

Dynamic Expression of HIV-1 Drug Resistance Mutations during Acute Infection

JT Lipscomb, SM Owen,
Jeffrey A Johnson

HIV Drug Resistance and Diagnostics Development Unit

Division of HIV/AIDS Prevention
Centers for Disease Control and Prevention

The findings and conclusions are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention



Introduction

- ❖ **Mutations associated with drug resistance can serve as unique genetic markers for evaluating HIV-1 expression**
- ❖ **Using sensitive testing we targeted resistance mutations during acute viremia to examine the breadth of virus expression following transmission – above noise**
- ❖ **We examined for M41L and K70R, which were prevalent during the time of seroconversion, and we also evaluated an unlikely mutation, K65R, for which no occurrences of transmission had been identified during that period by conventional genotyping**

Methods

Study Population

- 13 longitudinal subtype B acute seroconverters totaling 105 samples collected between 1997-2000

Reverse transcriptase-PCR

- HIV-B RT templates for real-time PCR testing were generated by reverse transcriptase-PCR with high-fidelity DNA polymerase

Real-time PCR

- Screened with real-time PCR-based drug resistance assays for M41L, K65R, and K70R

Detection limits:

M41L= 0.8%, K65R= 0.3%, K70R= 2.0%

Methods

Positive real-time PCR amplicons were sequenced to confirm the results, as well as to identify any linked mutations

Clonal sequencing

Positive 65R results and variant genotypes were further supported by clonal sequencing

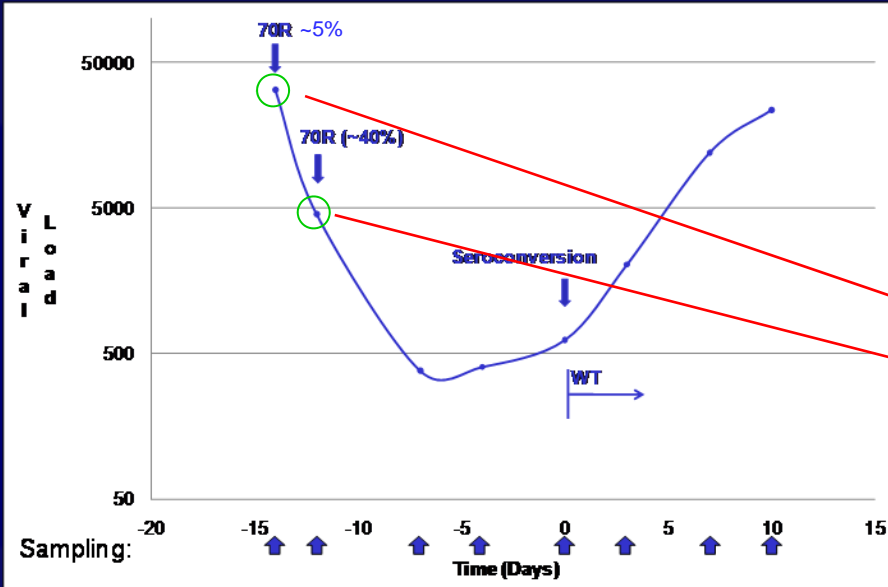
***Seroconversion* was defined at time point positive with Ag-only assay**

3 of 13 seroconverters

screened positive for drug resistance
mutations during acute infection

ID 9017 (1998)

(2/8 time points screened positive for *K70R*: 14 & 12 days pre seroconversion)



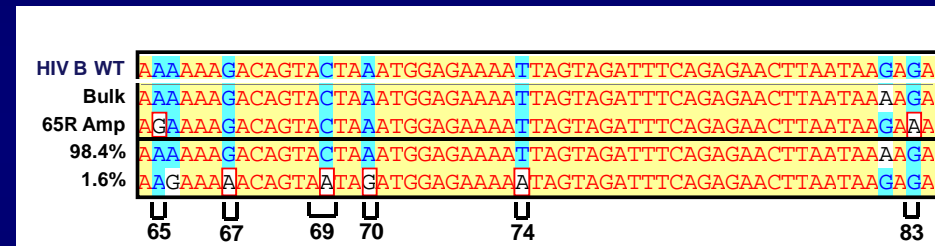
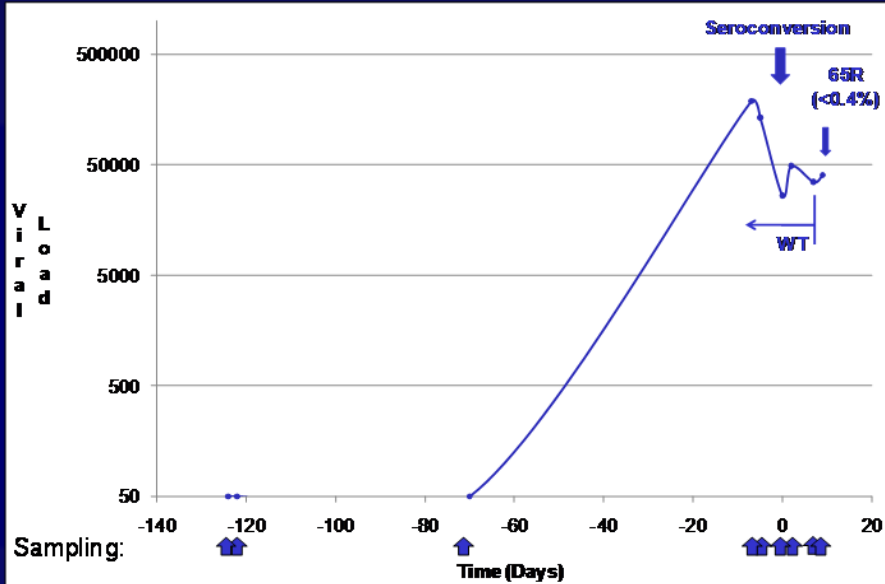
HIV B WT	A	A	T	A	C	C	A	G	T	A	T	T	T	G	C	C	A	T	A	A	A	G	A	A	A	A	A	A	A	G	A	C	A	G	T	A	C	T	A	A	A
-14 days Bulk	A	A	T	A	C	C	A	G	T	A	T	T	T	G	C	C	A	T	A	A	A	G	A	A	A	A	A	A	A	G	A	C	A	G	T	A	C	T	A	A	A
-14 days 70R	A	A	T	A	C	C	A	G	T	A	T	T	T	G	C	C	A	T	A	A	A	G	A	A	A	A	A	A	A	G	A	C	A	G	T	A	C	T	A	A	G

U
70

❖ **Virus rebound at seroconversion had no detectable *K70R***

ID 12007 (1999)

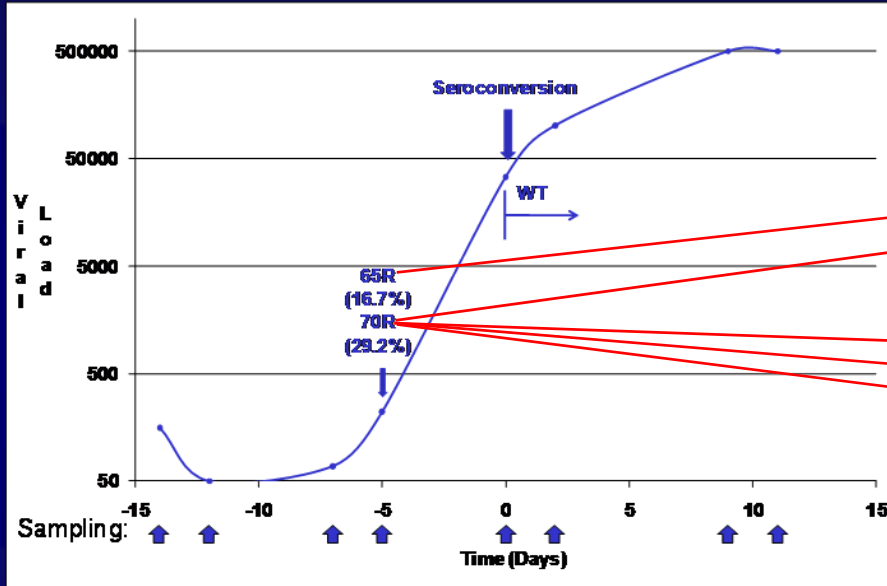
(1/6 time points screened positive for *K65R* variant: 9 days post seroconversion)



- ❖ TAM/ddl resistance mutations identified in a separate clone at 1.6%
- ❖ 65R amplicon sequencing revealed linkage to R83K

ID 9012 (1997)

(1/6 time points screened positive for *K65R* and *K70R* variants: Day 10 of RNA escalation)



WT	A A A A A A G A C A G T A C T A A A T G G A G A A A A T T A G T A G A T T T C A G A G A A C T T A A T A A G A G A
Bulk	A A A A A A R A C A G T R M Y A R A T G G A G R A A A W T A R T A G A T T T Y A G A G A A C T Y A A T A A R A R A
65R Amp	A G A A A A G A C A G T A C T A A A T G G A G A A A A T T A G T A G A T T T C A R A R A A C T T A A T A A R A A A
25.0%	A A A A A A G A C A G T A C T A A A T G G A G A A A A T T A G T A G A T T T C A G A G A A C T T A A T A A G A A A
16.7%	A G A A A A G A C A G T A C T A A A T G G A G A A A A T T A G T A G A T T T C A G A G A A C T T A A T A A G A A A
16.7%	A A G A A A A A C A G T A T A T A G A T G G A G A A A A T T A G T A G A T T T C A G A G A A C T T A A T A A G A G A
8.3%	A A G G A A A A C A G T G A T A A A T G G A G A A A A T T A G T A G A T T T T A G A G A A C T T A A T A A G A G A
4.2%	A A A A A A G A C A G T A C T A A A T G G A G A A A A T T A G T A G A T T T C A G A G A A C T T A A T A A G A G A
4.2%	A A G A A A A A C A G T G A T A A A T G G A G A A A A T T A G T A G A C T T T A G A G A A C T T A A T A A G A G A
4.2%	A A G A A A A A T A G T A T A T A G A T G G A G A A A A T T A G T A G A T T T C A G A G A A C T T A A T A A G A G A
4.2%	A A A A A A G A C A G T A C T A G A T G G A G A A A A T T A G T A G A T C T T A G A G A A C T T A A T A A G A G A
4.2%	A A A A A A G A C A G T A C T A A A T G G A G A A A A T T A G T A G A T T T C G G A G A A C T T A A T A A G A A A
4.2%	A A A A A G G A C A G T A C T A A A T G G A G A A A A T T A G T A G A T T T C A G A G A A C T T A A T A A G A A A
4.2%	A A A A A A G A C G G T A C T A A A T G G A G A A A A T T A G T A G A T T T C A G A G A A C T T A A T A A G A A A
	U 65 U 67 U 69 U 70 U 74 U 83

- ❖ Five distinct TAM/ddl resistance variants comprising 41.8% of the clones were present unlinked to K65R
- ❖ 65R amplicon sequencing revealed linkage to R83K

Summary

Subject 9017

- ❖ Amplicon sequencing revealed intact 70R mutations in two samples pre seroconversion
- ❖ K70R never went above 50% and was no longer detectable in subsequent samples from the time of seroconversion

Subject 12007

- ❖ K65R was detected after seroconversion in the final time point of the panel and was at the limit of detection (~0.3%)
- ❖ A separate multi-TAM variant in this individual was also present at a minority level

Subject 9012

- ❖ Clonal sequencing revealed a K65R variant that was distinct from the genomes with TAM/ddl resistance mutations
- ❖ The subsequent time point coincided with the time of seroconversion and no longer had detectable K65R nor K70R

In subjects 12007 and 9012, K65R subpopulations were linked to the R83K polymorphism, a mutation enriched by d4T.

Conclusions

- Sensitive resistance analysis during acute infection revealed transmissions of drug-resistant variants that remained at low-frequency
- In the acute subtype B seroconverters, low-level *K65R* was only associated with transmitted drug resistance and decayed very rapidly (<2 months)
- The presence only at low levels suggests that the *K65R* variants were minor constituents of the infecting swarm and the co-existence with other mutations associated with resistance supports that multiple, distinct minority resistant variants were able to infect

Conclusions

- **The brief and low-level appearance of transmitted K65R, which the mutation patterns reveal could have been d4T-selected, may explain its lack of detectable transmission in the 1990s**
- **The data suggest that the breadth of resistance mutation selection and of variant composition during transmission may be greater than currently realized**