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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Background: In 2011, Malawi launched Option B+, a program of universal antiretroviral therapy (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 3 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 viral loads > 1000 copies/ml), confirmed HIV resistance was found in 35% (19/55), primarily to the nonnucleoside reverse transcriptase inhibitor 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% target, but the observed confirmed resistance rates suggest that

adherence support should be the primary approach for early failure in option B+.

Key Words: 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 after initiation[3,4], 96% of retained women had HIVRNA < 400 copies at 6 months after initiation,[5] and the HIV infection rate among exposed children tested at < 2 months was 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 and 2 years after ART initiation 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 because of pre-existing transmitted ART resistance.

The prevalence of ART drug resistance among option B+ women in Malawi has not yet been described. A previous survey in Malawi found high virological suppression at 12 months (91%).[7,8] However, the estimated prevalence of pretreatment 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% and 15% of pregnant women have transmitted HIV drug resistance using the WHO threshold survey (personal communication, Nellie Wadonda Kabondo), predominantly to the nonnucleoside reverse transcriptase inhibitor (NNRTI) class of drugs which forms the back bone of the most popular first line therapy worldwide.[9]

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 3 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 breastfeeding women initiate tenofovir/lamivudine/efavirenz 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 pretreatment 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 before 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–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 3 entire spots (depending on availability) were eluted in 1.7 mL 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 reverse transcriptase (RT) gene (codons 41-116 and 135-230) were used. RNA was concentrated using RNA Clean and Concentrator columns (Zymo Research), eluting into 10 μl 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 Polymerase Chain Reaction using the Expand High Fidelity Polymerase Chain Reaction System (Roche Life Science). The 2 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 2 RT regions did not amplify, so the 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 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 nucleoside reverse transcriptase inhibitor (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 (interquartile range: 22–31) (Table 1). Only 6 women (0.4%) had ever received any previous Prevention of Mother to Child Transmission (PMTCT) intervention. At the 6 months evaluation time (512 person years), 950/1269 (75%) were retained in care, of whom 833/950 (88%) had VL testing conducted, with arm 1 (standard of care) having a lower proportion of VL specimens collected (Table 2). Notably, 92 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 (interquartile range: 232, 295) and was similar across arms

TABLE 1. Baseline Characteristics of 1269 Women Participating in the 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)
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)

(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 who (16%) had VL ≥ 1000 copies/mL were eligible for resistance testing (Fig. 1). A total of 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).

TABLE 2. Virologic and Resistance Outcomes at 6 Months According to Study Arm

Outcome	Arm 1	Arm 2	Arm 3	*P*
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, interquartile range)	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, interquartile range)	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

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. Although 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
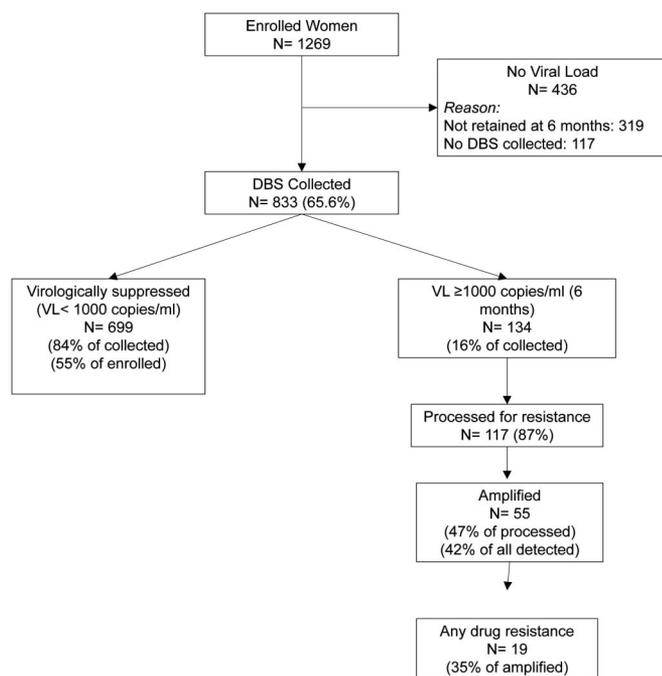

FIGURE 1. Flow algorithm highlighting participant and sample flow to evaluate HIV drug resistance.

continuous ART use [14/741 (2%)] by virtue of the higher rate of detectable HIVRNA (see Figure 3, Supplemental Digital Content, http://links.lww.com/QAI/A994).

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), and L100i (1), whereas the NRTI resistance included the K65R (3), M184V (3), A62 (2), K70 (1), Y115 (1), and T69N (1) (see Table, Supplemental Digital Content 1, http://links.lww.com/QAI/A994). 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) were 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 pretreatment ART exposure of this individual was unknown.

Overall, arms 2 and 3 had a higher proportion of suppressed VL representing resistance prevention (Fig. 2) with arm 1 having 46% [95% confidence interval (CI): 42 to 51] suppression, whereas arm 2 and arm 3 had 59% (95% CI: 55 to 64) and 60% (95% CI: 55 to 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 (odds ratio: 0.23; 95% CI: 0.14 to 0.38).

Also, to further understand the potential of previous unreported ART exposure including single dose Nevirapine
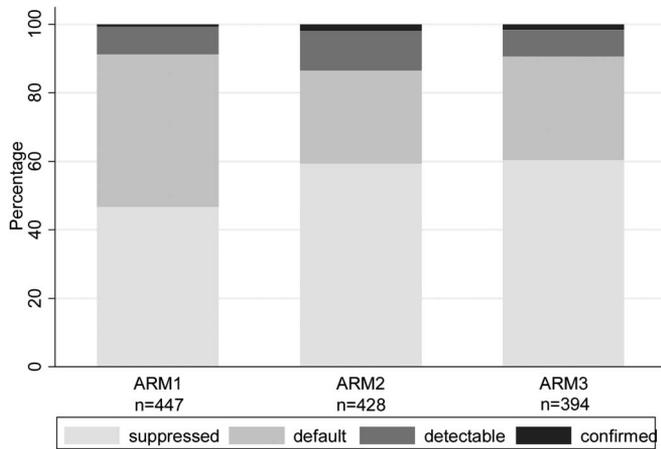
FIGURE 2. HIV drug resistance prevention outcomes at 6 months according to the study arm. HIV viral suppression (resistance prevention) was higher in arm 2 and arm 3.

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 primigravida women vs multiparous women [84% (61/73) vs 84% (429/511), $P = 0.932$]. Regarding confirmed resistance, 1/7 (14.3%) primigravida women had resistance vs 9/32 (28.1%) multigravida women, $P = 0.448$.

DISCUSSION

In this description of HIV VL suppression and drug resistance among HIV-infected women in option B+ program,

TABLE 3. Adjusted and Unadjusted Logistic Regression Showing Factors Associated With Virologic Suppression at 6 Months

Characteristic	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)
Study arm		
Arm 1 (referent)	1.0	1.0
Arm 2	0.81 (0.45 to 1.40)	0.76 (0.43 to 1.30)
Arm 3	1.13 (0.61 to 2.12)	1.17 (0.65 to 2.10)
Age		
<25 (referent)	1.0	1.0
≥25	0.91 (0.63 to 1.33)	1.01 (0.68 to 1.49)
WHO stage		
Stage 1 (referent)	1.0	1.0
Stage 2, 3 or 4	1.87 (0.82 to 4.27)	2.01 (0.84 to 4.81)
Pregnancy status*		
Pregnant (ref)	1.0	1.0
Lactating	0.83 (0.49 to 1.40)	0.71 (0.41 to 1.22)
Default event		
Yes (referent)	1.0	1.0
No	0.25 (0.15 to 0.42)	0.23 (0.14 to 0.38)

*Refers to status at the time of enrollment into the study, not at the time of HIVRNA collection.

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 before the development of HIV drug resistance.

Compared with other cohorts in Malawi[6] and elsewhere, suppression rates were lower than the UNAIDS target 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 with 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 VLs may not yet have been fully suppressed. Inadequate time for suppression is supported by the association between defaulting from care and increased likelihood of virological failure. Although 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 VL testing was missed at the clinic. These retained but nontested 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 nontested group. By contrast, our finding of lower rates of suppression in those with defaulting episodes suggests that those not retained in care are more likely to have nonsuppression. Hence, at a population level, the suppression rate including women lost to follow-up is likely to be lower than that 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 VLs and with successful amplification for resistance sequencing, NRTI and/or NNRTI resistance was detected in 35% of the women. This confirmed that resistance rate is relatively low compared with 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 because of 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 that 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 that 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 that 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 VL which includes enhancing adherence counseling and rechecking VL 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 Nevirapine use. Reported previous ART use for PMTCT or general health was extremely low in our cohort. Among the 2 individuals we identified with pretreatment drug resistance, there was no reported ART exposure. We saw no difference in suppression according to gravidity status, suggesting that previous unreported PMTCT exposure was less likely. However, we acknowledge the need to further evaluate pretreatment 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] there may be significant gains in resistance prevention overall.

Our study includes a 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 time points and challenges with maintaining appropriate storage conditions for DBS. DBS can be sensitive to RNA degradation, and hence we found that using the 2 primer approach as 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 nonadherent 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 that supporting retention and adherence interventions

for women engaged in the option B+ program in Malawi can achieve treatment and suppression targets.

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