Original article

**A 72-week randomized study of the safety and efficacy of a stavudine to zidovudine switch at 24 weeks compared to zidovudine or tenofovir disoproxil fumarate when given with lamivudine and nevirapine**

**Nittaya Phanuphak**1,2,*, Jintanat Ananworanich1,2,3,4,5, Nipat Teeratakulpisarn2, Tanate Jadwattanakul6, Stephen J Kerr5,7, Nitiya Chomchey1, Piranun Hongchookiat2, Pornpen Mathajittiphun6, Suteeraporn Pinyakorn1,2,5, Patcharawee Rungrajorit1, Pairopa Praisuranyakit1, Mariana Gerschenson8, Praphan Phanuphak1,2,4,5, Victor Valcour1,9, Jerome K H kim1,10, Cecilia Shikuma1,3, the SEARCH 003 Study Group

1South East Asia Research Collaboration with Hawaii (SEARCH), Bangkok, Thailand
2Thai Red Cross AIDS Research Centre, Bangkok, Thailand
3Department of Medicine, University of Hawaii, Honolulu, HI, USA
4Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
5HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT), Bangkok, Thailand
6Queen Savang Vadhana Memorial Hospital, Chonburi, Thailand
7The Kirby Institute for Infections and Immunity in Society, University of New South Wales, Sydney, NSW, Australia
8Department of Cell and Molecular Biology, University of Hawaii, Honolulu, HI, USA
9University of California at San Francisco, San Francisco, CA, USA
10US Military HIV Research Program, Rockville, MD, USA

*Corresponding author e-mail: Nittaya.P@SearchThailand.org

**Background:** Due to superior long-term toxicity profiles, zidovudine (AZT) and tenofovir disoproxil fumarate (TDF) are preferred over stavudine (d4T) for first-line antiretroviral regimens. However, short-term d4T use could be beneficial in avoiding AZT-induced anaemia.

**Methods:** We randomized (1:1:1) 150 treatment-naive Thai HIV-infected adults with CD4+ T-cell count <350 cells/mm³ to arm 1 (24-week GPO-VIR S30® [d4T plus lamivudine (3TC) plus nevirapine (NVP)] followed by 48-week GPO-VIR Z250® [AZT plus 3TC plus NVP]), arm 2 (72-week GPO-VIR Z250®) or arm 3 (72-week TDF plus emtricitabine [FTC] plus NVP). Haemoglobin (Hb), dual energy x-ray absorptiometry, neuropathic signs, estimated glomerular filtration rate (eGFR), CD4+ T-cell count, plasma HIV RNA and adherence were assessed.

**Results:** In an intention-to-treat analysis, mean Hb decreased from baseline to week 24 in arm 2 compared with arm 1 (-0.19 versus 0.68 g/dl; \( P=0.001 \)) and arm 3 (0.48 g/dl; \( P=0.010 \)). Neuropathic signs were more common in arm 2 compared with arm 3 (20.4 versus 4.2%; \( P=0.028 \)) at week 24. There were no differences in changes in peripheral fat and eGFR from baseline to weeks 24 and 72 among arms. CD4+ T-cell count increased more in arm 1 than arms 2 and 3 from baseline to week 24 (168 versus 117 and 118 cells/mm³; \( P=0.01 \) and 0.02, respectively) but the increase from baseline to week 72 was similar among arms.

**Conclusions:** A 24-week d4T lead-in therapy caused less anaemia and greater initial CD4+ T-cell count increase than initiating treatment with AZT. This strategy could be considered in patients with baseline anaemia or low CD4+ T-cell count. If confirmed in a larger study, this may guide global recommendations on antiretroviral initiation where AZT is more commonly used than TDF.

**Introduction**

The 2010 World Health Organization guidelines for HIV treatment preferred first-line three-drug antiretroviral (ARV) regimens for resource-limited settings are zidovudine (AZT) or tenofovir disoproxil fumarate (TDF) plus lamivudine (3TC) or emtricitabine (FTC) as the two nucleoside reverse transcriptase inhibitor (NRTI) backbones plus a non-NRTI (NNRTI), nevirapine (NVP) or efavirenz (EFV) [1]. This represents...
a change from the 2006 guidelines, which included stavudine (d4T) plus 3TC as one of the preferred NRTI options [2]. d4T is an effective drug that is the least expensive among the NRTIs. Its availability has allowed for rapid scale-up of antiretroviral therapy (ART) in the poorest resourced settings [3]. It is now being phased out due to the risk of neuropathy and lipoatrophy with long-term use [1,4]. Currently, >5 million people are receiving ART worldwide, but there are still 10 million who are in need of ART and have yet to receive it [5]. Scaling up AZT and, in particular, TDF as first-line therapy is a major cost burden for resource-poor countries. Additionally, these drugs are not without side effects [6,7].

The adverse events associated with d4T are linked to dose and duration of use [8,9], and certain strategies could be employed for the safe use of d4T. One option may be to reduce the d4T dose to reduce toxicity, but a randomized study to test such a strategy would be needed. Another approach is to use d4T for a short lead-in time before switching to a different NRTI, such as AZT, a strategy endorsed by the 2010 Thai Ministry of Public Health HIV treatment guidelines where lead-in time before switching to a different NRTI, such as AZT was given at 200 mg or 300 mg twice-daily for body weight ≤60 or >60 kg, respectively. GPO-VIR S30®, GPO-VIR Z250® and Neravir® are generic ARVs produced by the Thai Government Pharmaceutical Organization (Bangkok, Thailand). Truvada® was provided by Gilead (Foster City, CA, USA).

Entry criteria included documented HIV infection, age ≥18 years, CD4+ T-cell count <350 cells/mm³, and ART-naive status except that females with past exposure to ARV drugs associated with pregnancy were allowed to enroll as long as the exposure was ≥3 months prior to entry. Subjects were excluded for abnormal laboratory values (absolute neutrophil count <750 cells/mm³, Hb<8.0 g/dl, serum alanine aminotransferase >5× the upper limit of normal or serum creatinine >2× the upper limit of normal), active AIDS-defining illness, other active medical illness, current use of immunomodulator therapy or any experimental therapy, pregnancy or breastfeeding, or the presence of an active malignancy.

Participants underwent a neuropathy examination during the screening process utilizing the AIDS Clinical Trials Group/Neurology and Neurologic AIDS Research Consortium methodology [11]. Neuropathy examinations were completed on all study participants and most were completed by one of two physicians (NT and TJ). Quality assurance was performed every 6 months on all study physicians who performed neuropathy examination at both study sites to ensure consistency. Participants diagnosed to have possible peripheral neuropathy (absent or diminished ankle reflex, or either diminished vibratory, pin or temperature sensation, or contact allodynia) were excluded from the study because of the potential risk of randomization to the d4T-containing arm. HBV-coinfected patients were excluded to avoid randomization to the non-TDF containing arm.

Data collection
Clinical assessments including the neuropathy examination were performed at baseline, weeks 4, 8 and 12, and every 3 months thereafter. We measured Hb and alanine aminotransferase at baseline and every 3 months thereafter. Lymphocyte CD4+ T-cell counts, plasma HIV RNA and serum creatinine levels were assessed at baseline and every 3 months. We estimated the glomerular filtration rate (eGFR) using the Levey Modification of Diet in Renal Disease (MDRD) formula. Serum lactate, fasting plasma glucose, fasting lipid profiles and full body dual energy x-ray absorptiometry (DEXA) were performed at baseline and every 6 months. Self-reported adherence

Methods
Study setting and population
SEARCH 003 was a 72-week randomized clinical trial comparing the safety and efficacy of three initial ARV regimens (ClinicalTrials.gov identification NCT00669487). ART-naive Thai HIV-infected adults were enrolled from two sites in central Thailand: the Thai Red Cross AIDS Research Centre in Bangkok and the Queen Savang Vadhana Memorial Hospital in Chonburi. The study was approved by four Institutional Review Boards from Chulalongkorn University, Queen Savang Vadhana Memorial Hospital, University of Hawaii, and University of California San Francisco. Informed consents were obtained in Thai language from all participants.

Participants were randomized (1:1:1) to arm 1 (24-week d4T 30 mg plus 3TC 150 mg plus NVP 200 mg twice daily [GPO-VIR S30®] followed by 48-week AZT 250 mg plus 3TC 150 mg plus NVP 200 mg twice daily [GPO-VIR Z250®]), arm 2 (72-week GPO-VIR Z250® twice daily) and arm 3 (72-week TDF 300 mg plus FTC 200 mg [Truvada®] once daily plus NVP 200 mg [Neravir®] twice daily). Randomization was conducted centrally using a block design stratified by baseline haemoglobin (Hb)<10 or ≥10 g/dl. During the NVP lead-in period, AZT was given at 200 mg or 300 mg twice-daily for body weight ≤60 or >60 kg, respectively. GPO-VIR S30®, GPO-VIR Z250® and Neravir® are generic ARVs produced by the Thai Government Pharmaceutical Organization (Bangkok, Thailand). Truvada® was provided by Gilead (Foster City, CA, USA).

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using 30-day visual analogue scale was assessed at weeks 4, 8 and 12, and then every 3 months. Clinical and laboratory toxicities were graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (December 2004). An independent Data Safety and Monitoring Board (DSMB) reviewed the overall quality of the trial and undertook two interim analyses. The first analysis occurred in accordance with the protocol when two-thirds of study participants had completed 12 weeks on the study. Stopping criteria were based on virological failure, resistance and safety data. The DSMB recommended a second interim analysis to occur when all patients had reached 24 weeks of follow-up.

Data analysis
We based our study sample size on the conservative assumption that Hb levels among patients in the arms treated with d4T-based or TDF-based regimens would not change, whereas the estimated mean decrease in Hb would be -1.09 g/dl (sd 1.54) after 24 weeks of AZT treatment based on data derived from the HIV-NAT 001 study [12]. A power analysis demonstrated that a sample size of 44 in each arm would provide 90% power to detect, at the 0.05 significance level, a 1.09 g/dl change in Hb between arms. Estimating a 10% loss to follow-up, we enrolled a sample size of 50 per arm.

We employed intention to treat (ITT) analyses, including all participants who were assigned to a particular treatment arm who had ≥1 follow-up visit according to their allocated treatment arm at baseline. Primary outcomes were changes in Hb, peripheral fat by DEXA, eGFR by MDRD, and the development of neuropathic signs or neuropathy at weeks 24 and 72. Secondary outcomes were changes in CD4+ T-cell count and plasma HIV RNA from baseline to weeks 24 and 72. All hypotheses were tested using two-sided, 

Results
Participant characteristics
Between April 2008 and August 2009, 214 HIV-infected patients were evaluated for eligibility at the two study sites (Figure 1). In total, 64 patients were excluded with 55 not meeting inclusion criteria, 3 declining to participate and 6 excluded for other reasons. The remaining 150 patients were randomized into one of the 3 study arms: 51 in arm 1, 50 in arm 2 and 49 in arm 3. Two participants did not receive allocated study drugs; 1 in arm 2 who had asymptomatic peripheral neuropathy revealed at the baseline visit and 1 in arm 3 who did not return for the baseline visit.

Among 150 randomized participants, 55% were female and the mean ±sd age was 34 ±8 years (Table 1). Clinical characteristics were comparable among treatment groups with baseline mean ±sd CD4+ T-cell count of 161 ±94 cells/mm³, log₁₀ plasma HIV RNA of 4.9 ±0.65 copies/ml, Hb of 12.5 ±1.6 g/dl and an eGFR of 83.0 ±15.5 ml/min/1.73 m².

Antiretroviral-therapy-related toxicities
By week 72, ≥1 ARV switch due to drug toxicities occurred in 30 participants, but the majority of these did not involve NRTIs (28 for NNRTI and 6 for NRTIs). NVP-related toxicities, mostly NVP-associated rash, caused ARV changes from NVP to lopinavir/ritonavir in 2 cases and from NVP to EFV in 26 cases and 6 of these 26 cases subsequently needed a change from EFV to lopinavir/ritonavir. The NNRTI switches due to toxicities occurred in all arms: 8 in arm 1, 13 in arm 2 and 7 in arm 3. One case had d4T changed to AZT due to numbness in extremities. AZT/3TC was switched to TDF/FTC in four cases (three for anaemia and one for lipoatrophy). In addition, one case initially had AZT/3TC switched to TDF/FTC due to hepatotoxicity and needed further switch to AZT/didanosine due to rash. AZT dose was reduced from 250 mg to 200 mg twice daily in one anaemic case, and from 250 mg twice daily to 200 mg twice daily to 100 mg thrice daily in one patient with neutropaenia.

Haemoglobin
After 24 weeks of treatment, there was a significant difference between the mean change in Hb between patients treated with AZT (-0.19 g/dl) compared with
Allocated to GPO-VIR S/GPO-VIR Z \((n=51)\)
- Received allocated intervention \((n=51)\)

Allocated to GPO-VIR Z \((n=50)\)
- Received allocated intervention \((n=49)\)
- Not allocated to intervention \((n=1)\; patient found to have neuropathy at baseline visit and withdrew\)

Allocated to NVP/Truvada \((n=49)\)
- Received allocated intervention \((n=48)\)
- Not allocated to intervention \((n=1)\; patient did not return for week 0 visit\)

Lost to follow-up
- LTFU could not contact patient \((n=1)\)
- Patient requested to withdraw from study \((n=1)\)

Discontinued intervention
- NNRTI substitution for AE \((n=8)\)
- Switched d4T to AZT at week 23 for numbness \((n=1)\)
- NNRTI switched to LPV/r due to pregnancy \((n=1)\)
- Patient HBV-positive and switched to TDF \((n=1)\)
- Did not switch to AZT at week 24 due to anaemia \((n=1)\)
- Virological failure \((n=3)\)

Analysed \((n=51)\)

Analysed \((n=49)\)
Excluded from analysis:
- Baseline neuropathy, no ARV started/patient withdrawn \((n=1)\)

Analysed \((n=48)\)
Excluded from analysis:
- Randomized but did not return for baseline visit \((n=1)\)

Randomized \((n=150)\)

Assessed for eligibility \((n=214)\)
- Excluded \((n=64)\)
  - Had not met inclusion criteria \((n=55)\)
  - Declined to participate \((n=3)\)
  - Other reasons \((n=6)\)

GPO-VIR S30® is a fixed-dose combination that contains stavudine (d4T) 30 mg, lamivudine (3TC) 150 mg, and nevirapine (NVP) 200 mg. GPO-VIR Z250® is a fixed-dose combination that contains zidovudine (AZT) 250 mg, 3TC 150 mg and NVP 200 mg. Truvada® is a fixed-dose combination that contains tenofovir disoproxil fumarate (TDF) 300 mg and emtricitabine (FTC) 200 mg. Arm 1 received GPO-VIR S30® twice daily for 24 weeks followed by GPO-VIR Z250® twice daily for 48 weeks. Arm 2 received GPO-VIR Z250® twice daily for 72 weeks, and arm 3 received Truvada® once daily and NVP 200 mg twice daily for 72 weeks. AE, adverse event; ARV, antiretroviral; LPV/r, lopinavir/ritonavir; LTFU, lost to follow-up; NNRTI, non-nucleoside reverse transcriptase inhibitor.
Table 1. Baseline characteristics of SEARCH 003 participants, by study arm

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Arm 1: GPO-VIR S30®/GPO-VIR Z 250® (n=51)</th>
<th>Arm 2: GPO-VIR Z250® (n=49)</th>
<th>Arm 3: Truvada®/NVP (n=48)</th>
<th>All arms (n=148)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bangkok, n (%)</td>
<td>29 (57)</td>
<td>29 (59)</td>
<td>29 (60)</td>
<td>87 (59)</td>
</tr>
<tr>
<td>Chonburi, n (%)</td>
<td>22 (43)</td>
<td>20 (41)</td>
<td>19 (40)</td>
<td>61 (41)</td>
</tr>
<tr>
<td>CDC classification</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, n (%)</td>
<td>25 (49)</td>
<td>26 (53)</td>
<td>27 (56)</td>
<td>78 (53)</td>
</tr>
<tr>
<td>B, n (%)</td>
<td>23 (45)</td>
<td>21 (43)</td>
<td>21 (44)</td>
<td>65 (44)</td>
</tr>
<tr>
<td>C, n (%)</td>
<td>3 (6)</td>
<td>2 (4)</td>
<td>-</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>28 (55)</td>
<td>28 (57)</td>
<td>25 (52)</td>
<td>81 (55)</td>
</tr>
<tr>
<td>Mean age, years (s)</td>
<td>35 (7)</td>
<td>34 (8)</td>
<td>35 (9)</td>
<td>34 (8)</td>
</tr>
<tr>
<td>Mean weight, kg (s)</td>
<td>56.7 (12.0)</td>
<td>58.2 (11.2)</td>
<td>58.9 (9.6)</td>
<td>57.9 (10.9)</td>
</tr>
<tr>
<td>Mean CD4 T-cell count, cells/mm³ (s)</td>
<td>154 (91)</td>
<td>174 (97)</td>
<td>157 (94)</td>
<td>161 (94)</td>
</tr>
<tr>
<td>Mean plasma HIV RNA, log₁₀ copies/ml (s)</td>
<td>4.8 (0.67)</td>
<td>4.9 (0.63)</td>
<td>4.9 (0.68)</td>
<td>4.9 (0.65)</td>
</tr>
<tr>
<td>Mean haemoglobin, g/dl (s)</td>
<td>12.4 (1.7)</td>
<td>12.4 (1.6)</td>
<td>12.8 (1.6)</td>
<td>12.5 (1.6)</td>
</tr>
<tr>
<td>Haemoglobin &lt;10 g/dl, n (%)</td>
<td>3 (6)</td>
<td>3 (6)</td>
<td>2 (4)</td>
<td>8 (5)</td>
</tr>
<tr>
<td>Mean peripheral fat by DEXA scan, g (s)</td>
<td>7,380 (3,792)</td>
<td>7,460 (3,229)</td>
<td>7,850 (2,586)</td>
<td>7,559 (3,237)</td>
</tr>
<tr>
<td>Mean eGFR by MORD, ml/min/1.73 m² (s)</td>
<td>82.5 (12.8)</td>
<td>83.3 (15.5)</td>
<td>83.0 (18.3)</td>
<td>83.0 (15.5)</td>
</tr>
<tr>
<td>Fasting lipids and glucose*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean total cholesterol, mg/dl (s)</td>
<td>164.3 (33.1)</td>
<td>168.2 (34.5)</td>
<td>177.8 (34.8)</td>
<td>170.0 (34.4)</td>
</tr>
<tr>
<td>Mean HDL cholesterol, mg/dl (s)</td>
<td>38.9 (11.0)</td>
<td>40.5 (12.6)</td>
<td>41.4 (10.3)</td>
<td>40.2 (11.3)</td>
</tr>
<tr>
<td>Mean LDL cholesterol, mg/dl (s)</td>
<td>96.1 (24.8)</td>
<td>98.2 (26.7)</td>
<td>107.3 (25.6)</td>
<td>100.4 (26.0)</td>
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<tr>
<td>Mean triglycerides, mg/dl (s)</td>
<td>112.9 (80.4)</td>
<td>111.4 (55.7)</td>
<td>108.0 (37.4)</td>
<td>110.8 (60.5)</td>
</tr>
<tr>
<td>Mean glucose, mg/dl (s)</td>
<td>82.1 (8.0)</td>
<td>82.7 (11.6)</td>
<td>83.5 (8.6)</td>
<td>82.8 (9.4)</td>
</tr>
</tbody>
</table>

*Includes one patient in arm 3 who did not fast. GPO-VIR S30® is a fixed-dose combination that contains stavudine 30 mg, lamivudine (3TC) 150 mg, and nevirapine (NVP) 200 mg. GPO-VIR Z250® is a fixed-dose combination that contains tenofovir disoproxil fumarate 300 mg and emtricitabine 200 mg. Truvada® is a fixed-dose combination that contains tenofovir diosproxil fumarate 300 mg and emtricitabine 200 mg. All arms received standard doses of antiretrovirals. Arm 1 received GPO-VIR S30® twice daily for 24 weeks followed by GPO-VIR Z250® twice daily for 48 weeks. Arm 2 received GPO-VIR Z250® twice daily for 72 weeks, and arm 3 received Truvada® once daily and NVP 200 mg twice daily for 72 weeks. DEXA, dual energy x-ray absorptiometry; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MORD, modification of diet in renal disease.

Those on d4T (0.68 g/dl; P=0.001) or TDF (0.48 g/dl; P=0.010; Table 2 and Figure 2A). The mean absolute Hb at week 24 was 13.1 g/dl in arm 1, 12.2 g/dl in arm 2 and 13.2 g/dl in arm 3. Arm 1 patients had a sharp decline in Hb at week 36 after switching from d4T to AZT at week 24 (mean change from baseline of -0.15 g/dl). By week 72, there was a significant increase in mean Hb from baseline in arm 3 (0.90 g/dl) compared with arm 1 (0.17 g/dl; P=0.006). Both arms 1 and 2 patients on AZT had lower Hb than patients treated with TDF in arm 3 (Figure 2A). The mean absolute Hb at week 72 was 12.6 g/dl in arm 1, 12.8 g/dl in arm 2 and 13.7 g/dl in arm 3 (P=0.009). Throughout the study, there were no differences between the proportion of patients with Hb<10 g/dl across arms.

Peripheral fat by DEXA
Across treatment arms, patients had similar increases in peripheral fat by week 24 (mean changes of 6.5 g in arm 1, 9.7 g in arm 2 and 68 g in arm 3; Table 2 and Figure 2B). However, by week 72, there was a trend towards a decrease in peripheral fat in patients who switched from d4T to AZT in arm 1 (mean change of -301 g) compared with those who were on TDF in arm 3 (281 g; P=0.086), but not for those on AZT in arm 2 (143 g; P=0.187). Clinical lipodystrophy was reported in only one patient in arm 2 at week 48, at which time the patient had AZT replaced by TDF. The mean changes in lean body mass were similar across arms.

Estimated glomerular filtration rate by Modification of Diet in Renal Disease formula
At week 24, the mean changes in eGFR did not differ among arms, at -0.8 ml/min/1.73 m² in arm 1 compared with -1.37 ml/min/1.73 m² in arm 2 and -3.1 ml/min/1.73 m² in arm 3 (P=0.05; Table 2 and Figure 2C). The eGFR values varied over time across arms, and by week 72, there was a non-significant decline in eGFR from baseline in arm 3 (mean change of -8.7 ml/min/1.73 m²) compared with arm 2 (-3.7; P=0.143) and arm 1 (-2.80; P=0.071). In PP analysis, mean eGFR change from baseline to week 72 was significantly greater in arm 3 (-10.6 ml/min/1.73 m²) compared with arm 1 (-2.3 ml/min/1.73 m²; P=0.04) but not to arm 2 (-3.8 ml/min/1.73 m²; P=0.12). Urinalysis was normal throughout the study except for grade 2 proteinuria in two patients (an arm 1 patient at week 48 and an arm 3 patient at arm 3 week 48).
Truvada® is a fixed-dose combination that contains tenofovir disoproxil fumarate 300 mg and emtricitabine 200 mg. Arm 3 received Truvada® once daily and NVP 200 mg twice daily for 72 weeks. eGFR, estimated glomerular filtration rate.

GPO-VIR S30® is a fixed-dose combination that contains stavudine 30 mg, lamivudine (3TC) 150 mg, and nevirapine (NVP) 200 mg. GPO-VIR Z250® is a fixed-dose combination that contains zidovudine (AZT) 250 mg, 3TC 150 mg, and NVP 200 mg.

Plasma HIV RNA<50 copies/ml

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Week 0</th>
<th>Week 24</th>
<th>Week 72</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T-cell count</td>
<td>40 (83.3)</td>
<td>45 (91)</td>
<td>44 (86.3)</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Neuronal crease</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Peripheral fat</td>
<td>7,850 (2,586)</td>
<td>7,460 (3,229)</td>
<td>7,446 (3,620)</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>13.2 (1.8)</td>
<td>12.5 (1.7)</td>
<td>13.1 (1.7)</td>
</tr>
</tbody>
</table>

Table 2. Changes in haemoglobin, peripheral fat, neuronal crease, peripheral neuropathy, renal function, CD4+ T-cell count and plasma HIV RNA, from baseline to weeks 24 and 72.
patient at baseline, weeks 12 and 24) and glucosuria in an arm 2 patient at baseline and week 72.

Neuropathic signs and neuropathy by detailed neuropathy examination
At week 24, the proportions of patients with neuropathic signs, defined as diminished sensation by any modality detected bilaterally in the lower extremities or absent or diminished ankle reflexes relative to knees, was higher in arm 2 compared with arm 3 (20.4% versus 4.2%; \( P = 0.028 \)) but was not statistically different from arm 1 (9.8%; \( P = 0.168 \); Table 2 and Figure 2D). This significant difference between arm 2 and arm 3 at week 24 did not persist to week 72; however, there was a trend for arm 2 to have higher proportion of patients with neuropathic signs than arm 1 (24.5% versus 9.8%; \( P = 0.064 \)). In PP analysis, the proportion of patients with neuropathic signs in arm 2 (13.3%) at week 24 was not higher than those in arm 1 (4.3%; \( P = 0.15 \)) and arm 3 (2.3%; \( P = 0.11 \)). At week 72, however, a significantly higher proportion of patients in arm 2 had neuropathic signs than in arm 1 (14.3% versus 0.0%; \( P = 0.01 \)). There were no differences by study arm in the proportion of patients who developed peripheral neuropathy, defined as reduced vibration sensation in both great toes or absent or diminished ankle reflexes bilaterally relative to

Figure 2. Comparison of toxicity primary end points between treatment arms by intention-to-treat analysis

(A) Mean haemoglobin change from baseline. (B) Mean peripheral fat change from baseline assessed by dual energy x-ray absorptiometry. (C) Mean estimated glomerular filtration rate change from baseline assessed by modification of diet in renal disease formula. (D) Proportions of patients with neuropathic signs assessed by detailed neuropathy examination. GPO-VIR S30® is a fixed-dose combination that contains stavudine 30 mg, lamivudine (3TC) 150 mg and nevirapine (NVP) 200 mg. GPO-VIR Z250® is a fixed-dose combination that contains zidovudine 250 mg, 3TC 150 mg and NVP 200 mg. Truvada® is a fixed-dose combination that contains tenofovir disoproxil fumarate 300 mg and emtricitabine 200 mg. All arms received standard doses of antiretrovirals. Arm 1 received GPO-VIR S30® twice daily for 24 weeks followed by GPO-VIR Z250® twice daily for 48 weeks. Arm 2 received GPO-VIR Z250® twice daily for 72 weeks, and arm 3 received Truvada® once daily and NVP 200 mg twice daily for 72 weeks.
knees. The rates were 7.8%, 10.2% and 2.1% at week 24 and 9.8%, 16.3% and 8.3% at week 72 for study arms 1, 2 and 3, respectively.

Other adverse events
The mean total cholesterol rise after 24 weeks of treatment was significantly less in arm 3 compared with arm 1 (17.3 versus 36.8 mg/dl; \( P = 0.01 \)) but not less than that in arm 2 (25.1 mg/dl; \( P = 0.3 \)). This trend remained present at week 72 with the mean total cholesterol rise being less in arm 3 than in arm 1 (16.4 versus 38.4 mg/dl; \( P = 0.005 \)) and in arm 2 (32.1 g/dl; \( P = 0.043 \)). The mean high-density lipoprotein (HDL) cholesterol level in arm 1 increased significantly more at week 24 than that seen in arm 2 (14.7 versus 8.9 mg/dl; \( P = 0.021 \)) and in arm 3 (7.2 mg/dl; \( P = 0.003 \)). We also observed an increase in the mean HDL cholesterol level at week 72 in arm 1 compared with that in arm 3 (mean change 19.4 versus 12.2 mg/dl; \( P = 0.022 \)). Among arms, there were no differences in changes of low density lipoprotein (LDL) cholesterol, triglyceride or glucose, and of the frequency of grade 2, 3 and 4 lipid and glucose abnormalities. The white blood cell count decreased more at weeks 24 and 72 in arm 2 (-0.90 and -0.67 \times 10^3\) cells/mm\(^3\)) compared with that in arm 1 (0.78 and 0.14 \times 10^3\) cells/mm\(^3\); \( P = 0.001 \)) and in arm 3 (0.56 and 0.68 \times 10^3\) cells/mm\(^3\); \( P < 0.001 \); data not shown). The absolute neutrophil counts decreased more at week 24 in arm 2 (-0.55 \times 10^3\) cells/mm\(^3\)) compared with that in arm 1 (0.63 \times 10^3\) cells/mm\(^3\); \( P < 0.001 \)) and in arm 3 (0.41 \times 10^3\) cells/mm\(^3\); \( P = 0.001 \)) and at week 72 in arm 2 (-0.29 \times 10^3\) cells/mm\(^3\)) compared with that in arm 3 (0.53 \times 10^3\) cells/mm\(^3\); \( P = 0.004 \); data not shown).

Immunological and virological responses
At week 24, the absolute CD4\(^+\) T-cell count increased more in arm 1 (mean change of 168 cells/mm\(^3\)) than in arm 2 (117 cells/mm\(^3\); \( P = 0.01 \)) and in arm 3 (118 cells/mm\(^3\); \( P = 0.01 \)), but the overall change by week 72 did not differ across arms (207 cells/mm\(^3\) in arm 1, 167 cells/mm\(^3\) in arm 2 and 198 cells/mm\(^3\) in arm 3; \( P > 0.05 \); Figure 3A). In PP analysis, in addition to higher CD4\(^+\) T-cell count change in arm 1 compared with arm 2 (\( P = 0.01 \)) and arm 3 (\( P = 0.03 \)) by week 24, CD4\(^+\) T-cell count change from baseline was also significantly higher in arm 1 versus arm 2 at week 72 (\( P = 0.03 \)). From baseline to week 24 and week 72, mean absolute CD4\(^+\) T-cell count increased from 154 to 322 and 361 cells/mm\(^3\) in arm 1, from 174 to 322 and 361 cells/mm\(^3\) in arm 2, and from 157 to 274 and 355 cells/mm\(^3\) in arm 3.

The proportion of patients with plasma HIV RNA<50 copies/ml was similar among arms at weeks 24 and 72 (Figure 3B). At week 24, these were 86.3% in arm 1, 81.6% in arm 2 and 79.2% in arm 3 (\( P = 0.05 \)). At week 72, 84.3% of patients in arm 1 versus 91.8% in arm 2.
versus 83.3% in arm 3 had plasma HIV RNA<50 copies/ml (P>0.05).

The proportion of participants who reported <95% adherence was similar among arms at all study visits (4.1%, 2.1% and 0.0% in arms 1, 2 and 3, respectively at week 72).

Discussion

In this 72-week randomized comparison of haematological, metabolic, renal and neurological toxicities of the three most commonly used first-line ARV regimens worldwide, we demonstrated that short-term d4T use for 24 weeks before switching to AZT did not result in greater toxicities compared with the other two regimens. By contrast, after 24 weeks of treatment, d4T-treated patients had significantly higher Hb levels than that seen in the AZT arm, and had a larger CD4+ T-cell count increase than that observed in both the AZT and TDF arms. These changes could be pertinent in settings where patients start ART with low CD4+ T-cell counts, since the magnitude of CD4+ T-cell count rise following treatment is associated with improved clinical outcomes in patients with low nadir CD4+ T-cell counts [13]. Our findings may have important implications for treatment programs in resource-limited settings where patients generally start treatment late and anaemia is frequent. The benefit of this treatment strategy may even be greater for HIV-infected patients in malarious areas with high burden of anaemia. As expected, we noted lower Hb levels in patients treated with AZT. More neuropathic signs were identified in patients treated with AZT compared with those on TDF; but, unexpectedly, the frequency of neuropathic signs was not greater for the d4T arm compared with the other arms. Virological suppression did not differ among arms.

Our data concur with a meta-analysis of six prospective, randomized, comparative studies identifying a greater negative impact on haematological parameters with AZT-based relative to d4T-based ARV regimens [14]. The use of d4T is being phased out globally because of long-term risks of peripheral neuropathy and lipoatrophy. In our study, we did not observe an increased risk of d4T-related neuropathic signs or neuropathy. In fact, in both ITT and PP analyses, a higher proportion of subjects in the AZT arm had abnormal neurological examinations compared with either the d4T or TDF arms. This suggests that there is a low risk for neuropathy with short-term use of d4T in patients carefully screened to not have neuropathic signs or symptoms before initiation of the medication. Our ongoing study to quantify epidermal nerve fiber density before and after ART in this cohort may shed some light on why neuropathic signs were more common with AZT than with d4T.

After 24 weeks of treatment, clinical lipodystrophy was not seen in patients on d4T and the peripheral fat mass was similar across arms. However, treatment with 24 weeks of d4T is likely too short a duration to cause significant changes in fat. In the GS-903 and GS-934 studies, clinical lipodystrophy and peripheral fat mass loss were observed in patients treated with 3 years of d4T compared with those on TDF or AZT [15,16]. Unexpectedly, we identified trends toward lower peripheral fat mass at week 72 in the d4T lead-in arm when compared with the AZT and TDF arms despite transition off d4T at 24 weeks. The peripheral fat mass loss in the d4T switched arm was small with differences in fat mass loss among arms being approximately 500 g or less, which is not likely to be clinically relevant. The GS-903 study reported a 2.9 kg difference in peripheral fat mass between d4T and TDF arms at 3 years. The lack of standardized DEXA criteria to diagnose lipodystrophy further limits our ability to identify the clinical impact of such findings [17].

Similar to other reports, the increase in total cholesterol was less with TDF than observed with AZT or d4T [15,16]. In contrast to the GS-903 study [15], we saw a more favourable HDL cholesterol profile with d4T than TDF. The HDL cholesterol increase was also higher with d4T than with AZT in our study. Other studies have not identified differences in the HDL cholesterol profile when participants on d4T were compared with those on AZT [18,19]. These findings need to be confirmed by larger studies.

AZT- and TDF-based first-line regimens are currently recommended in resource-limited settings, including Thailand [1,10]. We found TDF to be a safe and well-tolerated drug. Some studies have reported an unexpectedly high early virological failure rate of 25–43% with TDF plus 3TC or FTC plus NVP regimen [20,21], especially in patients with high baseline plasma HIV RNA, but these findings were not found by others [22,23]. Our study was not powered to compare virological efficacy between treatment arms. Nevertheless, treatment failures were uncommon in all arms. We saw a small but non-significant decline in eGFR with TDF that is consistent with previous reports [15,16]. Acute renal failure after TDF, particularly in patients with advanced HIV disease has been reported [6]. It is possible that in such patients, short-term d4T could be used as the initial NRTI before switching to TDF to lessen such risk. Thus, with the expanded use of TDF in developing countries, regular and long-term monitoring of renal function is necessary.

Our study was designed and powered to test short-term outcomes associated with a d4T lead-in strategy so our ability to comment on less common toxicities, such as lactic acidosis, and hepatic steatosis, or those that occur after long-term ART is limited due to a...
small sample size and short duration of follow-up. In addition, our patients who were mostly well, lacked neuropathic signs and had nadir CD4+ T-cell counts close to 200 cells/mm³, may not represent the majority of patients in developing countries who because of advanced disease may be at greater risk for ARV-related toxicities. Nevertheless, we included equal proportions of female and male participants, which more closely reflects the patient population needing treatment in developing countries, and the prospective, systematic and detailed evaluation of ARV-related toxicities in subjects randomly assigned to one of three commonly used NRTIs strengthens the validity of our findings.

In summary, we demonstrated that initiating ART with d4T for 6 months before switching to AZT was safe among subjects without neuropathic signs, and resulted in greater initial CD4+ T-cell count and Hb rise when compared with initiating treatment with AZT. TDF was also safe, and resulted in higher Hb and lower neuropathic signs than observed with AZT; however, its higher cost may limit its scale-up in resource-limited settings. Our results provide important information which, if confirmed in a larger study, will be useful for the refinement and further development of existing global recommendations for initiating ART.

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Disclosure statement

JA has received speakers’ fees or honoraria from Gilead, Abbott and ViiV. NP and JA are advisory committee members for the Thai Government Pharmaceutical Organization. All other authors declare no competing interests.

Additional file

Additional file 1: A supplementary table displaying changes in Hb, peripheral fat, neuropathic signs, peripheral neuropathy, renal function, CD4+ T-cell count and plasma HIV RNA, from baseline to weeks 24 and 72, by study arm can be accessed via http://www.intmedpress.com/uploads/documents/AVT-12-0A-2481_Phanuphak_Add_file1.pdf

References


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