

Improvements in Subcutaneous Fat, Lipid Profile and Parameters of Mitochondrial Toxicity in Patients with Peripheral Lipotrophy when Stavudine is Switched to Tenofovir (LIPOTEST Study)

ABSTRACT

Background: Stavudine-associated mitochondrial toxicity may play a role in the etiology of lipotrophy. We hypothesized that switching from stavudine to tenofovir will result in an increase in mtDNA in peripheral blood mononuclear cells (PBMCs), with improvements in the lipid profile and peripheral lipotrophy.

Methods: The study included 53 patients with HIV-1 RNA <50 copies/mL on stavudine-based ART regimens with lipotrophy. Stavudine was discontinued for lipotrophy, whereas the other drugs were maintained. Patients were followed-up prospectively. Fasting plasma samples were obtained to measure the lipid profile and lactatemia. Real-time PCR was used to quantify the mtDNA:nDNA ratio in PBMCs. Fat mass and subcutaneous malar fat thickness were determined by bioelectrical impedance analysis and ultrasonography, respectively.

Results: Baseline characteristics: mean age was 40 years, 68% were males, median CD4 count was 434 cells/ μ L. Over 18 months of treatment, withdrawal of the drug due to adverse effects of virological failure was not required in any case. In three patients with virological failure, viral isolates remained sensitive to tenofovir. There were no significant differences in CD4 cell counts. Median lactate values, mtDNA ratio, lipid profile, fat mass and malar fat (Bichat) thickness are shown in the table.

Conclusions: Switching from stavudine to tenofovir was well-tolerated and virological and immunological success was maintained. Patients showed rapid improvements in the lipid profile, decreased lactate levels and a slight increase of mtDNA content in PBMCs. In addition, they presented a slow but significant recovery of the fat mass and malar fat thickness.

Parameter	Baseline	Month 6	Month 12	Month 18
Lactate level (mM/L)	1.7 (1.1-2.7)	1.3 (0.8-1.7)*	1.2 (0.9-1.7)*	1.1 (0.8-1.8)*
PBMC mtDNA ratio	42 (32-63)	57 (39-78)	57 (44-78)	66 (45-112)*
Total cholesterol (mg/dL)	225 (172-262)	195 (161-229)	202 (158-248)*	202 (158-248)*
Triglycerides (mg/dL)	264 (145-497)	205 (123-334)	209 (119-414)*	212 (130-368)*
Fat (kg)	18.4 (14.1-23.1)	19.2 (14.2-25.1)	20.2(16.3-26.2)*	21.2(16.2-26.6)*
Malar fat thickness (mm)	2.9 (2.6-4.1)	3.1 (2.4-3.6)	3.1 (2.3-3.9)	3.7 (2.8-4.1)*

INTRODUCTION

- Peripheral lipotrophy is common in HIV-infected patients receiving antiretroviral therapy (ART) and is associated with dyslipidemia and lactic acidemia.
- The type and duration of nucleoside reverse transcriptase inhibitor (NRTI) therapy, especially stavudine, has been related to lipotrophy.
- Lipotrophy in these patients may be a result of NRTI mitochondrial toxicity. Mitochondrial DNA (mtDNA) depletion has been found in adipocytes and PBMCs.
- Clinical improvement in fat loss was demonstrated after replacing stavudine with abacavir or zidovudine, without changes in metabolic profile.
- The potential for tenofovir to interfere with mitochondrial functions is low.
- We hypothesized that switching from stavudine to tenofovir will result in an increase in mtDNA in PBMCs and an improvement in lipid profile and peripheral lipotrophy.

SUMMARY / CONCLUSIONS

- Switching from stavudine to tenofovir was well-tolerated, and virological and immunological success was maintained.
- Patients showed rapid improvement of the lipid profile, with a decrease in total cholesterol and in the total/HDL cholesterol ratio.
- We observed a decrease in lactate levels in patients with hyperlactatemia at baseline, and slight increase of mtDNA content in PBMCs.
- In addition, a slow but significant recovery of the fat mass and the subcutaneous malar fat thickness was observed.

METHODS

Study design:

- On-going prospective, open-label, one-arm, single-center switch-study.

Inclusion criteria:

- Moderate/severe lipotrophy at least in the face on physical examination.
- VL <50 copies/mL at screening and for at least the preceding 6 months.
- Stable ART therapy for at least 6 months, with standard stavudine doses.
- No prior tenofovir therapy.
- Absence of an active AIDS-defining condition.

Treatment switch:

- Stavudine was switched to tenofovir, while continuing all other drugs.

Study visits:

- Screening, baseline (n=53), months 1 (n=41), 3 (n=53), 6 (n=48), 9 (n=41), 12 (n=39), 18 (n=28). The first 12 patients enrolled were not evaluated at month 1.

Measurements:

- Clinical data and adverse events, fasting blood testing (blood cell count, liver enzymes, urea, creatinine, glucose, total, HDL and LDL cholesterol, and triglycerides), HIV-RNA (VL) (lower limit of detection: 50 copies/mL) and CD4 cell count, venous lactate (sample collection and processing performed carefully to avoid false-positive results), mtDNA:nDNA ratio in PBMCs (real-time PCR), and measures of lipotrophy (every 6 months).

mtDNA assessment:

- To determine mitochondrial DNA levels in PBMCs, we extracted total DNA with a commercial DNA extraction kit.
- We developed a single-tube real time PCR targeting a mitochondrial and a nuclear gene (FAM probe, 12S ribosomal gene; and VIC probe, RNase P gene).

- An ABIPrism 7700 Sequence Detector (Applied Biosystems) was used.
- Results expressed as number of mtDNA copies per nuclear DNA copy.

Assessment of body fat changes:

- Malar fat thickness was assessed by ultrasonography (mean value of two measurements on the right malar bone, without pressing the underlying skin).

- Total body fat was assessed by bioelectrical impedance.

- Anthropometry and body mass index.

Statistical analysis:

- SPSS for Windows (version 12.0).
- All analyses were performed by nonparametric tests, as follows:
 - Quantitative variables: median and IQR (central tendency and dispersion), Wilcoxon rank sum test (paired comparisons).
 - Qualitative variables: number of patients in each category and %, chi-square or Fisher exact test.
- All statistical tests were two-tailed. Statistical significance was set at P<0.05.

RESULTS

Baseline Characteristics (n=53)

Characteristic	Value	Current ART (+ tenofovir)	Value
Male	36 (68%)	3TC + NNRTI	31 (58%)
Age, y	40 (37-45)	3TC + PI	17 (32%)
HIV exposure group: IDU	17 (32%)	Abacavir + NNRTI	4 (8%)
Homosexual	11 (21%)	Abacavir + PI	4 (8%)
Heterosexual	20 (38%)	dIdI + NNRTI	4 (8%)
Other	5 (9%)	dIdI + PI	1 (2%)
Time since HIV diagnosis, y	7.4 (4.3-11.7)	3TC + abacavir	3 (6%)
Time on ART, y	5.4 (1.4-7.1)	dIdI + abacavir	1 (2%)
Time on d4T treatment, y	4.2 (1.2-5.4)		
AIDS category	28 (53%)		
Body mass index	22.5 (20.5-24.3)		
CD4 cell count, cells/ μ L	434 (297-733)		

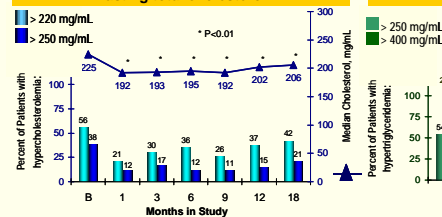
Patients disposition (end of follow-up)

HIV RNA < 50 copies/mL (ITT): 50 (94%)	
Virologic failure	3 (6%)
Discontinued tenofovir	0
Discontinued any drug	3 (6%)
3TC alone	1 (2%)
3TC and abacavir	1 (2%)
3TC and efavirenz	1 (2%)
Discontinuation due to adverse events	0
Withdraw consent	0
Lost to follow-up	0
Opportunistic diseases	0
Death	0

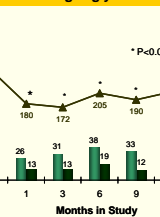
Patients with virologic failure (follow-up)

Baseline treatment	Mutations	New treatment	VL follow-up
TDF + ABC + 3TC	K65R, M184V	TDF + AZT + EFV	<50 copies/mL
TDF + ABC + 3TC	M184I	TDF + ABC + EFV	<50 copies/mL
TDF + 3TC + EFV	K65R, M184V, K103N	TDF + AZT + LPV/r	<50 copies/mL

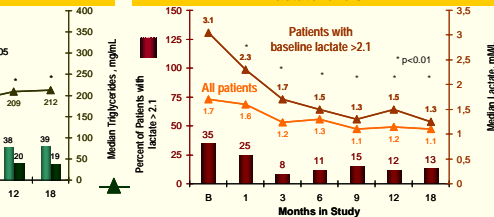
Fasting total cholesterol



Fasting triglycerides

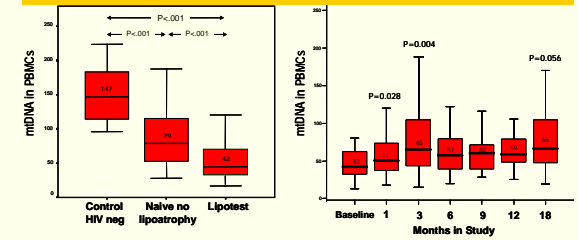


Lactate levels

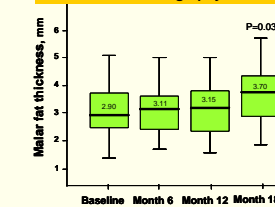


- Significant decrease in LDL cholesterol (median values: 114, 107, 110, 108, 101, 109 and 109 mg/dL) and total cholesterol/HDL cholesterol ratio (median values: 5.3, 4.9, 4.7, 4.7, 4.8, 4.9 and 4.9)
- No changes in HDL cholesterol

Real-time PCR



Ultrasonography



Bioelectrical Impedance

