Clinical Use of Drug Resistance Testing in HIV-1 Infection

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HIV-1 drug resistance

- Emergence of drug-resistant virus is an inevitable consequence of the failure to fully suppress HIV-1 replication.

- Drug resistance is a major factor contributing to the failure of antiretroviral therapy.
Possible Causes of Treatment Failure

- Poor adherence
- Pharmacologic factors
- Limited drug/regimen potency
- Host factors
- Drug resistance
Viral Population in an RNA Virus Infected Person

- A *quasispecies*
- Genetically distinct viral variants evolve from initial virus inoculum
- Variants are generated due to error-prone nature of RT
Resistance-associated mutations

- For some drugs (e.g., 3TC, NNRTI’s), single mutations can confer high-level resistance.

- For other drugs, high-level resistance requires 3 or more mutations within a single genome (e.g., ZDV, PI’s).

- Accumulation of additional resistance mutations after initial treatment failure suggests continued HIV-1 adaptation to growth in presence of drugs.
Rapid turnover of viral quasispecies

- Approximately half of the virus population in plasma is cleared and replaced each day.

- Rapid turnover allows rapid emergence of drug-resistant variants under selective pressure.

- Resistant variants may be replaced by residual wild-type virus if selective pressure is removed.

- Resting latently infected cells may continue to harbor drug-resistant provirus.
Nucleoside Resistance Mutations

AZT
- 41
- 67 70
- 215

ddI
- 65 74
- 210 219

ddC
- 65 69 74
- 184

3TC
- 65 74
- 115
- 184

ABC
- 62 69 75
- 116
- 151
- 210 215

MNR
- 62 69 75 77
- 116
- 151
- 210 215

SSS
Mutational interactions in HIV-1 RT

- M184V
- L74V
- M41L T215Y
- Y181C
NNRTI resistance mutations

NVP
103 108 181 190
106 188

DLV
103 181 236

EFV
103 190 225
100 108 188
Protease inhibitor resistance mutations

IDV: 10 20 24 32 46 54 63 71 73 82 84 90

RTV: 10 20 32 33 36 46 54 63 71 73 82 84 90

SQV: 10 20 46 48 54 63 71 73 82 84 90

NFV: 30 36 46 63 71 77 84 88 90

APV: 10 46 47 50 84
PR mutations in PI-naïve patients

n=45
Primary drug resistance in HIV-1

- **Wegner et al**
  - Recent (3 yr) seroconverters in the military (N=114)
  - NRTI - 1%; NNRTI - 5-7.7%; PI - 1% (Virco)
  - up to 20% if you include “intermediate” category

- **Little et al**
  - New seroconverters or patients with primary infection (N=133)
  - NRTI - 2%; NNRTI - 1%; PI - 2% (ViroLogic)

- **Boden et al**
  - Newly infected gay men in NYC, LA (N=80)
  - AZT or 3TC, 5-7.5%; NNRTI - 7.5%; PI - 2.5% (Virco)

- **Verbiest et al**
  - Survey of 133 treatment-naïve subjects in 5 cities
  - NRTI - 1%; NNRTI 2%; PI 2% (ViroLogic)
Not all PI failure is due to resistance

- Resistance to PI’s develops more slowly than resistance to other components of a regimen.
  - 3TC, EFV

- Initial failure of triple-therapy regimens associated with emergence of M184V mutation, not PI resistance mutations.
  - ACTG 343, ACTG 347, Trilège

- A regimen may fail without resistance to all components of that regimen.
Detecting drug resistance

- Genotypic assays
- Phenotypic assays
Genotypic assays for drug resistance

- Determine presence or absence of specific changes in HIV-1 genes (PR, RT).

- Pre-suppose knowledge of critical mutations.
  - Drug resistance is *inferred* by presence of known mutations.

- Various methods and platforms
  - automated dideoxynucleotide sequencing
    - ABI, Alf, VGI, “home brew”
  - hybridization-based sequencing
    - GeneChip, LiPA
QC of HIV-1 genotyping (ENVA 2)

- Coded panel of plasma specimens with wt or mutant HIV-1 strains in different proportions
  - Five mutations in PR and RT, respectively
- WT specimens correctly identified in most labs
  - RT 100%
  - PR 94%
- Mutant sequences identified less often
  - RT 66%
  - PR 71%
- In samples that contained 50:50 mix of WT:MUT
  - 37% detected all five mutations in RT
  - 49% detected all five mutations in PR

Schuurman et al. Rancho Bernardo, 1999 [Abstract 58].
Novel genotypes

- Survey of >9000 samples by Antivirogram and VircoGen sequencing.

- New mutations associated with resistance identified for each class of drugs.
  - require confirmation by site-directed mutagenesis

- Continued discovery of new resistance mutations complicates interpretation of genotypic assays.

Phenotypic assays of drug resistance

- Measure the $IC_{50}$ or $IC_{90}$ for a drug by recombinant virus assay.
  - Antivirogram (Virco)
  - PhenoSense (ViroLogic)

- Changes >2.5- to 4-fold reliably detected.

- Clinically relevant “break points” have not been determined for most drugs.
  - Assays measure drug susceptibility
  - Definition of “resistance” requires clinical correlation
Problems in defining drug resistance
HIV-1 drug resistance assays

RNA extraction → HIV-1 RNA → RT → HIV-1 cDNA → PCR → PR-RT amplicon

Plasma

HIV-1 recombinants

transfection → recombination

infectious HIV clone

HIV-1 plasmid

susceptibility assay

IC50 data
Technical limitations of resistance assays

- Generally, plasma samples with >500-1000 copies/mL of HIV-1 RNA are needed to generate results.

- Species constituting $\geq 20\%$ of amplified product can usually be detected.

- False positive and negative results possible from carryover from other HIV-1 samples or from random polymerase errors during PCR.
Relative Advantages of Assays

Genotypic Assays
- Availability
- Shorter time to results (days)
- Less technically demanding
- Mutations may precede phenotypic resistance

Phenotypic Assays
- Direct measure of susceptibility
- More familiar results (eg, IC$_{50}$ or IC$_{90}$)
Limitations of genotypic assays

- Indirect measure of susceptibility
- May not correlate with phenotype
- Expert interpretation may be required
- Insensitive for detecting minor species
Limitations of Phenotypic Assays

- Restricted availability
- Longer time to results (weeks)
- Clinically significant cut-offs not defined
- Insensitive for detecting minor species
Evidence supporting clinical benefits of resistance testing

- Retrospective studies
  - Genotype
  - Phenotype

- Prospective randomized trials
  - Viradapt
  - GART
Retrospective drug resistance studies

- **Deeks et al**
  - Phenotype predicts response to RTV/SQV salvage therapy.

- **Lanier et al**
  - Phenotype and genotype predict response to abacavir

- **Harrigan et al**
  - Baseline genotype and phenotype are significant predictors of response to RTV/SQV after PI failure

- **Zolopa et al**
  - Genotype is a significant *independent* predictor of response to salvage therapy after controlling for treatment history

- **Katzenstein et al**
  - Number of RT resistance mutations associated with failure

- **Lorenzi et al**
  - Number of PR and RT mutations independent predictor
HIV RNA Response: Number of Active Drugs

Deeks et al 1999
Effect of zidovudine and lamivudine mutations on HIV-1 RNA response to abacavir by week 16

*(Lower limit of detection = 100 copies/mL)*

Proportion of subjects with virologic response (%)

Baseline mutations
- Wild-type
- 1–2 ZDV only
- 1–2 ZDV + 184V
- 3 or more ZDV only
- 3 or more ZDV + 184V

R. Lanier et al
VIRADAPT

- Randomized trial of genotyping for management of patients failing antiretroviral therapy
- 108 patients (mean plasma HIV-1 RNA = 4.8 log)

<table>
<thead>
<tr>
<th>△ plasma HIV-1 RNA</th>
<th>genotyping</th>
<th>control</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>3 mos</td>
<td>-1.3</td>
<td>-0.6</td>
<td>0.021</td>
</tr>
<tr>
<td>6 mos</td>
<td>-1.3</td>
<td>-0.5</td>
<td>0.038</td>
</tr>
<tr>
<td>% &lt;200 copies/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mos</td>
<td>33%</td>
<td>16.7%</td>
<td>0.039</td>
</tr>
<tr>
<td>6 mos</td>
<td>39.1%</td>
<td>9.5%</td>
<td>0.047</td>
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GART (CPCRA 046)

- Randomized trial of genotyping vs clinical management.
- Expert advice regarding choice of regimen provided to patients in genotyping arm, but not to controls.
- Virologic failure defined as 3-fold increase in plasma HIV-1 RNA from baseline after $\geq 16$ wk treatment with 2 NRTI + PI.
- N = 153 patients
- Follow-up limited to 12 weeks.

Baxter et al. 6th CROI LB 8, Chicago, 1999.
GART (CPCRA 046) Results

- 73% of patients had major RT and PI resistance mutations
  - 20% had RT mutation w/o PI mutation
  - 4.6% had no resistance mutations

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<thead>
<tr>
<th></th>
<th>GART</th>
<th>Std of Care</th>
<th>p</th>
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<tbody>
<tr>
<td>ΔRNA</td>
<td>-1.17 log</td>
<td>-0.62 log</td>
<td>0.0001</td>
</tr>
<tr>
<td>% &lt;500</td>
<td>29%</td>
<td>17%</td>
<td>0.15</td>
</tr>
<tr>
<td># sens drugs</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>ΔRNA per drug (log/mL)</td>
<td>-0.1</td>
<td>-0.58</td>
<td>-1.02</td>
</tr>
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</table>

Baxter et al. 6th CROI LB 8, Chicago, 1999.
Each additional new drug to which virus was “sensitive” added 0.26 log decrease in HIV-1 RNA.

86% in GART arm received ≥3 active drugs vs 30% in control arm.

GART resulted in a recommended change in regimen in 85% of patients, but only 54% followed through on this advice.

Baxter et al. 6th CROI LB 8, Chicago, 1999.
Individualized approach to treatment

VIRAL LOAD

RESISTANCE MONITORING

Rx1

VL Detection limit

Rx2

Resistance limit

TIME

Resistance limit

VL Detection limit
Possible uses for drug resistance testing

- Primary HIV Infection
- Before starting therapy
- Changing therapy
  - Early failure
  - Late failure
- Pregnancy
- Post-exposure prophylaxis
Confirmed increase in plasma HIV-1 RNA level should be the main trigger for considering change in therapy.

No substitute for thorough treatment history in choosing new regimens.

If resistance to a drug is detected, use of that drug in a regimen should be avoided (if possible).
Drug Resistance Testing: Caveats

- Resistance tests are most accurate in assessing resistance to the *current* regimen.

- Absence of resistance to a previously used drug does not rule out reservoirs of resistant virus that may emerge after re-initiation of that drug.

- If resistance to a given drug has *ever* been detected, that drug should probably not be used again, even if current test results suggest viral susceptibility.