Risk Factors and Mortality Associated With Resistance to First-Line Antiretroviral Therapy: Multicentric Cross-sectional and Longitudinal Analyses

Loretxu Pinoges, MSc, Birgit Schramm, PhD, MSc,* Elisabeth Poulet, MSc,* Suna Balkan, MBBS,† Elisabeth Szumilin, MSc,† Cecilia Ferreyra, MBBS,‡ and Mar Pujades-Rodríguez, PhD, MSc*§*

Background: Understanding the factors associated with HIV drug resistance development and subsequent mortality is important to improve clinical patient management.

Methods: Analysis of individual electronic health records from 4 HIV programs in Malawi, Kenya, Uganda, and Cambodia, linked to data from 5 cross-sectional virological studies conducted among patients receiving first-line antiretroviral therapy (ART) for ≥6 months. Adjusted logistic and Cox-regression models were used to identify risk factors for drug resistance and subsequent mortality.

Results: A total of 2257 patients (62% women) were included. At ART initiation, median CD4 cell count was 100 cells per microliter (interquartile range, 40–165). A median of 25.1 months after therapy start, 18% of patients had ≥400 and 12.4% ≥1000 HIV RNA copies per milliliter. Of 180 patients with drug resistance data, 83.9% had major resistance(s) to nucleoside or nonnucleoside reverse transcriptase inhibitors, and 74.4% dual resistance. Resistance to nevirapine, lamivudine, and efavirenz was common, and 6% had etravirine cross-resistance. Risk factors for resistance were young age (<35 years), low CD4 cell count (<200 cells/μL), and poor treatment adherence. During 4978 person-years of follow-up after virological testing (median = 31.8 months), 57 deaths occurred [rate = 1.14/100 person-years; 95% confidence interval (CI): 0.88 to 1.48]. Mortality was higher in patients with resistance (hazard ratio = 2.08; 95% CI: 1.07 to 4.07 vs. <400 copies/mL), and older age (hazard ratio = 2.41; 95% CI: 1.24 to 4.71 for ≥43 vs. ≤34 years), and lower in those receiving ART for >30 months.

Conclusions: Our findings underline the importance of optimal treatment adherence and adequate virological response monitoring and emphasize the need for resistance surveillance initiatives even in HIV programs achieving high virological suppression rates.

Key Words: HIV infection, resistance, longitudinal studies, mortality, outcome evaluation

(*J Acquir Immune Defic Syndr* 2015;68:527–535)

INTRODUCTION

Access to HIV antiretroviral therapy (ART) in sub-Saharan Africa and South-East Asia has increased considerably in the past decade. By the end of 2012, the estimated coverage in these geographical areas was 63% and 55%, respectively.[1] The widespread use of ART has greatly improved the life expectancy and quality of life of people infected with HIV in low- and middle-income countries.[2,3] The need for lifelong therapy has important implications for patients, clinicians, and program managers. Development of HIV drug resistance is frequently associated with suboptimal treatment adherence, leads to treatment failure, and compromises future treatment options, for individuals who acquired resistance as well as for those to whom resistant virus is transmitted.[4,5] Identifying the main factors associated with the development of drug resistance and associated mortality is therefore critical to design, improve, and evaluate therapeutic and program management strategies that facilitate the use and effectiveness of existing and new second- and third-line regimens. This is especially important in settings where a public health approach is adopted for the clinical management of HIV-infected patients because access to new treatment options is limited. Because most resource-constrained countries have low access to regular viral load monitoring and/or drug resistance genotyping, data on virological response to ART primarily come from research settings, or more recently, from drug resistance surveys supported by the WHO Global HIV Drug Resistance Network initiative (HIVResNet).[6,7]

To inform patient management strategies that prevent drug resistance development, we analyzed cross-sectional data from 3 sub-Saharan African countries with high HIV prevalence and from Cambodia [8–13] to identify risk factors for drug resistance to first-line ART among adult and pediatric patients. Linking these data to patient electronic health

Received for publication July 22, 2014; accepted November 21, 2014.

From the *Clinical Research, Epicentre, Paris, France; †Medical Department, Médecins Sans Frontières, Paris, France; ‡Medical Department, Médecins Sans Frontières, Barcelona, Spain; and §Department of Epidemiology and Public Health, University College London, London, United Kingdom.

Supported by Médecins Sans Frontières.

The authors have no conflicts of interest to disclose.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jaids.com).

L.P. and B.S. contributed equally to this study. M.P.-R. conceived the analyses. L.P. and M.P.-R. prepared the plan of analysis and performed the data management. L.P. performed the statistical analyses. B.S., L.P., E.P., and M.P.-R. wrote the article. All authors contributed to interpret the data and critically reviewed the article for intellectual content.

Correspondence to: Mar Pujades-Rodríguez, PhD, MSc, University College London, 222 Euston Road, London NW1 2DA, United Kingdom (e-mail: mar.pujades@ucl.ac.uk).

records (EHRs), we also assessed mortality and related risk factors during the 4 years after virological testing.

METHODS

Data Sources and Study Inclusion Criteria

Between 2004 and 2008, 8 cross-sectional virological and genotype testing studies were conducted in Médecins Sans Frontières-supported HIV programs to determine virological outcomes in patients who received ART for 6 months or more. After excluding 3 studies with unclear patient inclusion selection criteria, data from 5 studies conducted in Chiradzulu, Malawi (January to April 2004[10]); Phnom Penh, Cambodia (December 2004 to March 2005[11] and December 2006 to April 2007[13]); Arua, Uganda (November 2005 to May 2006[8,9]); and Busia, Kenya (April to September 2008[12]) were pooled (Table; see also Figure S1, Supplemental Digital Content, http://links.lww.com/QAI/A622). Adult and pediatric patients who at the time of the virological evaluation were receiving protease inhibitor (PI)-containing regimens, antiretroviral drug generally prescribed for second-line therapy (n = 45), were excluded. For those who participated in the 2 studies conducted in Cambodia (n = 309), only the data from the second study (evaluation performed after 48 months of ART use) were included. The cross-sectional data were linked to individual EHRs from the appropriate HIV programs using their unique patient identification number (2010 update). Data recorded in EHRs were prospectively collected in the HIV programs using the FUCHIA software (Follow-Up and Care of HIV Infection and AIDS, Epicentre, Paris).

Laboratory Testing Methods

Plasma HIV RNA was measured using Amplicor HIV-1 monitor v1.5 RT-PCR (Roche Diagnostics, Meylan, France),[10,11] the ANRS generic real-time PCR test,[8,9,13] or the NucliSENS EasyQ HIV version 1.2 (bioMerieux, Marcy l'Etoile, France).[12] The viral load detection thresholds were 400 RNA copies per milliliter,[8–11] 250 copies per milliliter,[13] and 50 copies per milliliter.[12] HIV-1 resistance genotyping was performed by sequencing the reverse transcriptase region from patients who had a minimal viral load of 400–5000 copies per milliliter (≥1000 in Refs. 8–10,13; ≥400 copies/mL in Ref. 11 and ≥5000 copies/mL in Ref. 14). Drug resistance mutations and level of drug resistance to nucleoside reverse transcriptase inhibitors (NRTI) and/or non-NRTI (NNRTI) drugs were interpreted using the genotypic resistance database and interpretation algorithm of the Stanford University.[15] CD4 T-cell counts were quantified using semi-automated (Cyflow counter; Partec, Münster, Germany), manual (Dynabead; Dynal Biotech SA, Compiègne, France) techniques[8–10], or flow cytometry (Facscount; Beckton Dickinson, Franklin Lakes, NJ).[11–13]

Treatment Adherence

Patient treatment adherence was assessed using a proxy indicator previously validated in our cohorts[16,17] and based on the timeliness of patient attendance to scheduled clinic visits. Briefly, the number of appointments attended with at least 1 day delay was divided by the number of months of follow-up between the date of ART initiation and the date of virological testing, and multiplied by 100. Adherence was classified as good if patients had less than 5% delayed appointments, moderate if they had between 5% and 19%, and poor if they had 20% or more delayed appointments.

Statistical Analysis

Patient characteristics and treatment outcomes were presented by the duration of follow-up on ART at the date of virological testing: 6 to <18, 18 to <30, 30 to <42, and ≥42 months. The Cuzick nonparametric test for trend across ordered groups was used to assess whether the percentage of patients with good adherence increased with higher duration of ART. The Wilcoxon rank-sum test allowed comparing median CD4 cell counts by the duration of ART. In cross-sectional analyses, multivariable logistic regression was used to investigate risk factors for resistance. In this analysis, patients without resistance mutations and those with viral load of <400 copies per milliliter were considered to have "no resistance." Those with unsuccessful genotype reactions or missing genotype data, or with viral load of 400–999 copies per milliliter were excluded from the primary analysis but were considered to have resistance in sensitivity analyses.

In longitudinal analyses, multivariable proportional Cox models were fitted to investigate the association between virological status of patients and all-cause mortality in the following 4 years and to assess associations with other individual-level factors. Patient follow-up was started at the date of virological testing and was censored at the earliest of the following dates: death, last clinic visit or 4 years. The virological status of patients was classified into 4 categories: undetectable viral load, viral load detectable with resistance, viral load detectable without resistance, and viral load detectable with unknown resistance. In sensitivity analyses, patients with detectable viral load (>400 copies/mL) and unknown resistance were included in the "virological failure with resistance" subgroup. All models were adjusted for study. Factors considered as potential confounders were sex, WHO clinical stage (1 or 2 vs. 3 or 4), body mass index (BMI, <18 and ≥18.5 kg/m^2, calculated using the age- and sex-specific cutoff values proposed by Cole et al[18] for patients aged 2–18 years), and CD4 cell count at ART start (<100, ≥100 cells/μL, or missing); CD4 cell count (<200, ≥200 cells/μL, missing), and age (categorized into tertiles: ≤34, 35–42, and ≥43 years), at the date of virological testing; duration of ART (6–29 and ≥30 months), and treatment adherence. Factors associated with the outcomes (P from likelihood ratio test <0.20) were included in final models. Statistical significance was determined using 2-tailed tests, with $P < 0.05$ considered as significant. Sensitivity analyses restricted to patients with complete CD4 cell count data, and analyses excluding the 84 pediatric patients were also performed. Statistical analyses were performed in Stata 13 (Stata Corporation, College Station, TX).

Ethical Review

Ethical approval was obtained before conducting all virological studies and written informed consent had been provided by all participating patients.

RESULTS

A total of 2302 patients participated in at least 1 of the 5 virological studies, 309 of them had participated in the 2 studies conducted in Cambodia (Table 1). After excluding 45 patients who were receiving a PI-containing antiretroviral regimen, data from the remaining 2257 patients were analyzed. The majority of patients were women (61.9%, Table 2). At ART initiation, 98.0% of individuals had no history of ART use, median CD4 cell count was 100 cells per microliter [interquartile range (IQR): 40–165], 83.8% were in advanced clinical stage, and 31.7% had a BMI <18.5 kg/m^2. At the time of virological testing, patients were receiving ART for a median of 29.5 months (IQR: 13.9–44.1); 76.0% of individuals were treated with stavudine (d4T)-based regimens and 22.7% with zidovudine (AZT)-containing therapy. The proportion of patients classified with good adherence increased with longer duration of ART (trend test $P < 0.001$). The median CD4 cell count at the time of the cross-sectional evaluation was 368 cells per microliter (IQR: 241–532) and counts were higher among patients with longer duration of ART (Wilcoxon rank-sum test, $P < 0.001$).

Viral load was undetectable (<400 copies/mL) in 81.9% of patients, 78.0% in those treated for 6 to <18 months and 89.4% in patients treated for ≥ 42 months. Eighteen percent of individuals (407/2254) had detectable viral load, 280 (76%) of them had virological failure (viral load >1000 copies/mL), and 176 (43.2%) had ≥ 5000 copies per milliliter. Resistance genotyping was available for 180 patients with virological failure. Ninety-one percent of these had at least 1 mutation in the reverse transcriptase. The median number of mutations identified per patient was 3 (IQR: 2–5), ranging from 1 (12.8%) to 9 (1.8%). The most frequent NRTI-associated mutations were M184V (n = 133), T215Y (n = 23), and M41L (n = 19). Two-hundred ninety-five NNRTI-associated mutations were detected, the most frequent being K103N (n = 63), Y181C (n = 56), and V179I (n = 54). Median viral load was higher in patients who had mutations to both NRTI and NNRTI drugs than in those without dual mutations (35,495 vs. 3483 copies/mL; P from nonparametric K-sample on the equality of medians = 0.02); and it increased with higher numbers of mutations (from 3550 copies/mL in patients with no or 1 mutation to 58,647 copies/mL in those with 5–9 mutations; $P = 0.07$).

Resistance and Risk Factors

A total of 151 of the 180 patients who had virological failure and resistance genotyping results (151/180, 83.9%) had mutations conferring high-level drug resistance. One hundred thirty-four patients (74.4%) had both NNRTI and NRTI drug resistance, 2 (1.1%) had only NRTI drug-resistant virus, and 15 (8.3%) only NNRTI drug-resistant virus. The proportion of patients with drug resistance was similar regardless of ART duration. However, there was a higher

TABLE 1. Study Sites and Patient Characteristics

	Virological Study				
Study site	1	2	3	4	5
Country	Malawi	Cambodia*	Uganda	Cambodia*	Kenya
Type of setting	Rural	Urban	Rural	Urban	Rural
HIV prevalence†	11.0	0.5	6.5	0.5	6.3
Virological evaluation					
Study inclusion period	January to April 2004	December 2004 to March 2005	November 2005 to May 2006	December 2006 to April 2007	April to September 2008
Duration of ART, mo‡	≥6	24	12 and 24	48	≥12
Patient selection	Random	All patients	Random (12 mo) All patients (24 mo)	All patients	All patients
No. patients	398	346	592	349	926
Men, n (%)	123 (30.9)	199 (57.5)	229 (38.7)	212 (60.7)	303 (32.7)
Median age, yrs (IQR)	36 (30–42)	36 (32–40)	37 (30–43)§	38 (35–43)	42 (35–48)
Median duration of ART, mo (IQR)	10 (7–15)	24 (23–24)	22 (11–23)	48 (47–49)	39 (34–45)
PI-based regimen, n (%)	5 (1.3)	12 (3.5)	2 (0.3)	36 (10.3)	0
Patients included in analyses					
No. patients	393	35	590	313	926
Median duration of follow-up after virological testing, mo (IQR)	80 (39–81)	58 (54–58)	56 (53–58)	32 (30–33)	8 (6–11)

*Three hundred nine patients participated in the 2 studies conducted in Cambodia.
†Source, UNAIDS prevalence estimates in 15- to 49-year-old population, 2009.
‡Survey inclusion criteria.
§This study included 84 children (median age = 7 years; IQR, 2–13).

TABLE 2. Patient Characteristics by Duration of ART at the Time of the Virological Evaluation

		Duration of ART at Virological Testing (mo)			
	All Patients	**6 to <18**	**18 to <30**	**30 to <42**	**≥42**
Patients, n (%)	2257 (100)	611 (27.1)	527 (23.3)	468 (20.7)	651 (28.8)
Women, n (%)	1398 (61.9)	410 (72.9)	325 (61.7)	317 (79.3)	346 (53.1)
Characteristics at ART initiation					
Median age, yrs (IQR)	35.7 (30.4–42.1)	34.8 (28.1–41.1)	35.5 (29.9–41.8)	39.1 (32.1–45.1)	35.5 (30.9–40.7)
Clinical stage 3 or 4, n (%)	1858 (83.8)	506 (86.5)	433 (83.1)	362 (77.7)	557 (86.5)
Median CD4 cell count, cells/μL (IQR)*	100 (40–165)	114 (64–172)	112 (48–171)	134 (66–189)	57 (11–133)
BMI <18.5 kg/m²†, n (%)	704 (31.7)	201 (34.0)	155 (29.9)	115 (24.6)	233 (36.3)
Characteristics at virological evaluation					
Median age, yrs (IQR)	39 (33–45)	36 (29–42)	38 (32–44)	42 (35–48)	39 (35–45)
ART regimen, n (%)					
3TC d4T NVP	1601 (70.9)	530 (86.7)	390 (74.0)	385 (82.3)	296 (45.5)
3TC AZT NVP	379 (16.8)	29 (4.8)	88 (16.7)	72 (15.4)	190 (29.2)
3TC AZT EFV	132 (5.9)	10 (1.6)	17 (3.2)	4 (0.8)	101 (15.5)
3TC d4T EFV	116 (5.1)	42 (6.9)	27 (5.1)	7 (1.5)	40 (6.1)
Other	29 (1.3)	0	5 (0.9)	0	24 (3.7)
Treatment adherence, n (%)					
Good	1319 (58.6)	222 (36.6)	264 (50.5)	301 (64.3)	532 (81.7)
Moderate	786 (34.9)	288 (47.5)	224 (42.8)	159 (34.0)	115 (17.7)
Poor	144 (6.4)	97 (16.0)	35 (6.7)	8 (1.7)	4 (0.6)
Median CD4 cell count, cells/μL* (IQR)	368 (241–532)	253 (175–396)	288 (192–413)	473 (323–615)	436 (313–580)
Plasma HIV RNA, copies/mL	n = 2254	n = 610	n = 527	n = 466	n = 651
<400	1847 (81.9)	476 (78.0)	400 (75.9)	389 (83.5)	582 (89.4)
400–999	127 (5.6)	48 (7.9)	41 (7.8)	24 (5.1)	14 (2.1)
1000–4999	104 (4.6)	36 (5.9)	23 (4.4)	25 (5.4)	20 (3.1)
≥5000	176 (7.8)	50 (8.2)	63 (11.9)	28 (6.0)	35 (5.4)
Genotype testing results, n (%)	n = 180	n = 79	n = 71	n = 10	n = 20
≥1 mutation	164 (91.1)	70 (88.6)	67 (94.4)	10 (100.0)	17 (85.0)
>1 mutation	143 (87.2)	59 (74.7)	58 (86.6)	9 (90.0)	17 (85.0)
Median no. mutations per patient (IQR)	3 (2–5)	3 (2–4)	4 (2–5)	4 (3–7)	4 (3–6)
Major drug resistance, n (%)	151 (83.9)	66 (83.5)	59 (83.1)	9 (90.0)	17 (85.0)
Only NRTI resistance	2 (1.1)	0	1 (1.4)	0	1 (5.0)
Only NNRTI resistance	15 (8.3)	12 (15.2)	3 (4.2)	0	0
NRTI and NNRTI resistance	134 (74.4)	54 (68.4)	55 (77.5)	9 (90.0)	16 (80.0)
TAMs	46 (25.6)	5 (63.3)	24 (33.8)	5 (50.0)	12 (60.0)

Patients with missing clinical stage (n = 41), BMI (n = 38), treatment adherence (n = 8), CD4 cell count (n = 399 at ART start; n = 355 at virological testing).
*Among patients aged >5 years.
†Among patients aged >2 years.
3TC, lamivudine; AZT, zidovudine; d4T, stavudine; EFV, efavirenz; NRTI, nucleoside reverse transcriptase inhibitor drug; NNRTI, non-NRTI drug; NVP, nevirapine.

percentage of patients with only NNRTI drug resistance among those treated for 6–18 months (15.2%) than in individuals receiving therapy for longer (4.2% for 18–30 months and none after 30 months). According to the resistance interpretation algorithms of the Stanford University, 133 (73.9%) patients presented with high-level resistance to 3TC/FTC, 14 (7.8%) to abacavir, 12 (6.7%) to stavudine, 11 (6.1%) to didanosine, 9 (5.0%) to ziduvudine, and 2 (1.1%) to tenofovir. Besides, 149 (82.8%) patients had high-level resistance to nevirapine, 111 (61.6%) to delavirdine, 98 (54.4%) to efavirenz, and 11 (6.1%) to etravirine. Forty-six individuals (25.6%) had thymidine analog mutations (TAMs). The proportion of patients with at least 1 TAM was higher in those receiving AZT-containing regimens (48.4%, 15/31) than in individuals receiving stavudine-based therapy (20.9%, 31/148) at the time of virological testing (test on the equality of proportions $P = 0.002$). The median viral load was higher in patients with than without TAMs (73,785 vs. 13,806 copies/mL; $P = 0.001$).

Of 2027 patients included in the analyses of risk factors for drug resistance, 151 had high-level drug resistance and 1876 no major drug resistance (1847 with <400 HIV RNA copies/mL, and 29 with >1000 copies/mL but no genotype resistance). Adjusted analyses showed higher risk of resistance in patients who had low or moderate treatment adherence [odds ratio (OR) = 2.91, 95% confidence interval (CI): 1.60 to 5.29 for poor; and OR = 1.79, 95% CI: 1.16 to 2.76 for moderate, vs. good adherence; Table 3], and in patients with low CD4 cell count at the date of virological testing (OR = 4.60, 95% CI: 2.86 to 7.40 for <200 vs. ≥200

TABLE 3. Associations Between the Presence of Drug Resistance and Individual Patient Factors

	No. Patients With Resistance (%)	Site-Adjusted Model OR (95% CI)	Multivariable Model 1 OR (95% CI)	Multivariable Model 2 OR (95% CI)	Multivariable Model 3 OR (95% CI)
Sex		$P = 0.0710$	$P = 0.1313$		$P = 0.0872$
Women	83 (6.7)	1	1		1
Men	68 (8.6)	1.38 (0.97 to 1.95)	1.34 (0.92 to 1.94)		1.57 (0.94 to 2.65)
Factors measured at ART start					
Clinical stage		$P = 0.7784$			
1 or 2	22 (6.9)	1			
3 or 4	124 (7.4)	0.93 (0.57 to 1.52)			
BMI, kg/m^2		$P = 0.8759$			
<18.5	49 (7.8)	1			
≥18.5	100 (7.3)	1.03 (0.71 to 1.49)			
CD4 cell count, cells/μL		$P < 0.0001$	$P = 0.0121$	$P = 0.12$	$P = 0.1766$
≥100	39 (4.8)	1	1	1	1
<100	72 (8.4)	2.01 (1.32 to 3.05)	1.43 (0.92 to 2.24)	0.91 (0.69 to 1.19)	1.50 (0.83 to 2.71)
Unknown	40 (11.4)	2.97 (1.85 to 4.78)	2.29 (1.37 to 3.82)	1.28 (0.94 to 1.75)	—
Factors measured at virological evaluation					
Duration of ART, mo		$P = 0.665$			
6–29	125 (12.2)	1			
≥30	26 (2.6)	0.80 (0.29 to 2.18)			
Age, yrs		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
≤34	93 (14.5)	1	1	1	1
35–42	29 (4.1)	0.32 (0.20 to 0.49)	0.31 (0.19 to 0.49)	0.52 (0.39 to 0.70)	0.19 (0.10 to 0.36)
≥43	29 (4.2)	0.38 (0.24 to 0.60)	0.37 (0.23 to 0.60)	0.63 (0.47 to 0.83)	0.23 (0.12 to 0.44)
CD4 cell count, cells/μL		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
≥200	47 (3.3)	1	1	1	1
<200	55 (19.6)	4.26 (2.73 to 6.64)	4.60 (2.86 to 7.40)	2.55 (1.85 to 3.51)	5.20 (2.92 to 9.28)
Unknown	49 (14.4)	6.10 (2.61 to 14.27)	3.26 (1.48 to 7.18)	2.20 (1.13 to 4.27)	—
Treatment adherence		$P = 0.0003$	$P = 0.0011$	$P < 0.0001$	$P = 0.0016$
Good	52 (4.3)	1	1	1	1
Moderate	74 (10.7)	1.89 (1.25 to 2.87)	1.79 (1.16 to 2.76)	1.40 (1.08 to 1.81)	2.75 (1.48 to 5.09)
Poor	25 (19.8)	3.03 (1.70 to 5.41)	2.91 (1.60 to 5.29)	2.48 (1.60 to 3.84)	3.92 (1.39 to 11.02)

Model 1 corresponds to estimates from the primary analysis excluding patients with unsuccessful genotype reactions or missing genotype results; model 2 corresponds to estimates from models in which patients without genotype results and those with viral load of 400–999 copies per milliliter were classified as having drug resistance; model 3 corresponds to estimates from analyses restricted to patients with complete CD4 cell count data; OR, odds ratio. Multivariable analyses are adjusted for sex, age, CD4 cell counts (at ART start and at the date of virological testing) and treatment adherence.

cells/μL). A lower risk of resistance was seen in older patients (OR = 0.31, 95% CI: 0.19 to 0.49 for the 35 to 42-year group; and OR = 0.37, 95% CI: 0.23 to 0.60 for the ≥43-year group, vs. <35 years). Although the risk of resistance was higher in patients with CD4 count <100 cells per microliter at ART start, the estimate was not statistically significant. Results from sensitivity analyses classifying patients without genotype results and those with viral load of 400–999 copies per milliliter as having resistance, those restricted to patients with complete CD4 cell count data, and those excluding pediatric patients were consistent.

Mortality and Risk Factors

Fifty-seven deaths were recorded during the 4978 person-years of study follow-up after virological testing (see Table S1, Supplemental Digital Content, http://links.lww.com/QAI/A622). The overall mortality rate was 1.14 per 100 person-years (95% CI: 0.88 to 1.48) and was higher during the second and third years of follow-up. Mortality was lower in patients who were receiving ART for longer periods at the time of virological testing [rates = 0.82 vs. 1.27 per 100 person-years; hazard ratio (HR) = 0.24, 95% CI: 0.09 to 0.67 for >30 vs. 6–30 months; Table 4]. In contrast, mortality was higher in men than in women (rates = 1.76 vs. 0.76 per 100 person-years; HR = 2.48, 95% CI: 1.42 to 4.32), and increased with age (HR = 1.20, 95% CI: 0.60 to 2.41 for 35–42 years; and HR = 2.41, 95% CI: 1.24 to 4.71 for ≥43 vs. <35 years). Compared with patients with undetectable viral load, mortality was higher among patients with detectable viral load and drug resistance (rates 2.42 vs. 1.02 per 100 person-years; HR = 2.08, 95% CI: 1.07 to 4.07), and higher but not statistically significant for patients with virological failure without resistance (rate = 1.15 per 100 person-years;

TABLE 4. Association Between Virological Outcome and Individual Patient Factors, and All-Cause Mortality

	No. Deaths	Death Rate per 100 PY (95% CI)	Site-Adjusted Model HR (95% CI)	Multivariable Model 1 HR (95% CI)	Multivariable Model 2 HR (95 CI)	Multivariable Model 3 HR (95% CI)
Factors measured at ART start						
Sex			$P = 0.0005$	$P = 0.0012$	$P = 0.009$	$P = 0.2000$
Women	23	0.76 (0.50 to 1.14)	1	1	1	1
Men	34	1.76 (1.26 to 2.46)	2.59 (1.51 to 4.45)	2.48 (1.42 to 4.32)	2.52 (1.44 to 4.39)	1.70 (0.75 to 3.86)
Clinical stage			$P = 0.5444$			
1 or 2	6	0.87 (0.39 to 1.93)	1			
3 or 4	50	1.20 (0.91 to 1.58)	1.29 (0.55 to 3.02)			
BMI, kg/m^2			$P = 0.2974$			
<18.5	21	1.24 (0.81 to 1.90)	1			
≥18.5	33	1.03 (0.73 to 1.45)	0.74 (0.43 to 1.29)			
CD4 cell count, cells/μL			$P = 0.1349$	$P = 0.2475$	$P = 0.2120$	$P = 0.3828$
≥100	16	0.79 (0.48 to 1.29)	1	1	1	1
<100	28	1.29 (0.89 to 1.86)	1.62 (0.85 to 3.07)	1.31 (0.68 to 2.53)	1.34 (0.69 to 2.59)	1.54 (0.58 to 4.08)
Unknown	13	1.67 (0.97 to 2.88)	2.02 (0.97 to 4.19)	1.94 (0.91 to 4.18)	2.01 (0.69 to 2.59)	—
Factors measured at virological evaluation						
Virological status			$P = 0.0797$	$P = 0.2355$	$P = 0.1868$	$P = 0.5671$
Undetectable viral load	40	1.02 (0.74 to 1.38)	1	1	1	1
Virological failure with resistance	12	2.42 (1.37 to 4.26)	2.55 (1.32 to 4.93)	2.08 (1.07 to 4.07)	1.79 (0.97 to 3.33)	1.75 (0.55 to 5.51)
Virological failure without resistance	1	1.15 (0.16 to 8.15)	1.91 (0.26 to 14.20)	1.85 (0.25 to 13.91)	1.85 (0.25 to 13.94)	—
Detectable viral load with unknown resistance	3	0.66 (0.21 to 2.05)	1.10 (0.33 to 3.72)	1.14 (0.34 to 3.84)	—	1.57 (0.43 to 5.67)
Age, yrs			$P = 0.0190$	$P = 0.0221$	$P = 0.0254$	$P = 0.0428$
≤34	16	0.84 (0.51 to 1.36)	1	1	1	1
35–42	17	0.99 (0.61 to 1.59)	1.20 (0.60 to 2.39)	1.20 (0.60 to 2.41)	1.18 (0.59 to 2.38)	1.24 (0.38 to 4.00)
≥43	24	1.79 (1.20 to 2.67)	2.46 (1.29 to 4.68)	2.41 (1.24 to 4.71)	2.01 (1.44 to 4.39)	3.09 (1.08 to 8.83)
Duration of ART, mo			$P = 0.0189$	$P = 0.0095$	$P = 0.009$	$P = 0.0110$
6–30	46	1.27 (0.95 to 1.69)	1	1	1	1
>30	11	0.82 (0.45 to 1.47)	0.28 (0.10 to 0.75)	0.24 (0.09 to 0.67)	0.24 (0.09 to 0.66)	0.21 (0.07 to 0.65)
Treatment adherence			$P = 0.7842$			
Good	31	1.17 (0.82 to 1.66)	1			
Moderate	19	1.03 (0.66 to 1.62)	0.80 (0.43 to 1.51)			
Poor	7	1.44 (0.69 to 3.03)	0.83 (0.34 to 2.02)			
CD4 cell count, cells/μL			$P = 0.0054$	$P = 0.1210$	$P = 0.1052$	$P = 0.1846$
≥200	17	0.59 (0.37 to 0.95)	1	1	1	1
<200	12	1.34 (0.76 to 2.35)	3.21 (1.46 to 7.07)	2.09 (0.93 to 4.73)	2.15 (0.95 to 4.83)	1.84 (0.76 to 4.48)
Unknown	18	2.32 (1.61 to 3.37)	4.32 (1.19 to 15.69)	2.27 (0.73 to 6.99)	2.31 (0.74 to 7.20)	—

Model 1 corresponds to estimates from the primary analysis; model 2 corresponds to estimates from models in which patients with detectable viral load and unknown resistance were classified as having virological failure with resistance; model 3 correspond to estimates from analyses restricted to patients with complete CD4 cell count data; PY, person-years of follow-up. Multivariable analyses are adjusted for sex, age, CD4 cell counts (at ART start and at the date of virological testing), duration of ART, and virological status.

HR = 1.85, 95% CI: 0.25 to 13.91). However, the number of deaths in the last group was very small, and these results should be interpreted with caution. Sensitivity analyses classifying patients with detectable viral load and unknown resistance as having "virological failure with resistance" showed similar results although associations with virological status did not reach statistical significance. Analyses excluding patients with missing CD4 count data showed also consistent results, but associations with sex and virological status were no longer significant. Results from analyses excluding pediatric patients were consistent.

Antiretroviral Regimen Modification in Patients Diagnosed With Resistance

After virological testing, the 151 patients presenting with resistance mutations were followed for a median time of 56.8 months (IQR: 32.7–58.7). One hundred forty-two of them changed their antiretroviral regimen: 123 initiated second-line PI-based therapy (79 with lopinavir and 44 with nelfinavir), a median of 6.4 months after the date of virological testing, 18 changed only the NRTI component of the regimen, and 1 replaced nevirapine by efavirenz. Median time to second-line start was similar regardless of the

CD4 count level at the time of the virological evaluation [3.6 vs. 3.8 months for patients with <200 (n = 45, 81.8%) and ≥200 cells/μL (n = 36, 76.6%), respectively] but was higher in patients with unknown CD4 cell count [median = 6.2 months, IQR: 7.4–8.7, (n = 42, 85.7%)]. Of patients who did not change their antiretroviral regimen (n = 9), 1 died after 13.2 months of follow-up, 4 were lost to follow-up after a median of 3.1 months (IQR: 1.2–18.6), 1 was transferred outside the program after 20.6 months, and 1 was still followed after 80.9 months. Two patients interrupted their regimen for noncompliance and restarted it after 14 and 26 days, respectively (1 died).

DISCUSSION

The objective of this multicentric analysis was to assess factors associated with HIV drug resistance development and subsequent mortality among patients treated with first-line ART in 3 sub-Saharan African countries and in Cambodia. Characteristics of study participants were typical of individuals treated and followed in HIV programs in resource-limited countries, mostly starting therapy at an advanced stage of HIV disease and without previous exposure to ART. In each site, good long-term virological outcomes were achieved,[8–13] and globally, 88% of all patients had <1000 copies per milliliter after a median treatment duration of 29.5 months. This percentage meets the recently established HIV drug resistance surveillance target for patients treated for 1 year (minimum of 70%, ideally ≥85%[19]). Consistent with findings from sites in sub-Saharan Africa and Thailand,[20–30] NNRTI and NRTI drug resistance prevalence was high among individuals diagnosed with virological failure, and the majority presented with dual-resistance to NNRTI and NRTI drugs. The most common resistances detected were to the most frequently prescribed drugs, lamivudine and nevirapine (73.9% and 82.8% of patients, respectively); and TAMs, known to potentially lead to resistance to a wider range of NRTIs, were frequent. Cross-resistance to the NNRTI etravirine was also detected, as found in other studies conducted in Sub-Saharan Africa.[20,22,23,29,31] Globally, the results of this multicentric study show that most of the drugs affected by resistance are key components of currently recommended first- or second-line regimens, and that cross-resistance to new generation NNRTIs, such as etravirine, may compromise third-line salvage regimens.

The findings presented are based on the analyses of data collected at a time when no routine access to viral load testing was available in the HIV programs. All patients who started ART routinely received 3 counseling sessions before ART initiation and those who came late for their appointments received enhanced counseling in which the importance of both taking treatment and attending follow-up visits on time were discussed, as well as the reasons for late attendance and potential solutions to prevent treatment interruptions (eg, increase of refilling period when travel was planned). Identification of patients with treatment failure was based on clinical and CD4 count monitoring, which are known to be relatively inefficient medical monitoring tools.[32,33] It is therefore likely that a number of patients with resistance were receiving a failing regimen for a considerable length of time before the cross-sectional virological assessment. Prolonged treatment with a failing regimen is known to increase the likelihood of multiclass drug resistance, and higher prevalence of NRTI and NNRTI resistance has been described in less frequently monitored patient cohorts.[34]

HIV drug resistance development is influenced by multiple factors, including virus characteristics, genetic resistance barrier of drugs, and patient- and/or program-level factors.[4,35] Suboptimal adherence is an important determinant of virological treatment response and virological failure,[4,5,8,16,17,24,27,36–38] and has been reported to be associated with drug resistance development.[24,36,39,40] In our analysis, a recently validated indicator based on the proportion of missed clinic visits since ART initiation was used as a proxy for patient adherence.[16] Most patients were classified as moderately or well-adherent patients, and those with moderate or poor adherence were 2–3 times more likely to have drug resistance than patients with good adherence. Similar to a recent study in Senegal,[36] we found that younger patients were more likely to have drug resistance. Low CD4 cell count at failure diagnosis was also associated with drug resistance, which is likely explained by immune system deterioration of patients treated with a failing regimen for a longer time. Globally, our findings highlight the need for routine virological monitoring of patients treated with ART, and for strengthening adherence and patient support, especially in younger adults.

The overall death rate during the 4 years after the virological evaluation was low, 1.14 deaths per 100 person-years after a median follow-up time of 31.8 months. Similar to the findings of a recent study in China,[41] we found some evidence that patients with detectable viral load and drug resistance had an increased risk of mortality. Limitations of our analysis were the relatively small number of patients with virological failure, the availability of resistance genotype results for about fifty percent of patients, and the absence of information about the reasons for delays in switching. Further studies comparing survival outcomes between patients with virological failure with or without drug resistance are needed to confirm our results. Notably, increased mortality among patients with failure and drug resistance may also be a consequence of delayed failure diagnosis,[32] or to delayed switch despite of failure diagnosis. In study sites, results of viral load testing were sent to the medical coordinators of each project who then communicated the information to the health care providers so as to adapt patient management according to program protocols. Clinicians were often reluctant to switch patients with first-line failure to second-line therapy unless barriers to adherence were satisfactorily addressed, given the higher complexity of second-line regimens and cost (eg, higher pill burden and absence of further options for treatment). Previous studies showed that suboptimal adherence was strongly associated with mortality after ART start.[38,42–44] In this study, moderate or poor adherence was associated with drug resistance at virological failure, but we found no evidence of association between suboptimal adherence and mortality. A likely explanation is that our adherence indicator was calculated for the time between ART initiation and time of virological assessment (ie, failure and/or resistance detection) and the extent of adherence to therapy might have changed in

the following years of treatment. In line with studies in Ethiopia[42] and Cameroon,[45] we noted a 3-fold increased mortality among patients with higher age (≥43 years at virological assessment), higher mortality risk in men, and a significantly lower mortality for patients receiving ART for >30 months. The decline in mortality with time on ART has been described in previous studies.[46–48] Globally, these findings emphasize the need to adopt targeted mortality prevention measures during ART follow-up, with special focus on the first years of ART, older patients, and men. Mathematical models using data from Malawi, Zambia, and South Africa, suggest that use of routine viral load monitoring during the first 5 years of ART will significantly reduce mortality under the assumption that improved adherence is achieved.[49]

This multicentric study used data collected in HIV programs providing ART to patients for over a decade in 4 African countries and in an urban setting in Cambodia. Patient monitoring strategies implemented by Médecins sans Frontières were similar in all programs and analyses were adjusted for study to take into account site-specific characteristics. Cross-sectional virolological data were linked to EHRs prospectively collected to study risk factors for resistance and to assess mortality up to 4 years after the virological assessment, and factors associated with death. Other limitations of the analyses not mentioned before include the fact that changes in the virological status of patients could not be studied because viral load and resistance testing were not routinely implemented. Furthermore, it was not possible to distinguish between HIV-related and unrelated death from routine monitoring data. It can also not be excluded that some of the patients recorded as lost to follow-up after the virological assessment (n = 116) were actual deaths, and this misclassification of deaths may have introduced bias in the mortality risk analysis.[50,51] Despite adjustment for factors known to be major confounders for treatment failure and mortality, such as clinical stage and CD4 cell count level, residual confounding by factors not available in the data sets (eg, baseline hemoglobin) cannot be excluded.

CONCLUSIONS

Resistance development remains a major challenge, even in HIV programs with good treatment response rates. Our findings emphasize that a key element of the management of patients receiving ART is ensuring continuous adherence support and monitoring, and that special attention should be given to younger patients. They also highlight the importance of promptly diagnosing and treating patients with virological failure and drug resistance. Current initiatives to increase access to routine viral load monitoring are expected to support clinical decision making and adherence, and thereby delay and limit the development of treatment-emergent resistance and improve long-term treatment outcomes.

ACKNOWLEDGMENTS

The authors thank the epidemiologists who conducted the cross-sectional evaluations in the field, the national and international MSF staff working in the projects, and their partners in the ministries of health of the participating sites for their support. They also thank Jihane Ben-Farhat for statistical support with sensitivity analyses.

REFERENCES

1. WHO, UNICEF, UNAIDS. *Global Update on HIV Treatment 2013: Results, Impact and Opportunities*. Geneva, Switzerland: WHO Document Production Services; 2013.
2. Johnson LF, Mossong J, Dorrington RE, et al. Life expectancies of South African adults starting antiretroviral treatment: collaborative analysis of cohort studies. *PLoS Med.* 2013;10:e1001418.
3. Mills EJ, Bakanda C, Birungi J, et al. Life expectancy of persons receiving combination antiretroviral therapy in low-income countries: a cohort analysis from Uganda. *Ann Intern Med.* 2011;155:209–216.
4. Bertagnolio S, De LA, Vitoria M, et al. Determinants of HIV drug resistance and public health implications in low- and middle-income countries. *Antivir Ther.* 2012;17:941–953.
5. Stadeli KM, Richman DD. Rates of emergence of HIV drug resistance in resource-limited settings: a systematic review. *Antivir Ther.* 2013;18: 115–123.
6. Bennett DE, Bertagnolio S, Sutherland D, et al. The World Health Organization's global strategy for prevention and assessment of HIV drug resistance. *Antivir Ther.* 2008;13(suppl 2):1–13.
7. Jordan MR, Bennett DE, Wainberg MA, et al. Update on World Health Organization HIV drug resistance prevention and assessment strategy: 2004-2011. *Clin Infect Dis.* 2012;54(suppl 4):S245–S249.
8. Ahoua L, Guenther G, Pinoges L, et al. Risk factors for virological failure and subtherapeutic antiretroviral drug concentrations in HIV-positive adults treated in rural northwestern Uganda. *BMC Infect Dis.* 2009;9:81.
9. Ahoua L, Guenther G, Rouzioux C, et al. Immunovirological response to combined antiretroviral therapy and drug resistance patterns in children: 1- and 2-year outcomes in rural Uganda. *BMC Pediatr.* 2011;11:67.
10. Ferradini L, Jeannin A, Pinoges L, et al. Scaling up of highly active antiretroviral therapy in a rural district of Malawi: an effectiveness assessment. *Lancet*. 2006;367:1335–1342.
11. Ferradini L, Laureillard D, Prak N, et al. Positive outcomes of HAART at 24 months in HIV-infected patients in Cambodia. *AIDS*. 2007;21: 2293–2301.
12. Ferreyra C, Yun O, Eisenberg N, et al. Evaluation of clinical and immunological markers for predicting virological failure in a HIV/AIDS treatment cohort in Busia, Kenya. *PLoS One*. 2012;7:e49834.
13. Pujades-Rodriguez M, Schramm B, Som L, et al. Immunovirological outcomes and resistance patterns at 4 years of antiretroviral therapy use in HIV-infected patients in Cambodia. *Trop Med Int Health*. 2011 Feb;16 (2):205–13 2010.
14. Arnedo M, Alonso E, Eisenberg N, et al. Monitoring HIV viral load in resource limited settings: still a matter of debate? *PLoS One*. 2012;7: e47391.
15. Stanford University. *HIV Drug Resistance Database*: Stanford University. Available at: http://hivdb.stanford.edu. Accessed on February 2, 2015.
16. Bastard M, Pinoges L, Balkan S, et al. Timeliness of clinic attendance is a good predictor of virological response and resistance to antiretroviral drugs in HIV-infected patients. *PLoS One*. 2012;7:e49091.
17. Pujades-Rodriguez M, Balkan S, Arnould L, et al. Treatment failure and mortality factors in patients receiving second-line HIV therapy in resource-limited countries. *JAMA*. 2010;304:303–312.
18. Cole TJ, Flegal KM, Nicholls D, et al. Body mass index cut offs to define thinness in children and adolescents: international survey. *BMJ*. 2007; 335:194.
19. World Health Organization (WHO). *Global Strategy for the Surveillance and Monitoring of HIV Drug Resistance*. 2012. http://www.who.int/hiv/pub/drugresistance/drug_resistance_strategy/en/.
20. Aghokeng AF, Kouanfack C, Eymard-Duvernay S, et al. Virological outcome and patterns of HIV-1 drug resistance in patients with 36 months' antiretroviral therapy experience in Cameroon. *J Int AIDS Soc.* 2013;16:18004.
21. Barth RE, van der Loeff MF, Schuurman R, et al. Virological follow-up of adult patients in antiretroviral treatment programmes in

sub-Saharan Africa: a systematic review. *Lancet Infect Dis*. 2010;10: 155–166.
22. Dagnra AY, Vidal N, Mensah A, et al. High prevalence of HIV-1 drug resistance among patients on first-line antiretroviral treatment in Lome, Togo. *J Int AIDS Soc.* 2011;14:30.
23. El-Khatib Z, DeLong AK, Katzenstein D, et al. Among HIV-1 subtype C infected patients receiving non-nucleoside reverse transcriptase inhibitors in South Africa. *J AIDS Clin Res.* 2011;2:117.
24. El-Khatib Z, Ekstrom AM, Ledwaba J, et al. Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa. *AIDS*. 2010;24:1679–1687.
25. Hamers RL, Sigaloff KC, Wensing AM, 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.
26. Hosseinipour MC, Gupta RK, Van ZG, et al. Emergence of HIV drug resistance during first- and second-line antiretroviral therapy in resource-limited settings. *J Infect Dis.* 2013;207(suppl 2):S49–S56.
27. Khienprasit N, Chaiwarith R, Sirisanthana T, et al. Incidence and risk factors of antiretroviral treatment failure in treatment-naive HIV-infected patients at Chiang Mai University Hospital, Thailand. *AIDS Res Ther.* 2011;8:42.
28. Maldonado F, Biot M, Roman F, et al. Viraemia and HIV-1 drug resistance mutations among patients receiving antiretroviral treatment in Mozambique. *Trans R Soc Trop Med Hyg.* 2009;103:607–612.
29. Messou E, Chaix ML, Gabillard D, 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 Cote d'Ivoire, West Africa. *J Acquir Immune Defic Syndr.* 2013;64:211–219.
30. Wadonda-Kabondo N, Hedt BL, van Oosterhout JJ, et al. A retrospective survey of HIV drug resistance among patients 1 year after initiation of antiretroviral therapy at 4 clinics in Malawi. *Clin Infect Dis.* 2012;54 (suppl 4):S355–S361.
31. van Zyl GU, van der Merwe L, Claassen M, et al. Antiretroviral resistance patterns and factors associated with resistance in adult patients failing NNRTI-based regimens in the Western Cape, South Africa. *J Med Virol.* 2011;83:1764–1769.
32. Keiser O, Tweya H, Boulle A, et al. Switching to second-line antiretroviral therapy in resource-limited settings: comparison of programmes with and without viral load monitoring. *AIDS*. 2009;23:1867–1874.
33. Sigaloff KC, Hamers RL, Wallis CL, et al. Unnecessary antiretroviral treatment switches and accumulation of HIV resistance mutations; two arguments for viral load monitoring in Africa. *J Acquir Immune Defic Syndr.* 2011;58:23–31.
34. Gupta RK, Hill A, Sawyer AW, 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 Infect Dis.* 2009;9:409–417.
35. Hamers RL, Sigaloff KC, Kityo C, et al. Emerging HIV-1 drug resistance after roll-out of antiretroviral therapy in sub-Saharan Africa. *Curr Opin HIV AIDS*. 2013;8:19–26.
36. DeBeaudrap P, Thiam M, Diouf A, 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.
37. Ford N, Darder M, Spelman T, et al. Early adherence to antiretroviral medication as a predictor of long-term HIV virological suppression: five-year follow up of an observational cohort. *PLoS One*. 2010;5:e10460.
38. Nachega JB, Hislop M, Dowdy DW, et al. Adherence to highly active antiretroviral therapy assessed by pharmacy claims predicts survival in HIV-infected South African adults. *J Acquir Immune Defic Syndr.* 2006; 43:78–84.
39. Hanson DL, Adje-Toure C, Talla-Nzussouo N, et al. HIV type 1 drug resistance in adults receiving highly active antiretroviral therapy in Abidjan, Cote d'Ivoire. *AIDS Res Hum Retroviruses.* 2009;25:489–495.
40. Harrigan PR, Hogg RS, Dong WW, et al. Predictors of HIV drug-resistance mutations in a large antiretroviral-naive cohort initiating triple antiretroviral therapy. *J Infect Dis.* 2005;191:339–347.
41. Liao L, Xing H, Su B, et al. Impact of HIV drug resistance on virologic and immunologic failure and mortality in a cohort of patients on antiretroviral therapy in China. *AIDS*. 2013;27:1815–1824.
42. Hambisa MT, Ali A, Dessie Y. Determinants of mortality among HIV positives after initiating antiretroviral therapy in Western Ethiopia: a hospital-based retrospective cohort study. *ISRN AIDS.* 2013;2013:491601.
43. Kiwuwa-Muyingo S, Oja H, Walker AS, et al; Dart Trial Team. Dynamic logistic regression model and population attributable fraction to investigate the association between adherence, missed visits and mortality: a study of HIV-infected adults surviving the first year of ART. *BMC Infect Dis.* 2013;13:395.
44. Vinikoor MJ, Schuttner L, Moyo C, et al. Short communication: late refills during the first year of antiretroviral therapy predict mortality and program failure among HIV-infected adults in urban Zambia. *AIDS Res Hum Retroviruses.* 2014;30:74–77.
45. Poka-Mayap V, Pefura-Yone EW, Kengne AP, et al. Mortality and its determinants among patients infected with HIV-1 on antiretroviral therapy in a referral centre in Yaounde, Cameroon: a retrospective cohort study. *BMJ Open.* 2013;3.
46. Brinkhof MW, Boulle A, Weigel R, et al. Mortality of HIV-infected patients starting antiretroviral therapy in sub-Saharan Africa: comparison with HIV-unrelated mortality. *PLoS Med.* 2009;6:e1000066.
47. Etard JF, Ndiaye I, Thierry-Mieg M, et al. Mortality and causes of death in adults receiving highly active antiretroviral therapy in Senegal: a 7-year cohort study. *AIDS*. 2006;20:1181–1189.
48. Spillane H, Nicholas S, Tang Z, et al. Incidence, risk factors and causes of death in an HIV care programme with a large proportion of injecting drug users. *Trop Med Int Health.* 2012;17:1255–1263.
49. Estill J, Egger M, Johnson LF, et al. Monitoring of antiretroviral therapy and mortality in HIV programmes in Malawi, South Africa and Zambia: mathematical modelling study. *PLoS One*. 2013;8:e57611.
50. Brinkhof MW, Pujades-Rodriguez M, Egger M. Mortality of patients lost to follow-up in antiretroviral treatment programmes in resource-limited settings: systematic review and meta-analysis. *PLoS One*. 2009;4:e5790.
51. Henriques J, Pujades-Rodriguez M, McGuire M, et al. Comparison of methods to correct survival estimates and survival regression analysis on a large HIV African cohort. *PLoS One*. 2012;7:e31706.