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HIV

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Wohl DA, Spinner CD, Flamm J, et al. HIV-1 infection kinetics, drug resistance, and long-term safety of pre-exposure prophylaxis with emtricitabine plus tenofovir alafenamide (DISCOVER): week 144 open-label extension of a randomised, controlled, phase 3 trial. *Lancet HIV* 2024; published online July 12. https://doi.org/10.1016/S2352-3018(24)00130-9.

SUPPLEMENTARY APPENDIX

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HIV-1 Infection Kinetics, Drug Resistance, and Long-term Safety of Pre-exposure Prophylaxis with Emtricitabine and Tenofovir Alafenamide (DISCOVER): Week 144 Open-Label Extension

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Supplementary Methods: Inclusion and Exclusion Criteria

Inclusion criteria

Participants who had the potential for higher exposure to HIV-1 through sexual contact, and met all of the following inclusion criteria, were eligible for participation in the study:

1. HIV-1 negative
2. Men who have sex with men (MSM) or transgender women (TGW) (male at birth) who had at least one of the following:
    a. Condomless anal intercourse with at least two unique male partners in the previous 12 weeks (partners were either HIV-1 infected or of unknown HIV-1 status)
    b. Documented history of syphilis in the past 24 weeks
    c. Documented history of rectal gonorrhoea or chlamydia in the past 24 weeks
3. Age ≥18 years
4. Estimated GFR (eGFR) ≥60 mL/min according to the Cockcroft-Gault formula for creatinine clearance (Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; **16**: 31–41).
5. Adequate liver and haematologic function:
    a. Aspartate transaminase (AST) and alanine transaminase (ALT) ≤2·5 x upper limit of normal (ULN); and total bilirubin ≤1·5 mg/dL, or normal direct bilirubin
    b. Absolute neutrophil count ≥1000/mm³, platelets ≥75 000/mm³, haemoglobin ≥10 g/dL
6. Willing and able to comply with study procedures

Exclusion criteria

Participants with any of the following exclusion criteria were not eligible for participation in the study:

1. Known hypersensitivity to the investigational medicinal product, the metabolites, or formulation excipient
2. Suspected or known active serious infection(s)
3. Acute viral hepatitis A, B, or C, or evidence of chronic hepatitis infection. Participants found to be susceptible to HBV infection were referred for HBV vaccination. Participants found to be positive for HCV at screening must not have had active infection or must have completed treatment and achieved a sustained virologic response
4. Need for continued use of any contraindicated concomitant medications
5. Implanted defibrillator or pacemaker
6. History of osteoporosis or bone fragility fractures
7. Current alcohol or substance abuse judged by the investigator to be problematic such that it potentially could interfere with participant study compliance
8. Grade 3 or Grade 4 proteinuria or glycosuria that was unexplained or not clinically manageable
9. Any other clinical condition or prior therapy that, in the opinion of the investigator, would have made the participant unsuitable for the study or unable to comply with dosing requirements

10. Received investigational agents for the treatment or prevention of HIV-1 infection in the 30 days prior to screening
11. Participation in any other clinical trial (including observational trials) without prior approval from the sponsor was prohibited while participating in this trial

Study Procedures

This study was done in accordance with recognised international scientific and ethical standards, including but not limited to the International Council for Harmonisation (ICH) guideline for Good Clinical Practice (GCP) and the Declaration of Helsinki. The protocol and any amendments were approved by central or site-specific independent ethics committees (IECs) or institutional review boards (IRBs). All participants provided written informed consent.

HIV-1 laboratory testing

- A rapid HIV blood test was conducted locally for all participants at each study visit: screening, baseline (start of study drug, less than 30 days since the screening visit), at Weeks 4, 8, and 12, and then every 12 weeks until 96 weeks. The type of rapid test (eg, 3rd generation antibody or 4th generation antibody/antigen) was determined at each study site and was not always recorded. HIV testing was repeated (with the exception of the baseline time point) using plasma and laboratory-based antibody (3rd generation) or antigen/antibody (4th generation) HIV serological tests performed at a central laboratory (Covance, Indianapolis, IN, US), with positive results confirmed using an HIV-1/2 antibody discrimination assay. Discordant results (ie, positive screen and negative differentiation) were further evaluated using qualitative HIV-1 RNA testing (APTIMA® HIV-1 RNA Qualitative Assay, performed at Covance) according to the CDC diagnostic algorithm.[1] Plasma specimens were not collected at the baseline timepoint. Efficacy was assessed by fourth-generation HIV-1 antigen–antibody or third-generation HIV-1 antibody test conducted at each site.
- Plasma and dried blood spot (DBS) specimens were collected at screening, at Weeks 4, 8, and 12, and then every 12 weeks until 96 weeks. HIV-1 RNA viral load (COBAS® TaqMan® HIV-1 Test, performed by Covance; lower limit of detection: 20 copies/mL) was measured from plasma for any participant with a positive rapid or laboratory HIV test, symptoms consistent with acute HIV infection, a recent high-risk exposure, or confirmed HIV infection. HIV viral load was also assessed retrospectively for the visits prior to HIV diagnosis among participants with available banked plasma specimens (n=22). Four of the five participants with suspected baseline HIV infection did not have banked plasma specimens available.
- Genotypic resistance testing was performed for all participants diagnosed with HIV infection and HIV-1 RNA >400 copies/mL (GenoSure MG, Monogram Biosciences, South San Francisco, CA, US). TFV and FTC resistance-related mutations were as defined by Monogram.
- We calculated the number of HIV-1 RNA tests that would have been needed in DISCOVER to detect an HIV-1 infection before seroconversion by dividing the number

of participants who were diagnosed with HIV-1 and had a detectable viral load before seroconversion by the total number of HIV tests conducted in this study.

- Tenofovir diphosphate (TFV-DP) levels were measured in DBS as previously described[2-5] at the Colorado Antiviral Pharmacology Laboratory (Aurora, CO, US). TFV-DP concentrations in DBS reflect cumulative adherence over the preceding 8–12 weeks and are measured from a single punch for TDF and two punches for TAF. Adherence was categorised as high (≥900 fmol/two punches for F/TAF, ≥700 fmol/punch for F/TDF), moderate (≥450 to <900 fmol/two punches for F/TAF, ≥350 to 700 for F/TDF), or low (<450 fmol/two punches for F/TAF, <350 fmol/punch for F/TDF). High adherence reflects an average of at least 4 doses per week in the prior 8–12 weeks, moderate adherence reflects an average of 2–3 doses per week, and low adherence is indicative of less than 2 doses per week.[3,4] Adherence was also assessed at all post-baseline visits by returned pill count.

Safety assessments

- For a subset of consenting participants, dual-energy x-ray absorptiometry scans of the hip and lumbar spine were done every 48 weeks during the blinded and open-label phases of the study. These were read and interpreted by a masked third party (BioClinica, Newtown, PA, USA)
- Safety was assessed by physical examinations, laboratory tests (Covance Laboratories, Indianapolis, IN, USA), asking about concomitant drug use, and ascertaining adverse events, which were coded by use of the Medical Dictionary for Regulatory Activities (version 21.1)

Sexual activity and sexually transmitted infections

- At each visit, computer-assisted self-interview (CASI) questionnaires of sexual behaviour were administered; gonorrhoea and chlamydia nucleic acid amplification testing were done from rectal, pharyngeal, and urine specimens; and syphilis testing was done by local laboratories in accordance with local guidelines

1. US Centers for Disease Control and Prevention. Laboratory testing for the diagnosis of HIV infection: updated recommendations. June 27 2014. https://stacks.cdc.gov/view/cdc/23447 (accessed August 20 2019).
2. Anderson PL, Glidden DV, Liu A, et al. Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in men who have sex with men. *Sci Transl Med* 2012; **4**(151): 151ra25.
3. Yager J, Castillo-Mancilla J, Ibrahim ME, et al. Intracellular Tenofovir-Diphosphate and Emtricitabine-Triphosphate in Dried Blood Spots Following Tenofovir Alafenamide: The TAF-DBS Study. *J Acquir Immune Defic Syndr* 2020; **84**(3): 323-30.
4. Anderson PL, Liu AY, Castillo-Mancilla JR, et al. Intracellular Tenofovir-Diphosphate and Emtricitabine-Triphosphate in Dried Blood Spots following Directly Observed Therapy. *Antimicrob Agents Chemother* 2018; **62**(1).
5. Zheng JH, Rower C, McAllister K, et al. Application of an intracellular assay for determination of tenofovir-diphosphate and emtricitabine-triphosphate from erythrocytes using dried blood spots. *J Pharm Biomed Anal* 2016; **122**: 16-20.

Supplementary Table S1: Baseline Demographics and Clinical Characteristics

Baseline Demographics and Clinical Characteristics of Participants Initially Randomised to Emtricitabine and Tenofovir Alafenamide and Those Who Continued on Emtricitabine and Tenofovir Alafenamide in the Open-Label Extension

	Initially Randomised to F/TAF **n=2694**	**Continued on F/TAF in the OLE** **n=2070**
Median age, years (Q1, Q3)	34 (28, 43)	35 (29, 44)
Race, n (%)		
White	2264 (84)	1777 (86)
Black/mixed Black	240 (9)	153 (7)
Asian, Pacific Islander, or Native Hawaiian	142 (5)	106 (5)
Other (Non-Black)	45 (2)	34 (2)
Hispanic/Latinx ethnicity, n (%)	635 (24)	491 (24)
Cisgender MSM, n (%)	2649 (98)	2034 (99)
Self-reported behavioural characteristics, n (%)		
≥2 receptive condomless sex partners in last 12 weeks	1660 (62)	1239 (60)
Binge drinking*	618 (23)	476 (23)
Recreational drug use in past 12 weeks	1785 (67)	1360 (66)
Taking PrEP at baseline	465 (17)	393 (19)

*≥6 drinks on ≥1 occasion at least monthly. Includes participants from the full analysis set.

F/TAF, emtricitabine and tenofovir alafenamide; MSM, men who have sex with men; OLE, open-label extension; PrEP, pre-exposure prophylaxis; Q, quartile.

Supplementary Table S2: Summary of HIV Testing Results for Participants Who Acquired HIV

No.	Arm	Discontinuation day	Visit timing	Day	Adherence*	Rapid HIV test at site**	Central lab HIV test†	Supplemental HIV test	Qualitative NAT	Viral load (copies/mL)	Retrospective viral load (copies/mL)	Genotype
A. Participants with suspected baseline infection												
1	F/TAF	40	**Prior**	1	..	Neg	..	..	..	..	..	..
			Diagnosis	29	High	Neg	..	..	..	Detected (<20)	Neg	..
			Post diagnosis	41	..	Neg	Pos	Ind	Pos	Detected (<20)	..	..
2	F/TDF	34	**Prior**	..	..	Neg	..	..	..	..	..	..
			Diagnosis	29	High	Pos	..	..	Pos	1000	..	M184I/V
3	F/TDF	37	**Prior**	1	..	Neg	..	..	..	..	..	..
			Diagnosis	29	High	Neg	Pos	Pos	..	..	114 000	M184I/V
			Post diagnosis	32	..	..	Pos	Pos	..	33 300	..	..
4	F/TDF	36	**Prior**	..	..	Neg	..	..	..	..	..	..
			Diagnosis	36	High	Pos	Pos	Pos	Pos	18 700	..	M184V
5	F/TDF	90	**Prior**	29	High	Neg	..	..	..	..	1150††	..
			Diagnosis	85	High	Pos	..	..	..	407	..	M184V
			Post diagnosis	169	..	..	Pos	Pos	..	..	..	..
B. Participants who discontinued study drug prior to HIV infection												
6	F/TAF	37	**Prior**	30	High	Neg	Neg	..	..	..	Neg	..
			Diagnosis	82	..	..	Pos	Pos	Pos	2780	..	No res
7	F/TAF	49	**Prior**	31	High	Neg	Neg	..	..	..	Neg	..
			Diagnosis	95	Medium	Pos	Pos	Pos	Pos	141 000	..	No res
8	F/TDF	121	**Prior**	94	Low	Neg	Neg	..	..	..	..	..
			Prior	121	..	Neg	Neg	..	..	..	Neg	..
			Prior	149	..	Neg	Neg	..	..	..	Neg	..
			Diagnosis	161	..	..	Pos	Pos	Pos	..	..	..

No.	Arm	Discontinuation day	Visit timing	Day	Adherence*	Rapid HIV test at site**	Central lab HIV test†	Supplemental HIV test	Qualitative NAT	Viral load (copies/mL)	Retrospective viral load (copies/mL)	Genotype
			Post diagnosis	219	..	Pos	..	..	..	162	..	..
9	F/TDF	125	**Prior**	88	Medium	Neg	Neg	..	..	..	Neg	..
			Diagnosis	184	Low	Pos	Pos	Ind	..	..	..	..
			Post diagnosis	191	..	..	..	..	Pos	5040	..	No res
10	F/TAF	74	**Prior**	74	High	Neg	Neg	..	..	..	..	..
			Prior	186	..	Neg	Neg	..	..	..	Neg	..
			Diagnosis	234	..	..	..	..	Pos	592 000	..	No res
11	F/TDF	168	**Prior**	254	Low	Neg	Neg	..	..	..	Neg	..
			Prior	338	Low	Neg	Neg	..	..	..	220,000††	..
			Diagnosis	372	..	Pos	..	..	Pos	8610	..	No res
12	F/TAF	363	**Prior**	337	Medium	Neg	Neg	..	..	..	Neg	..
			Diagnosis	408	..	..	Pos	Neg	Pos	..	..	..
			Post diagnosis	421	..	Pos	Pos	Pos	Pos	199 000	..	No res
13	F/TDF	334	**Prior**	335	Low	Neg	Neg	..	..	..	Neg	..
			Diagnosis	420		Neg	Pos	Ind	..	..	6,320,000	..
			Post diagnosis	425	..	..	..	..	Pos	211 000	..	No res
C. Participants on study drug with low adherence												
14	F/TDF	96	**Prior**	37	Low	Neg	Neg	..	..	..	Neg	..
			Diagnosis	126	Low	Pos	Pos	Ind	Pos	8 070 000		No res
15	F/TDF	209	**Prior**	144	Low	Neg	Neg	..	..	..	Neg	..
			Diagnosis	228	..	Pos	Pos	Pos	..	79 145 ‡	..	No res
16	F/TDF	420	**Prior**	250	Low	Neg	Neg	..	..	..	Neg	,,
			Prior	342	Low	Neg	Neg	..	..	..	332††	..
			Diagnosis	420	Low	Pos	Pos	Pos	Pos	5340	..	No res
17	F/TDF	510	**Prior**	420	Low	Neg	Neg	..	..	..	Neg	..

No.	Arm	Discontinuation day	Visit timing	Day	Adherence*	Rapid HIV test at site**	Central lab HIV test†	Supplemental HIV test	Qualitative NAT	Viral load (copies/mL)	Retrospective viral load (copies/mL)	Genotype
			Diagnosis	506	Low	Neg	Pos	Neg	..	..	3 630 000	..
			Post diagnosis	511	..	Pos	Pos	Pos	Pos	1 280 000	..	No res
18	F/TAF	589	**Prior**	505	Low	Neg	Neg	..	..	..	Neg	..
			Diagnosis	589	Low	Pos	Pos	Pos	..	2900	..	No res
19	F/TAF	589	**Prior**	499	Low	Neg	Neg	..	..	..	Neg	..
			Diagnosis	589	Low	Pos	Pos	Pos	Pos	242 000	..	No res
20	F/TDF	615	**Prior**	583	Low	Neg	Neg	..	..	0	Neg	..
			Diagnosis	615	..	Pos§	..	..	..	..	..	..
			Post diagnosis	667	Low	Neg	Pos	Pos	..	567	..	No res
			Post diagnosis	674	..	Pos	Pos	Pos	Pos	792	..	..
21	F/TAF	678	**Prior**	598	Low	Neg	Neg	..	..	..	Neg	..
			Diagnosis	679	Low	Pos	Pos	Pos	Pos	21 700	..	No res
22	F/TDF	753	**Prior**	670	Low	Neg	Neg	..	..	..	Neg	..
			Diagnosis	754	Low	Pos	Pos	Pos	Pos	15 900	..	No res
23	F/TDF	760	**Prior**	672	Low	Neg	Neg	..	..	..	Neg	..
			Diagnosis	755	Low	Neg	Pos	Pos	..	..	7170	..
			Post diagnosis	760	..	Pos	..	..	Pos	..	..	..
			Post diagnosis	762	Low	..	..	..	Pos	359	..	..
24	F/TAF	1102	**Prior**	979	High	Neg	Neg	..	..	..	..	..
			Prior	1026	Low	Neg	..	..	Neg	0	..	..
			Diagnosis	1097	Low	Neg	Pos	Neg	..	138 200	..	..
			Post diagnosis	1102	Low	Neg	..	..	Pos	5630	..	No res
25	F/TDF	1121	**Prior**	937	..	Neg	Neg	..	..	..	Neg	..
	-> F/TAF		**Prior**	1027	Low	Neg	Neg	..	..	..	257††	..
			Diagnosis	1127	Low	Pos	Pos	Pos	..	..	..	No res

No.	Arm	Discontinuation day	Visit timing	Day	Adherence*	Rapid HIV test at site**	Central lab HIV test†	Supplemental HIV test	Qualitative NAT	Viral load (copies/mL)	Retrospective viral load (copies/mL)	Genotype
			Post diagnosis	1102	..	..	..	..	Pos	33 500	..	..
26	F/TAF	1219	**Prior**	1177	High	Neg	Neg	..	..	..	Neg	..
			Diagnosis	1220	..ϵ	Pos	..	..	..	..	..	..
			Post diagnosis	1225	Low	..	Pos	Pos	..	190 000	..	No res
D. Participants on study drug with uncertain adherence												
27	F/TDF	476	**Prior**	425	High	Neg	..	..	..	..	Neg	..
			Diagnosis	474	..	Pos	..	Neg	Pos	..	..	..
			Post diagnosis	489	..	..	..	..	..	1 070 000	..	No res

Shading indicates treatment arm (F/TAF in green, F/TDF in grey); darker shading highlights the visit at which HIV was first diagnosed.

* Adherence assessment based on TFV-DP concentration in DBS. High: ≥4 doses/week, medium: 2–3 doses/week, low: <2 doses/week; corresponding to TFV-DP levels: F/TAF high ≥900, medium ≥450–<900, low <450 fmol/two punches; F/TDF high ≥700, medium ≥350–<700, low <350 fmol/punch). ** Version of rapid test (3rd or 4th generation) varied between sites and was not always recorded. † 4th generation tests were used for all participants except #1 and #5.
†† Retrospective positive viral load test result from stored specimen (red font). ‡ Specimen for HIV viral load was collected on Day 229. § Day 615: participant reported a positive HIV test at an outside clinic, no details available, no specimens available for testing. ϵ Adherence in the 8–12 weeks preceding HIV diagnosis was assessed as low based on a TFV-DP concentration of 302 fmol/punches in DBS collected five days after diagnosis.
DBS, dried blood spot; F/TAF, emtricitabine and tenofovir alafenamide; F/TDF, emtricitabine and tenofovir disoproxil fumarate; Ind, indeterminate; NAT, nucleic acid test; Neg, negative; No res, no resistance-associated mutations detected; Pos, positive; TFV-DP, tenofovir diphosphate.

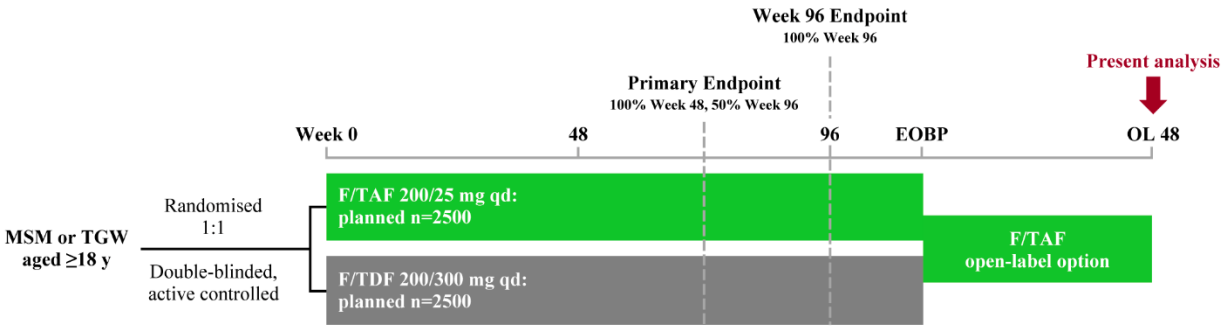
Supplementary Table S3: Lipid profiles

Measurement	Group	Median of OLE baseline	Median of OLE Week 48	Median of change from baseline	p-value[a]
Total cholesterol (mg/dL)	Stay on F/TAF	171	181	+9	<0·001
	F/TDF → F/TAF	159	183	+22	
LDL cholesterol (mg/dL)	Stay on F/TAF	99	107	+7	<0·001
	F/TDF → F/TAF	92	106	+13	
HDL cholesterol (mg/dL)	Stay on F/TAF	48	48	0	<0·001
	F/TDF → F/TAF	45	48	+3	
Triglycerides (mg/dL)	Stay on F/TAF	95	105	+8	<0·001
	F/TDF → F/TAF	87	106	+16	
Fasting glucose (mg/dL)	Stay on F/TAF	94	96	+2	0·86
	F/TDF → F/TAF	94	96	+1	
Total cholesterol:HDL ratio	Stay on F/TAF	3·55	3·71	+0·15	0·015
	F/TDF → F/TAF	3·48	3·73	+0·20	
Body weight (kg)	Stay on F/TAF	82·3	83·7	+1.2	<0·001
	F/TDF → F/TAF	81·0	82·4	+2.0	

[a]Lipid and glucose p-values from 2-sided Wilcoxon rank sum test to compare 2 study arms, weight p-values from ANOVA including treatment as fixed effect.

F/TAF, emtricitabine and tenofovir alafenamide; F/TDF, emtricitabine and tenofovir disoproxil fumarate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OLE, open-label extension.

Supplementary Figure S1: Study Design

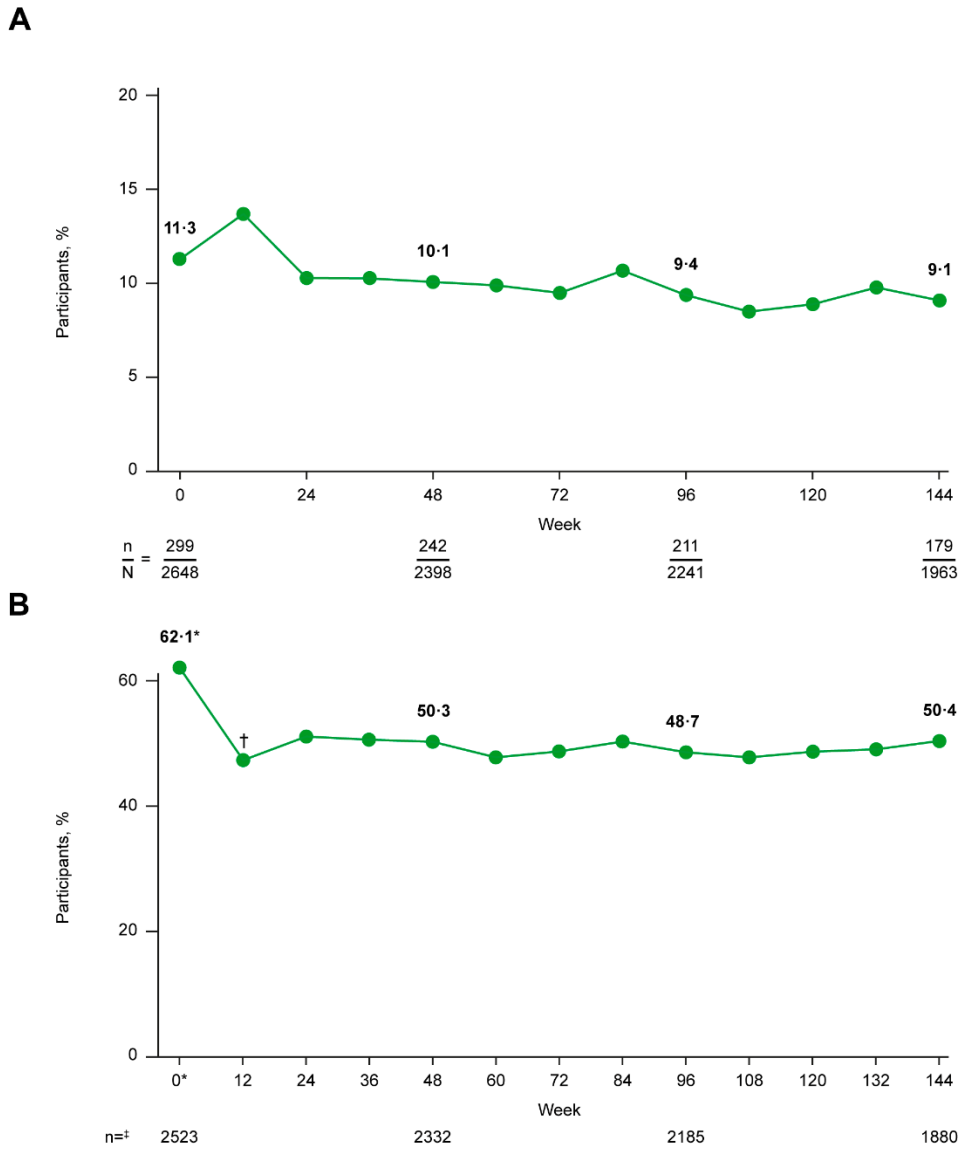


Blinded phase did not end at Week 96; amount of time between Week 96 and end of blinded phase (EOBP) varied between participants; most reached Week 144 on F/TAF during first 48 weeks of open-label phase.

F/TAF, emtricitabine and tenofovir alafenamide; F/TDF, emtricitabine and tenofovir disoproxil fumarate; EOBP: end of blinded phase; MSM: men who have sex with men; TGW, transgender women; qd, daily dosing; OL, open-label.

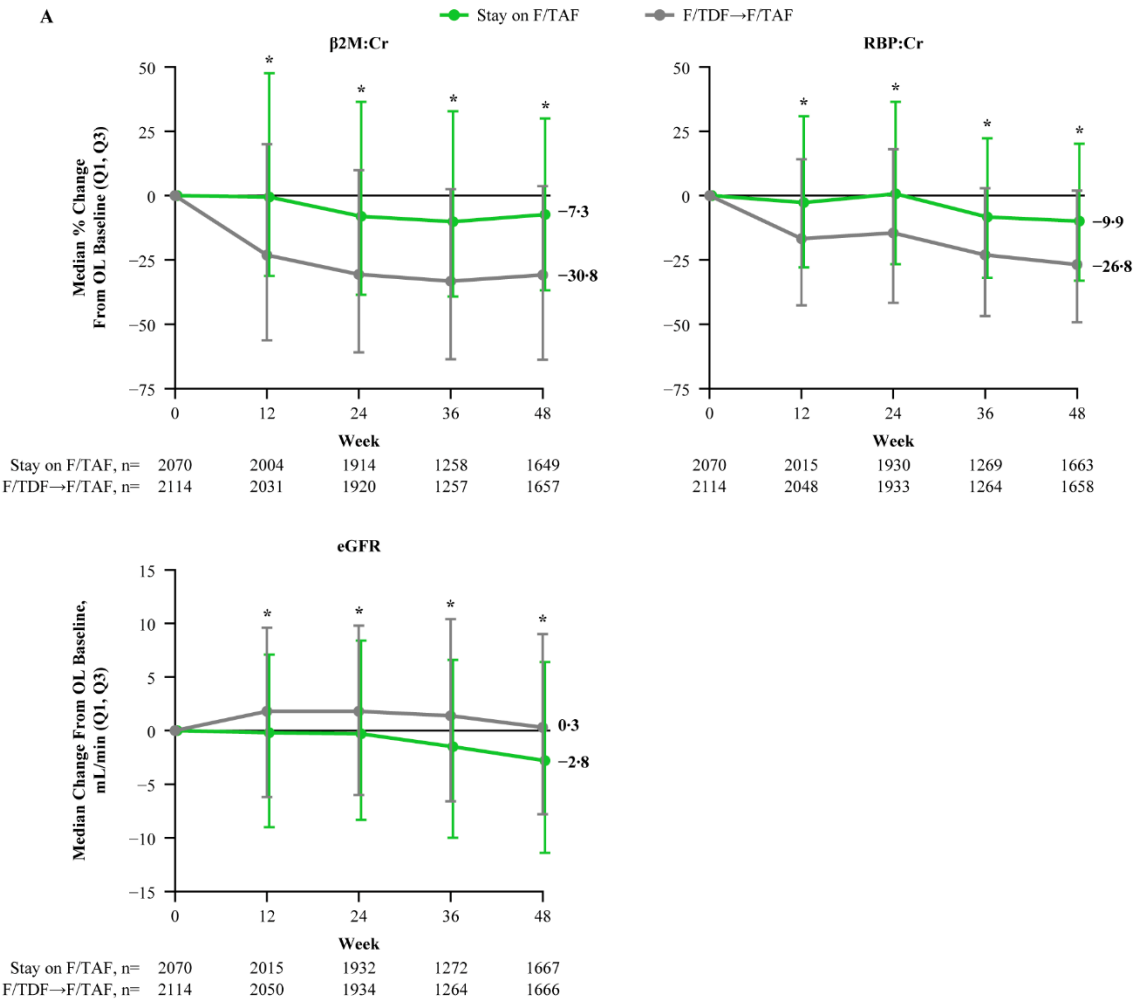
Supplementary Figure S2: Sexually Transmitted Infections and Condomless Sex.

(A) Incidence of rectal gonorrhoea or chlamydia and (B) participant-reported condomless receptive anal sex with ≥2 unique partners since the prior visit through 144 weeks of emtricitabine and tenofovir alafenamide



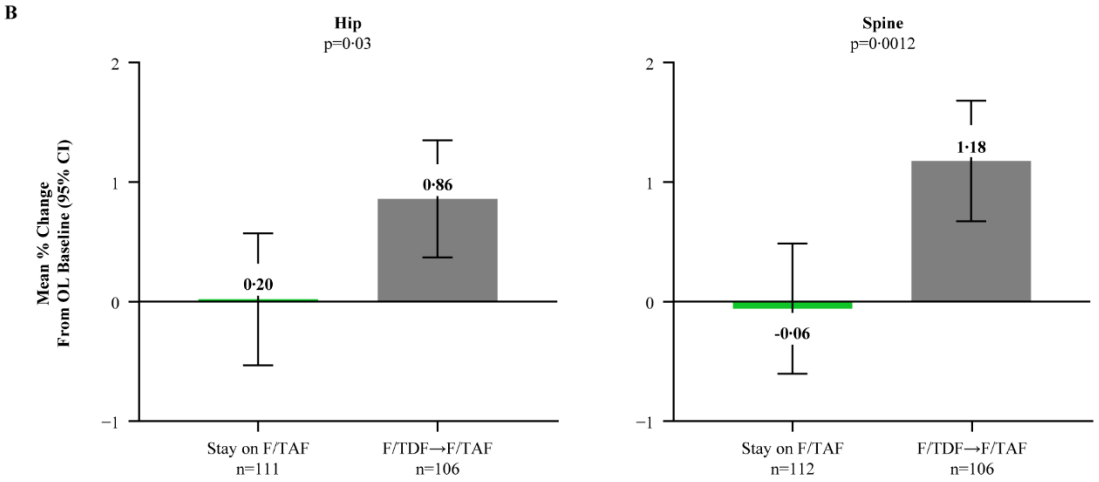
*Participants with ≥2 partners in the 90 days prior to screening; †Participants with ≥2 partners between Weeks 4 and 12; ‡Number of participants with available data for condomless receptive anal sex partners.

Supplementary Figure S3: Markers of Proximal Renal Tubule Dysfunction (A) and Bone Mineral Density (B)



*p <0.0001, from CMH test with row mean scores to compare two study arms.
eGFR, estimated glomerular filtration rate; F/TAF, emtricitabine and tenofovir alafenamide. F/TDF, emtricitabine and tenofovir disoproxil fumarate; OL, open-label; RBP:Cr, retinol-binding protein:creatinine ratio; β2M:Cr, β2-microglobulin:creatinine ratio.

B



p-values from ANOVA with treatment as fixed effect.

CI, confidence interval; F/TAF, emtricitabine and tenofovir alafenamide. F/TDF, emtricitabine and tenofovir disoproxil fumarate; OL, open-label.

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