

# Choice of co-nucleoside analog in d4T-treated subjects may influence the pattern of thymidine analog mutations (TAMs) and multi-nucleoside resistance mutations (MNRs)

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## Introduction

In vivo, stavudine (d4T) and zidovudine (ZDV) resistance develops through similar pathways, by selecting for thymidine analog mutations (TAMs) and/or multiple nucleoside resistance (MNR) mutations that are associated with decreased susceptibility to both drugs. In the clinic, d4T is often combined with either lamivudine (3TC) or didanosine (ddl) for the treatment of HIV-1 infection. Although the L74V mutation is most commonly associated with ddl resistance, the effect of TAMs/MNR mutations on phenotypic susceptibility to ddl has not been thoroughly investigated. Data are also limited on the type and frequency of TAMs when d4T is combined with different co-nucleoside analogs such as ddl and 3TC.

To investigate whether these mutations affect ddl phenotypic susceptibility, or if the choice of co-nucleoside analog influences the mutational pattern observed, HIV-1 genotypic mutations and phenotypic susceptibilities to different NRTIs were examined in samples from 147 viremic subjects who had been on d4T-based regimens and were ZDV naïve. These data were collected from baseline samples from subjects meeting these criteria who were enrolled in clinical trials NZTA4005, VIR3001 or NZT40012 from 1998 through 2000.

## Methods

Plasma HIV-1 samples from 147 viremic d4T-treated, ZDV-naïve patients from multiple US sites were examined for resistance mutations following RT-PCR amplification and ABI sequencing. Phenotypic drug susceptibility of HIV-1 from these samples was analyzed by Antivirogram® (Virco Group NV, Belgium).

Figure 1 • HIV-1 from d4T-treated, ZDV-naïve patient samples containing TAMs and/or MNR mutations (N = 147)

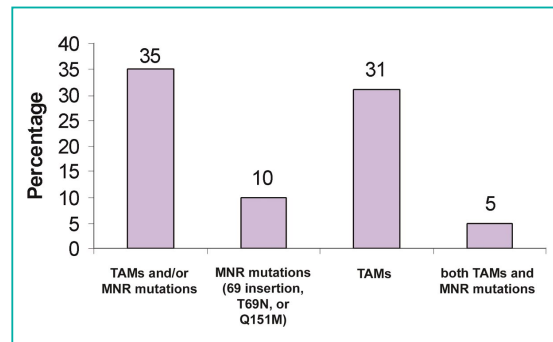


Table 1 • Summary of HIV-1 fold resistance data to ddl, d4T, ZDV, and 3TC in the presence or absence of TAMs, MNR mutations, or both TAMs and MNR mutations, for patient samples where both genotypic and phenotypic data were obtained (n = 115)

Mean fold drug resistance	Mean fold drug resistance with TAMs either present or absent*			Mean fold drug resistance with MNR mutations either present or absent			Mean fold drug resistance with TAMs and/or MNR mutations either present or absent		
	Present n = 30	Absent n = 74	P value <sup>†</sup>	Present n = 11	Absent n = 104	P value <sup>†</sup>	Present n = 41	Absent n = 74	P value <sup>†</sup>
ddl	2.32	1.65	0.040	7.34	1.84	0.005	3.67	1.65	0.003
d4T	1.7	0.9	0.062	7.79	1.13	<0.001	3.33	0.90	<0.001
ZDV	6.01	1.10	<0.001	21.19	2.52	<0.001	10.1	1.10	<0.001
3TC	53.36	44.01	0.22	35.64	46.71	0.74	48.6	44.01	0.31

\* Samples with MNRs excluded (n=104)

<sup>†</sup> Comparisons were made using the two-sample t test

## Results

### Baseline Patient Characteristics:

- 100% of patients d4T treated, ZDV naïve
- 84% male, 16% female
- 59% Caucasian, 30% African-American, 7% Hispanic, 4% other
- Mean age: 40.5 years
- Mean plasma HIV-1 RNA: 3.81 log<sub>10</sub> copies/mL
- Mean CD4 count: 454 cells/mL
- Length of prior treatment: <6 months: 9%, 12%, and 32%; 6 months-1 year: 24%, 20%, and 34%; >1 year: 67%, 68%, and 34% for d4T, 3TC, and ddl, respectively

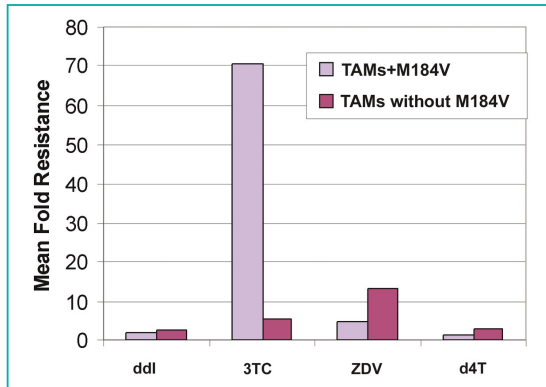
- HIV-1 mutation data were obtained from 147 baseline samples and phenotypic data were obtained from 115/147 samples.

- For the 147 samples with genotypic data, TAMs (M41L, K70R, L210W, T215Y/F, and K219Q/E) were present in HIV-1 from 31% (45/147) of patient samples. MNR mutations (defined as either the Q151M mutation, T69N, or the 69 insertion mutations) were present in HIV-1 from 10% (14/147) of the patient samples. Only 5% (7/147) of the HIV-1 isolated from patient samples contained both MNR mutations and TAMs, and 35% (52/147) had TAMs and/or MNR mutations (Figure 1).

- Table 1 summarizes the mean fold drug resistance values for the 115 samples with both genotypic and phenotypic data with respect to the presence of TAMs, MNR mutations, or both. HIV-1 from patient samples containing TAM, MNR mutations or TAMs plus MNR mutations had significantly higher phenotypic resistance to ddl than HIV-1 from patient samples without these mutations. The mean ddl fold resistance for samples containing TAMs alone was 2.32 compared to 7.34 for samples containing MNR mutations alone or 3.67 for samples containing either TAMs or MNR mutations. HIV-1 containing TAMs had higher levels of resistance to all NRTIs (except 3TC) than those samples lacking TAMs. Higher levels of resistance to all NRTIs (except 3TC) was also observed for samples containing MNRs, as well as for samples containing both TAMs and MNR mutations.

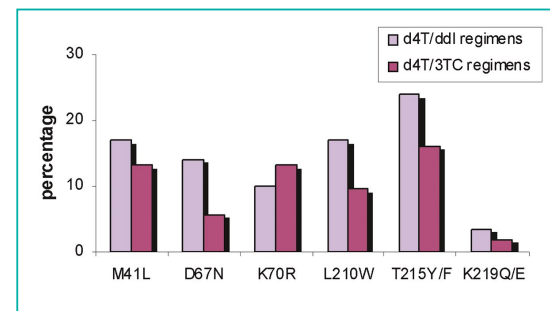
- When analyzing for the effect of TAMs and/or MNR mutations on the mean fold level of 3TC resistance, there was no significant difference. The samples were reanalyzed for the presence of TAMs with or without the M184V mutation, and the mean fold resistance to NRTIs was calculated.

Figure 2 • The M184V mutation does not affect the level of phenotypic fold resistance seen for ddl in samples with TAMs, but increases the fold resistance seen for 3TC, and decreases the level of phenotypic resistance seen for ZDV or d4T



- In the presence of TAMs, the M184V mutation significantly (P < 0.001) increased the level of 3TC fold resistance, but not ddl fold resistance compared to samples with TAMs and without M184V. The presence of the M184V mutation appeared to decrease the levels of resistance to ZDV and d4T (Figure 2) in the presence of TAMs.
- The incidence of specific TAMs in samples from patients on either d4T/ddl-containing regimens (n = 29) or d4T/3TC-containing regimens (n = 106) was examined. HIV-1 from patients on d4T/ddl-containing regimens were more likely to contain TAMs (42.9%) than patients on d4T/3TC-containing regimens (33.0%). As shown in Figure 3, an approximately two-fold or greater increase in incidence was observed for the D67N and L210W mutations in the ddl-containing arm as compared to the 3TC-containing arm (14.0% vs. 5.7% and 17% vs. 9.4%, respectively).
- While subjects on 3TC-containing regimens were on prior ART longer than subjects on ddl-containing regimens, TAMs and MNRs were more common in ddl/d4T (41% TAMs and 20.6% MNRs) than in 3TC/d4T-containing regimens (33% TAMs and 5.6% MNRs).

Figure 3 • Percentage of specific TAMs in HIV-1 from patients on d4T/ddl-containing regimens (n = 29) or on d4T/3TC-containing regimens (n = 106)



## Discussion

- In this study we have noted that TAMs (and/or MNR mutations) can be selected by HIV-1 from viremic patients receiving d4T-based therapies. While the majority (57%) were on d4T/3TC-based therapies, many were receiving d4T/ddl-based therapies. Our data suggest that the TAMs selected for in this d4T-treated population may also produce a low level of cross resistance to ddl. Lacking a population group in this study that was ddl treated and ZDV and d4T naïve, we are unable to determine if ddl can also select for TAMs. Other investigators have also recently noted decreased ddl susceptibility in HIV-1-containing TAMs plus other mutations, including MNR mutations such as the 69 insertions.<sup>1</sup>

- The patients in this study were viremic, with a mean HIV-1 RNA of 3.81 log<sub>10</sub> copies/mL. While samples with TAMs had significantly higher levels of cross resistance to ddl than did samples without these mutations, they were still below the Virco resistance cutoff. The HIV-1 evaluated by the Virco phenotypic assay is categorized as being either within the normal range of susceptible virus or above the range of susceptible virus—i.e., reduced susceptibility. This is a biologic cutoff and not a cutoff based upon clinical relevance. These data, like other retrospective analyses<sup>2</sup> comparing the utility of phenotypic testing vs standard of care, suggest the need for establishing clinically relevant cutoffs for antiretroviral drugs.

- TAMs were observed more frequently in HIV-1 from patients receiving d4T/ddl-based regimens (41.4%) than in HIV-1 from patients receiving d4T/3TC-based regimens (33.0%). This may be due to the presence of the M184V mutation, which can impair the fitness of virus and lead to reduced replication. The mean fold resistance levels for both d4T and ZDV were lower for samples containing TAMs plus M184V compared to samples containing TAMs without M184V. These findings are similar to those reported for CNA3002, where lower frequency and number of ZDV/d4T-associated mutations (TAMs) were observed in baseline virus from 3TC-experienced vs 3TC-naïve patients.<sup>3</sup>

- The D67N and L210W mutations also occurred more frequently in HIV-1 from the d4T/ddl-treated group than from the d4T/3TC-treated group. Moyle and colleagues<sup>4</sup>, a study of 24 patients receiving d4T/ddl and 23 patients receiving d4T/3TC at the time of first failure, also saw mutations at codons 41, 67, 210, 215, and 219 occur more frequently in the d4T/ddl group, although only the T215Y mutation had a two-fold increase in incidence in this group compared to the d4T/3TC-treated group (only a 1.5 fold increase in our study). The K70R mutation was more common in the d4T/3TC-treated group in their study than in this study, which may reflect the differences between sampling at time of first failure and after prolonged viremia, with the K70R mutation being replaced over time by the T215Y/F mutation in the d4T/3TC-treated group.

## Conclusions

- ♦ Like ZDV, d4T can select for TAMs and MNR mutations, and these mutations reduce susceptibility to d4T, ZDV and ddl. While the mean fold resistance to ddl was significantly higher for HIV-1 samples containing TAMs than for samples without them, it was still below the current Virco phenotypic cutoff.
- ♦ The mean fold resistance to ddl was significantly higher for samples containing MNR mutations and/or TAMs than for those without these mutations; it was only slightly above the current Virco phenotypic cutoff of 3.5.
- ♦ Specific TAMs, D67N and L210W, were observed more frequently in the d4T/ddl-containing regimens than in d4T/3TC-containing regimens.
- ♦ Both TAMs and MNR mutations occurred more frequently in samples from patients on d4T/ddl-based regimens than in samples from patients on d4T/3TC-based regimens. These results may suggest additional benefit from inclusion of 3TC in an initial antiretroviral regimen, as the presence of the M184V mutation may delay acquisition of TAMs, thus allowing ZDV or d4T to remain active.

## References

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