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Efficacy and safety of three second-line antiretroviral regimens in HIV-infected patients in Africa

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Objective: WHO recommends ritonavir-boosted protease inhibitor with two nucleoside reverse transcriptase inhibitors in HIV-infected patients failing non-nucleoside reverse transcriptase inhibitor-based first-line treatment. Here, we aimed to provide more evidence for the choice of nucleoside reverse transcriptase inhibitor and boosted protease inhibitor.

Design: ANRS 12169 is a 48-week, randomized, open-label, non-inferiority trial in three African cities, comparing efficacy and safety of three second-line regimens.

Methods: Patients failing non-nucleoside reverse transcriptase inhibitor-based antiretroviral therapy with confirmed plasma HIV-1 viral load above 1000 copies/ml were randomly assigned to tenofovir/emtricitabine + lopinavir/ritonavir (control group as per WHO recommendations), abacavir + didanosine + lopinavir/ritonavir (ABC/ddI group) or tenofovir/emtricitabine + darunavir/ritonavir (DRV group) regimens. The primary endpoint was the proportion of patients with plasma vral load below 50 copies/ml at week 48 in the modified intention-to-treat population. Non-inferiority was pre-specified with a 15% margin.

Results: Of the 454 randomized patients, 451 were included in the analysis. Globally, 294 (65.2%) and 375 (83.2%) patients had viral load below 50 and 200 copies/ml, respectively, at week 48. The primary endpoint was achieved in 105 (69.1%) control group patients versus 92 (63.4%) in the ABC/ddI (difference 5.6%, 95% confidence interval −5.1 to 16.4) and 97 (63.0%) in the DRV (difference 6.1%, 95% confidence interval −4.5 to 16.7) groups (non-inferiority not shown). Overall, less number of patients with baseline viral load at least 100 000 copies/ml ($n = 122$) had a viral load below 50 copies/ml at week 48 (37.7 versus 75.4%; $P < 0.001$).

Conclusions: The three second-line regimens obtained similar and satisfactory virologic control and confirmed the WHO recommendation (TDF/FTC/LPVr) as a valid option. However, the suboptimal response for patients with high viral load warrants research for improved strategies.

AIDS 2015, **29**:1473–1481

Keywords: Africa, HIV, randomized clinical trial, second-line antiretroviral therapy

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Received: 16 February 2015; revised: 2 April 2015; accepted: 2 April 2015.

DOI:10.1097/QAD.0000000000000709

ISSN 0269-9370

Introduction

Access to antiretroviral treatment (ART) for HIV infection in resource-limited settings (RLS) has increased remarkably in the past decade, with HIV morbidity and mortality consequently decreasing [1]. Globally, only an estimated 3% of patients in RLS receive second-line ART [2]. The reasons include limited access to viral load monitoring, which impedes timely diagnosis of treatment failure and lack of convenient, co-formulated and safe second-line treatments. Guidelines issued by the WHO in 2013 [3] recommend a second-line regimen based on boosted protease inhibitors (bPIs), with two nucleoside reverse transcriptase inhibitors (NRTIs), after failure of a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based first-line treatment. This strategy was recently validated in three randomized clinical trials [4–6], but data on the NRTI and bPI options are lacking. In the WHO recommendations, the possible choice of bPI is between ritonavir-boosted atazanavir (ATZ/r) and ritonavir-boosted lopinavir (LPV/r), whereas ritonavir-boosted darunavir (DRV/r), considered as an option by the WHO expert panel, is finally not recommended due to non-availability as a generic co-formulation, its high price and limited registration [3]. The choice of the NRTI backbone is based on previous NRTI exposures: patients having received thymidine analogues should receive a combination with tenofovir disoproxil fumarate (TDF), whereas those who failed on TDF should receive zidovudine (ZDV). Lamivudine (3TC) or emtricitabine (FTC) is always continued. The NRTI combination with didanosine (ddI) and abacavir (ABC) recommended in WHO 2006 guidelines [7] as an alternative for the possible residual activity in patients with long-term virological failure was never evaluated, and was discarded in 2010 because of complexity and cost criteria [8]. No direct comparison of these possible combinations is available, and evidence for the choice is deemed of low quality [9].

In this study, we compared the efficacy and safety of one of the current WHO standard regimens (TDF/FTC and LPV/r), chosen for its availability in national programmes with two alternatives: TDF/FTC and DRV/r – a new-generation protease inhibitor with a once-daily dose, providing improved tolerability [10]; and ABC, ddI and LPV/r – a combination still in use in RLS due to availability of the drugs and lack of exposure to these two NRTIs in first line.

Methods

Study design and sites

We conducted a 48-week randomized, parallel, open-label, multicentre, non-inferiority trial to compare efficacy and safety of three bPI-based second-line antiretroviral combinations in three African countries: the HIV services of the Central and Military Hospitals of Yaoundé in Cameroon; the Clinical Research Centre and the Day Care Centre of the Fann Hospital of Dakar in Senegal; and the Day Care Hospital of the University Hospital of Bobo Dioulasso in Burkina Faso. The institutional Ethics Committee of the Institut de Recherche pour le Développement (IRD) in France and the participating countries' national Ethic Committees approved the protocol. All participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki [11] and the Good Clinical Practice Guidelines [12]. This study is registered with ClinicalTrial.gov with the number NCT00928187.

Participants

Patients over 18 years old, HIV-1-positive, failing an NNRTI-based first-line ART after minimum 6 months and without recent (3 months) ART switch were eligible. Treatment failure was defined as confirmed HIV-1 RNA plasma viral load above 1000 copies/ml, after 1 month of adherence support. The following were excluded: pregnant and nursing women, HIV-1 group O, N or P-infected patients, those previously exposed to study drugs (except 3TC/FTC), those with renal [creatinine clearance (Cockcroft–Gault) $<$ 50 ml/min] or hepatic failure (prothrombine time $<$ 50%), or with severe ongoing AIDS-related illness, including tuberculosis.

Randomization and masking

Randomization was stratified by study site. The computer-generated randomization sequence was concealed from the study personnel all along the inclusion period. After randomization, study allocation was not masked from clinicians or patients.

Interventions

Eligible patients were randomized 1 : 1 : 1 to LPV/r (co-formulated LPV 200 mg/ritonavir 50 mg two tablets twice daily) with TDF 300 mg/FTC 200 mg (Truvada, Gilead Sciences; Nycomed, Germany; Patheon, Canada; Cork, Ireland; one tablet daily with food) (control group); ABC (600 mg tablet) with ddI (enteric-coated capsule of 250 or 400 mg based on body weight) once daily fasting, and LPV/r twice daily (ABC/ddI group) or DRV 800 mg (Prezista, Janssen - Borgo S. Michele, Italy, two 400 mg tablets) boosted with ritonavir (100 mg tablet) and TDF/FTC (Truvada), all taken with food once daily (DRV group). Participants in the ABC/ddI group with chronic hepatitis B continued 3TC 150 mg after enrolment. All drugs were originators (donated by Gilead Sciences and Janssen) or WHO-prequalified generics provided by the National Programmes. Human leucocyte antigen B*5701 routine screening was not performed because it was not recommended by WHO, was unavailable in the national laboratories and the allele have a low prevalence in the African population [13].

Outcomes

Primary endpoint was the proportion of patients with viral load below 50 copies/ml at week 48 in the modified intention-to-treat (mITT) population. Secondary endpoints were the proportions of patients with viral load below 200 and 1000 copies/ml at week 48. Immunological ($CD4^+$ cell count changes) and clinical (occurrence of HIV and non-HIV-related events) endpoints were also evaluated. Safety and tolerance were measured using clinical and laboratory assessments. The number of adverse and serious adverse events, together with study drug interruptions, was also reported.

Procedures

Patients on first-line ART having viral load above 1000 copies/ml underwent structured interviews to support adherence (details about intervention in supplemental material page 4, http://links.lww.com/QAD/A701). One month later, after providing written, informed consent, viral load control and screening visit were proposed. Randomization occurred if the control viral load was still above 1000 copies/ml. Follow-up visits were scheduled at weeks 4, 12, 24, 36 and 48, and every 6 months thereafter, until the end of the study (week 48 visit of last included patient). Visits included clinical evaluation, renal and liver function tests, total blood count and plasma storage. Plasma HIV-1 viral load using m2000rt RealTime HIV-1 assay (Abbott, Abbott Park, Illinois, USA) and $CD4^+$ cell count (Becton Dickinson, Franklin Lakes, New Jersey, USA) measurements were performed at all time points (except $CD4^+$ at week 4) in national reference laboratories. Baseline genotypic drug resistance was performed retrospectively for all patients, at the end of the study, on stored plasma. Samples from Senegal and Burkina Faso were tested in the IRD WHO-accredited laboratory in Montpellier, France. Samples from Cameroon were analysed locally at Centre de Recherche sur les Maladies Emergentes et Réémergentes/ Institut de Recherches Médicales et d'Etudes des Plantes Médicinales (CREMER/IMPM) – a WHO-accredited laboratory for antiretroviral resistance surveillance in Yaoundé.

Participants attended the pharmacy quarterly for prescribed drugs. Pharmacists administered a standard questionnaire [14] and performed pill count to measure adherence. Intensive adherence support was proposed to patients with increased viral load.

Participants were followed using standardized medical protocols: for tuberculosis, DRV/r was replaced with LPV/r with increased ritonavir doses (+300 mg twice as from WHO recommendations [3]). For virological failure, that is, confirmed viral load above 1000 copies/ml after adherence support, resistance testing was performed on baseline and last visit samples, and patients were managed accordingly. A third-line regimen including DRV, etravirine and raltegravir was available.

An independent Data and Safety Monitoring Board supervised the study. Two non-blinded interim analyses were performed, the Board approving study continuation on each occasion.

Statistical analysis

Cross-arm comparison of patient proportions with viral load below 50 copies/ml at week 48 was performed using a mITT analysis, which included patients who received at least one dose of the assigned treatment and excluded patients with major protocol violations. The following were considered failures: patients with viral load at least 50 copies/ml at week 48, non-completers at week 48 for whatever reason and those who switched ART before week 48 for whatever reason, except pregnancy and tuberculosis. We also performed per protocol comparison of the three groups which included patients still receiving the assigned treatment at week 48 and those who changed ART because of virological failure before week 48. Patients who interrupted the assigned treatment for at least 15 days were excluded from the per protocol analysis. Those with viral load at least 50 copies/ml at week 48 or who switched ART because of virological failure before week 48 were deemed failures in the per protocol analysis.

The differences between proportions of patients with viral load below 50 copies/ml at week 48 in the control group and in the two other groups, respectively, were calculated, and the non-inferiority was thus tested by comparing the upper limits of the 95% confidence intervals (CIs) of these differences with the pre-defined non-inferiority margin of 15%. Similar analyses were performed for the proportion of patients with viral load below 200 and 1000 copies/ml at week 48. In a subgroup exploratory analysis, we compared the proportion of patients with viral load below 50 copies/ml at week 48 across all arms stratifying by baseline viral load ($<100\,000$ copies/ml versus $\geq 100\,000$ copies/ml). All other endpoints were tested for superiority. Analysis of variance (ANOVA) or Kruskal–Wallis test was used for continuous variables, and chi-square or Fisher's exact test was used for categorical ones. Factors associated with baseline viral load at least 100 000 and 50 at week 48 were identified using logistic regression models. STATA version 12 (STATA Corp., College Station, Texas, USA) was used for statistical analyses.

Sample size

Hypothesizing 80% efficacy at the 50 copies/ml viral load threshold in the control group at week 48, we calculated a required sample size of 150 participants per group to show non-inferiority of ABC/ddI and DRV groups compared with the control group in ITT analysis, with a non-inferiority margin of 15%, a power of 90% and a two-sided α of 5%.

Results

Baseline characteristics

Between January 2010 and September 2012, of the 584 patients assessed for eligibility, 130 were excluded, primarily [81 (13.9%)] because control viral load decreased below 1000 copies/ml after adherence support. Three of the 454 randomized patients were excluded from the analysis (Fig. 1): two withdrew before study drug administration and one was excluded for protocol violation (HIV-1 group O identified at genotyping).

Baseline characteristics were balanced among the three groups (Table 1) except for fewer participants with viral load at least 100 000 copies/ml in the control group and a lower median $CD4^+$ cell count in the DRV group: these differences were not significant. Globally, the median age was 38 years [inter-quartile range (IQR) 32–46] and 72% of the participants were women. At ART initiation, 282 (62%) were at clinical WHO stage 3 or 4, with a median $CD4^+$ cell count of 118 (IQR 57–184) cells/μl. Median ART duration was 49 months (IQR 33–69). Thirty-eight (8%) participants were positive for the surface antigen of hepatitis B virus (HBsAg).

At inclusion, participants were mainly asymptomatic [411 (91%)], despite a low $CD4^+$ cell count [median 183 (IQR 87–290) cells/μl] and a median viral load of 4.5 $\log_{10}$ (IQR 4.0–5.1); 122 (27%) had a viral load at least 100 000 copies/ml. At failure, 85, 15, 29 and 71% of the participants were taking ZDV, stavudine, efavirenz and nevirapine, respectively, as first-line drugs. All combinations included 3TC. At baseline, 429 of 446 (96%) participants had resistance mutations to both NNRTI and NRTI drugs (Table 1). Interestingly, 249 (56%) enrolled patients harboured a virus with major mutations conferring high-level resistance to all their first-line drugs [Agence Nationale de Recherche sur le SIDA et les hépatites virales (ANRS) algorithm, version 2014].

Virological and immunological outcomes

At week 48, 451 participants were included in the mITT analyses and 441 (97.8%) were still followed up (Fig. 1). For the primary endpoint (Fig. 2), 294 (65.2%)

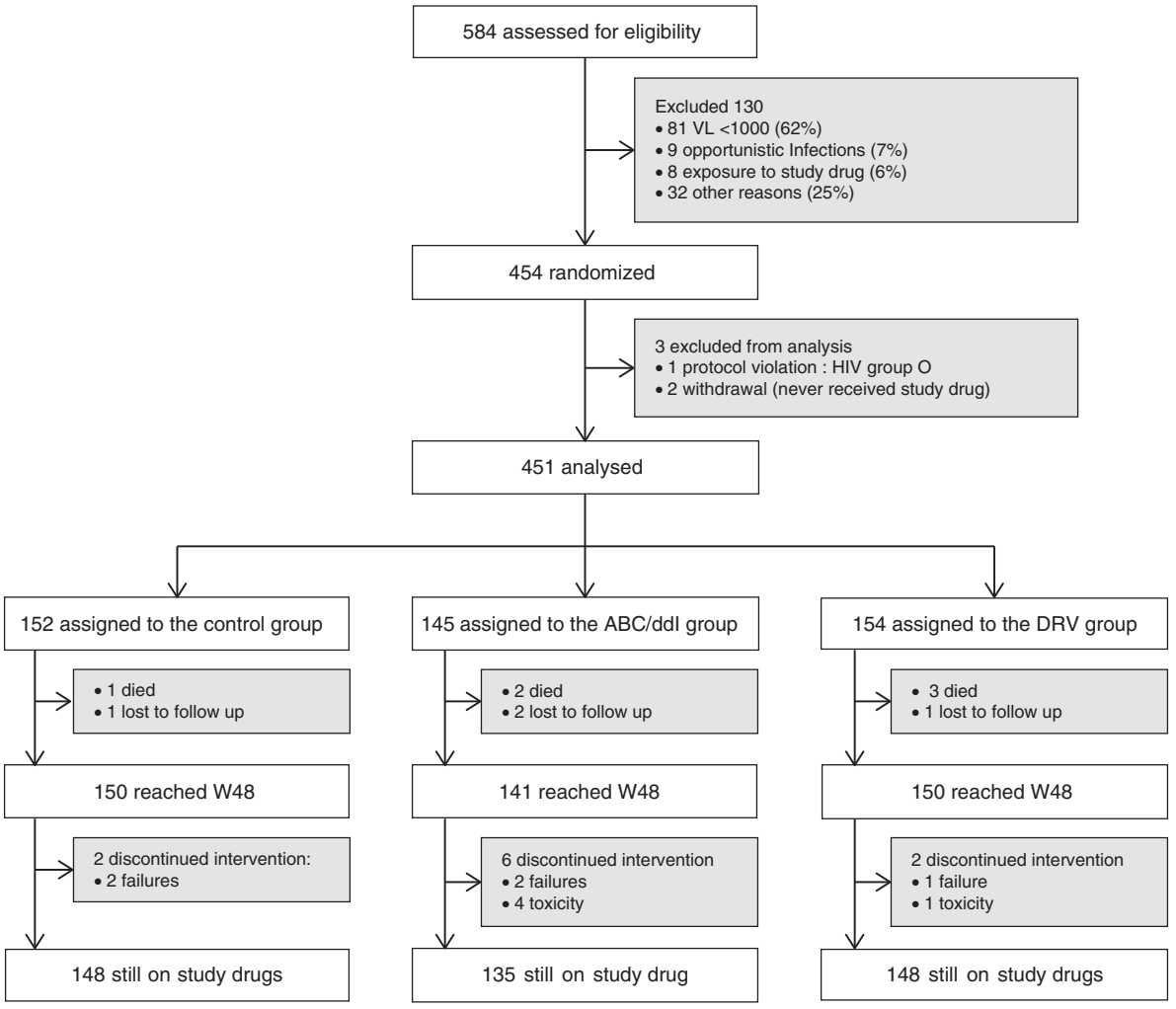

Fig. 1. Trial profile. ABC, abacavir; ddI, didanosine; DRV, darunavir; VL, viral load.

Table 1. Baseline characteristics.

	TDF/FTC LPV/r ($n=152$)	ABC ddI LPV/r ($n=145$)	TDF/FTC DRV/r ($n=154$)	Total ($n=451$)
Site				
Bobo Dioulasso	30	28	32	90 (20%)
Dakar	21	18	20	59 (13%)
Yaoundé	101	99	102	302 (67%)
Women	113 (74%)	105 (72%)	106 (69%)	324 (72%)
Age (years)	38 (34–45)	38 (33-47)	36 (32–45)	38 (32–46)
Weight (kg)	64 (55–71)	65 (55–75)	65 (58–71)	65 (56–72)
BMI (kg/m^2)	22.9 (20.4–25.4)	22.9 (21.0–27.3)	23.6 (21.5–25.9)	23.1 (21.0–26.0)
WHO classification at ART initiation				
1	16	33	26	75 (17%)
2	41	26	27	94 (21%)
3	74	67	76	217 (48%)
4	21	19	25	65 (14%)
Asymptomatic at treatment switch	136 (89%)	133 (92%)	142 (92%)	411 (91%)
Duration (months) first ART	50 (31–68)	52 (37–68)	45 (32–69)	49 (33–69)
HBsAg positive	13 (9%)	9 (6%)	16 (10%)	38 (8%)
VL $\log_{10}$	4.4 (4.0–5.0)	4.6 (4.1–5.1)	4.5 (4.0–5.1)	4.5 (4.0–5.1)
VL $>$ 5000 copies/ml	130 (86%)	126 (87%)	133 (86%)	389 (86%)
VL $\geq$ 10 000 copies/ml	114 (75%)	112 (77%)	116 (75%)	342 (76%)
VL $\geq$ 100 000 copies/ml	36 (24%)	42 (29%)	44 (29%)	122 (27%)
CD4$^+$ (cell/μl), median (IQR)	199 (92–318)	195 (100–288)	153 (81–261)	183 (87–290)
eGFR (ml/min), median (IQR)	94 (79–114)	96 (76–118)	98 (82–119)	96 (82–119)
Resistances (ANRS algorithm)[a]	$n=150$	$n=143$	$n=153$	$n=446$
No mutation	1 (1%)	1 (1%)	4 (3%)	6 (1%)
Resistance to at least one first-line drug	149 (99%)	142 (99%)	149 (99%)	440 (99%)
NRTI only	1 (1%)	4 (3%)	2 (1%)	7 (2%)
NNRTI only	2 (1%)	3 (2%)	1 (1%)	6 (1%)
NRTI + NNRTI	146 (97%)	135 (94%)	146 (95%)	427 (96%).
Three first-line drugs	84 (56%)	77 (54%)	88 (58%)	249 (56%).
Resistance to at least one second-line drug	147 (98%)	58 (41%)	147 (96%)	352 (79%)
ABC only	37 (25%)	41 (29%)	50 (33%)	128 (29%)
ddI only	0	0	0	0
ABC + ddI	10 (7%)	10 (7%)	13 (8%)	33 (7%)
FTC only	118 (79%)	114 (80%)	107 (70%)	339 (76%)
TDF only	0	0	0	0
FTC + TDF	29 (19%)	25 (17%)	41 (27%)	95 (21%)

Data are presented as *n* (%) or median (IQR). ABC, abacavir; ART, antiretroviral therapy; ddI, didanosine; DRV, darunavir; eGFR, estimated glomerular filtration rate (Cockcroft–Gault); FTC, emtricitabine; HBsAg, hepatitis B surface antigen; IQR, inter-quartile range; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside or nucleotide reverse transcriptase inhibitor; TDF, tenofovir disoproxil fumarate; VL, viral load.

[a]Intermediate and high-level resistance.

participants had a viral load below 50 copies/ml. Primary mITT analyses (Fig. 3) results showed a difference of 5.6% (95% CI −5.1, 16.4) and 6.1% (95% CI −4.5, 16.7) between the control group, and the ABC/ddI and DRV groups, respectively, with no evidence for non-inferiority. In the per protocol analysis, 294 (68.1%) of the 432 participants had viral load below 50 copies/ml at week 48. The differences between the control group, and the ABC/ddI and DRV groups were 2.3% (95% CI −8.4, 13.1) and 4.9% (95% CI −5.7, 15.5), respectively (Supplementary Table S1 for detailed results, http://links.lww.com/QAD/A701).

A mITT analysis of secondary virological endpoints at week 48 was also performed (Figs. 2 and 3), and showed that 375 (83.2%) and 410 (90.9%) participants had a viral load below 200 and 1000 copies/ml, respectively. In the subgroup of patients with baseline viral load at least 100 000 copies/ml, the proportion of participants with viral load below 50 copies/ml at week 48 was only 46 of 122 (37.7%) compared to 248 of 329 (75.4%) for those with lower viral load ($P<0.001$), making high viral load the most important prognostic factor for successful second-line treatment (Supplementary Table S2, http://links.lww.com/QAD/A701). In this population, patients in the DRV group had the worst results [10/44 (22.7%) with viral load <50 copies/ml]. Compared with patients with baseline viral load below 100 000 copies/ml (Supplementary Table S3, http://links.lww.com/QAD/A701), the 122 with baseline viral load at least 100 000 copies/ml were significantly more often men [adjusted odds ratio (aOR) 1.9, 95% CI 1.1–3.2], had lower CD4$^+$ cell counts (aOR 1.9, 95% CI 1.1–3.2) and had more often virus with intermediate-high resistance to two drugs of their second-line combination (aOR 4.7, 95% CI 2.6–8.5), but were not less adherent during the study (adherence was 94 versus 92% in the group with viral load above or below 100 000 copies/ml at baseline, respectively; $P=0.34$). During the 48 weeks, 5 of the 451 patients had virological failure (confirmed viral load

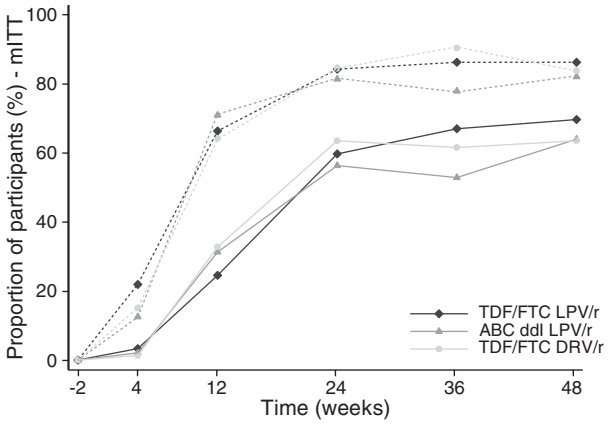

Fig. 2. Proportion of patients in each group with VL <50 (solid line) and <200 copies/ml (dashed line) in the mITT population. mITT, modified intention-to-treat; VL, viral load.

>1000 copies/ml). Resistance testing was performed and compared with genotype at baseline: no protease inhibitor resistance or change in NRTI mutations were detected (data not shown). Median gain in $CD4^+$ at week 48 was 127 cells/μl (IQR 72–203) with no significant differences across groups.

Clinical outcomes and safety

Eighty-seven HIV-related events occurred in 70 (16%) participants (Table 2), mainly in the first 3 months after second-line initiation: only two were stage 4 (extra-pulmonary tuberculosis). Six (1%) patients died during the 48 weeks. One died from liver cancer and another from severe tuberculosis (TB). The other four patients died at home of unknown causes (two were on the ABC/ddI arm, but the short treatment duration before death – 3 to 4 days – excluded an ABC hyper-sensibility reaction). Grade 3 or 4 adverse events occurred in 58 (13%) patients, with no difference between groups (Table 2). Only five patients stopped assigned treatment because of adverse events: one for suspected ABC reaction (not confirmed by the expert review committee), one in DRV group for severe kidney failure not related to study drug, two for progressive neuropathy on ddI in patients with pre-existent stavudine-related neuropathic pain and one patient requesting to reduce pill burden (ABC/ddI group on TB treatment).

Gastrointestinal complaints were common and significantly different between the DRV/r and the LPV/r groups [26 (17%) versus 48 and 50 (33%); $P = 0.001$]. Twenty-four (5%) participants reported symptoms of neuropathy, mainly from baseline: 11 were in ABC/ddI group. No statistically significant differences were recorded across groups for liver and kidney toxicity: only one patient had increased alanine aminotransferase (>5 × upper limit of normal). Sixty-one (14%) participants experienced a 25% decrease in estimated glomerular filtration rate (eGFR – Cockcroft–Gault formula) between baseline and week 48, with a higher frequency in those taking TDF. Globally, adherence was good throughout the study, with only 29 of 439 (7%) having a score at questionnaire of less than 80% at least once between inclusion and week 48 (Supplementary Table S4, http://links.lww.com/QAD/A701).

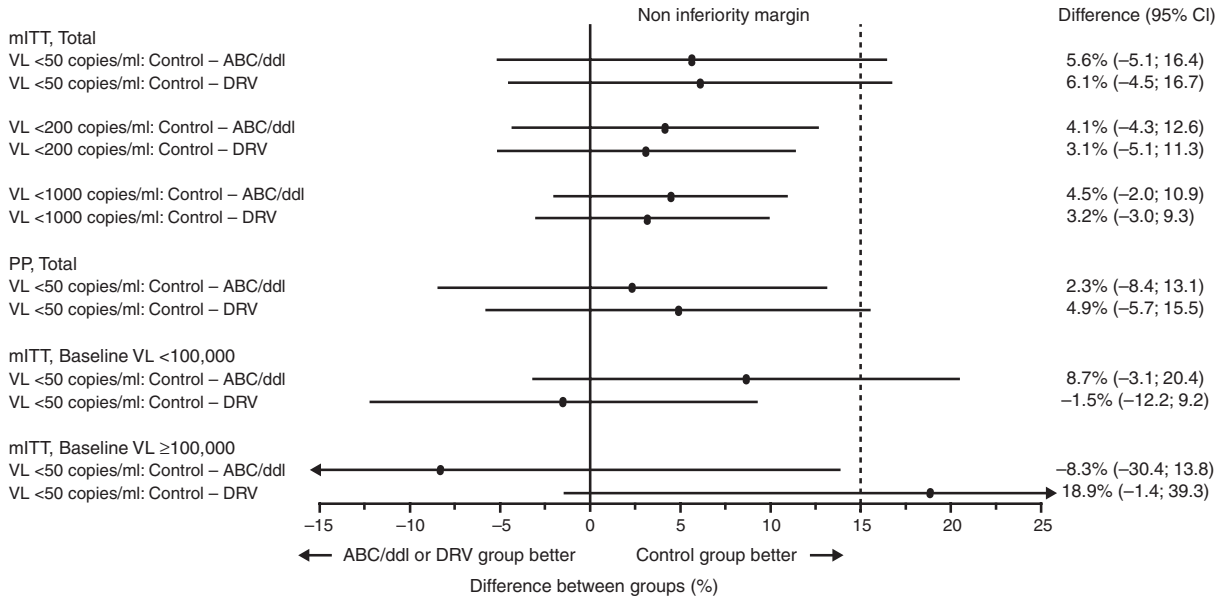

Fig. 3. Differences (% with 95% CI) between the control group (TDF/FTC LPV/r), and ABC/ddI (ABC ddI LPV/r) and DRV (TDF/FTC DRV/r) groups at week 48 in the mITT and PP populations; and for subgroups (patients with VL below and above 100 000 copies/ml at switch to second line). ABC, abacavir; CI, confidence interval; ddI, didanosine; DRV, darunavir; FTC, emtricitabine; LPV/r, ritonavir-boosted lopinavir; mITT, modified intention-to-treat; PP, per protocol; TDF, tenofovir disoproxil fumarate; VL, viral load.

Table 2. Participants experiencing mortality, Severe Adverse Event, HIV-related events and toxicity in the three groups and in the total population.

	TDF/FTC LPV/r ($n=152$)	ABC ddI LPV/r ($n=145$)	TDF/FTC DRV/r ($n=154$)	Total ($N=451$)	*P* value
Death [*n* (%)]	1 (1%)	2 (1%)	3 (2%)	6 (1%)	0.7
Grade 3 and 4 adverse events [*n* (%)]	17 (11%)	22 (15%)	19 (12%)	58 (13%)	0.6
WHO grade 3 and 4 HIV-related events [*n* (%)]	17 (11%)	23 (16%)	30 (19%)	70 (16%)	0.13
Gastrointestinal events (grade 1 to 4) [*n* (%)]	50 (33%)	48 (33%)	26 (17%)	124 (27%)	0.001
Neuropathy symptoms (grade 1 to 4) [*n* (%)]	5 (3%)	11 (8%)	8 (5%)	24 (5%)	0.26
Reduction in eGFR between baseline and week 48 $\geq$25% [*n* (%)]	28 (18%)	14 (10%)	19 (12%)	61 (14%)	0.076

ABC, abacavir; ddI, didanosine; DRV, darunavir; eGFR, estimated glomerular filtration rate; FTC, emtricitabine; TDF, tenofovir disoproxil fumarate.

Discussion

WHO's preferred strategy for second-line ART, based on two NRTIs + bPI combination, when NNRTI-containing regimens are used in first line, has been validated in recent clinical trials [4–6], but evidence for the choice of combinations is deemed of low quality and is derived from settings in high-resource countries [9].

The 2LADY study aimed at providing more evidence for the choice of NRTIs and bPI for second-line ART in RLS settings. The study was conducted in field conditions very similar to those of national programmes, with late diagnosis of first-line ART failure and no resistance testing to inform decisions about drug choice. Importantly, in our study, patients were selected after a confirmed virological failure following an adherence intervention, as recommended by the WHO. Accordingly, retrospective genotypic testing showed that 95% of the participants had a virus resistant to at least two first-line drugs.

Until recently, data on second-line treatment efficacy had mainly been based on observational retrospective cohorts in multiple settings, with diverse populations, follow-up durations and viral load thresholds. Reported success rates varied from 65 to 86% [15–19]. Different factors associated with second-line efficacy had been identified and were mainly adherence [20] or accumulation of mutations due to late diagnosis of failure [21]. Recently, two clinical trials [4,5], testing the combination of an integrase inhibitor (raltegravir) + boosted LPV versus the standard WHO recommendation, validated the WHO choice with response rate of 70.5 and 75%, respectively, for viral load below 50 copies/ml, showing that the alternative combination was not superior to NRTI + LPV/r. Monotherapy with bPI was also less efficacious than the combination with NRTI in two trials [5,6].

In our study, we observed a response rate of 65.2% for the protocol-defined primary endpoint of viral load below 50 copies/ml at week 48, failing to demonstrate non-inferiority of the tested regimens to the standard WHO 2013 recommended second line, therefore confirming once more WHO choice as a valid option.

Globally, at week 48, 83.2% of enrolled patients had a viral load below 200 copies/ml. We thus showed that bPI-based regimens provide satisfactory results in bPI-naive patients even with NRTI-resistant viral strains.

Moreover, we observed good immune recovery and reassuring results of safety and tolerance for all three regimens, with very few interruptions or adverse events due to drug toxicity. Nevertheless, the worst toxic profile (especially neuropathy) and more complicated schedule of the combination with ABC/ddI with no advantages in terms of efficacy argue for its elimination from the WHO recommendations.

The three regimens shared comparable efficacy for viral load thresholds of 200 and 1000 copies/ml, suggesting that the efficacy of the combination is independent from the choice of regimen and the presence of resistance to NRTI [22]. In fact, despite differences in adherence (the DRV group having a significantly better score than the ABC/ddI group, but not the control group despite the once-daily regimen) and in resistances to one or two drugs of the second-line combination, response rate to treatment was similar among arms.

For patients with baseline viral load at least 100 000 copies/ml, we, however, observed a suboptimal response despite no difference in adherence during the study. In multivariate analysis, viral load at least 100 000 copies/ml at baseline was associated with the presence of mutations, conferring intermediate-high resistance to two drugs of the second-line combination (aOR 4.7, 95% CI 2.6–8.5) (Supplementary Table S3, http://links.lww.com/QAD/A701). This may suggest that patients with more mutations have been longer on failing first-line treatment and had uncontrolled viral replication with high viral load at baseline and worst results therefore on the long run, which may be partly explained by a higher frequency of resistance to backbone in this group. For the TDF/FTC + DRV/r combination, we observed a lower response (22.7%) at the 50 copies/ml threshold. Our patient population differed from those of other trials using DRV-based regimens [23]. We may hypothesize that once-daily dose of 800/100 mg might be insufficient for patients with partially active triple therapy,

despite results from the Once-daily Darunavir in Treatment-experienced Patients (ODIN) trial which showed non-inferiority of once-daily to twice-daily doses [24], irrespective of viral load thresholds at baseline or number of active NRTI in the backbone [25]. In addition, we could not verify the actual intake with food assuring therapeutic blood concentrations. These results suggest that use of DRV/r could in fact be more strategic in third line due to the residual activity of this drug on protease inhibitor-resistant viral strains with no advantages in second line.

Patients with high viral load at switch are at high risk of early failure and may deserve special strategies: to note that both clinical trials testing combinations with raltegravir [4,5] showed a trend towards a better response in patients with high viral load using raltegravir than NRTI in combination with bPI.

Our study has some limitations as the open-label design may have introduced bias and the random imbalance at baseline in group characteristics could have complicated interpretations. We could not show non-inferiority of alternative regimens, but we believe our findings are influenced by the over-optimistic working hypothesis of 80% viral success (50 copies/ml) for the standard regimen which was not reached (69% in the control group). Therefore, the study power was reduced (80%).

The study includes populations representative of those followed in National HIV Programmes in Africa where viral load monitoring and genotyping are not readily available in routine care. Therefore, we believe that the results can be applied to other settings with these constraints and provide critical information for second-line strategies in different RLS. The results appear reliable, as very few patients were lost to follow-up, and randomization sequence was concealed from study personnel.

Finally, although results about second-line regimen efficacy are, for the moment, reassuring, our results suggest that patients with high viral load at first-line failure may need special management to avoid early second-line failure. Additional studies are requested to define an approach compatible with public health and programmes' constrains, but ensuring to those patients a second line of long duration.

Acknowledgements

Authors' contribution: L.C., S.K.S., A.S., C.T.N. and E.D. contributed to the conception and design of the study, overviewed the conduct of the project and participated in the interpretation of the data and critical revision of the manuscript. V.L.M. contributed to the conception and design of the study, participated in the analysis and the interpretation of the data and in critical revision of the manuscript. S.E.D. analysed all the data, prepared figures and tables and participated in the interpretation of the data. S.I. overviewed the data collection, managed the data throughout the project. C.K. participated in the clinical follow-up of the patients in Yaoundé, the interpretation of the data and critical revision of the manuscript. N.F.N.G. collected and participated in the interpretation of the data and critical revision of the manuscript. A.A.F. contributed to the biological monitoring of the patients, the interpretation of the data and critical revision of the manuscript. J.R. and A.C. contributed to the conception and design of the study, interpretation of the data and critical revision of the manuscript. All authors gave the final approval for this version of the manuscript.

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Funding: This work was supported by the Agence National de Recherche sur le SIDA – Inserm ANRS – (Grant number 12169) and by European and Developing Countries Clinical Trials Partnership (EDCTP) (grant number IP.2007.33011.004). Gilead Sciences and Janssen provided study drugs, the latter partially funding the study and had no role in study design, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors had access to study data and all approved study publication.

Conflicts of interest

E.D. and L.C. report grants from ANRS and EDCTP during the conduct of the study; E.D. and A.S. had travel grant from Gilead outside the submitted work; L.C. had travel grant from Gilead and Janssen outside the submitted work. J.R. reports personal fees and travel grants from

Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen, Merck, Pfizer, Splicos, and ViiV outside the submitted work. V.L.M. reports personal fees and non-financial support from Gilead, Abbvie, Janssen, MSD, outside the submitted work. The other authors declare that they have no conflicts of interest.

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