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Virological efficacy of 24-week fozivudine-based regimen in ART-naive patients from Tanzania and Côte d'Ivoire

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Objective: Use of zidovudine (ZDV) in antiretroviral therapy is limited by toxicity and twice daily (b.i.d.) dosing. Fozivudine (FZD) is a ZDV prodrug, which is activated intracellularly to ZDV-monophosphate especially in mononuclear cells but not in bone marrow cells. FZD promises improved myelotoxicity and once daily (o.d.) dosing.

Design: Randomized clinical trial.

Methods: We conducted an open-label, phase II, proof-of-concept trial investigating three different FZD doses (800 mg o.d., 600 mg b.i.d., 1200 mg o.d.) versus ZDV (300 mg b.i.d.) in combination with lamivudine and efavirenz in HIV-infected, ART-naive patients from Tanzania and Côte d'Ivoire. The primary objective was to demonstrate virological efficacy after 24 weeks in intent-to treat and per-protocol analysis. Secondary endpoints included safety and pharmacokinetic outcomes.

Results: Of 119 participants included in the intent-to treat analysis, HIV RNA less than 50 copies/ml at 24 weeks was observed in 64 of 88 (73%) patients in the combined FZD arms versus 24 of 31 (77%) in the ZDV arm (RR 0.94, 95% confidence interval 0.75–1.18). In the per-protocol analysis, responses were 64 of 77 (87%) versus 23 of 29 (79%), respectively (RR 1.09, 95% confidence interval 0.89–1.34). Outcomes were similar between FZD arms. Overall, treatments were well tolerated. Severe or worse anaemia occurred in two cases (one related to FZD, one to ZDV), grade III/IV neutropenia was less frequent in FZD compared with ZDV arms (22 versus 42%, $P = 0.035$). Pharmacokinetic analysis supported o.d. administration of FZD.

Conclusion: Virological 24-week efficacy was demonstrated in b.i.d. and o.d. administered FZD-based regimens. Reduced myelotoxicity of FZD needs to be confirmed in a larger trial.

AIDS 2017, **31**:501–509

Keywords: Africa, antiretroviral therapy, fozivudine, HIV, zidovudine

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Received: 31 August 2016; revised: 25 November 2016; accepted: 26 November 2016.

DOI:10.1097/QAD.0000000000001362

ISSN 0269-9370

Introduction

Increased effectiveness of HIV-1 treatment through optimizing antiretroviral regimens for simplification and reduced toxicity is a priority in HIV treatment recommendations [1].

Zidovudine (ZDV) has been widely used as part of antiretroviral regimens, but is no longer recommend as first choice because its use is limited by twice daily (b.i.d.) dosing and haematologic toxicity [1–3]. However, ZDV exhibits important characteristics, notably related to drug resistance pattern relevant for antiretroviral treatment strategies in nonsubtype B HIV-1 strains from Africa when compared with other nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) [4,5]. To overcome the above limitations of ZDV, the availability of an improved thymidine-analogue NRTI would be of great value, especially in African countries where anaemia and neutropenia are frequent, and monitoring for toxicity and adherence is often limited [6,7].

Fozivudine tidoxil is a thioether lipid–ZDV conjugate. After intake it is split intracellularly into the lipid moiety and ZDV-monophosphate, which is subsequently phosphorylated to the active metabolite ZDV-triphosphate. The rationale behind the development of fozivudine (FZD) was to take advantage of the high cleavage activity in mononuclear cells and other organs resulting in increased amounts of intracellular ZDV available for phosphorylation to the active metabolite, and a very low activity in red blood and stem cells, which should result in reduced haematologic toxicity [unpublished data on file at Boehringer Mannheim].

FZD was studied in four phase I/II clinical trials in HIV-infected, treatment-naive patients in Europe and the United States. FZD o.d. and b.i.d. daily administered single oral doses were evaluated up to 1800 mg/day over 1 week, showing a dose linear increase of pharmacokinetic parameters and good tolerability [8,9]. A bioavailability study of FZD under fed and fasting conditions revealed no clinically relevant pharmacokinetic differences, indicating that the drug can be given without regard to timing of meals [unpublished data on file at Boehringer Mannheim]. In a single FZD, multiple dose-finding, phase II study, the largest viral load reduction after 4 weeks was seen with 600 mg b.i.d. (−0.67 log) with a similar reduction observed in the 800 mg o.d. group [10].

The further development of FZD was halted during the early millennium mainly because of marketing considerations at this time. Based on an expected greatest benefit of the drug in African HIV-infected populations, we designed a study with the objective to demonstrate treatment efficacy, tolerability, and pharmacokinetics of o.d. and b.i.d. administered FZD-based antiretroviral regimens in African treatment-naive patients.

Methods

Study design and population

The Fozivudine in Africa Trial Initiative (FATI-1) was an open-label, multicentre, prospective, randomized, phase IIa, proof-of-concept study, aiming to demonstrate 24-week treatment efficacy comparing three different doses of FZD (600 mg b.i.d., 800 mg o.d., 1200 mg o.d.) and standard ZDV (300 mg b.i.d.) in combination with lamivudine (3TC either 150 mg b.i.d. or 300 mg o.d.) and efavirenz (EFV 600 mg o.d.) in HIV-infected, ART-naive patients. The study was conducted at two African centres: the National Institute for Medical Research (NIMR)-Mbeya Medical Research Center in collaboration with the Mbeya Referral Hospital in Mbeya, Tanzania, and the Service de Maladies Infectieuses et Tropicales (SMIT) at the CHU Treichville Hospital in collaboration with the PACCI Program in Abidjan, Côte d'Ivoire.

The study protocol aimed to include 120 patients aged 18 years or above, with an indication to start ART according to WHO and/or country guidelines. Laboratory inclusion criteria were: $CD4^+$ cell count at least 100 cells/μl, haemoglobin (Hb) at least 9.5 g/dl, platelets at least 50 000 cells/μl, neutrophils at least 500 cells/μl, bilirubin and alanine aminotransferase (ALT) less than 2.5-fold upper limit of normality, no severe hepatic insufficiency (prothrombin time < 50%), creatinine clearance calculated by Cockroft's formula at least 50 ml/min, and only trace or below protein and blood in the urine dipstick test. Women were excluded if pregnant or breastfeeding. Furthermore, we excluded patients with evidence of an HIV-2 infection (Abidjan only), hepatitis B coinfection, and presence of a severe uncontrolled, ongoing disease.

The investigational FZD was provided as 200 mg film-coated tablets. ZDV, 3TC, and EFV were distributed by each country's National AIDS Control Programme. A master randomization list containing 60 sequential randomization slots was provided to each study centre and block randomization was performed containing four assignments per block for each of the study arms. The sequence of each arm per block was randomly distributed using the certified web-based custom software system ALEA (FormsVision BV, 1391 GT Abcoude, The Netherlands). Randomization was stratified by centre and sex with 30 patients per arm (15 patients per arm and site). A minimum of 30% representation for each sex was requested per arm and site.

This study was registered with ClinicalTrials.gov (NCT01714414) and the WHO International Clinical Trials Registry Platform (PACTR201205000384379). Ethical and regulatory approval was obtained from the institutional review board of the Mbeya Medical Research Ethics Committee, NIMR Ethics Committee and the Tanzania Food and Drugs Authority (TFDA) in Tanzania, the Comité National d'Ethique et de la Recherche (CNER) and the Direction de la Pharmacie

et du Medicament, Ministère de la Santé in Côte d'Ivoire, and the Ethics Committee of Munich University (Ludwig-Maximilians-Universität) in Germany. All patients received detailed oral and written study information and provided written informed consent.

Procedures

All patients were requested to take their drugs with or without food at about the same times each day. Study drugs were dispensed in monthly intervals and pill counts for each drug were performed at the next visit to assess treatment adherence. Follow-up visits were performed at 2, 4, 8, 12, and 24 weeks after treatment initiation. Primary outcome assessments at baseline and subsequent visits included plasma HIV RNA (COBAS TaqManV2; Roche Molecular Systems, Branchburg, New Jersey, USA) and $CD4^+$ cell counts (FACSCount system; BD Bioscience, San Jose, California, USA), the latter not performed at week 2. Virological failure was defined as two consecutive HIV RNA measurements more than 1000 copies/ml at week 12 or later. Genotypic resistance testing was performed at the time of virological failure and from stored baseline samples in these cases to assess preexisting resistance before treatment. At baseline and each subsequent visit, safety assessments were performed, including physical assessments, Hb, complete blood count and platelets, biochemistry (creatinine, ALT, aspartate aminotransferase, gamma-glutamyltransferase, bilirubin, amylase, and glucose) and urine pregnancy testing in women. Metabolic (total cholesterol, triglycerides, and lactate) were collected at baseline, week 12, and 24. All laboratory tests were performed at the Centre de Diagnostic et de Recherches sur le SIDA (CeDReS) in Abidjan and at the NIMR-Mbeya Medical Research Center main laboratory in Mbeya. Both laboratories implement strict internal quality control programmes and participate in external proficiency testing programmes, including accreditation by the College of American Pathologists (CAP) in Mbeya and by AfriQualab (AFQL) in Abidjan.

Treatment-emergent clinical adverse events were defined as any new or worsening previous clinical condition after ART initiation and reported according to severity and relationship to study treatments. Laboratory events were considered as clinical adverse events if they required intervention (e.g. treatment for anaemia, neutropenia-associated interruption of cotrimoxazole prophylaxis), serious adverse events reporting included grade 4 laboratory events. Clinical and laboratory adverse events were graded using the Agence Nationale de Recherches sur le Sida (ANRS) Toxicity Scale (Version 1.0 translated to English from the French Version 6.0) [11]. Because we found large discrepancies between ANRS toxicity and site-specific reference ranges for lactate, values were graded retrospectively using local reference ranges. At week 24, all patients receiving FZD were switched to either ZDV or tenofovir at the discretion of the investigator and the local HIV clinic.

Pharmacokinetics assessments at baseline and week 4 for steady-state analysis were planned for 24 participants (six per study arm) on a first come, first-serve basis. Pharmacokinetics assignments were balanced by study site (three patients per arm and site) and sex with a least 30% representation of each sex per arm. Pharmacokinetics sampling included repeated blood and urine collections over a 12-h period for b.i.d. regimens (arms A and D) and a 24-h period for o.d. regimens (arms B and C; pharmacokinetics details in the supplement, http://links.lww.com/QAD/B27).

Outcomes

The primary outcome was the proportion of patients with plasma HIV RNA less than 50 copies/ml at week 24. Secondary efficacy outcomes included the proportion of patients with plasma HIV RNA less than 400 copies/ml, and change in $\log_{10}$ viral load and in $CD4^+$ cell counts through week 24. The primary safety analyses included grade III/IV clinical and laboratory adverse events, focused analysis involved events related to myelotoxicity. Pharmacokinetics outcomes included plasma and urine concentrations of FZD, ZDV, and ZDV–glucuronide after the first treatment dose and at steady state (week 4).

Statistical analysis

The study was designed as proof of concept to demonstrate treatment efficacy of FZD-based ART. Intent-to-treat (ITT) analyses included all patients who received at least one dose of study drug, with missing outcome data classified as failure. Per-protocol analyses included all patients who stayed on treatment until week 24 without substantial treatment interruption and moderate or good adherence for at least 65% of visits. Safety analyses included all participants who received at least one dose of study regimen and all time points up to 14 days after the last dose of study regimen (for patients who discontinued treatment) or until week 24. To facilitate comparison of FZD and ZDV, safety and other analyses were not only performed separately for each arm, but also on pooled data for all FZD arms. Treatment adherence was calculated as the number of pills taken divided by the number of pills supposed to be taken, expressed as a percentage.

Descriptive analyses report the median and interquartile range (IQR) for continuous variables and the number and percentage of participants in each stratum for binary and categorical variables, confidence intervals for percentages were calculated using the exact or Clopper–Pearson binomial formula. To compare binary outcomes between arms, we report risk ratios and their accompanying confidence interval or Fisher's exact P value. All reported P values are two sided and for all statistical tests an α level of less than 0.05 denotes significance. Stata statistics software (version 14; StataCorp, College Station, Texas, USA) was used to perform statistical analyses and to draw graphs. Pharmacokinetics noncompartmental analysis was performed using Phoenix WinNonlin 6.4 software (Certara USA, Inc., Princeton, New Jersey, USA).

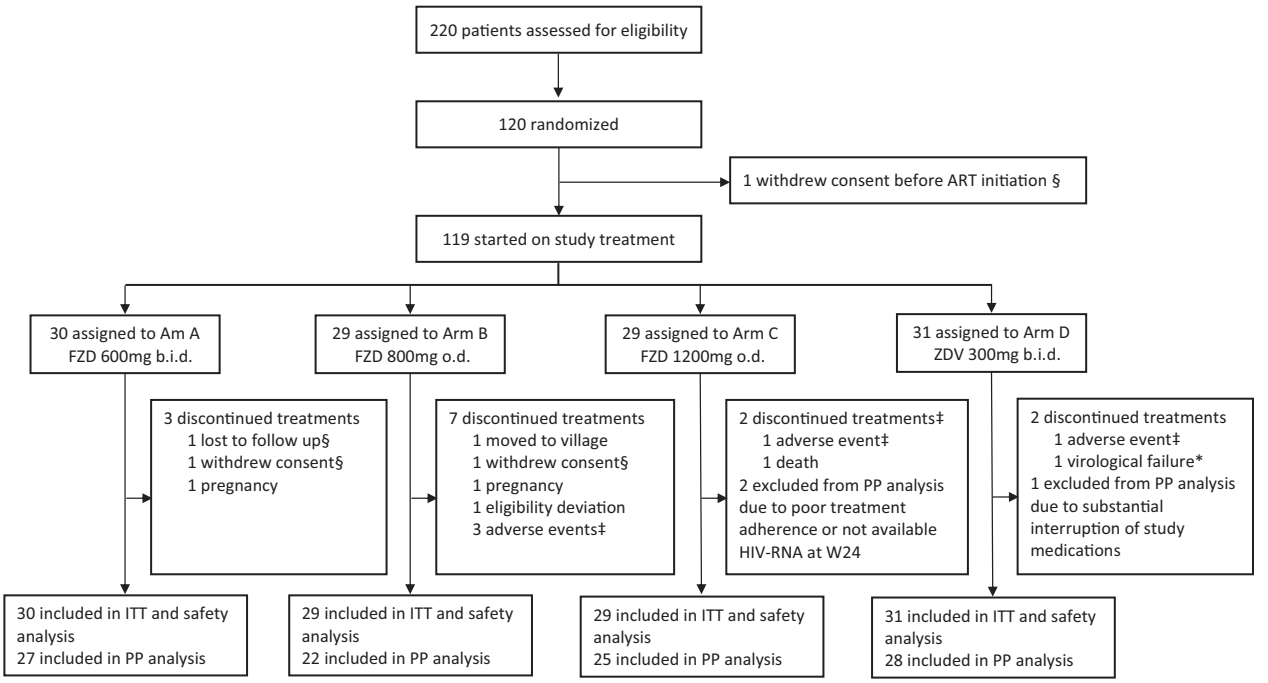

Fig. 1. Study profile. FZD, fozivudine; ZDV, zidovudine; b.i.d., twice daily; o.d., once daily; PP, per-protocol; ITT, intent-to-treat. *Retrospectively detected transmitted drug resistance prior to randomization. ‡Adverse event included: FZD Arm B: IV anaemia (1), IV GGT elevation (1), Kaposi's sarcoma and chemotherapy (1); FZD Arm C: death due to cholangiocellular carcinoma (1), severe rash probably related to efavirenz (1), ZDV arm: IV anaemia (1). §Lost to follow-up and withdrawn consent between study week 2 and 8 were mainly due because of being not ready to take antiretroviral therapy.

Results

Between 7 January 2013 and 8 January 2014, we recruited 120 patients who were randomly assigned to the four study arms (Fig. 1). One patient who withdrew consent prior to first intake of study regimen was excluded from analysis. Of the remaining 119 patients, 14 (11.8%) patients did not complete the study treatments with reasons indicated in Fig. 1. Baseline demographic and HIV disease characteristics were balanced between study arms (Table 1). Overall 38 patients were in WHO stage 3 or 4 and more often assigned to arms A and C (40 and 38%, respectively). Diseases included tuberculosis (TB) ($N=10$, of those six patients were still on stable TB treatment at inclusion), sever weight loss ($N=8$), unexplained diarrhoea ($N=6$), unexplained fever ($N=9$), persistent oral thrush ($N=4$), sever bacterial infection ($N=7$), or chronic herpes simplex virus infection ($N=1$). In all patients, disease was either controlled or not considered to be severe at the time of inclusion.

Treatment outcomes

At week 24, the proportion of patients with HIV RNA loads less than 50 and with less than 400 copies/ml was similar in the four treatment arms, both, according to the ITT and the per-protocol analysis (Table 2). The ITT analysis resulted in 73% virological responses less than 50 copies/ml in the combined FZD arms versus 77% in the ZDV arm. Response rates were balanced between FZD arms, with the lowest proportions of responders seen in arm B. Here, the difference in the response for a threshold less than 400 copies/ml was lower than in the ZDV arm (69 versus 90%). According to the per-protocol analysis, virological responses less than 50 copies/ml were 87% for the combined FZD arms versus 79% in the ZDV arm, with outcomes balanced between FZD arms.

The median HIV $\log_{10}$ decrease from baseline to week 24 was −3.7 (IQR −4.3 to −3.2) in the combined FZD arms versus −4.0 (IQR −4.3 to −3.4) in the ZDV arm (Fig. 2), the median absolute $CD4^+$ cell count increase was 99 cells/μl (IQR 52–181) versus 79 cells/μl (IQR 65–144), respectively, with similar values seen across all treatment arms (suppl. Figure 1, http://links.lww.com/QAD/B26). Confirmed virological treatment failure was observed in four (3.4%) patients until week 24, with one case each in arms B and C, and two cases in arm D. Genotypic resistance testing revealed the selection of the K103N non-nucleoside reverse transcriptase inhibitors mutations but no NRTI mutations in all of these patients at the time of virological failure (two patients with subtype CRF02_AG from Côte d'Ivoire and two with subtype C from Tanzania). In one patient receiving ZDV confirmed virological failure occurred already at week 12 and retrospective analysis from baseline samples revealed preexistence of the K103N mutation prior to treatment initiation.

Table 1. Baseline characteristics and HIV status of the safety population.

	Arm A FZD 600 mg b.i.d. ($n=30$)	Arm B FZD 800 mg o.d. ($n=29$)	Arm C FZD 1200 mg o.d. ($n=29$)	Arm D ZDV 300 mg b.i.d. ($n=31$)	All ($n=119$)
Study site					
Abidjan	15 (50%)	14 (48%)	14 (48%)	16 (52%)	59 (50%)
Mbeya	15 (50%)	15 (52%)	15 (52%)	15 (48%)	60 (50%)
Sex					
Women	19 (63%)	19 (66%)	19 (66%)	21 (68%)	78 (66%)
Men	11 (37%)	10 (34%)	10 (34%)	10 (32%)	41 (34%)
Age (years)	37 (32–41)	36 (34–45)	38 (34–46)	39 (32–46)	38 (32–45)
BMI (kg/m^2)	22.8 (20.9–25.4)	22.1 (21.0–24.5)	23·0 (20.1–26.0)	23.9 (21.5–27.2)	23.0 (20.7–25.8)
WHO Stage					
Stage 1 or 2	18 (60%)	22 (76%)	18 (62%)	23 (74%)	81 (68%)
Stage 3 or 4	12 (40%)	7 (24%)	11 (38%)	8 (26%)	38 (32%)
Started or on cotrimoxazole prophylaxis	28 (93%)	27 (93%)	26 (90%)	29 (97%)	110 (93%)
HIV RNA $\log_{10}$ (copies/ml)	5.2 (4.7–5.5)	5.2 (4.4–5.5)	4.9 (4.5–5.3)	5.1 (4.5–5.6)	5.1 (4.5–5.4)
HIV RNA[a]					
<100 copies/ml	13 (43%)	12 (41%)	17 (59%)	12 (39%)	54 (45%)
≥100 copies/ml	16 (53%)	17 (59%)	12 (41%)	19 (61%)	64 (54%)
CD4$^+$ cell count (cells/μl)	241 (172–295)	223 (161–291)	257 (220–303)	205 (142–264)	235 (167–297)
CD4$^+$ cell count categories					
≥200 cells/μl	19 (63%)	17 (59%)	22 (76%)	17 (55%)	75 (63%)
<200 cells/μl	11 (37%)	12 (41%)	7 (24%)	14 (45%)	44 (37%)
Haemoglobin (g/dl)	12.1 (11.2–13.3)	11.9 (10.7–13.0)	11.3 (10.9–13.1)	11.7 (11.0–14.0)	11.9 (11.0–13.3)
Neutrophils (cells/ml × 10^3)	1.50 (1.35–2.01)	1.49 (1.02–2.02)	1.73 (1.38–2.94)	1.79 (1.35–2.33)	1.66 (1.28–2.31)
Grade I	12 (40%)	8 (28%)	7 (24%)	11 (35%)	38 (32%)
Grade II	3 (10%)	6 (21%)	1 (3%)	2 (6%)	12 (10%)
Grade III	0	1 (3%)	2 (7%)	0	3 (3%)

Data are median (IQR) or *n* (%). b.i.d., twice daily; FZD, fozivudine; o.d., once daily; ZDV, zidovudine.
[a]$N=1$ HIV RNA at baseline missing for arm A.

Table 2. Proportion of patients with HIV RNA less than 50 and less than 400 copies/ml in intent-to-treat and per protocol analysis.

	Arm A FZD 600 mg b.i.d.	Arm B FZD 800 mg o.d.	Arm C FZD 1200 mg o.d.	FZD arms combined	Arm D ZDV 300 mg b.i.d.	All arms combined
Intent-to-treat analysis						
HIV RNA < 50 copies/ml[a]	24/30 (80%; 61–92%)	19/29 (66%; 46–82%)	21/29 (72%; 53–87%)	64/88 (73%; 62–82%)	24/31 (77%; 59–90%)	88/119 (74%; 65–82%)
Risk ratio (95% CI)[b]	1.03 (0.80–1.34)	0.85 (0.61–1.17)	0.94 (0.70–1.26)	0.94 (0.75–1.18)	NA	NA
Risk difference (%; 95% CI)[b]	2.6 (−18.0 to 23.1)	−11.9 (−34.6 to 10.8)	−5.0 (−26.9 to 16.9)	−4.7 (−22.1 to 12.7)	NA	NA
HIV RNA < 400 copies/ml[a]	25/30 (83%; 65–94%)	20/29 (69%; 49–85%)	23/29 (79%; 60–92%)	68/88 (77%; 67–86%)	28/31 (90%; 74–98%)	96/119 (81%; 72–87%)
Risk ratio (95% CI)[b]	0.92 (0.76–1.12)	0.76 (0.58–1.00)	0.88 (0.71–1.09)	0.86 (0.73–1.01)	NA	NA
Risk difference (%; 95% CI)[b]	−7.0 (−23.9 to 9.9)	−21.4 (−41.2 to −1.6)	−11.0 (−29.1 to 7.0)	−13.1 (−26.7 to 0.6)	NA	NA
Per protocol analysis						
HIV RNA < 50 copies/ml[a]	24/27 (89%; 71–98%)	19/22 (86%; 65–97%)	21/25 (84%; 64–96%)	64/74 (87%; 77–93%)	23/29 (79%; 60–92%)	87/103 (85%; 76–91%)
Risk ratio (95% CI)[b]	1.12 (0.89–1.41)	1.09 (0.85–1.40)	1.06 (0.82–1.36)	1.09 (0.89–1.34)	NA	NA
Risk difference (%; 95% CI)[b]	6.6 (−9.3 to 28.5)	7.1 (−13.5 to 27.6)	4.7 (−15.9 to 25.3)	7.2 (−9.5 to 23.9)	NA	NA
HIV RNA < 400 copies/ml[a]	25/27 (93%; 76–99%)	20/22 (91%; 71–99%)	23/25 (92%; 74–99%)	68/74 (92%; 83–97%)	27/29 (93%; 77–99%)	95/103 (92%; 85–97%)
Risk ratio (95% CI)[b]	0.99 (0.86–1.15)	0.98 (0.83–1.15)	0.99 (0.85–1.15)	0.99 (0.88–1.11)	NA	NA
Risk difference (%; 95% CI)[b]	−0.5 (−14.0 to 13.0)	−2.2 (−17.3 to 13.0)	−1.1 (−15.2 to 13.0)	−1.2 (−12.3 to 9.9)	NA	NA

b.i.d., twice daily; CI, confidence interval; FZD, fozivudine; NA, not applicable; o.d., once daily; ZDV, zidovudine.
[a]Data shown are *n*/*N* (%; 95% exact/Clopper–Pearson confidence interval).
[b]Compared with arm D.

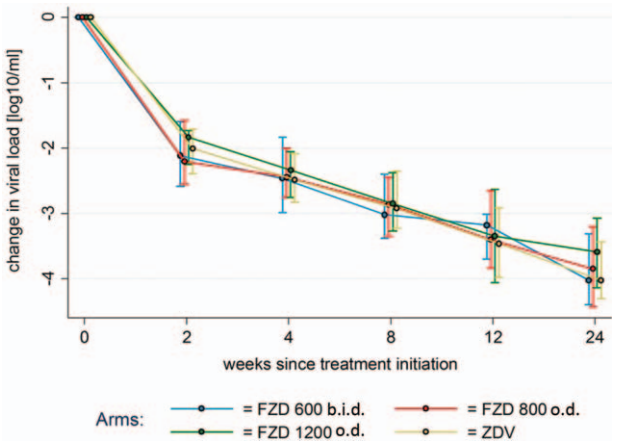

Fig. 2. Change in median HIV-1 RNA log_{10} viral load in the intent-to-treat population since baseline (only study treatment emergent values reported, error bars indicate interquartile range). b.i.d., twice daily; FZD, fozivudine; o.d., once daily; ZDV, zidovudine.

Safety assessments

Mean time of exposure to study regimen was similar in the combined FZD and the ZDV arms (Table 3). Overall, 299 treatment-emergent clinical adverse events were reported, which occurred in 86% of patient receiving FZD and in 84% receiving ZDV, with most events being mild or moderate. Type and frequencies of common clinical adverse events were balanced between all four arms. Six patients were taken off study treatments because of adverse events with reasons indicated in Table 3. Of note, two patients experienced possibly treatment-related grade IV anaemias, which required blood transfusion and were reported as serious adverse events. One of these anaemic patients received ZDV and the other FZD (arm B), the latter was diagnosed with a sickle cell trait, implying other possible triggers for anaemia. In total, 19 serious adverse events (SAEs) were reported in 17 patients and 12 SAEs were considered as treatment related. Of those, seven were based on clinically asymptomatic, transient grade IV neutropenia (five related to ZDV and two to FZD), which prompted temporary interruption of cotrimoxazole prophylaxis. The remaining five included the two cases of anaemia mentioned above, two cases were related to immune reconstitution syndromes associated with TB (arm A) and Kaposi's sarcoma (arm B), which required hospitalization, and one case (arm B) was based on transient grade IV γ-glutamyl transferase, and mild-to-moderate liver transaminases elevation mainly because of extensive alcohol abuse. This case was reported as treatment related based on possibly added antiretroviral drug toxicity.

Table 3. Treatment emergent adverse events.

	FZD arms ($N=88$)	ZDV arm ($N=31$)	P value[a]
Weeks of treatment exposure, mean (SD)	21.7 (5.99)	23.5 (2.48)	0.117
Any adverse event	76 (86%)	26 (84%)	0.768
Adverse events related to study treatment	45 (51%)	15 (48%)	0.837
Grade 3 or 4 adverse events	12 (14%)	6 (19%)	0.560
Serious adverse events	10 (11%)	7 (23%)[b]	0.142
Serious adverse events related to study treatment	6 (7%)[c]	6 (19%)[c]	0.077
Adverse events leading to study treatment discontinuation	5 (6%)[d]	1 (3%)[d]	0.606
Most common treatment emerging adverse events (>5% overall)			
Nausea, vomiting	16 (18%)	5 (16%)	1
Dizziness, vertigo	15 (17%)	3 (10%)	0.396
Nasopharyngitis, tonsillitis	11 (13%)	4 (13%)	1
Upper respiratory tract infection	7 (8%)	7 (23%)	0.048
Asthenia, fatigue, somnolence	12 (14%)	1 (3%)	0.180
Headache	10 (11%)	3 (10%)	1
Gastritis, dyspepsia, abdominal pain	8 (9%)	4 (13%)	0.508
Gastroenteritis, diarrhoea	8 (9%)	2 (7%)	1
Influenza-like illness	7 (8%)	3 (10%)	0.719
Cough	4 (5%)	3 (10%)	0.375
Rash	6 (7%)	1 (3%)	0.675
Genital infection, pelvic inflammatory disease	7 (8%)	2 (7%)	1
Grade 3–4 laboratory abnormalities (new or worsening)			
Anaemia, haemoglobin, <7 g/dl	1 (1%)	1 (3%)	0.455
Leukopenia, <2000 cells/μl	3 (3%)	4 (13%)	0.074
Neutropenia, <750 cells/μl	19 (22%)	13 (42%)	0.035
Thrombocytopenia, <50 000 cells/μl	1 (1%)	0	1
Aspartate aminotransferase, >5 × ULN	1 (1%)	1 (3%)	0.455
γ-Glutamyl transferase, >5 × ULN	6 (7%)	1 (3%)	0.606

FZD, fozivudine; SAE, serious adverse event; ULN, upper limit of normal; ZDV, zidovudine.
[a]Calculated using Fisher's exact formula.
[b]One patients reported with three SAEs (septicaemia secondary to urinary tract infections, cataract surgery, and IV neutropenia).
[c]FZD arms: IV anaemia (1), IV GGT elevation (1), TB-IRIS (1), KS-IRIS (1), IV neutropenia (2); ZDV arm: IV anaemia (1), IV neutropenia (5).
[d]FZD arms: IV anaemia (1), IV GGT elevation (1), death because of cholangiocellular carcinoma (1), severe rash probably related to efavirenz (1), Kaposi's sarcoma and chemotherapy (1); ZDV arm: IV anaemia (1).

Laboratory adverse events related to myelotoxicity were especially focused. Comparing the combined FZD arms with the ZDV arm, anaemia of any grade was observed in 11 versus 23% ($P = 0.142$), grade III/IV anaemia in 1 versus 3% ($P = 0.455$), and grade III/IV neutropenia in 22 versus 42% ($P = 0.035$), respectively. A decrease of Hb levels and absolute neutrophil counts was observed in all arms, with lowest values seen at 4 weeks after treatment initiation with a median Hb decrease from baseline of 0.4 versus 0.9 g/dl ($P = 0.033$) in patients receiving FZD and ZDV, and a median neutrophil decrease of 269 versus 864 cells/μl ($P = 0.004$), respectively (Supplemental Figures 2 and 3, http://links.lww.com/QAD/B26). Transient mild or moderate creatinine elevation was seen in 3 versus 10%, and liver transaminase elevation in 10 versus 13% of patients receiving FZD and ZDV, respectively. Severe or worse liver transaminases were additionally observed in two patients which was associated with diseases (alcohol induced hepatitis, septicaemia). Elevated γ-glutamyl transferases were frequent (35% of patients). Mild or moderate transient hyperglycaemia was overall observed in 13%, hypoglycaemia in 12%, hypercholesterinaemia in 11%, and hypertriglyceridemia in 1% of patients; events were balanced between arms (Supplemental Table 1, http://links.lww.com/QAD/B26). Clinically asymptomatic hyperlactaemia was observed in two patients receiving FZD.

Pharmacokinetic assessments

Pharmacokinetics results for FZD, ZDV, and ZDV–glucuronide were available for 24 patients (Supplemental Table 1, http://links.lww.com/QAD/B26). A delay of FZD absorption after the first dose was observed with a mean time to quantifiable plasma concentration of about 2 h in the 600 mg b.i.d. and 800 mg o.d. arms, and 1 h in the 1200 mg o.d. arm. The time to maximum FZD concentration (T_{max}) did not vary depending on the dose or the kinetic (first dose or steady state) with a mean of 6.8 (SD ± 0.6) versus 2.2 h for the ZDV. The FZD elimination half-life was around 5.4 h for arms A and B regardless of the kinetic and slightly higher for arm C (7.2 and 7.5 h for week 0 and 4, respectively). Although we found dose linearity from 600 to 1200 mg by maximum FZD concentration after initial drug administration, linearity was not demonstrated at steady state for both o.d. FZD regimens, which was likely because of interpatient variability. Two patients from the 800 mg arm presented with very high maximum concentrations and without these two high values the previous observed linearity would have been conserved. Comparison between average concentrations, calculated after the first dose and at steady state, showed a weak accumulation factor of about 1.35 for FZD in the two o.d. arms. As expected, a greater accumulation factor around 2.1 was found for the b.i.d. regimen because of the higher dosing frequency. Similarly, trough concentrations at steady state were lower in the o.d. (mean 1.31 and 1.69 μg/ml for arms B and C, respectively) than those in the b.i.d. arm (mean 3.42 μg/ml), but still much higher than the 50% inhibitory concentration of FZD for HIV-1 (15–150 ng/ml).

A delayed T_{max} and prolonged elimination half-life was also observed for ZDV and for the ZDV–glucuronide metabolite within the FZD arms, with values very close to those seen in the FZD pharmacokinetic, indicating a slow FZD absorption and constant transformation of FZD into ZDV. The metabolization of ZDV to ZDV–glucuronide did not present a limiting step at any dose since the observed T_{max} and elimination half-lives of ZDV–glucuronide were very close to those in the ZDV arm. FZD was not detectable in urine. At steady state, around 4.5% of the parent compound and 64% of the glucuronide form was recovered in the urine in the ZDV arm. On a molar basis, mean urinary ZDV and ZDV–glucuronide excretion was found around 2.2 and 21%, respectively, of the dose in the FZD arms.

Discussion

Our study demonstrates treatment efficacy and overall good tolerability of FZD-based antiretroviral treatment regimens over a period of 24 weeks in an HIV-infected, ART-naive, African population. Response rates in FZD and ZDV arms were similar, especially when analysed per-protocol, which best reflects treatment potency. For our primary prespecified ITT endpoint, the response rate was lowest in the FZD 800 mg o.d. arm because of higher numbers of noncompletion compared with the other study arms. Because sample size was low, we think that this observation should be interpreted with caution. We also want to point out that one patient receiving ZDV with early virological treatment failure had a preexisting non-nucleoside reverse transcriptase inhibitors mutation, which was not an exclusion criterion in our ITT analysis. If this patient would have been excluded from analysis, the response rate for the ZDV arm would have been slightly better (80% with a virological response below 50 copies/ml); however, this would not significantly impact the primary endpoint. Our response rates are comparable with those reported from previously published studies reporting 24-week data in treatment-naive patients receiving ZDV, 3TC, and EFV, such as the Prospective Evaluation of Antiretrovirals in Resource Limited Setting study (PEARLS) [12] and outcomes from the 934 Study Group [13,14].

Our data furthermore demonstrate the potential of o.d. FZD administration as virological response rates of the o.d. and the b.i.d. arms were similar, which was further supported by pharmacokinetic outcomes. As shown in previous studies [8–10], a long FZD elimination half-life and a delayed FZD absorption was observed. This is consistent with a delayed release of the drug from the formulation or slow absorption in the upper part of the gut possibly because of weak permeability or membrane transporters efflux activity. These characteristics point out an umbrella-like pharmacokinetic profile which provides constant drug levels allowing for once daily dosing.

In line with previous reports, concentrations of ZDV and ZDV–glucuronide in the FZD arms were much lower

than those measured in the ZDV arms. This, apart from the FZD selectivity for mononuclear cells and very low activity in red blood and stem cells, probably explain a reduced risk for haematologic toxicity in patients receiving FZD. Our study found a lower prevalence of severe or worse neutropenia and a lower decrease of Hb levels and absolute neutrophil counts within the first weeks after FZD exposure compared with patients receiving ZDV. Although these observations might be of marginal clinical relevance and need to be cautiously interpreted because of low sample size and inter-patient variability, they might indicate lower haemotoxicity for FZD-based regimens.

Intracellularly, NRTIs undergo three sequential phosphorylations leading to the therapeutically active triphosphate metabolites [15]. Preclinical data showed that FZD is directly cleaved intracellularly to ZDV-monophosphate, indicating that the first phosphorylation step is not necessary for FZD. Given a slow transformation rate from FZD to ZDV and assuming that, after FZD exposure, conversion to ZDV-monophosphate is not the limiting step, intracellular accumulation of ZDV-monophosphate seems likely. ZDV-monophosphate has been associated with increased cell or mitochondrial toxicity [16–18] linked to metabolic toxicity and lipodystrophy syndrome. In our study, metabolic abnormalities were transient and infrequent, with similar proportions seen in the study arms. Given the relatively short drug exposure time, we did not investigate for clinical features of lipoatrophy. Samples for the analysis of intracellular phosphorylated ZDV metabolite levels after FZD exposure were collected in our study, and will be published later.

Generalizability of our results is limited by small sample size, and favouring a o.d. regimen we cannot clearly conclude if 800 or 1200 mg o.d. FZD would be better. However, our study provides promising evidence to further advance into larger efficacy trials investigating FZD as a o.d. thymidine analogue NRTI. FZD has the potential to replace ZDV as a potentially toxicity improved part of NRTI backbones in antiretroviral therapy, maintaining the distinct characteristics of thymidine analogue NRTI drug resistance patterns relevant in antiretroviral treatment strategies, thus providing an alternative to tenofovir or abacavir-based regimens in first and second-line antiretroviral treatments.

Acknowledgements

The Ludwig-Maximilians-Universität (LMU) in Munich, Germany in affiliation with the French Agence Nationale de Recherches sur le Sida (ANRS) sponsored the trial. We thank for the contributions made to this study by all patients, their partners, and families. We also thank Dr Roki Mugeniwalwo, Dr Joseph Mabusila, Dr Omar Salehe, Dr Zelica Diallo, Jerry Kupungu, and Nice Mwinuka for their devoted clinical work; Emième Arlette, Hervé Ménan, Cornelia Luer, and Triphonia Mbena for excellent laboratory management and technical assistance; Revocatus Kunambi and Romuald Konan for pharmacy work; Dickens Kowuor, Peter Edwin, and Larissa N'guessan for data management. We especially thank Ulrich Braun for his dedicated study training and monitoring at the sites, followed by Otto Geisenberger and Eric Ouattara, and Tina Purnat for setting up the study database and monitoring system. We thank Dr Jan Schmidt-Brand from Heidelberg Pharma AG, Germany and Mr. Yan-Ho Choo from STADA Pharmaceuticals, Hong Kong, for supporting the trial with the fozivudine.

F.E., J.M., T.L., R.M., L.M., and S.E. enrolled patients, reviewed and interpreted data, and edited drafts of the report. A.K., X.A., M.H., and C.D. designed the study. A.K. and C.D. oversaw data collection. R.Z. and F.VM. produced and provided the fozivudine IMP. A.P. performed the pharmacokinetic analyses. E.S. and A.K. analysed the data. A.K. wrote the first draft of the manuscript. All authors contributed to edits of the final manuscript and A.K. served as the corresponding author.

The trial was funded by the European and Developing Countries Clinical Trials Partnership (EDCTP, Grant ID MS.2010.10800.001), the German Ministry for Science and Education (BMBF, Grant ID 01KA1201) and the German Center for Infection Research (DZIF, Grant ID TTU 04.703), and the French Agence Nationale de Recherches sur le Sida (ANRS). The fozivudine IMP substance was provided by Heidelberg Pharma AG, Germany, produced by Chiracon, Luckenwalde, Germany and manufactured, packed, labelled and provided by Stada, Vietnam. Zidovudine, lamivudine, and efavirenz were provided by each country's National AIDS Control Programme.

Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization. *Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection.* Geneva, Switzerland: World Health Organization; 2013.
2. Richman DD, Fischl MA, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, *et al*. **The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial.** *N Engl J Med* 1987; **317**:192–197.
3. Cretton EM, Xie MY, Bevan RJ, Goudgaon NM, Schinazi RF, Sommadossi JP. **Catabolism of 3′-azido-3′-deoxythymidine in hepatocytes and liver microsomes, with evidence of formation of 3′-amino-3′-deoxythymidine, a highly toxic catabolite for human bone marrow cells.** *Mol Pharmacol* 1991; **39**:258–266.
4. Martinez-Cajas JL, Pai NP, Klein MB, Wainberg MA. **Differences in resistance mutations among HIV-1 nonsubtype B infections: a systematic review of evidence (1996-2008).** *J Int AIDS Soc* 2009; **12**:11.

5. Montes B, Vergne L, Peeters M, Reynes J, Delaporte E, Segondy M. **Comparison of drug resistance mutations and their interpretation in patients infected with non-B HIV-1 variants and matched patients infected with HIV-1 subtype B.** *J Acquir Immune Defic Syndr* 2004; **35**:329–336.
6. Moh R, Danel C, Sorho S, Sauvageot D, Anzian A, Minga A, *et al*. **Haematological changes in adults receiving a zidovudine-containing HAART regimen in combination with cotrimoxazole in Côte d'Ivoire.** *Antivir Ther* 2005; **10**:615–624.
7. Bussmann H, Wester CW, Ndwapi N, Grundmann N, Gaolathe T, Puvimanasinghe J, *et al*. **Five-year outcomes of initial patients treated in Botswana's National Antiretroviral Treatment Program.** *AIDS* 2008; **22**:2303–2311.
8. Bogner JR, Boerner D, Mühlhöfer A, Thoma-Greber E, Herrmann DB, Hoegl L, *et al*. **Single dose, dose escalating trial with fozivudine tidoxil (BM 21.1290).** *Antiviral Ther* 1997; **2**:249–256.
9. Bogner JR, Roecken M, Herrmann DB, Boerner D, Kaufmann B, Gürtler L, *et al*. **Phase I/II trial with fozivudine tidoxil (BM 21.1290): a 7 day randomized placebo controlled dose-escalating trial.** *Antiviral Ther* 1997; **2**:257–264.
10. Girard PM, Pegram PS, Diquet B, Anderson R, Raffi F, Tubiana R, *et al*. **Phase II placebo-controlled trial of fozivudine tidoxil for HIV infection: pharmacokinetics, tolerability and efficacy.** *J Acquir Immune Defic Syndr* 2000; **23**:227–235.
11. ANRS scale to grade the severity of adverse events in adults, Version 1.0, 4 November 2008 (Translation of the French version 6.0, 9 September 2003). http://www.anrs.fr/content/download/2242/12805/file/ANRS-GradeEI-V1-En-2008.pdf [Accessed 26 August 2016].
12. Campbell TB, Smeaton LM, Kumarasamy N, Flanigan T, Klingman KL, Firnhaberet C, *et al*. **Efficacy and safety of three antiretroviral regimens for initial treatment of HIV-1: a randomized clinical trial in diverse multinational settings.** *PLoS Med* 2012; **9**:e1001290.
13. Gallant JE, DeJesus E, Arribas JR, Pozniak AL, Gazzard B, Campo RE, Study 934 Group. **Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV.** *N Engl J Med* 2006; **354**:251–260.
14. Arribas JR, Pozniak AL, Gallant JE, Dejesus E, Gazzard B, Campo RE, *et al*. **Tenofovir disoproxil fumarate, emtricitabine, and efavirenz compared with zidovudine/lamivudine and efavirenz in treatment-naive patients: 144-week analysis.** *J Acquir Immune Defic Syndr* 2008; **47**:74–78.
15. Bazzoli C, Jullien V, Le Tiec C, Rey E, Mentré F, Taburet AM. **Intracellular pharmacokinetics of antiretroviral drugs in HIV-infected patients, and their correlation with drug action.** *Clin Pharmacokinet* 2010; **49**:17–45.
16. Tan CK, Civil R, Mian AM, So AG, Downey KM. **Inhibition of RNase H activity of HIV reverse transcriptase by azidothymidilate.** *Biochemistry* 1991; **30**:4831–4835.
17. Bridges EG, Faraj A, Sommadossi JP. **Inhibition of mammalian DNA polymerases-associated 38 to 58 exonuclease activity by 58-monophosphates of 38-azido-38-deoxythymidine and 38-amino-38-deoxythymidine.** *Biochem Pharmacol* 1993; **45**:1571–1576.
18. Harrington JA, Reardon JE, Spector T. **38-Azido-38-deoxythymidine (AZT) monophosphate: an inhibitor of exonucleotide repair of AZT-terminated DNA.** *Antimicrob Agents Chemother* 1993; **37**:918–920.