Clinical Use of Drug Resistance Testing in HIV-1 Infection

Daniel R. Kuritzkes, MD

Associate Professor of Medicine & Microbiology

University of Colorado Health Sciences Center

HIV-1 drug resistance

- Emergence of drug-resistant virus is an inevitable consequence of the failure to fully supress 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

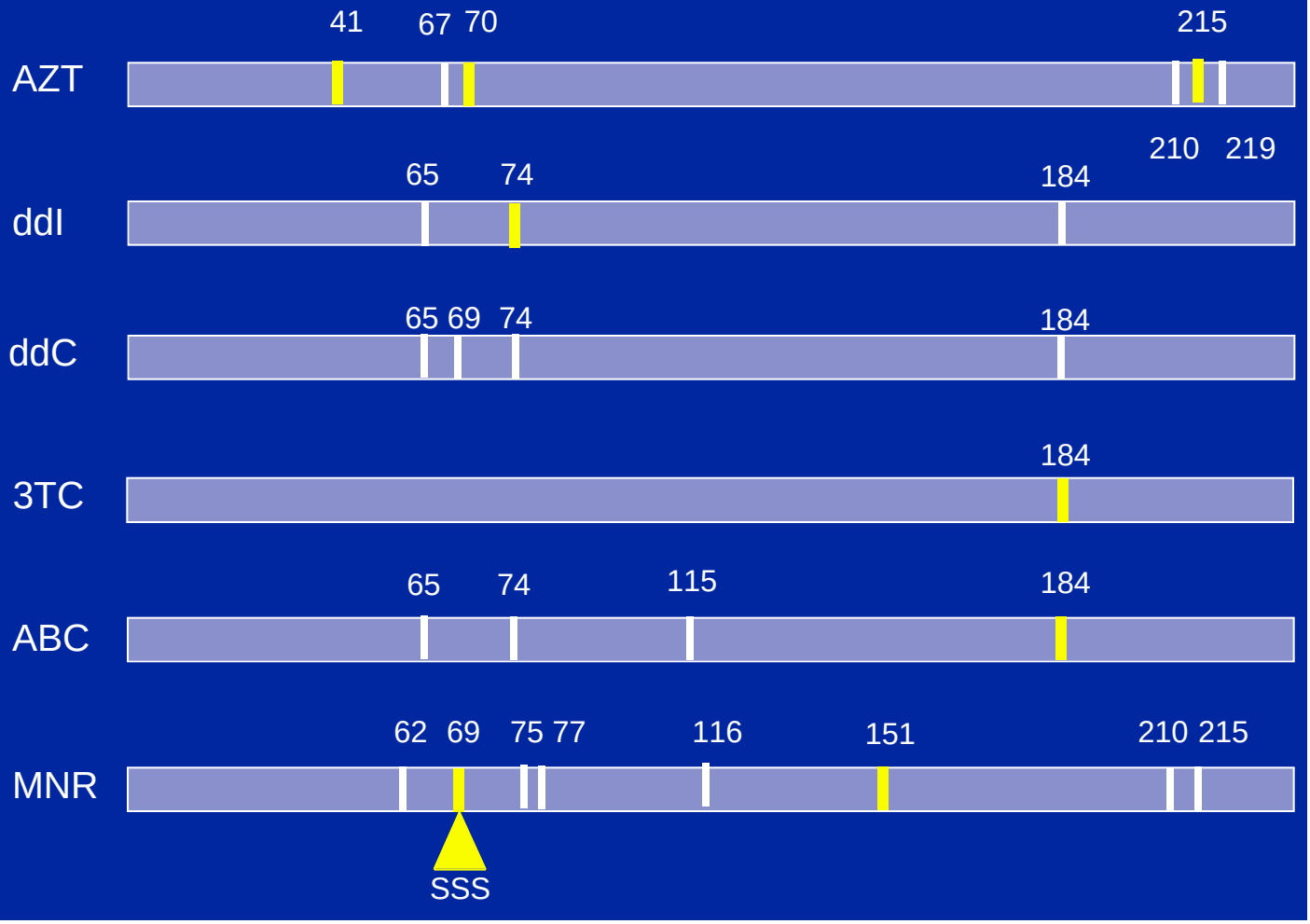
Resistance-associated mutations

- For some drugs (eg, 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 (eg, ZDV, PI's).
- Accumulation of additional resistance mutations after initial treatment failure suggests continued HIV-1 adaptation to growth in presence of drugs.

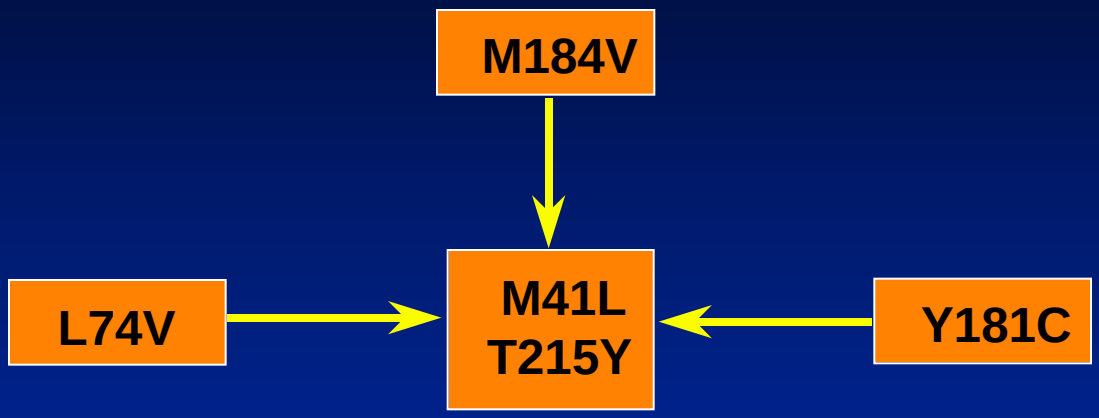
Rapid turnover of viral quasispecies

- 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.

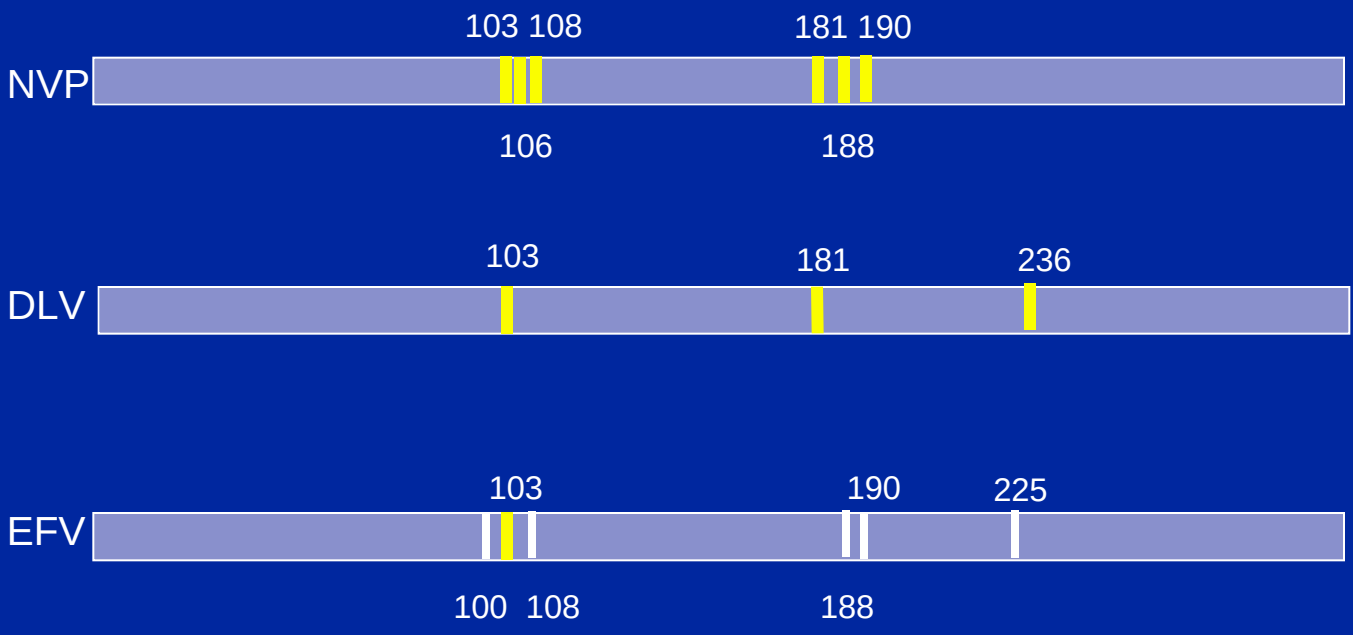
Nucleoside Resistance Mutations



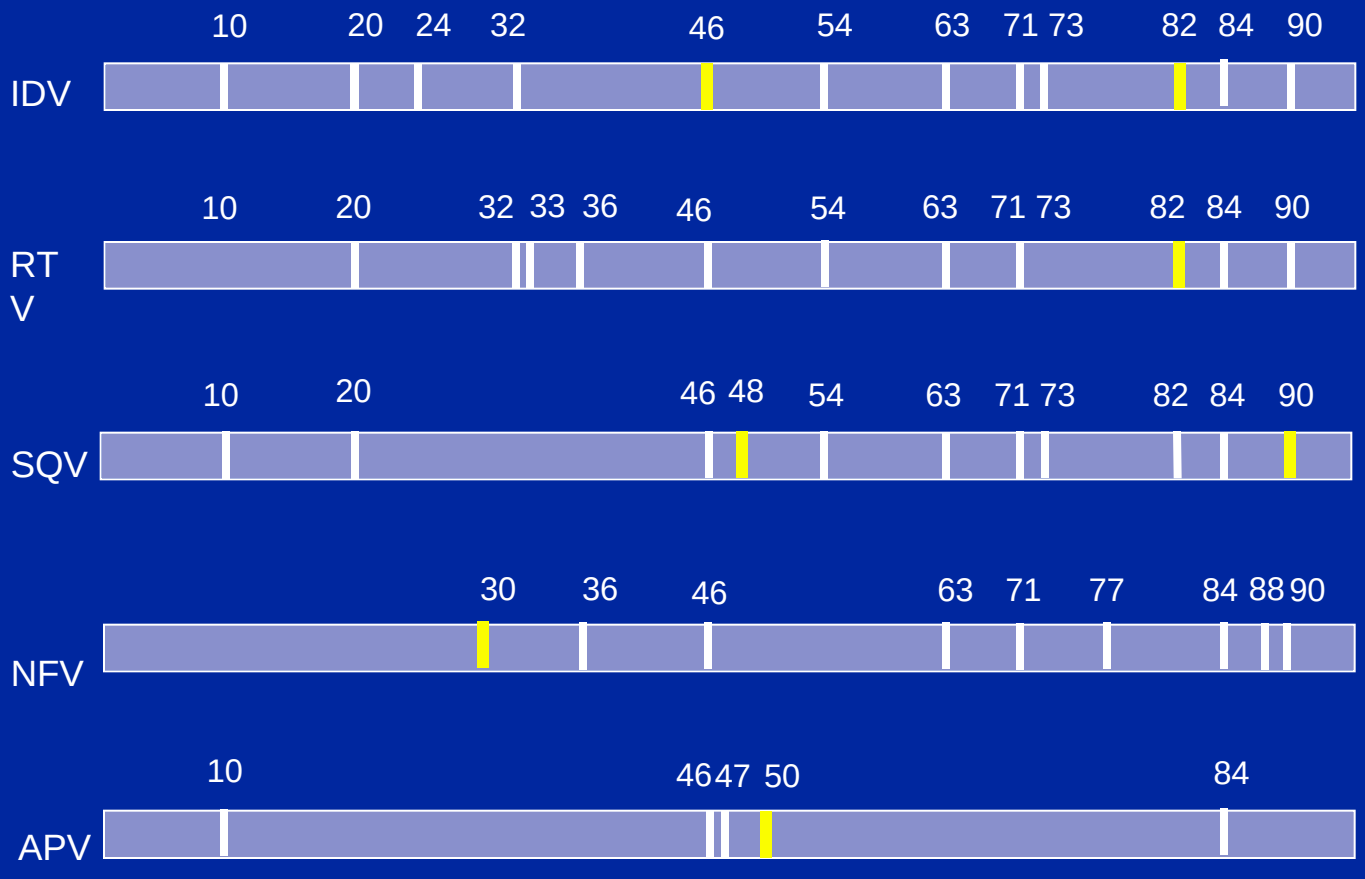
Mutational interactions in HIV-1 RT



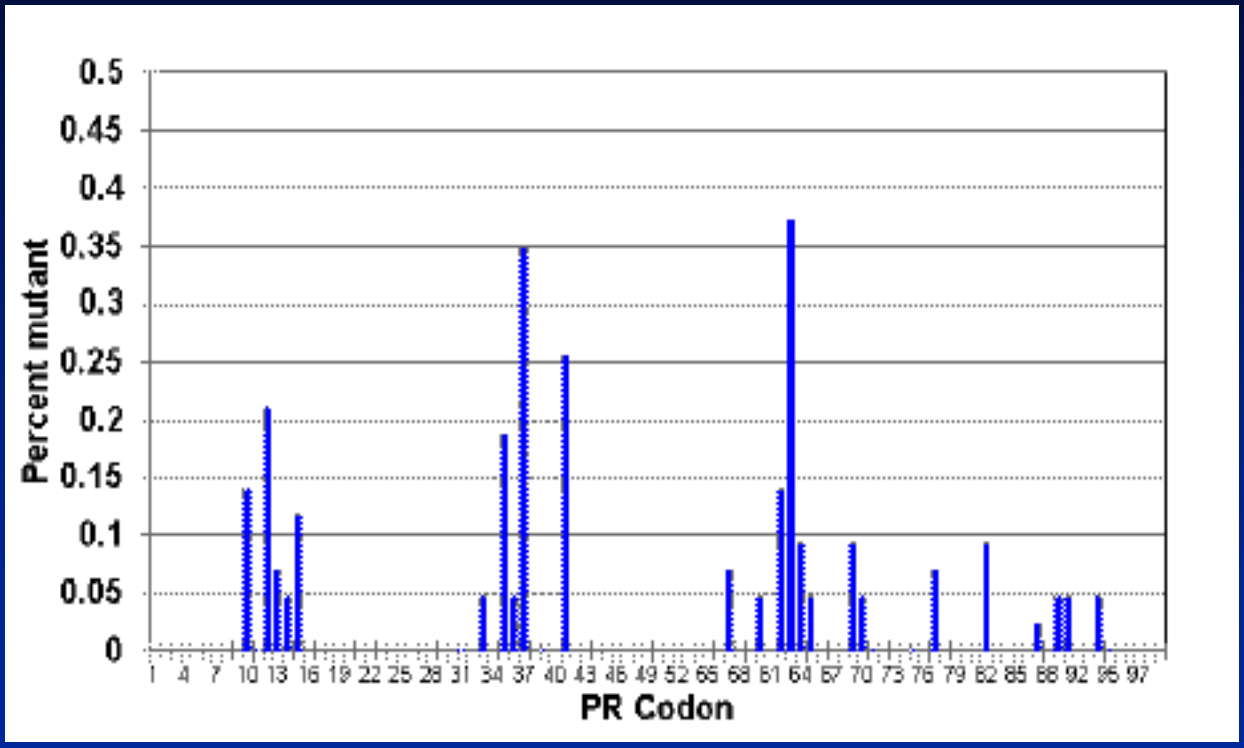
NNRTI resistance mutations



Protease inhibitor resistance mutations



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

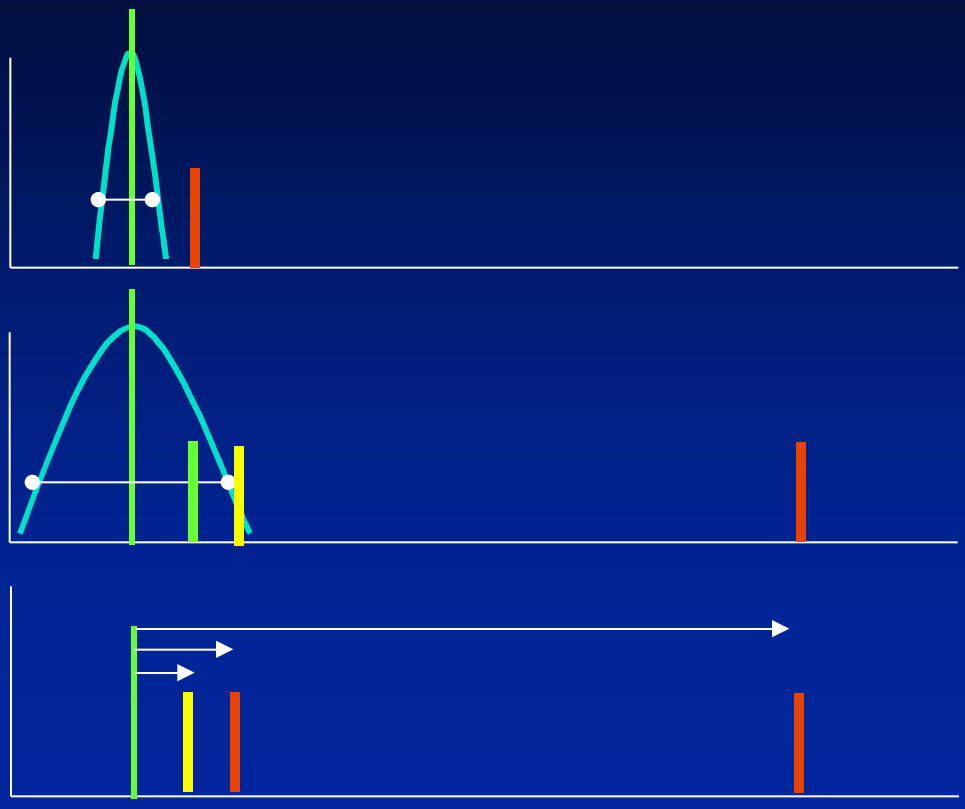
- Survey of >9000 samples by Antivirogram and VircoGen sequencing.
- New mutations associated with resistance identified for each class of drugs.
  - require confirmation by site-directed mutagenesis
- Continued discovery of new resistance mutations complicates interpretation of genotypic assays.

Hertogs et al, Rancho Bernardo, 1999.

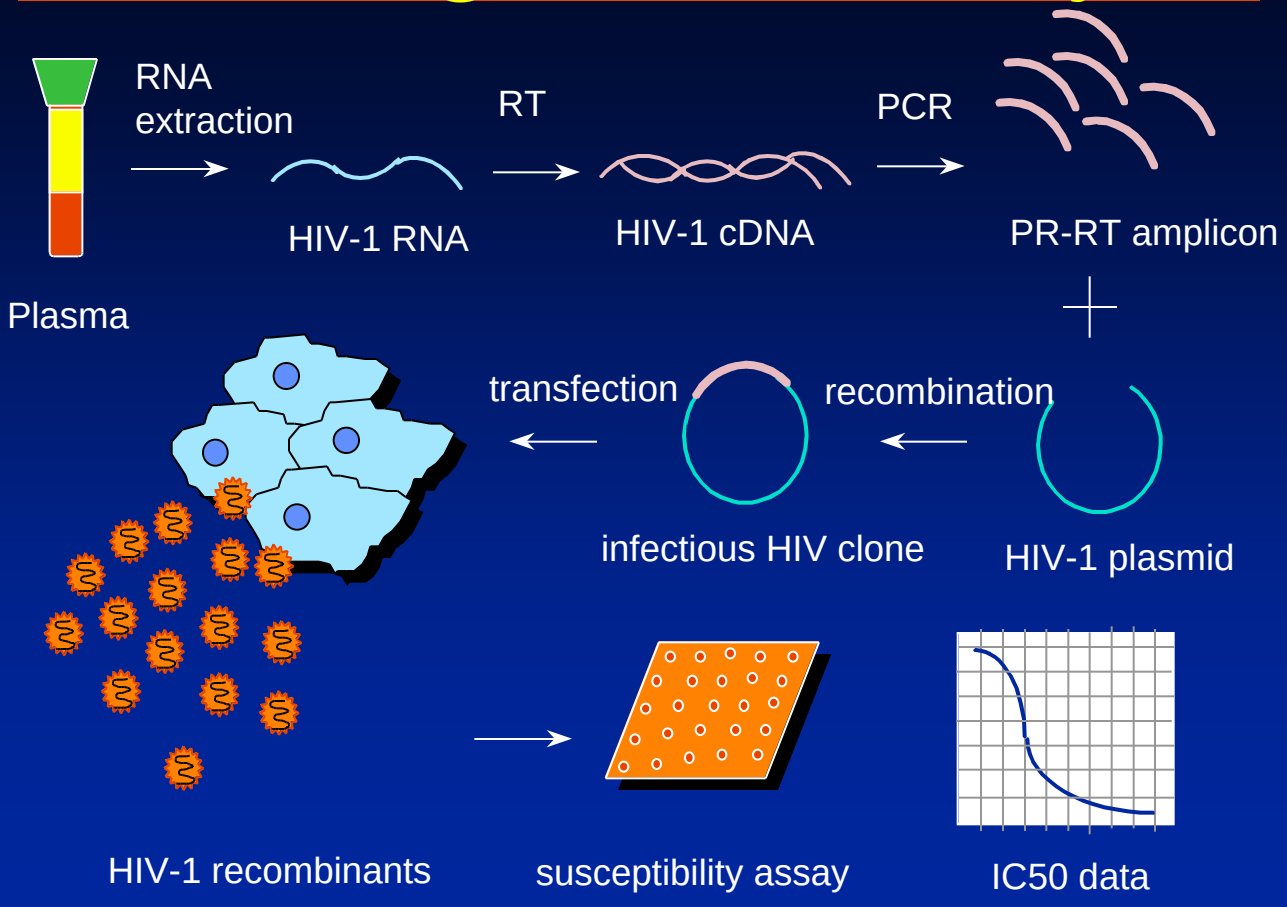
Phenotypic assays of drug resistance

- 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



Technical limitations of resistance assays

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

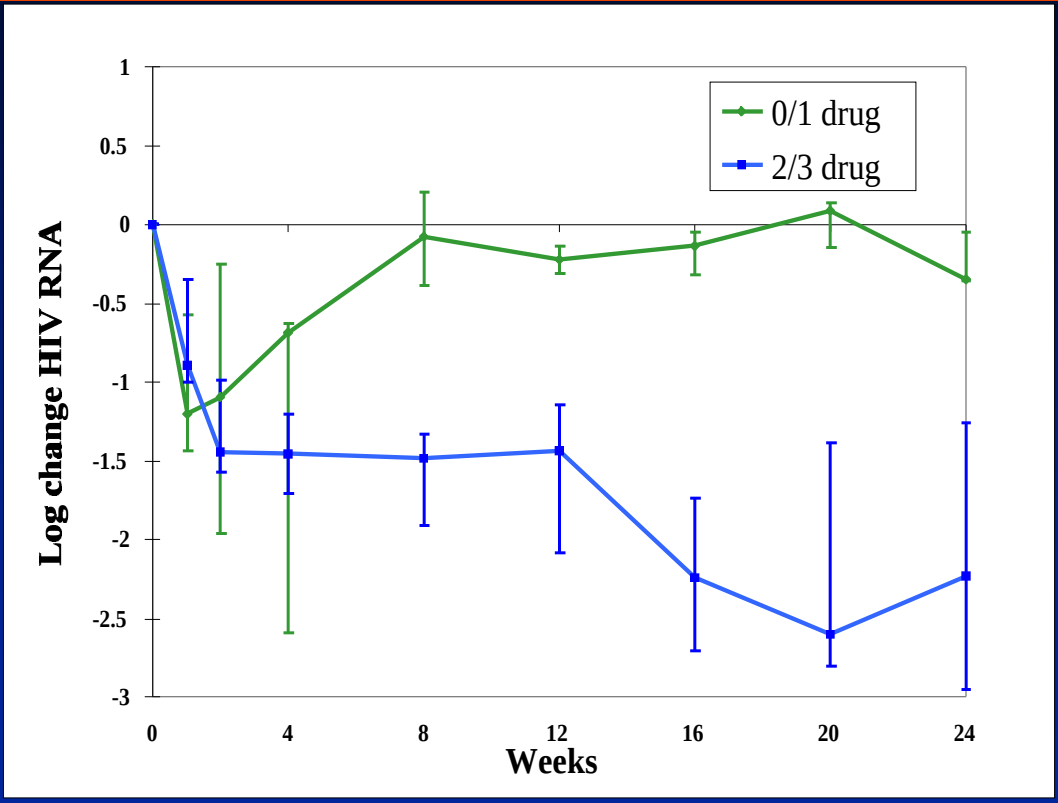
Evidence supporting clinical benefits of resistance testing

- Retrospective studies
  - Genotype
  - Phenotype
- Prospective randomized trials
  - Viradapt
  - GART

Retrospective drug resistance studies

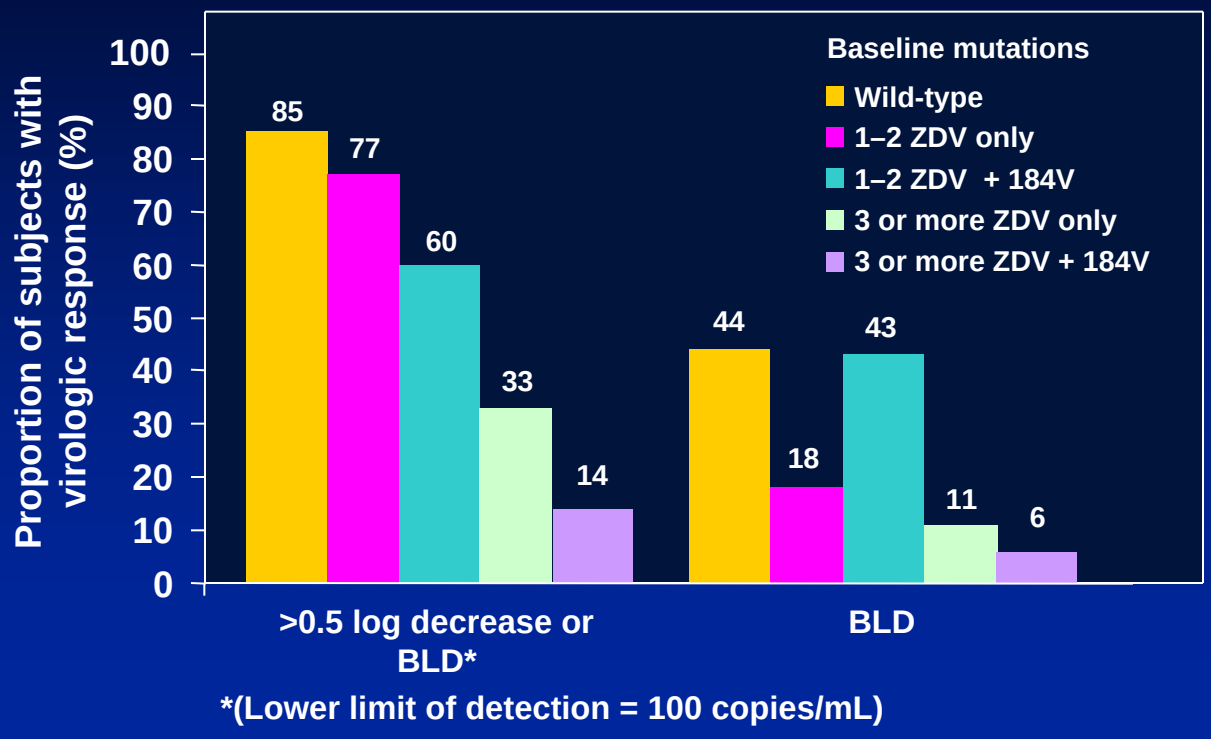
- **Deeks et al**
  - Phenotype predicts response to RTV/SQV salvage therapy.
- **Lanier et al**
  - Phenotype and genotype predict response to abacavir
- **Harrigan et al**
  - Baseline genotype and phenotype are significant predictors of response to RTV/SQV after PI failure
- **Zolopa et al**
  - Genotype 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)

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)

	genotyping	control	p
Δ plasma HIV-1 RNA			
3 mos	-1.3	-0.6	0.021
6 mos	-1.3	-0.5	0.038
% <200 copies/mL			
3 mos	33%	16.7%	0.039
6 mos	39.1%	9.5%	0.047

Durant et al Lancet 1999.

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 ≥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

	GART	Std of Care	p
ΔRNA	-1.17 log	-0.62 log	0.0001
% <500	29%	17%	0.15

# sens drugs	1	2	3	≥4
ΔRNA per drug (log/mL)	-0.1	-0.58	-1.02	-1.25

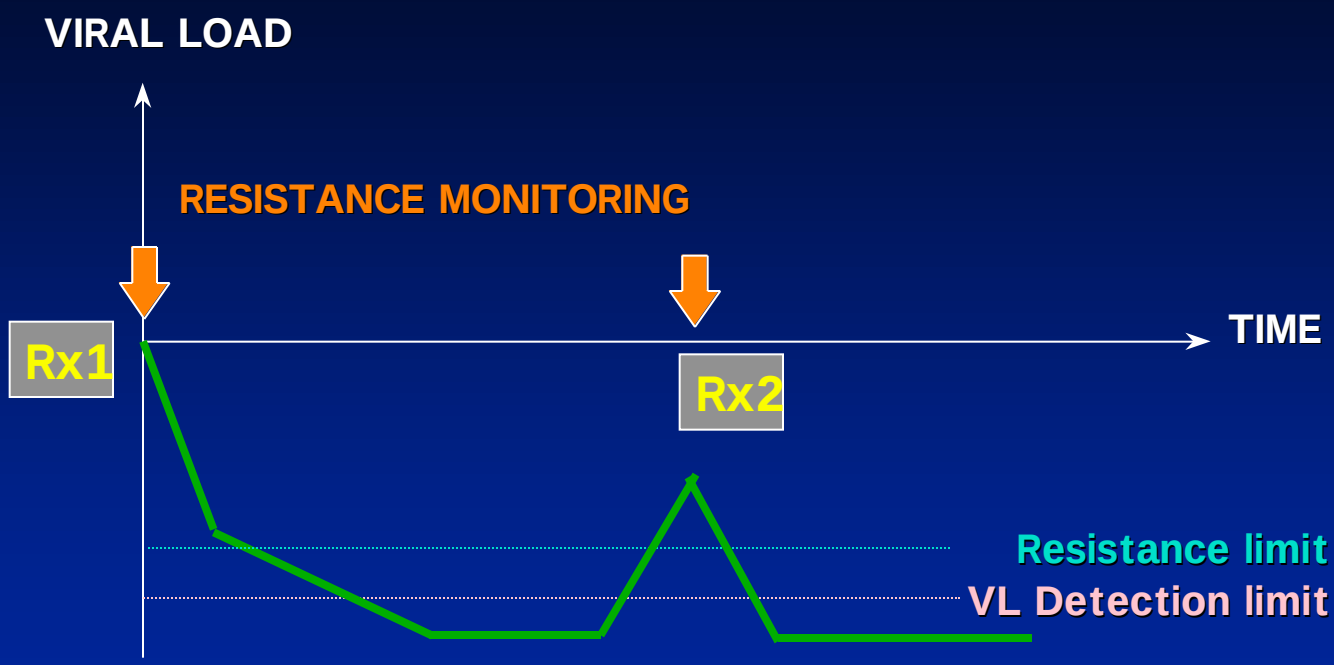
Baxter et al. 6th CROI LB 8, Chicago, 1999.

GART (CPCRA 046) comments

- 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



Possible uses for drug resistance testing

- **Primary HIV Infection**
- **Before starting therapy**
- **Changing therapy**
  - Early failure
  - Late failure
- **Pregnancy**
- **Post-exposure prophylaxis**

Use of Drug Resistance Testing When Changing Therapy

- 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.