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Point-of-Care Tenofovir Urine Testing for the Prediction of Treatment Failure and Drug Resistance During Initial Treatment for Human Immunodeficiency Virus Type 1 (HIV-1) Infection

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Background. Viral rebound during antiretroviral treatment (ART) is most often driven by suboptimal adherence in the absence of drug resistance. We assessed the diagnostic performance of point-of-care (POC) tenofovir (TFV) detection in urine for the prediction of viral rebound and drug resistance during ART.

Methods. We performed a nested case-control study within the ADVANCE randomized clinical trial (NCT03122262) in Johannesburg, South Africa. Adults with human immunodeficiency virus (HIV) and newly initiating ART were randomized to receive either dolutegravir or efavirenz, tenofovir disoproxil fumarate or alafenamide, and emtricitabine. All participants with rebound ≥200 copies/mL between 24 and 96 weeks of follow-up were selected as cases and matched to controls with virological suppression <50 copies/mL. Rapid POC urine-TFV detection was performed retrospectively.

Results. We included 281 samples from 198 participants. Urine-TFV was detectable in 30.7% (70/228) of cases and in 100% (53/53) of controls. Undetectable urine-TFV predicted rebound with a sensitivity of 69% [95% confidence interval {CI}: 63–75] and specificity of 100% [93–100]. In cases with virological failure and sequencing data (n = 42), NRTI drug resistance was detected in 50% (10/20) of cases with detectable urine-TFV versus in 8.3% (2/24) of cases with undetectable urine-TFV. Detectable urine-TFV predicted NRTI resistance (odds ratio [OR] 10.4 [1.8–114.4] $P = .005$) with a sensitivity of 83% [52–98] and specificity of 69% [50–84].

Conclusions. POC objective adherence testing using a urine-TFV test predicted viral rebound with high specificity. In participants with rebound, urine-TFV testing predicted the selection of drug resistance. Objective adherence testing may be used to rapidly provide insight into adherence, suppression, and drug resistance during ART.

Keywords. HIV; antiretroviral treatment (ART); objective adherence testing; point-of-care testing.

Current guideline recommendations for first-line antiretroviral therapy (ART) include the integrase strand-transfer inhibitors (InSTI) dolutegravir (DTG) or bictegravir (BIC), combined with either tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF) and either lamivudine (3TC) or emtricitabine (FTC) [1, 2]. In low- and middle-income countries (LMIC) with high human immunodeficiency virus (HIV) disease burden, access to objective adherence measurement and drug resistance testing is limited and monitoring of treatment success is mainly performed through annual measurement of quantitative human immunodeficiency virus type 1 (HIV-1) RNA (viral load [VL]) [1].

Despite rising trends of virological suppression on treatment, viral rebound during ART still occurs at an annual rate of approximately 10% in LMIC [3]. Viral rebound has detrimental effects on individual patient health and increases the risk of HIV transmission to others [4–8]. Rebound during InSTI-based ART in treatment-naive populations is very rarely accompanied by the emergence of InSTI-resistance [9, 10]. Most cases of rebound are instead driven by suboptimal adherence alone and could be resolved by adherence interventions without necessitating a switch to an alternate regimen [9–15]. Objective adherence testing has the potential to improve clinical assessment of adherence and provide improved insight into adherence to both clinicians and

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patients. Such testing could be used to guide adherence interventions and to monitor the response to such interventions, ultimately leading to increased rates of treatment success.

It has recently been demonstrated that qualitative measurement of tenofovir (TFV), a metabolite of TDF and TAF, can be reliably performed using rapid lateral flow immunoassays on urine [16, 17]. TDF and TAF are commonly prescribed components of ART and are prodrugs that rapidly convert to TFV via esterase hydrolysis after administration and absorption. TFV is converted to its active form TFV-diphosphate once inside HIV-infected CD4+ T cells [18]. TFV is subject to renal clearance, resulting in high urine TFV concentrations, which are strongly correlated with plasma as well as dried blood spot TFV concentrations, providing a reliable marker of recent TFV exposure [19, 20]. Recent studies have demonstrated that drug level measurement of tenofovir can be used for risk stratification and to improve adherence outcomes in individuals on TDF/FTC pre-exposure prophylaxis (PrEP) [19, 21–23].

We hypothesized that a point-of-care (POC) qualitative urine TFV lateral flow assay can be used for objective adherence testing in people with HIV (PHIV) on TDF-/TAF-containing ART. We evaluated the diagnostic performance of the POC urine-TFV assay to predict viral rebound and selection of drug resistance during ART.

METHODS

Study Design

This was a nested case-control study within the ADVANCE randomized clinical trial (RCT). The ADVANCE RCT (NCT03122262) was an open label randomized, non-inferiority phase 3 study to assess the efficacy and safety of DTG (50 mg once daily [QD]) administered in combination with tenofovir alafenamide (TAF) (25 mg QD) and FTC (200 mg QD) compared to DTG (50 mg QD) administered in combination with TDF (300 mg QD) and FTC (200 mg QD) and compared to EFV (600 mg QD) administered in combination with TDF (300 mg QD) and FTC (200 mg QD) over 96 weeks in adult PHIV eligible for first-line ART. Viral load testing and urine sampling was performed at 12-week intervals from start of ART. The study results and protocol are reported elsewhere [10, 24].

Case and Control Selection

Cases were defined as all study participants with viral rebound ≥200 copies/mL in any of the 3 study arms at week 24 of follow-up or later. Each 12 week timepoint with a VL ≥200 copies/mL for which a urine sample was available was counted as a separate case episode, allowing case participants to contribute multiple case episodes. For every 4 case episodes, 1 control participant without rebound and with follow-up until week 96 was selected. Controls were matched to cases by trial arm and analyzed at the timepoint at which the matching case developed viremia (Figure 1).

Study Samples

Urine samples were collected and stored at all scheduled study visits. Samples were refrigerated immediately after collection and shipped to a laboratory where they were centrifuged and stored in either 1.5 mL or 10 mL aliquots at −80°C for further analysis. Urine samples from week 24 onward were eligible for selection in this study.

Data Collection

Clinical data, CD4+ T-lymphocyte (CD4) counts, VL measurements, and prescribed ART were sourced for all participants. For participants with a VL ≥1000 copies/mL after week 24 who underwent drug resistance testing as per the RCT protocol, drug resistance testing results were included.

Rapid Qualitative TFV Urine Detection

Urine-TFV detection was performed using the SureQuick Rapid Tenofovir Adherence Test (OraSure Technologies Inc., USA) [16, 25]. This lateral flow immunoassay detects levels of TFV around cut-offs associated with no TFV intake for >48 hours. A negative test line indicates detectable TFV and a positive test line indicates undetectable TFV. For clarity, results were reported as TFV detectable/detected or undetectable/undetected.

Drug Resistance Testing

Genotypic drug resistance testing was performed in all participants with at least 2 consecutive VL ≥1000 copies/mL. Drug resistance testing was also performed on a pretreatment sample for all participants with failure. Drug resistance testing was performed using population-based sequencing of reverse transcriptase and integrase regions of the viral *pol* gene according to previously described methods [26, 27]. The results were interpreted using the 2019 IAS-USA drug resistance guidelines and figures [28].

Statistical Analysis

Diagnostic accuracy of qualitative urine-TFV testing was assessed on the following study outcomes: (1) Viral rebound ≥200 copies/mL; (2) Drug resistance mutations conferring resistance to the InSTI DTG or to the non-nucleoside reverse transcriptase inhibitor (NNRTI) EFV, depending on the regimen used; (3) Drug resistance mutations conferring resistance to nucleos(t)ide reverse transcriptase inhibitors (NRTIs) present in the regimen used. For the viral rebound outcome, analysis was performed using a per-sample and per-participant approach. For per-participant analysis, where each record represents a unique participant, only the urine-TFV level result measured at first detection of viral rebound was used as the predictor variable (Figure 1). For drug resistance outcomes, analysis was performed using the per-participant approach and included participants with a confirmed VL ≥1000 copies/mL and available drug resistance testing results. For this analysis, having ≥1 detectable urine-TFV result at any rebound timepoint was used as the predictor variable (Figure 1). Sensitivity analyses for this outcome were performed considering

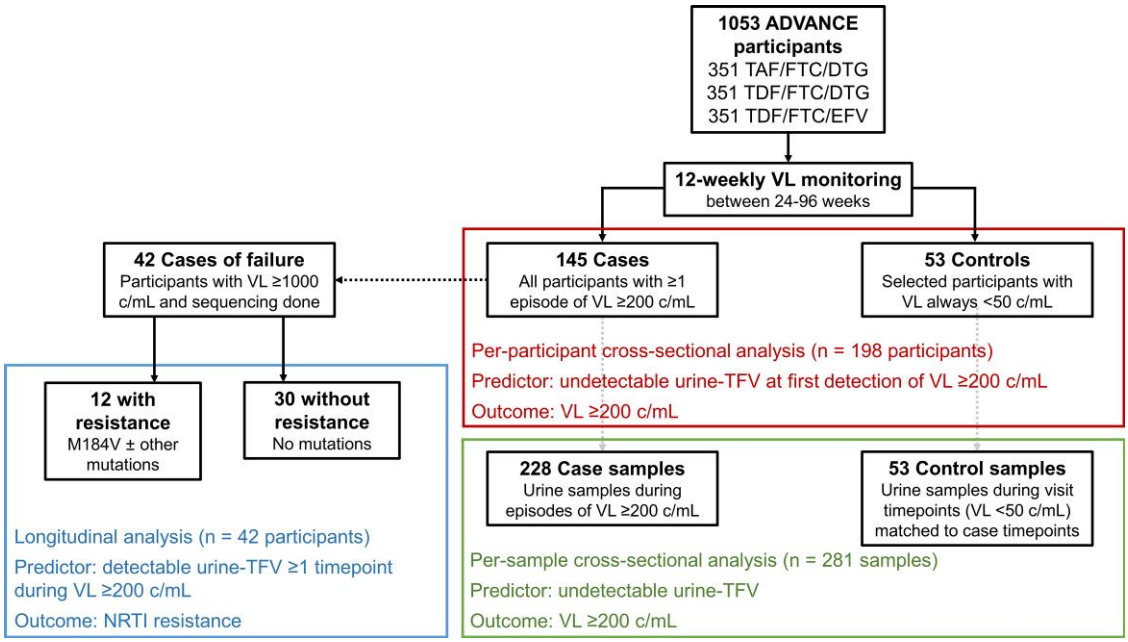

Figure 1. Study flowchart. Abbreviations: DTG, dolutegravir; EFV, efavirenz; FTC, emtricitabine; NRTI, nucleos(t)ide reverse transcriptase inhibitor TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; TFV, tenofovir; VL, viral load.

only the urine-TFV level result measured at first detection of rebound and including only participants without detected pretreatment drug resistance mutations.

Diagnostic performance was reported using standard measures of diagnostic accuracy (sensitivity, specificity, positive predictive value, negative predictive value) which were reported with 95% confidence intervals (95% CI). For univariate analysis of outcomes and continuous covariables, Student *t* test was used in case of normally distributed covariables and the Mann–Whitney *U*-test was used in case of non-normally distributed covariables. For categorical covariables the χ^2 test was used to identify association between outcome variable and clinical characteristics. Multivariable analysis was performed using logistic regression to identify correlates of virological outcomes and drug level testing results. Adjusted odds ratios (aOR) were reported with 95% CI.

Study sample size was driven by the amount of available case samples. With at least 200 available case samples, the selection of 50 control samples would result in the ability to detect a 15% difference or greater in the positivity rate of urine-TFV, presuming a 90% urine-TFV positivity rate in the control group, assuming an α of 0.05 and a 1-β of 0.9, in a 1-sided comparison of proportions.

RESULTS

Participant Characteristics

A total of 1053 participants were enrolled in the ADVANCE RCT. Of 152 participants with viral rebound at week 24 or later, 145 (95.4%) participants with available samples (n = 228 samples) were selected as cases. Fifty-three matched control participants were selected (n = 53 samples). A cumulative total of 281 samples from 198 participants were available for analysis. Participants had a median age of 30.0 years [interquartile range {IQR}: 25.0–35.0] and 61.6% were female. Median CD4 count at ART initiation was 321 cells/mm^3 [IQR: 168–474]. Of all participants, 28.8% (57/198) received EFV + TDF + FTC, 36.9% (73/198) received DTG + TDF + FTC, and 34.3% (68/198) received DTG + TAF + FTC. The mean number of episodes of viral rebound per case participant was higher in the EFV + TDF + FTC arm than in the DTG + TDF + FTC or DTG + TAF + FTC arms (2.05 [sd 1.43]; 1.36 [0.72]; and 1.47 [0.77] respectively; $P = 0.003$) (Table 1).

TFV Urine Testing and Virological Outcomes

Urine-TFV testing was performed successfully on all 281 samples. TFV was detected in 30.7% (70/228) of case samples and in 100% (53/53) of control samples. In per-sample analysis, undetectable urine-TFV was significantly correlated with viral rebound ≥200 copies/mL ($P < .001$) and predicted viral rebound with a sensitivity of 69% (95% CI: 63–75) and specificity of 100% (95% CI: 93–100). In per-participant analysis, 34.5% (50/145) of case participants had detectable urine-TFV at the first timepoint of viral rebound and 39.3% (57/145) had at least 1 detectable urine-TFV at any timepoint with rebound. Having undetectable urine-TFV at the first timepoint predicted rebound with a sensitivity of 66% (95% CI: 57–73) and specificity of 100% (95% CI: 93–100) (Table 2).

Table 1. Baseline Characteristics of Participants

	Cases (n = 145)	Controls (n = 53)	*P* value
Female (%)	88 (60.7)	34 (64.2)	.781
Age, median [IQR]	30.0 [24.0- 35.0]	31.0 [26.0–37.0]	.095
Visit (%)			.434
Week 24	24 (16.6)	12 (22.6)	
Week 36	21 (14.5)	10 (18.9)	
Week 48	26 (17.9)	9 (17.0)	
Week 60	8 (5.5)	5 (9.4)	
Week 72	15 (10.3)	5 (9.4)	
Week 84	16 (11.0)	6 (11.3)	
Week 96	24 (16.6)	6 (11.3)	
Unscheduled visit	11 (7.6)	0 (0.0)	
Treatment arm (%)			.802
EFV-TDF-FTC	40 (27.6)	17 (32.1)	
DTG-TAF-FTC	50 (34.5)	18 (34.0)	
DTG-TDF-FTC	55 (37.9)	18 (34.0)	
CD4-count, median cells/mm^3 [IQR]	309 [148–446]	365 [244–530]	**.027**
VL at failure, median 10log copies/mL [IQR]	3.5 [2.9–4.3]	NA	NA
VL at failure (%)			NA
200–399 copies/mL	24 (16.6)	NA	
400–999 copies/mL	21 (14.5)	NA	
≥1000 copies/mL	100 (69.0)	NA	
Number of VL results ≥200 copies/mL, mean (SD)	1.59 (1.02)	NA	NA
Resuppression VL <200 copies/mL, mean (SD)	98 (67.6)	NA	NA
Urine-TFV detectable at index visit	50 (34.5)	53 (100)	**<.001**
Urine-TFV detectable at ≥1 timepoint	57 (39.3)	53 (100)	**<.001**

Statistically significant *P* values <.05 are marked in bold.
Abbreviations: CD4-count, CD4+ T-lymphocyte count; DTG, dolutegravir; EFV, efavirenz; FTC, emtricitabine; IQR, interquartile range; NA, not available; SD, standard deviation; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate; TFV, tenofovir; VL, viral load.

In sub-analysis stratified by regimen, participants on DTG + TAF + FTC or DTG + TDF + FTC had detectable urine-TFV in 30.4% (45/148) of case samples and 100% (36/36) of control samples, for a sensitivity of 70% (95% CI: 62–77) and a specificity of 100% (95% CI: 90–100). Participants on EFV + TDF + FTC had detectable urine-TFV in 31.3% (25/80) of case samples and 100% (17/17) of control samples, for a sensitivity of 69% (95% CI: 57–79) and a specificity of 100% (95% CI: 80–100) (Table 2).

TFV Urine Testing and Drug Resistance

Forty-two case participants developed confirmed virological failure and had available sequencing data at failure. Of these, NRTI resistance was detected in 55.6% (10/18) of cases with at least 1 detectable urine-TFV result during rebound versus in 8.3% (2/24) of cases with continuously undetectable urine-TFV. Having detectable urine-TFV at any timepoint predicted presence of NRTI resistance (OR 12.8 [2.1–144.8], $P = .001$) with a sensitivity of 83% (52–98) and specificity of 73% (54–88) (Table 3). This association remained significant in sensitivity analyses where only the urine-TFV result at first detection of rebound was considered (Supplementary Table 2) and including only participants without pre-treatment drug resistance (Supplementary Table 3).

In sub-analysis of drug resistance data stratified by regimen, participants with virological failure on DTG + TAF + FTC or DTG + TDF + FTC (n = 24) harbored drug resistance in 16.7% (4/24) of cases. Encountered drug resistance consisted of NRTI resistance including the M184V mutation in all cases. No cases of InSTI-resistance were detected. In participants with a detectable urine-TFV at any timepoint, drug resistance was more frequent (37.5% [3/8] versus 6.7% [1/16]), but this was not statistically significant (OR 8.1 [0.5–501.1] $P = .09$). In participants with virological failure on EFV + TDF + FTC (n = 18), drug resistant HIV was detected in 61.1% (11/18). NNRTI resistance mutations were detected in all cases of resistance and NRTI resistance mutations were detected in 44.4% (8/18) of cases. Having detectable urine-TFV at any timepoint predicted presence of NRTI resistance (OR 13.5 [1.0–832.6], $P = .025$) with a sensitivity of 88% (47–100) and specificity of 70% [35–93] but not the presence of NRTI/NNRTI resistance (OR 5.9 [0.6–96.8], $P = .14$). (Supplementary Table 1) Assay diagnostic performance in subgroups remained similar when only the urine-TFV result at first detection of rebound was considered (Supplementary Table 2).

Table 2. Diagnostic Accuracy of Undetectable Urine-TFV for Viral Rebound

All Participants—Participant Level Cross-Sectional Analysis							
All Participants (n = 198)	Cases	Controls		Sensitivity	66%	[57–73]	$P < .001$
Urine-TFV undetectable	95	0	95	Specificity	100%	[93–100]	
Urine-TFV detectable	50	53	103				
	145	53	198				
All Participants—Sample Level Cross-Sectional Analysis							
All samples (n = 281)	Cases	Controls		Sensitivity	69%	[63–75]	$P < .001$
Urine-TFV undetectable	158	0	158	Specificity	100%	[93–100]	
Urine-TFV detectable	70	53	123				
	228	53	281				
EFV-Receiving Participants—Sample Level Cross-Sectional Analysis							
All samples (n = 97)	–	Controls		Sensitivity	69%	[57–79]	$P < .001$
Urine-TFV undetectable	55	0	55	Specificity	100%	[80–100]	
Urine-TFV detectable	25	17	42				
	80	17	97				
DTG-Receiving Participants—Sample Level Cross-Sectional Analysis							
All samples (n = 184)	cases	controls		Sensitivity	70%	[62–77]	$P < .001$
Urine-TFV undetectable	103	0	103	Specificity	100%	[90–100]	
Urine-TFV detectable	45	36	81				
	148	36	184				

Abbreviations: DTG, dolutegravir; EFV, efavirenz; TFV, tenofovir.

Correlates of TFV Urine Testing

In multivariable analysis of participants experiencing viral rebound (n = 145), participants with at least one detectable urine-TFV result during rebound had a significantly lower VL at rebound (adjusted odds ratio [aOR] 0.44 [95% CI: .27–.68];
$P < .001$) and had a significantly lower CD4 count at ART initiation (aOR 0.75 [95% CI: .62–.90]; $P = .003$). There were no statistically significant correlations between urine-TFV result and age or treatment group (Supplementary Table 4).

DISCUSSION

In this study, an undetectable urine-TFV POC result predicted viral rebound on TDF- or TAF-containing ART. All cases of undetectable urine-TFV were accompanied by rebound, rendering it predictive of rebound with 100% specificity. A minority of PHIV with viral rebound had detectable urine-TFV. Detectable urine-TFV during viral rebound predicted the presence of treatment-emergent NRTI resistance. These results show that objective adherence testing provides valuable insights into virological suppression status during ART and into the potential presence of drug resistance in case of viral rebound.

The results of this study have important implications for clinical management of PHIV on ART. Routine viral load testing forms the bedrock of ART monitoring and is often combined with regular assessment of patient adherence through methods such as self-reported adherence questionnaires and pill count-based assessments. These subjective methods vary in their effectiveness, are reliant on patient compliance, and might not improve adherence even if performed regularly [29–33]. The performance of urine-TFV testing in this study, as well as its rapid point-of-care format, relative cost-effectiveness, and low complexity render this type of testing highly suitable for integration into routine adherence assessment protocols in HIV treatment programs.

Although an undetectable urine-TFV test result was highly predictive of viral rebound, just under one-third of rebound episodes were accompanied by a detectable urine-TFV. This finding highlights that objective adherence testing is likely to miss cases of rebound and is therefore not to be considered equivalent to viral load testing. Interestingly, the profile of participants with a rebound episode that had undetectable urine-TFV was different to those with rebound and detectable urine-TFV results. First, participants with detectable urine-TFV during rebound had a lower viral load during their rebound episode. This may be explained by the effect of drug pressure, which is likely lower in participants with undetectable urine-TFV owing to a longer period of non-adherence. Second, a detectable urine-TFV result during rebound was associated with a higher risk of drug resistance to the NRTI backbone. NRTI resistance patterns in this study included the M184V mutation in all cases. This mutation confers high-level resistance to 3TC and FTC and rapidly reverts to wild type if treatment with these drugs is stopped [34]. It is therefore likely that presence of M184V correlates with other markers of recent drug intake, an effect that has been observed in other studies [35].

In subgroup analysis of participants on DTG, the correlation between a detectable urine-TFV result and NRTI resistance was

Table 3. Diagnostic Accuracy of Detectable Urine-TFV for Drug Resistance Participant Level Longitudinal Analysis

All Participants and NRTI Resistance				OR	12.8	[2.1–144.8]	$P = .001$
All participants (n = 42)	NRTI resistance	No NRTI resistance		Sensitivity	83%	[52–98]	
Urine-TFV detectable at ≥1 timepoint	10	8	18	Specificity	73%	[54–88]	
Urine-TFV undetectable at all timepoints	2	22	24	PPV	56%	[31–78]	
	12	30	42	NPV	92%	[73–99]	

Here "urine-TFV detectable at ≥1 timepoint" indicates participants who had a detectable urine tenofovir level during at least 1 of their rebound episodes. Resistance data is cumulative for participants with more than 1 drug resistance test. Drug resistance testing was performed after the first instance of rebound.

Abbreviations: DTG, dolutegravir; EFV, efavirenz; NPV, negative predictive value; NRTI, nucleos(t)ide reverse transcriptase inhibitor; NNRTI, non-NRTI; OR, odds ratio; PPV, positive predictive value; TFV, tenofovir.

also observed but was not statistically significant, likely owing to low sample size. The emergence of NRTI resistance in this subgroup may be of important clinical relevance. Whereas the development of InSTI resistance in treatment-naive populations receiving DTG- or BIC-based regimens has been extremely rare, clinical trials evaluating the use of DTG in second-line ART with compromised NRTI backbones have shown that InSTI resistance emerges more readily in these populations [36, 37]. It could therefore be postulated that emergent NRTI resistance during treatment with DTG may put individuals at risk for the subsequent selection of InSTI resistance.

Having consistently undetectable urine-TFV results during rebound was associated with a very low risk of harboring NRTI resistance. The value of TFV detection as a screening test to essentially rule out the presence of drug resistance has significant potential clinical benefit. As a result of the very low likelihood of treatment-emergent InSTI resistance during first-line treatment with DTG-based regimens, viral rebound on these regimens is usually solely due to non-adherence in the absence of drug resistance. In high-income settings, drug resistance testing is routinely performed to detect potential InSTI resistance. Given the frequent occurrence of rebound and the very low rate of InSTI resistance, current capacity for resistance testing in LMIC is not sufficient, and this strategy is not likely to be (cost-) effective. Empiric switching to other regimens is likely not beneficial due to the low likelihood of drug resistance as well as the non-inferiority or superiority of DTG-based ART compared to protease inhibitor-based second-line ART [36, 37]. In order to allocate the limited capacity for resistance testing in LMIC appropriately, alternative strategies for risk stratification of patients with rebound are urgently needed.

Drug detection after detection of viral rebound would be able to identify those at highest risk of developing drug resistance and allow for rationalized targeting of resistance testing, while avoiding resistance tests in individuals with negative drug levels, who should rather be prioritized for adherence interventions. The clinical value of such a strategy has been demonstrated in two retrospective studies that used liquid chromatography-tandem mass spectrometry detection of LPV level to predict LPV/r-resistance in adults with rebound on LPV/r-based second-line ART [35, 38]. To our knowledge, our study is the first to demonstrate that drug detection can predict drug resistance during DTG-based ART, and the first to use a POC drug detection test to do so. POC TFV detection would give clinicians the added ability to gain insight in the cause of viral rebound in real-time and use these results to guide adherence interventions. The time to TFV plasma washout after cessation of TDF-based PrEP ranges from 1 to 3 weeks, allowing clinicians to accurately estimate the minimum duration of non-adherence in case of a negative qualitative test result with a known threshold value [20, 39]. Furthermore, use of a POC TFV test was associated with a near-doubling of adherence in one clinical trial including participants on PrEP with TDF/FTC, suggesting that serial POC TFV testing could potentially be used to improve adherence in PHIV on ART [23].

Several limitations of this work need to be mentioned. Although TDF and TAF are very frequently prescribed components of ART, some patients receive combinations without these drugs, and drug detection tests for other antiretrovirals would be required to ensure coverage of all PHIV on ART. Furthermore, this analysis demonstrated the value of urine-TFV detection retrospectively, and in a limited patient sample. Larger scale prospective studies are required to evaluate the clinical efficacy and cost-effectiveness of urine-TFV detection. Operational research is needed to guide implementation and evaluate whether the test can be performed and interpreted reliably in a clinical environment. Such research should also consider the hypothetical possibility of "white coat adherence" effects, whereby patients take treatment on the day of testing to generate a detectable test result. Regional variation in the way that VL monitoring and adherence counselling is performed may impact on the way that urine-TFV testing is integrated within existing treatment monitoring algorithms. Finally, the applicability of urine-TFV testing in settings where drug resistance testing is routinely provided requires further study.

In summary, objective adherence testing using POC TFV urine detection was highly predictive of viral rebound and selection of drug resistance during ART in this study. These results support clinical implementation of POC TFV urine detection to rapidly provide insight into adherence, suppression, and drug resistance during ART.

Notes

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Ethics and data privacy statement. This analysis and the ADVANCE RCT were approved by the Human Research Ethics Committee at the University of the Witwatersrand (Protocol reference numbers M190418 and 160606B, respectively). The study was performed in concordance with the standards of the Helsinki Declaration and the World Medical Association. Participant study data were dissociated from personal identifiers using anonymized study numbers.

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References

1. World Health Organization. *Consolidated Guidelines on HIV Prevention, Testing, Treatment, Service Delivery and Monitoring.* (2021).
2. Saag MS, Gandhi RT, Hoy JF, et al. Antiretroviral drugs for treatment and prevention of HIV infection in adults: 2020 Recommendations of the International Antiviral Society-USA Panel. JAMA **2020**; 324:1651–1669.
3. Hermans LE, Carmona S, Nijhuis M, et al. Virological suppression and clinical management in response to viremia in South African HIV treatment program : a multicenter cohort study. PLoS Med **2020**; 17:e1003037.
4. Quinn TC, Wawer MJ, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. N Eng J Med **2000**; 342: 921–929.
5. Cohen MS, Chen YQ, McCauley M, et al. Antiretroviral therapy for the prevention of HIV-1 transmission. N Engl J Med **2016**; 375:830–839.
6. Brault MA, Spiegelman D, Hargreaves J, Nash D, Vermund SH. Treatment as prevention: concepts and challenges for reducing HIV incidence. J Acquir Immune Defic Syndr **2019**; 82:S104–S112.
7. Ssempijja V, Nakigozi G, Chang L, et al. Rates of switching to second-line antiretroviral therapy and impact of delayed switching on immunologic, virologic, and mortality outcomes among HIV-infected adults with virologic failure in Rakai, Uganda. BMC Infect Dis **2017**; 582:1–10.
8. Petersen ML, Tran L, Geng EH, et al. Delayed switch of antiretroviral therapy after virologic failure associated with elevated mortality among HIV-infected adults in Africa. AIDS **2014**; 28:2097–107.
9. Walmsley S, Baumgarten A, Berenguer J, et al. Dolutegravir plus Abacavir/lamivudine for the treatment of HIV-1 infection in antiretroviral therapy-naive patients: week 96 and week 144 results from the SINGLE randomized clinical trial. J Acquir Immune Defic Syndr **2015**; 70:515–519.
10. Venter WDF, Sokhela S, Simmons B, et al. Dolutegravir with emtricitabine and tenofovir alafenamide or tenofovir disoproxil fumarate versus efavirenz, emtricitabine, and tenofovir disoproxil fumarate for initial treatment of HIV-1 infection (ADVANCE): week 96 results from a randomised, phase 3, non-inferiority trial. Lancet HIV **2020**; 7:e666–76.
11. Rhee SY, Grant PM, Tzou PL, et al. A systematic review of the genetic mechanisms of dolutegravir resistance. J Antimicrob Chemother **2019**; 74:3135–3149.
12. Lubke N, Jensen B, Hüttig F, et al. Failure of dolutegravir first-line ART with selection of virus carrying R263K and G118R. N Engl J Med **2019**; 381:887–889.
13. Fulcher JA, Du Y, Zhang T-H, Sun R, Landovitz RJ. Emergence of integrase resistance mutations during initial therapy containing dolutegravir. Clin Infect Dis **2018**; 67:791–794.
14. Pena MJ, Chueca N, D'Avolio A, Zarzalejos JM, Garcia F. Virological failure in HIV to triple therapy with dolutegravir-based firstline treatment: rare but possible. Open Forum Infect Dis **2019**; 6:12–14.
15. Lepik KJ, Harrigan PR, Yip B, et al. Emergent drug resistance with integrase strand transfer inhibitor-based regimens. AIDS **2017**; 31:1425–1434.
16. Daughtridge G, Hebel S, Fischl M, et al. Development and validation of a point-of-care, urine assay to measure adherence to PrEP and ART. in *International AIDS Society Conference* (International AIDS Society Conference, 2019).
17. Gandhi M, Wang G, King R, et al. Development and validation of the first point-of-care assay to objectively monitor adherence to HIV treatment and prevention in real-time in routine settings. AIDS **2020**; 34:255–260.
18. Kearney BP, Flaherty JF, Shah J. Tenofovir disoproxil fumarate clinical pharmacology and pharmacokinetics. Clin Pharmacokinet **2004**; 43:595–612.
19. Spinelli MA, Glidden DV, Rodrigues WC, et al. Low tenofovir level in urine by a novel immunoassay is associated with seroconversion in a preexposure prophylaxis demonstration project. AIDS **2019**; 33:867–872.
20. Drain PK, Kubiak RW, Siriprakaisil O, et al. Urine tenofovir concentrations correlate with plasma and relate to tenofovir disoproxil fumarate adherence: a randomized, directly observed pharmacokinetic trial (target study). Clin Infect Dis **2020**; 70:2143–2151.
21. Landovitz RJ, Beymer M, Kofron R, et al. Plasma tenofovir-levels to support adherence to TDF/FTC Pre-exposure prophylaxis for HIV prevention in MSM in Los Angeles, California HHS public access. J Acquir Immune Defic Syndr **2017**; 76:501–511.
22. Spinelli MA, Glidden DV, Anderson PL, et al. Brief report: short-term adherence marker to PrEP predicts future nonretention in a large PrEP demo project: implications for point-of-care adherence testing. J Acquir Immune Defic Syndr **2019**; 81:158–162.

23. Joseph Davey DL, Dovel K, Mvududu R, et al. Pre-exposure prophylaxis recent adherence with real-time adherence feedback and partner human immunodeficiency virus self-testing: a pilot trial among postpartum women. Open Forum Infect Dis **2022**; 9:ofab609. https://doi.org/10.1093/ofid/ofab609.
24. Venter WDF, Moorhouse M, Sokhela S, et al. Dolutegravir plus two different prodrugs of tenofovir to treat HIV. N Engl J Med **2019**; 381:803–815.
25. Daughtridge G, Hebel S, Larabee L, et al. Development and Clinical Use Case of a Urine Tenofovir Adherence Test. in *HIV Diagnostics Conference* (2019).
26. Wallis CL, Viana RV, Saravanan S, et al. Performance of celera RUO integrase resistance assay across multiple HIV-1 subtypes. J Virol Methods **2017**; 241:41–45.
27. Wallis CL, Papathanasopoulos MA, Lakhi S, et al. Affordable in-house antiretroviral drug resistance assay with good performance in non-subtype B HIV-1. J Virol Methods **2010**; 163:505–508.
28. Wensing AM, Calvez V, Ceccherini-Silberstein F, et al. 2019 Update of the drug resistance mutations in HIV-1. Top Antivir Med **2019**; 27:111–121.
29. Arnsten JH, Demas PA, Farzadegan H, et al. Antiretroviral therapy adherence and viral suppression in HIV-infected drug users : comparison of self-report and electronic monitoring. Clin Infect Dis **2001**; 33:1417–1423.
30. Stirratt MJ, Dunbar-Jacob J, Crane HM, et al. Self-report measures of medication adherence behavior: recommendations on optimal use. Transl Behav Med **2015**; 5:470–482.
31. Hebel S, Kahn-Woods E, Malone-Thomas S, et al. Brief report: discrepancies between self-reported adherence and a biomarker of adherence in real-world settings. J Acquir Immune Defic Syndr **2020**; 85:454–457.
32. Kalichman SC, Amaral C, Swetsze C, et al. Monthly unannounced pill counts for monitoring HIV treatment adherence: tests for self-monitoring and reactivity effects. HIV Clin Trials **2010**; 11:325–331.
33. Ndege RC, Okuma J, Kalinjuma AV, et al. Failure to return pillbox is a predictor of being lost to follow-up among people living with HIV on antiretroviral therapy in rural Tanzania. HIV Med **2022**; 23:661–672.
34. Devereux HL, Youle M, Johnson MA, Loveday C. Rapid decline in detectability of HIV-1 drug resistance mutations after stopping therapy. AIDS **1999**; 13:123–127.
35. Hermans LE, Steegen K, Ter Heine R, et al. Drug level testing as a strategy to determine eligibility for drug resistance testing after failure of ART: a retrospective analysis of South African adult patients on second-line ART. J Int AIDS Soc **2020**:e25501.
36. Aboud M, Kaplan R, Lombaard J, et al. Dolutegravir versus ritonavir-boosted lopinavir both with dual nucleoside reverse transcriptase inhibitor therapy in adults with HIV-1 infection in whom first-line therapy has failed (DAWNING): an open-label, non-inferiority, phase 3b trial. Lancet Infect Dis **2019**; 19:253–264.
37. Paton NI, Musaazi J, Kityo C, et al. Dolutegravir or darunavir in combination with zidovudine or tenofovir to treat HIV. N Engl J Med **2021**; 385:330–341.
38. Court R, Gordon M, Cohen K, et al. Random lopinavir concentrations predict resistance on lopinavir-based antiretroviral therapy. Int J Antimicrob Agents **2016**; 48:158–162.
39. Cressey TR, Siriprakaisil O, Kubiak RW, et al. Plasma pharmacokinetics and urinary excretion of tenofovir following cessation in adults with controlled levels of adherence to tenofovir disoproxil fumarate. Int J Infect Dis **2020**; 97:365–370.