translate into aminoacids
- Compare wild type vs sample
- Identify mutational patterns (rules, expert opinion, etc.)

Automatic sequencing
Target genome regions

P7/P1  P1/P6

GAG  PRO  polymerase  RNaseH  INT

RT

Affymetrix  242
Bayer  300
Celera  330
ViroLogic  303
Virco  400

560
**Resistance associated RT Mutations:** No relevant mutations detected.

### Nucleoside and Nucleotide RT Inhibitors

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Resistance Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>zidovudine (AZT)</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>didanosine (ddI)</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>lamivudine (3TC)/emtricitabine (FTC)</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>stavudine (d4T)</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>abacavir (ABC)</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>tenofovir (TDF)</td>
<td>No Evidence of Resistance</td>
</tr>
</tbody>
</table>

### NonNucleoside RT Inhibitors

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Resistance Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>nevirapine (NVP)</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>efavirenz (EFV)</td>
<td>No Evidence of Resistance</td>
</tr>
</tbody>
</table>


### Protease Inhibitors

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Resistance Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>saquinavir + ritonavir (SQV/ir)</td>
<td>Resistance</td>
</tr>
<tr>
<td>indinavir (IDV)</td>
<td>Possible Resistance</td>
</tr>
<tr>
<td>IDV/ir **</td>
<td>Possible Resistance</td>
</tr>
<tr>
<td>nelfinavir (NFV)</td>
<td>Resistance</td>
</tr>
<tr>
<td>amprenavir (APV)/fosamprenavir (FPV)</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>APV/ir or FPV/ir **</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>lopinavir + ritonavir (LPV/ir)</td>
<td>Possible Resistance</td>
</tr>
<tr>
<td>atazanavir (ATV)</td>
<td>Possible Resistance</td>
</tr>
<tr>
<td>atazanavir + ritonavir (ATV/ir) **</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>tipranavir + ritonavir (TPV/ir)</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>darunavir + ritonavir (DRV/ir)</td>
<td>Possible Resistance</td>
</tr>
</tbody>
</table>

**Protease Inhibitors administered with low-dose ritonavir for pharmacological boosting**

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**Resistance interpretation is based upon interpretation by an international expert panel (The Consensus Panel) of in vitro and in vivo data including phenotypic and virologic response data available as of June 2006 for correlation of Protease and RT sequences to antiretroviral-drug resistance. These include primary and secondary mutations.**

* Comments marked with an asterisk pertain to Comment(s) in italics in the Mutation Details sections.
HIV Drug Resistance Mutations

The HIV Drug Resistance Mutations Figures and User Notes are regularly revised and disseminated by the IAS–USA Drug Resistance Mutations Group, an independent volunteer panel of experts focused on identifying key HIV-1 drug resistance mutations. The group strives to provide current, accurate, and unbiased information on these mutations for HIV practitioners. The mutations figures and accompanying text are published in Topics in HIV Medicine. The most recent revision is available in the April/May 2008 issue.

The IAS–USA has recently compiled a list of resources related to HIV drug resistant mutations. This list will be expanded in the upcoming months.

The Mutations Figures and User Notes are available as downloadable PowerPoint file. The figures will soon be available in pocket-sized folding cards, available in both Spanish and English. To request folding cards, complete the card request form and return via fax at (415) 544-9401, or mail to the address shown on the card. You may also send an e-mail to resistance2008 “at” iasusa.org, or call the IAS-USA at (415) 544-9400.
## Mutations Selected by nRTIs

<table>
<thead>
<tr>
<th></th>
<th>65</th>
<th>74</th>
<th>115</th>
<th>184</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abacavir</strong></td>
<td>V</td>
<td>M</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Didanosine</strong></td>
<td>V</td>
<td>M</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>M</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Emtricitabine</strong></td>
<td>V</td>
<td>M</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lamivudine</strong></td>
<td>V</td>
<td>M</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Stavudine</strong></td>
<td>M</td>
<td>D</td>
<td>K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>N</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td><strong>Tenofovir</strong></td>
<td>M</td>
<td>D</td>
<td>K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>N</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td><strong>Zidovudine</strong></td>
<td>M</td>
<td>D</td>
<td>K</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>N</td>
<td>R</td>
<td></td>
</tr>
</tbody>
</table>
Interpreting Resistance from Genotypic Reports

- Single mutation can confer resistance
  - M184 (3TC)  D30N (NFV)  I50L (ATV)

- Pairs of mutations

- Multiple or stairwise accumulation of mutations
  - LPV/r  DRV/r  AZT (NAMs)  ETR

- Multi-NRTI mutations: K65R, Q151M
Advantages and Disadvantages of Genotype Testing

**Advantages**
- Rapid turnaround (1-2 weeks)
- Less expensive
- Widely available
- More sensitive than conventional phenotype for detecting mixtures of resistant and wild-type virus

**Disadvantages**
- Indirect measure of resistance
- Relevance of some mutations unclear
- Complex mutational patterns may be difficult to interpret
- No standardized algorithms for interpretation of sequences
PHENOTYPE
Conventional Phenotype Testing

- Measures laboratory susceptibility of an HIV isolate to a given drug
- Measures the concentration of drug needed to inhibit the replication of a patient's virus
- Degree of resistance is quantified
  - Compares the fold-change in drug concentration required to inhibit the replication of the patient's virus compared to a representative, wild type, sensitive virus isolate
Conventional Phenotype Antivirogram® (Virco)

Creation of deleted molecular clone

WT molecular clone (HXB2)

Delete 3’ end of gag, PRO & RT genes

gag, PRO, RT

Deleted molecular clone

Gene transfer (nucleofection)

CD4+ T-cells (MT4)

Susceptibility assay (2-3 complete viral replication cycles)

Titrated to equivalent concentration for assays

Infectious RECOMBINANT virus
Conventional Phenotype Antivirogram® (Virco)

Monitoring of individual infected cells in 384 well plates

AZT

<table>
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<tr>
<th>0</th>
<th>0.0064</th>
<th>0.032</th>
<th>0.16</th>
<th>0.8</th>
<th>4</th>
<th>20</th>
<th>100 µM</th>
</tr>
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<tr>
<td>Res</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Phenotypic Susceptibility: Relationship Between Drug Concentration and Viral Inhibition

- Wild-type IC\textsubscript{50} = 10\textmu M
- Resistant IC\textsubscript{50} = 100\textmu M

Fold Change = 10

Fold resistance

IC\textsubscript{50} 100\textmu M
IC\textsubscript{50} 10\textmu M

= 10
Numbers need to be interpreted according to cut-points: Biological cut offs

Wild-type distribution

BCO = 97.5th percentile

Fold-change

Numbers need to be interpreted according to cut-points: Biological cut offs.
# Antivirogram® phenotype

<table>
<thead>
<tr>
<th>Patient/Sample Details</th>
<th>Physician Details</th>
</tr>
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<tbody>
<tr>
<td>Original ID</td>
<td>Sample Type</td>
</tr>
<tr>
<td>virco ID</td>
<td>Purified Amplicon</td>
</tr>
<tr>
<td></td>
<td>Collection Date</td>
</tr>
<tr>
<td></td>
<td>25-Jan-2008</td>
</tr>
<tr>
<td></td>
<td>Received by Virco</td>
</tr>
<tr>
<td></td>
<td>NA</td>
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<tr>
<td></td>
<td>Visit</td>
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<tr>
<td></td>
<td>26-Feb-2008</td>
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<tr>
<td>Virco ID</td>
<td>258991</td>
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</table>

## Drug Susceptibility

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Generic name</th>
<th>Susceptibility</th>
<th>Fold change in IC&lt;sub&gt;50&lt;/sub&gt; (Cut-off for normal susceptible range)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Fold change</strong> in IC&lt;sub&gt;50&lt;/sub&gt;, relative to reference virus (log&lt;sub&gt;10&lt;/sub&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NRTI / NRTI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrovir®</td>
<td>Zidovudine</td>
<td>![Green](Sample within normal susceptible range)</td>
<td><strong>10.2</strong> (2.5)</td>
<td></td>
</tr>
<tr>
<td>Epivir®</td>
<td>Lamivudine</td>
<td>![Green](Sample within normal susceptible range)</td>
<td><strong>2.2</strong> (2.1)</td>
<td></td>
</tr>
<tr>
<td>Videx®</td>
<td>Didanosine</td>
<td>![Green](Sample above normal susceptible range)</td>
<td><strong>0.6</strong> (2.3)</td>
<td></td>
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<tr>
<td>Zerit®</td>
<td>Stavudine</td>
<td>![Green](Sample above normal susceptible range)</td>
<td><strong>1.4</strong> (2.2)</td>
<td></td>
</tr>
<tr>
<td>Ziagen®</td>
<td>Abacavir</td>
<td>![Green](Sample above normal susceptible range)</td>
<td><strong>1.0</strong> (2.0)</td>
<td></td>
</tr>
<tr>
<td>Emtriva®</td>
<td>Emtricitabine</td>
<td>![Green](Sample above normal susceptible range)</td>
<td><strong>4.4</strong> (3.1)</td>
<td></td>
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<tr>
<td>Viread® *</td>
<td>Tenofovir DF</td>
<td>![Green](Sample above normal susceptible range)</td>
<td><strong>1.8</strong> (2.2)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>![Green](Sample above normal susceptible range)</td>
<td>![Red](Sample above normal susceptible range)</td>
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<tr>
<td><strong>NNRTI</strong></td>
<td></td>
<td></td>
<td>![Red](Sample above normal susceptible range)</td>
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</tr>
<tr>
<td>Viramune®</td>
<td>Nevirapine</td>
<td>![Green](Sample above normal susceptible range)</td>
<td>![Red](Sample above normal susceptible range)</td>
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<tr>
<td>Sustiva® , Stocrin®</td>
<td>Efavirenz</td>
<td>![Green](Sample above normal susceptible range)</td>
<td>![Red](Sample above normal susceptible range)</td>
<td></td>
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<tr>
<td>Intenence™</td>
<td>Etravirine</td>
<td>![Green](Sample above normal susceptible range)</td>
<td>![Red](Sample above normal susceptible range)</td>
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<tr>
<td><strong>PI</strong></td>
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<td></td>
<td>![Red](Sample above normal susceptible range)</td>
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<tr>
<td>Criviwan®</td>
<td>Indinavir</td>
<td>![Green](Sample above normal susceptible range)</td>
<td>![Red](Sample above normal susceptible range)</td>
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<tr>
<td>Viracept®</td>
<td>Nelfinavir</td>
<td>![Green](Sample above normal susceptible range)</td>
<td>![Red](Sample above normal susceptible range)</td>
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<td>Invirase®</td>
<td>Saquinavir</td>
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<td>![Red](Sample above normal susceptible range)</td>
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<tr>
<td>Lexiva®, Telzir®, a prodrug of</td>
<td>Amprenavir</td>
<td>![Green](Sample above normal susceptible range)</td>
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<tr>
<td>Kaletra®</td>
<td>Lopinavir</td>
<td>![Green](Sample above normal susceptible range)</td>
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<tr>
<td>Reyataz®</td>
<td>Atazanavir</td>
<td>![Green](Sample above normal susceptible range)</td>
<td>![Red](Sample above normal susceptible range)</td>
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<tr>
<td>Aptivus®</td>
<td>Tipranavir</td>
<td>![Green](Sample above normal susceptible range)</td>
<td>![Red](Sample above normal susceptible range)</td>
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<tr>
<td>Prezista™</td>
<td>Darunavir</td>
<td>![Green](Sample above normal susceptible range)</td>
<td>![Red](Sample above normal susceptible range)</td>
<td></td>
</tr>
</tbody>
</table>
Advantages and Disadvantages of Conventional Phenotype Testing

Advantages

- Provides direct and quantitative measure of resistance
- Often uses clinical cutoffs (CCO) derived from clinical cohorts to define spectrum of resistance
- Indicates which drugs may have partial activity
- Can assess interactions among mutations

Disadvantages

- Clinical cut-offs not defined for some agents
- Complex technology with longer turnaround (~ 3 wks)
- More expensive than genotyping
- Limited laboratories perform testing
VIRTUAL
PHENOTYPE
Correlative and Clinical Outcomes Databases*

- Routine clinical testing
- Clinical trials
- Research collaborations

Genotypic data >314,000
Phenotypic data >86,000
Correlative database >53,000 G/Ps

VirtualPhenotype™-LM engine

Clinical Outcomes Database
>21,000 patients or
>8,800 Treatment Change Episodes

Nucleotide sequence (...AAGTC TCCGCAT GCATA...)

Calculated fold change values in IC₅₀
Clinical Cut-Offs

*Status July 07
Creating the Phenotype from the Genotype

- Define mutations/pairs which impact the Fold Change for each drug
- Two factors in Virco G/P database:
  - The “Weight”: *How much* this mutation/pair changes the phenotypic Fold Change
  - The “Direction”: Does this mutation/pair lead to more resistance or more drug susceptibility?
- Each mutation (single and pairs) analyzed for these two factors
- A total “score” is created from the sum of these factors → the calculated “fold change”
### FC Assessment: Example of Tipranavir PI Mutation Analysis

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Decrease in FC</th>
<th>Increase in FC</th>
</tr>
</thead>
<tbody>
<tr>
<td>10C &amp; 33F</td>
<td>13A &amp; 47V</td>
<td>20T &amp; 73T</td>
</tr>
<tr>
<td>10F</td>
<td>13V &amp; 15V</td>
<td>20T &amp; 84V</td>
</tr>
<tr>
<td>10F &amp; 47V</td>
<td>13V &amp; 34Q</td>
<td>20V</td>
</tr>
<tr>
<td>10F &amp; 54M</td>
<td>13V &amp; 36I</td>
<td>22V</td>
</tr>
<tr>
<td>10F &amp; 58E</td>
<td>13V &amp; 43S</td>
<td>24F &amp; 60E</td>
</tr>
<tr>
<td>10F &amp; 82C</td>
<td>13V &amp; 71L</td>
<td>24I</td>
</tr>
<tr>
<td>10F &amp; 82F</td>
<td>13V &amp; 71V</td>
<td>24I &amp; 33F</td>
</tr>
<tr>
<td>10F &amp; 82L</td>
<td>13V &amp; 82F</td>
<td>24I &amp; 50V</td>
</tr>
<tr>
<td>10F &amp; 84A</td>
<td>13V &amp; 84V</td>
<td>24I &amp; 82T</td>
</tr>
<tr>
<td>10F &amp; 84V</td>
<td>14T</td>
<td>30N</td>
</tr>
<tr>
<td>10I &amp; 13A</td>
<td>15V &amp; 43I</td>
<td>30N &amp; 50V</td>
</tr>
<tr>
<td>10I &amp; 33M</td>
<td>15V &amp; 95F</td>
<td>30N &amp; 88D</td>
</tr>
<tr>
<td>10I &amp; 82F</td>
<td>16A</td>
<td>33.1Q</td>
</tr>
<tr>
<td>10I &amp; 82I</td>
<td>18H</td>
<td>35D</td>
</tr>
<tr>
<td>10V</td>
<td>20R &amp; 35D</td>
<td>33F &amp; 36L</td>
</tr>
<tr>
<td>10V &amp; 34A</td>
<td>20R &amp; 35D</td>
<td>33F &amp; 48A</td>
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<td>10V &amp; 88D</td>
<td>20T &amp; 33F</td>
<td>33F &amp; 60E</td>
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<tr>
<td>10Y &amp; 13V</td>
<td>20T &amp; 41K</td>
<td>33F &amp; 66L</td>
</tr>
<tr>
<td>12S &amp; 69K</td>
<td>20T &amp; 53L</td>
<td>33F &amp; 82F</td>
</tr>
</tbody>
</table>

**Legend:**
- **Decrease in FC:** Mutations leading to a decrease in drug efficacy.
- **Increase in FC:** Mutations leading to an increase in drug efficacy.
FC Assessment: Example of Tipranavir Mutation Analysis: Defining Fold-Change

PI Mutations Detected and Evaluated
3I, 10I, 14R, 19I, 24I, 37N, 41K, 46I, 53Y, 54V, 55R, 63P, 64V, 71V, 82T, 84V

<table>
<thead>
<tr>
<th>Mutations</th>
<th>RWF</th>
</tr>
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<tbody>
<tr>
<td>24I</td>
<td>-0.18198</td>
</tr>
<tr>
<td>41K</td>
<td>-0.06075</td>
</tr>
<tr>
<td>55R</td>
<td>0.05418</td>
</tr>
<tr>
<td>82T</td>
<td>0.36480</td>
</tr>
<tr>
<td>84V</td>
<td>0.16181</td>
</tr>
<tr>
<td>24I &amp; 82T</td>
<td>-0.10545</td>
</tr>
<tr>
<td>41K &amp; 54V</td>
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</tr>
<tr>
<td>54V &amp; 84V</td>
<td>0.20493</td>
</tr>
<tr>
<td>Intercept (no mutations)</td>
<td>-0.06039</td>
</tr>
</tbody>
</table>

Resistance Weight Factor: weight and direction for mutations which impact TPV
Log(FC) = 0.415
FC = 10^{0.415} = 2.6

Linear Models DB0704 implemented on DEC18, 2007
Types of Cut-Offs

● Biological Cut-Offs (BCO)
  ● Define what is resistant and non-resistant based on how a patient’s virus responds to a drug \textit{in vitro}

● Clinical Cut-Offs (CCO)
  ● Define what is resistant and non-resistant based on how a patient’s virus responds to a drug \textit{in vivo}
Defining Clinical Cut-Offs Based on Loss of Drug Effect

- Identify two cut-offs/drug as the virco®TYPE HIV-1 report predicted Fold Change associated with:
  - 80% loss of the contribution to response demonstrated by a wild type virus
  - 20% loss of the contribution to response demonstrated by a wild type virus
# Example Report

**vircoTYPE HIV-1**

The Complete Resistance Analysis

## ANALYZED SEQUENCE REGION

<table>
<thead>
<tr>
<th>DRUGS</th>
<th>DIV</th>
<th>CLADE</th>
<th>PATIENT ID</th>
<th>VIRCO ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEF</td>
<td>0.5</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

## RESISTANCE ANALYSIS

**DRUGS**

<table>
<thead>
<tr>
<th>DRUG</th>
<th>RESISTANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF</td>
<td>RESISTANT</td>
</tr>
<tr>
<td>TBV</td>
<td>RESISTANT</td>
</tr>
<tr>
<td>EFV</td>
<td>RESISTANT</td>
</tr>
<tr>
<td>NVP</td>
<td>RESISTANT</td>
</tr>
<tr>
<td>RAL</td>
<td>RESISTANT</td>
</tr>
</tbody>
</table>

## SUMMARY REPORT

**DRUGS**

<table>
<thead>
<tr>
<th>DRUG</th>
<th>RESISTANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF</td>
<td>RESISTANT</td>
</tr>
<tr>
<td>TBV</td>
<td>RESISTANT</td>
</tr>
<tr>
<td>EFV</td>
<td>RESISTANT</td>
</tr>
<tr>
<td>NVP</td>
<td>RESISTANT</td>
</tr>
<tr>
<td>RAL</td>
<td>RESISTANT</td>
</tr>
</tbody>
</table>

## ADDITIONAL CLINICAL NOTES

Note 1: The results are based on in vitro assays and may not necessarily predict the patient's response in vivo. The resistance profile is not a substitute for clinical judgment and should be considered in conjunction with other clinical information. For more information about the resistance profile, please visit [virco-type.com](http://virco-type.com).
Advantages and Disadvantages of Calculated Phenotype Testing

**Advantages**

- Require less interpretation of complex genotypes
- Less expensive, quicker than conventional phenotyping
- Assess impact of interactions between mutations
- Equivalent virologic outcomes in clinical trials to conventional phenotyping
- Available from many reference labs
- Rapid turnaround

**Disadvantages**

- Not an actual measured phenotype; a calculated phenotype based on genotypic information
- Reliability will depend on the accuracy of the genotype
- Only one source but widely distributed through many labs that perform genotyping
- Slightly more expensive than genotype alone
Overview

- Review basic concepts on Resistance

- Determine how resistance tests work and are interpreted

- When should resistance tests be used?

- What is the relevance of the Resistance phenomenon and what do we know about resistance to new classes?
**When to Use Resistance Testing**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary/acute</strong></td>
<td>Recommend</td>
<td>Recommend</td>
<td>Recommend</td>
</tr>
<tr>
<td><strong>Postexposure prophylaxis</strong></td>
<td>--</td>
<td>--</td>
<td>Recommend*</td>
</tr>
<tr>
<td><strong>Chronic, Rx naive</strong></td>
<td>Recommend</td>
<td>Recommend</td>
<td>Recommend</td>
</tr>
<tr>
<td><strong>Failure</strong></td>
<td>Recommend</td>
<td>Recommend</td>
<td>Recommend</td>
</tr>
<tr>
<td><strong>Pregnancy</strong></td>
<td>Recommend</td>
<td>Recommend</td>
<td>Recommend</td>
</tr>
<tr>
<td><strong>Pediatric</strong></td>
<td>--</td>
<td>Recommend</td>
<td>Recommend</td>
</tr>
</tbody>
</table>

Limitations of Resistance Testing

- High cost compared with other tests routinely used in HIV care
- Cannot be reliably performed when HIV RNA <500-1,000 copies
- May not be able to detect minority populations of resistant virus (<20%)
  - Especially common after drug discontinuation
- Resistant strains in viral reservoirs are not detected
Which Resistance Test and When?

- The utility of phenotypic resistance information increases with treatment experience/mutations complexity.
When To Use Resistance Testing

Naïve Patients / Starting Therapy

- If resistance mutations are not detected, it is still possible that the patient has been infected with a drug-resistant strain.

- The lack of drug pressure can cause the wild-type strain to dominate and minor resistant species may not be detected.
When To Use Resistance Testing

On Therapy

- Virologic failure: assist in selecting active drugs for next regimen
- Suboptimal viral load reduction
- Perform while patient is taking ARV agents or immediately after discontinuing therapy (within 4 weeks)
- All pregnant women prior to initiation of therapy and for those entering pregnancy with detectable viremia while on therapy

Resistance Testing in Clinical Practice

- **Genotype** preferred for:
  - Treatment naive: acute or chronic infection
  - Early virologic failure
  - Patient no longer receiving therapy

- **Phenotype or combined phenotype/genotype** preferred for:
  - High-level resistance to NRTIs or PIs on genotype
  - Multiple regimen failure with limited treatment options

- **Virtual phenotype** also used in settings where phenotypic testing would be preferred
  - Virtual phenotype is a type of complex interpretation of genotype data, intended to predict phenotypic response
Overview

- Review basic concepts on Resistance
- Determine how resistance tests work and are interpreted
- When should resistance tests be used?
- What is the relevance of the Resistance phenomenon and what do we know about resistance to new classes?
Resistance is associated with worse clinical outcomes

- In multivariable analyses, patients with drug resistance mutations to ≥ 2 classes during first 2 years of HAART at significantly higher risk of AIDS progression or death

<table>
<thead>
<tr>
<th>Definition of Resistance</th>
<th>Adjusted RH (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug-class resistance mutations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- ≥1 NRTI mutation</td>
<td>1.52 (1.14-2.03)</td>
<td>.004</td>
</tr>
<tr>
<td>- ≥1 NNRTI mutation</td>
<td>1.95 (1.28-2.95)</td>
<td>.002</td>
</tr>
<tr>
<td>- ≥1 PI mutation (major and minor)</td>
<td>1.50 (1.14-1.97)</td>
<td>.004</td>
</tr>
<tr>
<td>- ≥1 PI mutation (major only)</td>
<td>1.79 (1.28-2.50)</td>
<td>.0007</td>
</tr>
<tr>
<td>Cumulative drug-class resistance (major and minor PI mutations counted)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Virologic failure with no resistance</td>
<td>1.32 (0.57-3.06)</td>
<td>.52</td>
</tr>
<tr>
<td>- Single-class resistance</td>
<td>1.03 (0.65-1.63)</td>
<td>.90</td>
</tr>
<tr>
<td>- Double-class resistance</td>
<td>1.55 (1.15-2.08)</td>
<td>.004</td>
</tr>
<tr>
<td>- Triple-class resistance</td>
<td>1.80 (1.20-2.70)</td>
<td>.005</td>
</tr>
</tbody>
</table>
Summary of Key Conclusions

- Emergence of drug resistance within 2 years of initiating HAART in EuroSIDA cohort associated with long-term clinical outcomes
  - 55% increased risk of new AIDS events or death among individuals who developed double-class resistance
  - 80% increased risk of new AIDS events or death among individuals who developed triple-class resistance
- Mechanism driving association unknown
  - Possibly due to suboptimal adherence or direct effect of resistance

Resistance Scoring

- **Genotypic susceptibility** score = sum of genotypic resistance scores for each drug in regimen
  - Based on rule-based algorithms using predefined drug-resistance mutations
    - 1.0: susceptible
    - 0.5: possibly resistant
    - 0: resistant

- **Phenotypic susceptibility** score = sum of phenotypic resistance scores for each drug in regimen
  - Based on FC in susceptibility of test sample relative to control (wild-type) isolate
    - 1: susceptible
    - 0: resistant
    - Between 0 and 1: partially susceptible
Focus on Number of Active Agents

- DHHS antiretroviral guidelines: ≥2, preferably 3, fully active agents in new regimen
- Highest rate of virologic suppression in patients receiving investigational drug plus OBR containing ≥1 other active agent\(^{[1-4]}\)
- Trend toward greater benefit with 3 vs 2 fully active agents\(^{[1-4]}\)
  - Not statistically significant
  - Must also consider potential drug-drug interactions, adverse events, pill burden, absence of future options
  - Contribution of “partially active” agents (eg, 3TC) difficult to calculate
- No added benefit from using 4 vs 3 fully active agents

BENCHMRK-1 & -2: HIV-1 RNA < 50 c/mL at Week 48, Overall and by GSS

DUET-1 and -2: HIV-1 RNA < 50 c/mL at Week 48, by Active Agents in OBR

BENCHMRK 1 & 2: RAL Resistance at Virologic Failure

- 492 patients treated with RAL
- 105 (23%) had virologic failure
- Genotype available at baseline and after virologic failure for 94 patients
  - 68% (64/94) had genotypic evidence of RAL resistance
    - Nearly all (62/64) had mutations at position 143, 148, and/or 155

Integrase Inhibitor Cross-Resistance

- In RAL study, resistance to ELV with mutations at positions 148 and 155\(^1\)
  - Patterns associated with high-level resistance to both RAL and ELV
    - G140S/Q148H
    - G140S/Q148R
- In ELV study, mean decrease in susceptibility\(^2\)
  - To ELV: > 151-fold (range: 1.02- to 301-fold)
  - To RAL: > 28-fold (range: 0.78- to > 256-fold)
- No significant short-term virologic response to RAL in 2 patients switched from ELV/RTV to RAL following failure\(^3\)
- Cross-resistance is a clear issue with first-generation of agents

# Phenotypic Biological Cutoff for RAL

<table>
<thead>
<tr>
<th>DRUG</th>
<th>PHENOSENSE™ SUSCEPTIBILITY</th>
<th>ASSESSMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generic Name</td>
<td>Brand Name</td>
<td>Cutoffs (Lower - Upper)</td>
</tr>
<tr>
<td>Raltegravir</td>
<td>Isentress</td>
<td>(1.5)</td>
</tr>
</tbody>
</table>

- **Generic Name**: Raltegravir
- **Brand Name**: Isentress
- **Cutoffs (Lower - Upper)**: (1.5)
- **Fold Change**: >MAX
- **Increasing**: Hypersusceptibility Cutoff
- **Drug Susceptibility**: Reduced Susc.
- **Decreasing**: Reduced Susceptibility

**Drug Assessment**: RAL
Conclusions

- The resistance phenomenon is a relevant factor in choosing antiretroviral treatment regimens.
- Knowledge of the different methods to detect resistance is important in order to correctly interpret their results.
- Resistance has been demonstrated in new drug classes and cross-resistance may be a limiting factor in the future.
- Resistance is one of the multiple factors that influence treatment response.