

Persistence of Primary Genotypic Resistance Following HIV-1 Seroconversion for up to Three Years Post Infection

P. Cane¹, G. Dean², M. Fisher², D. Pao², S. Drake³, D. Pillay⁴

¹ Health Protection Agency Antiviral Susceptibility Unit, Birmingham, UK ² Royal Sussex County Hospital, Brighton, UK ³ Heartlands Hospital, Birmingham, UK ⁴ University College, London, UK

Abstract

Background: Transmission of HIV-1 drug resistance is well documented. The public health impact of this phenomenon will be further determined by the stability of these viruses as the majority population within such individuals. We have therefore undertaken prospective monitoring of primary infections for drug resistance, and follow-up in those infected with such viruses.

Methods: Primary infection was defined as an evolving serology response or, seroconversion within an 18 month period. 16 patients experienced primary infection with evidence of resistance-associated mutations and were followed for up to 3 years. Genotypes were determined using an in-house assay. One patient received 1 year of treatment post diagnosis.

Results: Drug resistance persisted over time in most patients studied. In particular, M41L, T69N, K103N and T215 variants within RT, and multidrug resistance (MDR) demonstrated little reversion to/outgrowth of wild type virus. By contrast, Y181C and K219Q in RT, occurring alone, disappeared within 25 and 9 months respectively. MDR (NRTI, NNRTI & PI) in 3 patients was found to be stable for up to 2 years, the maximum period studied, and to be associated with low viral loads, except for one patient showing a change from T215Y to T215C associated with a marked increase in viral load. An additional patient was found to have virus showing M41L and T215S 10 years after known seroconversion, although she had received 5 months of AZT after 2 years, so complicating interpretation of this result in the absence of a seroconversion sample.

Conclusions: Certain resistance-associated mutations are highly stable long term, and could be utilised as indicators of infection with drug resistant virus. Persistence of MDR viruses is of concern, and lack of reversion may be due to the presence of compensatory mutations within these viruses. However, other mutations may disappear rapidly possibly due to the fitness gain of reversion. These data demonstrate that all new HIV diagnoses in areas where primary resistance may occur should undergo genotyping irrespective of whether the date of seroconversion is known. The long-term persistence of primary resistance indicates a significant risk of transmission of resistant virus from patients who are not necessarily antiretroviral experienced.

Introduction

The use of antiretroviral therapy (ARV) has dramatically improved the life expectancy of individuals infected with HIV-1.

However, virus with reduced susceptibility to available drugs emerges in many individuals on therapy and consequently there is an ever-increasing pool of potential transmitters of resistant virus.

Primary infection with HIV-1 that carries resistance-associated mutations has been reported from many countries. Initially, only very low levels of transmitted drug resistance were observed in the 1990s but these had increased to up to 25% of new cases of primary infection in some populations by 2001^{1,2}. Infection with virus that has genotypic resistance has been shown to prolong the period required for virologic suppression. Consequently, it is important to consider the resistance profile of a new infection in order to optimise initial therapy, as recommended by current guidelines.

Most patients are diagnosed some time after primary infection: similar levels of resistance are found in these treatment naïve chronic infections as in the primary infection patients, but comparisons between studies can be confounded by inconsistencies in criteria for defining resistance.

The purpose of this study was to monitor the persistence of resistance associated mutations following transmission, particularly in untreated patients. The study included patients with primary infection with virus showing NRTI or NNRTI resistance or MDR, with follow-up periods for up to 10 years.

Methods

Study samples: Patients were identified as having primary HIV-1 infection either through having had a negative antibody test within 18 months of a positive test, or laboratory evidence of acute seroconversion illness, including the serological testing algorithm for recent HIV seroconversion (STARHS) along with clinical markers indicated recent infection³.

Follow-up samples were obtained 1-120 months following the date of the initial sample in which resistant virus was identified..

Details of dates of initial and subsequent samples are shown in Table 1

Analysis: Sequence was derived from the entire protease gene and first 230 codons of reverse transcriptase. Differences from subtype B consensus sequence were derived using the Stanford database⁴.

Results

Drug resistance associated mutations were observed in 16 patients for whom follow-up samples were available. The mutations observed in the initial and follow-up samples are summarised in Table 1, together with the time intervals between samples. All patients were infected with subtype B virus.

NRTI mutations

M41L was detected in the initial samples from 5 patients, and was still present in the last samples tested from all these patients, which were obtained between 7 and 33 months later.

A62V was observed alone in one patient. This mutation is usually associated with the multi-nRTI resistance complex based on Q151M. It is unlikely that this mutation alone confers resistance and its presence may not represent transmitted resistance. Nevertheless, the mutation rapidly disappeared, becoming undetectable within 2 months.

T69N was observed in the first sample from 4 patients, including one with multidrug resistant virus. This mutation was still present in all subsequent samples between 15 and 32 months later. One of the patients showed **V118I** in addition to T69N (patient O), which was also unchanged after 16 months.

Codon 215 variants were observed in 6 patients at diagnosis. Patient C had T215L along with M41L and no change was observed after 7 months. Patient D had T215Y in conjunction with multi-drug resistant virus and no change was observed after 17 months. Patient E had T215Y together with M41L and this was replaced with T215C in a sample taken 21 months later. Patient F had T215Y with multi-drug resistant virus: this patient had 12 months of treatment of treatment starting at diagnosis; a sample tested a further 12 months later after stopping treatment showed T215C. Patients M and P showed T215D alone at diagnosis and this was still present 11 and 13 months later respectively.

K219Q was observed in 2 patients and **K219R** in one. One patient with 219Q and one with 219R, both with many other resistance mutations, showed retention of these mutations after 18 and 9 months respectively. One patient with 219Q alone showed loss of the mutation within 12 months; further testing of samples at 24 and 26 months showed continued absence of this mutation.

An additional patient (patient J) also showed NRTI mutations M41L and T215S. A seroconversion sample was not available from this patient who was known to have seroconverted during pregnancy in 1992 following contact with an AZT treated source. Two years after seroconversion she had treatment with AZT alone for 5 months during a second pregnancy. The sample tested for genotypic resistance was from February 2002 prior to commencement of HAART. This sample showed M41L and T215S: it seems most likely that these mutations were derived from the original infection. Although there was the short period of AZT treatment which could have allowed development of M41L and T215Y/F, it is more likely that had the mutations arisen only as a result of that treatment then they would have been replaced by overgrowth of an original wild-type virus within the subsequent 8 years, as is well documented to occur usually within weeks of cessation of treatment. This patient showed no such evidence of wild-type virus after 8 years without treatment so it is most probable that the founding virus was AZT resistant. Thus it is likely that these AZT resistance mutations had persisted for over 10 years albeit with a brief period of possibly reinforcing treatment after 2 years.

NNRTI & PI mutations

Two of the patients studied had NNRTI resistance alone: patient A showed only Y181C which had disappeared 25 months later. The second patient (patient G) showed K103N which was still present after 23 months. Three patients were infected with MDR virus including K103N and V179L (patient D), K103N and Y188L (patient F) and A98G, V106I, and Y188L (patient N), and these mutations were unchanged after 17, 24 and 18 months respectively.

No patients in the study had PI resistance alone. The three MDR patients showed L10I, L24F, L33F, I54V, L63P, A71V, G73S, V77I, V82A, and L90M (patient D), V82C (patient F) and L10I, I54L, L63P, A71V, G73T, I84V, L90M, and I93L (patient N) in protease, and as with the NNRTI mutations in these patients, these were unchanged 17, 24 and 18 months after diagnosis respectively.

MDR virus and viral load

As described above, three patients (D, F and N) were infected with virus showing resistance to NRTIs, NNRTIs and PIs. Patient F received 12 months of treatment with AZT, 3TC and EFV following diagnosis, the other patients received no treatment. The viral loads and CD4 counts for these patients are shown in Figures 1-3. Viral loads remained low off treatment for some time for all three patients.

Patient D's viral load on diagnosis was 2500 copies/ml, fell to 150 copies/ml after 2 months (no treatment) and has remained below 1000 copies/ml for the subsequent 18 months. His CD4 count has declined from a peak of 1100 to 670 cells/ml during this period. No change in resistance associated mutations was observed in this period.

Patient F had a high viral load on diagnosis (>100000 copies/ml) but responded to AZT, 3TC, and EFV treatment with a viral load of <50 copies/ml despite the genotypic resistance results indicating that the only active drug was likely to be 3TC. Treatment continued for 12 months after diagnosis. After 8 months off treatment the viral load had rebounded to only 707 copies/ml. However, 4 months later the viral load rose abruptly to 18,200 copies/ml: this sample showed the single change in sequence of T215Y to T215C, all other resistance mutations remaining the same. This patient's viral load remained between 18,200 copies/ml and 4,430 copies/ml for the following 18 months while his CD4 cell count dropped from 1260 cells/ul to 576 cells/ul.

Patient N's viral load was 4140 copies/ml at diagnosis and 456 copies/ml 18 months later, while his CD4 count showed little change during this period (from 685 to 527 cells/ul). As with patient D, no change was observed in the resistance associated mutations during this period.

Discussion

This poster describes the duration of persistence of drug resistance associated mutations following transmission. Resistance mutations that arise in wild-type virus following treatment may rapidly become undetectable when treatment is stopped. This may mainly be due to overgrowth of wildtype virus originating in the viral reservoirs rather than true reversion of the mutant virus *per se*.

As previously described, virus from patients infected with AZT resistant virus showing mutations at codon 215 of RT frequently showed onward mutation at this codon resulting in T215S/D/C. These variants were shown in this study to be very stable, persisting in one patient for over 10 years, albeit with a brief period of AZT treatment. Most other mutations were also found to persist over the period of study. The exceptions were A62V, Y181C and K219Q where these mutations occurred singly. The disappearance of mutations could either be due to reversion and selection of a fitter virus, or alternatively, due to overgrowth of wild-type virus that was present in the original infecting inoculum as a minority population. In the case of Y181C, it is unlikely that this mutation is highly unstable *per se* since we have observed this mutation in a new diagnosis of a patient with AIDS (data not shown).

Samples from patients with MDR virus showed no change over the period studied other than T215Y to T215C in one patient. The slow evolution of MDR virus to fitter wildtype virus may be a consequence of the multiple mutations needing replacement in these viruses. It is possible that reversion of each site leads to only a small increase in replicative capacity and thus replacement with fitter virus lacking all mutations will be a stepwise process, possibly with a contribution by recombination, and so may take a very long time. Further, since MDR viruses may already contain fitness compensating mutations, the route to reversion to wildtype may require the virus to travel through a fitness trough. The exception to this was observed in patient F who showed change in MDR virus of T215Y to T215C, presumably indicating that in this context the increase in fitness conferred by this change is sufficient to allow outgrowth of this virus. Indeed, the patient's viral load had remained below 1000 copies per ml for 2 years post infection (including one year on treatment), with a rise to 18,200 copies/ml concurrent with the T215Y to T215C change.

Of note, the CD4 cell count decline in patients infected with MDR virus does not appear attenuated, suggesting equivalent pathogenicity to wild type virus.

Dr P.A. Cane, HPA AVSRU, Division of Immunity & Infection, University of Birmingham, Birmingham B15 2TT
p.cane@bham.ac.uk
44-121 414 6972



Table 1. Persistence of drug resistance associated mutations over time following primary infection

Patient	Months after 1 st sample	NRTI mutations	NNRTI mutations	PI mutations
A	0			L63P, V77I, I93L
	25		Y181C	L63P, A71T, V77I, I93L
B	0	T69N		M36L, L63P
	15	T69N		M36L, P63P/L
C	0, 7	M41L, T215L		L63P, V77I/V, I93L
D	0, 1, 9, 17	M41L, E44D, V118I, 210W, T215Y, K219R	K103N, V179L	L10I, L24F, L33F, I54V, L63P, A71V, G73S, V77I, V82A, L90M
	E	0	M41L, T215Y	D60E, L63P
E	21	M41L, T215C		D60E, L63P/S
	33	M41L, T215C		D60E, L63P
F	0	M41L, K43E, T215Y	K103N, Y188L	L10I, L63P, V77I, V82C
	24	M41L, K43E, T215C	K103N, Y188L	L10I, L63P, V77I, V82C
G	0		K103N, V108I/V	L33V
	23		K103N	L33V
H	0	T69N		L63P
	32	T69N		L63P, V77I
I	0, 28	M41L		L10I, L33M, L63N, I93L
J	0	N/a	N/a	N/a
	120	M41L, T215S		L63P
K	0	A62V		L63P
	2			L63P
L	0	K219Q		L10V, M36I
	9, 22, 36			L10V, M36I
M	0, 11	T215D		M36I
N	0, 18	T69N, K219Q	A98G, V106I, Y188L	L10I, I54L, L63P, A71V, G73T, I84V, L90M, I93L
	O	0, 16	T69N, V118I	L63P, V77I, I93L
P	0, 13	T215D		M36I

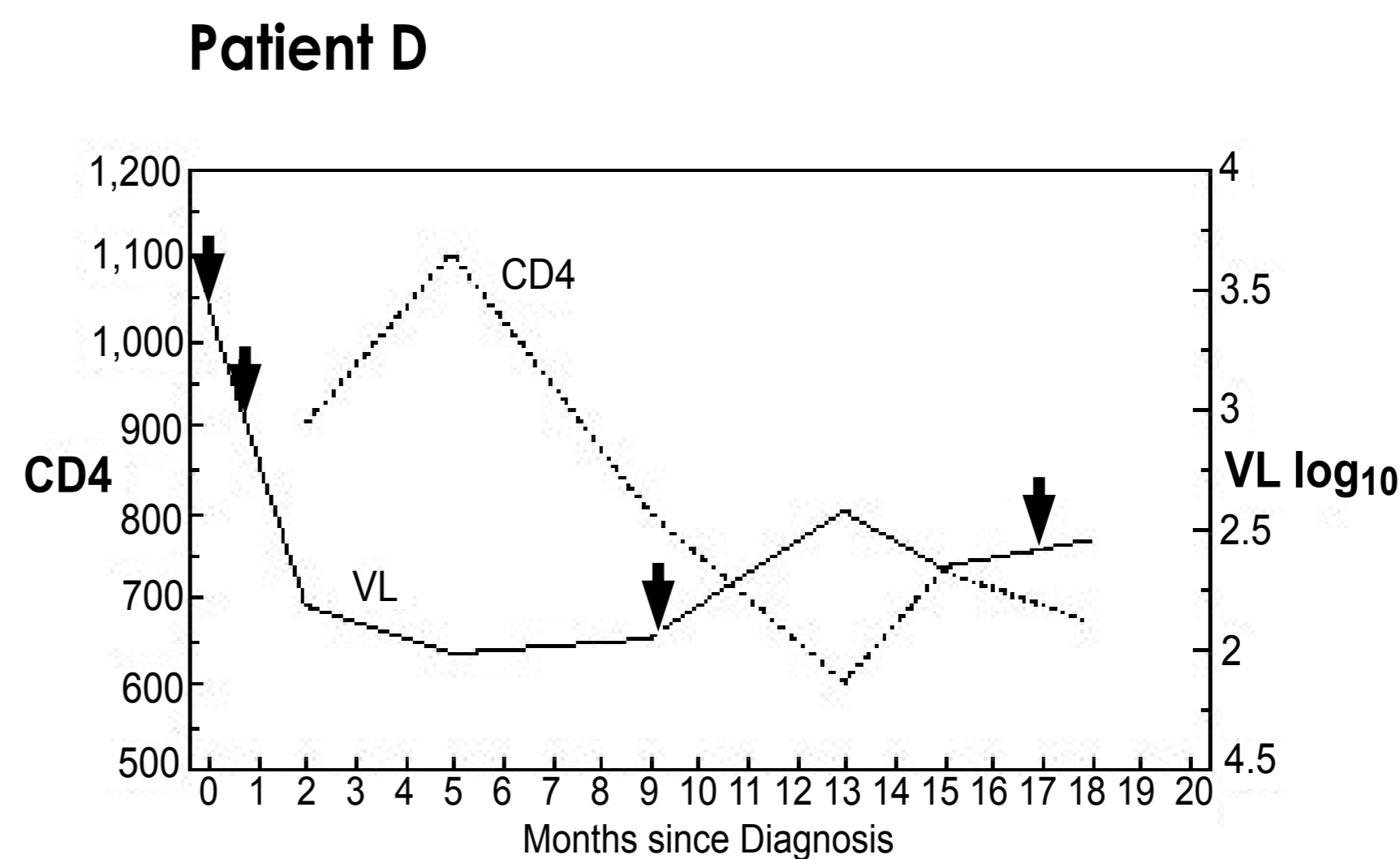


Figure 1. Viral load and CD4 counts of patient D infected with MDR HIV-1. Arrows indicate when genotypic resistance testing was performed. No changes were observed in mutations during period of study.

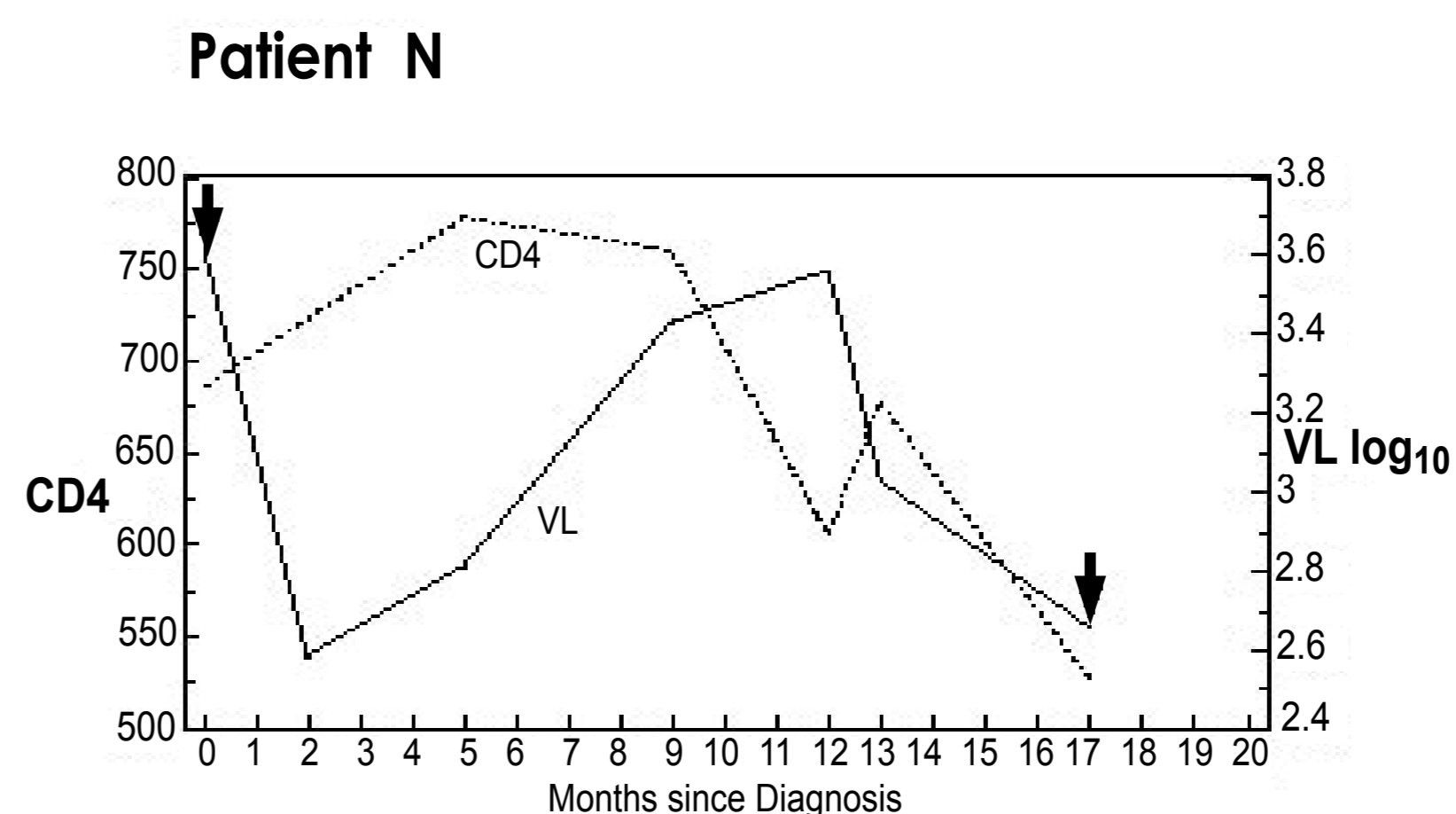


Figure 2. Viral load and CD4 counts of patient F infected with MDR HIV-1. Arrows indicate when genotypic resistance testing was performed. Virus tested at month 24 showed T215Y had changed to T215C.

Conclusions

- Drug resistance mutations in HIV-1 often persist for a considerable time following primary infection.
- There is potential for onward transmission of resistant virus from untreated patients, as has already been observed⁵. Thus, even if the majority of treated patients are virologically suppressed, primary resistance may increase if it is introduced into a community with high risk/rates of onward transmission.
- It is useful to perform a resistance test at diagnosis, even where evidence of recent infection is not available.

Acknowledgements

Many thanks to Judith Workman and Daina Ratcliffe for carrying out the genotyping assays. We also thank our collaborators: Drs U. Andradý, J. Clarke, G. Dean, T. Green, M. Murphy, K. Sivakumar, A. Tang, S. Taylor, D. White, & G. Underhill

References
1. UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance (2001). <i>BMJ</i> 322:1087-1088.
2. Grant RM <i>et al</i> (2002). <i>JAMA</i> 288:181-188.
3. Murphy G <i>et al</i> (2001). <i>Commun Dis Public Health</i> 4:33-37
4. Shafer RW <i>et al</i> (2000). <i>Nucleic Acids Res</i> 28:346-348.
5. Taylor S <i>et al</i> (2003). <i>AIDS Res Hum Retroviruses</i> 19:353-61