Pretreatment HIV Drug Resistance and HIV-1 Subtype C Are Independently Associated With Virologic Failure: Results From the Multinational PEARLS (ACTG A5175) Clinical Trial

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(See the Editorial Commentary by Wainberg on pages 1550–1.)

Background. Evaluation of pretreatment HIV genotyping is needed globally to guide treatment programs. We examined the association of pretreatment (baseline) drug resistance and subtype with virologic failure in a multinational, randomized clinical trial that evaluated 3 antiretroviral treatment (ART) regimens and included resource-limited setting sites.

Methods. *Pol* genotyping was performed in a nested case-cohort study including 270 randomly sampled participants (subcohort), and 218 additional participants failing ART (case group). Failure was defined as confirmed viral load (VL) >1000 copies/mL. Cox proportional hazards models estimated resistance–failure association.

Results. In the representative subcohort (261/270 participants with genotypes; 44% women; median age, 35 years; median CD4 cell count, 151 cells/μL; median VL, 5.0 $\log_{10}$ copies/mL; 58% non-B subtypes), baseline resistance occurred in 4.2%, evenly distributed among treatment arms and subtypes. In the subcohort and case groups combined (466/488 participants with genotypes), used to examine the association between resistance and treatment failure, baseline resistance occurred in 7.1% (9.4% with failure, 4.3% without). Baseline resistance was significantly associated with shorter time to virologic failure (hazard ratio [HR], 2.03; $P = .035$), and after adjusting for sex, treatment arm, sex–treatment arm interaction, pretreatment CD4 cell count, baseline VL, and subtype, was still independently associated (HR, 2.1; $P = .05$). Compared with subtype B, subtype C infection was associated with higher failure risk (HR, 1.57; 95% confidence interval [CI], 1.04–2.35), whereas non-B/C subtype infection was associated with longer time to failure (HR, 0.47; 95% CI, .22–.98).

Conclusions. In this global clinical trial, pretreatment resistance and HIV-1 subtype were independently associated with virologic failure. Pretreatment genotyping should be considered whenever feasible.

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Keywords. HIV; drug resistance; subtype; clinical trial.

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Resource-limited settings disproportionately bear the global burden of human immunodeficiency virus (HIV) [1]. The development and transmission of antiretroviral (ARV) drug resistance are major hurdles to HIV care [2]. As ARV therapy (ART) becomes more accessible in resource-limited settings [3], it is essential to investigate ARV drug resistance in diverse HIV type 1 (HIV-1) subtypes and settings to guide clinical care [4–6].

In resource-rich settings, resistance prior to ART is associated with a shorter time to and higher rate of treatment failure [7, 8]. In such settings, baseline resistance testing is recommended and cost effective. In resource-limited settings, the World Health Organization (WHO) recommends surveillance of baseline resistance only on a population level to guide programmatic strategies [9]. With escalating ART rollout, a significant increase in ARV drug resistance is expected over time. Recent reports show moderate rates of ARV drug resistance (5%–15%) [10] that are at least partly related to limited monitoring of patients receiving ART, late identification of ART failure, and accumulation of resistance that can be transmitted [11, 12]. Further increases in transmitted and acquired HIV drug resistance could compromise use of ARV drugs for HIV treatment and prevention [3].

Global data on the effect of HIV drug resistance on virologic response to first-line ART, particularly in persons infected with HIV-1 non-B subtypes, are limited [13, 14], and have not been reported from clinical trials in adults. The Prospective Evaluation of Antiretrovirals in Resource-Limited Settings (PEARLS) AIDS Clinical Trials Group (ACTG) study was a clinical trial of initial ART conducted between 2005 and 2010 in 9 countries spanning 4 continents; most participants were in resource-limited settings [15]. Here, we present results of preplanned analyses from PEARLS of the association of pre-ART resistance with treatment outcome.

METHODS

Participants and Study Design

PEARLS (ClinicalTrials.gov NCT00084136) was a multinational, phase 4, randomized, open-label clinical trial, conducted in Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, the United States, and Zimbabwe [15]. Major enrollment criteria included age >18 years, HIV infection, CD4 count <300 cells/μL, and ART naive by self-report and medical records review (<7 days of cumulative drug exposure prior to enrollment). Single-dose nevirapine (sdNVP) or zidovudine (ZDV) for prevention of mother-to-child transmission (PMTCT) was allowed. During 2005–2007, 1571 eligible participants were enrolled and randomly assigned to 1 of 3 treatment arms: arm A: efavirenz (EFV) + coformulated ZDV-lamivudine (3TC), n = 519; arm B: atazanavir (ATV) + didanosine (ddI) + emtricitabine (FTC), n = 526; arm C: EFV + coformulated FTC–tenofovir disoproxil fumarate (TDF), n = 526. For management of ARV toxicity, the study provided the following drugs as substitutes for randomized therapy: stavudine or TDF for ZDV, ddI for TDF, and NVP for EFV. Follow-up was completed in May 2010.

Personnel at all sites received standardized training in adherence counseling and adherence assessment prior to study initiation. Trained site personnel provided adherence counseling at study entry and as needed thereafter using a checklist that included covered information about adherence, motivational training, and adherence skills (eg, scheduling and techniques for remembering). Adherence counseling was provided to participants with virologic failure, but the protocol did not specify that the adherence counseling occur between the 2 viral load (VL) measurements [15]. Adherence data at each visit were collected via self-report using a standardized questionnaire administered in local languages (ACTG QOL0061, adapted from Chesney et al [16]). Adherence data over the first 12 weeks of study follow-up (ie, prior to initial measurement of virologic failure at week 16) were summarized into a dichotomous indicator variable representing either no problems or any/some adherence problems.

Low prevalence of baseline resistance was assumed. Therefore, genotyping was not performed for the entire study population (n = 1571). Instead, a nested case-cohort study design (Figure 1) was employed to address the study objectives in a large cohort where the outcome (ie, viral failure) was relatively rare. Baseline samples were obtained from 2 groups: (1) subcohort group: a subset of 270 participants randomly selected from the total study population (stratified by treatment arm and

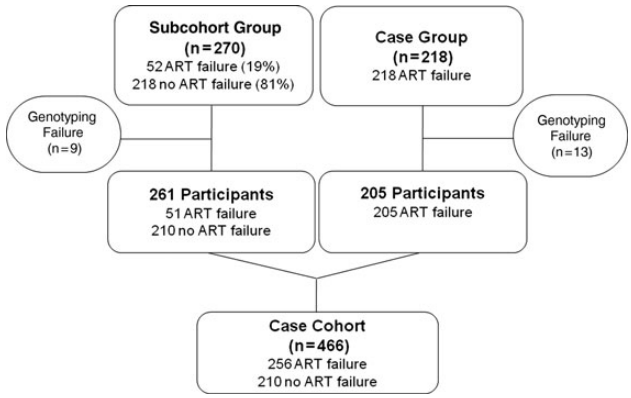

Figure 1. A flow diagram of eligible participants included in the case-cohort analysis. The 270 participants in the subcohort group are a representative random sample of the 1571 participants enrolled in the Prospective Evaluation of Antiretrovirals in Resource-Limited Settings (PEARLS) study. The 218 participants in the case group are participants who are not part of the subcohort group, who experienced virologic failure. Abbreviation: ART, antiretroviral treatment.

country to provide similar estimation precision). This group was included in the estimation of baseline resistance and its correlates for the entire study cohort; and (2) case group: 218 participants not included in the subcohort group who experienced virologic failure. The case group was only included in the assessment of associations between baseline resistance and virologic failure. For this analysis, the case group was combined with the subcohort group to form the full case cohort sample (488 participants, 466 who had genotyping results). Covariate information (including baseline genotype) was collected from the full case cohort. Because the subcohort group was a random sample chosen prospectively without regard for virologic failure, the sampling design simultaneously provided unbiased estimates of baseline resistance.

Laboratory Methods

VL and CD4 cell count were measured at enrollment and every 8 weeks throughout the study, as previously described [15]. Plasma samples were stored for genotyping. *Pol* genotyping was performed retrospectively at 4 regional laboratories that participated in the National Institute of Allergy and Infectious Diseases (NIAID) Division of AIDS Virology Quality Assurance program [17] using the ViroSeq HIV-1 Genotyping System (Celera Diagnostics, Alameda, California). The HIV Prevention Trials Network (HPTN) Laboratory Center coordinated the genotyping.

Sequence Analysis

Resistance mutations were assessed using the 2009 WHO mutation list [18]. HIV-1 subtypes were determined by phylogenetic analysis, using PHYLIP DNADIST and neighbor-joining programs [19], with reference sequences from the Los Alamos National Laboratory (LANL) Database [20]. Based on the resulting output matrix of genetic distance coefficients, the subtype of the closest reference sequence was taken as the probable subtype. Sequences with ambiguous subtype calls were reviewed and manually classified by the LANL recombinant identification program [20]. Sequence quality control was performed with SQUAT [21].

Statistical Methods

The association of baseline resistance with other baseline characteristics of participants was assessed in the subcohort group. Characteristics included sex, race, age, pretreatment CD4 cell count and VL, subtype, country, history of AIDS or tuberculosis, hepatitis B serology, and prior ARV drug use for PMTCT. Fisher exact tests (for categorical variables) or Wilcoxon rank-sum tests (for continuous variables) tested association between characteristics and presence of baseline resistance. Prevalence estimates used exact methods (Clopper–Pearson) for confidence interval (CI) calculations.

Confirmed virologic failure was defined as having 2 consecutive VLs >1000 copies/mL ≥14 weeks after randomization. Association between resistance and initial confirmed virologic failure was tested in the full case cohort (Figure 1). To handle the case-cohort sampling design appropriately, a Cox proportional hazards model using inverse probability weighting was fit to study data. Multivariable models were built purposefully (ie, investigator request for covariate inclusion), and testing whether resistance modified the treatment effect for virologic failure used an interaction term and 2 degrees of freedom Wald test.

The planned size of the subcohort group (n = 270) was based on the following assumptions: (1) 5% prevalence of resistance in the full study cohort; (2) virologic failure in 29% of the 1571 participants in the full study cohort; and (3) type 1 error of 5%. Based on these assumptions, the case-cohort design had 80% power to detect a hazard ratio (HR) of 2.3 or higher for virologic failure between those with vs those without resistance. Using the same assumed prevalence of resistance, the estimated precision on prevalence of resistance within any country (n = approximately 30 participants) was estimated to be approximately 13 percentage points (or approximately 5 percentage points across countries). All statistical analyses were performed using SAS version 9.2 on the UNIX platform.

RESULTS

Study Participants

Of 1571 participants, 488 (31%) were included in the full case cohort (Figure 1). Genotyping was successful for 261 of 270 (97%) participants in the subcohort group (51 failed ART) and for 205 of 218 (94%) additional participants in the case group (all failed ART). Baseline characteristics for participants with genotyping results in the randomized subcohort group and full case cohort are shown in Table 1.

Baseline Resistance in the Subcohort Group and Case Group

In the subcohort group, at least 1 resistance mutation was identified in 11 of 261 (4.2%) participants (95% [CI], 2.1%–7.4%) (IDs 1–11, Table 2); 7 had nucleoside reverse transcriptase inhibitor (NRTI) mutations, 2 had nonnucleoside reverse transcriptase inhibitor (NNRTI) mutations, and 2 had protease inhibitor (PI) mutations. Six of the 11 (55%) were infected with non-B subtypes; none of the 11 reported prior ART. The prevalence of resistance did not significantly vary by country (Table 3). None of the baseline characteristics examined (listed in Table 1) were significantly associated with resistance, overall or by ARV drug class. There was no significant association with resistance and early self-reported adherence to ART.

In the case group, at least 1 resistance mutation was identified in 22 of 205 (11%) participants (IDs 12–33, Table 2). All 22

Table 1. Baseline Demographics and Clinical Characteristics of Study Participants With Genotyping Results

Variable	Random Subcohort (n = 261)	Full Case Cohort (N = 466)
Male sex	147 (56%)	259 (56%)
Race		
Asian	57 (22%)	91 (20%)
Black or African American	133 (51%)	257 (55%)
White	38 (15%)	65 (14%)
Native American	0 (0%)	1 (0%)
Other	31 (12%)	50 (11%)
Unknown	2 (1%)	2 (0%)
Age, y, mean (range)	36 (18–65)	35 (18–65)
VL, $\log_{10}$ copies/mL, mean (range)	5.0 (2.6–5.9)	5.0 (2.6–5.9)
CD4 count, cells/μL, mean (range)	151 (3–297)	151 (2–298)
Treatment arm		
A (EFV+3TC+ZDV)	88 (34%)	150 (32%)
B (ATV+ddI+FTC)	86 (33%)	172 (37%)
C (EFV+FTC+TDF)	87 (33%)	144 (31%)
Geographic region		
Africa	86 (33%)	179 (38%)
South America	59 (23%)	93 (20%)
Asia	56 (23%)	88 (19%)
United States	30 (11%)	66 (14%)
Caribbean	30 (11%)	40 (9%)
Subtype/CRF		
C	119 (46%)	243 (52%)
B	109 (42%)	181 (39%)
CRF01_AE	24 (9%)	26 (6%)
F1	4 (2%)	5 (1%)
CRF02_AG	2 (1%)	3 (1%)
Other[a]	3 (1%)	8 (2%)

Abbreviations: 3TC, lamivudine; ATV, atazanavir; CRF, circulating recombinant form; ddI, didanosine; EFV, efavirenz; FTC, emtricitabine; TDF, tenofovir disoproxil fumarate; VL, viral load; ZDV, zidovudine.

[a] Random subcohort: CRF12_BF (1), CRF31_BC (2); full case cohort: A1 (1), CRF12_BF (1), CRF15_01B (1), CRF31_BC (5).

participants had ≥1 NNRTI mutation, 9 (41%) had ≥1 NRTI mutation, and 9 (4.4%) had dual-class resistance. Four of the 22 participants reported having received ARV drugs for PMTCT.

Association of Baseline Resistance and Virologic Failure

Virologic failure outcomes by resistance, subtype, and treatment arm were evaluated using the full case cohort (N = 466; Table 4). At least 1 resistance mutation was detected in 24 of 256 (9.4%) samples from participants with virologic failure and 9 of 210 (4.3%) samples from participants without virologic failure. Among the 24 participants with resistance who failed ART, 23 (96%) had NNRTI resistance, 9 (38%) had NRTI resistance, and 1 (4%) had PI resistance. Furthermore, 83% (20/24) of participants with resistance who failed ART had mutations associated with drugs in their initial ART regimen. The 4 participants who did not (IDs 7, 16, 17, and 22) were in treatment arm B and had the baseline EFV-associated mutation, K103N. Three of these 4 participants changed to an EFV-containing regimen prior to ART failure.

Eighty-five of the 256 (33%) participants who failed ART experienced ART failure at the earliest time (week 16 study visit). Eleven of these 85 (13%) had baseline resistance (Table 2). Median time to virologic failure among those 256 who failed was 32 weeks (75th percentile: 68 weeks) and the median time to confirmation of ART failure was 4 weeks (interquartile range [IQR], 2–7 weeks). Among 232 participants without baseline resistance, median VL at failure was 4.2 $\log_{10}$ copies/mL (IQR, 3.6–5.0 $\log_{10}$ copies/mL). Among 24 participants with resistance, the median VL at failure was 4.2 $\log_{10}$ copies/mL (IQR, 3.4–4.7 $\log_{10}$ copies/mL). VL at initial or confirmatory time points was not significantly associated with resistance or treatment arm.

Among 256 participants who failed ART, median CD4 count at failure was 241 cells/μL (IQR, 167–359 cells/μL), and median change from pretreatment CD4 cell count was 98 cells/μL (IQR, 24–181 cells/μL). Among 232 participants without resistance who failed ART, these values were 242 cells/μL (IQR, 171–368 cells/μL) and 102 cells/μL (IQR, 26–189 cells/μL), respectively. Among 24 participants with resistance, these values were 211 cells/μL (IQR, 145–248 cells/μL) and 69 cells/μL (IQR, 13–121 cells/μL), respectively. The distribution of changes in CD4 cell count from pretreatment to virologic failure was not significantly associated with resistance ($P = .08$).

In a univariable weighted Cox proportional hazards model, baseline resistance was significantly associated with virologic failure and shorter time to virologic failure (HR, 2.03 [95% CI, 1.05–3.92; $P = .035$]). After adjusting for sex, treatment arm, sex–treatment arm interaction, pretreatment CD4 cell count, baseline VL, and subtype, significance remained (HR, 2.1 [95% CI, 1.0–4.6]; $P = .05$). In the same multivariable model, subtype was independently associated with virologic failure ($P = .001$). Compared to subtype B, non–subtype C/non–subtype B infection was protective against failure (HR, 0.39 [95% CI, .19–.81]), whereas there was a nonsignificant trend toward an association between subtype C infection and increased risk of failure (HR, 1.4 [95% CI, .98–2.1]).

In a multivariable model, additionally adjusting for CD4 cell count change and self-report of nonadherence within the first 12 weeks, baseline resistance remained significantly associated with virologic failure and time to failure (HR, 2.26 [95% CI, 1.03–4.95]). Subtype was again independently associated with

Table 2. Baseline Drug Resistance Mutations According to Treatment Arm, Country, and Subtype

ID	Treatment Arm	Country	Subtype	NRTI	NNRTI	PI
Subcohort group						
1	A (EFV+3TC+ZDV)	India	C		L100IL	
2		Thailand	AE	D67N, K219Q		
3		US	B			M46LM
4		US	B	M41LM		
5	B (ATV+ddI+FTC)	Haiti	B	M184I		
6[a]		Malawi	C			I85IV
7[a]		Malawi	C		K103N	
8		South Africa	C	T69ADNT		
9	C (EFV+FTC+TDF)	Peru	B	M41L, L210W, T215D		
10		Thailand	AE	T215S		
11		US	B	T215S		
Case group						
12	A (EFV+3TC+ZDV)	Malawi	C	M184V, T215NSTY	K103N, 181C	
13		Malawi	C		K103KN	
14		South Africa	C		K103KN	
15[b]		South Africa	C		K103KN	
16	B (ATV+ddI+FTC)	Brazil	B		K103N	
17		Haiti	B		K103KN	
18		India	C	M184V	K101EK, Y188CY, G190S	
19		Malawi	C	M184V	V106AV	
20[b]		Malawi	C	M184V	Y181C	
21		Malawi	C	M184V	K103N	
22		US	B		K103KN	
23	C (EFV+FTC+TDF)	Brazil	B		K103KN	
24		Brazil	F1		K103KN	
25		Haiti	B	T215FIST	Y188L	
26		Malawi	C	M184V	K101E, G190A	
27		Malawi	C	K65R, Y115FY, M184V	K103N, V106M	
28[b]		Peru	B		K103RS	
29[b]		South Africa	C		K101E	
30		Thailand	AE		K103N, G190AG	
31		US	B	K65R	L100I	
32		US	B		Y188L	
33		US	B		K103N	

Subcohort group: ID 1–11; case group: ID 12–33. The identification numbers of participants who failed at the earliest available time point (16 weeks) are underlined.

Participants with mutations not associated with initial regimens: ID 7, switched to ZDV+3TC+EFV after 79 weeks on the study regimen and failed 95 weeks after the regimen switch; ID 17, switched to ddI+FTC+EFV after 19 weeks on the study regimen and failed 5 weeks after that regimen switch; ID 22, switched to ddI+FTC+EFV after 16 weeks on the study regimen and failed 3 weeks after the regimen switch; ID 16, remained on study drug and failed treatment after 16 weeks.

The following participants received antiretroviral drugs for prevention of mother-to-child transmission (PMTCT) of HIV prior to enrollment: ID 15, received single-dose nevirapine (sdNVP) 16 weeks before enrollment; ID 20, received sdNVP 11 weeks before enrollment; ID 28, received ZDV monotherapy 65–70 weeks before enrollment; ID 29, received sdNVP 29 weeks before enrollment.

Abbreviations: 3TC, lamivudine; ATV, atazanavir; ddI, didanosine; EFV, efavirenz; FTC, emtricitabine; HIV, human immunodeficiency virus; ID, participant number; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

[a] Participants in the subcohort group who subsequently failed treatment.

[b] Participants with prior exposure to antiretrovirals for PMTCT (see text in footnote above for details).

virologic failure and time to virologic failure: subtype C infection was associated with shorter time to failure (HR, 1.57 vs subtype B [95% CI, 1.04–2.35]), and infection with other subtypes (non-B, non-C) was associated with longer time to failure (HR, 0.47 vs subtype B [95% CI, .22–.98]). There was no statistically significant evidence that the treatment effect for VL that was

Table 3. Estimated Prevalence of Baseline Resistance in the Subcohort

	No.	Prevalence Estimate	95% CI for Prevalence
Overall	261	4.2%	(2.1%–7.4%)
Country			$P = .7$
Brazil	29	0%	NA
Haiti	30	3.3%	(0%–10.2%)
India	28	3.6%	(0%–10.9%)
Malawi	29	6.9%	(0%–16.7%)
Peru	30	3.3%	(0%–10.2%)
South Africa	29	3.5%	(0%–10.5%)
Thailand	28	7.1%	(0%–17.3%)
United States	30	10.0%	(0%–21.4%)
Zimbabwe	28	0%	NA
Subtype			$P = .7$
B	109	4.6%	(.6%–8.6%)
C	119	3.4%	(.1%–6.7%)
Non-B, non-C	33	6.1%	(0%–14.7%)

Abbreviations: CI, confidence interval; NA, not applicable.

originally observed in the study [15]) was modified by baseline resistance ($P = .4$ for interaction).

DISCUSSION

This report presents results of preplanned analyses of baseline HIV drug resistance in a randomized controlled clinical trial. Baseline resistance was relatively uncommon (4.2%) but was significantly associated with virologic failure and time to virologic failure. The strict enrollment criteria, study oversight, and monitoring of the PEARLS trial strengthen the conclusions of the study. Inclusion of participants with equal sex distribution from geographically, ethnically, racially, and economically diverse regions strengthens the relevance of the findings to the global HIV epidemic.

The observed associations between resistance and ART failure in the report are consistent with previous findings from both resource-limited and resource-rich settings [7, 13, 22, 23], including a report by Hamers et al that analyzed baseline resistance in sub-Saharan Africa [13]. That study and ours both reached similar conclusions, but some differences between the studies should be noted. First, PEARLS was a randomized, controlled trial, whereas the prior study was based on analysis of a prospective cohort. Second, PEARLS was conducted on 4 continents; the prior study was conducted in Africa only. Third, we used the WHO mutation list designed specifically for transmitted resistance surveillance in multiple subtypes [18], whereas

Table 4. Virologic Failure Outcomes by Baseline Resistance, Subtype, and Treatment Arm

	Subcohort Group		Case Group	
Outcome	No VL Failure	VL Failure	VL Failure	Total
Full case cohort				
No resistance	201	49	183	433
Any resistance	9	2	22	33
Total	210	51	205	466
Model, resistance only: HR, 2.03 [95% CI, 1.05–3.92]				
Subtype				
Subtype B				
All	88	21	72	181
No resistance	83	21	63	167
Any resistance	5	0	9	14
Subtype C				
All	92	27	124	243
No resistance	90	25	113	228
Any resistance	2	2	11	15
Other subtypes				
All	30	3	9	42
No resistance	28	3	7	38
Any resistance	2	0	2	4
Model, resistance controlling for subtype: HR, 2.16 [95% CI, 1.11–4.23]				
Treatment arm				
Arm A: EFV+3TC+ZDV				
All	72	16	62	150
No resistance	68	16	58	142
Any resistance	4	0	4	8
Arm B: ATV+ddI+FTC				
All	67	19	86	172
No resistance	65	17	79	161
Any resistance	2	2	7	11
Arm C: EFV+FTC+TDF				
All	71	16	57	144
No resistance	68	16	46	130
Any resistance	3	0	11	14
Model, resistance controlling for treatment arm: HR, 2.01 [95% CI, 1.04–3.88]				

Abbreviations: 3TC, lamivudine; ATV, atazanavir; CI, confidence interval; ddI, didanosine; EFV, efavirenz; FTC, emtricitabine; HR, hazard ratio; TDF, tenofovir disproxil fumarate; VL, viral load; ZDV, zidovudine.

the prior study used the International AIDS Society–USA [24] and Stanford [25] mutation lists. Fourth, PEARLS had stringent eligibility criteria regarding previous ART exposure, which reduced the likelihood for confounding by prior exposure. Finally, the longitudinal design of the PEARLS trial, in which participants were monitored for virologic failure in real time at 8-week intervals, allowed identification of the association between baseline resistance and time to virologic failure, which was not feasible in the prior study. Our findings thus significantly expand

and strengthen previously observed associations between baseline resistance and ART outcomes in resource-limited settings.

Interestingly, we did not observe a significant association between the prevalence of baseline resistance and either HIV-1 subtype or country. However, we did find that those infected with subtype C were more likely to fail ART, and to fail earlier than those infected with subtype B. Although HIV-1 subtype is not currently part of clinical care considerations, some reports suggest that it might be associated with disease progression and drug resistance development [4, 6, 26], whereas others disagree [27, 28]. Similar controversy exists particularly for subtype C [29–31]. We present the first clinical trial data to support the association of HIV-1 subtype, particularly the globally predominant subtype C, with viral failure. Subtype C has been associated with a unique NNRTI mutation, V106M [32], increased occurrence of the NRTI K65R mutation [33], and overall higher NRTI and NNRTI resistance prevalence [34]. Differences in the types and frequency of specific drug resistance mutations have been noted for other HIV-1 subtypes as well [35, 36]. These differences likely reflect subtype-based differences in the sequences of HIV protease, reverse transcriptase, and other viral proteins targeted by ARV drugs. These differences may impact treatment outcomes and the prevalence of drug resistance in regions where different subtypes predominate. Ongoing studies are analyzing resistance at failure in the PEARLS study; other studies are planned to evaluate the frequency and impact of minority resistance variants [37]. Continued research on subtype-related differences in treatment failure and drug resistance is warranted, especially as subtype may be confounded with other factors that were not controlled in this study. The genetic barrier to resistance varies among ARV drugs. The impact of specific drug resistance mutations also varies. Mutations such as K103N and M184V, which confer high-level drug resistance and resistance to multiple drugs, have more significant implications for treatment options and outcomes than mutations associated with low-level resistance and/or resistance to single ARV drugs.

Pre-ART genotyping is cost-effective in the United States where the estimated prevalence of transmitted drug resistance is >1% [8], but is not routine in most resource-limited settings. This primarily reflects infrastructure and cost constraints, but also the lack of data documenting utility and cost-effectiveness of pre-ART resistance testing, particularly in settings with low prevalence of drug-resistant HIV. The benefit of pre-ART resistance testing is expected to increase in resource-limited settings as levels of resistance rise with ART scale-up. It is important to note that this study was conducted in the context of a clinical trial, with frequent VL monitoring, as well as other adherence assessments and adherence counseling. It will also be important to conduct similar analyses of baseline resistance and ART outcome in nontrial (clinical) settings in resource-limited settings where key components of HIV care, such as VL and CD4 cell count monitoring, are not routine [38].

One limitation of our study is that some participants may have had prior exposure to ARV drugs and either did not know this or chose not to disclose this information at time of enrollment. Thus, it is not possible to conclude that all cases of baseline resistance were due to transmitted drug resistance. Failure to disclose ARV drug use has been documented in other studies, including a trial performed at some of the same sites as PEARLS [39–41]. Another limitation of this study is that pre-ART resistance may have been underestimated due to mutation decay and reversion in study participants with long-standing HIV infection [42]. Genotyping was also performed using population sequencing, which may not detect low-level HIV variants with drug resistance mutations [43]. Additionally, PEARLS baseline samples were collected between 2005 and 2007, relatively early in the roll-out of ART, which might have affected the prevalence of pretreatment drug resistance. Last, because the frequency of baseline resistance was relatively low in the subcohort, some analyses designed to identify subpopulations that would be most likely to benefit from pre-ART genotyping (eg, analyses of the association of baseline resistance with subtype and geography) were limited due to the small absolute numbers of persons with the outcome of baseline resistance.

In summary, this report demonstrates a significant negative impact of baseline resistance on ART outcome in adults with diverse subtypes in diverse settings. Although baseline resistance prevalence remains low in many settings, continued surveillance is necessary, especially in regions with programs or plans for enhanced ART scale-up. With expected increases in the prevalence of transmitted resistance worldwide, this study will help inform policy decisions related to the use of resistance testing in clinical practice in resource-limited settings. The findings in the report support use of genotyping prior to ART initiation in resource-limited settings, when feasible.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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