

Population Genetics in HIV-1 Superinfection

S. Palmer^{1*}, M. Kearney¹, V. Boltz¹, F. Maldarelli¹, G. Achaz³, J. Mellors², E. Daar⁴ and J. Coffin¹

¹HIV Drug Resistance Program, NCI, NIH, Frederick, MD; ²University of Pittsburgh, Pittsburgh, PA; ³Dept of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA; ⁴Harbor-UCLA Medical Center, UCLA School of Medicine, Torrance, CA



HIV-1 Superinfection

Frequent Isolation of Intersubtype Recombinant Viruses

- ❖ Implies double infection
- ❖ Co-infection or Superinfection?

Recent Superinfection Cases

- ❖ Intersubtype superinfection (AE and B) two injection drug users: *Ramos et al., 2002* one sexual transmission: *Jost et al., 2002*
- ❖ Intrastubtype superinfection (B) after primary infection with MDR virus: *Daar et al., 2002*; *Koelsch et al., 2003* during STI: *Atfeld et al., 2002*
- ❖ Increase in viremia
- ❖ Lack of immune control

Objectives of the Study

A detailed genetic analysis of HIV-1 populations in a patient with superinfection was performed to examine:

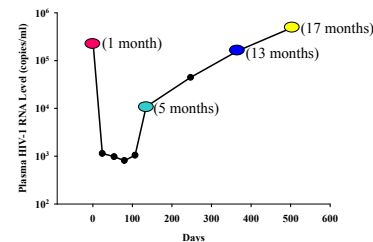
- ❖ Competition between two virus populations
- ❖ Changes in virus populations and their diversity
- ❖ Possible recombination between the two virus genotypes

Patient Studied

- ❖ 32 y. o. homosexual Asian male who presented with 10 days of fever, rash, myalgia, headache, lymphadenopathy, and oral ulcers
- ❖ No history of HIV-1 infection
- ❖ Laboratory data at time of presentation:
 - HIV ELISA negative
 - WB indeterminate (gp160, p24)
 - HIV RNA –301,000 copies/ml
 - HLA-A3,24; B35,40

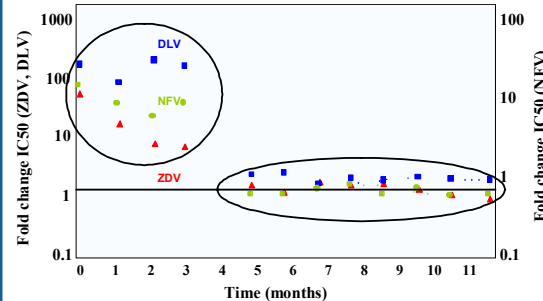
Preliminary Data

Viral RNA Level and Composite Sequence of Patient Samples



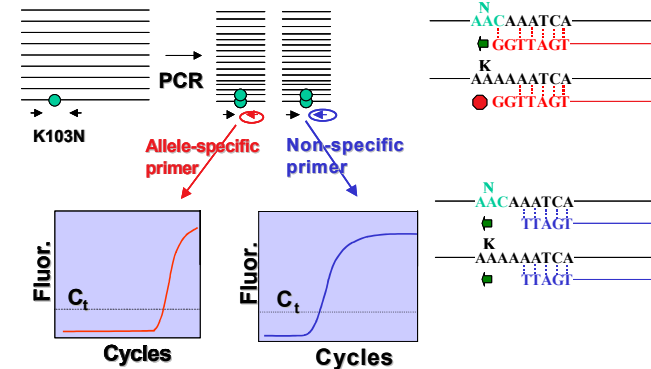
Virus	Resistance Mutations	
	Reverse Transcriptase	Protease
MDR	M41L, 688S69, W88S, K103N, Y181C, T215F	L10I, M46I, L90M, I93L
WT	none	L63P
WT	none	L63P
WT	none	L63P

Switch in Drug Susceptibility

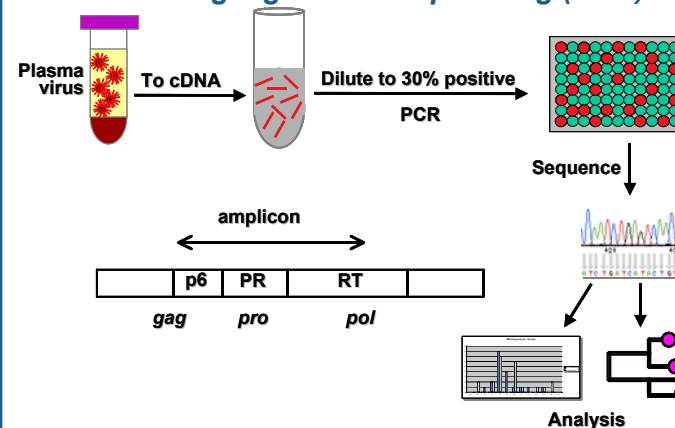


Methods

103N/K Allele-Specific PCR

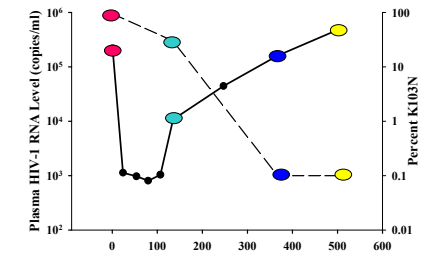


Limiting Dilution PCR Sequencing AKA: single genome sequencing (SGS)



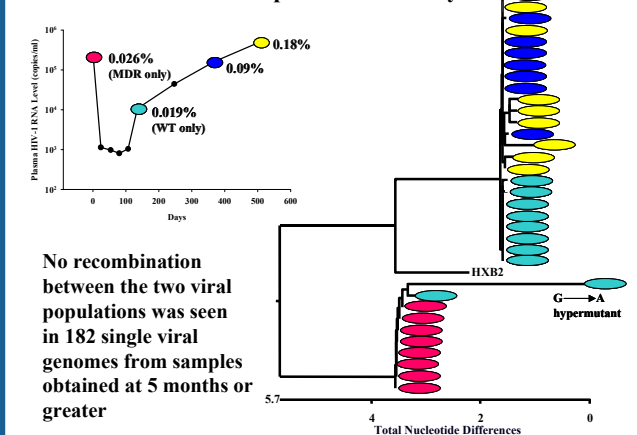
Results

Percentage of K103N by Allele-Specific PCR



Virus	K103N	K103
MDR	94 ± 18%	0.04 ± 0.02%
WT	31 ± 7%	68 ± 10%
WT	0.1 ± 0.03%	67 ± 6%
WT	0.1 ± 0.008%	64 ± 6%
Background	0.03 ± 0.004%	0.09 ± 0.02%

HIV-1 Population Diversity



No recombination between the two viral populations was seen in 182 single viral genomes from samples obtained at 5 months or greater

Conclusions: Our results indicate that this patient was initially infected with a multidrug resistant virus, and was subsequently superinfected with a wildtype virus, which dominated the replicating virus population, leaving only a very small proportion of resistant virus (0.1% or less). Longitudinal samples showed the diversification of the wildtype superinfecting virus from a monomorphic population to a more heterogeneous one by 1-1.5 years after infection. The diversification of the superinfecting wildtype virus occurred slowly by de novo mutation rather than recombination with the initially infecting virus.