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Zidovudine and Stavudine Sequencing in HIV Treatment Planning: Findings From the CHORUS HIV Cohort

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Background: Optimal sequencing of zidovudine and stavudine in antiretroviral therapy has not been elucidated.

Objective: To examine the impact of the sequence of therapeutic regimens containing zidovudine and stavudine on HIV-1 RNA and CD4 lymphocyte counts over 12 months.

Design: Observational, multicenter, longitudinal cohort study.

Setting: Four large outpatient, HIV practices participating in the community-based Collaborations in HIV Outcomes Research—U.S. (CHORUS) cohort study.

Participants: 940 HIV-infected patients.

Methods: Comparison of HIV-1 RNA and CD4 lymphocyte responses in patients sequenced from zidovudine to stavudine or from stavudine to zidovudine using repeated measures regression models fit to outcomes by application of generalized estimating equation (GEE) methodology.

Results: Patients treated with zidovudine prior to stavudine ($n = 834$) achieved a greater mean drop from baseline HIV-1 RNA ($p = .01$) and higher proportion of undetectable HIV-1 RNA results ($p = .05$) over 12 months than those sequenced from stavudine to zidovudine ($n = 106$). $CD4^+$ lymphocyte increases did not differ between the groups ($p = .6$).

Conclusions: Prior zidovudine therapy was not associated with long-term attenuation of HIV-1 RNA or CD4 response to subsequent stavudine-containing regimens. Zidovudine before stavudine may have benefit in a strategic long-term therapeutic plan.

Key Words: Nucleoside reverse transcriptase inhibitor sequencing—Zidovudine—Stavudine—NRTI—HIV-1 RNA—CD4 cell count.

The goal of antiretroviral therapy (ART) is to produce clinically significant immune reconstitution including return of HIV- and other pathogen–specific lymphoproliferative responses and increases in naive $CD4^+$ cells (1). Potent ART has been shown to achieve this goal through prolonged virus suppression (2–4). Increasingly, the challenge to physicians is to maximize therapeutic effectiveness over time with a strategic antiretroviral plan. Consideration of timing, sequence, and therapeutic combinations represent areas of heightened concern. Current recommendations by the U.S. National Institutes of Health (5)and the International AIDS Society–USA Panel (1) are to treat patients with HIV-1 infection aggressively with an initial regimen that combines three or more antiretroviral therapies. Many of these combina-

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tions resulting in significant reductions in HIV-1 RNA have included zidovudine or stavudine (1,6). However, the optimal sequence of these antiretroviral therapies has not yet been elucidated.

Regrettably, few clinical or epidemiologic data are available from which to draw firm conclusions about the optimal sequencing of these two nonnucleoside reverse transcriptase inhibitors (NRTIs) in HIV treatment. Previously, ALTIS (7) and two small intracellular phosphorylation studies (8,9) have suggested that zidovudine may impair subsequent stavudine therapy. In ALTIS, a stavudine/lamivudine regimen proved more effective virologically and immunologically in patients who had never received zidovudine-containing ART ($n = 42$) compared with zidovudine-experienced patients ($n = 41$; a difference of approximately 1 log (10) HIV-1 RNA reduction and +60 cells/(L in the CD4 cell count). Sommadossi et al. (8) and Turriziani et al. (9) have suggested that zidovudine may impair the intracellular phosphorylation of stavudine.

To expand the scope of these initial studies, an evaluation of patients from the Collaborations in HIV Outcomes Research—United States (CHORUS) cohort was undertaken. CHORUS is an ongoing, multicenter, community-based observational study that began in August, 1997. It is designed to follow clinical, epidemiologic, economic, and humanistic outcomes on a population of patients with HIV-1 infection during their routine physician visits and hospital stays at four U.S. practice sites. From the date of enrollment, data are systematically collected 12 months' retrospectively and 3 years prospectively for each patient through an electronic medical record. Additionally, key laboratory and medication data are collected from the beginning of medical care for HIV/AIDS when available. The objective of the present analysis was to examine the impact of zidovudine and stavudine sequencing on plasma HIV-1 RNA and CD4 lymphocyte responses in the CHORUS cohort of 3,997 patients with HIV infection.

METHODS

Study Population

Patients who tested seropositive for HIV-1 infection were consecutively enrolled in the CHORUS Study at four large outpatient specialty practices in the United States, including Comprehensive Care Center (Nashville, TN), Liberty Medical Group (New York City, NY), Pacific Horizon Medical Group (San Francisco, CA), and Pacific Oaks Medical Group (Los Angeles, CA). All patients provided written informed consent to participate in this study; the study protocol was approved by the Institutional Review Boards at Research Triangle Institute (Research Triangle Park, NC) and Vanderbilt University (Nashville, TN).

To be included in this analysis, patients must have demonstrated a sequence of thymidine analogue use defined as receiving at least two antiretroviral treatment regimens, one containing either zidovudine or stavudine followed by another regimen containing the other thymidine analogue. These regimens were not necessarily consecutive. If a patient had more than one sequence, the first sequence was used in the analysis. Patients were excluded if they had received zidovudine and stavudine concurrently in either regimen of the sequence or if they lacked key laboratory data at initiation of the second regimen or during 1 year of follow-up.

The present analysis was designed to assess the impact of zidovudine and stavudine sequencing on plasma HIV-1 RNA and CD4 lymphocyte responses among patients in the CHORUS observational cohort. Patients with a thymidine analogue sequence were divided into two groups respectively; patients with a stavudine to zidovudine sequence (S–Z sequencing) and patients with a zidovudine to stavudine sequence (Z–S sequencing).

Procedures

Baseline was defined as the start date of the second regimen in the sequence. Follow-up was compared for the second regimen to evaluate potential impairment or synergism of one sequence over the other. Baseline plasma HIV-1 RNA levels and CD4 counts used were those observed no more than 3 months before therapy initiation. Subsequent laboratory values were determined at quarterly intervals (±1 month) over a 1-year period from baseline. When more than one laboratory value was available for each interval, the last value was used. HIV-1 RNA response was examined first as mean change in $\log_{10}$ HIV-1 RNA from baseline. For this outcome, undetectable HIV-1 RNAs (<500 copies/ml) were assigned a value of 499 then $\log_{10}$ transformed. Secondly, an additional binary outcome (HIV-1 RNA <500 copies/ml versus HIV-1 RNA (<500 copies/ml) was created to examine the percentage of patients with an undetectable HIV-1 RNA at each time point. CD4 lymphocyte count response was evaluated as the mean change in CD4 counts from baseline.

Plasma HIV-1 RNA levels were measured by the assay used by the laboratory that provided regular clinical care. These varied by provider and payer and included reverse transcriptase polymerase chain reaction (RT-PCR), branched DNA (bDNA) signal amplification assay, ultrasensitive PCR, ultrasensitive bDNA, and nucleic acid sequence–based amplification procedure (NASBA). For the purpose of achieving comparability in HIV-1 RNA analyses, HIV-1 RNA levels reported as RT-PCR values were converted to bDNA values using the following formula published by Mellors et al. (10): bDNA value (copies/ml) = 0.2 × (RT-PCR copies/ml) (11). Additionally, ultrasensitive and NASBA tests represented <1% of all HIV-1 RNA tests available and were excluded from analysis because a conversion equation was not available. All CD4 counts reported were used for analysis.

Medical information for each patient in the cohort is maintained on a computerized patient record system developed by HealthMatics, Inc. (Cary, NC, U.S.A.) for use in physicians' offices at the time of each patient encounter. This system electronically captures detailed demographics; laboratory and procedure data; history and physical reporting; and prescriptions ordered. The computerized patient record is made anonymous and electronically transferred through a secure connection to an independent data management and analysis facility at Research Triangle Institute in Research Triangle Park, NC. The anonymous data is aggregated into a database for analysis. Patient confidentiality is maintained at every step of data collection, transfer, management, and analysis. Data quality is maintained through a quality management plan encompassing acceptance testing, ongoing site monitoring, best entry practices training, data edit checks, and data validation.

An independent advisory board oversees the program and analysis of the aggregate anonymous data. The Board is composed of physicians and researchers from participating sites, HIV community activists (nonmedical by profession), academic experts, personnel from the analysis center, and the sponsor.

Statistical Analysis

Statistical significance for unadjusted comparisons of baseline characteristics was determined by Fisher exact test for categoric variables and the Wilcoxon rank-sum test for continuous variables. Comparisons of HIV-1 RNA response and CD4 counts between the exposure groups were investigated using linear repeated measures models and logistic repeated measures models (for undetectable HIV-1 RNA, yes/no). The models were fit to outcomes across all quarterly assessments using generalized estimating equation (GEE) methodology, which accounts for the correlation of measures obtained on the same patient and allows for missing data (11). Robust variance estimators were used based on a working independence correlation structure. In addition to the exposure group indicator variable, models included: effects for time (quarterly assessment points starting with month 3), baseline CD4 count (categorized as 0–99, 100–199, 200–349, and ≥350 cells/μl), baseline HIV-1 RNA ($\log_{10}$ scale; continuous), number of antiretroviral medications included in the treatment regimen at initiation of zidovudine or stavudine, presence of a protease inhibitor in the regimen (yes/no), age at therapy initiation, time since HIV infection, and site. Models were fit initially with an interaction term between time and exposure group to look for changes in the prior exposure effect across time, and were refit with no interaction term to obtain an overall test between exposure groups. Patients remained in the analysis once selected regardless of changes in ART regimens during the 12-month analysis period after initiation of zidovudine or stavudine (an "intent-to-treat" approach). To evaluate misclassification that may have occurred with this methodology, the analysis was rerun using only data during which the patient continued without interruption on the regimen of interest (an "as-treated" approach).

Additionally, the main effects models were refit to two subsets of patients defined based on duration of zidovudine or stavudine use before beginning the second drug in the sequence. First, the models were fit using data from patients whose use of the first thymidine analogue in the sequence was less than 6 months and next using data from patients with prior use less than 12 months. This permitted examination of the effect of the duration of prebaseline therapy on the results.

A separate secondary analysis of sequenced or thymidine analogue–experienced patients versus patients with exposure to only one thymidine analogue or thymidine analogue–naive patients was carried out to replicate work previously published (7,12–15). This included a comparison of patients on zidovudine with patients S–Z sequenced and a comparison of patients on stavudine with patients Z–S sequenced. A two-sided p value of ≤.05 was considered statistically significant.

RESULTS

Patient Characteristics

The CHORUS cohort, which comprised 3,997 patients consenting to the project as of August 4, 1998, was predominantly male, white, homosexual, and averaged approximately 40 years of age. The subcohort eligible for the primary and secondary analysis was not different than the subcohort not eligible with the exception of age (Table 1).

A total of 1,870 patients (46.8%) had ever received both zidovudine and stavudine, of whom 940 (50.3%) were evaluable. The remaining 930 patients were excluded from evaluation for the following reasons: 421 had received stavudine and zidovudine concurrently, 261 did not have HIV-1 RNA or CD4 data available during the period of interest, and 248 did not have an HIV-1 RNA test result available at baseline. Of those with unavailable laboratory data, 80% to 90% had their first exposure to thymidine analogues before 1996. Of the 940 evaluable patients, 106 (11.3%) had been S–Z sequenced and 834 (88.7%) had been Z–S sequenced.

Patients in both groups were similar in age, gender, and race (Table 2). Patients in the Z–S sequenced group had a longer duration of prior exposure to ART (108 median weeks versus 43 median weeks; $p < .001$). The S–Z sequenced group more frequently included a protease inhibitor at baseline (77% vs. 60%; $p < .001$). Baseline laboratory data were not significantly different between the groups.

HIV-1 RNA Response

The comparison of HIV-1 RNA response favored the Z–S sequence in both the change from baseline and percent achieving undetectable levels in the univariate comparison (Figs. 1 and 2) and multivariable models (change from baseline, $p = .01$; percentage undetectable, $p = .05$) Overall, 31.0% of patients S–Z sequenced achieved an undetectable HIV-1 RNA level with a mean drop from baseline of −0.47 copies/μl on their second regimen in the sequence. In contrast, 42.1% of patients Z–S sequenced achieved an undetectable HIV-1 RNA level with a mean drop from baseline of −0.76 copies/μl in the adjusted analysis.

Overall and quarterly comparisons between treatment groups in mean change from baseline and percentage undetectable HIV-1 RNA are shown in Table 3. Statistically significant time by exposure group interactions were not detected, justifying the use of an overall test for each comparison.

At individual time points, Z–S sequenced patients experienced a greater drop in HIV-1 RNA from baseline in both the adjusted and unadjusted comparisons. However, limited numbers prevented statistical significance at 9 and 12 months in the adjusted model (9 months, $p = .08$; 12 months, $p = .6$). Similarly, Z–S sequenced patients were more likely to achieve an undetectable HIV-1 RNA at every time point, but the comparison lacked power to achieve statistical significance for all but the overall

TABLE 1. *Characteristics of patients at consent*

Characteristic	Total cohort (n = 3,997)	Analysis subcohort[a] (n = 1,841)	Nonanalysis subcohort (n = 2,156)	p value[b]
Age, y				
Mean ± SE	40.3 ± 0.131	39.9 ± 0.193	40.6 ± 0.178	<.001
Range	18–77	18–77	18–74	
Sex, no. (%)				
Male	3,648 (91)	1,686 (92)	1,962 (91)	.5
Race, no. (%)				
White	3,033 (77)	1,408 (77)	1,625 (76)	.8
Black	521 (13)	239 (13)	282 (13)	—
Hispanic	290 (7)	127 (7)	163 (8)	—
Other	117 (3)	56 (3)	61 (3)	—
Log_{10} HIV-1 RNA, copies/ml[b,c]				
Mean ± SE	3.30 ± 0.019	3.32 ± 0.028	3.28 ± 0.027	.5
Median	3.00	3.03	2.97	—
Range	1.93–6.86	2.16–6.86	1.93–6.19	—
CD4 cell count, cells/μl				
Mean ± SE	394.9 ± 4.49	400.2 ± 6.41	389.8 ± 6.28	.2
Median	365	368	362	—
Range	0–1900	1–1615	0–1900	—
AIDS (%)	1762 (44)	795 (43)	967 (45)	.3
Probable route of infection (%)				
Homosexual contact	2,488 (78)	1,158 (77)	1,330 (78)	.2
Bisexual contact	101 (3)	56 (4)	45 (3)	—
Heterosexual contact	365 (11)	170 (11)	195 (11)	—
Blood products	50 (2)	28 (2)	27 (2)	—
Intravenous drug use	187 (6)	79 (5)	108 (6)	—
Other	15 (<1)	10 (<1)	5 (<1)	—

[a] The analysis subcohort (1,841 patients) results from 106 S–Z sequenced patients, 834 Z–S sequenced patients, 752 zidovudine without prior stavudine patients, and 417 stavudine without prior zidovudine patients in which 44 patients were analyzed in both S–Z sequenced and stavudine without prior zidovudine groups and 224 were analyzed in both Z–S sequenced and zidovudine without prior stavudine groups. Comparisons were never made between groups with shared patients.

[b] The p values for comparisons between the analysis and nonanalysis subcohorts are from Fisher exact or χ^2 tests for categoric variables and the Wilcoxon test for continuous variables.

[c] Log_{10} HIV-1 RNA values according to bDNA scale.

SE, standard error.

comparison (3 months, p = .2; 6 months, p = .1; 9 months, p = .3; 12 months, p = .7).

CD4 Count Response

No difference was found in CD4 response between the sequencing groups in either the univariate comparison (Fig. 3) or the multivariable model (p = .6). This observation was consistent across all timepoints. The overall adjusted mean increase in CD4 cell count observed in the S–Z sequenced group was 57.24 cells/ml and 64.26 cells/ml for the Z–S sequenced group. Overall and quarterly adjusted mean change from baseline CD4 counts from repeated linear regression models are shown in Table 3.

Analysis of Confounding

Inferences were largely unchanged when group comparisons were made using data collected only while patients remained on the stavudine or zidovudine regimen of interest, an as-treated approach. An exception was percentage undetectable HIV-1 RNA for which the difference between sequences became more significant (intent-to-treat: p = .05, as-treated: p = .006). When the data were reanalyzed among the subsets of patients with <6 months' and <12 months' duration of the first thymidine regimen in the sequence, the results were similar (data not shown).

Secondary Analysis

Thymidine Analogue-Experienced Versus Thymidine Analogue-Naive

In general, thymidine analogue-naive patients had better virologic responses than thymidine analogue-experienced patients did. Thus, zidovudine-treated patients with no previous stavudine exposure (n = 752) had a greater decrease in HIV-1 RNA from baseline

TABLE 2. *Baseline characteristics of treatment groups*[a]

Characteristic	S–Z sequenced (n = 106)	Z–S sequenced (n = 834)	p value[b]
Age, y			
Mean ± SE	39 ± 0.68	39 ± 0.28	.44
Range	27–60	20–75	
Sex, no. (%)			
Male	102 (96)	788 (94)	.65
Race, no. (%)			
White	77 (73)	656 (79)	.39
Black	14 (13)	87 (10)	—
Hispanic	11 (10)	58 (7)	—
Other	3 (3)	30 (4)	—
Time since HIV infection: Mean/median mo	81/80	74/72	.12
Prior ART exposure: Duration (mean/median wk)	64/43	147/108	<.001
Current ART Exposure (% of patients)			.07
2 Concurrent ARTS	28 (26)	282 (34)	—
3 Concurrent ARTs	77 (73)	519 (62)	—
Use of Concurrent Pls	82 (77)	500 (60)	<.001
Log_{10} HIV-1 RNA, copies/ml[c]			
Mean ± SE	3.9 ± 0.107	3.9 ± 0.035	.64
Median	4.02	4.1	
Range	2.16–6.66	2.16–6.56	
CD4 cell count, cells/μl			
Mean ± SE	283 ± 23.95	300 ± 7.89	.22
Median	246	270.5	
Range	2–1180	0–1374	

[a] Baseline defined as initiation of the second thymidine analogue in a sequence.

[b] The p values for comparisons between treatment groups are from Fisher Exact or χ^2 tests for categoric variables and the Wilcoxon test for continuous variables.

[c] Log_{10} HIV-1 RNA values according to bDNA scale.

S–Z sequenced, stavudine to zidovudine sequenced; Z–S sequenced, zidovudine to stavudine sequenced; SE, standard error; ART, antiretroviral therapy; PI, protease inhibitors.

($p < .001$) (Fig. 1) and a greater frequency of undetectable HIV-1 RNA ($p < .001$) than S–Z sequenced patients (Fig. 2). Similarly, stavudine-treated patients with no previous zidovudine exposure ($n = 417$) had a greater decrease in HIV-1 RNA from baseline ($p = .01$) and a greater frequency of undetectable HIV-1 RNA ($p = .003$) than Z–S sequenced patients. The improvement in CD4 cell counts observed in each treatment group roughly paralleled the HIV-1 RNA reduction (Fig. 3). The results of the multivariable analysis were largely the same (data not shown).

DISCUSSION

In this comparison of Z–S to S–Z sequencing, evidence suggests that zidovudine does not adversely impact subsequent stavudine use. This finding was seen at every timepoint and overall. Further, consideration of sequence in developing a strategic therapeutic plan may be of importance to patients beginning ART because there is an indication that Z–S sequencing may be favored. Evaluation of optimal sequencing is best accomplished in a comparison of groups that have both been sequenced between two regimens. Consistent with other studies, we found that antiretroviral treatment–naive patients have better response to ART than treatment-experienced patients (1,12–14). To determine the effect of sequencing, we concentrated on treatment-experienced patients.

Other work in this area includes data reported from a Johns Hopkins' prospective observational study (15) and AIDS Clinical Trials Group (ACTG)-370 (16). The Johns Hopkins' study showed no difference in virologic or CD4 cell responses to a stavudine-containing regimen between zidovudine-naive ($n = 98$) and zidovudine-experienced ($n = 130$) patients over a 1-year period (15). In the ACTG-370 study, patients switched from zidovudine/lamivudine dual therapy to stavudine/delavirdine/indinavir triple therapy had a virologic response equivalent to that observed in patients switched from stavudine/lamivudine to zidovudine/delavirdine/indinavir (16).

Our finding that zidovudine does not impair subsequent stavudine efficacy counters hypotheses based on two intracellular phosphorylation studies (8,9). Sommadossi et al. (8) suggested that previous zidovudine treat-

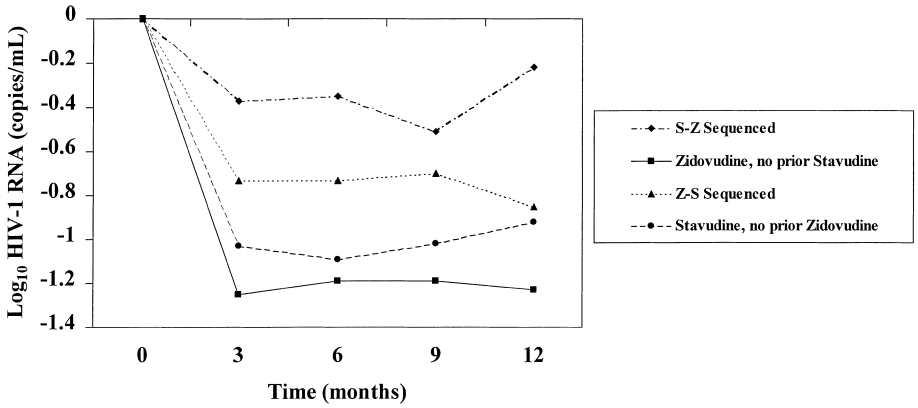

FIG. 1. Unadjusted mean change in $\log_{10}$ HIV-1 RNA levels from baseline over 12 months .

N's (Unadjusted mean change in HIV-1 RNA)					
	0	3	6	9	12
S-Z	106	51	54	33	20
Zid, no prior Stav	752	403	344	311	277
Z-S	834	492	442	388	316
Stav, no prior Zid	417	219	183	164	141

ment resulted in impaired stavudine phosphorylation through zidovudine-induced downregulation of thymidine kinase. Turriziani et al. (9) speculated that long-term treatment with zidovudine could induce an in vivo defect in thymidine kinase activity, reducing the efficiency of this enzyme to phosphorylate stavudine.

Several recent intracellular studies (17–19), taken in conjunction with the results of CHORUS, the Johns Hopkins' study (15), and ACTG-37016, suggest that the phosphorylation hypotheses do not explain differences that may exist in the clinical response of stavudine-treated patients who are zidovudine-experienced com-

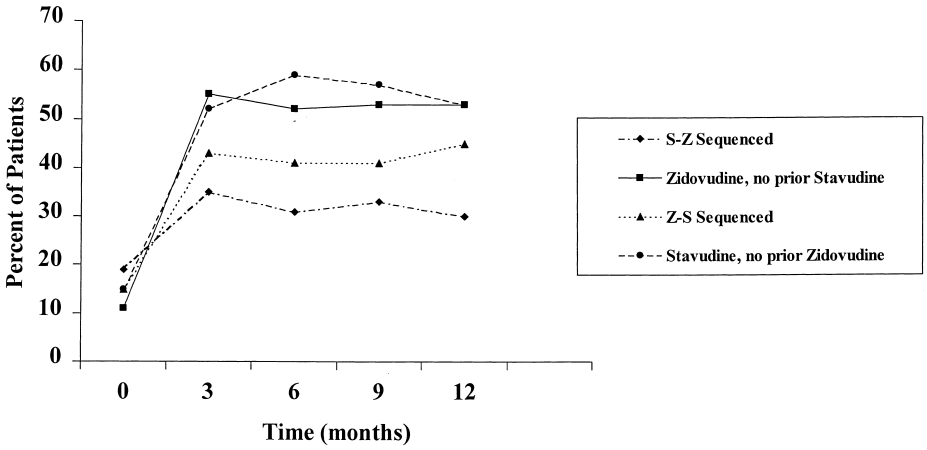

FIG. 2. Unadjusted proportion of undetectable HIV-1 RNA (<500 copies/ml) over 12 months.

N's (Unadjusted % undetectable HIV-1 RNA)					
	0	3	6	9	12
S-Z	106	51	54	33	20
Zid, no prior Stav	752	403	344	311	277
Z-S	834	492	442	388	316
Stav, no prior Zid	417	219	183	164	141

TABLE 3. *Comparison of sequenced groups: Overall and quarterly adjusted means and significance (*p *values) from repeated linear and logistic regression models*[a]

Time point	S–Z sequenced (n = 106)	Z–S sequenced (n = 834)	p value
Mean change from baseline $\log_{10}$ HIV-1 RNA (copies/ml)			
Overall	–0.47	–0.76	.01
3 Mo	–0.45	–0.79	.04
6 Mo	–0.43	–0.76	.03
9 Mo	–0.43	–0.70	.08
12 Mo	–0.67	–0.79	.6
Proportion undetectable HIV-1 RNA (<500 copies/ml)			
Overall	.310	.421	.05
3 Mo	.309	.420	.2
6 Mo	.301	.417	.1
9 Mo	.306	.418	.3
12 Mo	.324	.426	.7
Mean change from baseline in CD4 counts (cells/μl)			
Overall	57.24	64.26	.6
3 Mo	42.01	48.64	.7
6 Mo	47.98	60.55	.5
9 Mo	63.29	68.52	.8
12 Mo	91.78	87.86	.9

[a] Adjusted for the following variables: time effects, baseline CD4, baseline HIV-1 RNA, number of antiretroviral medications included in the treatment regimen at initiation of zidovudine or stavudine, presence of a protease inhibitor in the regimen, age at therapy initiation, time since HIV infection, and site.

S–Z sequenced, patients sequenced from a stavudine-containing regimen to a zidovudine-containing regimen; Z–S sequenced; patients sequenced from a zidovudine-containing regimen to a stavudine-containing regimen.

pared with those who are zidovudine-naive. The half-life of the active zidovudine triphosphate anabolite is 3 to 6 hours (20), implying that its effect on thymidine kinase should be gone within 2 days. Long-term studies in which zidovudine phosphorylation was monitored in patients treated continuously with zidovudine for 12 months (17) or >18 months (18) have indicated no negative effect on thymidine kinase activity. Furthermore, using a validated assay for determining intracellular stavudine triphosphate concentrations, Phiboonbanakit et al. (19) have recently demonstrated no significant difference between zidovudine-naive (n = 8) and zidovudine-experienced HIV-1–infected patients (n = 17). It is worth noting that stavudine is capable of being phosphorylated by other enzymatic pathways besides that involving thymidine kinase (9,21).

The apparent better response of the Z–S sequenced group could be explained by recent findings regarding thymidine analogue resistance. Although it is uncertain how stavudine might impair subsequent zidovudine efficacy, increasing evidence exists for zidovudine-associated resistance mutations (e.g., 215) being selected for in vivo in zidovudine-naive patients during treatment with NRTIs (22–24) including stavudine (25). The selection by stavudine of mutations responsible for zidovudine resistance (thymidine analogue mutations [TAMs]) might be the primary mechanism by which stavudine impairs subsequent zidovudine efficacy. In addition, several investigators have recently demonstrated that stavudine treatment is associated with the insertions of two amino acids at codon 69 that could confer resistance to zidovudine (26–29). Although most studies have shown that mutations associated with phenotypic resistance to zidovudine are not associated with high-level phenotypic resistance to other NRTIs, including stavudine (30–36), recent data demonstrate that they may be responsible for low level resistance to stavudine, which may be sufficient to compromise clinical response.

The limitations of this analysis are those common to observational studies. Differences between treatment groups and adequate control of these factors are of paramount concern. Simplified indicators of treatment and past exposure may not provide adequate adjustment for complexities of ART regimens between groups. One strategy for assessing potential bias is sensitivity analysis. For example, pretreatment experience was greater in the Z–S sequenced exposure group than in the S–Z sequenced exposure group. To assess whether this difference produced any impact on our results, we conducted sensitivity analyses among subgroups with <12 months and then <6 months of exposure to the first regimen in the sequence, respectively. Similar results were obtained from both analyses suggesting that pretreatment differences did not explain our results.

Selection bias may also have resulted from the nonrandomized nature of the study. In particular, many patients were excluded from the analysis because their historical laboratory data were not available at baseline owing to the limited availability of HIV-1 RNA tests before 1996. As a result, the analysis reflected regimens that were more recently prescribed and inferences should be restricted to patients treated since 1996. Further, study participants were predominantly white men. All were treated at HIV specialty clinics. It is possible that the findings may differ for a more diverse sample. Additionally, observational data potentially suffer from confounding by "intention to treat," meaning there may be ancillary factors affecting the decision-making process regarding treatment, which may put a subset of patients at a greater risk of having an inferior outcome. The similarities of the treatment groups at baseline and the analysis subcohort to the nonanalysis subcohort argue against substantial bias from these sources. However, residual confounding can not be entirely ruled out, suggesting caution in interpretation of these results.

Of note, surrogate markers were used in this analysis and may not equate to clinical outcomes. Plasma HIV-1

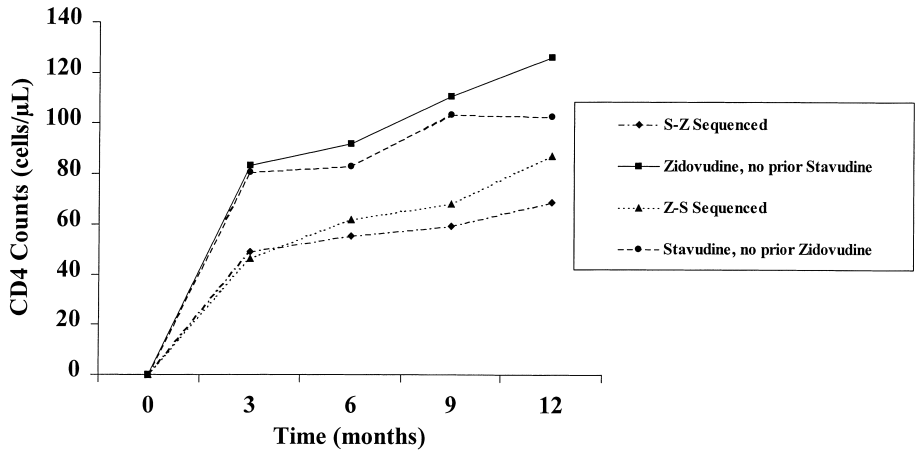

FIG. 3. Unadjusted mean change in CD4 cell counts from baseline over 12 months.

N's (Unadjusted mean change in CD4)					
	0	3	6	9	12
S-Z	97	42	45	35	18
Zid, no prior Stav	696	388	319	308	276
Z-S	768	436	417	357	294
Stav, no prior Zid	378	207	170	162	121

RNA assays available for analysis were predominantly not ultrasensitive. More recently, an assay has become available that has a much lower quantitation limit of 20 copies/ml (37). Use of ultrasensitive assays could potentially affect the percentage of patients reported to have achieved undetectable HIV-1 RNA levels. As more data are collected on this cohort, the question of optimal thymidine analogue sequencing could be addressed using clinical outcomes.

Observational studies have the advantage of reflecting the real world experience of large numbers of patients over long periods, uniquely providing clinically relevant information about the variables that influence regimen success. The strengths of this analysis included a large population observed during a follow-up of 12 months and use of an intent-to-treat methodology. Patients with adverse reactions and patients who experienced treatment failures remained in the analysis regardless of treatment changes providing potentially more conservative and robust results. When the data were reanalyzed with an "as treated" methodology to ascertain potential misclassifications in the intent-to-treat analysis, the inferences remained.

In conclusion, prior zidovudine therapy was not associated with long-term attenuation of HIV-1 RNA or CD4 response to subsequent stavudine-containing regimens. These findings support the need for consideration of thymidine analogue sequence in developing a strategic therapeutic plan as zidovudine prior to stavudine may have benefit. Further studies are needed to replicate these results and evaluate the variables influencing the optimal sequence and time to drug failure, as well as the mechanisms behind these observations.

APPENDIX

The following are members from participating sites in the CHORUS Observation Study:

The Comprehensive Care Center, Nashville, Tennessee: Stephen Raffanti, principal investigator; Wendy Mangialardi; Anne Maier; *Liberty Medical Group, New York, New York:* Douglas T. Dieterich, principal investigator; James F. Braun; Kirk Lawson; Rose Tirelli; *Pacific Horizons Medical Group, San Francisco, CA:* Stephen Becker, principal investigator; Jeremy Berge; Thomas Bond; *Pacific Oaks Medical Group, Los Angeles, CA:* Anthony Scarsella, principal investigator; Laurie Shaker-Irwin.

The following are consultants for the CHORUS Observational Study:

Amy C. Justice, University of Pittsburgh and VA Pittsburgh Healthcare System, Pittsburgh, PA; Michael S. Saag, University of Alabama-Birmingham, Birmingham, AL: Richard Moore, Johns Hopkins University, Baltimore, MD; Kevin Frost, American Foundation for AIDS Research, New York City, NY; Dawn Averitt, AIDS Treatment Advocate, Raleigh, NC; Wintson Liao, Research Triangle Institute; Neil Graham, Jeff Chulay, and Ebere F. Igboko, Glaxo Wellcome, Inc.

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