Diagnostic Test
HIV Resistance Test

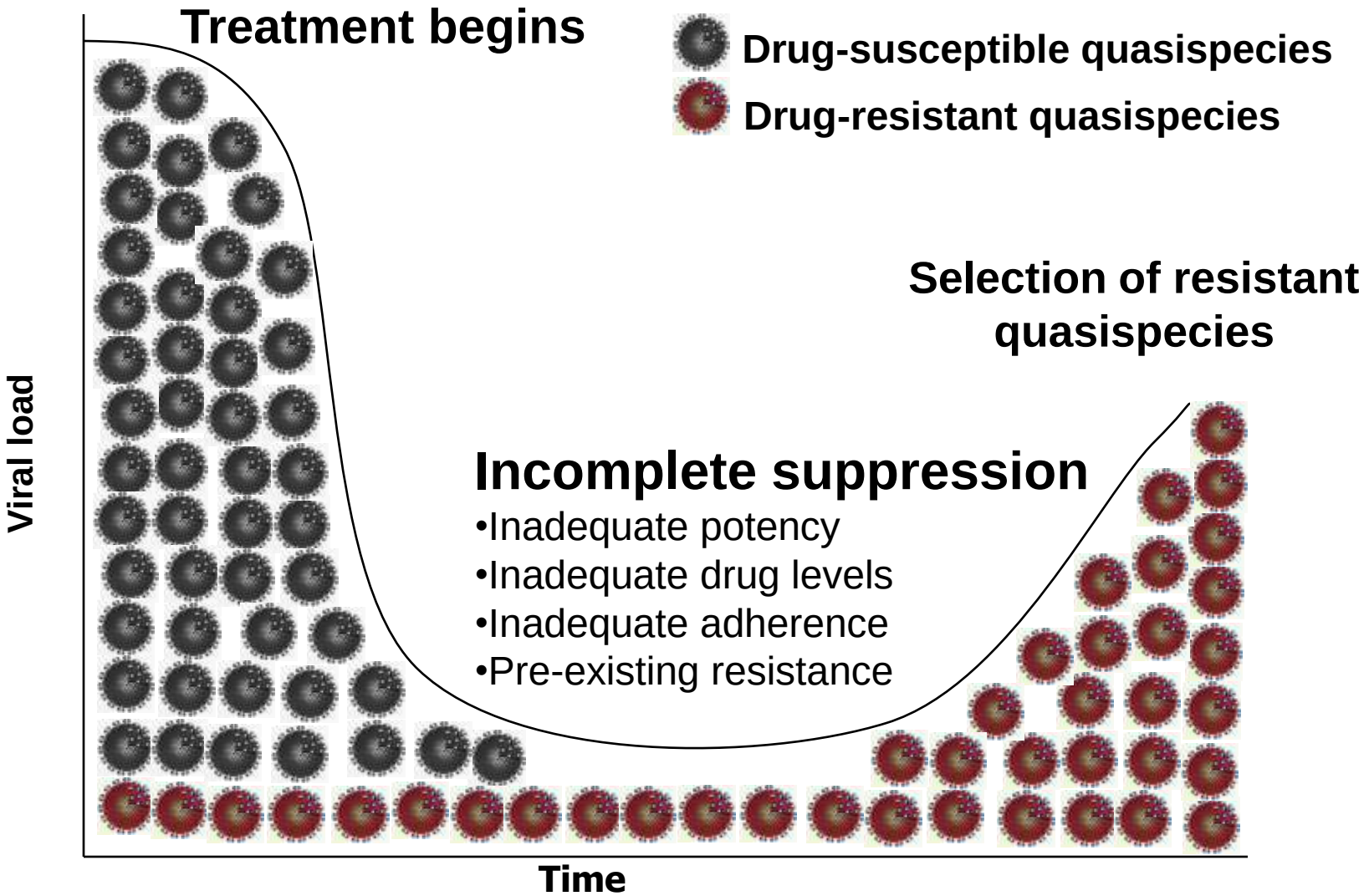
Thumbi Ndung'u, BVM, PhD.

Nelson R. Mandela School of Medicine

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 $\rightarrow$ quasispecies
- Every possible mutation present in quasispecies before ARV therapy

Selection of Resistant strains



Mutations (cont)

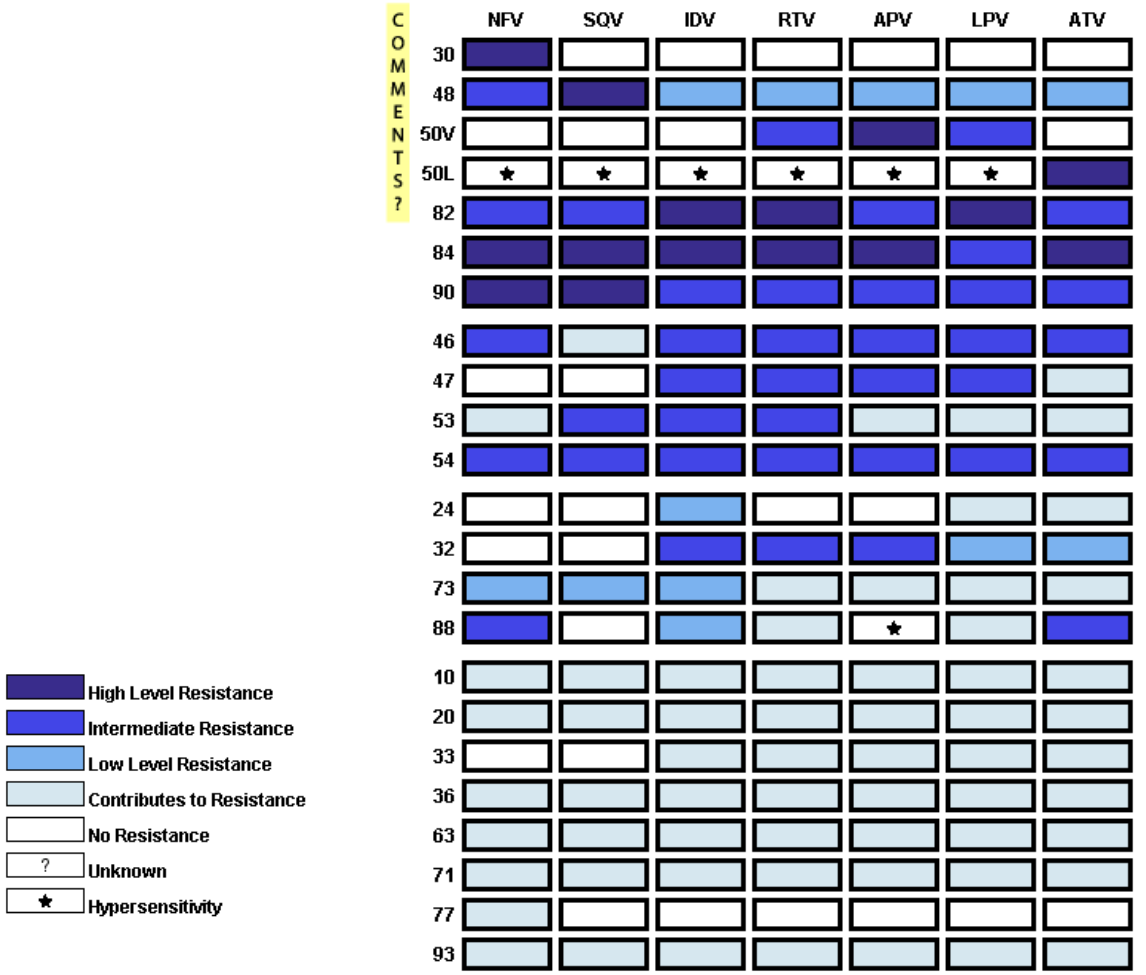
Mutations described in ff way: e.g. M184V

- 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**

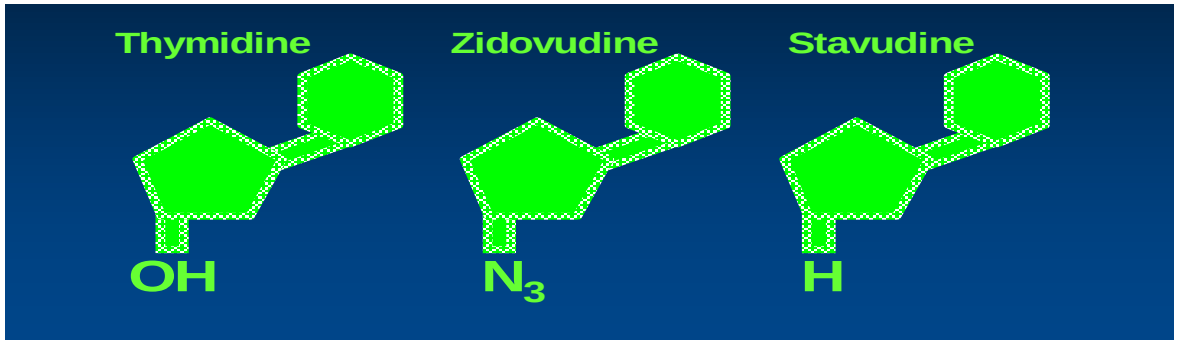
Mutations associated with resistance to PIs



Patterns of resistance (cont)

2. NRTIs

- **Thymidine Analog Mutations (TAMS)**



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

  - **Selected by AZT and/or d4T**
  - **Accumulation of mutations**
    - **41L, 67N, 70R, 210W, 215Y/F, 219Q/E**

- **3TC**
  - **M184V**

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

Patterns of resistance (cont)

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?

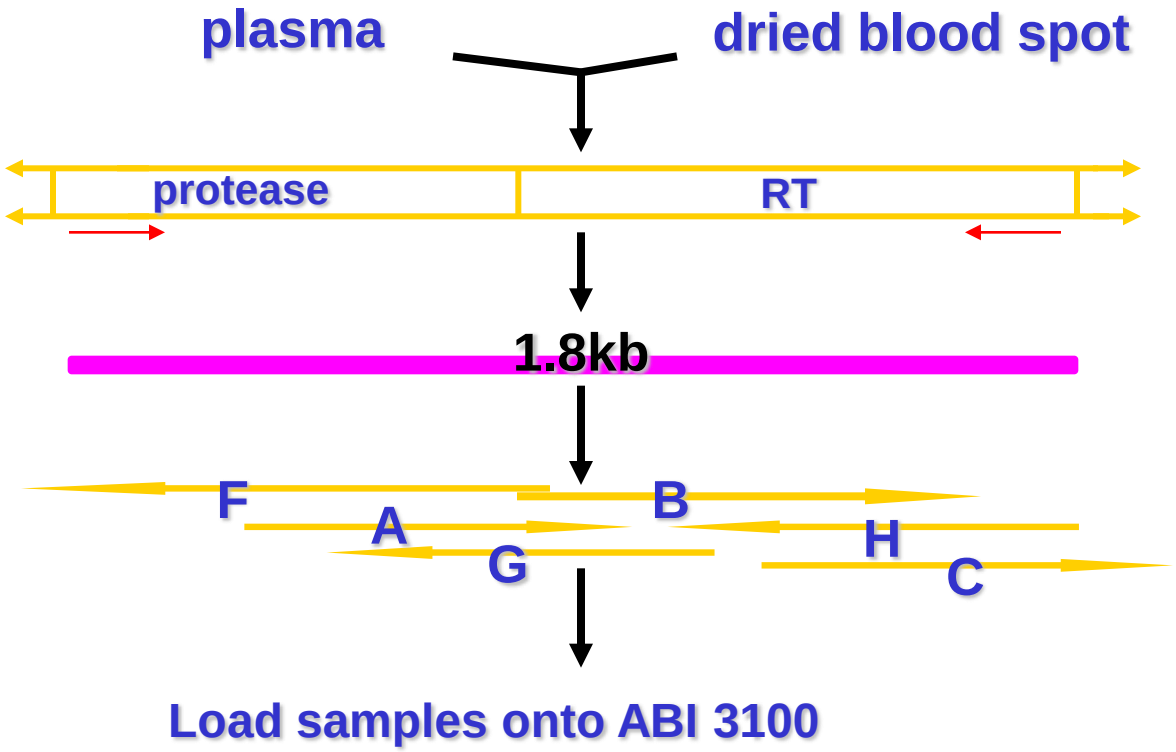
Genotyping:

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

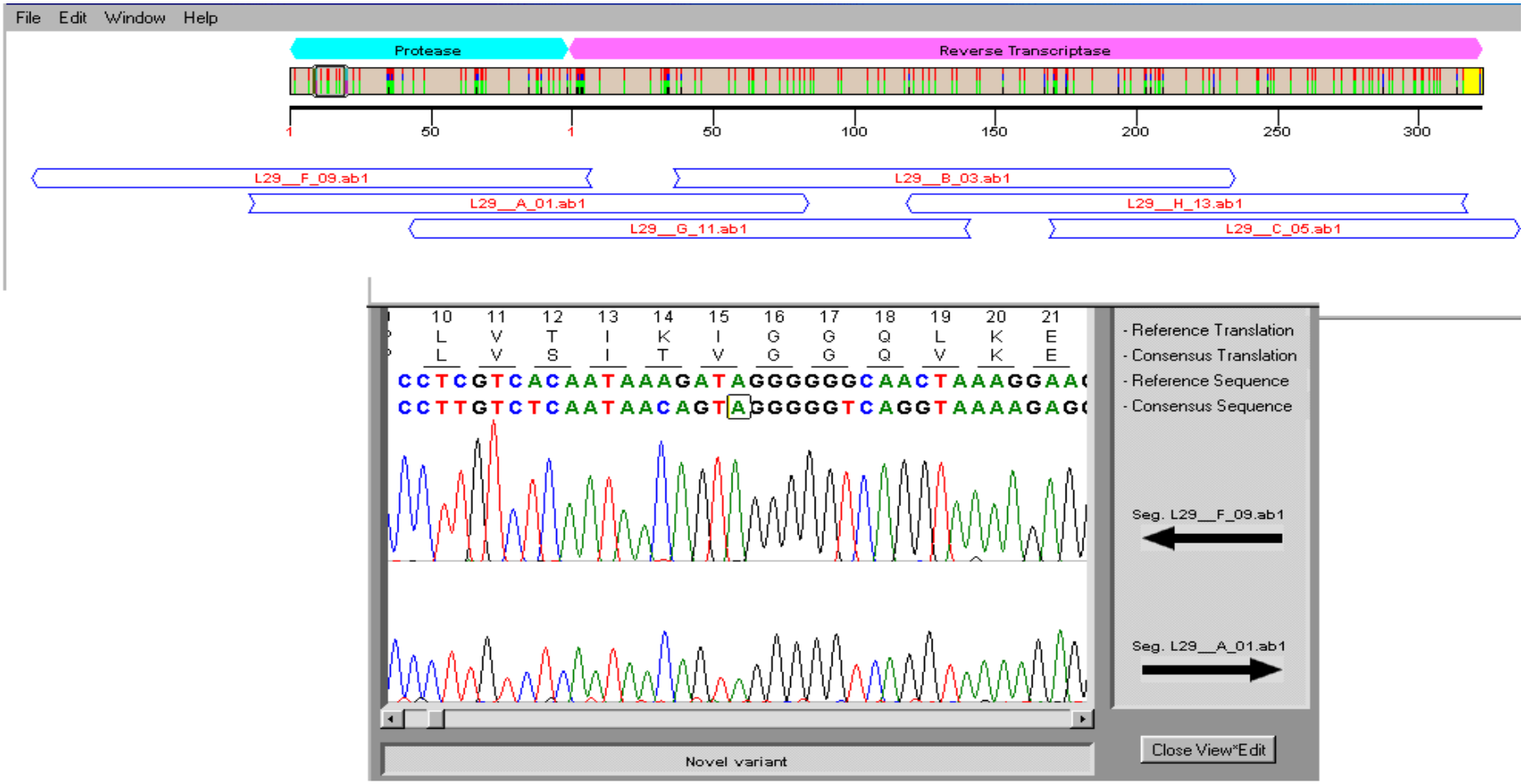
Phenotyping:

Direct assay: Measures the ability of the virus to grow in various concentrations of antiretroviral drugs.

Genotyping using the Viroseq Kit



Sequence Analysis



http://hivdb.stanford.edu/

Stanford HIV Drug Resistance Database

A curated database containing nearly all published HIV RT and protease sequences: a resource for researchers studying evolutionary and drug-related variation in the molecular targets of anti-HIV therapy.

Home | Seq Analysis | Database Queries | Resistance Notes | User Guide

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 (**HIValg**)

Drug Resistance Notes

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

Protease inhibitors mutations		
	Major resistance	**M461, I54V, L76V, V82A, L90M**
	Minor resistance	**L10FI, Q58E, A71V**
Interpretation	**High level resistance**	**Atazanavir [ATV], fosamprenavir [FPV], indinavir [IDV], lopinavir [LPV], nelfinavir [NFV], saquinavir [SQV]**
	Intermediate resistance	**Darunavir [DRV] and tipranavir [TPV]**
Reverse transcriptase inhibitors (RTI) mutations		
	Nucleoside reverse transcriptase inhibitor (NRTI)	**M41L, D67N, M184V, L210W, T215Y, G333E**
	Non-nucleoside reverse transcriptase inhibitor (NNRTI)	**None**
Interpretation	**High level resistance**	**Lamivudine [3TC], abacavir [ABC], zidovudine [AZT], stavudine [D4T], emtricitabine [FTC]**
	Intermediate resistance	**Didanosine [DDI] , tenofovir [TDF]**
	Susceptible	**Delavirdine [DLV], efavirenz [EFV], etravirine [ETR], and nevirapine [NVP]**

Final Diagnosis

Multidrug resistant HIV

References

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