Supplemental digital content for:

A study of the pharmacokinetics, safety, and efficacy of bictegravir/emtricitabine/tenofovir alafenamide in virologically suppressed pregnant women with HIV

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Table 1. IRBs/IECs. Sites listed alphabetically by PI last name (final PI listed for study sites with multiple PIs); addresses reflect IRB/IEC physical location and not a mailing address.

Geographic region / country Principal investigator (site number)	**IRB or IEC**
APAC / Thailand	
Aurpibul, Linda, MD (16541)	1. Human Experimentation Committee Research Institute for Health Sciences, Chiang Mai University, 110 Intavaroros Road, Muang, Chiang Mai, 50200, Thailand 2. Research Ethics Committee, Faculty of Medicine, Chiang Mai University, 110 Intavaroros Road, Muang, Chiang Mai, 50200, Thailand
Avihingsanon, Anchalee, MD (01936)	1. Institutional Review Board, Faculty of Medicine, Chulalongkorn University, 1873 Rama 4 Road, 3rd Floor of Anuntamahidol Building, Pathumwan, Bangkok, 10330, Thailand 2. Central Research Ethics Committee (CREC) 196 Moo 5, Phaholyothin Road, Ladyao, Building 2, 5th Floor, The National Research Council of Thailand, Chatuchak, Bangkok, 10900, Thailand
Chetchotisakd, Ploenchan, MD (04127)	1. The Khon Kaen University Ethics Committee for Human Research, 123 Mittraphap Road, Muang, Khon Kaen, 40002, Thailand 2. Central Research Ethics Committee (CREC) 196 Moo, 5, Phaholyothin Road, Ladyao, Building 2, 5th Floor, The National Research Council of Thailand, Chatuchak, Bangkok, 10900, Thailand
Chokephaibulkit, Kulkanya, MD (07546)	Siriraj Institutional Review Board Wang Lang Rd., 2nd Floor, His Majesty the King's 80th Birthday Anniversary 5 Dec2007 Bldg. Bangkok Noi, Bangkok, 10700, Thailand
Siripassorn, Krittaecho, MD (08217)	1. The Institutional Review Board of Bamrasnaradura Infectious Diseases Institute, 38 Tiwanon Road, Muang, Nonthaburi, 11000, Thailand 2. Central Research Ethics Committee (CREC) 196 Moo 5, Phaholyothin Road, Ladyao, Building 2, 5th Floor, The National Research Council of Thailand, Chatuchak, Bangkok, 10900, Thailand
Latin America / Dominican Republic	
Koenig, Ellen Levi, MD (00986) Peynado, Monica Thormann, MD (08422)	Asociación de Bioética, Estudios Protocolares y Seguimientos -(ABIEPROSE) Dr. Piñeyro 211, Zona Universitaria. Santo Domingo, 10103, Dominican Republic

North America / United States	
Batra, Jagmohan, MD (08275) Osiyemi, Olayemi, MD (02106) Ramgopal, Moti, MD (01950) Rodriguez, Carina, MD (07409)	Advarra Institutional Review Board, 6100 Merriweather Dr, Ste 600, Columbia, MD 21044, United States[a]
McKellar, Mehri Sadri, MD (04735)	Duke University Health System Institutional Review Board, Hock Plaza, Suite 405 2424 Erwin Road, Durham, NC 27705, United States
Zorrilla, Carmen D., MD (07487)	Western Institutional Review Board (WIRB), 1019 39th Ave SE, Ste 120, Puyallup, WA 98374, United States

[a]Current address as of 1 March 2021.

APAC, Asia-Pacific; IEC, independent ethics committee; IRB, institutional review board; PI, principal investigator.

Table 2. Baseline characteristics of pregnant women and neonates.

Adult participants	***N* = 33**
Age, years, median (Q1, Q3)	30 (26, 34)
Race, *n* (%)	
Asian	25 (76)
Black	6 (18)
White	1 (3)
Other	1 (3)
Ethnicity, *n* (%)	
Hispanic or Latinx	4 (12)
HIV-1 RNA <50 copies/mL, *n* (%)	33 (100)
CD4 count, cells/μL, median (Q1, Q3)	558 (409, 720)
CD4, %, median (Q1, Q3)	32 (27, 40)
Neonate participants	***N* = 29**
Female sex at birth, *n* (%)	10 (35)
Race, *n* (%)	
Asian	24 (83)
Black	4 (14)
Other	1 (3)
Ethnicity, *n* (%)	
Hispanic or Latinx	4 (14)
HIV-1 RNA, n (%)	
<50 copies/mL	2 (7)
Missing	27 (93)
Apgar score, median (Q1, Q3)[a]	9 (9, 10)

[a]Used to assess status of newborn infants using five measures (appearance of skin color, pulse, grimace response, activity, and respiration) on a scale of 0 to 2 for each measure with 10 being the maximum overall Apgar score.

Q, quartile.

Table 3. Statistical comparison of pharmacokinetic parameters.

	Second trimester vs. Week 6 postpartum, %GLSM (90% CI)	Second trimester vs. Week 12 postpartum %GLSM (90% CI)	Third trimester vs. Week 6 postpartum %GLSM (90% CI)	Third trimester vs. Week 12 postpartum %GLSM (90% CI)
BIC parameters				
AUC_{tau}, h·µg/mL	44.7 (40.0, 49.8)	41.2 (36.7, 46.3)	44.4 (40.0, 49.3)	40.6 (36.8, 44.8)
Unbound AUC_{tau},[a] h·µg/mL	61.8 (55.3, 69.0)	59.7 (52.5, 68.0)	62.4 (55.7, 69.9)	58.8 (52.7, 65.7)
C_{max}, µg/mL	57.7 (52.5, 63.4)	51.9 (46.5, 58.0)	54.4 (48.4, 61.2)	48.2 (43.0, 53.9)
C_{trough}, µg/mL	27.0 (22.2, 32.8)	26.2 (21.5, 31.9)	30.0 (26.5, 33.9)	29.0 (25.7, 32.7)
FTC parameters				
AUC_{tau}, h·µg/mL	64.3 (61.0, 67.8)	67.4 (63.5, 71.6)	65.1 (61.8, 68.6)	69.2 (65.9, 72.7)
C_{max}, µg/mL	77.8 (68.8, 88.1)	75.6 (66.7, 85.7)	77.1 (69.8, 85.2)	77.5 (70.3, 85.3)
C_{trough}, µg/mL	42.9 (36.6, 50.3)	64.2 (54.6, 75.5)	46.9 (39.6, 55.6)	64.7 (59.3, 70.6)
TAF parameters				
AUC_{tau}, h·µg/mL	62.5 (50.8, 77.0)	77.6 (65.4, 92.1)	56.5 (46.3, 69.0)	69.7 (58.6, 82.9)
Unbound AUC_{tau},[a] h·µg/mL	83.6 (72.9, 95.9)	89.3 (79.0, 100.8)	86.2 (71.9, 103.2)	89.2 (78.2, 102)
C_{max}, µg/mL	66.6 (53.8, 82.3)	69.9 (56.2, 87.0)	55.3 (44.7, 68.4)	57.1 (46.0, 70.9)

[a]Unbound AUC_{tau} = AUC_{tau} × fraction unbound.

%GLSM, percentage geometric least-squares mean; AUC_{tau}, area under the plasma drug concentration–time curve over the dosing interval; BIC, bictegravir; CI, confidence interval; C_{max}, maximum observed plasma concentration of drug; C_{trough}, trough concentration; FTC, emtricitabine; TAF, tenofovir alafenamide.

Table 4. Percentage of protein-unbound plasma BIC and TAF.

	Second trimester	Third trimester	Week 6 postpartum	Week 12 postpartum
BIC				
n	21	30	31	32
Percentage protein unbound, mean (%CV)	0.351 (17.2)	0.365 (16.0)	0.261 (15.8)	0.252 (16.5)
TAF				
n	21	29	30	32
Percentage protein unbound, mean (%CV)	7.39 (30.9)	8.27 (24.5)	5.29 (27.7)	6.05 (22.4)

%CV, percentage coefficient of variation; BIC, bictegravir; TAF, tenofovir alafenamide.

Table 5. Pharmacokinetic parameters for FTC and TAF.

	Second trimester (*n* = 21)	Third trimester (*n* = 30)	Week 6 postpartum (*n* = 31)	Week 12 postpartum (*n* = 32)	Non-pregnant adults with HIV-1[a]
FTC parameters					(*n* = 77)
AUC_{tau}, h·µg/mL, mean (%CV)	10.3 (20.0)	10.4 (20.3)	16.3 (24.7)	15.3 (21.9)	12.3 (29.2)
C_{max}, µg/mL, mean (%CV)	2.64 (36.6)	2.59 (26.5)	3.39 (28.0)	3.36 (26.9)	2.13 (34.7)
C_{trough}, µg/mL, mean (%CV)	0.0598 (103.9)	0.0514 (27.2)	0.152 (178.5)	0.0811 (33.7)	0.096 (37.4)
T_{max}, h, median (Q1, Q3)	1.50 (1.00, 2.00)	1.50 (1.00, 2.00)	1.50 (1.00, 1.55)	1.00 (1.00, 1.75)	-
$t_{½}$, h, median (Q1, Q3)	6.43 (5.62, 6.70)	6.41 (5.59, 6.90)	6.27 (5.65, 6.76)	5.76 (5.29, 6.58)	-
CL_{ss}/F, mL/h, mean (%CV)	20,200 (19.7)	20,000 (21.1)	13,000 (23.9)	13,600 (20.7)	-
V_Z/F, mL, mean (%CV)	182,000 (20.2)	185,000 (30.5)	117,000 (30.1)	118,000 (28.3)	
TAF parameters					(*n* = 486)
AUC_{tau}, h·µg/mL, mean (%CV)	0.236 (45.6)	0.212 (45.0)	0.374 (41.0)	0.296 (31.8)	0.142 (17.3)
Unbound AUC_{tau}, h·µg/mL, mean (%CV)[b]	0.015 (28.2)	0.016 (28.4)	0.018 (33.8)	0.017 (23.4)	-
C_{max}, µg/mL, mean (%CV)	0.332 (52.1)	0.271 (42.1)	0.506 (49.2)	0.495 (52.5)	0.121 (15.4)
C_{last}, µg/mL, mean (%CV)	0.00449 (114.2)	0.00480 (84.4)	0.00313 (59.1)	0.00336 (59.5)	-
T_{max}, h, median (Q1, Q3)	0.75 (0.50, 1.50)	1.00 (0.75, 1.50)	0.75 (0.50, 1.00)	0.75 (0.50, 1.00)	-
$t_{½}$, h, median (Q1, Q3)	0.30 (0.25, 0.46)	0.28 (0.22, 0.35)	0.40 (0.35, 0.51)	0.35 (0.30, 0.43)	-
CL_{ss}/F, mL/h, mean (%CV)	123,000 (36.1)	135,000 (33.2)	76,900 (37.9)	92,900 (31.7)	-
V_Z/F, mL, mean (%CV)	62,300 (59.7)	53,200 (31.4)	44,400 (30.8)	49,800 (44.2)	-

[a]The PK parameter values for non-pregnant adults with HIV-1 are based on intensive PK analysis in Studies GS-US-380-1489, GS-US-380-1490, GS-US-380-1844, and GS-US-380-1878 for FTC (*n* = 77) and population PK analysis in Studies GS-US-380-1489 and GS-US-380-1490 for TAF (*n* = 486) (4, 13).

[b]Unbound values were calculated by correcting the individual AUC_{tau} estimates by the percentage unbound fraction for BIC and TAF.

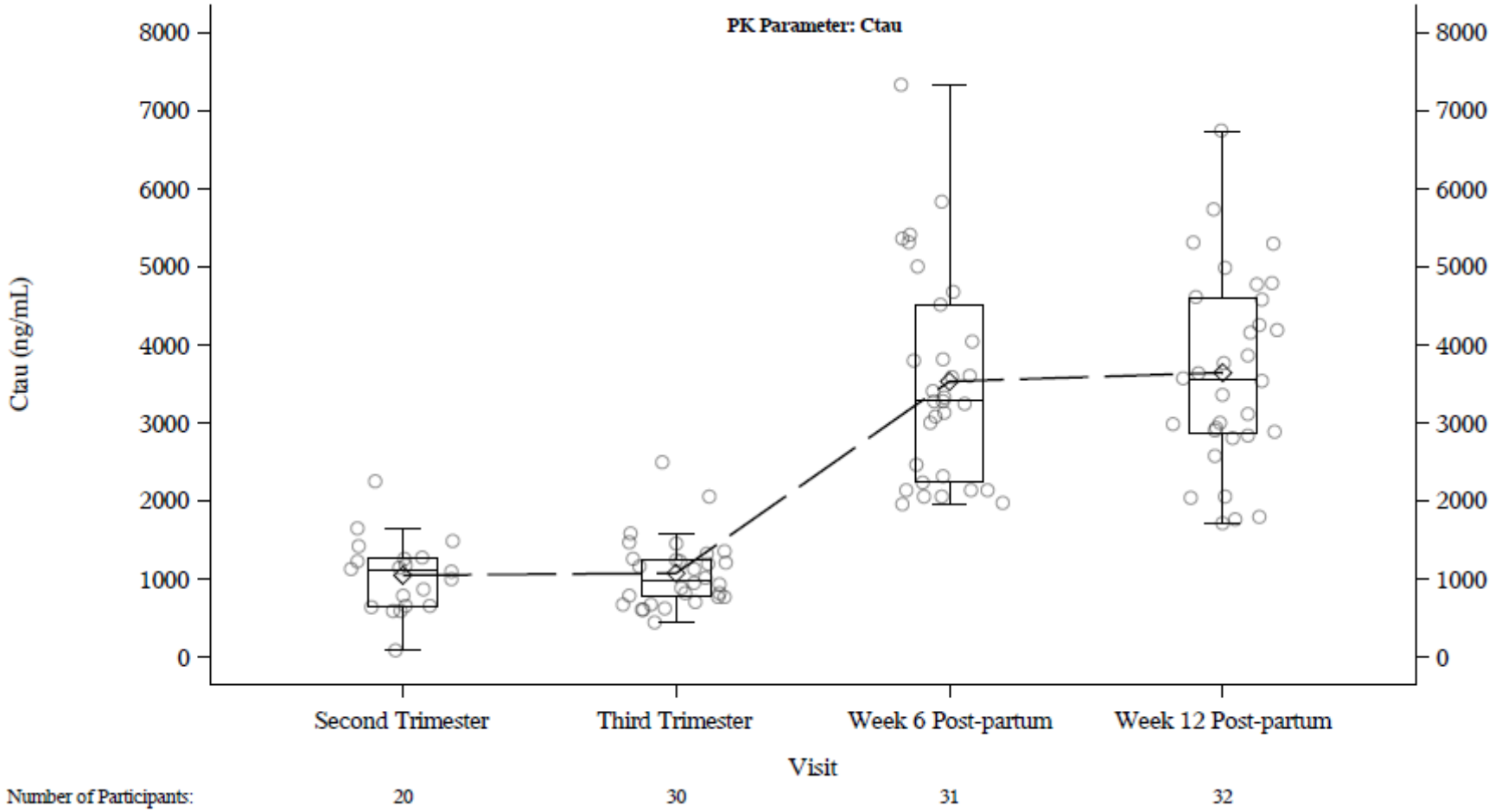
%CV, percentage coefficient of variation; AUC_{tau}, area under the plasma drug concentration–time curve over the dosing interval; BIC, bictegravir; C_{last}, last observed quantifiable concentration of the drug; CL_{ss}/F, apparent oral clearance of the drug at steady state; C_{max}, maximum observed plasma concentration of drug; C_{trough}, trough concentration; FTC, emtricitabine; PK, pharmacokinetic; Q, quartile; $t_{½}$, terminal elimination half-life; TAF, tenofovir alafenamide; T_{max}, observed time point of C_{max}; V_z/F, apparent volume of distribution.

Table 6. Trough TFV-DP concentrations in PBMCs.

	Second trimester	Third trimester	Week 6 postpartum	Week 12 postpartum
TFV-DP C_{trough}				
n	11	19	15	18
µmol/L, mean (%CV)	2.47 (106.87)	2.33 (82.03)	2.80 (82.91)	2.79 (72.86)

C_{trough}, trough concentration; %CV, percentage coefficient of variation; PBMC, peripheral blood mononuclear cell; TFV-DP, tenofovir diphosphate.

Figure 1. Boxplot summary of BIC C_{tau} values.



BIC, bictegravir; C_{tau}, observed plasma drug concentration at the end of the dosing interval.

Extended methodology

Study locations, recruitment, and screening

The study was conducted at eight centers, which include clinical research units, universities, hospitals, and institutes in the Dominican Republic, Thailand, and the USA. Recruitment strategies included pre-screening patients, referrals (from other departments/hospitals/clinics/private physicians), word of mouth, as well as approved posters and leaflets.

Number of participants and subject selection

As per the protocol, up to 35 participants who met all eligibility criteria were planned for enrollment. A minimum of 25 participants were planned to be enrolled in the study in order to obtain at least 20 evaluable pairs of PK assessments between the second trimesters and post-partum and at least 20 evaluable pairs of PK assessment between the third trimesters and post-partum. Replacement participants could be enrolled in the event that participants did not complete all intensive PK visits as expected. Infants born to women participating in the study were to be followed from birth to 4–8 weeks of age if consent was obtained from the parents/legal guardian.

Inclusion criteria

Participants must meet all of the following inclusion criteria to be eligible for participation in this study:

1. The ability to understand and sign a written informed consent form, which must be obtained prior to initiation of study procedures
2. Female participants of age ≥18 to <40 years with singleton pregnancy, at least 12 weeks but not more than 31 weeks pregnant at the time of screening
3. Agree not to breastfeed for the duration of the study
4. Currently on a stable antiretroviral regimen for ≥6 months preceding the screening visit
    a. Documented plasma HIV-1 RNA levels of <50 copies/mL for ≥6 months preceding the Screening Visit and have HIV-1 RNA <50 copies/mL at the Screening Visit
    b. In the preceding 6 months prior to screening, one episode of “blip” (HIV-1 RNA ≥50 copies/mL and <400 copies/mL) is acceptable, only if HIV-1 RNA is <50 copies/mL immediately before and after the blip
5. To determine virologic suppression in the preceding 6 months prior to screening, the lower limit of quantification (LLOQ) by the local HIV-1 RNA assay may be used, only if its LLOQ is >50 copies/mL (e.g. LLOQ of 75 copies/mL)
6. Have no documented or suspected resistance to emtricitabine (FTC), tenofovir (TFV), or integrase strand transfer inhibitors including, but not limited to, the reverse transcriptase resistance mutations K65R or M184V/I.
    a. Historic genotype reports will be collected if available

7. Have a normal ultrasound, completed locally prior to the Day 1 visit, with no evidence of any fetal malformation or structural abnormality affecting either fetus or placenta
8. Normal maternal alfa-fetoprotein level at the Screening Visit
9. Estimated glomerular filtration rate (eGFR) ≥90 mL/min according to the Cockcroft–Gault (C-G) formula:

$$\frac{(\mathbf{140 - age\ in\ years}) \times (\mathbf{wt\ in\ kg}) \times \mathbf{0.85}}{\mathbf{72} \times (\mathbf{serum\ creatinine\ in\ mg/dL})} = \mathbf{CLcr\ (mL/min)}$$

$$\frac{(\mathbf{140 - age\ in\ years}) \times (\mathbf{wt\ in\ kg}) \times \mathbf{0.85}}{\mathbf{72} \times \left(\mathbf{serum\ creatinine\ in\ \frac{mg}{dL}}\right) \times \mathbf{0.6786}} = \mathbf{CLcr\ (mL/min)}$$

10. Normal electrocardiogram (or if abnormal, determined by the investigator not to be clinically significant)
11. Hepatic transaminases (aspartate aminotransferase and alanine transaminase) ≤5 × upper limit of normal (ULN)
12. Total bilirubin ≤1.5 mg/dL (≤26 µmol/L), or normal direct bilirubin
13. Adequate hematologic function (absolute neutrophil count ≥750/mm^3 (≥0.75 GI/L); platelets ≥50,000/mm^3 (≥50 GI/L); hemoglobin ≥9.5 g/dL (≥95 g/L))
14. Serum amylase ≤5 × ULN (participants with serum amylase >5 × ULN will remain eligible if serum lipase is ≤5 × ULN)
15. Participants of childbearing potential must agree to utilize protocol recommended highly effective contraceptive methods or be non-heterosexually active or practice sexual abstinence during the postpartum period of the study, and for 7 days following the last dose of study drug

Exclusion criteria

Participants who meet any of the following exclusion criteria are not to be enrolled in this study:

1. Have chronic hepatitis B virus (HBV) as determined by either:
    a. Positive HBV surface antigen (HBsAg) at the Screening Visit
    b. Negative HBV surface antigen, negative HBV surface antibody, positive HBV core antibody and quantifiable HBV DNA (HBV DNA ≥20 IU/mL) at the Screening Visit
2. Have active hepatitis C virus (HCV) infection
    a. Positive anti-HCV antibody and negative HCV polymerase chain reaction (PCR) results are acceptable
3. An opportunistic illness indicative of stage 3 HIV diagnosed within the 30 days prior to screening
4. Participants experiencing decompensated cirrhosis (e.g., ascites, encephalopathy, or variceal bleeding)
5. Have been treated with immunosuppressant therapies or chemotherapeutic agents within 3 months of study screening, or expected to receive these agents

or systemic steroids during the study (e.g., corticosteroids, immunoglobulins, and other immune- or cytokine-based therapies)

6. Malignancy within 5 years of screening other than cutaneous Kaposi's sarcoma, completely resected non-melanoma skin cancer (basal cell carcinoma or non-invasive cutaneous squamous carcinoma), or completely resected carcinoma in-situ of the cervix (CIN 3) or anus (AIN 3). A prior malignancy treated with curative therapy and for which there has been no evidence of disease for at least 5 years prior to screening is allowed
7. Current alcohol or substance use judged by the Investigator to potentially interfere with participant study compliance.
8. Active, serious infections (other than HIV-1 infection) requiring antibiotic or antifungal therapy within 30 days prior to Day 1
9. Participation in any other clinical trial, including observational studies, without prior approval from the sponsor is prohibited while participating in this trial
10. Any other clinical condition, including pregnancy complications such as gestational diabetes or prior therapy that, in the opinion of the Investigator, would make the participant unsuitable for the study or unable to comply with the dosing requirements
11. Active tuberculosis infection
12. Known hypersensitivity to B/F/TAF, their metabolites, or formulation excipient
13. Participants receiving ongoing therapy with any of the following medications in the table below, including drugs not to be used with B/F/TAF

Drug class	Agents disallowed[a]
Antiarrhythmic agent	Dofetilide
Anticonvulsants	Phenobarbital, phenytoin, carbamazepine, oxcarbazepine
Antimycobacterials	Rifampin, rifapentine, rifabutin
Antiretrovirals	Any antiretroviral drug that is not part of the study regimen
Herbal/natural supplements	St. John's Wort

[a]Administration of any of the above medications must be discontinued at least 30 days prior to the Day 1 visit and for the duration of the study

Enrollment criteria for neonates

Neonates must meet the following inclusion criterion to be eligible for participation in this study:

1. Parent/legal guardian consent obtained for neonate participation

Neonates who meet any of the following exclusion criteria are not to be enrolled in this study:

1. If the mother has discontinued study drug >24 hours prior to delivery
2. At birth, the neonate has a medical condition that, in the opinion of the Investigator, precludes them from participating

PK assessments

In adults, steady-state intensive PK sample collection was performed over 24 hours post-dose during the second trimester (at or between 20 and 28 weeks gestation), if applicable, during the third trimester (at or between 30 and 38 weeks gestation), and at 6 and 12 weeks postpartum.

Optional trough peripheral blood mononuclear cell (PBMC) samples for tenofovir diphosphate (TFV-DP) as well as protein binding samples for BIC and TAF were collected at the intensive PK visits. For BIC and TAF, a single maternal blood sample and an umbilical cord sample were collected at the time of delivery, and sparse PK blood samples were collected for up to 7 days after birth in enrolled neonates with maternal consent. Protein binding of BIC and TAF from maternal intensive PK samples was assessed at 24 and 3 hours post-dose, respectively, in each of the four periods.

Concentrations of BIC, FTC, and TAF in plasma samples were quantified using validated assays involving protein precipitation extraction followed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). Concentrations of TFV-DP in PBMC samples were determined using validated methods, including cell lysis extraction of TFV-DP and fluorescent quantitation of PBMC-derived DNA in conjunction with LC-MS/MS. Plasma protein binding for BIC and TAF were measured by equilibrium dialysis. Plasma, PBMC, and protein binding assays were developed by QPS, LLC (Newark, DE, USA). For further information on PK assessments, please see the study protocol (Supplemental Digital Content Study Protocol). PK parameters for BIC, FTC, and TAF were calculated using standard non-compartmental analyses (Phoenix WinNonlin v8.3.5).

Statistical analyses

With 20 evaluable participants, the study had at least 74% power to show that the lower bound of the 90% confidence interval (CI) of the ratio for AUC_{tau} of BIC during pregnancy relative to postpartum was >50%, assuming a decrease of 40% in AUC_{tau} of BIC during pregnancy relative to postpartum. It was assumed that a standard deviation of BIC AUC_{tau} would be no more than 0.34 on a natural logarithm scale.

Statistical comparisons of BIC, FTC, and TAF PK parameters were performed using SAS® PROC MIXED using a mixed-effects analysis of variance model fitted to the natural logarithmic-transformed maternal PK parameters. Two-sided 90% CIs were calculated for the ratios of geometric least-squares means (GLSMs) between pregnancy (second or third trimester) and postpartum (6 or 12 weeks postpartum). The cross-trial comparison with data from non-pregnant adults with HIV was a qualitative numeric comparison of means to contextualize the findings.

The proportion of adult participants with plasma HIV-1 RNA <50 copies/mL at each visit was summarized using the missing=excluded approach for imputing missing values. CD4 cell counts and CD4% at each visit and changes from baseline were summarized descriptively using observed on-treatment data.