OPEN

# First-line antiretroviral therapy with nevirapine versus lopinavir-ritonavir based regimens in a resource-limited setting

Nathan Clumeck[a], Claude Mwamba[b], Kabamba Kabeya[a], Serge Matanda[b], Dolorès Vaira[c], Coca Necsoi[a], David Kadiebwe[b], Marc Delforge[a], Eric Kasamba[b], Chantal Milolo[b], Joe Ilunga[b] and Liévin Kapend[b]

**Objective:** To compare WHO first-line antiretroviral therapy (ART) with nonnucleoside reverse transcriptase inhibitors (NNRTI)-based regimen with a boosted protease inhibitor (bPI) regimen in a resource-limited setting regarding treatment outcome and emergence of drug resistance mutations (DRMs).

**Methods:** Treatment-naive adults were randomized to nevirapine (NVP) or ritonavir-boosted lopinavir (LPV/r) regimens each in combination with tenofovir (TDF)/emtricitabine (FTC) or zidovudine (ZDV)/lamivudine (3TC). Primary endpoint was the incidence of therapeutical (clinical and/or virologic) failure at week 48 with follow-up till week 96.

**Results:** Four hundred and twenty-five patients (120 men; 305 women) received at least one dose of the study drug. mITT analysis showed no difference in proportion of therapeutical failure between treatment arms [67/209 (32%) in NVP vs. 63/216 (29%) LPV/r at week 48 ($P = 0.53$); 88/209 (42%) in NVP vs. 83/216 (38%) in LPV/r at week 96 ($P = 0.49$)]. Per-protocol analysis demonstrated significantly more virologic failure with NVP than with LPV/r regimens [at week 48: 19/167 (11%) vs. 7/166 (4%), $P = 0.014$; at week 96: 27/158 (17%) vs. 13/159 (8%), $P = 0.019$)]. Drug resistance mutations to NNRTI were detected in 19 out of 22 (86.3%) and dual-class resistance to nucleoside reverse transcriptase inhibitor (NRTI) and NNRTI in 15 out of 27 (68.2%) of NVP failing patients. K65R mutation was present in seven out of 14 patients failing NVP-TDF/FTC regimen. No major protease inhibitor-DRM was detected among LPV/r failing patients. Discontinuation for adverse events was similar between treatment groups.

**Conclusion:** In resource-limited settings, first-line NNRTI-NRTI regimen as compared with bPI-based regimen provides similar outcome but is associated with a significantly higher number of virologic failure and resistance mutations in both classes that jeopardize future options for second-line therapy.



*AIDS* 2014, **28**:1143–1153

**Keywords: Africa, antiretroviral therapy, boosted protease inhibitor, nevirapine, resistance mutations, virologic failure**

[a]Saint-Pierre University Hospital, 1000 Brussels, Belgium, [b]Lubumbashi Network & PNMLS, Bureau de Coordination PNMLS/Katanga, Avenue Likasi, Lubumbashi, Congo RDC, and [c]CHU Liège, 1 Avenue de l'Hôpital, Domaine Universitaire du Sart Tilman, Liège 1, Belgium.

Correspondence to Nathan Clumeck, MD, PhD, Division of Infectious Diseases, Saint-Pierre University Hospital, 1000 Brussels, Belgium.

Tel: +32 2 535 41 20; e-mail: nclumeck@stpierre-bru.be

Received: 13 September 2013; revised: 15 January 2014; accepted: 15 January 2014.

DOI:10.1097/QAD.0000000000000214

ISSN 0269-9370 

## Introduction

In developed countries, HIV guidelines recommend a nonnucleoside reverse transcriptase inhibitor (NNRTI), a boosted protease inhibitor (bPI) or an integrase inhibitor associated with a nucleos(t)ide reverse transcriptase inhibitor (N(t)RTI) backbone as first-line therapy for individuals with HIV-1 infection [1].

In resource-limited settings (RLS), the WHO recommends the use of two N(t)RTIs and one NNRTI as first-line antiretroviral therapy (ART). The new 2013 recommendations state that EFV should be the first choice as NNRTI and ART should be started when $CD4^+$ cell count is below 500 cells/μl (http://www.who.int/hiv/pub/guidelines/arv2013). However, in countries where NVP remains the first choice, the later criteria will be an issue owing to its contraindication for women with $CD4^+$ cell count above 250 cells/μl and men with more than 400 cells/μl.

In RLS, three key problems are of concern with the use of NNRTI-based ART. First, there is evidence for an increasing baseline resistance to N(t)RTIs and NNRTIs among treatment-naive patients, which may compromise the efficacy of these treatments [2], particularly in sub-Saharan Africa where the estimated prevalence of primary NNRTI resistance reached 3.4% in 2009 [3]. Second, due to limited or no monitoring for plasma HIV RNA during therapy, treatment failures remain undetected for months or years and drug resistance mutations (DRMs) accumulate precluding a successful switch to a second-line therapy, and third, patients developing NNRTI/NRTI drug resistance may transmit resistant virus to sexual partners [4,5]. Therefore, in RLS, the need to maximize the effectiveness of available first-line regimens and to sustain reduced morbidity and mortality remains a major challenge for ART programmes [2]. When compared with NNRTIs, bPI regimens are more forgiving when adherence is relatively low [6] and protect against emergence of resistance of NRTIs in long-term failing patients [7], even in settings with low or absent virologic monitoring [8]. NVP being the NNRTI of choice in Democratic Republic of Congo (DRC) in 2008, the Lubumbashi trial was designed to assess the efficacy and safety of initial ART with NVP compared with lopinavir/ritonavir (LPV/r) with two backbones recommended by WHO, either tenofovir disoproxyl fumarate/emtricitabine (TDF/FTC) or zidovudine/lamivudine (ZDV/3TC).

## Materials and methods

### Study design and individuals

Patients were randomized in an open-label, multicentre, parallel group superiority study conducted in Lubumbashi (DRC), the capital of the Katanga region, a city of one million inhabitants and an estimated HIV prevalence of 6% in adults in 2006. Eligible participants were adults, recruited through advertisements, who were ART-naive (previous single NVP dose for prevention of mother-to-child transmission was allowed) with an indication to start ART according to the Congolese National HIV treatment guidelines (WHO clinical stage 3 and $CD4^+$ cell count <350 cells/μl, or clinical stage 4 or a $CD4^+$ cell count <200 cells/μl regardless of WHO clinical stage). Main exclusion criteria were active tuberculosis, haemoglobin less than 8.5 g/dl in women and less than 9.0 g/dl in men, alanine aminotransferase or aspartate aminotransferase more than three times the upper limit of normal and creatinine clearance less than 50 ml/min.

Eligible individuals were randomized (1 : 1) with a simple randomization procedure based on computerized random numbers to receive: LPV/r (Aluvia; Abbott Laboratories) 800/200 mg/day [taken daily (q.d.) or twice daily (b.i.d.) at the patient choice] or NVP 200 mg b.i.d. combined with either TDF/FTC (300/200 mg) fixed-dose combination (FDC) (Truvada; Gilead Sciences Inc.) or ZDV/3TC, 300/150 mg generic fixed-dose combination. TDF/FTC replacing ZDV/3TC in individuals with positive hepatitis B surface antigen. These two backbones are recommended by the WHO and are available through the National Programme in DRC. Therefore, the allocated backbone was also randomly chosen with no *a priori* hypothesis about their respective therapeutic effect. Patients treated with NVP started with a 2-week lead-in period at 200 mg daily. Nevirapine was chosen because in 2008 it was the first choice in association with ZDV and 3TC in the national guidelines in DRC. Following the new recommendations of the WHO, EFV is now the preferred NNRTI. Trimethoprim/sulfamethoxazole (80/400 mg) daily prophylaxis was systematically given. Patients failing on NVP or LPV/r were allowed to switch to the other arm on the basis of the genotypic analysis. In case of treatment-limiting toxicity to the randomized dual N(t)RTI or to NVP or LPV/r, a switch to the alternate drug(s) was allowed.

Patients who developed active tuberculosis were discontinued from the study and switched to an efavirenz and rifampin-based treatment.

### Study oversight

The study was approved by the National Ethical Committee in DRC and by the Ethical Committees of Saint-Pierre University Hospital, Brussels and the University of Liège in Belgium. Written informed consent was obtained from all participants. An independent data and safety monitoring committee blindly reviewed the efficacy and safety data after all patients have completed 24 weeks in the study. LPV/r and TDF/FTC, respectively, were donated by Abbott and Gilead Laboratories who played no role in the final design decisions of the study, the analysis of data or preparation of

the manuscript. All others drugs were available through the National AIDS Program in DRC.

## Study assessments

Clinical assessments were performed at screening, baseline, week 2, 4, 12 and every 12 weeks thereafter for 96 weeks. The treating physician assessed patient's adherence at each visit on the basis of pill counts and patients self-administered questionnaires. Counselling and support were given in case of adherence difficulties and detectable HIV-1 viral load.

Laboratory safety assessments were performed every 12 weeks. Hepatitis B and C serologies were assessed at baseline.

$CD4^+$ cell counts and plasma HIV-1 RNA (Amplicor HIV-1 Monitor assay, 1.5; F. Hoffman-La Roche Ltd) were done at baseline, week 12 and every 24 weeks thereafter by a central local university laboratory. Virologic failure was defined as two consecutive (taken 15–30 days apart) plasma HIV-1 viral load at least 1000 copies/ml after week 24 after starting therapy. We used a threshold of 1000 copies/ml because it is commonly used in most routine laboratory for genotypic determination. Since 2013, this limit is considered by WHO as an early site-based warning indicator of treatment failure in developing countries (http://www.who.int/hiv/pub/guidelines/arv2013), with the goal of ART remaining to achieve and sustain viral suppression. For all individuals with confirmed virologic failure, genotype sequencing was performed centrally on baseline sample and on sample taken at the time of VF, using the TRUGENE HIV-1 genotyping kit (Global Siemens Healthcare) and OpenGene DNA sequencing system. DRMs and HIV clades were interpreted using the HIVdb Program, 2011, from Stanford (http://hivdb.stanford.edu). For minority variant determination, DNA sequencing clonal analysis of the reverse transcriptase and protease amplified products sequencing at least 200 clones per sample was performed. Antiretroviral plasma levels were determined by liquid chromatography coupled with tandem mass spectrometry in samples collected at the time of virologic failure.

## Study endpoints

The primary objective of the study was to assess whether lopinavir/ritonavir is superior to nevirapine at week 48 when both are combined with one of the WHO-recommended NRTI backbones (TFD/FTC or ZDV/3TC). Follow-up was made until week 96. The primary endpoint was the number of patients who have reached a therapeutic failure endpoint, a composite of clinical or virologic failure.

Clinical failure was defined as the occurrence of a WHO stage 4 or WHO stage 3 event or death after at least 24 weeks of therapy, or a discontinuation of study drugs for toxicity at any time. Virologic failure was defined as confirmed plasma HIV-1 viral load of at least 1000 copies/ml after week 24 after starting therapy.

Secondary endpoints included the proportion of patients with plasma HIV-1 RNA viral load below 50 copies/ml, $CD4^+$ cell counts changes from baseline, HIV-1 resistance mutations, adherence and safety of the four regimens. The proportion of patients with a viral load below a threshold of 400 copies/ml was also assessed (exploratory analysis). A separate analysis between patients with baseline viral load above 100 000 copies/ml was also performed (exploratory analysis).

## Statistical analyses

Assuming a rate of 15% of patients with clinical or virological failure in the LPV/r arm and 30% in the NVP arm at week 48, a sample size of at least 174 evaluable patients by arm was considered necessary to detect a difference of 15% with a 90% power and a significant alpha level of 0.05 using a Fisher's exact two-sided test. Assuming a rate of 25% of loss to follow-up, a proportional oversampling was made.

The rate of therapeutic failure defined as a composite of clinical and virological endpoint in randomized treatment groups was compared for superiority using a modified intention-to-treat (mITT) analysis, which included data for all patients who were assigned to a treatment group and who have taken at least one dose of study medication regardless of any reason of discontinuation. In this analysis, patients who died before week 24 had tuberculosis before week 24, withdrew their consent, had a protocol violation or were lost to follow-up were counted as failure. Missing HIV-1 RNA viral load data were imputed as failure irrespective of the reason of absence.

We also did a per-protocol analysis on all the individuals who received study drugs up to 48 and 96 weeks and who did not discontinue the study treatment for another reason than therapeutic failure as defined above.

The differences between treatment groups (nevirapine-lopinavir/ritonavir) are presented in terms of risk difference and associated two-sided 95% confidence intervals. We used the Cochran–Mantel–Haenszel test to assess virologic and overall therapeutic responses adjusted according to the backbones of the patients. All other *P*-values are unadjusted.

Simple descriptive statistics were used for summarizing characteristics of patients by arm at baseline using median and interquartile ranges (IQRs) for continuous data, and frequencies and percentages for categorical data.

Other analyses of efficacy or safety between groups of patients were performed at considered week with the

Mann–Whitney *U* test if the data were continuous and Fisher's exact test if the data were categorical.

Clinical and laboratory-associated adverse events occurring when the patients were still on the treatment assigned at the randomization were included in the safety analyses. Adverse events were recorded as drug-related if they were judged by the investigator to be definitely, probably or possibly related to any of the study drugs. Intensity grading was based on the Division of AIDS (DAIDS) toxicity guidelines for adults (2004).

The comparison between the backbones was only exploratory and focused on the safety data.

A posthoc analysis was performed to assess the sexual difference outcomes between treatment groups. Breslow–Day test for homogeneity of odds ratio was used to verify the existence of an interaction between sex, treatment groups and endpoints. All reported *P*-values are two-sided.

Analysis and graphs were produced using SAS statistical software (version 9.2; SAS Institute, Cary, North Carolina, USA).

## Results

### Patients disposition and baseline characteristics

We screened 724 individuals between December 2008 and October 2009 of whom 299 (41.2%) were not eligible. Four hundred and twenty-five (58.7%) patients were randomized [209 (49.1%) in NVP and 216 (50.8%) in LPV/r groups] and took at least one dose of study drugs. The number of individuals who have discontinued the study before week 96 was similar in both groups (Fig. 1). At week 96, 28 (6.6%) patients were lost to follow-up and 25 (5.9%) had a major protocol violation (20/25 individuals were found to be not treatment-naive at the inclusion).

Demographics and baseline characteristics were well balanced across both treatment groups (Table 1). Median $CD4^+$ cell count (cells/μl) was 181 (IQR: 77–256) and 165 (IQR: 92–250) in men and women, respectively. In the NVP group, 24% of women (35/148) had $CD4^+$ cell count more than 250 cells/μl. Thirty-four out of 216 (15.7%) patients started with LPV/r once daily.

### Primary endpoints

In the mITT analysis that considers all patients who received at least one dose of study medication together with the other reasons of discontinuation, there was no difference in the rate of therapeutic failure between NVP and LPV/r-based regimens: at week 48, 32% (67/209) in NVP compared with 29% (63/216) in LPV/r ($P = 0.53$); and at week 96, 42% (88/209) in NVP compared with 38% (83/216) in LPV/r ($P = 0.49$) (Table 2a). For the overall group, death occurred in 42 out of 425 (9.9%) patients mostly before week 24 (30/42, 71%) with no difference between treatment groups. Causes of deaths are reported in supplemental page Table 1, http://links.lww.com/QAD/A485.

In the per-protocol analysis at week 48, the rate of therapeutic failure in the nevirapine arm was twice the one in the ritonavir-boosted lopinavir arm: 15% (25/167) in NVP compared with 8% (13/166) in LPV/r ($P = 0.04$) (Table 2b). This difference was mostly due to a higher rate of virologic failure among the individuals treated with nevirapine (19/167; 11.4%) versus those receiving LPV/r (7/166; 4.2%) ($P = 0.015$). At week 96, the higher proportion of patients with virologic failure in the NVP arms persisted: 27/158 (17.1%) as compared with 13/159 (8.2%) in LPV/r groups ($P = 0.018$). This difference remained statistically significant ($P = 0.04$) when the three patients with a baseline resistance to NVP were excluded from the analysis. mITT analysis among the individuals with baseline viral load more than 100 000 copies/ml did not find any difference in treatment outcomes at week 48 ($P = 0.54$) and week 96 ($P = 0.15$).

The analysis carried out on all patients who took at least one dose of study drugs and adjusted to backbone showed no differences in therapeutic failure endpoints at week 48 (adjusted $P = 0.538$; Cochran–Mantel–Haenszel test) and at week 96 (adjusted $P = 0.456$).

### Secondary endpoints

Secondary endpoints have been assessed in all patients who received at least one dose of study medication. The median change from baseline in $CD4^+$ cell count at week 48 was 119 cells/μl (range: 49–198) for NVP vs. 125 cells/μl (range: 66–199) for LPV/r ($P = 0.473$), and at week 96 was 161 cells/μl (range: 66–255) for NVP vs. 181 cells/μl (range: 69–312) for LPV/r ($P = 0.181$).

No difference was observed between the treatment groups in the proportion of patients with HIV-1 RNA less than 50 copies/ml at week 48: 68% (NVP) vs. 67% (LPV/r) ($P = 0.699$) and at week 96: NVP (63%) vs. LPV/r (60%) ($P = 0.597$) (supplemental figure, http://links.lww.com/QAD/A485). When considering a cut-off of 400 copies/ml, the proportion of individuals above this threshold was still significantly higher in the nevirapine than in lopinavir/ritonavir-based treatments at week 48 [10% (21/209) vs. 4% (9/216), $P = 0.012$] but not at week 96 [14% (29/209) vs. 8% (17/216), $P = 0.060$].

### Resistance data

At baseline, major DRMs were found in three out of 27 (two women, one man) NVP-failing patients and in zero

out of 13 patients who failed in the LPV/r group. In addition, no minority variants with resistance mutations were found in any patients who developed virologic failure during the trial.

At the time of failure, DRMs to NNRTI, N(t)RTIs or both classes were detected among 19 out of 22 (86.3%), 15 out of 22 (68.2%) and 15 out of 22 (68.2%) of patients receiving NVP, respectively (Table 3). Pattern of

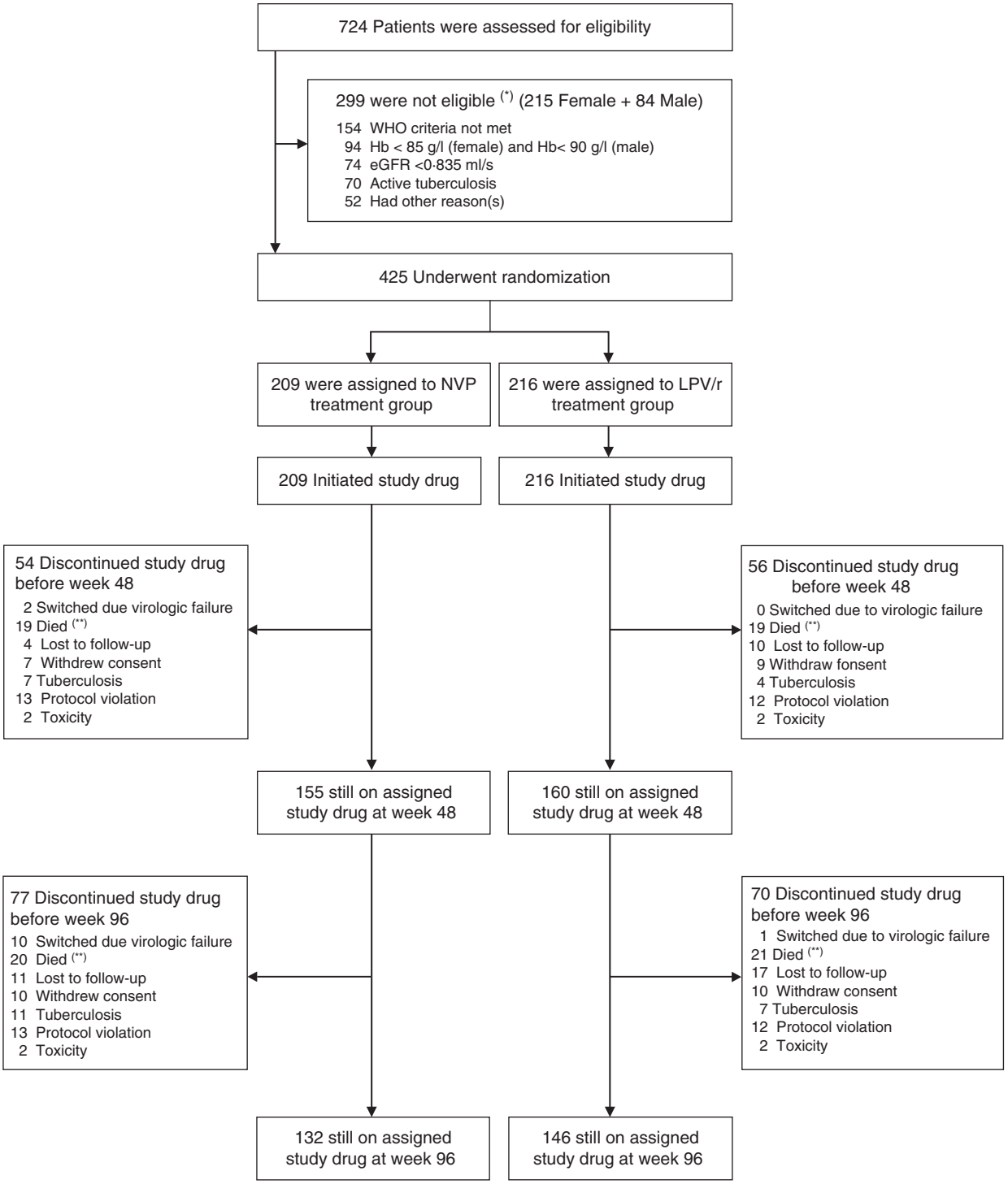


**Fig. 1. Individual's disposition.** [a]A patient may have multiple reasons. [b]Data represent the total number of patients who died including those who died after reaching a study endpoint after week 24.

**Table 1. Baseline characteristics.**

| Randomized patients | NVP $n=209$ | LPV/r $n=216$ |
|---|---|---|
| Age (years), median (IQR) | 38 (33–45) | 38 (33–45) |
| Female | 148 (70.8%) | 157 (72.7%) |
| Previous exposure to NVP single dose for PMTCT | 2 | 1 |
| Black race | 209 (100%) | 216 (100%) |
| Weight (kg), median (IQR) | 55 (50–62) | 54 (49–62) |
| BMI (kg/m$^2$), median (IQR) | 21 (19–23) | 20 (18–23) |
| WHO stage | | |
| CD4$^+$ cell count <200 cells/μl | 121 (58%) | 128 (59%) |
| Stage III, *n* (%) | 190 (90.9%) | 202 (93.5%) |
| Stage IV, *n* (%) | 10 (4.8%) | 5 (2.3%) |
| CD4$^+$ cell count (cells/μl) – median (IQR) | 164 (84–253) | 168 (84–253) |
| CD4$^+$ cell count <100 cells/μl | 60 (28.7%) | 61 (28.2%) |
| Plasma HIV RNA (log copies/ml), median (IQR) | 5.17 (4.5–5.6) | 5.13 (4.4–5.6) |
| Plasma HIV RNA >100 000 copies/ml (%) | 115 (55%) | 115 (53%) |
| Clades (*n*) | | |
| Clade C (%) | 60.6 | 60.0 |
| Clade A (%) | 10.6 | 11.2 |
| Clade G (%) | 7.3 | 7.2 |
| Clade K (%) | 8.1 | 6.4 |
| Other (%) (B, D, F, H, J, CRF01, CRF01_AE, CRF02_AG) | 13.4 | 15.2 |
| Hep B surface antigen, positive, *n* (%) | 18 (8.6%) | 22 (10.2%) |
| Hep C antibody, positive, *n* (%) | 7 (3.3%) | 8 (3.7%) |
| Hb, mmol/l, median (IQR) | 6.8 (6.2–7.4) | 6.8 (6.2–7.4) |
| eGFR (Cockcroft–Gault; ml/s), median (IQR) | 1.17 (1.00–1.34) | 1.12 (0.97–1.30) |

IQR, interquartile range; PMTCT, prevention of mother-to-child transmission.

mutations selected in the NVP group (Y181C/V, K101E) showed a predicted decreased susceptibility to etravirine or rilpivirine in 50% of individuals. In the subgroup of patients on NVP-TDF/FTC, K65R was found in seven out of 14 patients (three clade C, two clade G, one clade A, one clade K). In the bPI group, no major protease inhibitor mutations emerged and NRTI-DRM (M184V/I) occurred in two out of 10 patients.

### Adherence

The proportion of patients with adherence at least 95% at each study visit was similar between the treatment groups up to 48 weeks; 75.4 vs. 74.6% ($P=0.906$) and up to 96 weeks; 75.9 vs. 75.5% ($P=1$) for NVP and LPV/r, respectively.

The proportion of individuals with an adherence of at least 95% was lower in patients with a virologic failure than in those without virologic failure (60 vs. 77.4%; $P=0.019$) up to 96 weeks. Measures of plasma drug concentrations confirmed the poor adherence in failing patients; NVP plasma concentrations were inadequate (<3000 ng/ml) in 14 out of 22 (63.6%) patients and LPV plasma level was below the limit of quantification in 10 out of 10 failing patients.

### Safety

Most adverse events were observed during the first 48 weeks. There were more patients with adverse events in the LPV/r arm (59/216; 27.3%) than in the NVP arm (34/209; 16.2%) (at week 48 $P\leq 0.0069$) (Table 4a). Gastrointestinal side effects were more frequent in the LPV/r groups, whereas rashes were significantly higher among patients on NVP. Two patients on LPV/r and two patients on NVP changed their therapy for grade 3 toxicity.

The preplanned exploratory analysis focused on the backbone safety comparison is shown on Table 4b. Zidovudine/lamivudine was discontinued in seven (3.5%) individuals (all for anaemia) compared with tenofovir/emtricitabine in only one (0.4%) individual (for renal failure), $P=0.029$.

Nausea, vomiting and neutropenia were more frequently reported with ZDV/3TC than with TDF/FTC.

No difference was seen between backbones in median estimated glomerular filtration rate (eGFR) changes from baseline (which remained within the normal range).

### Posthoc sexual analysis

At week 48, sexual analysis did not show any difference in outcomes.

At week 96, men, as compared with women had a lower median CD4$^+$ cell count increase [105 cells/μl (IQR: 34–188) vs. 197 cells/μl (IQR: 84–319) $P<0.0001$] and developed more tuberculosis (6/120; 5% vs. 4/305; 1.3%; $P=0.034$).

## Discussion

In this prospective trial comparing a NNRTI-based first-line ART with a bPI regimen, mITT analysis demonstrated equivalent outcomes of both strategies.

**Table 2. Treatment outcome at week 48 and week 96.**

| (a) Comparison between NVP vs. LPV/r-based regimens; mITT analysis | Week 48 | | | | Week 96 | | | |
|---|---|---|---|---|---|---|---|---|
| | NVP $n=209$ | LPV/r $n=216$ | Risk difference (95% CI) | *P* | NVP $n=209$ | LPV /r $n=216$ | Risk difference (95% CI) | *P* |
| Primary endpoints, *n* (%) | | | | | | | | |
| Clinical failure | | | | | | | | |
| New or recurrent WHO clinical stage III or IV event | 4 (1.9) | 2 (0.9) | −1% (−3 to 1) | | 7 (3.3) | 8 (3.7) | 0.4% (−3 to 3) | |
| Death after 24 weeks | 0 (0) | 2 (0.9) | 1% (−0.4 to 2) | | 1 (0.4) | 3 (1.4) | 1% (−1 to 3) | |
| Changing of treatment for toxicity | 2 (1) | 2 (0.9) | −0.3% (−2 to 2) | | 2 (1) | 2 (0.9) | −0.1% (−2 to 2) | |
| Virologic failure (plasma VL >1000 copies/ml) | 19 (9.1) | 7 (3.2) | −5.9% (−10 to −1) | | 27 (12.9) | 13 (6) | −6.9% (−12.4 to −1.4) | |
| Other reasons off treatment, *n* (%)[a] | 42 (20.1) | 50 (23.1) | 3% (−5 to 11) | 0.48 | 51 (24.4) | 57 (26.4) | 2% (−6 to 10) | |
| Total outcomes, *n* (%) | 67 (32.1) | 63 (29.2) | −2.9% (−11.7 to 5.9) | 0.52 | 88 (42.1) | 83 (38.4) | −3.7% (−13 to 5.6) | 0.48 |

| (b) Comparison between NVP vs. LPV/r-based regimens; per-protocol analysis | Week 48 | | | | Week 96 | | | |
|---|---|---|---|---|---|---|---|---|
| | NVP $n=167$ | LPV/r $n=166$ | Risk difference (95% CI) | *P* | NVP $n=158$ | LPV/r $n=159$ | Risk difference (95% CI) | *P* |
| Primary endpoints, *n* (%) | | | | | | | | |
| Clinical failure | | | | | | | | |
| New or recurrent WHO clinical stage III or IV event | 4 (2) | 2 (1.2) | −1.2% (−4 to 1.7) | | 7 (4) | 8 (5) | 0.6% (−4.1 to 5.3) | |
| Death after 24 weeks | 0 (0) | 2 (1.2) | 1.2% (−0.4 to 2.9) | | 1 (1) | 3 (2) | 1.3% (−1.2 to 3.7) | |
| Changing of treatment for toxicity | 2 (1.2) | 2 (1.2) | 0% (2.3 to −2.3) | | 2 (1) | 2 (1) | 0% (1.3 to −1.3) | |
| Virologic failure (plasma VL >1000 copies/ml) | 19 (11) | 7 (4.2) | −7% (−13 to −1) | 0.01 | 27 (17) | 13 (8) | −9% (−16 to −2) | 0.02 |
| Total outcomes, *n (%)* | 25 (15) | 13 (7.8) | −7.1% (−13.9 to −0.4) | 0.04 | 37 (23%) | 26 (16%) | −7% (−16 to 2) | 0.11 |

[a]Death before week 24, tuberculosis before week 24, consent withdrawal, protocol violation, lost to follow-up, missing data.

**Table 3. Virologic results and drug resistance mutations (at week 96).**

| | NVP $n=209$ | LPV/r $n=216$ | $P$ |
|---|---|---|---|
| Number of patients with VF, *n* (%) | 27 (13) | 13 (6) | 0.019 |
| Genotype available at VF, *n* (%) | 22 (82) | 10 (77) | |
| Any resistance-associated mutation at baseline, *n* | 3[a] | 0 | |
| Resistance-associated mutations at VF (excluding minor protease mutation), *n* (%) | 19 (86) | 2 (20) | <0.0001 |
| NNRTI-associated mutation, *n* (%) | 19 (86) | 0 | <0.0001 |
| K103N | 13 (59) | 0 | |
| Y181C/V | 10 (45) | 0 | |
| Other[b] | 12 (54) | 0 | |
| N(t)RTI-associated mutation, *n* (%) | 15 (68) | 2 (20) | 0.020 |
| M184V/I | 15 (68) | 2 (20) | 0.020 |
| K65R | 7 (32) | 0 | 0.069 |
| Thymidine analogue associated mutation[c] | 4 (18) | 0 | |
| N(t)RTI along with NNRTI associated mutation, *n* (%) | 15 (68) | 0 | <0.0001 |
| Any protease mutation, *n* (%) | 0 | 1 (10) | |
| Major protease mutation, *n* | 0 | 0 | |

Resistance-associated mutations according to IAS-USA, 2011 list. N(t)RTI, nucleoside or nucleotide reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; VF, virologic failure.
[a]NNRTI mutations: K103N, Y181C, V108I; N(t)RTI mutations: M184V, L210W.
[b]Other NNRTI mutations: K101E, V106I/L, V108I/L, Y188C, G190A, F227L, M230L, P236H.
[c]Thymidine analogue-associated mutation: 41L, 67N, 70R, 210W, 215F/Y, 219Q/E.

Per-protocol analysis demonstrated more virologic failures and DRMs among patients who received a NNRTI-based regimen than those who received a bPI-based regimen. Although discontinuation for severe adverse events was similar in both groups, NVP-based regimen was better tolerated than LPV/r regimen. No difference in outcomes was seen in relation with the NRTI backbones.

Few randomized trials have been conducted in RLS to compare NNRTI vs. bPI-based regimen as first-line ART in adults [9,10]. Among 500 African women who

**Table 4. Safety analysis through 96 weeks.**

| | Week 0 to week 48 | | | Week 48 to week 96 | | |
|---|---|---|---|---|---|---|
| (a) Comparison between NVP vs. LPV/r-based regimens | NVP $n=209$ | LPV/r $n=216$ | $P$ | NVP $n=209$ | LPV/r $n=216$ | $P$ |
| Patients with at least one clinical adverse event, *n* (%) | 34 (16.2) | 59 (27.3) | 0.007 | 0 | 3 (1.4) | 0.249 |
| Adverse events leading to study drug discontinuation, *n* (%) | 2 (0.9) | 2 (0.9) | 1 | 0 | 0 | |
| Clinical events (grade 1–4)[a], *n* (%) | | | | | | |
| Rash | 8 (3.8) | 0 | 0.003 | 0 | 0 | |
| Vomiting | 15 (7.2) | 29 (13.4) | 0.038 | 0 | 2 (0.9) | 0.499 |
| Diarrhoea | 0 (0) | 32 (14.8) | <0.0001 | 0 | 3 (1.4) | 0.249 |
| Nausea | 20 (9.6) | 40 (18.5) | 0.008 | 0 | 0 | |
| Laboratory abnormalities (grade 3–4)[a] | | | | | | |
| Hepatic aminotranferase (>5.1× ULN) | 5 (2.4) | 3 (1.4) | 0.497 | 2 (0.9) | 1 (0.46) | 0.618 |
| Haemoglobin (<4.65 mmol/l) | 17 (7.9) | 17 (8.1) | 0.863 | 2 (0.9) | 1 (0.46) | 0.618 |
| eGFR (<0.835 ml/s) | 3 (1.4) | 2 (0.9) | 0.681 | 0 | 0 | |

| | Week 0 to week 48 | | | Week 48 to week 96 | | |
|---|---|---|---|---|---|---|
| (b) Backbones comparison | TDF/FTC $n=225$ | ZDV/3TC $n=200$ | $P$ | TDF/FTC $n=225$ | ZDV/3TC $n=200$ | $P$ |
| Patients with at least one clinical adverse event, *n* (%) | 34 (15.1) | 56 (28) | 0.001 | 5 (2.2) | 0 | 0.063 |
| Adverse events leading to study drug discontinuation, *n* (%) | 1 (0.4) | 7 (3.5) | 0.029 | 0 | 0 | |
| Clinical events (grade 1–4)[a], *n* (%) | | | | | | |
| Rash | 6 (2.7) | 2 (1.0) | 0.291 | 0 | 0 | |
| Vomitting | 15 (6.7) | 28 (14.0) | 0.015 | 2 (0.88) | 0 | 0.501 |
| Diarrhoea | 17 (7.6) | 15 (7.5) | 1 | 3 (1.3) | 0 | 0.147 |
| Nausea | 17 (7.6) | 42 (21.0) | <0.0001 | 0 | 0 | |
| Laboratory abnormalities (grade 3–4)[a] | | | | | | |
| Hepatic aminotranferase (>5.1× ULN) | 6 (2.7) | 1 (0.5) | 0.126 | 2 (0.88) | 0 | 0.501 |
| Haemoglobin (<4.65 mmol/l) | 12 (5.3) | 18 (9.0) | 0.183 | 2 (0.88) | 1 (0.05) | 1 |
| Neutropenia ($<0.750\times10^9$/l) | 1 (0.4) | 12 (6.0) | 0.0014 | 0 | 1 (0.05) | 0.471 |
| eGFR (<0.835 ml/s) | 1 (0.4) | 2 (1.0) | 0.603 | 0 | 1 (0.05) | 0.471 |
| Median change in eGFR from baseline (ml/s) | +0.08 | +0.13 | 0.201 | +0.27 | +0.24 | 0.832 |

eGFR, estimated glomerular filtration rate; ULN, upper limit of normal.
[a]Grading according with Division of AIDS Table for grading the severity of adult and paediatric adverse events, December 2004.

had no prior exposure to single-dose NVP, initial NVP-TDF/FTC was as effective as LPV/r-TDF/FTC in terms of virologic failure and death [9]. In a South African trial, no differences in virological outcome were found between LPV/r and EFV treatment arms [10]. The apparent discrepancy in rate of virologic failure between the above-mentioned trials and our trial may be related to a different definition of virologic failure [11]. Virologic failure rates are highly sensitive to thresholds and the choice of HIV RNA cut-off to define viral failure may lead to an underestimation of the prevalence of resistance [12]. In the above-mentioned studies, a cut-off of 400 copies/ml was used to define virologic failure. With a threshold of 1000 copies/ml, our failure rate of 12.9% among patients treated with NVP-based regimens for 24 months is consistent with the results of observational studies. In 2008, in the Democratic Republic of Congo, 14.6% of patients had a plasma viral load more than 1000 copies/ml after a median time on NNRTI regimen of 25 months [13]. In the systematic review by Barth *et al.* [14] of 89 studies with 13 288 patients with a median duration of treatment of 10 months, the prevalence of virologic failure after 24 months was 33%.

In our study, virologic failure was not driven by pretreatment transmitted drug resistance. Indeed, only three out of 27 individuals who failed on NVP had NNRTI resistance at baseline. We found no minority resistance mutation at baseline in the remaining individuals. Our pharmacokinetic data and adherence measures are consistent with previous observations that suboptimal adherence is associated with an increased risk of emergence of DRMs in case of NNRTI-based therapy in contrast with bPI-based therapy [6,15].

No protease inhibitor mutations and only minimal NRTI resistance being found among LPV/r failing patients, our study also confirms that bPI-based regimens have a more 'forgiving' profile in terms of emergence of resistance [6–11,16,17]. Of those patients with virologic failure, 86% had drug-resistant mutation to NNRTI and 50% were predicted to harbour HIV strains with reduced susceptibility or drug resistance to etravirine or rilpivirine, a relevant finding if these drugs would be considered for future salvage therapy in RLS [18]. High frequency of NRTI mutations and dual resistance to NNRTI-NRTI were also found that limit the choice and response to a second-line therapy. Mutations associated with cross-resistance to NRTIs comprised multiple thymidine-associated mutations (18%) or K65R (32%). This high level of K65R in patients failing on TDF confirms the association with subtype C clades as compared with subtypes B and is consistent with a meta-analysis showing that genotypic resistance to NRTIs appears far greater in RLS [19–21].

Because our participants were evaluated for viral load every 6 months after week 24, it is not surprising that observed resistant patterns were more extensive for this trial than for cohorts that received intensive virologic monitoring [16,18]. In the absence of viral load monitoring, similar high frequencies of DRM (70–93%) were reported in persons with prolonged ART failure [22–26]. In most sub-Saharan countries, the diagnosis of therapeutic failure is still based on clinical and immunological criteria that are not sensitive or specific enough leading to late or inappropriate switch to second-line regimen [27]. A recent editorial has stressed the public health priority in RLS to implement viral load testing as a standard monitoring tool [28].

Although this study was not designed to evaluate differences between men and women, our results suggest also that men had a poorer clinical and immunological outcome in response to ART. In RLS, poorer outcomes have been constantly observed in men, including a higher risk of opportunistic infections, mortality and virologic failure [29,30]. So far, late access to care, differences in behaviour and lower adherence to therapy have been advocated to explain these sexual differences [31].

Our study has some limitations; we used NVP as NNRTI-based regimen. One cannot exclude that with an EFV-based regimen, rates of virologic failure would be lower [32]. However, in case of virologic failure, NNRTI resistance mutations have been shown to remain significantly higher with EFV than with bPI and our conclusions on the robustness of bPI regimen would not change [10,33].

This study was not designed to see differences in outcomes between backbone and no definitive conclusions could be driven from our results.

This trial was conducted at clinics in a large city and does not reflect the routine of care in smaller cities and rural healthcare facilities. Public health decisions cannot be solely based on randomized clinical trials in settings with fewer constraints and optimal follow-up.

Although resistance considerations need to be balanced against other factors, such as cost, coformulation as fixed-dose combinations, short and longer-term toxicity, administration of ART with tuberculosis medication and lack of second-line availability following protease inhibitor use as a first-line regimen, it remains that particularly in sub-Saharan Africa, important gaps in service delivery and programme performance affecting a considerable proportion of ART programmes contribute to substandard antiretroviral regimen and acquired drug resistance [34]. This worrying situation reinforces the need, among other public health interventions, to maximize the effectiveness of available first-line regimen taking into account unplanned treatment interruptions and patients with a risk of poor adherence particularly in the absence of baseline genotypic determination and viral load monitoring. This is even more important since the

implementation of the 2013 WHO guidelines, which will substantially increase the number of patients on ART and in the current settings of suboptimal therapies and lack of viral load monitoring may critically increase the level of resistance development. In such a setting, bPI-containing regimen might be a better suited first-line ART option in RLS that might improve rates of viral suppression and reduce treatment-emergent drug resistance. In case of virologic failure with bPI, due to the absence of major protease inhibitor mutations and infrequent minority variant resistant mutations [35], bPI could possibly be reused with a different antiretroviral backbone including integrase inhibitors [36]. In the next years, availability of other classes for second-line regimens, decreased prices and development of new drug formulation, following WHO prequalification and FDA approval of atazanavir/ritonavir FDC in late 2011, should facilitate strategic decisions regarding future switch of first-line combination ART to bPI.

## Acknowledgements

We thank Andrew Hill and Stéphane de Wit for reviewing the manuscript. We thank Prof. Michel Moutschen for his support. We thank the trial participants and all the staff (doctors, nurses, social workers, data and administrative workers) of the five clinics of Lubumbashi. We thank Prof Gilles Peytavin and Prof Vincent Calvez for performing the pharmacokinetic analysis (GP) and the minority variants analysis (VC). We also thank the data safety monitoring board and the institutional review boards (Ethics committee of Brussels, Kinshasa and Liège) for their technical advice and oversight of this study.

N.C. designed the study, interpreted data, searched literature, wrote the manuscript, presented the data and had final responsibility for the decision to submit for publication. C.M., lead recruiter, collected data. K.K. analysed data, searched literature and contributed to writing of the manuscript. D.V. contributed to perform the genotypic determination and analysed data. C.N. collected, reviewed data and searched literature. D.K., C.M., J.I. and S.M., recruiters, collected data. M.D. was the protocol statistician. E.K. performed the local laboratory determination. L.K., local medical leader, contributed to design the study.

This study is registered with ClinicalTrials.gov, number NCT01772940.

This project was funded by the Ministry of Cooperation of the Belgian government.

### Conflicts of interest

We declare that we do not have a commercial or other association that might pose a conflict of interest.

*These results have been partially presented to the 19th Conference on Retroviruses and Opportunistic Infections (CROI, Seattle 2012).*

## References

1. European Guidelines. http://eacsociety.org/Guidelines.aspx [accessed September 2013].
2. Gupta R, Jordan M, Sultan B, Hill A, Davis DHJ, Gregson J, *et al*. **Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis.** *Lancet* 2012; **380**:1250–1258.
3. WHO. WHO HIV Drug resistance report 2012. Geneva: World Health Organization; 2012. http://apps.who.int/iris/bitstream/10665/75183/1/9789241503938_eng.pdf. [Accessed 20 December 2013].
4. De Luca A, Marazzi M, Mancinelli S, Ceffa S, Doro Altan AM, Buonomo E, *et al*. **Prognostic value of virological and immunological responses after 6 months of antiretroviral treatment in adults with HIV-1 infection in sub-Saharan Africa.** *J Acquir Immune Defic Syndr* 2012; **59**:236–244.
5. Milloy M-J, Wood E. **Transmitted antiretroviral-resistant HIV: a coming anarchy?** *Lancet Infect Dis* 2011; **11**:336–337.
6. von Wyl V, Klimkait T, Yerly S, Nicca D, Furrer H. **Adherence as predictor of the development of class-specific resistance the Swiss HIV Cohort Study.** *PLoS One* 2013; **8**:e77691.
7. Scherrer A, Böni J, Yerly S, Klimkait T, Aubert V, Furrer H, *et al*. **Long-lasting protection of activity of nucleoside reverse transcriptase in protease inhibitors by boosted PI containing regimens.** *PLoS One* 2012; **7**:e50307.
8. Gupta R, Hill A, Sawyer A, Cozzi-Lepri A, von Wyl V, Yerly S, *et al*. **Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis.** *Lancet Inf Dis* 2009; **9**:409–417.
9. Lockman S, Hughes M, Sawe F, Zheng Y, Mcintyre J, Chipato T, *et al*. **Nevirapine-versus lopinavir/ritonavir-based initial therapy for HIV-1 infection among women in Africa: a randomized trial.** *PLoS Med* 2012; **9**:e1001236.
10. The Phidisa II Writing Team for Project Phidisa. **A randomized factorial trial comparing 4 treatment regimens in treatment-naive HIV-infected persons with AIDS and/or a CD4 cell count <200 cells/(L in South Africa.** *J Infect Dis* 2012; **202**:1529–1537.
11. Hill A, McBride A, Sawyer AW, Clumeck N, Gupta RK. **Resistance at virological failure using boosted protease inhibitors versus nonnucleoside reverse transcriptase inhibitors as first-line antiretroviral therapy: implications for sustained efficacy of ART in resource-limited settings.** *J Infect D* 2013; **207**:S78–84.
12. Fox MP, Van Cutsem G, Giddy J, Maskew M, Keiser O, Prozesky H, *et al*. **Rates and predictors of failure of first-line antiretroviral therapy and switch to second-line ART in South Africa.** *J Acquir Immune Defic Syndr* 2012; **60**:428–437.
13. Muwonga J, Edidi S, Butel C, Vidal N, Monleau M, Okenge A, *et al*. **Resistance to antiretroviral drugs in treated and drug-naive patients in the Democratic Republic of Congo.** *J Acquir Immune Defic Syndr* 2011; **57**:S27–S33.
14. Barth RE, van der Loeff MF, Schuurman R, Hoepelman AI, Wensing AM. **Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review.** *Lancet Infect Dis* 2010; **10**:155–166.
15. Lima VD, Gill VS, Yip B, Hogg RS, Montaner J, Harrigan PR. **Increased resilience to the development of drug resistance with modern boosted protease inhibitor-based highly active antiretroviral therapy.** *J Infect Dis* 2008; **198**:51–58.
16. Nomthandazo Dlamini J, Hu Z, Ledwaba J, Morris L, Maldarelli FM, Dewar RL, *et al*. **Genotypic resistance at viral rebound among patients who received lopinavir/ritonavir-based or efavirenz-based first antiretroviral therapy in South Africa.** *J Acquir Immune Defic Syndr* 2011; **58**:304–308.

17. Mtambo A, Chan K, Shen A, Lima V, Hogg R, Montaner J, *et al*. **Treatment limitations imposed by antiretroviral drug resistance mutations: implication for choices of first line regimens in resources-limited settings.** *HIV Med* 2012; **13**:141–147.
18. Wallis C, Mellors J, Venter W, Sanne I, Stevens W. **Varied patterns of HIV-1 drug resistance on failing first-line antiretroviral therapy in South Africa.** *J Acquir Immune Defic Syndr* 2010; **53**:480–484.
19. Sunpath H, Wu B, Gordon M, Hampton J, Johnson B, Moosa MY, *et al*. **High rate of K65R for antiretroviral therapy-naive patients with subtype C HIV infection failing a tenofovir-containing first-line regimen.** *AIDS* 2012; **26**:1679–1684.
20. Gupta R, Hill A, Sawyer A, Pillay D. **Emergence of drug resistance in HIV type 1-infected patients after receipt of first-line highly active antiretroviral therapy: a systematic review of clinical trials.** *Clin Infect Dis* 2008; **47**:712–722.
21. Van Zyl G, Liu T, Claassen M, Engelbrecht S, Oliveira T, Wood W. **Trends in genotypic HIV-1 antiretroviral resistance between 2006 and 2012 in South African patients receiving first- and second-line antiretroviral treatment.** *PLoS One* 2013; **8**:e67188.
22. Hamers RL, Sigaloff KCE, Wensing AM, Wallis CL, Kityo C, Siwale M, *et al*. **Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies.** *Clin Infect Dis* 2012; **54**:1660–1669.
23. Hosseinipour M, van Oosterhout J, Weigel R, Phiri S, Kamwendo D, Parkin N, *et al*. **The public health approach to identify antiretroviral therapy failure: high-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy.** *AIDS* 2009; **23**:1127–1134.
24. Aghokeng A, Monleau M, Eymard-Duvernay S, Kiana D, Ngo-Giang-Huong N, Toni TD, *et al*. **Extraordinary heterogeneity of virological outcomes in patients receiving highly antiretroviral therapy and monitored with the World Health Organisation public health approach in Sub-Saharan Africa and Southeast Asia.** *Clin Infect Dis* 2014; **58**:99–109.
25. De Beaudrap P, Thiam M, Diouf A, Toure-Kane C, Ngom-Guèye NF, Vidal N, *et al*. **Risk of virological failure and drug resistance during first and second-line antiretroviral therapy in a 10-year cohort in Senegal: results from the ANRS 1215 Cohort.** *J Acquir Immune Defic Syndr* 2013; **62**:381–387.
26. Messou E, Chaix M, Gabillard M-L, Yapo V, Toni TD, Minga A, *et al*. **Increasing rate of TAMs and Etravirine resistance in HIV-1-infected adults between 12 and 24 months of treatment: the VOLTART Cohort Study in Côte d'Ivoire, West Africa.** *J Acquir Immune Defic Syndr* 2013; **64**:211–219.
27. Sigaloff K, Hamers R, Wallis C, Kityo C, Siwale M, Ive P, *et al*. **Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa.** *J Acquir Infect Defic Syndr* 2011; **58**:23–31.
28. Ford N, Roberts T, Calmy A. **Viral load monitoring in resources-limited settings: a medical and public health priority.** *AIDS* 2012; **26**:1719–1720.
29. Alibhai A, Kipp W, Saunders LD, Senthilselvan A, Kaler A, Houston S, *et al*. **Gender related mortality for HIV-infected patients on highly active antiretroviral therapy (HAART) in rural Unganda.** *Int J Womens Health* 2010; **2**:45–52.
30. Druyts E, Dybul M, Kanters S, Nachega J, Birungi J, Ford N, *et al*. **Male sex and the risk of mortality among individuals enrolled in antiretroviral treatment programs in Africa: a systematic review and meta-analysis.** *AIDS* 2013; **27**:417–425.
31. Johannessen A. **Are men the losers of the antiretroviral treatment scale-up?** *AIDS* 2011; **25**:1225–1226.
32. The HIV-CAUSAL Collaboration. **The effect of Efavirenz versus Nevirapine-containing regimens on immunologic, virologic and clinical outcomes in a prospective observational study.** *AIDS* 2012; **26**:1691–1705.
33. Riddler SA, Haubrich R, DiRienzo G, Peeples L, Powderly WG, Klingman KL, *et al*. **Class-sparing regimens for initial treatment of HIV-1 infection.** *N Engl J Med* 2008; **358**:2095–2106.
34. Bennett DE, Jordan MR, Bertagnolio S, Hong S, Ravasi G, McMahon JH, *et al*. **HIV drug resistance early warning indicators in cohorts of individuals starting antiretroviral therapy between 2004 and 2009: World Health Organization Global Report from 50 Countries.** *Clin Infect Dis* 2012; **54 (S4)**:S280–S289.
35. Lataillade M, Chiarella J, Yang R, DeGrosky M, Uy J, Seekins D, *et al*. **Virologic failures on initial boosted-PI regimen infrequently possess low-level variants with major PI resistance mutations by ultra-deep sequencing.** *PLoS One* 2012; **7**: e30118.
36. Belloso W, Boyd MA, Cooper DA, Elliott J, Emery S, Gazzard B, *et al*. **Ritonavir-boosted lopinavir plus nucleoside or nucleotide reverse transcriptase inhibitors versus ritonavir-boosted lopinavir plus raltegravir for treatement of HIV-1 infection in adults with virological failure of a standard frist-line ART regimen (SECOND-LINE): a randomized, open-label, noninferiority study. SECOND-LINE Study Group.** *Lancet* 2013; **381**: 2091–2099.