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Chapter 9

Effect of pretreatment HIV-1 drug resistance on immunological, virological and drug resistance outcomes of first-line antiretroviral treatment in sub-Saharan Africa: a multicentre cohort study

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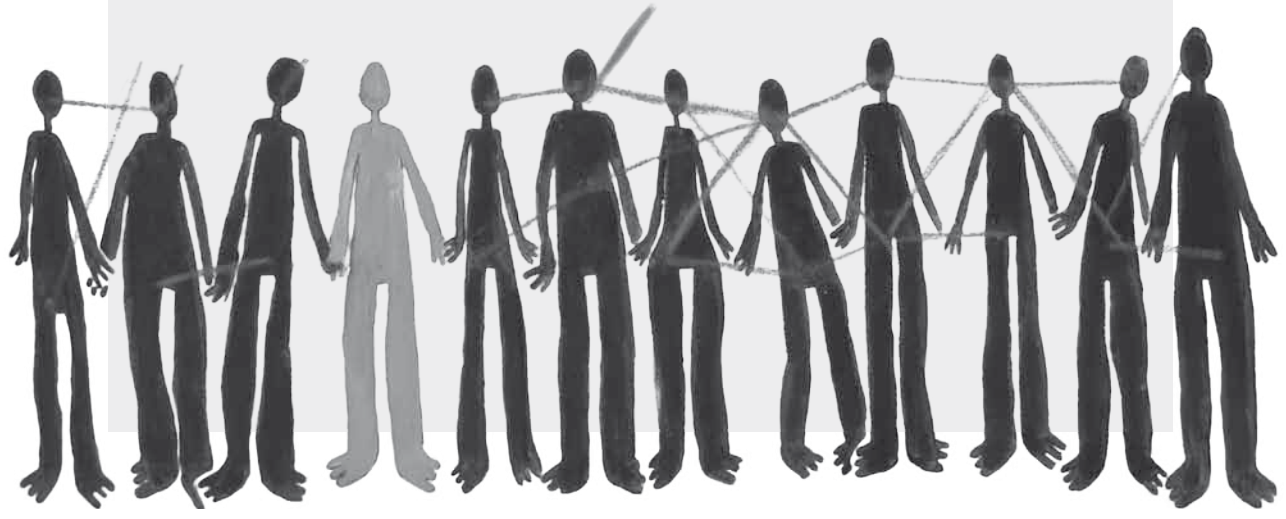


ABSTRACT

Background

The effect of pretreatment HIV-1 drug resistance on the response to first-line combination antiretroviral therapy (ART) in sub-Saharan Africa has not been assessed. We studied pretreatment drug resistance and virological, immunological, and drug resistance treatment outcomes in a large prospective cohort.

Methods

HIV-1 infected patients in the PharmAccess African Studies to Evaluate Resistance Monitoring (PASER-M) cohort started non-nucleoside reverse transcriptase inhibitor-based ART at 13 clinical sites in six countries, from 2007 to 2009. We used the International Antiviral Society-USA drug resistance mutation list and the Stanford algorithm to classify participants into three pretreatment drug resistance categories: no pretreatment drug resistance, pretreatment drug resistance with fully active ART prescribed, or pretreatment drug resistance with reduced susceptibility to at least one prescribed drug. We assessed risk factors of virological failure (≥400 copies per mL) and acquired drug resistance after 12 months of ART by use of multilevel logistic regression with multiple imputations for missing data. CD4 cell count increase was estimated with linear mixed models.

Findings

Pretreatment drug resistance results were available for 2579 (94%) of 2733 participants; 2404 (93%) had no pretreatment drug resistance, 123 (5%) had pretreatment drug resistance to at least one prescribed drug, and 52 (2%) had pretreatment drug resistance and received fully active ART. Compared with participants without pretreatment drug resistance, the odds ratio (OR) for virological failure (OR 2.13, 95% CI 1.44–3.14; p<0.0001) and acquired drug resistance (2.30, 1.55–3.40; p<0.0001) was increased in participants with pretreatment drug resistance to at least one prescribed drug, but not in those with pretreatment drug resistance and fully active ART. CD4 count increased less in participants with pretreatment drug resistance than in those without (35 cells per μL difference after 12 months; 95% CI 13–58; p=0.002).

Interpretation

At least three fully active antiretroviral drugs are needed to ensure an optimum response to first-line regimens and to prevent acquisition of drug resistance. Improved access to alternative combinations of antiretroviral drugs in sub-Saharan Africa is warranted.

INTRODUCTION

A public-health approach based on standardized, affordable drug regimens and limited laboratory monitoring has been crucial for the scale-up of combination antiretroviral therapy (ART) in sub-Saharan African countries with high prevalences of HIV-1.[1] By 2010, more than 4 million people with HIV-1 in sub-Saharan Africa were receiving ART—about 40% of those in need.[2] Mutations in the HIV genome that confer drug resistance can diminish the virological response to ART.[3-6] Drug resistant variants, acquired because of selective pressure in people receiving ART, can be transmitted to people newly infected with HIV.[7, 8] In east, central, and southern Africa, reports suggest that transmitted drug resistance has increased, in parallel with the widespread distribution of ART.[9-12]

In developed countries, drug resistance testing to guide first-line therapy choices mitigates virological failure in people who have a transmitted drug resistant strain.[3-6] By contrast, in the absence of drug resistance testing in resource-limited countries, standard first-line combinations of antiretroviral drugs including two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) are prescribed empirically.[13, 14] The effect of emerging pretreatment drug resistance on the virological and immunological responses to the standard first-line ART regimens in sub-Saharan Africa has not been assessed.

This study investigates the effect of pretreatment drug resistance on the virological and immunological responses and the acquisition of drug resistance after the first year of ART and additional risk factors for virological failure and acquisition of drug resistance in the PharmAccess African Studies to Evaluate Resistance Monitoring (PASER-M) cohort.[15]

METHODS

Study design and participants

PASER-M is a multicentre prospective cohort of people with HIV-1 receiving ART at 13 clinics in Kenya (two clinics), Nigeria (one), South Africa (three), Uganda (three), Zambia (three), and Zimbabwe (one). Cohort characteristics[15] and pretreatment drug resistance mutations in the antiretroviral-naive cohort[10] have been described previously.

Patients were included if they were aged 18 years or older, HIV-1 infected, and started first-line ART in accordance with national guidelines—i.e., when with advanced immunodeficiency (a CD4 cell count less than 200 cells per μL) or advanced HIV disease (WHO clinical stage 3 or 4).[13] To prevent recruitment of patients who were already receiving

ART, people who had received a first-line regimen in the previous 30 days were excluded. Use of ART (more than 30 days previously), or any previous monotherapy, dual therapy, or use of antiretroviral prophylaxis was allowed. Our analysis included participants who received a standard first-line regimen containing two NRTIs and an NNRTI, excluding those who had started receiving three NRTIs only or a regimen containing a protease inhibitor. Other exclusion criteria were pregnancy at study screening and—tested in Nigeria only—HIV-2 co-infection.

Participants provided written, informed consent at enrolment. The study protocol was approved by the appropriate national and local research ethics committees at the collaborating sites and by the Academic Medical Centre, University of Amsterdam, Netherlands.

Procedures

Participants were followed up in accordance with the local standard of care guidelines. Medical staff at each site extracted routine clinical and laboratory data recorded in medical records into standard case-report forms, which were then entered into a central online database. CD4 cell counts recorded closest to the ART start date were used as pretreatment counts. Throughout the study, a data monitoring team verified source data and reviewed study data.

Drug adherence was assessed at each follow-up clinic visit by two measures of self-reported adherence. For 3 day self-reports, we counted the number of follow-up visits at which the patients reported to have missed any pills during the previous 3 days. For the 30 day visual analogue scale, we averaged the number of pills taken at all follow-up visits and classified adherence accordingly as less than 80%, 80–94%, 95–99%, or 100%.[16, 17]

Patients were included in one of the following outcome categories: still on a first-line ART regimen after 12 months of follow-up, discontinued ART, transferred out, lost to follow-up, died, or switched to second-line ART because of treatment failure as judged by local criteria.[13] A single-drug substitution because of toxicity or intolerance was not classed as a regimen switch. Time was measured from the start of ART and ended at the earliest of either last follow-up visit or 12 months after starting ART (from 11 to 15 months); possible treatment interruptions were ignored.

Plasma was sampled at the baseline visit, after 12 months of therapy, and—if applicable—earlier, in case of treatment failure, and stored for later assessment of HIV RNA viral load and genotypic drug resistance. HIV viral load results after 12 months of follow-up

for participants who were still on a first-line regimen were classed as either suppression (<400 copies per mL) or failure (≥400 copies per mL).

All virological testing was done at either of two reference laboratories: one in South Africa or one in Uganda.[10] Viral loads were measured with NucliSens EasyQ real-time assay (version 2.0) or COBAS Ampliprep/COBAS Taqman assay. For baseline and follow-up specimens with a viral load greater than 1000 copies per mL, population-based genotyping of HIV protease and reverse transcriptase was done with in-house sequencing methods. HIV subtypes were inferred from the *pol* sequences with the STAR algorithm[18] and confirmed with REGA (version 2.0).[19] Genotypic drug resistance was defined as the presence of at least one major amino acid substitution included in the International Antiviral Society USA mutation list of December, 2010,[20] including revertant mutations at codon 215.[21] NRTIs, NNRTIs, and protease inhibitors were included.

Pretreatment drug resistance was defined in two steps. We used the International Antiviral Society USA mutation list to distinguish between participants with a virus with at least one drug resistance mutation and those with none.[20] For participants with a virus with at least one drug resistant mutation, we used the Stanford drug-susceptibility algorithm (version 6.0.9)[22] to classify participants into those receiving fully active ART (Stanford levels 1 [susceptible] or 2 [potential low-level resistance] for all prescribed drugs), or those receiving partly active ART (Stanford levels 3 [low-level resistance], 4 [intermediate resistance], or 5 [high-level resistance] for at least one of the prescribed drugs). All sequences have been deposited in GenBank (accession numbers HM119603–HM120150, HQ993572- HQ995497, JN630892-JN631033).

Statistical analysis

We estimated a required sample size of at least 190 people per site on the basis of anticipated virological failure and drop-out rates, and balanced against the cost of genotypic testing.[15] We did group comparisons for categorical data with a χ^2 test, and for continuous data with a one-way ANOVA or Kruskal-Wallis test,[23] as appropriate. We used multilevel analysis with random intercepts to assess the effect of baseline and prospective factors at the level of individuals and sites (while accounting for clustering of observations within sites) on two outcomes after 12 months of therapy—virological failure and acquired drug resistance (defined as the presence of at least one International Antiviral Society USA drug resistance mutation and viral load >1000 copies per mL). To account for missing data due to participant attrition, we did multiple imputations with the Markov chain Monte Carlo approach with five simulation datasets.

The main analysis was a model that incorporated the following baseline characteristics: pretreatment drug resistance, age, sex, clinical stage of disease, initial NRTI backbone (stavudine, zidovudine, tenofovir, or abacavir) and NNRTI drug (efavirenz or nevirapine), previous antiretroviral drug exposure (ART, single-dose nevirapine for prevention of mother-to-child transmission, combination prophylaxis for prevention of mother-to-child transmission, or other), pretreatment CD4 cell count (<50, 50–199, or ≥200 cells per μL), pretreatment viral load (log_{10}-transformed, fitted as a continuous variable), HIV subtype, anemia, body mass index (fitted as a continuous variable), cost to patient, year of ART initiation, government or non-government site, years of on-site ART experience, and patient to staff ratio. The prospective model also includes CD4 cell count increase 6 months after ART initiation (fitted as a categorical variable), single-drug substitution, 3 day and 30 day adherence (fitted as categorical variables), and routine viral-load test at 6 months after ART initiation (available at seven of 13 sites). All variables were assessed univariately and those associated ($p<0.10$) with the outcome were entered stepwise into the multivariate model. Biologically plausible interactions were examined in all multivariate models. We did several sensitivity analyses (webappendix table 3).

We estimated the mean changes in CD4 cell counts between the pretreatment drug resistance groups over intervals of 3 months by use of linear mixed models. All CD4 cell counts measured routinely before and after start of ART were used. The model was adjusted for age, sex, pretreatment CD4 cell count and viral load, HIV subtype, previous antiretroviral drug exposure, year of ART initiation, types of NRTI and NNRTI, and 30 day adherence. A sensitivity analysis included only participants who had viral suppression (HIV RNA <50 copies per mL) after 12 months of ART.

Reported p values are two-sided and a p value below 0·05 was judged significant. All analyses were done with Stata (version 11).

RESULTS

Between March, 2007, and September, 2009, 2733 participants were recruited (median 221 per site, range 116–239; figure 1). 2588 participants (95%) reported no previous exposure to antiretroviral drugs, 145 (5%) had previously had antiretroviral drugs, including ART (n=61), monotherapy or dual therapy (four), single-dose nevirapine for prevention of mother-to-child transmission (43), combination regimen for prevention of mother-to-child transmission (19), post-exposure prophylaxis (one), or unknown (23). Webappendix table 1 shows participant characteristics by site.

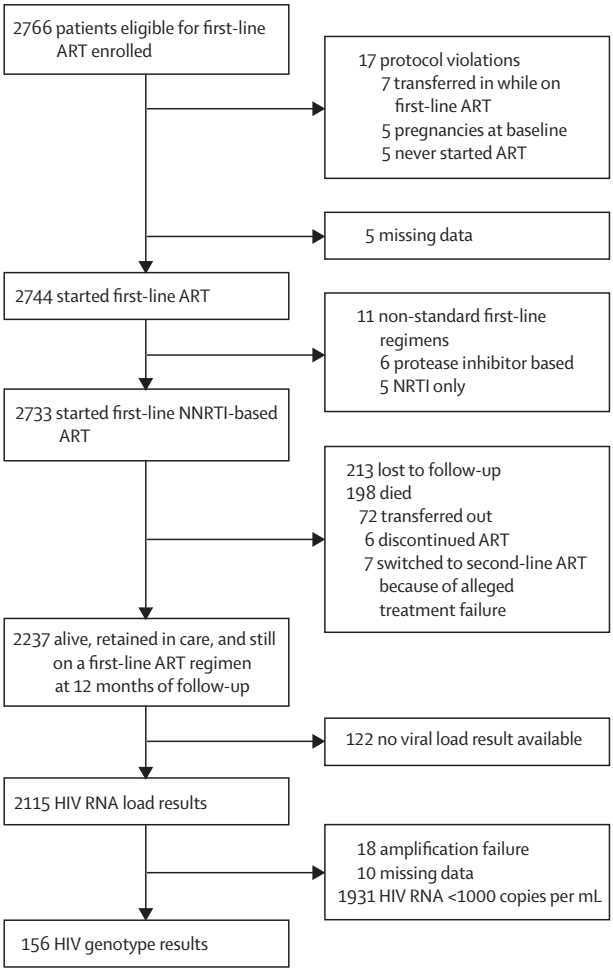


Figure 1. Study profile. ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

A pretreatment drug resistance test result was available for 2579 participants (94%; table 1). Pretreatment drug resistance was detected in 175 participants (7%), of whom 52 (30%) received fully-active ART and 123 (70%) had reduced susceptibility to at least one prescribed drug. Of these 123 participants, 99 (80%) had high-level resistance (Stanford 5), 11 (9%) had intermediate-level resistance (Stanford 4), and 13 (11%) had low-level resistance (Stanford 3) to at least one prescribed drug. The median number of active drugs for participants with pretreatment drug resistance to at least one prescribed drug was 2·0 (IQR 1.25–2.0). 1405 participants (54%) had HIV-1 subtype C, then 638 (25%) A, 296 (11%) D, 117 (5%) A/G recombinant, 68 (3%) G, 48 (2%) other recombinants, seven (<1%) other subtypes, and five (<1%) B. The distribution of pretreatment drug resistance and previous exposure to antiretroviral

Table 1. Patients' baseline characteristics

	All (n=2579)	No PDR (n=2404)	PDR (n=175)	P-value
Sex				0.494
Female	1493 (57.9)	1396 (58.1)	97 (55.4)	
Male	1086 (42.1)	1008 (41.9)	78 (44.6)	
Age – mean years (SD)	37.8 (9.0)	37.8 (9.0)	37.6 (9.3)	0.7361
18-29	506 (19.6)	470 (19.6)	36 (20.6)	0.947
30-39	1166 (45.2)	1088 (45.3)	78 (44.6)	
≥40	907 (35.2)	846 (35.2)	61 (34.9)	
Country				<0.0001
Zambia	551 (21.4)	522 (21.7)	29 (16.6)	
South Africa	601 (23.3)	566 (23.5)	35 (20.0)	
Uganda	606 (23.5)	530 (22.1)	76 (43.4)	
Kenya	424 (16.5)	405 (16.9)	19 (10.9)	
Zimbabwe	204 (7.9)	194 (8.1)	10 (5.7)	
Nigeria	193 (7.5)	187 (7.8)	6 (3.4)	
Calendar year of initiation				0.014
2007	618 (24.0)	585 (24.3)	33 (18.9)	
2008	1596 (61.9)	1470 (61.2)	126 (72.0)	
2009	366 (14.2)	349 (14.5)	16 (9.1)	
WHO clinical stage at initiation				0.936
1 or 2	1000 (38.8)	930 (38.7)	70 (40.0)	
3	1150 (44.6)	1073 (44.6)	77 (44.0)	
4	429 (16.6)	401 (16.7)	28 (16.0)	
Previous antiretroviral experience				<0.0001
No	2442 (94.7)	2302 (95.8)	140 (80.0)	
Yes	115 (4.5)	82 (3.4)	33 (18.9)	
Unknown	22 (0.9)	20 (0.8)	2 (1.1)	
Type of initial NRTI-backbone *				0.773
Zidovudine based	960 (37.2)	900 (37.4)	60 (34.3)	
Stavudine based	685 (26.6)	639 (26.6)	46 (26.3)	
Tenofovir based	870 (33.7)	805 (33.5)	65 (37.1)	
Abacavir based	64 (2.5)	60 (2.5)	4 (2.3)	
Type of initial NNRTI				0.459
Efavirenz	1542 (59.8)	1442 (60.0)	100 (57.1)	
Nevirapine	1037 (40.2)	962 (40.0)	75 (42.9)	
Pre-treatment body-mass index, median kg per m^2 (IQR) †	21.0 (18.8-24.0)	21.0 (18.3-23.7)	21.0 (18.4-23.6)	0.3553
Pre-treatment hemoglobin, median gram per dL (IQR) ‡	11.4 (9.9-12.9)	11.4 (9.9-12.9)	11.8 (10.5-13.2)	0.2300

Table 1 (continued)

	All (n=2579)	No PDR (n=2404)	PDR (n=175)	P-value
Pre-treatment CD4 cell count, median cells per µL (IQR) §	133 (62-204)	133 (62-204)	129 (53.5—204.5)	0.7148
<50	534 (20.8)	492 (20.5)	42 (24.0)	0.490
50-99	432 (16.8)	408 (17.0)	24 (13.7)	
100-199	910 (35.4)	845 (35.3)	65 (37.1)	
≥200	695 (27.0)	651 (27.2)	44 (25.1)	
Pre-treatment HIV-RNA, median log_{10} copies per mL (IQR) ¶	5.00 (4.36-5.59)	5.04 (4.46-5.62)	5.16 (4.58-5.74)	0.591 ||

Data are n (%), mean (SD), or median (IQR). Data are from participants who initiated an NNRTI-based first-line regimen and had a pretreatment genotypic resistance test result (n=154 excluded). p values refer to the comparison between PDR and no PDR groups. *Combined with lamivudine or emtricitabine. †Data available for 2562 patients. ‡Data available for 2526 patients. §Data available for 2568 patients. ¶Data available for 2561 patients. ||Adjusted for assay. PDR, pretreatment drug resistance; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor;

drugs did not differ between the initial ART regimens (data not shown). The prevalences of all pretreatment drug resistance mutations are listed in webappendix table 2.

Total follow-up was 2358 person-years, with a median of 11·9 months (IQR 11.2–12.2). Of 2733 participants, 2237 (82%) were retained in care and still receiving first-line ART after 12 months of follow-up. 489 (18%) were not retained, 198 (7%) because they had died, 213 (8%) lost to follow-up, 72 (3%) transferred out, and six (<1%) discontinued ART. Seven (<1%) patients switched to a second-line regimen in the first year of ART because of treatment failure (figure 1), one of whom had pretreatment drug resistance (webappendix figure 1). A slightly smaller proportion of patients with pretreatment drug resistance were retained on first-line ART (133 [76%]) than were those without (1979 [82%]; p=0·036); whereas all-cause mortality did not differ significantly between the groups (17 [10%] *vs.* 171 [7%]; p=0·201).

1942 participants (87%) were still taking their original regimen after 12 months, and 295 (13%) had a single-drug substitution because of toxicity, intolerance, or other reasons. 12 patients (1%) interrupted ART because of severe intolerance. 2218 participants (81%) had not missed any pills in the 3 days before any follow-up visit. 1517 patients (56%) had 100% 30 day adherence, 726 (27%) had 95–99% adherence, 201 (7%) had 80–94% adherence, and 37 (1%) had <80% adherence. Pretreatment drug resistance and adherence were not associated (data not shown).

Table 2. Risk factors of virological failure in the first year of first-line ART

	Number of events	Univariate			Multivariate					
					Baseline model			Prospective model		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Total	213									
Pre-treatment drug resistance										
No PDR	174	1			1			1		
PDR and fully-active ART	6	1.62	0.67, 3.92	0.282	1.01	0.55, 1.87	0.964	1.07	0.54, 2.11	0.853
PDR and partially-active ART	21	3.31	1.96, 5.59	<0.001	2.13	1.44, 3.14	<0.001	2.21	1.40, 3.50	0.001
Sex										
Women	108	1			1			1		
Men	105	1.53	1.15, 2.03	0.003	0.86	0.39, 1.86	0.695	0.52	0.21, 1.30	0.162
Age (yrs), mean (sd) *		0.76	0.64, 0.91	0.002						
For men					0.87	0.76, 1.01	0.06	0.87	0.61, 1.04	0.114
For women					0.74	0.64, 0.85	<0.001	0.67	0.74, 1.03	<0.001
Calendar year of ART initiation										
2007	64	1			1			1		
2008	116	0.55	0.36, 0.84	0.006	0.87	0.66, 1.13	0.291	0.72	0.53, 0.99	0.041
2009	33	0.60	0.33, 1.11	0.104	0.69	0.46, 1.04	0.075	0.67	0.42, 1.07	0.092
WHO clinical stage										
1 or 2	76	1								
3 or 4	137	1.34	1.00, 1.80	0.054						
Body Mass Index, kg/m^2 †		0.81	0.68, 0.97	0.019						
Pre-treatment viral load (log_{10} c/mL), median (IQR)		1.29	1.10, 1.52	0.002	1.20	1.08, 1.34	0.001	1.13	0.99, 1.28	0.071
Previous antiretroviral experience										
None	194	1			1			1		
ART	10	3.64	1.73, 7.69	0.001	2.62	1.50, 4.57	0.001	3.10	1.65, 8.81	<0.001
Single-dose nevirapine for PMTCT	5	1.95	0.73, 5.20	0.181	1.66	0.84, 3.30	0.148	2.04	0.94, 4.47	0.073
Other	3	2.01	0.57, 7.10	0.278	1.91	0.84, 4.36	0.124	1.86	0.75, 4.60	0.182
Type of initial NRTI-backbone ‡										
Zidovudine based	102	1								
Stavudine based	43	0.63	0.43, 0.91	0.015						
Tenofovir based	63	0.72	0.52, 1.01	0.057						
Abacavir based	5	1.04	0.40, 2.71	0.942						
Type of initial NNRTI §										
Efavirenz	104	1			1			1		
Nevirapine	109	1.50	1.13, 1.99	0.005	0.98 §	0.80, 1.20	0.864	1.12	0.88, 1.41	0.363

Table 2 (continued)

	Number of events	Univariate			Multivariate					
					Baseline model			Prospective model		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
CD4 gain six months after initiation, cells per µL ¶										
<0 (decrease)	18	1						1		
0-99	41	0.41	0.22, 0.76	0.004				0.36	0.22, 0.60	<0.001
100-199	28	0.30	0.16, 0.57	<0.001				0.27	0.16, 0.45	<0.001
≥200	17	0.27	0.13, 0.55	<0.001				0.31	0.18, 0.54	<0.001
3-day self-report adherence, no. ¶										
0	126	1						1		
1	52	2.39	1.76, 3.42	<0.001				1.31	0.94, 1.83	0.114
2	27	6.41	3.83, 10.73	<0.001				1.67	0.96, 2.88	0.068
3 or more	8	7.92	3.16, 19.83	<0.001				1.87	0.71, 4.94	0.208
30-day VAS adherence, %¶										
100	99	1						1		
95-99	57	1.10	0.76, 1.57	0.623				0.63	0.47, 0.85	0.003
94-80	38	3.69	2.39, 5.70	<0.001				1.75	1.14, 2.69	0.010
<80	10	9.44	3.97, 22.45	<0.001				3.70	1.71, 8.04	0.001

Table shows results of multilevel logistic regression, including the data from all participants who initiated a NNRTI-based first-line regimen. 3 day self-report is defined as the number of follow-up clinic visits at which the patient reported to have missed any pills in the 3 previous days. 30 day VAS is defined as the mean visual analogue score at all follow-up clinic visits. Total number of events=213. *Odds ratio for 10-year increase in age. †Odds ratio for 5 kg/m2 increase. ‡Combined with lamivudine or emtricitabine. §Inverse-probability weighting for NNRTI assignment: OR 0.91 (95% CI 0.69–1.19; p=0.475). ¶The baseline model did not include data for prospectively collected parameters. ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PMTCT, prevention of mother-to-child transmission; VAS, visual analogue score; OR, odds ratio; PDR, pretreatment drug resistance.

Viral load at 12 months' follow-up was assessed for 2115 (95%) of 2237 participants still taking first-line ART. 213 of 2733 patients (70%, 95% CI 68–71) who started first-line ART had viral suppression; 213 of 2115 (90%, 89–91) of those who were still on first-line ART had viral suppression (figure 1). Virological suppression at 12 months did not differ significantly between participants without pretreatment drug resistance (1697 [91%]) and those with pretreatment drug resistance and fully active ART (36 [86%]; p=0.274), but was significantly lower in participants with resistance to at least one prescribed drug (63 [75%]; p<0·0001).

Of the 184 participants with a viral load greater than 1000 copies per mL at 12 month follow-up, 156 (85%) specimens were successfully genotyped: 113 (72%) had one or

more major drug resistance mutation. Drug resistance mutations were associated with NRTIs (94, 60%), NNRTIs (98, 63%) and protease inhibitors (two, 1%). Dual-class resistance to NRTIs and NNRTIs was detected in 80 participants (51%), and no triple-class resistance was detected. Of the 113 participants who had one or more major drug resistance mutation after 12 months of ART, 20 (18%) had pretreatment drug resistance, 87 (77%) did not, and status was unknown in six (5%).

Compared with participants without pretreatment drug resistance, people who had resistance to at least one prescribed drug had an increased risk of virological failure (odds ratio [OR] 2.13, 95% CI 1.44–3.14; p<0·0001; table 2) and acquired drug resistance (2.30, 1.55–3.40; p<0.0001; table 3). By contrast, for those who had pretreatment drug resistance and were prescribed a fully active regimen, the ORs for virological failure (1.01, 0.55–1.87; p=0.964; table 2) or acquired drug resistance (0.97, 0.51–1.58; p=0.934; table 3) were not increased, compared with those without pretreatment drug resistance (webappendix table 3). Additionally, compared with participants with pretreatment drug resistance and reduced susceptibility to at least one prescribed drug, the risks of virological failure (0.48, 0.23–0.97, p=0.041) and acquired drug resistance (0.42, 0.20–0.88; p=0.022) were reduced in those with pretreatment drug resistance and fully active ART.

Independent of pretreatment drug resistance, participants who had virological failure or acquired drug resistance were more likely to have been previously exposed to ART or prevention of mother-to-child transmission, to have high pretreatment viral load, and to be younger (for women) at ART initiation, than were participants who did not (tables 2, 3). Participants who acquired drug resistance were also more likely to have a pretreatment CD4 count less than 50 cells per µL than were those who did not. Of the measures obtained prospectively after ART initiation, no CD4 cell count increase during the first 6 months of ART, and suboptimum (<95%) 30 day adherence were associated with virological failure (table 2) and acquired drug resistance (table 3) by month 12. In the univariate analysis the following covariables were not associated with virological failure: pretreatment CD4 cell count, treatment cost, severe anemia at initiation, HIV subtype, site administration, site ART experience, patient to staff ratio, single-drug substitution, and viral-load test at 6 months after initiation. In univariate analysis the following covariables were not associated with acquired drug resistance: free treatment, severe anemia at initiation, body-mass index, HIV-1 subtype, site administration, site ART experience, patient to staff ratio, single-drug substitution, viral-load test at 6 months after initiation. No significant effect modification across strata of pretreatment drug resistance and covariables occurred.

Table 3. Risk factors of acquired drug resistance in the first year of first-line ART

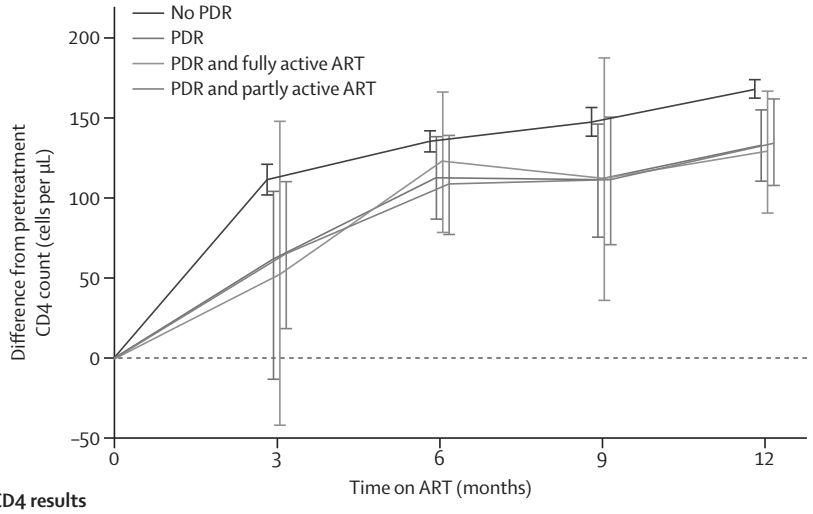
	Number of events	Univariate			Multivariate					
					Baseline model			Prospective model		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Total	113									
Pre-treatment drug resistance										
No PDR	87	1			1			1		
PDR and fully-active ART	4	2.11	0.73, 6.11	0.166	0.97	0.51, 1.58	0.934	1.05	0.52, 2.15	0.884
PDR and partially-active ART	16	5.03	2.77, 9.12	<0.001	2.30	1.55, 3.40	<0.001	2.42	1.53, 3.83	<0.001
Sex										
Women	56	1			1			1		
Men	57	1.54	1.05, 2.26	0.026	0.78	0.35, 1.76	0.555	0.45	0.17, 1.17	0.102
Age (yrs), mean (sd) *		0.75	0.59, 0.94	0.014						
For men					0.90	0.77, 1.04	0.151	0.91	0.76, 1.08	0.280
For women					0.75	0.65, 0.87	<0.001	0.67	0.56, 0.80	<0.001
Calendar year of ART initiation										
2007	33	1			1			1		
2008	62	0.56	0.32, 0.98	0.042	0.97	0.74, 1.27	0.819	0.78	0.57, 1.08	0.132
2009	18	0.63	0.29, 1.41	0.263	0.83	0.55, 1.27	0.394	0.76	0.47, 1.22	0.255
WHO clinical stage										
1 or 2	38	1								
3 or 4	75	1.46	0.97, 2.18	0.068						
Pre-treatment CD4 count, cells per µL										
<50	50	1			1			1		
50-199	43	0.62	0.40, 0.97	0.037	0.67	0.55, 0.83	<0.001	0.64	0.50, 0.81	<0.001
≥200	20	0.35	0.20, 0.63	<0.001	0.60	0.47, 0.76	<0.001	0.58	0.44, 0.77	<0.001
Pre-treatment viral load (log_{10} c/mL), median (IQR)		1.43	1.13, 1.80	0.002	1.12	1.00, 1.26	0.048	1.04	0.91, 1.19	0.594
Previous antiretroviral experience										
None	100	1			1			1		
ART	8	5.83	2.56, 13.31	<0.001	2.76	1.58, 4.85	<0.001	3.22	1.71, 6.05	<0.001
Single-dose nevirapine for PMTCT	3	2.16	0.63, 7.45	0.222	1.99	0.99, 3.99	0.052	2.34	1.06, 5.18	0.035
Other	1	2.21	0.16, 9.40	0.855	2.11	0.90, 4.93	0.084	1.98	0.78, 4.99	0.150
Type of initial NRTI-backbone †										
Zidovudine based	58	1			1			1		
Stavudine based	12	0.31	0.16, 0.58	<0.001	1.13	0.85, 1.50	0.398	1.05	0.76, 1.44	0.759
Tenofovir based	39	0.79	0.52, 1.20	0.272	1.08	0.84, 1.39	0.557	1.02	0.76, 1.38	0.883
Abacavir based	4	1.47	0.51, 4.28	0.478	1.99	1.13, 3.51	0.018	1.53	0.78, 3.00	0.221

Table 3 (continued)

	Number of events	Univariate			Multivariate					
					Baseline model			Prospective model		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Type of initial NNRTI										
Efavirenz	52	1			1			1		
Nevirapine	61	1.65	1.11, 2.46	0.014	0.96‡	0.77, 1.20	0.735	1.06	0.82, 1.38	0.636
CD4 gain six months after initiation, cells per µL §										
<0 (decrease)	11	1						1		
0-99	32	0.56	0.27, 1.16	0.117				0.34	0.20, 0.57	<0.001
100-199	13	0.24	0.10, 0.55	0.001				0.22	0.13, 0.38	<0.001
≥200	7	0.19	0.071, 0.51	0.001				0.27	0.15, 0.48	<0.001
3-day self-report adherence, no. §										
0	70	1						1		
1	25	1.87	1.16, 3.02	0.010				0.79	0.56, 1.13	0.205
2	12	3.90	2.00, 7.61	<0.001				0.64	0.35, 1.17	0.147
3 or more	6	8.44	3.15, 22.62	<0.001				1.03	0.37, 2.90	0.952
30-day VAS adherence, % §										
100	48	1						1		
95-99	35	1.50	0.93, 2.42	0.097				0.86	0.63, 1.16	0.325
94-80	24	4.46	2.60, 7.66	<0.001				2.46	1.57, 3.84	<0.001
<80	5	7.99	2.76, 23.11	<0.001				4.97	2.30, 10.74	<0.001

Table shows results of multilevel logistic regression, including the data from all participants who initiated a NNRTI-based first-line regimen. *OR for 10-year increase in age. †Combined with lamivudine or emtricitabine. ‡Inverse-probability weighting for NNRTI-assignment: OR 0.78 (95% CI 0.59–1.04; p=0.088). §The baseline model did not include data for prospectively collected parameters. PDR, pretreatment drug resistance; ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PMTCT, prevention of mother-to-child transmission; VAS, visual analogue score; OR, odds ratio.

The median gain in CD4 count between the start of ART and 6 months' follow-up was 110 (IQR 57–186) cells per µL and 149 (IQR 76–239) cells per µL at 12 months. Compared with participants without pretreatment drug resistance, CD4 count increased less in the first 3 months of ART (49 cells per µL; 95% CI 7–90; p=0.021) up to 12 months (35 cells per µL; 13–58; p=0.002) in all participants with pretreatment drug resistance; the difference was 40 cells per µL per 12 months (2–78; p=0.041) for those with pretreatment drug resistance and fully active ART, and 33 cells per µL (6–61; p=0.015) for those with resistance to at least one prescribed drug (figure 2).



Number of CD4 results					
No PDR	2396	442	1095	489	1527
PDR	175	24	80	38	113
PDR and fully active ART	52	4	26	10	39
PDR and partly active ART	123	20	54	28	72

Figure 2. Change in CD4 cell count, by pretreatment drug resistance. Linear mixed model (n=2439) adjusted for age, sex, pre-treatment CD4 count, pretreatment HIV RNA load, HIV-1 subtype, year of ART initiation, NRTI-backbone, NNRTI-drug, previous antiretroviral drug exposure, and adherence; not adjusted for HIV RNA loads at 12 months of therapy. Vertical bars=95% CI. PDR=pretreatment drug resistance. ART, antiretroviral therapy.

In a sensitivity analysis, which only included participants who had viral suppression (viral load <50 copies per mL) at 12 months' follow-up, the reduced CD4 increase for all participants with pretreatment drug resistance was of borderline statistical significance (28 cells per µL; 95% CI 1–55; p=0.039), compared with those without pretreatment drug resistance (webappendix figure 2).

DISCUSSION

Pretreatment drug resistance was strongly associated with virological failure and development of drug resistance after the first year of standard first-line NNRTI-based ART in patients in sub-Saharan Africa who received partly active regimens—i.e., that included at least one drug to which the virus had reduced susceptibility. By contrast, the response to ART was not reduced in patients who had a drug resistant virus when the prescribed regimen was predicted to be fully active. Our findings seem to be robust in sensitivity analyses that adjusted for possible effects of previous antiretroviral drug exposure and the M184V mutation (webappendix table 3). These findings are largely in agreement

with results from studies in developed countries (panel).[3-6] Notably, 70% of participants who had pretreatment drug resistance were empirically started on a suboptimum first-line regimen—nearly 5% of the total study population. In view of the limited potency and low genetic barrier of NNRTI-based regimens, our study emphasizes the need for at least three fully active antiretroviral drugs in first-line regimens in Africa to ensure an optimum virological response and to prevent the acquisition of NRTI and NNRTI drug resistance mutations.

Panel: Research in context

Systematic review

We searched PubMed for English-language studies, published between January, 2006, and September, 2011, with a sample size greater than 100, that assessed the effect of pretreatment HIV-1 drug resistance on response to first-line antiretroviral therapy. We identified seven cohort studies from Europe and none from resource-limited countries (appendix pp 9–10). Some studies showed no significant association between the presence of pretreatment drug resistance and virological [24-26] or immunological [24, 25] response to antiretroviral therapy. Other studies reported a decreased virological response in patients with pretreatment drug resistance compared with those without [3, 6]. A collaborative study including 10 056 patients from 25 European cohorts showed an increased risk of virological failure in patients with pretreatment drug resistance to at least one prescribed drug compared with those without pretreatment drug resistance, but no increased risk for those with pretreatment drug resistance who received fully active ART [5].

Interpretation

Our study confirms findings from studies done in high-income countries. The risk of virological failure and acquired drug resistance for patients in sub-Saharan Africa receiving non-nucleoside reverse transcriptase inhibitor-based regimens with pretreatment drug resistance to at least one prescribed drug was increased compared with those without pretreatment drug resistance and those with pretreatment drug resistance who received a fully active regimen. Improved access to alternative drugs with different modes of action and without cross-resistance to nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors is warranted in resource-limited countries to ensure continued effectiveness of HIV/AIDS treatment.

Overall, CD4 cell count recovered well during the first year of ART in patients with pretreatment drug resistance, although it was reduced compared with those without resistance. This difference is probably due to the higher proportion with virological failure among patients with pretreatment drug resistance, rather than by a direct effect of drug resistant virus on CD4 cell count. The small difference in CD4 gain after adjustment for differences in viral load at 12 months of ART could be due to the longer time needed to achieve viral suppression or by residual viral replication below the detection level of the HIV RNA assay in the pretreatment drug resistance group, compared with the group without. However, the clinical relevance of this difference might be small, because the overall immunological response in participants with pretreatment drug resistance was

favorable. By contrast with a European study (panel),[5] which showed a reduced CD4 cell count increase only in patients with transmitted resistance to at least one prescribed drug, but not in patients with transmitted resistance who received a fully active regimen, immunological outcomes did not differ between the ART susceptibility subgroups in our study, which might be because of a lack of statistical power.

Independent of pretreatment drug resistance, previous use of ART was strongly associated with virological failure and acquired drug resistance. Previous exposure to ART might result in minority-resistant viral strains not detected by population-based sequencing. Minority NNRTI resistance mutations might contribute to virological failure in patients starting an NNRTI-based regimen.[27-32] Furthermore, women who had had single-dose nevirapine to prevent perinatal transmission of HIV-1 had a slightly higher risk of virological failure than did antiretroviral-naive women. A randomized study in Botswana showed that previous exposure to peripartum single-dose nevirapine results in increased rates of virological failure if given less than 6 months before the start of subsequent NNRTI-based ART.[33] For this reason, the revised 2010 WHO HIV treatment guidelines[14] recommend initiating a regimen based on a protease inhibitor in women with previous exposure to antiretrovirals for prevention of mother-to-child transmission if they have received single-dose nevirapine alone or in combination with other drugs without an NRTI tail within 12 months of starting long-term ART, irrespective of the presence of NNRTI-associated pretreatment drug resistance. Because of the small numbers of women who had received ART for prevention of mother-to-child transmission in our study, we could not differentiate according to the time since prevention of mother-to-child transmission treatment exposure.

In the absence of routine virological monitoring, clinical proxy measures of treatment failure, obtained prospectively after ART initiation, might be helpful for clinical decision making as an early warning signal. In our study, we noted that failure to achieve an increase in CD4 cell count by month 6 as well as prolonged non-adherence below 95% was associated with virological failure and the acquisition of drug resistance by month 12. Sustained suboptimum drug concentrations leads to ongoing viral replication and selection for drug resistant variants. Suboptimum ART adherence predicts virological failure,[34] the development of drug resistance[35, 36] and death.[37] Our finding supports current recommendations for NNRTI-based regimens to strive for adherence levels above 95%. Average adherence was high in our cohort, which suggests that adequate rates of adherence can be achieved in African ART programmes; although self-reported adherence measures as used in our study can be affected by recall or social desirability bias.[38]

This study has some limitations. Attrition due to mortality and loss to follow-up in the first year of ART was high 489 (18% of patients enrolled), which is consistent with attrition estimates for ART programmes in sub-Saharan Africa.[2, 39] To account for the uncertainty introduced by these missing data, we used multiple imputations, which reduce bias and improve statistical efficiency. A sensitivity analysis that classed missing data as failures also suggests that our findings are robust. The overall virological failure after 12 months of therapy was 30% for all patients who started ART and 10% for those who were still on first-line ART, which compares favorably to a systematic review of 89 ART programmes in Africa.[40] Although the PASER network includes mostly free-access, routine ART programmes, non-government and urban sites were over-represented, and public and rural sites were under-represented. Therefore, caution is warranted when extrapolating the results to other settings where resource constraints might be even more substantial. Population-based sequencing cannot detect minority-resistant viral strains that are present below 25% of the viral population. Therefore, a proportion of participants with pretreatment drug resistance could have been misclassified as having none (false-negative), which might have led to underestimates of the effect on treatment outcome. Furthermore, the absence of serial blood sampling after ART initiation precluded a time-to-event analysis for virological failure and the development of pretreatment drug resistance.

Our study emphasizes the need for at least three fully active antiretroviral drugs to ensure an optimum response to first-line NNRTI-based ART regimens in sub-Saharan Africa and prevent the acquisition of HIV drug resistance. The findings have important implications for public health policy. Early mortality and attrition are major challenges to the success of ART programmes in Africa. Emerging drug resistance also jeopardizes the effectiveness of standard regimens. To maintain effective HIV/AIDS treatment in Africa, further studies and mathematical modeling are needed to establish optimum strategies for the prevention of drug resistance, ART monitoring, retention of patients, and use of protease-inhibitor-based regimens for specific high-risk groups, taking into account that pretreatment drug resistance frequencies in Africa will likely increase in the coming years.[9-12] Extended genotypic drug resistance testing as part of population-based surveillance is needed in regions where ART is scaled up. Improved access to alternative drugs with different modes of action and without cross-resistance to NRTIs and NNRTIs is warranted in resource-limited countries to ensure the continued effectiveness of HIV/AIDS treatment.

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WEBAPPENDIX

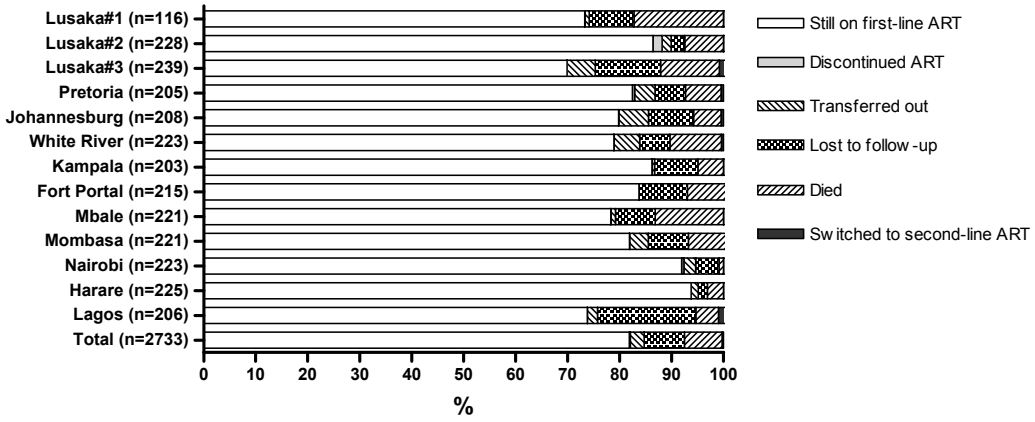

Figure 1. Bar chart of participants' clinical outcomes at 12 months of follow-up for each site. Bart chart shows data from all participants who initiated a NNRTI-based first-line regimen.

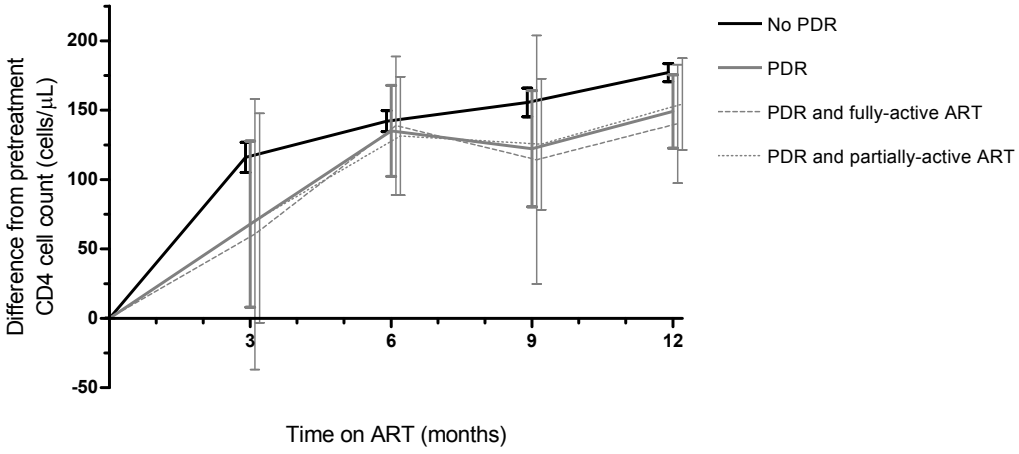

Figure 2. Change in CD4 cell count, by pre-treatment drug resistance. Sensitivity analysis: only includes participants with HIV-RNA<50c/mL after 12 months of therapy (n=1560). Linear mixed model adjusted for age, sex, pre-treatment CD4 count, pretreatment HIV-RNA load, HIV-1 subtype, calendar year of initiation, NRTI-backbone, NNRTI-drug, prior antiretroviral drug exposure, and adherence. Vertical bars = 95% confidence interval. PDR, pre-treatment drug resistance; ART, antiretroviral therapy.

Table 1. Baseline characteristics of all participants, by site.

Country			Zambia		South Africa			Uganda			Kenya		Zimbabwe	Nigeria
Sex														
Female	1592 (58.3)	53 (45.7)	133 (55.7)	133 (58.3)	113 (55.1)	155 (74.5)	132 (59.2)	113 (55.7)	120 (55.8)	117 (52.9)	131 (59.3)	121 (54.3)	147 (65.3)	124 (60.2)
Male	1141 (41.8)	63 (54.3)	95 (41.7)	106 (44.4)	92 (44.9)	53 (25.5)	91 (40.8)	90 (44.3)	95 (44.2)	104 (47.1)	90 (40.7)	102 (45.7)	78 (34.7)	82 (39.8)
Age – mean yrs (SD)	37.8 (9.0)	39.1 (8.9)	37.5 (8.7)	37.6 (9.4)	38.2 (8.3)	36.5 (7.4)	39.0 (10.0)	36.1 (8.8)	37.3 (10.3)	38.6 (9.6)	37.5 (8.6)	38.7 (8.7)	38.7 (8.7)	37.0 (8.2)
Calendar year of initiation														
2007	650 (23.8)	48 (41.4)	134 (58.8)	238 (99.6)	117 (57.1)	20 (9.6)	31 (13.9)	0 (0)	0 (0)	0 (0)	62 (28.1)	0 (0)	0 (0)	0 (0)
2008	1690 (61.8)	68 (58.6)	94 (41.2)	1 (0.4)	88 (42.9)	188 (90.4)	192 (86.1)	203 (100.0)	215 (100.0)	221 (100.0)	159 (72.0)	132 (59.2)	60 (26.7)	69 (33.5)
2009	393 (14.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	91 (40.8)	165 (73.3)	137 (66.5)
WHO clinical stage at initiation														
1 or 2	1078 (39.4)	74 (63.8)	92 (40.4)	97 (40.6)	84 (41.0)	136 (65.4)	59 (26.5)	76 (37.4)	77 (35.8)	46 (20.8)	81 (36.7)	66 (29.6)	102 (45.3)	88 (42.7)
3	1205 (44.1)	19 (16.4)	115 (50.4)	126 (52.7)	71 (34.6)	53 (25.5)	96 (43.1)	78 (38.4)	90 (41.9)	139 (62.9)	124 (56.1)	106 (47.5)	102 (45.3)	86 (41.8)
4	450 (16.5)	23 (19.8)	21 (9.2)	16 (6.7)	50 (24.4)	19 (9.1)	68 (30.5)	49 (24.1)	48 (22.3)	36 (16.3)	16 (7.2)	51 (22.9)	21 (9.3)	32 (15.5)
Type of initial NRTI-backbone [a]														
Zidovudine based	1017 (37.2)	13 (11.2)	60 (26.3)	10 (4.2)	20 (9.8)	10 (4.8)	18 (8.1)	122 (60.1)	66 (30.7)	130 (58.8)	171 (77.4)	106 (47.5)	159 (70.7)	132 (64.1)
Stavudine based	728 (26.6)	13 (11.2)	10 (4.4)	86 (36.0)	2 (1.0)	193 (92.8)	204 (91.5)	0	1 (0.5)	0	47 (21.3)	107 (48.0)	65 (28.9)	0 (0)
Tenofovir based	919 (33.6)	90 (77.6)	158 (69.3)	92 (38.5)	183 (89.3)	5 (2.4)	1 (0.5)	81 (39.9)	147 (68.4)	91 (41.2)	3 (1.4)	10 (4.5)	1 (0.4)	57 (27.7)
Abacavir based	69 (2.5)	0 (0)	0 (0)	51 (21.3)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	17 (8.3)
Type of initial NNRTI														
Efavirenz	1634 (59.8)	91 (78.5)	129 (56.6)	161 (67.4)	187 (91.2)	194 (93.3)	213 (95.5)	82 (40.4)	185 (86.1)	129 (58.4)	85 (38.5)	112 (50.2)	30 (13.3)	36 (17.5)
Nevirapine	1099 (40.2)	25 (21.6)	99 (43.4)	78 (32.6)	18 (8.8)	14 (6.7)	10 (4.5)	121 (59.6)	30 (14.0)	92 (41.6)	136 (61.5)	111 (49.8)	195 (86.7)	170 (82.5)
CD4 cell count at initiation -Median cells/μL (IQR) [b]	135 (63-206)	115.5 (47-184)	140.5 (83.5-197.5)	130 (58.5-201.5)	148 (60.5-235.5)	95.5 (28.3-163)	94.5 (34-155)	136 (53-219)	179 (99-259)	114 (50.5-177.5)	128 (61-195)	164 (104.5-223.5)	191 (114-268)	133 (66.5-199.5)

Table 1 (continued)

Country		Zambia		South Africa			Uganda			Kenya		Zimbabwe	Nigeria
HIV RNA at initiation - median log_{10} c/ml (IQR) [c]	5.00 (4.36-5.59)	4.88 (4.09-5.67)	4.99 (4.42-5.57)	5.20 (4.73-5.68)	4.56 (3.94-5.17)	4.71 (4.10-5.32)	4.99 (4.50-5.48)	5.40 (4.96-5.84)	5.23 (4.79-5.66)	5.59 (5.03-6.14)	4.62 (3.96-5.28)	4.66 (4.07-5.25)	4.68 (4.06-5.29)	5.10 (4.53-5.66)
Pre-treatment drug-resistance [d]														
No PDR	2404 (88.0)	103 (88.8)	207 (90.8)	212 (88.7)	189 (92.2)	181 (87.0)	196 (87.9)	170 (83.7)	175 (81.4)	185 (83.7)	203 (91.9)	202 (90.6)	194 (86.2)	187 (90.8)
PDR and fully-active ART	52 (1.9)	1 (0.9)	1 (0.44)	5 (2.1)	0 (0)	3 (1.4)	7 (3.1)	12 (5.9)	9 (4.2)	3 (1.4)	5 (2.3)	2 (0.9)	4 (1.8)	0 (0)
PDR and partially-active ART	123 (4.5)	5 (4.3)	9 (4.0)	8 (3.4)	8 (3.9)	6 (2.9)	11 (4.9)	12 (5.9)	18 (8.4)	22 (10.0)	6 (2.7)	6 (2.7)	6 (2.7)	6 (2.9)
PDR unknown	154 (5.6)	7 (6.0)	11 (4.8)	14 (5.9)	8 (3.9)	18 (8.7)	9 (4.0)	9 (4.4)	13 (6.1)	11 (5.0)	7 (3.2)	13 (5.8)	21 (9.3)	13 (6.3)
Previous antiretroviral experience	122 (4.5)													
No	2588 (94.7)	110 (94.8)	213 (93.4)	232 (97.1)	182 (88.8)	197 (94.7)	214 (96.0)	188 (92.6)	197 (91.6)	217 (98.2)	211 (95.5)	214 (96.0)	211 (93.8)	202 (98.1)
Yes	122 (4.5)	4 (3.5)	11 (4.8)	5 (2.1)	21 (10.2)	11 (5.3)	5 (2.2)	15 (7.4)	17 (7.9)	2 (0.9)	9 (4.1)	6 (2.7)	14 (6.2)	2 (1.0)
Unknown	23 (0.8)	2 (1.7)	4 (1.8)	2 (0.8)	2 (1.0)	0 (0)	4 (1.8)	0 (0)	1 (0.5)	2 (0.9)	1 (0.5)	3 (1.4)	0 (0)	2 (1.0)

Data are number (%) unless stated otherwise. [a] Combined with lamivudine or emtricitabine. [b] All n =2722; [c] All n =2706; [d] At least one drug-resistance mutation of the IAS-USA list, includes 52 participants who received a fully-active regimen (Stanford levels 1 or 2) and 123 who harbored drug-resistance to at least one drug in the prescribed regimen (Stanford levels 3, 4, or 5). PDR, pre-treatment drug-resistance; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; SD, standard deviation; IQR, interquartile range; WHO, World Health Organization.

Table 2. Pre-treatment prevalence of major IAS-USA drug-resistance mutations (n=2579).

NRTI mutations		NNRTI mutations		PI mutations	
Mutation	N (%)	Mutation	N (%)	Mutation	N (%)
M41L	19 (0.74)	L100I	4 (0.16)	D30N	1 (0.04)
A62V	3 (0.12)	K101P	0	V32I	0
K65R	4 (0.16)	K103N	57 (2.21)	M46I	5 (0.19)
D67N	3 (0.12)	V106A	0	M46L	6 (0.23)
T69INS	0	V106M	3 (0.12)	I47A	0
K70E	3 (0.12)	V108I	15 (0.58)	I47V	0
K70R	13 (0.50)	Y181C	25 (0.97)	G48V	0
L74V	1 (0.04)	Y181I	1 (0.04)	I50L	1 (0.04)
V75I	7 (0.27)	Y188L	1 (0.04)	I50V	1 (0.04)
F77L	1 (0.04)	Y188C	1 (0.04)	I54L	0
Y115F	1 (0.04)	Y188H	0	I54M	0
F116Y	0	G190A	22 (0.85)	Q58E	10 (0.39)
Q151M	0	G190S	1 (0.04)	T74P	0
M184I	0	P225H	2 (0.08)	L76V	2 (0.08)
M184V	32 (1.24)			V82A	0
L210W	7 (0.27)			V82F	0
T215C	0			V82L	0
T215D	2 (0.08)			V82S	0
T215E	0			V82T	0
T215F	6 (0.23)			N83D	1 (0.04)
T215G	0			I84V	0
T215H	0			N88S	0
T215I	4 (0.16)			L90M	7 (0.27)
T215L	0				
T215N	1 (0.04)				
T215S	1 (0.04)				
T215V	0				
T215Y	13 (0.50)				
K219E	4 (0.16)				
K219Q	6 (0.23)				

Pre-treatment genotypic drug-resistance was defined as the presence of at least one amino acid substitution included in the IAS-USA mutation list of December 2010, including the revertant mutations at codon 215. Drug-classes considered were NRTIs, NNRTIs and PIs. Drug-resistance mutations: the wild-type amino acid is given, followed by the codon of the reverse-transcriptase or protease gene, followed by the amino acid substitution conferring resistance. IAS-USA, International Antiviral Society of the USA; NRTI, nucleotide reverse transcriptase inhibitors; NNRTI, nonnucleotide reverse transcriptase inhibitors; PI, protease inhibitors. Amino acid abbreviations: A, alanine; C, cysteine; D, aspartate; E, glutamate; F, phenylalanine, G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

Table 3. Overview of sensitivity analyses on virologic endpoints in the PASER-M cohort.

Definition of virologic endpoint and/or patient sample	PDR groups	N	Virologic failure		Acquired drug-resistance	
			OR (95% CI)	p	OR (95% CI)	p
Virologic failure (≥400c/mL) (main analysis)		2733				
	No PDR		1		1	
	PDR and fully-active ART		1.01 (0.55, 1.87)	0.964	0.97 (0.51, 1.58)	0.934
	PDR and partially-active ART		2.13 (1.44, 3.14)	<0.001	2.30 (1.55, 3.40)	<0.001
Virologic failure (≥50c/mL)		2096				
	No PDR		1		n/a	
	PDR and fully-active ART		1.01 (0.45, 2.25)	0.981		
	PDR and partially-active ART		2.53 (1.53, 4.18)	<0.001		
Exclusion of patients with prior antiretroviral drug exposure		2588				
	No PDR		1		1	
	PDR and fully-active ART		0.97 (0.51, 1.85)	0.919	0.91 (0.46, 1.81)	0.797
	PDR and partially-active ART		2.11 (1.36, 3.25)	0.001	2.29 (1.48, 3.55)	<0.001
Exclusion of patients harbouring a virus with the M184V mutation before ART initiation		2701				
	No PDR		1		1	
	PDR and fully-active ART		1.02 (0.55, 1.88)	0.958	0.97 (0.51, 1.84)	0.929
	PDR and partially-active ART		2.61 (1.67, 4.06)	<0.001	2.75 (1.76, 4.31)	<0.001
Exclusion of patients with "possible drug-resistance"		2690				
	No PDR		1		1	
	PDR and fully-active ART		1.01 (0.54, 1.90)	0.965	0.99 (0.52, 1.88)	0.964
	PDR and partially-active ART		2.24 (1.51, 3.29)	<0.001	2.25 (1.52, 3.33)	<0.001
Missing equals failure *		2699				
	No PDR		1		1	
	PDR and fully-active ART		0.91 (0.47, 1.74)	0.768	0.89 (0.44, 1.78)	0.742
	PDR and partially-active ART		2.31 (1.44, 9.11)	<0.001	2.36 (1.58, 3.53)	<0.001

(table legend on next page)

Table 3 (legend)

Table shows results of multilevel logistic regression baseline models with multiple imputations for missing data. * Participants for whom no outcome HIV-RNA load result was available due to attrition (stopped ART, lost to follow-up, transferred out, died, switched to second-line ART) were classified as virologic failure (≥400 copies per mL). PDR=pre-treatment, drug-resistance; ART, antiretroviral therapy; CI, confidence interval; OR, odds ratio; n/a, not applicable

Summary of results

- Changing the definition of virological failure to ≥50 copies per mL showed the same results as the main analysis.
- Missing-equals-failure* analysis showed the same results as the main analysis.
- Exclusion of all participants with prior antiretroviral drug exposure from regression analysis showed the same results as the main analysis.
- Exclusion of all participants who harboured the M184V mutation (associated with recent prior use of ART) before start of treatment from regression analysis showed the same results as the main analysis.
- Participants with HIV-RNA load >1000 copies per mL but without major IAS-USA drug-resistance mutations after 12 months of ART have been classified as "No drug-resistance" in the main analysis. However, there might be a resistant virus below the sensitivity level of the population-based genotypic assay. Exclusion of this group of "possible drug-resistance" from regression analysis did not affect results.

Table 4. Overview of observational cohort studies investigating the effect of pretreatment drug resistance on treatment outcome.

Panel research in context: Systematic review

Reference	N	Cohort/ Study population	Year	Country	Definition of PDR	Main results
Wittkop *et al. (1)*	10,056	Eurocoord and CHAIN; Genotype was not used to guide treatment selection	≥1998	Europe	Stanford algorithm: at least low-level resistance to ≥1 drug	Patients with PDR to ≥1 prescribed drug had higher risk of VL≥500 c/mL; No risk difference for patients with PDR and fully-active ART.
Bansi *et al. (2)*	935	UK-CHIC; Genotype was not used to guide treatment selection	1999-2006	UK	Stanford algorithm: GSS calculated	Patients with GSS<3 had higher risk of VL≥50 c/mL (week 52)
Chaix *et al. (3)*	350	ANRS CO 06 PRIMO; Genotype was not used to guide treatment selection	1996-2005	France	ANRS: resistant to ≥1 drug	Proportion of patients with VL<400 c/mL lower in patients with PDR (week 24)
Bannister *et al. (4).*	277	EuroSIDA; Genotype was not used to guide treatment selection	1996-2004	Europe	Stanford: at least intermediate resistance to ≥1 drug	No difference for proportion of VL <500 c/mL (week 24)
Oette *et al. (5)*	269	RESINA; Genotype was used to guide treatment selection	2001-2003	Germany	Geno2pheno	No difference for proportion of VL <50 c/mL (week 48)
Pillay *et al. (6)*	201	Seroconverters CASCADE; Genotype was not used to guide treatment selection	1996-2003	Europe	Stanford: at least intermediate resistance to ≥1 drug	No difference in time to VL suppression <500 c/mL
Peuchant *et al. (7)*	172	ANRS CO3 Aquitaine Cohort	1996-2005	France	ANRS: resistant to ≥1 drug	Slower decrease in mean viral load in patients with PDR (one month)

Studies are sorted by descending sample size. ANRS, Agence Nationale de Recherches sur le Sida et les hépatites virales; GSS, Genotypic Sensitivity Score; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PDR, pretreatment drug-resistance; WHO, World Health Organization mutation list.

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