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Viral suppression and HIV drug resistance at 6 months among women in Malawi's Option B+ program: Results from the PURE Malawi Study

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Abstract

Background—In 2011, Malawi launched Option B+, a program of universal ART treatment for pregnant and lactating women to optimize maternal health and prevent pediatric HIV infection. For optimal outcomes, women need to achieve HIVRNA suppression. We report 6 month HIVRNA suppression and HIV drug resistance in the PURE study.

Methods—PURE study was a cluster-randomized controlled trial evaluating three strategies for promoting uptake and retention; Arm 1: Standard of Care, Arm 2: Facility Peer Support and Arm 3: Community Peer support. Pregnant and breastfeeding mothers were enrolled and followed according to Malawi ART guidelines. Dried blood spots for HIVRNA testing were collected at 6 months. Samples with ART failure (HIVRNA ≥1000 copies/ml) had resistance testing. We calculated odds ratios for ART failure using generalized estimating equations with a logit link and binomial distribution.

Results—We enrolled 1269 women across 21 sites in Southern and Central Malawi. Most enrolled while pregnant(86%) and were WHO Stage 1(95%). At 6 months, 950/1269 (75%) were retained; 833/950 (88%) had HIVRNA testing conducted and 699/833(84%) were suppressed. Among those with HIVRNA ≥1000 copies/ml with successful amplification (N=55, 41% of all VL> 1000 copies/ml), confirmed HIV resistance was found in 35% (19/55), primarily to the Non-nucleoside reverse transcriptase inhibitors(NNRTI) class of drugs. ART failure was associated with treatment default but not study arm, age, WHO stage, or breastfeeding status.

Conclusions—Virologic suppression at 6 months was <90% targets, but the observed confirmed resistance rates suggest adherence support should be the primary approach for early failure in Option B+.

Keywords

HIV drug Resistance; Option B+

Background

In 2011, Malawi launched Option B+, a program of universal antiretroviral therapy (ART) for pregnant and lactating women regardless of CD4+ cell count and/or any clinical stage to optimize maternal health and prevent pediatric HIV infections[1,2]. The early results of the Malawi program have been dramatic and positive: by 2012, the number of HIV infected women receiving ART increased by 7 fold[3], over 77% of women were retained in the program through 12 months post-initiation[3,4], 96% of retained women had HIV RNA < 400 copies at 6 months post initiation[5] and HIV infection rates among exposed children tested at < 2months were 2.4%[4].

Alongside the Option B+ program, the Malawi ART program has advanced routine virological monitoring to assess treatment success. To date, results suggest high levels of suppression among patients retained in care.[6] The current schedule for monitoring includes HIVRNA testing at 6 months post ART initiation, 2 years, and every 2 years thereafter. The 6 month collection time point, early in the treatment course, is designed to detect early adherence problems that may be addressed through appropriate counseling interventions. Alternatively, this early time point may detect individuals who are failing treatment due to pre-existing transmitted ART resistance.

The prevalence of ART drug resistance among Option B+ women in Malawi has not yet been described. A prior survey in Malawi found high virological suppression at 12 months (91%)[7,8]. However, the estimated prevalence of pre-treatment HIV resistance ranged from 2.8 and 6.5%[7] which accounted for 37.5% of the detected resistance at 12 months. A more recent report from Malawi suggests that between 5-15% of pregnant women have transmitted HIV drug resistance using the WHO threshold survey (personal communication, Nellie Wadonda Kabondo), predominantly to the non-nucleoside reverse transcriptase inhibitor (NNRTI) class of drugs which forms the back bone of the most popular first line therapy worldwide [9].

In order to understand the relative contributions of pre-existing resistance and ART adherence to treatment failure, this study evaluated the HIV drug resistance at 6 months among women experiencing treatment failure in the PURE (**P**MTCT **U**ptake and **RE**tention) study, a cluster randomized trial evaluating strategies for promoting uptake and retention in the Option B+ program in Malawi.

Methods

Study Design and Population

The PURE study was a cluster-randomized trial evaluating three strategies for promoting ART uptake and retention in care; Arm 1: Standard of Care, Arm 2: Facility Based peer support, and Arm 3: Community based peer support[10,11]. The PURE study was conducted in 21 sites in Central and Southern Malawi. Pregnant and Breastfeeding mothers were enrolled at the time of HIV diagnosis in the ANC or post-partum clinics and followed according to Malawi ART management protocols.

Clinical Follow up

The Malawi national guidelines recommend that newly diagnosed pregnant and breast feeding women initiate Tenofovir/Lamivudine/Efavirenz (TDF/3TC/EFV) therapy as a single fixed dose combination tablet. Women were seen monthly for the first 6 months, and then quarterly. Dried blood spots (DBS) were collected at the time of ART initiation, 6 months and 2 years for HIVRNA testing and possible resistance testing. For the viral load (VL) and resistance analysis, DBS collected through month 11 were considered as “6 month” specimens and all women with VL data were included regardless of whether they had any default episodes during the follow-up period. Default within the national program is defined as missing an antiretroviral appointment by 60 days but such individuals can return

to care. Demographics, WHO stage, and treatment outcomes were collected using the standard HIV monitoring tools, extracted and double entered into an Access database.

Sample Collection and Resistance Testing

HIVRNA ≥ 1000 copies/ml was considered ART failure and those samples were sent for additional resistance testing. Drug resistance testing was also conducted on pre-treatment samples of those with detected drug resistance at month 6.

All health care staff at the sites were trained in the collection of DBS for HIVRNA using DBS at the initial study training and received refresher training by the study coordinators prior to the 6 month follow-up time point. DBS cards were collected by ART clinic using fingerstick. Cards were dried, packaged with desiccant sachets, stored on-site, and transported at ambient temperature to the UNC Project laboratory in Lilongwe (15 minutes to 6 hours away depending on the study site). VL testing was conducted on DBS cards using Abbott RealTime HIV-1 Assay (Abbott Laboratories, Chicago, IL). After testing, cards were returned to plastic zip bags, desiccant was replaced as necessary, and cards were eventually transferred to a -20°C freezer. DBS cards where the VL was ≥1000 copies/ml and the corresponding baseline samples were shipped on dry ice to the United States for sequencing analysis.

Resistance testing

RNA was extracted from DBS cards using the Sample Preparation System for RNA on the Abbott m2000sp (Abbott Laboratories). One to three entire spots (depending on availability) were eluted in 1.7ml RNA Lysis Buffer (Promega) for 2 hours at ambient temperature and extracted using the Abbott 0.6 ml protocol. Previously described primers [12] did not produce any amplicons, so new nested primers for smaller regions of the RT gene (codons 41-116 and 135-230) were used. RNA was concentrated using RNA Clean and Concentrator columns (Zymo Research), eluting into 10ul water. The concentrated RNA was used to synthesize a single cDNA strand with 2 downstream primers and SuperScript III reverse transcriptase (Invitrogen). One-quarter of the reaction was used in a nested PCR using the Expand High Fidelity PCR System (Roche Life Science). The two RT regions were amplified and sequenced. Successful sequences were analyzed using Sequencher 5.3 software and the results were submitted to the Stanford University Drug Resistance Database (http://hivdb.stanford.edu) to determine resistance mutations. In some cases, one of the two RT regions did not amplify, so resistance was only scored for the region that was successfully amplified.

Statistical Analysis

Associations with ART failure (VL≥1000 copies/ml) were evaluated using generalized estimating equations (GEE) with a logit link and binomial distribution and exchangeable correlation structure, to account for clustering. Variables of interest included study arm, age (>=25 years vs <25 years), pregnancy vs lactating at ART initiation, and WHO stage (Stage 1 vs. other). Resistance patterns were described (any NRTI resistance, any NNRTI resistance, and any detected reverse transcriptase resistance) among amplified specimens. All Analyses were conducted using Stata Version 14.0.

Ethics Statement

This study was approved by the National Health Sciences Research Committee, the University of North Carolina Institutional Review Board, and the WHO Ethics Review Committee. All participants provided written informed consent.

Results

This study enrolled 1272 women across 21 sites in Southern and Central Malawi, of which 3 were deemed ineligible and not included in analysis. The majority enrolled while pregnant (86%), were WHO Stage 1(95%), and the median age was 26 years (IQR 22-31) (Table 1). Only 6 women (0.4%) had ever received any previous PMTCT intervention. At the 6 months evaluation time (512 person years), 950/1269 (75%) were retained in care, of whom 833/950 (88%) had viral load testing conducted, with Arm 1 (standard of care) having a lower proportion of viral load specimens collected (Table 2). Notably, ninety two individuals were documented to have defaulted from care but returned to receive HIVRNA during the evaluations period [Arm A: 23/447 (5.1%), Arm B: 33/428 (7.7%), and Arm C: 35/394 (8.9%), p=0.096]. The median duration of ART at the time of collection was 260 days (IQR 232, 295) and was similar across arms (p=0.326). At 6 months, 699/833 (84%) were suppressed with VL<1000 copies/ml. There was no difference between the arms (Table 2), but those with a default episode had lower rates of virologic suppression than those with continuous retention (56/92 (61%) vs. 643/742(87%), p<0.001).

Overall, 134 individuals (16%) had VL>=1000 copies/ml were eligible for resistance testing (Figure 1). 117/134 (87%) specimens were evaluated for drug resistance with some variation across study arms (Table 2).

Overall, 62/117 (53%) failed to amplify either amplicon (RT codons 41-116 and 135-230), which did not vary across arms. Failure to amplify was strongly associated with having lower HIVRNA at the time of failure evaluation. Specifically, 50/62 (81%) of samples that failed to amplify had HIVRNA < 5000 copies/ml. Of those samples that amplified (55), 26 amplified for both primer domains, 17 for region 1 (RT codons 41-116), and 12 for region 2 (RT codons 135-230).

Among the specimens that amplified, 19/55 (35%) had resistance detected with some samples having multiple mutations. NNRTI resistance was more common [19/55 (35%) of amplified specimens] than NRTI resistance [4/55 (7.4%) of amplified specimens]; NRTI resistance was only seen in combination with NNRTI resistance. While not statistically significant, higher rates of confirmed resistance were seen in both of the intervention arms. Also, there was no difference in detected drug resistance patterns among those with default episodes. However, overall more women with default episodes [5/92 (5%)] had confirmed resistance than women with continuous ART use[14/741 (2%)] by virtue of the higher rate of detectable HIVRNA (see Figure 3, Supplementary Digital Content).

The pattern of NNRTI resistance included the K103N (6), G190 (4), e138 (4), Y181C (3), v106 (3), v90 (3), K101E (2), P225H (2), A98G (1), V179D (1), Y188l (1), L100i (1) while the NRTI resistance included the K65R (3), M184V (3), A62 (2), K70 (1), Y115(1)and

T69N (1) (see table, Supplementary Digital Content 1). Overall, 13 individuals had major mutations that would confer reduced susceptibility to current first line and an additional 6 had minor mutations not expected to reduce susceptibility. Among those with detected ART resistance (19), 10 baseline samples were successfully amplified, of which 2 demonstrated baseline resistance. For one individual (Arm 1), both NNRTI (A98G, K101E, Y181C) and NRTI (M184V A62V) was present at baseline and this individual added the K65R mutation by month 6. No previous exposure to ART was reported. The other individual (Arm 2) had NNRTI resistance only and had no new mutations identified at month 6. The pre-treatment ART exposure of this individual was unknown.

Overall, according to arm, Arm 2 and 3 had a higher proportion of suppressed VL representing resistance prevention (Figure 2) with Arm 1 have 46% (95% CI 42-51%) suppression while Arm 2 and Arm 3 had 59% (95% CI 55-64) and 60% (95% CI 55-65) suppression, respectively. Confirmed resistance represented an extremely small fraction of the population.

Additionally, using VL<1000 copies as the failure definition, we found no association with ART failure rate according to age (< 25 vs. 25 and above), WHO stage (Stage 1 vs. other), or pregnant vs. breastfeeding status or treatment arm on unadjusted or adjusted analysis (Table 3). However, those without any default episodes were more likely to have achieved virological suppression (OR 0.23 95% CI 0.14-0.38).

Also, to further understand the potential of previous unreported ART exposure including single dose NVP on virologic suppression and resistance, we evaluated the results according to gravidity status of the women. 864/1269 (68%) of the women had recorded data on gravidity. There was no difference in virologic suppression rates among primagravida women vs. multiparous women (84% (61/73) vs 84% (429/511), p=0.932). With respect to confirmed resistance, 1/7 (14.3%) primigravida women had resistance versus 9/32 (28.1%) multi-gravida women, p=0.448.

Discussion

In this description of HIV viral load suppression and drug resistance among HIV infected women in Option B+ program, HIV virological suppression was below the 90% suppression desirable target and did not differ according to treatment support arm, age, WHO stage, or lactation status. However, among those with elevated HIVRNA, relatively low rates of confirmed resistance were observed and the majority of detected resistance was acquired during treatment, suggesting the potential for intervention to promote adherence prior to the development of HIV drug resistance.

Compared to other cohorts in Malawi[6] and elsewhere, suppression rates were lower than UNAIDS targets of 90% among individuals on ART thereby potentially compromising health gains for the mother and increasing transmission risk to the infant. In part, this higher rate was due to our failure definition of < 1000 copies/ml that was defined by Malawi guidelines and the appropriate threshold for DBS testing, but is not directly comparable to other surveys that have reported at lower VL thresholds. Additionally, our current evaluation

occurred at 6 months. It is possible that individuals with very high baseline viral loads may not yet have fully suppressed. This is supported by the association between defaulting from care and increased likelihood of virological failure. While we did not collect exact dates for resuming treatment, women returning from default may not have been resumed on ART for sufficient time to suppress.

Some women were in care but viral load testing was missed at the clinic. These retained but not-tested women were similar in demographics but more likely to have been seen at a standard of care clinic rather than the peer-supported intervention arms. The study arm interventions focused on retention more than adherence such that virological suppression may not be different in this retained but non-tested group. In contrast, our finding of lower rates of suppression in those with defaulting episodes suggests those not retained in care are more likely to have non-suppression. Hence, at a population level, the suppression rate including women lost to follow-up is likely to be lower than we found in the VL tested population. Overall, this underscores the importance of effective interventions that promote retention, as seen in the PURE study[11] to achieve the 90-90-90 targets.

Among patients with elevated viral loads and with successful amplification for resistance sequencing, NRTI and/or NNRTI resistance was detected in 35% of the women. This confirmed resistance rate is relatively low compared to previous studies evaluating drug resistance at 6 months[13] and markedly contrasts the higher prevalence of confirmed HIV drug resistance among populations tested at or after 12 months of treatment or at the time of ART switch. [9,14-16] This lower rate may be falsely low due to the approach for drug resistance evaluation, whereby we included samples for evaluation even if only 1 of the 2 amplicons amplified and a large number of samples failed to amplify. We conservatively included any mutation identified, including minor mutations that may not affect drug susceptibility such that this may have overestimated resistance. Regardless, in a study conducted in Malawi using the same resistance testing strategy among ART experienced patients retained in care, high rates (>90%) of confirmed resistance were detected[17] suggesting there is a notable distinction between the resistance patterns of Option B+ women at 6 months versus established patients on longer term treatment. Also, given we found defaulters had not necessarily yet developed an increased rate of resistance, programs designed to return these women to care as demonstrated in the PURE study can result in successful suppression. While some studies suggest that resuppression may occur in the presence of existing resistance[18], generally the finding of HIV resistance suggests the regimen should be modified as eventual failure is more likely. For this population, the relatively low rate of resistance supports Malawi's current advice for management of detectable viral load which includes enhancing adherence counseling and rechecking viral load in 3 months to confirm continued viral replication[19].

The pattern of ART resistance observed in our study was primarily due to NNRTI resistance. This may represent a combination of transmitted HIV drug resistance or acquired resistance from either current or previous ART use, including potentially previously single dose NVP use. Reported previous ART use for PMTCT or general health was extremely low in our cohort. Among the 2 individuals we identified with pre-treatment drug resistance, there was no reported ART exposure. We saw no difference in suppression according to gravidity

status suggesting previous unreported PMTCT exposure was less likely. However, we acknowledge the need to further evaluate pre-treatment drug resistance in this cohort, particularly according to primigravida status, to better understand the contribution of transmitted drug resistance toward early treatment failure.

We did not detect any difference between virological suppression and overall confirmed presence of drug resistance according to arm. However, noting that the uptake and retention of ART and HIVRNA collection at 6 months were improved in the peer supported arms[11], likely there are significant gains in resistance prevention overall.

Our study includes the large population of Option B+ mothers attending various sized clinics with the highest HIV prevalence within existing operations of ART clinics in the Malawi government program, thereby representing a highly generalizable population. Yet, our complete resistance evaluation is limited by challenges of working within these settings. These include failure to collect all specimens among retained women at the appropriate timepoints and challenges with maintaining appropriate storage conditions for DBS. DBS can be sensitive to RNA degradation and hence we found using the two primer approach described resulted in more potential amplification of expected drug resistance mutations than traditional sequencing. Low level viremia (<5000 copies/ml) was also less likely to amplify thereby limiting our conclusions to individuals with higher VL. Likewise, as with most resistance testing, our methods would not detect minority resistant variants and non-adherent patients can revert to wild-type in the absence of selective drug pressure. Overall, drug resistance may be somewhat underestimated in our study.

In summary, by 6 months, participants in the PURE study had not yet achieved 90% suppression. Relatively low rates of confirmed HIV resistance coupled with lower suppression rates among those with defaulting episodes suggest supporting retention and adherence interventions for women engaged in the Option B+ program in Malawi can achieve treatment and suppression targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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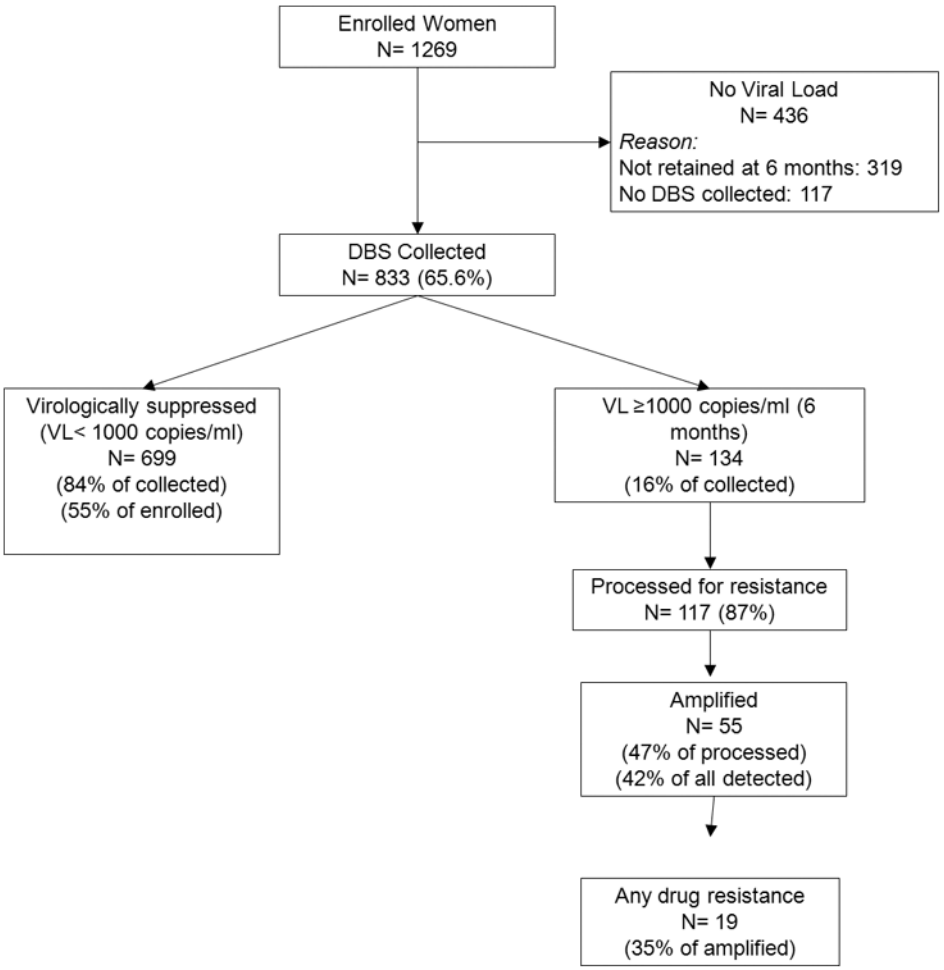

Figure 1.
Flow Algorithm highlighting participant and sample flow to evaluate HIV drug resistance.

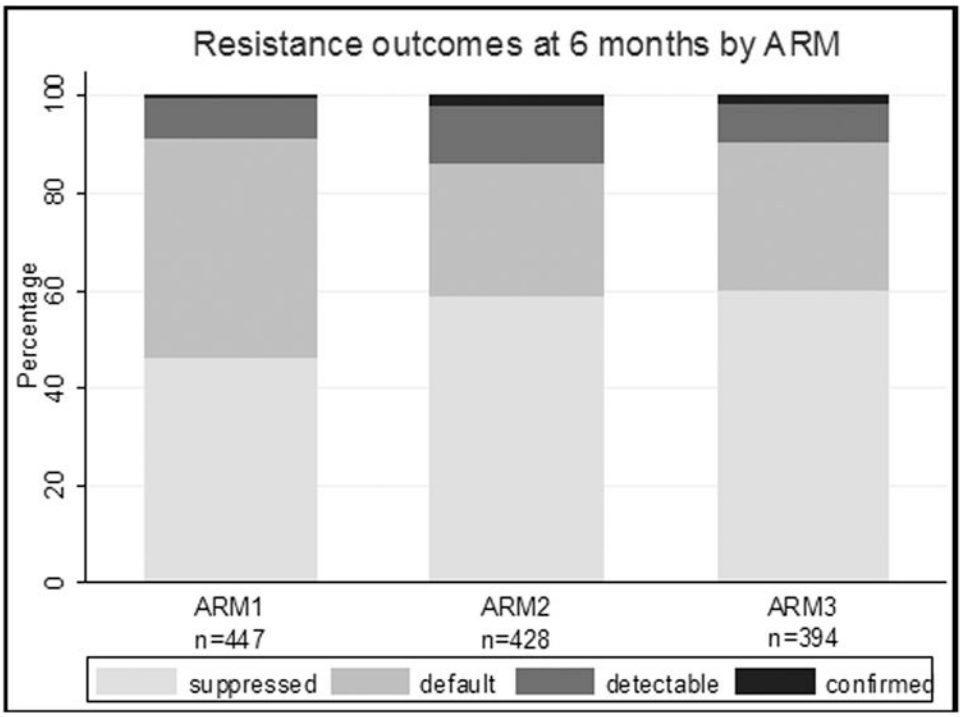

Figure 2.

HIV drug resistance prevention outcomes at 6 months according to study arm. HIV viral suppression (Resistance prevention) was higher in Arm 2 and Arm 3.

Table 1

Baseline Characteristics of 1269 Women participating in PURE study

	Arm 1	%	Arm 2	%	Arm 3	%
N	447		428		394	
Health Zone						
Central West	144	32.2%	40	9.3%	88	22.3%
South East	179	40.0%	177	41.4%	229	58.1%
South West	124	27.7%	211	49.3%	77	19.5%
Facility size						
smallest (20)	119	26.6%	122	28.5%	88	22.3%
small (50)	126	28.2%	103	24.1%	104	26.4%
medium (75)	75	16.8%	78	18.2%	77	19.5%
large(125)	127	28.4%	125	29.2%	125	31.7%
Pregnant/Lactating						
Pregnant	401	89.7%	360	84.1%	333	84.5%
Lactating	46	10.3%	68	15.9%	61	15.5%
Age						
15-21	79	17.7%	100	23.4%	87	22.2%
22-28	174	38.9%	175	40.9%	139	35.3%
29-35	157	35.1%	120	28.0%	129	32.7%
≥36	35	7.8%	31	7.2%	37	9.4%
Missing	2	0.4%	2	0.5%	2	0.5%
Marital Status						
Never married	7	1.5%	5	1.2%	9	2.3%
Married	419	93.7%	393	91.8%	363	92.1%
Divorced	12	2.7%	18	4.2%	19	4.8%
Widowed	5	1.1%	9	2.1%	3	0.8%
Missing	4	0.9%	3	0.7%		
Currently having a sexual partner						
Yes	426	95.3%	400	93.4%	364	92.4%
No	13	2.9%	20	4.7%	27	6.9%

J Acquir Immune Defic Syndr. Author manuscript; available in PMC 2018 June 01.

	Arm 1	%	Arm 2	%	Arm 3	%
Missing	8	1.8%	8	1.9%	3	0.8%
Current sexual partner living in catchment area						
Yes	355	83.3%	328	82.0%	308	84.6%
No	49	11.5%	50	12.5%	33	9.1%
Missing	22	5.2%	22	5.5%	23	6%

Table 2

Virologic and Resistance outcomes at 6 months according to study arm

Outcome	Arm 1	Arm 2	Arm 3	p-value
Number enrolled (N)	447	428	394	N/A
Retained at 6 mo (n/N, %)	312 (70%)	323 (75%)	315 (80%)	
Duration of ART use at time of sampling (median, IQR)	263(232-294)	252 (228-292)	267(239-302)	0.326
HIVRNA collected (n/N, %)	247 (55%)	312 (72%)	274 (70%)	<0.001
< 1000 Copies/ml (n, %)	208 (84%)	254(81 %)	237 (87%)	0.20
1000< 5000 copies/ml (n, %)	24 (10%)	26 (8 %)	21 (8 %)	
>5000 copies/ml (n, %)	15 (6 %)	32 (10%)	16 (6%)	
Log_{10} HIVRNA (median, IQR)	3.4 (3.2-4.4)	3.9 (3.3-4.6)	3.5 (3.3-4.6)	0.723
Eligible for Resistance testing	39	58	37	N/A
Conducted Resistance (n, %)	34 (87%)	56 (97%)	27 (73%)	0.003
Failed to Amplify (n, %)	20 (59%)	27 (48%)	15 (56%)	0.226
Amplified (n, %)	14 (41%)	29 (52%)	12 (44%)	0.226
“Any Resistance”, (n, %)	3 (8%)	9(24%)	7 (58%)	0.198
NRTI resistance	1 (7%)	1 (3%)	3 (25%)	0.130
NNRTI resistance	3 (8%)	9 (24%)	7 (58%)	0.198

Table 3

Adjusted and Unadjusted Logistic Regression showing factors associated with Virologic Suppression at 6 months

Characteristic	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Study Arm		
Arm 1 (Referent)	1.0	1.0
Arm 2	0.81 (0.45- 1.40)	0.76 (0.43-1.30)
Arm 3	1.13 (0.61 - 2.12)	1.17 (0.65-2.10)
Age		
<25 (Referent)	1.0	1.0
>= 25	0.91 (0.63-1.33)	1.01 (0.68-1.49)
WHO stage		
Stage 1 (Referent)	1.0	1.0
Stage 2, 3 or 4	1.87 (0.82-4.27)	2.01 (0.84-4.81)
Pregnancy status[†]		
Pregnant (Ref)	1.0	1.0
Lactating	0.83 (0.49-1.40)	0.71 (0.41-1.22)
Default Event		
Yes (Referent)	1.0	1.0
No	0.25 (0.15-0.42)	0.23 (0.14-0.38)

[†]Refers to status at the time of enrolment into the study, not at the time of HIVRNA collection.