

The Effect of a Potent, Four-drug Combination (Lopinavir/ritonavir, Efavirenz, Tenofovir DF and Lamivudine) on Reducing HIV Unspliced mRNA and Proviral DNA in PBMC

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INTRODUCTION

Analysis of the dynamics of the replication of human immunodeficiency virus type 1 (HIV-1) *in vivo* has provided substantial insight into the mechanisms of HIV pathogenesis and has influenced treatment strategies. Mathematical modeling of clinical trial data suggests that the decrease in plasma HIV-1 RNA in response to combination therapy occurs in three phases. In the first phase, effective antiviral therapy interrupts the replication of HIV-1, allowing the estimation of the half-lives of free virus ($t_{1/2}$ ~6 hour) and virus-producing CD4+ T cells ($t_{1/2}$ ~1 day).^{1,2} In the second phase of viral decay, the average half-life has been found to be ~14 days,³ reflecting the slower turnover of a distinct population of chronically infected cells. After several weeks of combination therapy, the level of plasma virus decreases to below the limit of detection in many patients. However, it has been demonstrated that there is a third, more stable compartment consisting of resting memory CD4+ T-cells harboring integrated, replication-competent HIV in patients with undetectable VL for years.^{4,5} In one study, this compartment was found to decay with a mean half-life of 6 months.^{6,7} In contrast, little decay ($t_{1/2}$ 44 months) was found in another study.⁸ To address this apparent discrepancy and understand the effect of maximal viral suppression on the decay rate of this persistent reservoir of latent infected cells, we analyzed the proviral DNA and unspliced mRNA (US mRNA) in longitudinal PBMC samples from 8 HIV infected adults who had received combination therapy with four highly active antiviral agents. This regimen has been previously demonstrated to be 20% more potent than other currently employed antiretroviral regimens based on first phase viral decay measurements.⁹

OBJECTIVE

To determine the effect of a potent 4-drug antiretroviral regimen on the decay rate of US mRNA and proviral DNA in patients with drug-sensitive HIV.

MATERIALS AND METHODS

- Patients: Eight HIV-infected adults were treated with lopinavir/ritonavir (LPV/r) (533/133 mg BID), efavirenz (600 mg QD), tenofovir DF (300 mg QD) and lamivudine (150 mg BID) for at least 9 months (Table 1). All patients were either treatment naïve or demonstrated no significant protease and reverse transcriptase mutations at baseline. Moreover, all patients had HIV RNA levels durably suppressed to <50 copies/ml after week 20.
- Peripheral blood mononuclear cells (PBMC) were collected at baseline and every 3 months during therapy.
- DNA was extracted from PBMC samples using a DNAase kit. Proviral DNA was quantified using a real-time PCR assay. The limit of quantitation of this assay was 48 copies/10⁶ PBMC.
- The levels of cell-associated HIV-1 US mRNA were measured by a quantitative real-time PCR assay with use of appropriate, precisely matched oligonucleotide primer pairs to identify unspliced viral mRNA. The limit of quantitation of this assay was 150 copies/10⁶ PBMC.
- Decay rates for proviral DNA were calculated using least-squares regression analysis of log-transformed data, using data from weeks 12 to 36. The half-life was calculated as $(-\log_{10}(2)/\text{average slope})$.
- Decay rates for US mRNA were calculated using log-transformed data from baseline to week 12, due to the high proportion of patients with undetectable US mRNA by week 12.

Table 1. Characteristics of Study Subjects

Patient ID	HIV Infection Status at Start of Rx	Baseline Plasma Viral RNA (copies/ml)	Baseline CD4 Count (cells/mm ³)
A	Acute	418800	394
B	Acute	70810	367
C	Chronic	31730	366
D	Chronic	443900	172
E	Chronic	20110	416
F	Chronic	55880	377
G	Chronic	20030	399
H	Chronic	23840	719

Figure 1. Changes in Plasma Viral Load During Therapy in the Eight Subjects

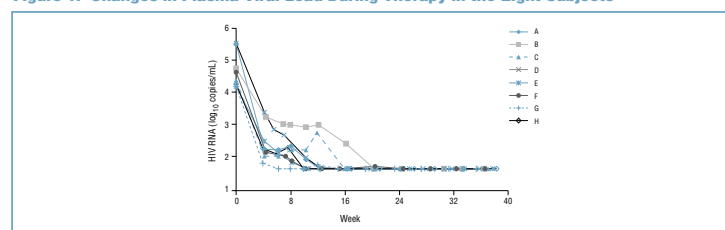


Table 2. Mean ± Standard Error for Proviral DNA and US mRNA at Each Visit

Visit	Proviral DNA* (log ₁₀ copies/10 ⁶ cells)	US mRNA# (log ₁₀ copies/10 ⁶ cells)
Baseline (n=8)	3.97 ± 0.19	3.66 ± 0.27
Week 12 (n=8)	3.54 ± 0.23	2.47 ± 0.19
Week 24 (n=8)	3.41 ± 0.23	2.21 ± 0.04
Week 36 (n=8)	3.20 ± 0.27	undetectable

* Limit of quantitation was 1.68 log₁₀ copies/10⁶ cells
 # Limit of quantitation was 2.16 log₁₀ copies/10⁶ cells

Figure 2. Mean (+ SEM) Proviral DNA and US mRNA Values

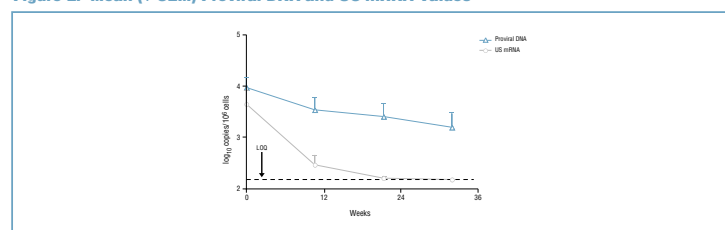


Table 3. Decay Rate of HIV-1 US mRNA in PBMC During the First 12 Weeks of Therapy

Patient ID	US mRNA (log ₁₀ copies/10 ⁶ PBMC)	Slope (log ₁₀ copies/10 ⁶ PBMC/day)	Half-life (days)	
A	3.51	≤-2.18	≤-0.0153	≤20
B	4.05	2.88	-0.0137	22
C	≤2.18	≤2.18	NA	NA
D	4.55	3.69	-0.0092	33
E	3.42	2.24	-0.0127	24
F	3.62	≤2.18	≤-0.0154	≤19
G	3.34	≤2.18	≤-0.0129	≤23
H	4.60	2.27	-0.0254	12
Mean (95% CI)	3.66	2.47	(-0.0112, -0.0187)	20 days
Half-life based on mean slope (95% CI)				(16 days, 27 days)

NA = not applicable

Figure 3. Changes in the Level of Cell Associated US mRNA During 12 Weeks of Therapy

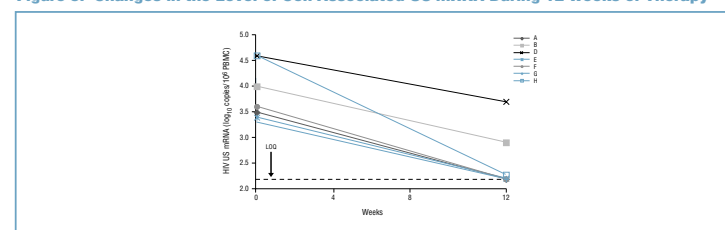


Table 4. Decay Rate of Proviral DNA Between Weeks 12 to 36 of Therapy

Patient ID	Proviral DNA (log ₁₀ copies/10 ⁶ PBMC)	Slope (log ₁₀ copies/10 ⁶ PBMC/day)	Half-life (days)		
A	3.68	3.48	3.34	-0.00209	144
B	4.50	4.22	4.15	-0.00211	142
C	3.33	3.18	3.02	-0.00176	171
D	4.12	3.87	3.97	-0.00093	323
E	3.24	2.95	3.09	-0.00092	328
F	2.43	2.13	<-1.68	≤-0.00453	≤66
G	3.18	3.50	2.77	-0.00261	115
H	3.82	3.93	3.60	-0.00114	265
Mean (95% CI)	3.54	3.41	3.20	(-0.00201, -0.00228)	150 days
Half-life based on mean slope (95% CI)					(106 days, 253 days)

Figure 4. Changes in the Levels of Proviral DNA in PBMC from Week 12 to Week 36

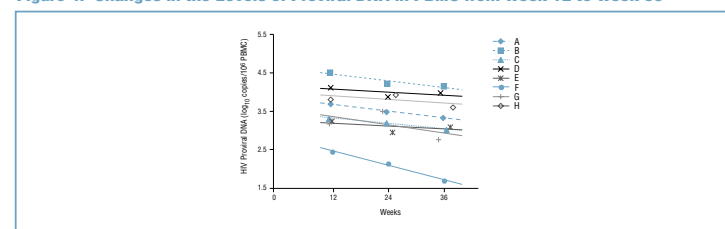


Table 5. Comparison of the Half-life of Latently Infected Cells Observed in Different Studies

Investigator	Number of Patients	Regimens	Method	Mean Half-life
Finzi D et al.	34	3 or 4 drugs, 1-2 PI and 2 RTIs	Quantitative viral culture	43.9 months
Ramratnam B et al.	33	3 or 4 drugs, 1-2 PI and 2 RTIs	Quantitative viral culture	18 months* 6.3 months*
Zhang L et al.	8	AZT/3TC with RTV/SQV or SQV or RTV	Quantitative viral culture and proportion of parental proviral sequence	6.2 months 6.3 months
Present Study	8	LPV/r with EFV/TFV/3TC	Quantitative proviral DNA	4.9 months

* Mean half-life of 33 subjects.
 * Mean half-life of 12 subjects with well-suppressed viral load.

SUMMARY OF RESULTS

- The first two phases of viral decay were observed in the plasma HIV RNA (Figure 1). The third phase of decay was observed in the proviral DNA between weeks 12 and 36 (Figure 4).
- A substantial reduction in cell-associated US mRNA was observed in all 8 subjects. During the initial 12 weeks of therapy, the mean concentration of HIV US mRNA dropped sharply from 3.66 to 2.47 log₁₀ copies/10⁶ PBMC (Table 2 and Figure 2). The mean slope for the decline during this period was -0.0149/log₁₀ copies/10⁶ PBMC/day, corresponding to a half-life of 20 days (Table 3 and Figure 3).
- By week 12 of therapy, plasma HIV RNA and US mRNA were low or undetectable in 6/8 and 7/8 patients, respectively, suggesting that the second phase of viral decay was largely finished.
- Similarly, the mean concentration of proviral DNA dropped from 3.97 to 3.54 log₁₀ copies/10⁶ PBMC during the initial 12 weeks of therapy (Table 2). Thereafter, a slower and relatively constant decline of proviral DNA was observed, suggesting that this decline is reflective of the third phase of viral decay. The mean slope for decline of proviral DNA was -0.0020 log₁₀ copies/10⁶ PBMC/day (Table 4), corresponding to a half-life of 150 days (Table 4, Figure 4).

CONCLUSIONS

- The decay of HIV US mRNA during the initial 12 weeks therapy observed in this study (mean $t_{1/2}$ 20 days) was faster than that observed in a previous study, in which the median half-life was 65 days during the first phase of viral decay.¹⁰
- Similarly, the half-life of latently infected CD4 cells estimated in this study (4.9 months) appears to be shorter than that found in previous studies (6.2 to 43.9 months) even in patients with well-suppressed plasma RNA.^{1,5,6}
- The difference between this study and other studies may be related to the degree of suppression of viral replication *in vivo* by the combination of four highly potent drugs.

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