

Virologic efficacy of tenofovir, lamivudine and dolutegravir as second-line antiretroviral therapy in adults failing a tenofovir-based first-line regimen

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Objective: Recycling tenofovir and lamivudine/emtricitabine (XTC) with dolutegravir would provide a more tolerable, affordable, and scalable second-line regimen than dolutegravir with an optimized nucleoside reverse transcriptase inhibitor (NRTI) backbone. We evaluated efficacy of tenofovir/lamivudine/dolutegravir (TLD) in patients failing first-line tenofovir/XTC/efavirenz or nevirapine.

Design: Single arm, prospective, interventional study.

Setting: Two primary care clinics in Khayelitsha, South Africa.

Participants: Sixty adult patients with two viral loads greater than 1000 copies/ml.

Intervention: Participants were switched to TLD with additional dolutegravir (50 mg) for 2 weeks to overcome efavirenz induction.

Primary outcome: Proportion achieving viral load less than 50 copies/ml at week 24 using the FDA snapshot algorithm.

Results: Baseline median CD4⁺ cell count was 248 cells/ μ l, viral load 10 580 copies/ml and 48 of 54 (89%) had resistance (Stanford score ≥ 15) to one or both of tenofovir and XTC. No participants were lost to follow-up. At week 24, 51 of 60 [85%, 95% confidence interval (CI) 73–93%] were virologically suppressed, six had viral load 50–100 copies/ml, one had viral load 100–1000 copies/ml, one no viral load in window, and one switched because of tenofovir-related adverse event. No integrase mutations were detected in the one participant meeting criteria for resistance testing. Virological suppression was achieved by 29 of 35 (83%, 95% CI 66–93%) with resistance to tenofovir and XTC, 11 of 13 (85%, 95% CI 55–98%) with resistance to XTC, and six of six (100%, 95% CI 54–100%) with resistance to neither.

Conclusion: A high proportion of adults switching to second-line TLD achieved virologic suppression despite substantial baseline NRTI resistance and most not suppressed had low-level viraemia (≤ 100 copies/ml). This suggests recycling tenofovir and XTC with dolutegravir could provide an effective second-line option.

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Introduction

Until recently, boosted protease inhibitor regimens were the mainstay of second-line antiretroviral therapy (ART) in low-income and middle-income settings. However, the DAWNING study demonstrated that in patients failing first-line nonnucleoside reverse transcriptase inhibitor (NNRTI)-based regimens, dolutegravir had superior efficacy and tolerability compared with a lopinavir–ritonavir regimen [1]. On balance with concerns of neural tube defects and long-term weight gain, the World Health Organization (WHO) has since recommended dolutegravir for second-line ART in programmatic settings [2].

Dolutegravir is available in a fixed-dose combination with tenofovir and lamivudine (TLD) to virologically suppressed patients on NNRTI-based first-line regimens. However, patients requiring second-line ART are not currently eligible for this treatment as most failed first-line regimens contain tenofovir and lamivudine/emtricitabine (XTC) as a backbone. A high proportion of patients failing first-line ART have nucleoside reverse transcriptase inhibitor (NRTI) resistance, particularly in sub-Saharan Africa [3,4], raising concern that recycling tenofovir and XTC from first to second-line could result in dolutegravir being the only fully active drug in the regimen, in turn risking the development of integrase resistance (which has been observed when dolutegravir is used as monotherapy [5,6]).

In DAWNING, at least one of the two NRTIs had to be fully active on resistance testing performed at screening. Resistance testing is not feasible at scale in lower resource settings because of cost and laboratory capacity, so the WHO currently recommends switching patients failing a tenofovir-based first-line NNRTI-based regimen to zidovudine, lamivudine and dolutegravir [2] (thereby ensuring at least one fully active NRTI as tenofovir does not select for zidovudine resistance mutations [7]).

However, using zidovudine has disadvantages compared with tenofovir: it is more expensive, less well tolerated, has a greater pill burden and requires more frequent initial laboratory monitoring [8]. TLD would thus be a more desirable second-line regimen than using zidovudine with dolutegravir, particularly in lower resource settings.

Despite concerns regarding recycling tenofovir and XTC, compromised NRTIs have proven effective in protease inhibitor regimens – several studies have demonstrated the virological efficacy of second-line ART that combines a ritonavir-boosted protease inhibitor with two NRTIs even in the presence of resistance to both NRTIs [9–12]. Dolutegravir's high barrier to resistance and the cost to viral fitness of NRTI mutations [13] mean that recycling NRTIs with dolutegravir could also result in TLD providing an efficacious second-line

regimen. Although there are clear advantages to patients and health services from such a strategy, there is currently insufficient evidence to recommend TLD in second-line regimens. We evaluated virological suppression at 24 weeks in a prospective cohort study of participants switched to TLD after failing a first-line regimen containing tenofovir, XTC and efavirenz or nevirapine.

Methods

Study design

We conducted a single arm, prospective, interventional study in two primary care clinics in Khayelitsha, a large, peri-urban informal settlement in Cape Town, South Africa, with nearly 50 000 patients accessing ART through the public health system. The protocol was approved by the University of Cape Town's Human Research Ethics Committee (039/2019) and is available with the statistical analysis plan on ClinicalTrials.gov (NCT03991013).

Participants and sample size

Eligible patients were HIV-positive adults who had failed a first-line regimen consisting of tenofovir, XTC and efavirenz or nevirapine, confirmed by two consecutive viral loads above 1000 copies/ml 2–24 months apart (the most recent at screening). They were recruited from the primary care clinics through clinician referral or identification from folders. Exclusion criteria were: CD4⁺ cell count less than 100 cells/ μ l, active or suspected tuberculosis, active AIDS-defining conditions, an estimated glomerular filtration rate less than 50 ml/min per 1.73 m², haemoglobin less than 7.5 g/dl, alanine aminotransferase greater than 100 IU/l, a previous or current diagnosis of malignancy or any condition judged to put the patient at increased risk if participating, a condition judged likely to impact adherence (active psychiatric disease or substance abuse), pregnancy, breastfeeding, intention to fall pregnant or unable to take the study medication (allergy, intolerance or contra-indicated drug interaction). Women of child-bearing potential were required to be on effective contraception.

A sample size of 57 was calculated to produce a 95% confidence interval (CI) of 72–92%, with the assumption that viral load suppression of 82% would be achieved in the modified intention-to-treat (mITT) analysis at week 24, as achieved by the dolutegravir arm at week 24 in the DAWNING study [1]. To account for patients discontinuing the regimen, we planned to enrol 65 participants. Due to coronavirus disease (COVID-19)-related recruitment challenges and as no participants had been lost to follow-up by June 2020, the data safety monitoring committee allowed early completion of enrolment after 62 participants. We report on the

outcomes of the first 60 participants who had completed 24 weeks follow-up.

Procedures

After providing written informed consent, participants were switched from first-line ART to oral TLD (tenofovir 300 mg, lamivudine 300 mg, dolutegravir 50 mg once daily). Participants received dolutegravir 50 mg twice daily for the first 14 days as efavirenz induces enzymes and transporters involved with dolutegravir transport and metabolism, resulting in a marked reduction in dolutegravir exposure [14]. The inducing effect of efavirenz is largely resolved 2 weeks after switching.

Study visits with clinicians occurred every 4 weeks to 24 weeks (with a $\pm$ 2-week visit window at each time point, but extended to 6 weeks after the 24 week time-point to accommodate COVID restrictions). Viral load was assessed at baseline and every subsequent visit, with a repeat viral load after 2 weeks of additional adherence counselling if viral load was more than 50 copies/ml after week 12. If the repeat viral load was more than 500 copies/ml, a genotypic resistance test was performed. Baseline genotypic resistance testing, as previously described [15], was performed retrospectively for all participants and was not available to inform treatment decisions.

CD4⁺ cell count was performed at baseline and 24 weeks, creatinine at baseline, 4 and 16 weeks and a pregnancy test at every visit for women of child-bearing potential. As insomnia, anxiety and depression are recognized side effects of dolutegravir, the insomnia severity index (ISI) [16] was conducted at each visit and mental health was assessed using the Modified Mini Screen (MMS) [17] at baseline, week 12 and week 24.

Tenofovir diphosphate (TFV-DP) concentrations on dried blood spots were conducted as an objective measure of adherence at weeks 0, 12 and 24 (batched and analysed after 24 weeks). An indirect method for the quantification of TFV-DP in 50 μ l human dried blood spots was adapted from the method developed by Castillo-Mancilla *et al.* [18] and validated at the Division of Clinical Pharmacology, University of Cape Town. It consisted of solid phase separation of tenofovir and TFV-DP, enzyme dephosphorylation of TFV-DP to tenofovir, followed by high performance liquid chromatography with tandem mass spectrometry detection of tenofovir [19].

During the COVID-19 pandemic national lockdown in April and May 2020, visits were rescheduled, participants given multimonth refills and follow-up conducted telephonically.

Outcomes and analysis

The primary outcome was viral load suppression (defined as viral load <50 copies/ml) at week 24, evaluated using a mITT analysis, according to the Food and Drug

Administration (FDA) snapshot algorithm [20]. We regarded the following as failure: those with measured viral load at least 50 copies/ml, missing viral load within the visit window, intolerance or adverse event because of any drug in the regimen requiring switch, and drug substitution not permitted by the protocol. Loss to follow-up and stopping or switching because of dolutegravir or NRTI intolerance or adverse events was regarded as failure. Switching because of stopping contraception, wish to become pregnant, becoming pregnant, transfer out for nonclinical reasons and death from non-HIV and nondrug causes (as assessed by the study doctor) were not regarded as failure.

Secondary outcomes included viral load suppression at week 12, proportion suppressed over time, time to suppression, development of new resistance mutations, adherence (determined by TFV-DP concentration) and safety (creatinine, mental health and sleep assessments, pregnancy, adverse events, and mortality).

Categorical variables were described using proportions and continuous variables using median and interquartile range. Proportions were presented with the corresponding exact binomial 95% CI. If the success proportion was 0 or 100%, a one-sided 97.5% CI was estimated. Time-to-event endpoints were analysed using survival analysis. A prespecified secondary analysis described viral load suppression defined as less than 400 copies/ml. A prespecified sensitivity analysis of viral load suppression at 12 and 24 weeks was conducted excluding certain participants included in the mITT analysis: those lost to follow-up or missing a viral load in the window, those with evidence of poor adherence at the visit (TFV-DP <350 fmol/punch) and those who stopped or were changed from the study drug for reasons other than treatment failure.

Definitions

Virological failure was defined as having two consecutive viral loads more than 1000 copies/ml after week 12. Genotypic resistance was classified using the Stanford algorithm (version 8.9-1), with a score of at least 15 indicating at least low-level resistance. Results were categorized as two fully active NRTIs (both with a Stanford score <15), resistance to one NRTI (one with a Stanford score <15 and one $\geq$ 15) and dual resistance to both NRTIs (both with a Stanford score $\geq$ 15) [21].

Adverse events were graded according to Division of AIDS (DAIDS) criteria [22]. TFV-DP concentrations were categorized using the thresholds defined by Anderson *et al.* [23] as <350 fmol/punch (men: <1.2 doses per week and women: <0.6 doses per week), 350–700 fmol/punch (men: 1.2–3.2 doses per week and women: 0.6–2.0 doses per week), 700–1250 fmol/punch (men: 3.2–6 doses per week and women: 2.0–5.3 doses per week) and >1250 fmol/punch (men: >6 doses per week and women: >5.3 doses per week).

Results

Between 8 August 2019 and 23 March 2020, 112 patients were screened and 62 participants were enrolled; 60 are included in this analysis (Fig. 1).

The enrolled participants were mostly women (70%) and had a median of 5.8 years of experience with ART (IQR 2.8–8.3). The median viral load was 10 580 copies/ml (IQR 2962–38 291) and one participant had a viral load greater than 100 000 copies/ml. Baseline resistance results were available for 54 of 60 participants (six samples failed sequencing) and 89% had at least low-level resistance to tenofovir or XTC: 20 of 54 (37%) participants had K65R and 45 of 54 (83%) had M184V/I (see Table 1 for baseline characteristics). Additionally, 19 of 54 (35%) had thymidine analogue mutations (Supplementary Table 1a and b, <http://links.lww.com/QAD/C145>).

Viral load outcomes

At week 24, 51 of 60 (85%, 95% CI 73.4–92.9%) participants achieved virologic suppression in the mITT analysis (Table 2). The outcomes for the other nine participants were: six had a viral load 50–99 copies/ml, one had a viral load 100–999 copies/ml, one had switched study drug because of a tenofovir-related adverse event, and one did not have a viral load performed in the window (Supplementary Table 2, <http://links.lww.com/QAD/C145>). One participant in

the 50–99 copies/ml category had a viral load reported as ‘<100 copies/ml’ by the laboratory because of a low-volume sample, which limited the assay range. If this participant is reclassified as suppressed, suppression would be 52 of 60 (87%, 95% CI 75.4–94.1%).

In a secondary mITT analysis defining viral load suppression as less than 400 copies/ml, 57 of 60 (95%, 95% CI 86.1–99.0%) were suppressed at week 24 (Table 2). Viral load suppression at each visit is illustrated in Fig. 2 and more granular viral load results are presented in Supplementary Tables 3 and 4, <http://links.lww.com/QAD/C145>. Viral load suppression dropped at week 20 as 11 participants missed this visit because of COVID-19 lockdown restrictions.

The prespecified sensitivity analysis at week 24 included 57 participants (three participants were removed from analysis, one for each of: missing a viral load within the ± 14 -day window, low TFV-DP concentration <350 fmol/punch, and switched because of an adverse event), and showed 51 of 57 participants had a viral load less than 50 copies/ml (89.5%, 95% CI 78.5–96.0%) and 57 of 57 participants had a viral load less than 400 copies/ml (100%, 95% CI 93.7–100.0%) (Table 2).

Median time to suppression less than 50 copies/ml was 4.0 weeks (95% CI 4.0–4.9), at which point 41 participants (68.3%) were suppressed (Supplementary Fig. 1, <http://links.lww.com/QAD/C145>).

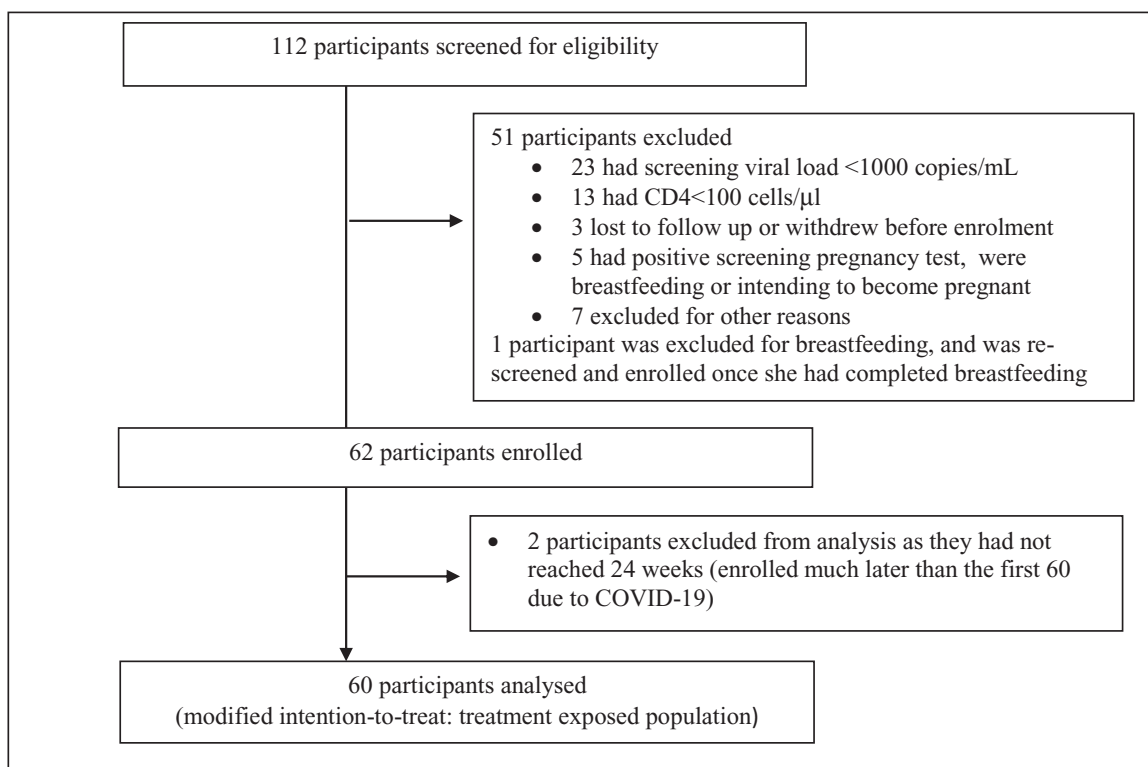


Fig. 1. Study recruitment and enrolment.

Table 1. Baseline characteristics (*n* = 60).

Female sex [<i>n</i> (%)]	42 (70%)
Age (years) [median (IQR)]	37 (31–46)
BMI (kg/m ²) [median (IQR)]	27.6 (23.4–32.5)
Weight (kg) [median (IQR)]	70 (62.3–81.2)
CD4 ⁺ cell count (cells/μl) [median (IQR)]	248 (175–346)
VL (copies/ml) [median (IQR)]	10580 (2 962–38 291)
NRTI genotypic resistance ^a [<i>n</i> (%)]	
Two fully active NRTIs	6/54 (11%)
Resistance to one NRTI	13/54 (24%)
Tenofovir, not XTC	0/54 (0%)
XTC, not tenofovir	13/54 (24%)
Resistance to both NRTIs	35/54 (65%)
Efavirenz and/or nevirapine genotypic resistance ^a [<i>n</i> (%)]	52/54 (96%)
ART history	
Duration of previous ART (years) [median (IQR)]	5.8 (2.8–8.3)
Efavirenz at enrolment [<i>n</i> (%)]	59 (98%)
Ever previously on zidovudine or stavudine as first-line ART [<i>n</i> (%)]	10 (17%)

ART, antiretroviral therapy; IQR, inter-quartile range; NRTI, nucleoside reverse transcriptase inhibitor; VL, viral load; XTC, lamivudine or emtricitabine.

^aResistance classified using a Stanford score of at least 15 for that drug. A Stanford score less than 15 indicates susceptible or potential low-level resistance to a drug, and a score of at least 15 indicates low-level, intermediate, or high-level resistance to a drug [21].

No participants had study-defined virological failure (two consecutive viral loads greater than 1000 copies/ml) by week 24. One participant (who reported periods of nonadherence and had a TFV-DP concentration <350 fmol/punch at week 24) had two consecutive viral loads greater than 500 copies/ml and had genotypic resistance testing as per protocol: there were no integrase-inhibitor or NRTI resistance mutations, but NNRTI resistance mutations were detected (K103N and P225H). The baseline resistance sample for this participant failed sequencing.

Participant characteristics were mostly similar in those who suppressed at week 24 and those who did not. However, baseline viral load was lower [8320 copies/ml (IQR 2608–24 971) versus 40 761 copies/ml (IQR 22 197–56 219)] and duration on ART was longer [6.4 years (IQR 3.0–8.5) versus 3.4 years (IQR 1.2–6.3)] in those who suppressed less than 50 copies/ml, with similar findings for those who suppressed less than 400 copies/ml (Supplementary Tables 5 and 6, <http://links.lww.com/QAD/C145>). There was no statistically significant difference between TFV-DP concentrations in those who had a suppressed viral load at week 24

compared with those who did not (*n* = 49, Fisher's exact *P* = 0.3, Supplementary Table 5, <http://links.lww.com/QAD/C145>).

Suppression was similar when stratified by baseline age, sex, viral load, CD4⁺ cell count and ART history for both the primary endpoint and defining suppression as viral load less than 400 copies/ml (Supplementary Figures 2 and 3, <http://links.lww.com/QAD/C145>). Virological suppression at week 24 was achieved by 29 of 35 (83%, 95% CI 66.4–93.4%) with resistance to tenofovir and XTC at baseline, 11 of 13 (85%, 95% CI 54.6–98.1%) with resistance to XTC only and six of six (100%, 95% CI 54.1–100.0%) with no NRTI resistance (Fisher's exact *P* value overall = 0.85, Supplementary Figure 2, <http://links.lww.com/QAD/C145>).

Other outcomes at week 24

At week 24, the median CD4⁺ cell count was 373 cells/μl (IQR 237–501), a median increase from baseline of 99 cells/μl (IQR 36–163). The median increase in BMI was 0.8 kg/m² (IQR 0.0–2.2) over 24 weeks, to 28.4 kg/m² (IQR 23.1–34.0) and the median increase in weight was 2.2 kg (IQR –0.1–6.2) to 74.8 kg (IQR 64.7–83.9)

Table 2. Viral load outcomes at weeks 12 and 24.

	VL suppression <50 copies/ml (<i>n</i> ; percentage, 95% CI)		VL suppression <400 copies/ml (<i>n</i> ; percentage, 95% CI)	
	mITT analysis ^a	Sensitivity analysis ^b	mITT analysis ^a	Sensitivity analysis ^b
Week 12	45/60; 75.0 (62.1–85.3)	45/54; 83.3 (70.7–92.1)	52/60; 86.7 (75.4–94.1)	52/54; 96.3 (87.3–99.5)
Week 24	51/60; 85.0 (73.4–92.9)	51/57; 89.5 (78.5–96.0)	57/60; 95.0 (86.1–99.0)	57/57; 100.0 (93.7–100.0)^c

CI, confidence interval. The primary outcome is viral suppression at week 24 (in bold).

^aModified intention-to-treat analysis (mITT) excludes those switching study drug for reasons of stopping contraception or wish to become pregnant, or becoming pregnant, transfer out for nonclinical reasons and death from non-HIV and nondrug causes.

^bSensitivity analysis excludes those excluded from mITT analysis, as well as lost to follow up, those missing a viral load (VL) within the window, participants who stopped or were changed from the study drug for reasons other than failure of the regimen and those who had evidence of poor adherence (TFV-DP <350 fmol/punch).

^cOne sided 97.5% confidence interval (CI).

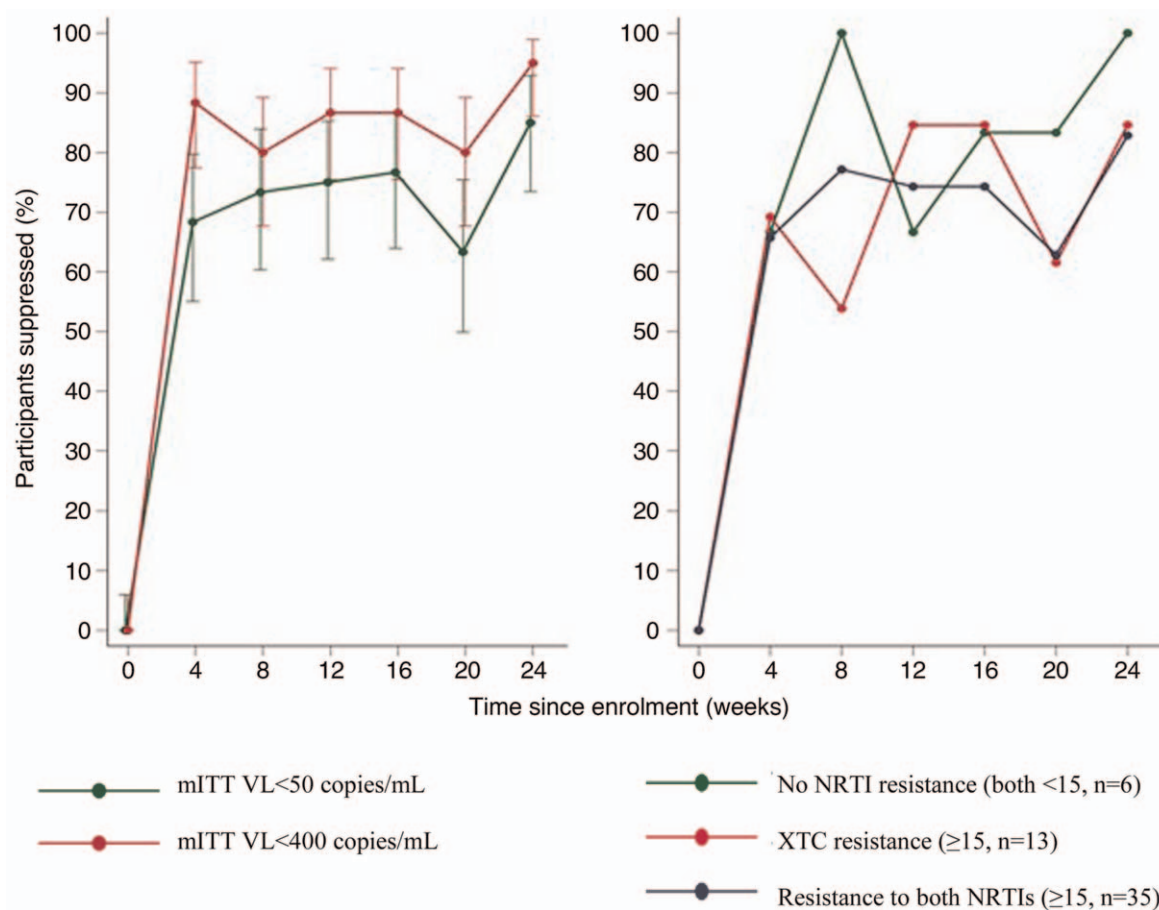


Fig. 2. Proportion suppressed at each study visit in the modified intention-to-treat analysis using the Food and Drug Administration snapshot algorithm. (a) Graph of the proportion with virological suppression of viral load less than 50 copies/ml and less than 400 copies/ml at each study visit. (b) Graph of the proportion suppressed (all mITT viral load <50 copies/ml) at each study visit categorized by baseline NRTI resistance*. mITT, modified intention-to-treat; NRTI, nucleoside reverse transcriptase inhibitor; VL, viral load; XTC, lamivudine or emtricitabine. *Genotypic resistance was classified using the Stanford algorithm (version 8.9-1), with a score at least 15 indicating at least low-level resistance. Results were categorized as 6 of 54 having two fully active NRTIs (both with a Stanford score <15), 13 of 54 with resistance to lamivudine (3TC) only (tenofovir with a Stanford score <15 and XTC with a Stanford score ≥15), 0 of 54 with resistance to tenofovir only and 35 of 54 with resistance to both NRTIs (both with a Stanford score ≥15) [7].

at 24 weeks. The median TFV-DP concentration was 1157 fmol/punch at baseline, 1939 fmol/punch at week 12 and 1350 fmol/punch at week 24 (Fig. 3 and Supplementary Table 7, <http://links.lww.com/QAD/C145>).

Safety and tolerability outcomes

TLD was generally well tolerated with seven of 60 (12%) of participants experiencing a DAIDS grade 3 or 4 adverse event and four of 60 (7%) experiencing a serious adverse event over 24 weeks (Supplementary Table 8, <http://links.lww.com/QAD/C145>). None of these were attributed to study drug and no participants died or became pregnant. One participant was switched from tenofovir to zidovudine after developing a creatinine elevation. No participants developed new or worsened scores indicating a DSM diagnosis of anxiety, mood or psychotic disorder

on the MMS score, or indicating insomnia on the ISI (Supplementary Figures 4 and 5, <http://links.lww.com/QAD/C145>). One participant was treated with rifampicin (for incident tuberculosis); dolutegravir was increased to 50 mg twice daily until 2 weeks after completing rifampicin as per protocol (Supplementary Table 9, <http://links.lww.com/QAD/C145>).

Discussion

Ours is the first prospective, interventional study to evaluate the recycling of tenofovir and XTC with dolutegravir in second-line ART, and demonstrated that a high proportion of participants achieved virological suppression by week 24. Our study was embedded in

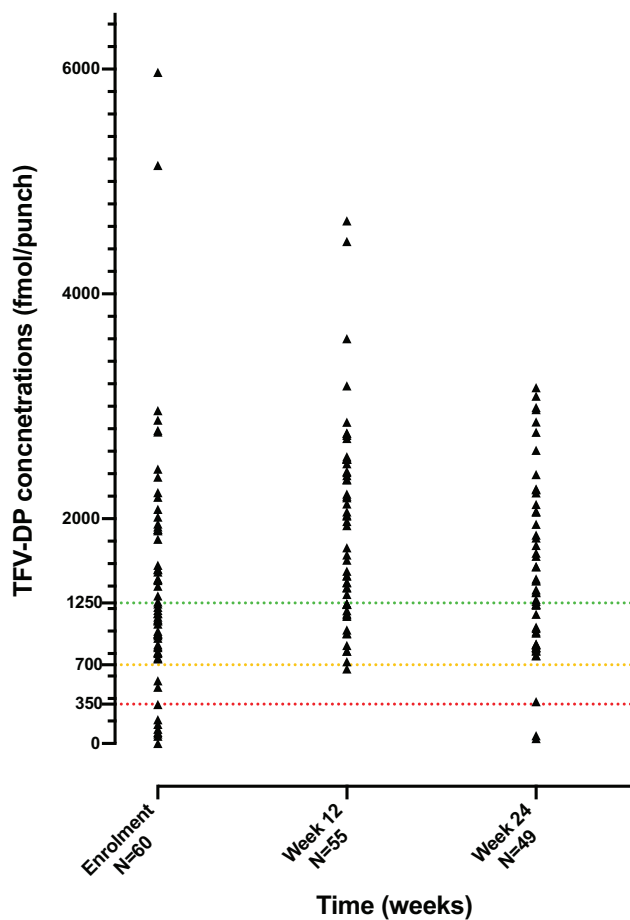


Fig. 3. Tenofovir diphosphate dried blood spot concentrations at baseline, week 12 and week 24. TFV-DP concentration, used as a marker of adherence, was categorized using the thresholds defined by Anderson *et al.* [23] as: 1, less than 350 fmol/punch (equivalent of men: <1.2 doses per week and women: <0.6 doses per week). 2, 350–700 fmol/punch (men: 1.2–3.2 doses per week and women: 0.6–2.0 doses per week). 3, 700–1250 fmol/punch (men: 3.2–6 doses per week and women: 2.0–5.3 doses per week). 4, 1250 fmol/punch (men: >6 doses per week and women: >5.3 doses per week). TFV-DP, Tenofovir diphosphate.

the primary healthcare system in an urban informal settlement in South Africa, representing real-world patient conditions. Our findings provide preliminary evidence that patients failing a first-line ART regimen containing tenofovir, XTC and efavirenz or nevirapine, can be switched to TLD as second-line ART.

We found that 85% of participants achieved a viral load less than 50 copies/ml at week 24, comparable with the 82% virologically suppressed at week 24 in the dolutegravir arm of the DAWNING study of second-line dolutegravir and an optimized NRTI backbone selected using resistance testing [1]. With a viral load threshold of 400 copies/ml, commonly used in programmatic settings, 95% of participants were suppressed at

week 24. None of our participants developed virological failure by week 24; only one participant met criteria for resistance testing, which detected no integrase resistance mutations and the patient reported poor adherence, corroborated by the TFV-DP concentration less than 350 fmol/punch at week 24. Resistance to both tenofovir and lamivudine was present in 65% of our participants at baseline, with only 11% of participants having two fully active NRTIs. Baseline NRTI resistance did not appear to affect the likelihood of virological suppression by week 24 but we were under-powered for this analysis.

It is well described that boosted protease inhibitors are effective with compromised NRTIs [9–12]. Our study builds the evidence base that dolutegravir might also be effective in second-line ART despite resistance to all NRTIs accompanying it. The ART-PRO study reported that no participants experienced virological failure whenever integrase-inhibitor-naïve, virologically suppressed patients were switched to dual therapy with dolutegravir and lamivudine, even though 21 of the 41 participants had lamivudine resistance mutations in historical plasma genotypes [24]. Similar findings were reported in switch studies evaluating lamivudine and dolutegravir dual therapy [25] and dolutegravir with companion drugs to which resistance was documented [26]. The efficacy of TLD in our study, despite resistance to one or both of tenofovir and lamivudine in 89% of our participants, may be attributable to the crippling effect that NRTI mutations have on viral fitness, resulting in residual antiviral activity [13]. In addition, there is in-vitro evidence that K65R and M184V/I mutations may protect against the development of the key dolutegravir resistance mutation R263K [27].

The median time to suppression was 4 weeks, at which point 68% of participants were suppressed, similar to the 66% of second-line participants on dolutegravir with an optimized NRTI backbone who suppressed at week 4 in DAWNING [1]. All of our participants who were not suppressed at week 24 had low-level viraemia (most of them <100 copies/ml). None of those who did have a viral load greater than 50 copies/ml at any visit after week 12 developed virological failure and most re-suppressed with enhanced adherence counselling, which has repeatedly been shown to result in high rates of re-suppression [28–30]. This is important to consider in interpreting whether unsuppressed viral loads represent the risk of failure because of resistance or rather poor adherence without development of resistance. Although sustained low-level viraemia can lead to accumulation of resistance mutations [31], virological failure is less likely to develop when the unsuppressed viral load is less than 200 copies/ml than at higher viral loads [32]. In the ADVANCE study, low-level viraemia at the primary endpoint visit did not predict later virological failure in the dolutegravir arms, leading the authors to suggest that the endpoints for ART trials may need to be rethought in

the era of second generation integrase inhibitors, which are more robust to the development of resistance [30].

Our study has limitations. Firstly, our sample size was small and we had no control arm. We had sufficient precision for our primary outcome but not for the important secondary outcome, as discussed in DAWN-ING, of virologic response stratified by level of baseline NRTI resistance mutations. Secondly, as the development of integrase-inhibitor resistance may be delayed and occur 24–48 weeks after switching to dolutegravir [5,6], we will continue to monitor our participants until 96 weeks. Thirdly, all our participants had supplemental dolutegravir doses for 2 weeks to overcome efavirenz induction effects, and our findings may not be generalizable to patients not given supplemental doses.

It is unknown whether this supplementary dosing strategy is necessary. Efavirenz induces enzymes and transporters involved in dolutegravir absorption and metabolism, reducing plasma dolutegravir concentrations at the end of the dosing interval up to 75% [14,33]. A pharmacokinetic sub-study of STRIIVING concluded that no dolutegravir dose adjustment is required when switching from efavirenz to dolutegravir but this trial was conducted in virologically suppressed individuals in whom efavirenz was likely still active [34]. As all patients entered our study with elevated viral loads and would likely have efavirenz resistance, we elected to include a 2-week supplementary dose of dolutegravir (50 mg twice daily). This dosing strategy has not been practiced in other second-line trials where small numbers of participants developed integrase-inhibitor resistance [35,36] or in low-income settings where infrequent cases of dolutegravir resistance have been reported as TLD has been introduced [37]. We will be exploring this strategy in stage 2 of our study, in which patients will be randomized to supplementary dolutegravir 50 mg or placebo for the first 2 weeks of second-line TLD.

Our findings that TLD was an efficacious second-line regimen and that baseline NRTI resistance did not impact virological suppression need to be confirmed in larger studies. This has significant implications for regimen choices as the aging HIV cohort gains experience on multiple ART regimens and pretreatment resistance rates increase [38]. Two large studies [Dolutegravir and Darunavir Evaluation in Adults Failing Therapy (D²EFT) [39] and Nucleosides and Darunavir/Dolutegravir in Africa (NADIA) [40]] are addressing the question of recycling NRTIs with dolutegravir in second-line – if these studies confirm our findings, there would be important implications for switching patients to dolutegravir-based regimens, particularly in low-resource settings with limited access to viral load testing or single-dose dolutegravir. Confirmation of our findings would suggest that all patients on first-line ART could be switched to TLD without first needing to

perform a viral load to confirm suppression, estimated to greatly simplify implementation and avert more DALYs than other more complex policy options that include performing a viral load before switch [41]. This model could show even more benefit if the emergence of dolutegravir resistance on second-line TLD is confirmed to be rare in larger trials, which our study suggests.

Our study demonstrated that a high proportion of patients failing first-line ART constituting tenofovir, XTC and efavirenz or nevirapine achieved virological suppression at week 24 on TLD, despite the presence of resistance to the recycled NRTIs in the majority of patients. Most of the participants who did not suppress had low-level viraemia, which may represent adherence rather than resistance issues. Our findings, if confirmed in larger ongoing controlled trials, create the opportunity to use TLD as a cheap, tolerable, single pill, second-line regimen. The switch of all patients on NNRTI-based regimens to TLD, regardless of viral load, would also reduce costs associated with second-line treatment and monitoring, and simplify the implementation of TLD rollout in ART programmes globally.

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Conflicts of interest

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