

Rate of Thymidine Analogue Resistance Mutation Accumulation With Zidovudine- or Stavudine-Based Regimens

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Abstract: Zidovudine (ZDV) and stavudine (d4T) select for the same set of thymidine analogue resistance mutations (TAMs). To compare the rate at which TAMs emerge, genotypic analysis of HIV-1 was performed on serial plasma samples from treatment-naive subjects randomly assigned to receive ZDV or d4T in combination with lamivudine. After 72 weeks of follow-up, TAMs were detected in samples from 50% of ZDV-treated subjects and 45% of d4T-treated subjects ($P = 0.79$). The frequency of K70R and T215Y or F mutations was similar in both groups, although M41L was observed more frequently in samples from ZDV-treated subjects. This randomized study shows that TAMs accumulate at similar rates during treat-

ment with ZDV or d4T, but the specific pattern of mutations may differ somewhat in patients treated with these thymidine analogues.

Key Words: zidovudine, stavudine, drug resistance, thymidine analogues

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Nucleoside analogue inhibitors of HIV-1 reverse transcriptase (RT) are a mainstay of antiretroviral therapy. The efficacy of these regimens may be limited, however, by the emergence of drug resistance. Particular interest has been focused on cross-resistance between the 2 thymidine analogues in clinical use, stavudine (d4T) and zidovudine (ZDV). Resistance to ZDV emerges in a step-wise manner by the ordered accumulation of mutations at RT codons 41, 67, 70, 210, 215, and 219 (reviewed in Hirsch et al.¹).

Analysis of HIV-1 isolates from subjects treated with d4T-containing regimens has identified the presence of mutations classically associated with ZDV resistance.^{2,3} It is now recognized that these mutations, referred to as nucleoside-associated mutations (NAMs) or thymidine analogue-associated mutations (TAMs), can result in a pattern of cross-resistance to all nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs).⁴ Although these studies demonstrate that TAMs can be selected by d4T-containing regimens, the frequency with which these mutations emerge in virus from subjects receiving d4T- or ZDV-based regimens has not directly been compared. To address this question we performed genotypic resistance testing of serial plasma virus samples from subjects randomly assigned to receive ZDV or d4T in Adult AIDS Clinical Trials Group (ACTG) protocol 306 and followed until entry into a rollover study, ACTG protocol 370.

METHODS

Subjects and Study Design

Adult ACTG protocol 306 was a multicenter, randomized, placebo-controlled trial conducted by 21 collaborating

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Informed consent was obtained from all subjects participating in this study. Conduct of this study conformed to human experimentation guidelines of the US Department of Health and Human Services.

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Adult AIDS Clinical Trials Units. That study compared the safety and efficacy of monotherapy with didanosine (ddI) or d4T to that of dual-nucleoside regimens consisting of either ZDV plus lamivudine (3TC), d4T + 3TC, or ddI + 3TC in treatment-naïve subjects with 200×10^6 to 600×10^6 CD4 cells/L.⁵ At week 24, 3TC was added to the regimen of subjects initially assigned to ddI or d4T monotherapy. Subjects who successfully completed at least 48 weeks of treatment in ACTG 306 and remained on their assigned study regimen were eligible for entry into a rollover study (ACTG 370).⁶ Baseline data from ACTG 370 are included to provide for longer follow-up on the ACTG 306 regimens. Genotypic resistance testing was performed on samples from subjects in the ZDV- and d4T-containing arms of ACTG 306 who had plasma HIV-1 RNA levels >500 copies/mL (Quantiplex HIV-1 RNA assay version 2.0; Bayer Nucleic Acid Diagnostics, Norwood, MA) at study entry and at one or more time points at weeks 24, 48, and/or week 72. In addition, genotypic resistance testing was performed on samples from subjects with plasma HIV-1 RNA levels > 500 copies/mL (ultrasensitive HIV-1 Monitor assay version 1.0, Roche Molecular Systems, Branchburg, NJ) at entry into ACTG 370.

Nucleotide Sequencing and Analysis

Sequencing of HIV-1 *pol* (codons 41–237 of RT) was performed using the TruGene HIV-1 Sequencing Kit and OpenGene DNA Sequence Analysis System (Bayer Nucleic Acid Diagnostics).⁷ Phylogenetic trees of the aligned, manually edited sequences were constructed using PHYLIP⁸ and Treeview⁹ to verify that each patient’s viral sequence was unique. ZDV resistance was assessed as the number of ZDV resistance-associated mutations (41L, 67N, 70R, 210W, 215Y or F, 219Q).

Statistical Analyses

Fisher exact tests were used to compare the proportion of samples containing TAMs at each time point. The distribution of the number of TAMs was compared between treatment groups using a Wilcoxon test.

RESULTS

Two hundred subjects were enrolled into the ZDV- or d4T-containing arms of ACTG 306; the analyses presented here are limited to the 120 subjects who had plasma HIV-1 RNA levels >500 copies/mL at the time points of interest. Similarly, 75 subjects from the ZDV- and d4T-containing arms of ACTG 306 enrolled into ACTG 370 with plasma HIV-1 RNA >500 copies/mL; genotypic resistance data from 65 subjects are presented here. (Resistance data from the remaining subjects were not obtained either because baseline, as opposed to screening, plasma HIV-1 RNA levels were <500 copies/mL, or because genotypic testing failed to yield a result.) Median plasma HIV-1 RNA levels for subjects included

in the resistance analyses were 4.54 log₁₀ copies/mL at entry into ACTG 306 and 3.73 log₁₀ copies/mL at entry into ACTG 370. Median CD4 cell counts for these subjects were 388×10^6 cells/L at entry into ACTG 306 and 497×10^6 cells/L at entry into ACTG 370. Median duration of NRTI therapy at entry into ACTG 370 was 79 weeks (range, 61–94 weeks). Baseline characteristics of subjects with resistance test results did not differ from those of the respective ACTG 306 or 370 study populations as a whole.

Accumulation of TAMs on Thymidine Analogue Therapy

Samples from 4 subjects (1 in the ZDV/3TC arm and 3 in the d4T or d4T/3TC arms) had ≥1 TAMs at entry into ACTG 306 (Table 1). At week 24, samples from 4 of 48 subjects (8%) in the ZDV-containing arms vs. 4 of 39 subjects (10%) in the d4T-containing arms had accumulated ≥1 TAM (*P* = 1.00). (Because results from the initial d4T monotherapy and d4T/3TC arms were similar, data from these 2 arms were pooled.) At week 48, samples from 7 of 40 ZDV-treated subjects (18%) vs. 6 of 29 d4T-treated subjects (21%) had ≥1 TAM (*P* = 0.76). At week 72, samples from 17 of 34 ZDV-treated subjects (50%) vs. 10 of 22 d4T-treated subjects (45%) had ≥1 TAM (*P* = 0.79). At entry into ACTG 370, ≥1 TAM had accumulated in samples from 17 of 34 ZDV-treated sub-

TABLE 1. Emergence of Thymidine Analogue Resistance Mutations During ACTG 306 According to Treatment Assignment

Treatment Arm	Study Week	Number of TAMs						Total
		0	1	2	3	4	5	
ZDV/3TC	0	65	0	0	1	0	0	66
	24*	44	4	0	0	0	0	48
	48	33	4	1	1	0	1	40
	72	17	10	6	0	0	1	34
	370 baseline†	17	7	8	1	0	1	34
d4T/3TC	0	31	1	1	0	0	0	33
	24*	21	1	0	0	0	0	22
	48	15	2	1	0	0	0	18
	72	8	2	1	1	0	0	12
d4T‡	370 baseline†	12	3	0	0	0	0	15
	0	20	1	0	0	0	0	21
	24*	14	3	0	0	0	0	17
	48	8	3	0	0	0	0	11
	72	4	5	1	0	0	0	10
370 baseline†	5	5	1	0	0	0	11	

*Data for weeks 24, 48, and 72 exclude TAMs present at week 0.
 †Entry into ACTG 370 (median time on ACTG 306 = 79 weeks, range 61–94 weeks).
 ‡3TC added at week 24.

jects (50%) and in samples from 9 of 26 d4T-treated subjects (35%) ($P = 0.30$).

Because of variable follow-up, data from all subjects were not available at each time point. Therefore, we also tabulated the cumulative frequency of subjects in whom TAMs were ever detected (excluding those with TAMs present at entry into ACTG 306). Although the overall frequency of TAMs was similar between the 2 groups (data not shown), differences emerged when the frequency of specific mutations was analyzed. The 70R and 215Y/F substitutions were found in samples from both groups with similar frequencies (25 and 12%, respectively), but the 41L substitution was found significantly more often in samples from the ZDV/3TC-treated group: 14 of 65 (22%) vs. 3 of 49 (6%) for the d4T/3TC group ($P = 0.032$). Other TAMs at codons 67, 210, and 219 occurred infrequently.

DISCUSSION

Although resistance to the NRTIs previously was thought to be drug specific, it is now clear that the accumulation of TAMs correlates strongly with increasing resistance to other NRTIs and to the nucleotide RT inhibitor tenofovir.^{10,11} In this study, RT mutations associated with ZDV resistance were detected in plasma specimens from similar proportions of subjects treated with ZDV- or d4T-based regimens. These results extend the findings of previous reports that document the emergence of mutations associated with resistance to ZDV in virus from patients receiving d4T and demonstrate cross-resistance between these drugs.^{2,11–15} Our study is unique because we were able to compare the relative frequency of emergence of these mutations in a previously treatment-naïve population randomized to regimens that included one or the other thymidine analogue.

Although the proportion of samples with ≥ 1 TAM was not significantly different between ZDV- and d4T-treated subjects, differences in the pattern of specific TAMs were found. Notably, the M41L mutation was significantly more common in the ZDV-treated group as compared with the d4T-treated group. Differences in the rate of 41L accumulation could be clinically significant. The combined presence of 41L and 215Y confers substantial ZDV resistance.¹ Moreover, presence of the 41L and 215Y mutations together was a significant predictor of increased risk of disease progression.¹⁶ However, the numbers of subjects in each group were relatively small, limiting the power of this study somewhat. In addition, selection of the 184V mutation by 3TC, a common component of contemporary combination regimens, can blunt the effects of the 41L and 215Y mutations by enhancing susceptibility to several of the NRTIs.¹⁰

Coadministration of 3TC reduces the rate of TAM accumulation in HIV from patients receiving ZDV plus 3TC.^{17,18} Although all subjects in this study eventually received 3TC-containing therapy, subjects randomly assigned to the d4T

monotherapy arm of ACTG 306 received 6 months of d4T prior to addition of 3TC. It is possible that inclusion of samples from those subjects overestimated somewhat the rate of TAM accumulation in subjects receiving d4T plus 3TC, but the rate of TAM accumulation in the 2 d4T arms was similar overall (Table 1).

The pattern of resistance mutations selected by a drug is only one of several factors to consider when selecting a nucleoside analogue for use in an initial regimen. Other important factors include potency, convenience, tolerability, and safety. It is important to bear in mind that the rates of TAM accumulation observed in our study apply to patients receiving dual-nucleoside therapy, which in most cases failed to suppress plasma HIV-1 RNA levels below the limits of detection. By contrast, TAMs are relatively infrequent at the time of virologic failure of a triple-drug regimen.¹⁹ These findings emphasize the importance of using a fully suppressive regimen when antiretroviral therapy is administered, so that selection of TAMs can be delayed or avoided if possible.

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