Diagnostic Test
HIV Resistance Test

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Evolution of Viral Mutations

- Mutations arise because HIV-1 RT makes spontaneous errors (1 in $10^4$)
- HIV-1 genome is 10,000 ($10^4$) bases long, therefore 1 error each time the genome is replicated
- Production of virus = $10^9$ to $10^{10}$ virions per day → quasispecies
- Every possible mutation present in quasispecies before ARV therapy
Selection of Resistant strains

Treatment begins

Viral load

Time

Drug-susceptible quasispecies

Drug-resistant quasispecies

Selection of resistant quasispecies

Incomplete suppression

- Inadequate potency
- Inadequate drug levels
- Inadequate adherence
- Pre-existing resistance
Mutations described in the following way: e.g. M184V

- Initial letter represents the wild-type amino acid
- Number represents the mutated codon
- End letter represents the mutant amino acid
Patterns of resistance

1. PIs
   - Primary mutations:
     - V32I, G48V, I50V, V82A/F/T/S, I84V and L90M.
   - Secondary/accessory mutations:
     - 46, 47, 53, and 54
   - Polymorphisms associated with resistance:
     - 10, 20, 36, 63, 71, 77, and 93
Mutations associated with resistance to PIs

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<th>Comments?</th>
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<td>30</td>
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<td>48</td>
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- High Level Resistance
- Intermediate Resistance
- Low Level Resistance
- Contributes to Resistance
- No Resistance
- Unknown
- Hypersensitivity
Patterns of resistance (cont)

2. NRTIs
   - Thymidine Analog Mutations (TAMS)

   - Selected by AZT and/or d4T
   - Accumulation of mutations

   - 3TC
     - M184V

The OH group (essential for continuation of the growing chain) is absent in AZT and d4T.
Effect of TAMS and M184V

- Cross-resistance with d4T, ddI, ddC, 3TC
- 2 TAMS + M184V significantly reduces potency of ABC
- ≥3 TAMs including M41L or L210W significantly reduces activity of TDF
- M184V (3TC mutation) reverses the effect of the T215Y/F
- but, M184V effect is lost with multiple TAMS
3. NNRTIs
   - NVP (Nevirapine)
     - L100I, K103N, V106A/M, V108I, Y181C/I, Y188C/L/H, G190A
   - EFV (Efavirenz)
     - L100I, K103N, V106M, V108I, Y181C/I, Y188L, G190A/S, G225H
How do you measure drug resistance?

Genotyping:
Indirect assay: Detects drug resistance mutations that are present in the relevant virus genes.

Phenotyping:
Direct assay: Measures the ability of the virus to grow in various concentrations of antiretroviral drugs.
Genotyping using the Viroseq Kit

Load samples onto ABI 3100
Sequence Analysis
http://hivdb.stanford.edu/
Stanford HIV Drug Resistance Database

Database Query Pages

- **Protease inhibitors**, **RT inhibitors**
  Retrieve sequences of isolates from persons receiving a selected antiretroviral therapy

- **Protease mutations**, **RT mutations**
  Retrieve sequences of isolates containing selected mutations

- **Protease inhibitor susceptibilities**, **RT inhibitor susceptibilities**
  Retrieve published drug susceptibility data for isolates with selected mutations

  Mutation profiles: **Protease**, **RT**, **Position summary**

  Retrieve summary mutation data according to treatment and subtype

Other pages: **References**, **Advanced query pages**, **GenBank**, ...

Sequence Analysis Programs

- **HIVseq**
  Compare new RT and protease sequences to published sequences with the same mutations.

- **HIVdb**
  Infer drug resistance to 17 available drugs using rules hyperlinked to data within the database.

  **Release notes** for the above programs, for creating algorithms using the **Algorithm Specification Interface (ASI)**, and for comparing algorithms (**HIValgs**)

Drug Resistance Notes

- **NRTI Notes**, **NNRTI Notes**, **PI Notes**
  Overview of HIV drug resistance with links to relevant database entries

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<th>Protease inhibitors mutations</th>
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<tr>
<td><strong>Major resistance</strong></td>
<td>M461, I54V, L76V, V82A, L90M</td>
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<td><strong>Minor resistance</strong></td>
<td>L10FI, Q58E, A71V</td>
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<td><strong>Interpretation</strong></td>
<td>High level resistance</td>
<td>Atazanavir [ATV], fosamprenavir [FPV], indinavir [IDV], lopinavir [LPV], nelfinavir [NFV], saquinavir [SQV]</td>
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<tr>
<td><strong>Reverse transcriptase inhibitors (RTI) mutations</strong></td>
<td>Intermediate resistance</td>
<td>Darunavir [DRV] and tipranavir [TPV]</td>
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<tr>
<td><strong>Non-nucleoside reverse transcriptase inhibitor (NNRTI)</strong></td>
<td>None</td>
<td></td>
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<tr>
<td><strong>Interpretation</strong></td>
<td>High level resistance</td>
<td>Lamivudine [3TC], abacavir [ABC], zidovudine [AZT], stavudine [D4T], emtricitabine [FTC]</td>
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<td><strong>Intermediate resistance</strong></td>
<td>Didanosine [DDI], tenofovir [TDF]</td>
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<td><strong>Susceptible</strong></td>
<td>Delavirdine [DLV], efavirenz [EFV], etravirine [ETR], and nevirapine [NVP]</td>
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Final Diagnosis

Multidrug resistant HIV
References

- http://hivdb.stanford.edu
- http://www.hivresistanceweb.com
- http://www.hivatis.org
- http://hiv-lanl.gov/seq-db.html