

## Viral efficacy maintained and safety parameters improved with a reduced dose of stavudine: a pilot study

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### Objectives

Stavudine (d4T) is a potent but potentially toxic nucleoside reverse transcriptase inhibitor that is still widely used in developing countries. This study's aim was to determine the efficacy and safety profile of lower-dose d4T.

### Methods

Multi-centre, open-label, single-arm, pilot, 48-week study in French patients weighing > 60 kg with viral load < 400 HIV-1 RNA copies/mL who were receiving d4T 40 mg twice daily and then switched to 30 mg twice daily. The primary endpoint was the proportion with plasma viral load < 400 copies/mL at week 24. Secondary endpoints included the proportion with < 50 copies/mL at weeks 24 and 48, changes in mitochondrial DNA, CD4 cell count and pharmacokinetics, and clinical and laboratory safety.

### Results

Fifty-seven patients enrolled. Baseline CD4 count was 584 cells/μL; viral loads were < 400 copies/mL and < 50 copies/mL in 100% and 89%, respectively. Prior antiretroviral drug exposure was 6.9 years, d4T exposure was 6.3 years. Fifty-six out of 57 (98%) patients had viral load < 400 copies/mL and 51 (89%) had viral load < 50 copies/mL at week 24. Median CD4 count increased by 63 cells/μL at week 48 ( $P = 0.006$ ). At 48 weeks, total cholesterol decreased by 0.24 mmol ( $P = 0.02$ ), high-density lipoprotein cholesterol by 0.15 mmol ( $P = 0.0001$ ) and alanine aminotransferase by 5.74 mg/dL ( $P = 0.01$ ). Paired baseline DNA and week 24 RNA mutations were unchanged. Mitochondrial DNA (copies/cell) content increased from  $672 \pm 254$  to  $682 \pm 269$ . d4T area under the plasma concentration time curve (AUC) decreased by 31% ( $P = 0.003$ ) and  $C_{max}$  by 44% ( $P = 0.004$ ). Clinical and laboratory parameters improved or were unchanged.

### Conclusions

Reduced-dose d4T is effective with improved safety parameters.

**Keywords:** combination antiretroviral therapy, mitochondrial toxicity, nucleoside reverse transcriptase inhibitor, reduced-dose stavudine, stavudine pharmacokinetics

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## Introduction

Stavudine (d4T) is a thymidine analogue nucleoside reverse transcriptase inhibitor (NRTI) that had been widely used in

Western countries, originally as part of a mono- or a dual-NRTI combination with either didanosine (ddI) or lamivudine (3TC). In the early era of combination antiretroviral therapy (cART), a protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI) was added to these nucleoside combinations. Over the past 5 years, d4T use in developed countries has declined significantly because of real or potential long-term toxicity concerns, while at the same time its use in resource-limited countries – typically

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as one of the active components of a fixed-dose combination tablet with 3TC and/or nevirapine (e.g. Triomune<sup>®</sup>) – has increased dramatically. The need for the rapid scaling up of cART in developing countries has led to the use of generic formulations of many antiretroviral (ARV) agents, which are potent and offer an acceptable adherence and treatment management profile in the context of limited resources [1,2]. Access to other NRTIs such as tenofovir and abacavir (ABC) has been limited because of relative cost and access concerns. In 2008, it is estimated that 2–3 million patients in developing countries are receiving cART. Many of the regimens include d4T at the standard dosages [3].

d4T and other NRTIs were developed in the pre-cART era when plasma viral load could not be measured routinely. In the context of sub-optimal mono- or dual-NRTI therapy, dosages of the NRTIs were originally pushed as high as possible in order to maximize the antiviral effect. Zidovudine (ZDV), ddI, zalcitabine and d4T were all studied at much higher doses than the ones currently recommended. d4T was originally studied at doses ranging from 0.1 to 12 mg/kg/day [4–6]. It is a fair assumption that the optimal dosage was not determined when d4T was approved. Peripheral neuropathy was the first significant dose-related toxicity associated with d4T with an incidence reported at 9.8 per 100 patient-years [7–9]. Toxicities such as lipodystrophy, fat accumulation and lactic acidosis were not recognized or were overlooked [10–18]. d4T was approved in part because of a 30-cell increase in CD4 cell count and a lower clinical endpoint rate (but no mortality difference) in patients failing ZDV monotherapy who switched to d4T [10]. While reduction of the d4T dose has been correlated with an improved safety profile, particularly in terms of peripheral neuropathy, its efficacy at these lower doses was not well evaluated in published studies [8].

d4T has several beneficial qualities that are important in the context of treatment in resource-limited settings. Firstly, d4T has been shown to be a virologically potent component of a cART regimen that is as effective as tenofovir. In a large randomized study in treatment-naïve patients, the percentage of patients with viral load <400 HIV-1 RNA copies/mL at 48 weeks was 84% in the d4T-containing arm compared to 80% in the comparator tenofovir-containing arm. Because this was the primary endpoint of the study it should be noted that the results were not equivalent, favouring the d4T group. However, looking at the proportion of patients with viral loads <50 copies/mL, the results were equivalent: 80% in the d4T arm and 76% in the tenofovir arm [19].

Another benefit of d4T is its favourable acute tolerability profile and limited need for intensive monitoring when therapy is initiated. Typically, anaemia, renal insufficiency

and hepatitis do not occur because of d4T treatment: hence initial monitoring requirements are quite minimal. This favourable acute tolerability profile translates into high adherence rates. In the study described earlier, adherence was estimated at 98% for those taking d4T – the same rate as for those receiving tenofovir-based therapy. Furthermore, the overall drug discontinuation rate was only 4% for d4T compared to 8% for tenofovir [19]. In contrast to ZDV, another thymidine analogue commonly used in resource-limited settings, the discontinuation rate for drug toxicity can be as high as 9%, primarily because of anaemia and leucopenia [20], uncommon side effects for those receiving d4T.

Finally, because of very low manufacturing costs compared to other ARV agents, the price of d4T-containing cART regimens is significantly lower than therapies that utilize the other commonly used nucleosides such as ZDV, tenofovir or ABC. This is a very important issue in many resource-limited countries. For all of these reasons, current guidelines in many countries coupled with guidelines published by the World Health Organization (WHO) still recommend the use of d4T in first-line therapy as a preferred or alternate choice [3]. A recent amendment to the WHO Guidelines recommends d4T use only at the reduced dosage [21]. Because of the existing clinical and economic pressures to continue using d4T in resource-limited settings, the key question is whether this drug can be used in a way that limits drug-induced toxicity, such as utilizing lower dosages. Furthermore, even in the context of a first-line strategy without d4T or ZDV, these thymidine analogues will remain a part of second-line and subsequent strategies in the event of tenofovir and/or ABC resistance or intolerance.

## Patients and methods

This was an open-label, single-arm, pilot, multi-centre 48-week study performed in France that evaluated the effect of switching d4T 40 mg twice daily to 30 mg twice daily in patients weighing >60 kg with optimally suppressed viral load. The primary endpoint was the proportion of patients with a plasma viral load <400 copies/mL at week 24. Secondary endpoints included the proportion of patients with viral load <50 copies/mL at weeks 24 and 48, changes in CD4 cell count, mitochondrial DNA and pharmacokinetic parameters over time, and clinical and laboratory safety.

Inclusion criteria consisted of documented HIV-1 infection, age >18 years, weight  $\geq$  60 kg, current administration of d4T 40 mg twice daily as part of a cART regimen for at least the last 3 months and viral load <400 copies/mL. There was no restriction in the other ARV drugs used in the

cART regimen. Exclusion criteria were: presence of an active opportunistic infection; pregnancy or breast feeding; alcohol abuse; and concurrent therapy with interferon, interleukine-2 or anti-cancer chemotherapy. The Institutional Review Boards of the Hôpital Pitié-Salpêtrière and Hôpital Tenon approved the study protocol; all patients gave their written informed consent.

All patients had their daily dose of d4T reduced at day 0 from 40 to 30 mg twice daily without modification of the other components of the cART regimen. Clinical and biological evaluations were carried out at the screening visit (within 30 days of enrolment), at baseline and at weeks 4, 12, 24, 36 and 48. Each visit included a physical examination, safety assessments with measurement of full blood cell count and chemistry, plasma viral load and CD4 cell count. Glucose, total cholesterol, triglycerides, direct low-density lipoprotein (LDL) and direct high-density lipoprotein (HDL) cholesterol were measured after an overnight fast at baseline, week 24 and week 48. Anthropometric measurement, including body weight, waist, hip and mid-thigh (15 cm above patella) circumferences were assessed at each visit. A neurological evaluation in a sub-set of patients was performed at baseline, week 24 and week 48; this included a neurological examination, completion of a self-reported questionnaire and (if needed) an electromyogram. Improvement of peripheral neuropathy was defined as a decrease in signs or symptoms as per the evaluation of a single independent neurologist and/or improved score in the patient questionnaire.

Plasma viral load was determined using the Amplicor HIV-1 Monitor™ assay version 1.5 (Roche Diagnostics, Basel, Switzerland) with a detection limit of 400 copies/mL or a bDNA assay version 3.0 (Bayer Healthcare Diagnostics Division, Puteaux, France). The Amplicor HIV-1 Monitor ultra-sensitive assay with a lower limit of detection of 50 copies/mL was performed on available plasma aliquots. Mitochondrial HIV DNA (mtDNA) was quantified from peripheral blood mononuclear cells (PBMC) at baseline and week 48 according to published techniques [22–24]. Both nuclear [23] and mitochondrial [24] genes were amplified by a real-time polymerase chain reaction assay using an ABI PRISM® 7000 Sequence Detector System (AB Applied Biosystems, Foster City, CA, USA). The resulting values were both taken into account to determine the number of copies of mtDNA per cell (copies/cell). All measurements were performed in a single batch.

Genotypic analyses of the reverse transcriptase (RT) and protease (PR) genes on proviral HIV-1 DNA was performed at baseline and in the event of virological rebound > 400 copies/mL. The viral DNA sequences were analysed using Sequence Navigator software [Area under the plasma (blood or serum) concentration time curve (AUC), half-life

( $T_{1/2}$ ), clearance of distribution (Cl/F), maximum concentration ( $C_{max}$ )] and the mutations detected in the PR and RT genes were considered to be associated with drug resistance according to the International AIDS Society – USA list ([www.iasusa.org](http://www.iasusa.org)).

### Pharmacokinetics

Plasma concentrations of d4T were determined at 0, 1, 3, 6 and 12 h after the last dosage was taken at baseline before the switch and at week 24 in a sub-group of 11 patients using a validated high-performance liquid chromatography assay coupled with ultraviolet detection [25]. The lower limit of quantification of the assay was 10 ng/mL. The assay was linear from 10 to 5000 ng/mL.

### Statistical analyses

Under the null hypothesis of a theoretical proportion of 92% of patients with plasma viral load < 400 copies/mL at week 24 (primary endpoint), we calculated that if at least 51 of 55 enrolled patients had virological suppression this would ensure a lower limit of the 95% confidence interval (CI), that the proportion of patients with virological suppression is significantly superior to 80% with a two-sided  $\alpha$  level of 0.05.

The primary efficacy population was the intent-to-treat population, which consisted of all patients who were included in the study. Patients who were lost to follow-up were considered as failures. Changes in quantitative measures from baseline to weeks 24 and 48 were tested for significance using Wilcoxon signed-rank test. Plasma pharmacokinetic parameters of d4T [Area under the plasma (blood or serum) concentration time curve (AUC), half-life ( $T_{1/2}$ ), clearance of distribution (Cl/F), maximum concentration ( $C_{max}$ )] were obtained by non-compartmental modelling using WinNonlin Software (Standard Edition, version 1.1; Pharsight Corporation, Mountain View, CA, USA).

## Results

Between June 2004 and March 2005, 57 patients were enrolled in the study. All patients were evaluated for the week 24 primary endpoint. Fifty-four patients completed 48 weeks of follow-up; three did not (two were also taking ddi and discontinued therapy; a third receiving indinavir changed the PI and was no longer followed).

The patient baseline characteristics are shown in Table 1. Patients were predominantly male, with a baseline median CD4 count of 584 cells/ $\mu$ L (range 374–752); all had plasma viral load < 400 copies/mL and 89% had a viral load < 50 copies/mL. The median duration of previous ARV

**Table 1** Patient baseline characteristics

	Study population (n = 57)
Age (years) (range)	43 (29–66)
Male sex, no. (%)	48 (84)
Median weight (kg) (IQR)	72 (64–76)
AIDS (category C disease), no. (%)	18 (22)
Mean (SD) CD4 count (cells/ $\mu$ L)	584 (271)
Viral load <50 HIV-1 RNA (copies/mL) (%)	50 (88)
Median time on antiretroviral therapy (years) (IQR)	6.9 (5.1–8.8)
Median time on stavudine (years) (IQR)	6.3 (4.6–7.4)
Antiretroviral therapy history	
Any NRTI, no. (%)	57 (100)
Median number of NRTIs (range)	4 (2–6)
Stavudine, no. (%)	57 (100)
Zidovudine, no. (%)	34 (60)
Lamivudine, no. (%)	53 (93)
Zalcitabine, no. (%)	8 (14)
Didanosine, no. (%)	28 (49)
Abacavir, no. (%)	16 (28)
Tenofovir, no. (%)	5 (9)
Any PI, no. (%)	38 (67)
Any NNRTI, no. (%)	33 (58)
Antiretroviral therapy at screening	
NRTI, no. (%)	9 (16)
NRTI + PI, no. (%)	22 (39)
NRTI + NNRTI, no. (%)	26 (45)

IQR, interquartile range; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SD, standard deviation.

therapy was 6.9 years [interquartile range (IQR) 5.1–8.8], median exposure to d4T was 6.3 years (IQR 4.6–7.4), and a prior history of NRTI monotherapy and/or dual therapy was present in 54%. As shown in Table 2, d4T 30 mg twice daily was combined with other NRTIs including 3TC in 43 patients, ddI in nine patients, ABC in four and none in one. d4T 30 mg was combined with an NNRTI in 26 patients, a PI in 22 patients, or one or two other NRTIs in nine patients (four with 3TC/ABC, three with 3TC/ddI and two with 3TC alone).

At baseline, 32 (56%) patients had a complete neurological examination. Among these patients, 11 (34%) had at least one symptom suggestive of peripheral neuropathy and six (19%) had clinically documented peripheral neuropathy as assessed by the neurologist.

By intent-to-treat analysis, 56/57 patients (98%) had a plasma viral load <400 copies/mL (95% CI 91–100%) at week 24. Using the ultra-sensitive quantification of the viral load assay, plasma viral load was <50 copies/mL in 51 (89%) at week 24. At week 48, 53/57 (93%) had plasma viral load <400 copies/mL (95% CI 83–98%); one patient had viral load >400 copies/mL and three were lost to follow-up. At week 48, 41/57 (72%) had plasma viral load <50 copies/mL (10 had 50–400 copies/mL, three were missing samples, two had no sample available for ultra-sensitive analysis and one had >400 copies/mL).

Baseline DNA and week 24 RNA genotypic analyses showed the same set of mutations in both RT and PR genes in the three patients with RNA >50 copies/mL that could be amplified at both of these timepoints. The baseline RT and PR DNA gene sequences were available for 42 patients, of whom nine had either the RT or PR gene sequence results (Table 2). The PR gene was wild-type in two patients. The number of mutations ranged between one and six. RT genes were either wild-type for 24 patients (63%) or mutated for 14 (27%).

The patient with virological failure (patient 17, Table 2) at week 24 had a viral load of 1400 copies/mL while receiving d4T/3TC plus lopinavir/ritonavir. This patient had adequate d4T, 3TC and lopinavir serum concentrations at baseline, week 4 and week 12, suggesting appropriate adherence. Baseline DNA and week 24 RNA genotypic analysis showed the same set of mutations in RT. No additional mutations were acquired in the PR gene at week 24 when comparing RNA genotyping results at week 24 to DNA genotyping at baseline.

To assess mtDNA changes from baseline to week 48, PBMC content was measured in 50 patients from whom cells could be collected at both timepoints. Mean mtDNA content in PBMCs were  $672 \pm 254$  and  $682 \pm 269$  copies/cell at baseline and week 48, respectively. Overall, mtDNA levels in PBMCs did not change over the year.

There was a significant increase in the CD4 count from baseline to week 24, which was sustained with an overall mean increase of 63 cells/ $\mu$ L at week 48 ( $P = 0.006$ ). A significant decrease in total cholesterol and HDL cholesterol was found after 24 weeks of d4T dose reduction; this was still observed at week 48 (Table 3). No significant difference was found with regard to mean LDL cholesterol, triglycerides and glucose when comparing values at baseline, week 24 and week 48. Lactate decreased during the year, with a mean variation of  $-0.10$  mg/mL at week 24 and  $-0.17$  mg/mL at week 48. Furthermore, a significant decrease in alanine aminotransferase (ALT) was observed both at week 24 and at week 48. No significant changes in body weight, body mass index, or waist, hip and thigh circumferences were noted during the study period.

Following the d4T dose reduction, 5/17 (23%) patients with symptomatic or clinical neuropathy experienced improvement. No deaths or progression of HIV disease or any serious adverse event were observed over the study period.

Plasma AUC,  $C_{max}$  and Cl/F of d4T were reduced significantly following the d4T dose reduction (Table 4). A decrease in AUC and  $C_{max}$  by 31 and 44%, respectively, was observed after the switch to the lower dose – suggesting an accumulation of d4T at the higher dose. Importantly, plasma half-lives were not modified by the change in the daily dosage ( $P = 0.42$ ).

**Table 2** Treatment regimen, baseline resistance mutations in proviral DNA and plasma viral load at baseline, week 24 and week 48

	Antiretroviral therapy additional to d4T	RT genotype	Protease genotype	Viral load, baseline (HIV-1 RNA copies/mL)	Viral load, week 24 (copies/mL)	Viral load, week 48 (copies/mL)
1	ABC + 3TC + NVP	M184I	G73S	< 50	< 50	< 50
2	3TC + NFV	M41L/M; L74V; T215Y	UTS	< 50	< 50	< 50
3	3TC + TDF + LPV/RTV	M41L/M; M184V/M; L210W/L; T215Y	L10I; M46L; L63P; A71V; L76V; V82A	< 50	< 50	< 50
4	3TC + EFV	M184I; V108I	I13V	< 50	< 50	< 50
5	3TC + EFV	UTS	I62V	< 50	< 50	< 50
6	3TC + NVP	K70R	G73S/G	< 50	< 50	< 50
7	3TC + EFV	WT	L10I; D60E	< 50	< 50	< 50
8	ddl + IDV/RTV	WT	L10I; I13V; K20I; M36I; V82I	< 50	< 50	389
9	3TC + LPV/RTV	WT	I13V	< 50	< 50	< 400*
10	3TC + NVP	WT	L63P; V77I	< 50	< 50	LTFU
11	3TC + EFV	WT	L10I; V11I; L63P	< 50	< 50	< 400*
12	3TC + NVP	M41L; L210W; T215Y	I13V; L63P	< 50	< 50	< 50
13	3TC + IDV/RTV	WT	L63P; A71T; V77I	< 50	< 50	LTFU
14	3TC + IDV/RTV	WT	I13V; D30N	< 50	< 50	< 50
15	3TC + TDF + LPV/RTV	M41L; D67N; K70R; M184V; T215Y; K219Q	I13V; K20I; M36I; H69K; V82I	< 50	< 50	< 50
16	3TC + NVP	WT	I13V; L63P	< 50	< 50	56
17	3TC + LPV/RTV	M41L; L74V; M184V; L210W; T215Y; L100I; K103N	I62V; L63P; V77I; L90M	< 50	1400	LTFU
18	3TC + IDV/RTV	WT	I13V; K20I; H69K; V82I	< 50	< 50	< 50
19	3TC + NVP	WT	D30N; M36V; G73S	< 50	< 50	< 50
20	3TC + IDV/RTV	M41L; K103N; V108I	M36I; I62V	< 50	< 50	< 50
21	3TC + NFV	WT	L63P	< 400*	< 400*	< 50
22	3TC + NVP	WT	I13V; K20I; M36I; H69K; V82I	< 50	< 50	< 50
23	3TC + EFV	WT	I13V; G16E; K20I; M36I; H69K	< 50	< 50	< 50
24	3TC + NVP	M41L; D67N; M184V; L210W; T215Y	UTS	< 50	< 50	< 50
25	3TC + SQV + RTV	WT	L63P	< 50	< 50	< 50
26	3TC + LPV/RTV	WT	L10V; I13V; G16E; K20I; M36I; H69K	< 50	< 50	< 50
27	3TC + NVP	WT	G16E; I62V; L63P; H69K	< 50	< 50	< 50
28	ddl + NVP	WT	L63P; V77I	< 50	< 50	< 50
29	3TC	UTS	I13V; V77I	234	113	110
30	ddl + NVP	UTS	I13V; L63P	< 50	< 50	< 50
31	3TC + SQV + RTV	A62V; V75I; F77L; Y115F; F116Y; Q151M; M184V; G190	M36I; I62V; L63P	< 50	< 50	< 50
32	TDF + ATV/RTV	WT	WT	< 50	< 50	65
33	3TC + ABC	WT	I62V; V77I	< 50	< 50	< 50
34	ddl + EFV	WT	UTS	< 50	< 50	118
35	3TC + ddl	UTS	L63P; V77I	< 50	< 50	< 50
36	3TC + EFV	WT	L63P	< 50	< 50	93
37	3TC + ATV	M41L/M; K70R/K	L33F/L; V77I	< 50	< 50	51
38	3TC + EFV	M184I	L10I; H69K	< 50	< 50	< 50
39	3TC + ABC	V118I	M36I; L63P	< 50	< 50	< 50
40	3TC + NVP	WT	UTS	< 50	< 50	< 50
41	3TC + TDF + EFV	WT	UTS	< 50	< 50	< 50
42	3TC + NVP	WT	WT	< 50	< 50	< 50
43	3TC + ddl	UTS	UTS	83	158	< 50
44	ddl + NFV	UTS	UTS	98	< 50	< 50
45	3TC + NVP	UTS	UTS	< 50	< 50	< 50
46	3TC + ddl	UTS	UTS	< 50	< 50	< 50
47	3TC + NVP	UTS	UTS	< 50	< 50	< 50
48	3TC + NFV	UTS	UTS	54	157	321

Table 2. (Contd.)

	Antiretroviral therapy additional to d4T	RT genotype	Protease genotype	Viral load, baseline (HIV-1 RNA copies/mL)	Viral load, week 24 (copies/mL)	Viral load, week 48 (copies/mL)
49	3TC + APV	UTS	UTS	338	190	< 50
50	3TC	UTS	UTS	< 50	< 50	< 50
51	3TC + APV/RTV	UTS	UTS	< 50	< 50	< 50
52	3TC + EFV	UTS	UTS	< 50	< 50	< 50
53	3TC + ABC	UTS	UTS	< 50	< 50	< 50
54	3TC + ABC	UTS	UTS	< 50	< 50	5772
55	3TC + LPV/RTV	UTS	UTS	< 50	< 50	136
56	3TC + SQV + RTV	UTS	UTS	< 50	< 50	< 50
57	ddl + NVP	UTS	UTS	< 50	< 50	55

\*Ultra-sensitive (< 50 copies/mL) assay not available.

d4T, stavudine; LTFU, lost to follow-up; RT, reverse transcriptase; UTS, unable to sequence; WT, wild type.

Antiretroviral drugs: ABC, abacavir; APV, amprenavir; ATV, atazanavir; ddl, didanosine; EFV, efavirenz; IDV, indinavir; LPV, lopinavir; NVP, nelfinavir; NVP, nevirapine; RTV, ritonavir; SQV, saquinavir; 3TC, lamivudine; TDF, tenofovir.

Table 3 Metabolic and biochemical measurements at baseline and changes over 24 and 48 weeks after stavudine dose reduction

	<i>n</i>	Mean (SD) at baseline	<i>n</i>	Mean (SD) change over 24 weeks	<i>P</i> -value*	<i>n</i>	Mean (SD) change over 48 weeks	<i>P</i> -value*
Cholesterol (mmol/L)	57	5.57 (1.28)	52	-0.26 (0.98)	0.02	50	-0.24 (0.63)	0.02
HDL cholesterol (mmol/L)	53	1.50 (0.56)	46	-0.12 (0.27)	0.006	45	-0.15 (0.23)	0.0001
LDL cholesterol (mmol/L)	52	3.37 (1.07)	45	-0.13 (0.65)	0.37	44	-0.18 (0.52)	0.07
Triglycerides (mmol/L)	57	1.96 (1.37)	52	+0.02 (2.32)	0.06	50	+0.09 (1.09)	0.79
Glucose (mmol/L)	55	5.49 (1.95)	52	-0.07 (1.24)	0.42	44	-0.23 (1.65)	0.60
Lactate (mmol/L)	55	1.71 (0.60)	52	-0.10 (0.41)	0.05	46	-0.17 (0.75)	0.07
AST	56	33.37 (19.21)	54	+4.26 (24.13)	0.27	47	+3.68 (17.09)	0.04
ALT	56	37.34 (17.24)	54	-3.17 (20.03)	0.02	47	-5.74 (14.91)	0.01

\*Wilcoxon matched pairs signed ranks test.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

Table 4 Steady-state plasma pharmacokinetics (PK) parameters of stavudine in 11 patients

	Baseline (40 mg b.i.d.)	Week 24 (30 mg b.i.d.)	<i>P</i> -value	Geometric mean ratio (95% CI)
Median AUC (ng h/mL) (IQR)	1693 (1502-2005)	1163 (976-1255)	0.003	0.62 (0.52-0.72)
Median <i>C</i> <sub>max</sub> (ng/mL) (IQR)	554 (461-573)	311 (245-361)	0.004	0.59 (0.47-0.73)
Median T <sub>1/2</sub> (h) (IQR)	1.9 (1.5-2.4)	2.1 (1.8-2.3)	0.42	0.95 (0.76-1.19)
Median Cl/F (mL/min) (IQR)	1.4 (1.2-1.6)	1.6 (1.4-1.8)	0.03	1.22 (1.04-1.42)

AUC, area under the plasma concentration time curve; b.i.d., twice daily; CI, confidence interval; IQR, interquartile range.

## Discussion

Because d4T was developed and approved before viral load testing was available, the optimal dosage was never determined. The aim of this study was to evaluate if a reduced and presumably safer dose of d4T could be effective in maintaining optimal viral suppression. Some pilot studies had suggested that a reduced dosage of d4T could be virologically effective [26-31]. In our study we showed that a reduction in d4T dosage from 40 to 30 mg

twice daily in adults at or above 60 kg body weight does not jeopardize the antiviral efficacy in the context of a cART regimen: all but one patient had a viral load <400 copies/mL at 24 weeks (the primary endpoint of the study) and 93% stayed suppressed for 48 weeks. When the more rigorous ultra-sensitive assay for viral load measurement was utilized, we found four patients with plasma viral load of 50-400 copies/mL at week 24 and 10 such patients at week 48; none of these plasma samples could be sequenced. It must be kept in mind that our patients were

heavily treatment-experienced and that sequencing performed at baseline from DNA revealed multiple mutations in 37/57 (65%) of samples.

The resistance profile of d4T is a favourable characteristic of this drug. d4T has been difficult to characterize in terms of specific resistance, but its effect appears to be similar to that of ZDV, the other commonly used thymidine analogue. This concept is supported by data that show ZDV-experienced patients do not have as favourable an antiviral response as do those who are treatment-naïve [18,32]. With regard to resistance selected at the time of virological failure, the resistance pattern is quite similar to that observed with tenofovir-based treatment with the exception of K65R, which tends to occur more frequently in tenofovir-treated patients ( $P = 0.06$ ) [19]. Despite thymidine analogue mutations identified in baseline DNA from nine patients and one other with the multi-drug-resistant mutation Q151M, the overall treatment success was robust in our cohort, all of whom received reduced-dosage d4T.

As mentioned earlier, the major problem with d4T is the long-term toxicities. The most important adverse events associated with d4T are the high rates attributed to mitochondrial toxicity (peripheral neuropathy, lipodystrophy and lactic acidosis), which are reported in up to 28% of patients randomized to d4T-containing cART regimens [19,20,33,34]. In our study, we attempted to measure the effect that d4T has on mitochondria by measuring mitochondrial DNA. When d4T is removed from a treatment regimen and substituted with a less toxic nucleoside such as ABC or tenofovir, mitochondrial DNA levels typically increase significantly [30]. When d4T dosage is decreased, mitochondrial toxicities have been reported to be reduced without compromising antiviral activity. In one study reporting on 11 patients, mtDNA was shown to increase by as much as two- to three-fold following d4T dose reduction. However, this patient group had received d4T for a mean of only 21 months, compared to our patients who had been treated for 6.3 years [35]. Overall, we only observed a slight and insignificant increase in mitochondrial DNA when the d4T dosage was decreased. The difference in the response may be caused by the higher cumulative d4T exposure in our patients, suggesting that patients with prolonged exposure may require a longer time to recover mtDNA or perhaps they may never recover completely.

This study had several limitations, the first being small sample size and pilot design. In 2004 (when this study was initiated), patients in most developed countries had already started moving away from d4T therapy. Our objective was to examine a lower dosage of d4T that had potentially better tolerability while retaining virological efficacy. To meet our objective in the timeframe of this trial, this study

was powered for virological outcome and key safety parameters and not for some of the well-known long-term toxicities of d4T such as lipodystrophy. Therefore, the sample size was relatively small and there was no control group, typical of the pilot nature of the trial making this study feasible in 2004–2005. Secondly, the patient population was highly treatment-experienced, including a median of 6.3 years of d4T use with optimal viral suppression, which may have introduced a selection bias that could have affected the results. However, it should be noted that 17/32 patients did have a sign or symptom compatible with peripheral neuropathy at baseline, suggesting the presence of a mitochondrial toxicity, and that 5/17 (29%) improved when the dose was reduced. None of these parameters should really be able to affect the antiviral response. Moreover, other recently published studies have shown similar results [30,31]. Another limitation of the study was the use of the 400 copies/mL plasma viral load assay as the primary endpoint. At the time this study was designed and enrolled, not all clinical services – even those in developed countries – utilized the more sensitive 50 copies/mL assay routinely like they do today. Even in some of the clinical trials enrolling patients in 2004, the 400 copies/mL assay was used as the primary endpoint in large clinical trials [20].

In conclusion, this study demonstrated that decreasing the dose of d4T from 40 to 30 mg twice daily in patients who have optimally controlled HIV infection is safe and is associated with continued suppression of HIV. Furthermore, several safety parameters were seen to improve including significant lowering of cholesterol and ALT, and approximately a quarter of patients with symptomatic neuropathy experienced clinical improvement. These data support the current recommendations to lower d4T doses in all patients currently receiving standard dosages. Our results do not directly answer the question as to whether this reduced dosage of d4T can be used when therapy is initiated in a treatment-naïve patient; although our results are suggestive, they are not conclusive. We hope that our findings will encourage generic manufacturers to focus on lower fixed-dose combination preparations for the many patients that still must rely on d4T as part of their therapeutic regimen. Further study of lower-dose d4T for efficacy and long-term complications such as lipodystrophy in treatment-naïve individuals is also important where d4T use is likely to continue.

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