

Unexpected interactions between dolutegravir and folate: randomized trial evidence from South Africa

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Objective: Dolutegravir exposure at conception was associated with a preliminary signal of increased infant neural tube defect risk. As low maternal folate levels are linked with neural tube defects, we aimed to assess serum folate concentrations in women starting dolutegravir.

Design: We analysed serum folate concentrations from stored plasma among women enrolled in the South African ADVANCE trial.

Methods: We compared changes in mean serum folate and occurrence of low serum folate (<14.0 nmol/l) at weeks 0, 12 and 24 across study arms. In ADVANCE, 1053 treatment-naïve participants were randomized to initiate tenofovir–alafenamide/emtricitabine + dolutegravir (TAF/FTC + DTG), tenofovir–disoproxil–fumarate (TDF)/FTC + DTG or TDF/FTC/efavirenz (EFV).

Results: Analysis includes 406 females, mean age 31.5 years and baseline CD4⁺ cell count 356 cells/μl. At baseline, folate concentrations were similar across treatment arms. However, serum folate increased over 12 weeks in the TAF/FTC + DTG arm (+4.0 ± 8.1 nmol/l), while folate concentrations decreased slightly in the TDF/FTC + DTG arm (−1.8 ± 8.9 nmol/l) and decreased in the TDF/FTC/EFV arm (−5.9 ± 8.1 nmol/l). Women taking TDF/FTC/EFV had low folate concentrations at both 12 and 24 weeks compared with the other arms (*P* < 0.001). Of 26 women who became pregnant on study before week 24, folate concentrations increased between baseline and 12 weeks by a mean 2.4 ± 7.1 nmol/l in the TAF/FTC + DTG arm and 2.3 ± 8.4 nmol/l in the TDF/FTC + DTG arm, but decreased by −3.3 ± 8.1 with TDF/FTC/EFV arm.

Conclusion: Unexpectedly, no declines were noted in the dolutegravir-containing arms, and concentrations were considerably higher than in the EFV arm. The possibility that dolutegravir may block cellular uptake of folate warrants investigation.

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Keywords: dolutegravir, efavirenz, HIV, neural tube defects, serum folate concentrations

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Introduction

Dolutegravir holds considerable advantages over efavirenz (EFV), the backbone of currently available antiretroviral therapy (ART) regimens in many low-income and middle-income countries (LMICs), in terms of resistance profile, tolerability and cost [1]. There are, however, concerns about its use in HIV-positive women of reproductive potential in the periconception period [2]. A systematic review that included data on 1200 birth outcomes and congenital anomalies did not detect any safety signals [3]. In 2018, however, preliminary findings from Botswana's Tsepamo study reported higher levels of neural tube defects (NTDs) following dolutegravir exposure at the time of conception, compared with women taking nondolutegravir containing ART at conception [4,5]. This signal persisted in a more recent analysis of Tsepamo data from a larger number of pregnancies, but with a lower difference in NTD prevalence between exposure groups than was initially observed [6]. The ramifications of these data have been immense. These findings have had major implications for ART programmes in many LMICs, where plans for rolling out dolutegravir-containing regimens are far advanced. There is now policy and programmatic uncertainty that has led to delays and differentiated implementation between men and women in the rollout of dolutegravir in many countries in Africa, where most people living with HIV are women of reproductive potential, and contraception services are under-resourced. However, offering dolutegravir to men but not to women of reproductive potential introduces programmatic complexity and poses equity issues for women [7,8]. Revised WHO guidelines endorse use of dolutegravir in first-line by all individuals, including women of reproductive age [9].

The neural tube, the embryonic basis of the nervous system, closes by day 28 postfertilization [10]. Sensitivity to teratogens, including drugs, is very high during this early developmental period, which can result in serious NTDs, such as anencephaly and encephalocele. Drug exposure in this period almost always occurs prior to recognition of pregnancy. The care of infants with NTDs is challenging and often neglected in resource-limited settings [11], and children with severe NTDs often do not survive.

Low folate levels in women before conception has been associated with several birth defects, including NTDs. If the association between dolutegravir and NTDs is confirmed, one hypothetical mechanism by which this may occur is by affecting folate transport and/or metabolism. Food fortification with folic acid is inexpensive, safe and programmatically has been relatively easy to implement [12]. The supplement is thought to be especially useful for women taking drugs that are associated with NTDs, such as sodium valproate, prior

to pregnancy, although the use of these supplements and recommended dosage thereof is subject to debate [13,14]. Several interactions have been noted between folate and other drugs, including antiretrovirals [15], and this could potentially be the case with dolutegravir [14,16]. Though much remains unknown about the underlying mechanisms of drug-induced folate deficiency, it appears that there are two main pathways. First, the drug may increase folate metabolism in the body and thus lower the amount of folate available for foetal metabolism [17]. Second, the drug may partially block folate uptake at the cellular level, meaning that the amount of folate available for foetal metabolism is reduced, while blood levels remain normal, or even elevated [18].

No studies on the effects of dolutegravir on folate concentrations in women have been done. These data might help resolve uncertainties about the drug's teratogenicity [19]. Using data from the ongoing randomized South African ADVANCE trial, we assessed whether dolutegravir initiation is associated with differential preconception serum folate concentrations in women.

Methods

We compared serum folate concentrations from the stored plasma samples in women enrolled in the ADVANCE trial (ClinicalTrials.gov number: NCT03048422). ADVANCE is a 96-week phase 3 clinical trial that enrolled 1053 ART-naïve HIV-positive adolescents and adults (age ≥ 18 years) at two sites in Johannesburg, South Africa. Enrolment commenced in February 2017, and 48-week results were reported in July 2019 [20]. In brief, exclusion criteria included pregnancy at screening, acute illness (including active tuberculosis and unstable liver disease), and a likelihood of relocation. Participants were randomly allocated with equal probability to one of three first-line ART regimens: tenofovir–alafenamide/emtricitabine + dolutegravir (TAF/FTC + DTG arm), tenofovir–disoproxil–fumarate/FTC + DTG (TDF/FTC + DTG arm) or tenofovir disoproxil/emtricitabine/EFV (TDF/FTC/EFV arm). The study included storage of plasma at all visit time points from enrolment, allowing for a comparison of serum folate concentrations between participants on dolutegravir-based ART versus those taking EFV-based regimens in the study. No instructions about food intake were given to patients prior to blood sampling, as results can be considered nonfasting serum folate.

Women who conceived on the study were continued on study drug until 11 June 2018, when the protocol was amended due to the findings from the Tsepamo study. All women on a dolutegravir-containing regimen who became pregnant after this date and were at gestational age 8 weeks

Table 1. Baseline characteristics of participants.

	TAF/FTC + DTG <i>n</i> = 148	TDF/FTC + DTG <i>n</i> = 133	TDF/FTC/EFV <i>n</i> = 125	Total <i>n</i> = 406
Characteristic				
Proportion of women in ADVANCE, <i>n</i> (%)	148/214 (69%)	133/208 (64%)	125/201 (62%)	406/623 (65%)
Female sex, <i>n</i> (%)	148 (100)	133 (100)	125 (100)	406 (100)
Age (years)	31.4 ± 7.5	30.8 ± 6.8	32.2 ± 6.7	31.5 ± 7.1
Black race, <i>n</i> (%)	148 (100)	133 (100)	125 (100)	406 (100)
South African, <i>n</i> (%)	82 (55)	79 (59)	76 (61)	237 (58)
Body weight (kg)	68.7 ± 14.6	67.9 ± 15.8	71.6 ± 17.3	69.3 ± 15.9
BMI (kg/m ²)	25.3 ± 4.9	25.1 ± 5.6	26.0 ± 6.2	25.5 ± 5.5
Haemoglobin (g/dl)	12.3 ± 1.7	12.4 ± 1.7	12.2 ± 1.7	12.3 ± 1.7
CD4 ⁺ cell count (cells/μl)	361.3 ± 238.5	336.5 ± 220.0	370.6 ± 206.3	356.1 ± 222.8
≤200 cells/μl, <i>n</i> (%)	42 (28)	37 (28)	28 (22)	107 (26)
HIV-1 RNA level (log ₁₀ copies/ml)	4.2 ± 0.7	4.2 ± 0.8	4.3 ± 0.8	4.2 ± 0.8
>100 000 (copies/ml), <i>n</i> (%)	24 (16)	24 (18)	25 (20)	73 (18)
Pregnancy				
Any point in trial, <i>n</i> (%)	21 (14)	20 (15)	19 (15)	60 (15)
Notified prior to week 24, <i>n</i> (%)	10 (7)	9 (7)	7 (6)	26 (6)

Results are mean ± SD unless otherwise stated. TAF/FTC + DTG, tenofovir–alafenamide/emtricitabine + dolutegravir; TDF/FTC/EFV, tenofovir–disoproxil–fumarate/emtricitabine/efavirenz; TDF/FTC + DTG, tenofovir–disoproxil–fumarate/emtricitabine + dolutegravir.

or less were offered the option to switch to TDF/FTC/EFV. As some pregnancies occurred prior to the protocol amendment, we were able to evaluate serum folate concentrations in a small number of pregnant women. All pregnant women received supplemental folic acid as recommended by local guidelines and the WHO [21].

Using simple random sampling, we selected serum samples from stored plasma of 486 of the 623 female participants enrolled in the trial. These criteria included female sex, age 15–49 years, consent to sample storage for future testing, availability of stored plasma samples at all study time points, and no change in baseline ART regimen up till 24 weeks on ART. To evaluate changes in folate concentrations, we analysed serum from stored plasma obtained at baseline, and at 12 and 24 weeks; only participants with results at all three time points were included in the analysis. All plasma samples were stored at –80 °C until the time of testing, and collection and storage of samples was standardized between the two sites. Both serum folate and red blood cell folate assays are valid measures of folate deficiency; while red blood cell folate is preferred, this was not feasible as we did not store the appropriate samples for this assay [22]. Serum folate concentrations were analysed using the Folate ARCHITECT Folate Reagent Kit (1P74–25, 1P74–35; Abbott Ireland, Diagnostics Division, Lisnamuck, Longford, Co. Longford, Ireland). Folate concentrations were defined as low if less than 14.0 nmol/l, combining both very low less than 7.0 nmol/l and marginal 7.0–14.0 nmol/l status [23,24].

Statistical analysis

Baseline participant characteristics and baseline serum folate concentrations were compared between study arms using Student's *t* tests for continuous variables and Chi-square test for categorical data. The primary outcome of this analysis was mean serum folate concentration in the

three treatment arms at week 12 of the study. We compared absolute folate levels as well as the change from baseline folate concentrations at weeks 12 and 24 between groups using unpaired Student's *t* test. These tests aimed to detect differences between the TDF/FTC/EFV arm and the two dolutegravir arms; between the TDF/FTC + DTG arm and the TDF/FTC/EFV arm, as these two arms are have the same nucleoside reverse transcriptase inhibitor backbone; and between the TAF/FTC + DTG and TDF/FTC + DTG arms. Paired Student's *t* tests were used to compare baseline and week 24 results within treatment arms. Ethical approval was granted by the Human Research Ethics Committee of the University of the Witwatersrand, South Africa (ethics no: 160606B), and all participants provided written informed consent for study participation and future testing of stored samples before any study procedures were performed.

Results

Four hundred and eighty-six of the women eligible for this study had serum folate levels tested, of which 406 participants had results available at each time point and were included in the analysis. Of these, 148 (36%) were randomized to the TAF/FTC + DTG arm, 133 (33%) to TDF/FTC + DTG arm and 125 (31%) to TDF/FTC/EFV arm. Baseline characteristics and laboratory screening tests were balanced across treatment arms and are summarized in Table 1. All participants were black African, 58% were South African and were a mean 31.5 years old (SD = 7.1). The mean haemoglobin was 12.3 g/dl (SD = 1.7). CD4⁺ cell counts at baseline were a median 356 cells/μl (SD = 222.8), with 26% having a count below 200 cells/μl. HIV-1 RNA level viral load measurements at baseline were similar across study arms,

Table 2. Serum folate concentrations (nmol/l) over time in women by randomized arm.

	TAF/FTC + DTG <i>n</i> = 148	TDF/FTC + DTG <i>n</i> = 133	TDF/FTC/EFV <i>n</i> = 125	Total <i>n</i> = 406	<i>P</i> value*	<i>P</i> value**	<i>P</i> value***
Baseline							
Folate (nmol/l), mean (SD)	22.8 ± 8.6	24.2 ± 9.0	23.4 ± 7.6	23.4 ± 8.4	0.903	0.406	0.175
Low folate, <i>n</i> (%)	26 (18)	18 (14)	12 (10)	56 (14)	0.102	0.325	0.353
Week 12							
Folate (nmol/l), mean (SD)	26.8 ± 8.2	22.4 ± 9.2	17.4 ± 7.5	22.5 ± 9.2	<0.001	<0.001	<0.001
Folate change from baseline, mean (SD)	4.0 ± 8.1	-1.8 ± 8.9	-5.9 ± 8.1	-1.0 ± 9.3	<0.001	<0.001	<0.001
Low folate, <i>n</i> (%)	7 (5)	30 (23)	50 (40)	87 (21)	<0.001	0.002	<0.001
Week 24							
Folate (nmol/l), mean (SD)	28.5 ± 8.0	24.1 ± 8.7	18.5 ± 7.7	24.0 ± 9.1	<0.001	<0.001	<0.001
Folate change from baseline, mean (SD)	5.7 ± 8.4	-0.1 ± 9.7	-4.8 ± 9.0	0.6 ± 10.0	<0.001	<0.001	<0.001
Low folate, <i>n</i> (%)	9 (6)	17 (13)	38 (30)	64 (16)	<0.001	0.001	0.053

P values are derived from Student's *t* tests for continuous outcomes and Chi-squared for categorical outcomes. The *n* is the same for each visit. Low folate is defined as a folate less than 14 nmol/l. IQR, interquartile range; TAF/FTC + DTG, tenofovir–alafenamide/emtricitabine + dolutegravir; TDF/FTC/EFV, tenofovir–disoproxil–fumarate/emtricitabine/efavirenz; TDF/FTC + DTG, tenofovir–disoproxil–fumarate/emtricitabine + dolutegravir.

**P* value compares the EFV arm with the two DTG arms combined.

***P* value compares the TDF/FTC + DTG arm with the TDF/FTC+EFV arm.

****P* value compares the TAF/FTC + DTG arm with the TDF/FTC + DTG arm.

with 18% of participants having more than 100 000 copies/ml.

At baseline, mean serum folate concentrations and the proportion with low serum folate at baseline were similar across treatment arms (Table 2). However, at 12 weeks mean folate concentrations were higher in the dolutegravir-containing arms: 26.8 nmol/l (SD = 8.2) in the TAF/FTC + DTG arm and 22.4 nmol/l (SD = 9.2) in the TDF/FTC + DTG arm, compared with 17.4 nmol/l (SD = 7.5) in the TDF/FTC/EFV arm (*P* < 0.001). Forty

percentage (50/125) of women on TDF/FTC/EFV had low serum folate at week 12 (<14.0 nmol/l), compared with 23% (30/133) on TDF/FTC + DTG and only 5% (7/148) on TAF/FTC + DTG (*P* < 0.001) (Table 2). Participants taking TAF/FTC + DTG showed a mean rise in serum folate concentrations of 5.7 nmol/l (SD = 8.4) from baseline to week 24 (*P* < 0.001). In the TDF/FTC + DTG arm, serum folate remained stable, whereas in the TDF/FTC/EFV arm there was a reduction in serum folate levels of 4.8 nmol/l (SD = 9.0) from baseline to week 24 (*P* < 0.001) (Table 2, and Fig. 1).

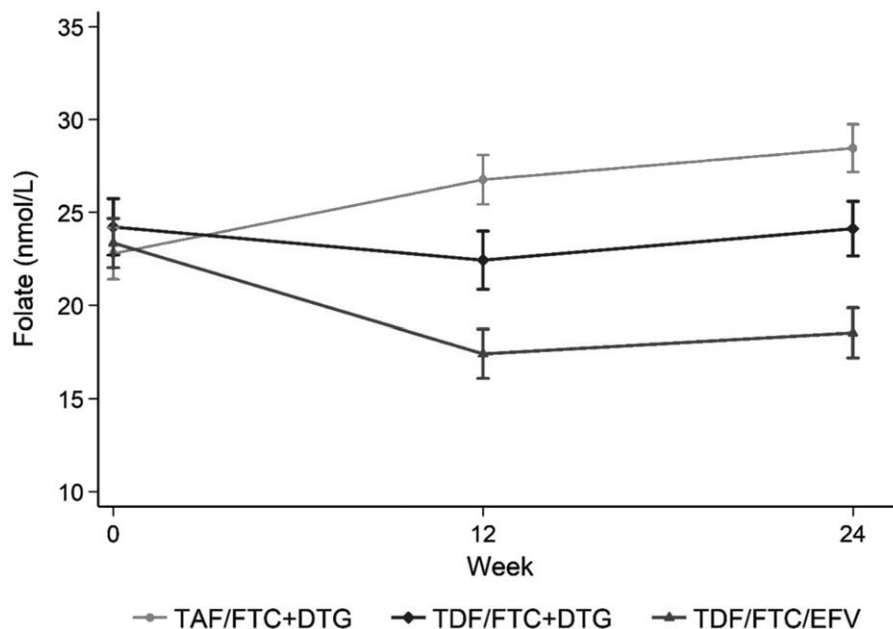


Fig. 1. Changes in serum folate concentrations, by antiretroviral trial arm and time on antiretroviral therapy. Significant changes were observed from baseline to week 24 within the tenofovir–alafenamide/emtricitabine + dolutegravir arm (*P* < 0.001) and tenofovir–disoproxil–fumarate/emtricitabine/efavirenz (*P* < 0.001), but not in the tenofovir–disoproxil–fumarate/emtricitabine + dolutegravir arm (*P* = 0.917). *P* less than 0.01 in comparisons between the dolutegravir arms and tenofovir–disoproxil–fumarate/emtricitabine/efavirenz at weeks 12 and 24. *N* = 406 at each time point.

Table 3. Changes in serum folate concentrations (nmol/l) in individuals with a pregnancy notification by week 24.

	TAF/FTC + DTG <i>n</i> = 10	TDF/FTC + DTG <i>n</i> = 9	TDF/FTC/EFV <i>n</i> = 7	Total <i>n</i> = 26
Baseline				
Folate (nmol/l), mean (SD)	25.4 ± 9.5	26.3 ± 9.0	23.0 ± 11.3	25.1 ± 9.5
Low folate, <i>n</i> (%)	0 (0)	1 (11)	2 (29)	3 (12)
Week 12				
Folate (nmol/l), mean (SD)	27.8 ± 8.5	28.6 ± 6.6	19.7 ± 8.2	25.9 ± 8.4
Folate change from baseline, mean (SD)	2.4 ± 7.1	2.3 ± 8.4	-3.3 ± 8.1	0.8 ± 7.9
Low folate, <i>n</i> (%)	1 (10)	0 (0)	1 (14)	2 (8)
Week 24				
Folate (nmol/l), mean (SD)	28.9 ± 9.2	28.7 ± 7.9	25.8 ± 12.2	28.0 ± 9.4
Folate change from baseline, mean (SD)	3.5 ± 8.1	2.3 ± 8.6	2.8 ± 13.4	2.9 ± 9.5
Low folate, <i>n</i> (%)	1 (10)	0 (0)	2 (29)	3 (12)

TAF/FTC + DTG, tenofovir–alafenamide/emtricitabine + dolutegravir; TDF/FTC/EFV, tenofovir–disoproxil–fumarate/emtricitabine/efavirenz; TDF/FTC + DTG, tenofovir–disoproxil–fumarate/emtricitabine + dolutegravir.

Among the cohort, 60 women became pregnant on-study at the time of this analysis and of these, 26 were found to be pregnant before week 24 on-study. No NTDs were identified. Mean serum folate concentrations increased in both dolutegravir-containing arms at 12 weeks on ART, with mean change of 2.4 (SD = 7.1) and 2.3 nmol/l (SD = 8.4) in the TAF/FTC + DTG arm and TDF/FTC + DTG arm, respectively. Similar to the trend observed in the overall population studied, serum folate concentrations decreased in the TDF/TFC/EFV arm at 12 weeks; however, levels increased to above baseline at 24 weeks (Table 3).

Discussion

The ADVANCE trial allowed us to perform what is, to our knowledge, the first analysis comparing serum folate concentrations in HIV-positive individuals starting dolutegravir-containing versus EFV-containing ART. In women taking dolutegravir-containing regimens the folate concentrations rose over time, and were considerably higher than in the EFV arm at weeks 12 and 24. Further, in the dolutegravir arms the folate concentrations were higher in the TAF-based arm than the TDF-based arm. It appears that changes in serum folate concentrations are smaller in pregnant than nonpregnant women, possibly due to folic acid supplementation in pregnant women. However, given the small number of pregnant women included in the analysis, it is difficult to draw definitive conclusions.

Our observations of serum folate declines in those in the standard-of-care arm (TDF/FTC/EFV) are consistent with those reported in other longitudinal studies among HIV-positive patients receiving ART, including those receiving EFV-based regimens, which indicate that serum micronutrient concentrations including folate concentrations, generally decline after ART initiation [15]. In the Breastfeeding, Antiretrovirals and Nutrition trial in

Malawi, pregnant women assigned to receive ART had lower folate concentrations than the non-ART group, and effects appeared to differ between women taking ritonavir-boosted lopinavir and those taking nelfinavir [15]. In our trial, however, no declines were noted with dolutegravir-containing regimens, and levels were considerably higher than with EFV. Though only few pregnancies occurred, it is notable that the folate concentrations did not decline during pregnancy in the dolutegravir arms, as normally occurs during pregnancy [25,26]. The findings of the study, in isolation, are hard to interpret. One possible explanation that might potentially account for the associations noted between dolutegravir and NTDs bears mention, namely that dolutegravir may block folate uptake at the cellular level (hence resulting in higher serum folate concentrations). This hypothesis is analogous to that believed to underlie valproate-induced NTDs, although exact mechanisms are uncertain. Most studies indicate that valproate does not reduce folate concentrations, unlike some antiepileptics [27,28]. Instead, valproate inhibits placental folate receptors, lowering the micronutrient's bioavailability [27,29]. Two studies using cell culture evaluated dolutegravir–folate interactions and found that dolutegravir was a partial blocker or inhibitor of folate receptor (FOLR1), one of which also showed that folic acid supplementation can lessen the toxic effects of dolutegravir in the early embryonic period in zebrafish [18,30].

Given the importance of the issue and the scale-up of dolutegravir-based ART for women of reproductive potential, we suggest that our findings be confirmed in longitudinal human studies that take into account supplemental folic acid intake. The differences between the TAF-based and TDF-based arms also warrant investigation. The TAF-based regimen was associated with higher folate concentrations than the TDF-based arm; however, it is unclear whether TAF increases or TDF reduces the possible interaction between dolutegravir and folate. Significantly, use of the TAF-based regimen compared with the TDF-based arm was

associated with other important differences such as raised levels of treatment emergent obesity in the ADVANCE trial [20]. Nevertheless, the variation in folate concentrations in the different groups makes it difficult to ascertain which drug is modulating folate, quite plausibly EFV and TAF both have important impacts. Irrespective of these findings, however, folic acid fortification of staple foods and folic acid supplements in women planning to conceive remain important, including in women taking dolutegravir-based ART.

The strengths of this study include its relatively large sample size and randomized, longitudinal study design. Furthermore, our study was conducted in a diverse female population, which includes women from neighbouring countries in southern African. There are, however, some limitations. Serum folate is a short-term indicator of folate status and may reflect recent dietary intake, though repeated low values suggest folate depletion. The findings may thus differ from measures of red blood cell folate, a longer term measure of intracellular folate. The clinical extrapolation of our findings to other settings is limited, as South Africa fortifies all staple food with folic acid, as reflected by the small numbers of women with folate deficiency.

In conclusion, data from an ongoing birth surveillance study identified evidence of potential increased risk for NTDs associated with maternal exposure to dolutegravir at conception. Folate is the largest known modifier of NTD risk, although the underlying mechanism of the impact of dolutegravir on folate concentrations is currently unknown. To better understand the associations between dolutegravir and NTDs, it is important to consider the biological mechanisms that might underpin these effects. This study may assist in understanding the concerns about the safety of dolutegravir in early pregnancy. We suggest, therefore, that as a next step, additional studies assess dolutegravir–folate interactions in both basic science and human studies.

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Conflicts of interest

N.C.C.: Research support: USAID, Unitaid, South African Medical Research Council, ViiV Healthcare, study drug donations from ViiV Healthcare and Gilead Sciences. Speaker's Bureau: Johnson & Johnson. Other: Conference/meeting sponsorship from Johnson & Johnson, Cipla. M.C., A.H., B.S., S.L.: No conflicts of interest. W.F.V.: Research support: USAID, Unitaid, South African Medical Research Council, ViiV Healthcare, study drug donations from ViiV Healthcare and Gilead Sciences. Board member/advisory panel: Gilead Sciences, AbbVie, Cipla, Pfizer, ViiV Healthcare, 3 Sixty Biopharmaceuticals. Speaker's Bureau: Gilead, ViiV, Mylan, Merck, Adcock-Ingram, Aspen, Abbott, Roche, J&J. Other: Conference/meeting sponsorship from Johnson & Johnson, Cipla. G.A.: Research support: USAID, Unitaid, South African Medical Research Council, ViiV Healthcare, study drug donations from ViiV Healthcare and Gilead Sciences. C.M.S.: Research support: USAID, Unitaid, South African Medical Research Council, ViiV Healthcare, study drug donations from ViiV Healthcare and Gilead Sciences. L.F.: Research support: USAID, Unitaid, South African Medical Research Council, ViiV Healthcare, study drug donations from ViiV Healthcare and Gilead Sciences. M.A.M.: Research support: USAID, Unitaid, South African Medical Research Council, ViiV Healthcare, study drug donations from ViiV Healthcare and Gilead Sciences. Speaker's Bureau: Merck, Spectrum Therapeutics, Gilead Sciences, AbbVie, Cipla, Johnson & Johnson, Sanofi, Pfizer, ViiV Healthcare, Mylan and Southern African HIV Clinicians Society. Board member/advisory panel: Gilead Sciences, AbbVie, Cipla, Pfizer, ViiV Healthcare, 3 Sixty Biopharmaceuticals.

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