


Durability of non-nucleotide reverse transcriptase inhibitor-based first-line ART regimens after 7 years of treatment in rural Uganda

A prospective cohort study

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Abstract

Most antiretroviral therapy (ART) programs in resource-limited settings have historically used non-nucleotide reverse transcriptase inhibitor (NNRTI)-based regimens with limited access to routine viral load (VL) testing. We examined the long-term success of these regimens in rural Uganda among participants with 1 measured suppressed VL.

We conducted a prospective cohort study of participants who had been on NNRTI-based first-line regimens for ≥ 4 years and had a VL < 1000 copies/mL at enrollment in Jinja, Uganda. We collected clinical and behavioral data every 6 months and measured VL again after 3 years. We quantified factors associated with virologic failure (VF) (VL ≥ 1000 copies/mL) using Wilcoxon Rank Sum, chi-square, and Fisher's Exact Tests.

We enrolled 503 participants; 75.9% were female, the median age was 45 years, and the median duration of time on ART was 6.8 years (IQR = 6.0–7.6 years). Sixty-nine percent of participants were receiving nevirapine, lamivudine, and zidovudine regimens; 22.5% were receiving efavirenz, lamivudine, and zidovudine; and 8.6% were receiving other regimens. Of the 479 with complete follow-up data, 12 (2.5%) had VL ≥ 1000 copies/mL. VF was inversely associated with reporting never missing pills (41.7% of VFs vs 72.8% non-VFs, $P = .034$). There were differences in distribution of the previous ART regimens ($P = .005$), but no clear associations with specific regimens. There was no association between having a VL of 50 to 999 copies/mL at enrollment and later VF ($P = .160$).

Incidence of VF among individuals receiving ART for nearly 7 years was very low in the subsequent 3 years. NNRTI-based regimens appear to be very durable among those with good initial adherence.

Abbreviations: 3TC = lamivudine, ART = antiretroviral therapy, AZT = azidothymidine, EFV = efavirenz, HIV = human immunodeficiency virus, NNRTI = non-nucleotide reverse transcriptase inhibitor, NRTI = nucleotide reverse transcriptase inhibitor, NVP = nevirapine, Q1–Q3 = quartile 1–quartile 3, TAMs = Thymidine Analogue Mutations, TASO = The AIDS Support Organisation, TDF = tenofovir disoproxil fumarate, VF = virologic failure, VL = viral load, WHO = World Health Organisation.

Keywords: antiretroviral therapy, drug resistance, non-nucleoside reverse transcriptase inhibitors, sub-Saharan Africa, treatment failure

1. Introduction

At the end of 2018, an estimated 37.9 million people were living with human immunodeficiency virus (HIV) and 24.5 million had access to antiretroviral therapy (ART),^[1] resulting in substantial

population-level reductions in HIV-associated morbidity, mortality, and the prevention of new infections.^[2,3] The highest burden of HIV has been in sub-Saharan Africa where the expansion of ART has been facilitated through a “public

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health approach.” Promoted by the World Health Organisation (WHO), this approach involves the use of standardized ART regimens, most often with 2 nucleotide reverse transcriptase inhibitors (NRTIs) and 1 non-nucleotide reverse transcriptase inhibitor (NNRTI).^[4] Furthermore, initial WHO guidelines did not recommend the use of viral load (VL) testing for routine clinical monitoring,^[4] given the high cost and lack of evidence regarding improved clinical outcomes associated with VL testing.^[5–7]

In 2013, WHO issued updated recommendations that VL testing should be conducted at 6 and 12 months after ART initiation and every 12 months thereafter for virologically suppressed persons.^[8] However, expansion of VL testing has been challenging for many ART programs in sub-Saharan Africa,^[9] and many programs continue to rely on clinical and immunological monitoring of patients on ART. Furthermore, NNRTI-based regimens have a lower physiologic barrier to selecting HIV resistance strains, in comparison to regimens based on protease-inhibitors.^[10] However, HIV care and treatment programs that have developed effective means to support participant retention and adherence to therapy have demonstrated low-levels of virologic failure, even in the absence of VL monitoring.^[11]

We designed a study to determine the durability of NNRTI-based regimens, in terms of sustained virologic suppression, among patients who have been receiving ART at a treatment site in rural Uganda for at least 4 years without previous VL monitoring. We then examined associations with virologic failure after an additional 3 years of follow-up.

2. Methods

We offered enrollment to clients of The AIDS Support Organisation (TASO) at the Jinja service centre between June 2012 and April 2013. Participants were aged ≥ 18 years, who had received ART for at least 4 years and were on first-line ART regimen which contained an NNRTI-based regimen (nevirapine or efavirenz). Study procedures were read aloud by a research assistant in 1 of 2 local languages (Luganda and Lusoga) or English and all participants provided written informed consent to participate. Participants continued to receive their regular HIV care through TASO. The clinic provides comprehensive HIV care, treatment and prevention services to over 7400 patients and their families, within a 75-km radius of Jinja town. HIV VL monitoring was not available for routine clinical care at TASO at the time of the study. We conducted an HIV VL test at enrollment. We had a planned enrollment of 500 participants with a VL of < 1000 copies/mL, based on the maximum number of participants we could feasibly follow every 6 months for 3 years while still allowing for a sufficient number of participants with virologic failure events to analyze. We also enrolled participants with VL measurements of ≥ 1000 copies/mL but report on these elsewhere.^[12,13] Study participants received an honorarium of 20,000 Ugandan shillings (approximately \$5 US) to offset travel costs and a meal at each study visit. This study was approved by the Research Ethics Board of the University of British Columbia, the Uganda Virus Research Institute and the Uganda National Council for Science and Technology.

Clinical and behavioral data were collected at enrollment and every 6 months for a period of 3 years, with data collection ending in September 2016. Collected variables included demographic information (age, gender, education level, and marital status),

adherence in terms of self-reported missed doses (never vs once per week or more vs more than once per week but less than once per month vs less than once per month), current and past ART regimens, and ART start dates. CD4 cell counts were measured every 6 months using a FACS Calibur instrument and samples for additional VL were drawn every 12 months. Plasma samples were stored in liquid nitrogen and shipped to the Uganda Virus Research Institute/Medical Research Council (United Kingdom) in Entebbe for VL testing. Genotypic antiretroviral drug resistance testing was conducted on samples collected after 36 months of follow-up for all those with a VL > 1000 copies/mL.

We calculated descriptive statistics of study participant characteristics. The primary outcome variable of interest was VL ≥ 1000 copies/mL at 36 months of follow-up. We conducted bivariate analyses comparing patients with an endpoint VL < 1000 copies/mL vs VL ≥ 1000 copies/mL using Fisher’s exact or chi-square tests for categorical variables and the Wilcoxon rank sum test for continuous variables. We reported on each variable individually and missing data were not imputed. As virologic failure was relatively uncommon, it did not allow us to conduct multivariable modelling to adjust for confounding. For participants with virologic failure at 36 months of follow-up, we calculated descriptive statistics (count and percentage) of Thymidine Analogue Mutations (TAMs), NNRTI mutations, and K65R mutations. All analyses were conducted using SAS Version 9.3 (SAS Corporation, Cary, NC).

3. Results

We screened 1091 TASO clients who had been receiving first-line ART for a minimum of 4 years, to identify 113 with VL ≥ 1000 copies/mL, whose outcomes are reported elsewhere.^[13] We enrolled the first 503 participants with VL < 1000 copies/mL, and 479 (95.2%) completed all study visits up to 36 months. Of those with incomplete data, 15 (3%) had died, 3 (1%) were lost to follow-up, and 5 (1%) withdrew voluntarily. Of the 479 patients, 364 (75.9%) were female, had a median age of 44 years (quartile 1 [Q1]–quartile 3 [Q3]=39–50 years), the median duration on ART at enrollment was 6.8 years (Q1–Q3=6.0–7.6 years), and the median baseline CD4 count was 572 (Q1–Q3=451–752 cells/ μ L). A total of 347 patients (69.0%) were receiving nevirapine with a backbone of azidothymidine and lamivudine (AZT/3TC/NVP), 113 (22.5%) were receiving efavirenz with the same backbone (AZT/3TC/EFV), 32 (6.7%) were receiving nevirapine with a backbone of tenofovir disoproxil fumarate and lamivudine (TDF/3TC/NVP), and 9 (1.8%) were receiving efavirenz with the same backbone (TDF/3TC/EFV). Of the 503 patients enrolled, 470 (93.4%) had an enrollment VL of < 50 copies, 32 (6.4%) had 50 to 500 copies/mL, and 1 (0.2%) had between 500 and 1000 copies/mL.

Table 1 shows the results of the bivariate analysis comparing 12 study participants (2.5%) with virologic failure (≥ 1000 copies/mL) at 36 months to the 467 (97.5%) without virologic failure. Participants who reported no missed pills significantly were less likely to have virologic failure at study exit (41.7% of failure patients vs 72.8% of non-failure patients; $P=.034$). There were no significant differences in the distribution of ART regimens at enrollment ($P=.071$), but there were observed differences in the distribution of previous regimens participants had received ($P=.005$). However, no specific clear association with a particular previous regimen was apparent. Notably, previous exposure to regimens containing stavudine (d4T) was

Table 1**Bivariable analysis of participant characteristics stratified by virologic failure at 36 mo of follow-up.**

Categorical variables	No virologic failure (n = 467)		Virologic failure (n = 12)		P
	n	(row %)	n	(row %)	
Gender					
Female	365	98.1%	7	1.9%	.151
Male	102	95.3%	5	4.7%	
Marital status					
Legally married	128	99.2%	1	0.8%	.002
Co-habiting	82	93.2%	6	6.8%	
Single/separated/divorced	73	94.8%	4	5.2%	
Widowed	184	99.5%	1	0.5%	
Education					
Less than primary completion	83	96.5%	3	3.5%	.688
Completed primary	225	97.8%	5	2.2%	
Completed secondary	159	97.5%	4	2.5%	
Frequency of missed pills					
Never	340	98.6%	5	1.4%	.034
Once a month or more	80	95.2%	4	4.8%	
Less than once a week but more than once a month	7	87.5%	1	12.5%	
Once a week or more	40	95.2%	2	4.8%	
ARV regimen at enrollment					
Nevirapine/3TC/ZDV	327	97.3%	9	2.7%	.071
Efavirenz/3TC/ZDV	102	99.0%	1	1.0%	
Nevirapine/3TC/TDF	29	96.7%	1	3.3%	
Efavirenz/3TC/TDF	8	100.0%	0	0.0%	
Other ARV regimen	1	50.0%	1	50.0%	
Previous ARV regimen					
Nevirapine/3TC/ZDV	21	95.5%	1	4.5%	.005
Efavirenz/3TC/ZDV	10	100.0%	0	0.0%	
Nevirapine/3TC/TDF	1	50.0%	1	50.0%	
Efavirenz/3TC/TDF	1	100.0%	0	0.0%	
Nevirapine/3TC/D4T	205	99.5%	1	0.5%	
Efavirenz/3TC/D4T	31	93.9%	2	6.1%	
D4T exposure					
No	96.3%	9	3.8%	96.3%	.141
Yes	98.7%	3	1.3%	98.7%	
Viral load at enrollment					
<50	440	97.8%	10		.173
50–500	26	92.9%	2		
500–1000	1	100.0%	0		
Continuous variables	Median (IQR)		Median (IQR)		P
Age (in years)	45 (40–50)		44 (38–50)		.509
Duration of ART at enrollment	6.8 (6.0–7.6)		6.4 (5.2–7.5)		.397
CD4 cell count at enrollment	577 (462–758)		450 (371–648)		.094
CD4 cell count at 36 mo	521 (404–700)		394 (176–630)		.103

3TC = lamivudine.

not associated with virologic failure ($P = .141$). Virologic failure was more common among individuals who were co-habiting, but not married (6.8% vs 0.5% of widowed patients; $P = .002$). Females were not more likely to have virologic failure at study exit compared to males ($P = .151$) and virologic failure was not significantly different by age (median = 45 years [Q1–Q3: 40–50] for non-failure patients vs median = 44 [Q1–Q3: 38–50] for failure patients; $P = .509$). Also, while CD4 cell count at enrollment was lower for those with virologic failure at study exit (median = 450 cells/ μ L; Q1–Q3: 371–648] than for those without failure (median = 577; [Q1–Q3: 462–759]), these difference was not statistically significant ($P = .094$).

Table 2 shows the results distribution of resistance mutations among those with virologic failure at study exit. Of the 12

Table 2**Frequency of resistance mutations among those with virologic failure (n = 12).**

Resistance mutation	n	%
Any mutation	11	91.7
TAMs		
0	10	83.3
1	1	8.3
3	1	8.3
NNRTI mutation	11	91.7
K65R	2	16.7

TAMs = Thymidine Analogue Mutations, NNRTI = non-nucleotide reverse transcriptase inhibitor.

patients with VL > 1000 copies/mL that had resistance data at 36 months, 11 (92%) had at least 1 mutation. All of these had an NNRTI mutation and 2 (16%) had a K65R mutation. With respect to TAMs, 10 (83%) had no TAMs, 1 (8%) had 1 TAM, and 1 (8%) had 3 TAMs.

4. Discussion

We observed a very low rate of virologic failure among patients who had been receiving an NNRTI-based first-line regimen for over 7 years and had a suppressed VL on their first ever VL test. Prevalence of virologic failure was 2.4% after an additional 36 months of observation, suggesting that for patients who are adherent to their ART, NNRTI-based regimens can provide very durable and effective treatment. These results provide reassurance that NNRTI-based ART can remain viable over the long-term, and as such, provide further support for current WHO ART guidelines which recommend efavirenz as an alternative first-line ART regimen component.^[14]

Given the low proportion of participants who developed virologic failure, we found very few differences between the failure and non-failure groups. We did, however, find differences in self-reported adherence to therapy in that 74% of the non-failure group reporting never missing any pills compared to only 40% in the failure group. This is interesting considering that all participants must have been sufficiently adherent to their therapy to achieve virologic suppression prior their first study visit and reinforces the need to re-visit supports for adherence to therapy even in individuals who have been receiving ART for long periods of time. We did find some differences in the distribution of previous ART regimens, but both failures and non-failures occurred across most of the regimens available. While we also found statistically significant differences in the distribution of marital status among those with virologic failure and those without, the absolute differences in terms of percentages were quite small. Individuals who were co-habiting but not legally married had the highest proportion of virologic failure (6.8%). However, given that 93% of co-habiting participants achieved virologic suppression, it would be hard to state definitely that co-habitation is a risk factor for subsequent virologic failure.

Another notable finding is the relatively few number of drug resistance mutations we found among those who developed treatment failure. Most of those with failure had only 1 drug resistance mutation (largely NNRTI mutations) and 10 of the 12 participants with virologic had no TAMs, the mutations which are of most concern in terms of their effects on second-line therapy. These are in stark contrast to the patients who we found to have virologic failure during enrollment,^[12] where we found that 83% had 2 or more mutations and 45% had ≥ 2 TAMs.

An important negative finding was that we did not find that low-level viremia at enrollment (between 50 and 999 copies/mL) predicted virologic failure at study exit. This observation has been shown previously in high-income countries^[15] and lends support to the principle that low-level viremia is of limited clinical consequence, provided that adequate adherence to therapy can be maintained. It also further supports the continued use of a VL > 1000 copies/mL as the WHO definition for virologic failure.^[16]

An ongoing challenge with the use of NNRTI-based regimens will be the steady increase in primary drug resistance to these medications. According to the WHO 2017 HIV Drug Resistance Report, the prevalence of pre-treatment NNRTI resistance ranges from 8% in Cameroon to 15% in Uganda^[17] and the prevalence of

pre-treatment drug resistance to NNRTIs was below 5% in South Africa until 2011, but increased to 10.0% by 2014.^[18] Whether the increased availability of VL testing will help to mitigate these increases remains to be seen, given that the ability to provide pre-treatment drug resistance testing is very limited in the region.^[17]

This study has a number of limitations. Firstly, we had very few participants who developed virologic failure, which limited our ability to detect factors associated with failure. Secondly, we had only 2 viral load result points at enrollment and 36 months of follow-up therefore because of this, we could not ascertain at what exact time of follow-up virologic failure occurred. However, overall retention this cohort was excellent with over 95% completing all follow-up visits and very low rates of mortality and loss-to-follow-up. It is also notable that the virologic suppression overall in this program was excellent, estimated to be 93% in among unselected patients receiving treatment for more than 4 years,^[11] even in the absence of prior VL testing. Clearly the ART programs implemented by TASO have effectively engaged their patients in long-term ART care. Lastly, adherence to therapy among participants was, by necessity very good or excellent prior to enrollment as they would have been unlikely to have achieved virologic suppression after 7 years on these regimens without excellence adherence. As such we have very limited ability to examine how lower levels of adherence in subsequent years may have altered these outcomes.

In summary, we found a very low incidence of virologic failure among patients who had been receiving NNRTI-based first-line ART for a median of 7 years at enrollment. This demonstrates that individuals who achieve virologic success while on NNRTIs can expect them to remain effective for very long periods of time, even without routine VL testing as long as they remain adherent.

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