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Supplementary appendix

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

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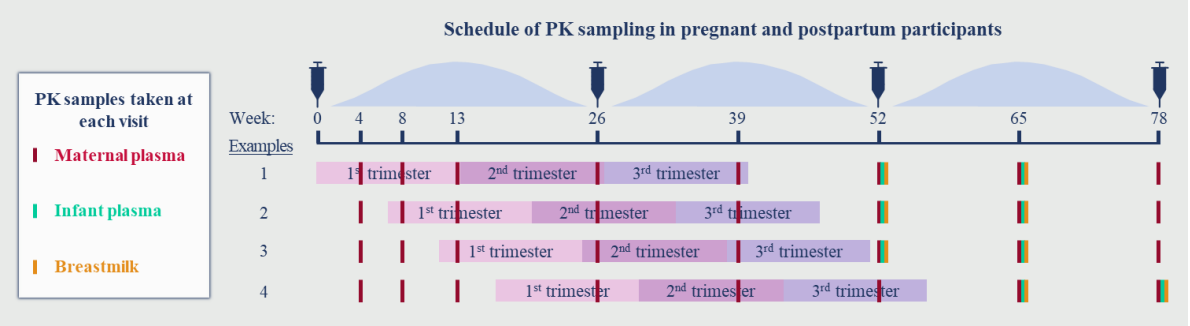
Supplementary methods

Study sites and ethics committees

Ethics Committee	Ethics Reference Number	Research Site	Location
The South African Medical Research Council (SAMRC)	EC029-7/2021	Botha's Hill Research Site	Botha's Hill, South Africa
		Chatsworth Research Site	Chatsworth, South Africa
		Tongaat Research Site	Tongaat, South Africa
		Phoenix Research Site	Phoenix, South Africa
		Verulam Research Site	Verulam, South Africa
University of Kwazulu-Natal, Biomedical Research Ethics Committee (BREC)	BREC/00002494/2021	Center for the AIDS Programme of Research in South Africa (CAPRISA) Vulindlela Research Clinic	KwaZulu-Natal, South Africa
	BREC/00003922/2022	Africa Health Research Institute (AHRI)	Durban, South Africa
	BREC/00002495/2021	Center for the AIDS Programme of Research in South Africa (CAPRISA) eThekwini Research Clinic	Berea, Durban, South Africa
	BREC/00002493/2021	Center for the AIDS Programme of Research in South Africa (CAPRISA) uMlazi Research site - Women's Health and HIV Unit	Umlazi, South Africa
University of Cape Town Human Research Ethics Committee (UCT HREC)	122/2021	Desmond Tutu Health Foundation (DTHF) Emavundleni Clinical Research Site	Kilpfontein, Cape Town, South Africa
	121/2021	Desmond Tutu Health Foundation (DTHF) Masiphumelele Research Site	Cape Town, South Africa
	612/2022	Desmond Tutu Health Foundation (DTHF) Groote Schuur Hospital	Cape Town, South Africa

	024/2023	Vuka Research Clinic, University of Cape Town	Khayelitsha, South Africa
	174/2022	Foundation for Professional Development	Ndevana, South Africa
University of the Witwatersrand Human Research Ethics Committee (Wits HREC)	210210	Setshaba Research Centre	Pretoria, South Africa
		The Aurum Institute Klerksdorp Clinical Research Site	Klerksdorp, South Africa
		Madibeng Centre for Research	Brits, South Africa
		Rustenburg Clinical Research Site	Rustenburg, South Africa
		MatCH Research Unit	Durban, South Africa
		Synergy Biomed Research Institute	East London, South Africa
		Wits Reproductive Health and HIV Institute	Johannesburg, South Africa
		The Aurum Institute Tembisa Clinical Research Site	Tembisa, South Africa
		Qhakaza Mbokodo Research Clinic	Ladysmith, South Africa
		The Aurum Institute Pretoria Clinical Research Site	Pretoria, South Africa
		Perinatal HIV Research Unit	Johannesburg, South Africa
Uganda Virus Research Institute	GC/127/863	Makerere University - Johns Hopkins University Research Collaboration	Mityana, Uganda
		Africa Medical and Behavioural Sciences Organization (AMBSO) - Masaka site	Masaka, Uganda
		Uganda Virus Research Institute/International Aids Vaccine Initiative Site	Kalangala, Uganda

***Figure S1*. Representative schematic to illustrate collection of maternal, infant, and breastmilk pharmacokinetic samples**



This figure illustrates the potential pharmacokinetic sampling windows for maternal, infant, and breastmilk samples for four theoretical participants.

PK=pharmacokinetic.

Lenacapavir bioanalysis

Lenacapavir concentrations in maternal plasma, breastmilk, and infant plasma were measured using a validated high-performance liquid chromatography–tandem mass spectrometry method with multiple reaction monitoring (MRM) and electrospray ionisation in the positive mode. The sample volume was 50 µL. Sample preparation was done using supported liquid extraction and Hamilton Star automation (Reno, NV, USA), and the calibration range was 0·5 to 500 ng/mL for maternal plasma, infant plasma, and breastmilk. Isotopically labelled internal standard (lenacapavir-d_6) was used for all the sample analyses. Response ratios of analyte:internal standard observed for calibration standards were subjected to linear regression with 1/(nominal concentration)^2 weighting, and the concentration of an analyte in a sample was determined by interpolation of its response ratio in the standard curve equation. Concentrations below the calibrated ranges of the methods were reported as below the quantification limit; samples with concentrations above the calibrated ranges of the methods were diluted with blank matrix prior to analysis and the appropriate dilution factor was applied to the result. The interassay precision range (% coefficient of variation [CV]) was 4·5 to 8·7, and the interassay accuracy range (% relative error [RE]) was −4·8 to 4·6 for both the maternal and infant plasma method validation study. The interassay precision range (%CV) was 2·9 to 9·8, and the interassay accuracy range (%RE) was −5·8 to 2·4 for the breastmilk method validation study. The method validation and bioanalysis were performed at Labcorp (Madison, WI, USA). All samples were analysed within the time frame supported by frozen stability storage data.

Population pharmacokinetic analysis

Lenacapavir plasma concentrations from 13 studies (N=1537 participants; N=18,716 samples) in healthy volunteers, people with HIV-1, or people who want or need pre-exposure prophylaxis (PrEP) were included in the population pharmacokinetic analysis. Data were used to support evaluation of lenacapavir pharmacokinetics during pregnancy.

To characterise lenacapavir disposition and absorption following oral and subcutaneous administration, data from healthy volunteers were incrementally incorporated in a stepwise manner during model development. Intravenous data were first used to characterise disposition. Intravenous and oral data were subsequently used to inform oral absorption, followed by intravenous and subcutaneous data to characterise subcutaneous absorption. Covariates including, but not limited to, body weight, age, sex assigned at birth, race, fed status, and lenacapavir dose were evaluated on model parameters, as appropriate.

After optimisation of the disposition and absorption components, data from people with HIV and people who want or need PrEP were incorporated to evaluate potential population-specific covariates. Time-varying pregnancy-related variables evaluated in the population pharmacokinetic analysis included gestational age, pregnancy trimester, and postpartum status. Trimester was determined based on estimated gestational age. Postpartum status was defined as within 13 weeks following the end of pregnancy, after either childbirth or pregnancy termination.

Covariate selection was guided by visual inspection of the data, improvement in model fit, statistical significance (alpha level of 0·01 based on change in objective function value), and clinical relevance. At each step of model development, random effect models were evaluated. NONMEM (version 7·5) was used to develop the population pharmacokinetic model.

Pregnancy data

In the PURPOSE 1 study, 601 participants were included in the population pharmacokinetic analysis. Participants who became pregnant in PURPOSE 1 and consented to remain on lenacapavir with continued study follow-up were eligible for inclusion. Overall, 296 women who became pregnant while on lenacapavir and had at least one quantifiable pharmacokinetic sample collected during pregnancy were included in the population pharmacokinetic analysis.

Supplementary results

***Table S1*. Additional details on reported congenital anomalies**

Participant	Randomised study group	Pregnancy term	Anomaly
1	Lenacapavir	Term	Umbilical hernia
2	Lenacapavir	Term	Polydactyly*
3	Lenacapavir	Term	Ventricular septal defect**
4	Lenacapavir	Term	Ventricular septal defect
5	Lenacapavir	Post-term	Infantile haemangioma
6	Lenacapavir	Term	Congenital reducible umbilical hernia
7	F/TAF	Term	Bilateral hydrocoele
8	F/TAF	Term	Umbilical and inguinal hernia
9	F/TAF	Term	Down syndrome
10	F/TAF	Term	Club foot

Pregnancy term was defined as: term, ≥37 to ≤42 weeks' gestation; preterm, <37 weeks' gestation; post-term, >42 weeks' gestation. All anomalies assessed by investigators as unrelated to study drug, unless otherwise noted.

*Strong maternal family history of polydactyly reported.

**Ventricular septal defect in participant 3 was assessed as related to study drug by the investigator.

F/TAF=emtricitabine/tenofovir alafenamide.

***Table S2*. Pregnancy and birth outcomes through the end of the randomised blinded phase**

	Lenacapavir (n=253)	**F/TAF (n=280)**	**F/TDF (n=132)**	**Total (n=665)**
Pregnancies				
Participants with confirmed pregnancies, n	253	280	132	665
Confirmed pregnancies, n	269	302	141	712
Pregnancy outcomes				
Total pregnancy outcomes,[*] n	272	304	141	717
Live births	172 (63%)	167 (55%)	84 (60%)	423 (59%)
Pregnancy losses	91 (33%)	123 (40%)	53 (38%)	267 (37%)
Stillbirths[†]	9 (3%)	8 (3%)	4 (3%)	21 (3%)
Induced/elective abortions	49 (18%)	71 (23%)	28 (20%)	148 (21%)
Spontaneous abortions[‡]	33 (12%)	44 (14%)	21 (15%)	98 (14%)
Pregnancies with unknown outcome[§]	9 (3%)	14 (5%)	4 (3%)	27 (4%)
Live birth outcomes	172	167	84	423
Term (≥37 to ≤42 weeks' gestation)	139 (81%)	127 (76%)	61 (73%)	327 (77%)
Preterm (<37 weeks' gestation)	22 (13%)	28 (17%)	18 (21%)	68 (16%)
Post-term (>42 weeks' gestation)	11 (6%)	12 (7%)	5 (6%)	28 (7%)
Congenital abnormality	6 (3%)	7 (4%)	1 (1%)	14 (3%)
Small for gestational age[¶]	22 (13%)	15 (9%)	10 (12%)	47 (11%)

Data presented as number (%) unless otherwise noted. The denominator for live births, pregnancy losses, and pregnancies with unknown outcome was number of pregnancy outcomes; the denominator for live birth outcomes was liveborn infants.

[*]A single pregnancy may include multiple outcomes/births (five sets of twins: three sets in participants randomised to lenacapavir and two sets in participants randomised to F/TAF). Birth outcomes are reported for each individual infant.

[†]Stillbirth/intrauterine foetal demise defined as occurring ≥20 weeks' gestation. There was one set of stillbirth twins in the lenacapavir group.

[‡]Spontaneous abortion defined as occurring at <20 weeks' gestation.

[§]Unknown due to participant discontinuation for the following reasons: pregnancy (lenacapavir, n=3; F/TAF, n=4; F/TDF, n=1); investigator's discretion (F/TAF, n=2); withdrew consent (lenacapavir, n=5; F/TAF, n=5); lost to follow-up (F/TAF, n=2; F/TDF, n=1); completed study (lenacapavir, n=1; F/TAF, n=1; F/TDF, n=1); ongoing pregnancy (F/TDF, n=1).

[¶]Small for gestational age was defined as weight <10th percentile by sex/gestational age.

F/TAF=emtricitabine/tenofovir alafenamide. F/TDF=emtricitabine/tenofovir disoproxil fumarate.

Table S3*. Summary of model-derived C_{max} and C_{trough} for participants stratified by pregnancy groups, for participants who took lenacapavir on time and were included in population pharmacokinetics analysis

	Did not become pregnant	**Non pregnant period**	**First trimester (EGA 1 to 84 days)**	**Second trimester (EGA 85 to 189 days)**	**Third trimester (EGA >189 days)**	**Postpartum (within 13 weeks after pregnancy)**
	Mean (% CV) [n]	Mean (% CV) [n]	Mean (% CV) [n]	Mean (% CV) [n]	Mean (% CV) [n]	Mean (% CV) [n]
Day 1 to Week 26[*]						
C_{max}, ng/mL	62·2 (18) [289]	63·5 (16) [228]	61·6 (16) [45]	64·3 (19) [21]	57·2 (··) [1]	63·0 (15) [17]
C_{trough}, ng/mL	33·3 (16) [289]	34·0 (14) [201]	34·0 (15) [44]	33·1 (13) [43]	34·0 (18) [14]	31·7 (16) [10]
Week 26 to Week 52						
C_{max}, ng/mL	75·4 (18) [250]	77·1 (15) [160]	76·8 (15) [27]	79·0 (16) [29]	74·9 (16) [22]	79·6 (18) [14]
C_{trough}, ng/mL	38·9 (16) [250]	39·6 (14) [138]	39·7 (13) [33]	39·7 (13) [30]	41·1 (15) [22]	39·3 (13) [29]
Week 52 to Week 78						
C_{max}, ng/mL	77·7 (18) [232]	78·4 (15) [126]	78·6 (14) [25]	81·5 (14) [29]	77·5 (18) [21]	85·7 (13) [22]
C_{trough}, ng/mL	39·8 (16) [232]	40·4 (13) [118]	40·5 (15) [30]	39·8 (14) [26]	41·1 (13) [23]	41·6 (13) [26]

Model-derived lenacapavir C_{max} and C_{trough} following on-time complete injections are shown. On-time complete injection was defined as both injections administered in full dose within ±2 weeks of the targeted day relative to the previous injection. Model-derived C_{max} and C_{trough} were based on simulated concentration-time profiles for each participant. Concentration-time profiles were only simulated up to 26 weeks following the last on-time complete injection or the start of oral bridging or oral reloading, whichever came first. Includes participants with available pharmacokinetic samples up to March 27, 2025.

[*]C_{trough} is trough concentration at Weeks 26, 52, 78.

C_{max}=maximum concentration. C_{trough}=trough concentration. CV=coefficient of variation. EGA=estimated gestational age.

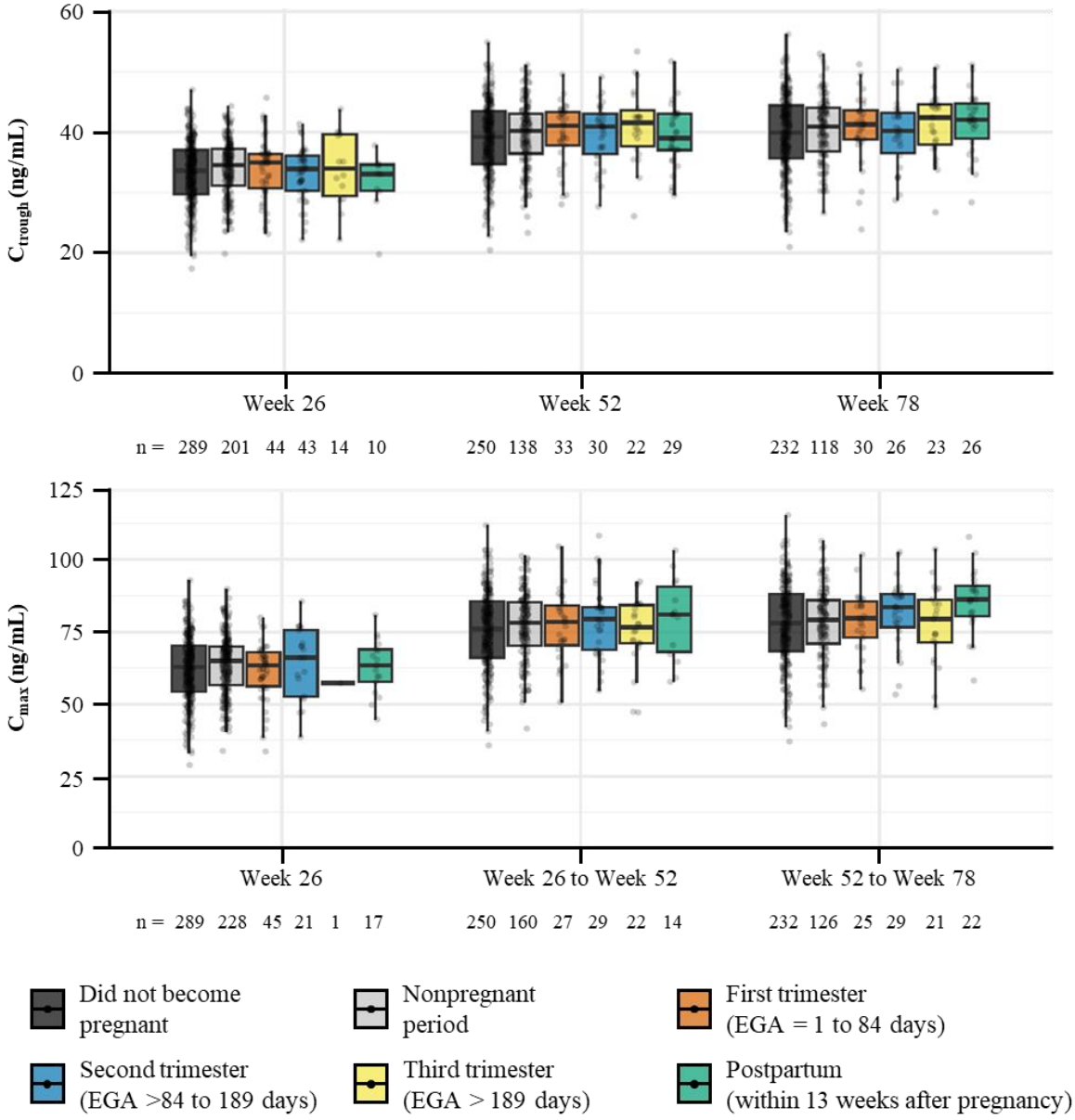
***Table S4*. Median observed lenacapavir concentrations in breastmilk-maternal plasma and breastfed-infant-maternal plasma pairs**

Breastmilk concentration (n=102)	Maternal plasma concentration (n=102)	Breastmilk-maternal plasma ratio (n=102 pairs)	Breastfed infant plasma concentration (n=98)*	Maternal plasma concentration (n=96)*	Breastfed-infant-maternal plasma ratio (n=98 pairs)
31·80 (21·40, 53·20)	62·35 (45·20, 89·00)	0·52 (0·38, 0·77)	1·63 (0·87, 2·85)	65·65 (46·00, 91·10)	0·02 (0·01, 0·05)

Data presented as median (Q1, Q3) concentration in ng/mL unless otherwise noted.

*There are 98 infants contributing breastfed infant plasma samples and 96 mothers contributing maternal plasma samples. This discrepancy reflects two mothers with twin infants who had pharmacokinetic samples paired with two infants each.

Figure S2*. Model-derived C_{max} and C_{trough} for participants stratified by pregnancy groups, for participants who took lenacapavir on time



Model-derived lenacapavir C_{max} and C_{trough} following on-time complete injections are shown. On-time complete injection was defined as both injections administered in full dose within ±2 weeks of the targeted day relative to the previous injection. Model-derived C_{max} and C_{trough} were based on simulated concentration-time profiles for each participant. Concentration-time profiles were only simulated up to 26 weeks following the last on-time complete injection or the start of oral bridging or oral reloading, whichever came first. Includes participants with available pharmacokinetic samples up to March 27, 2025.

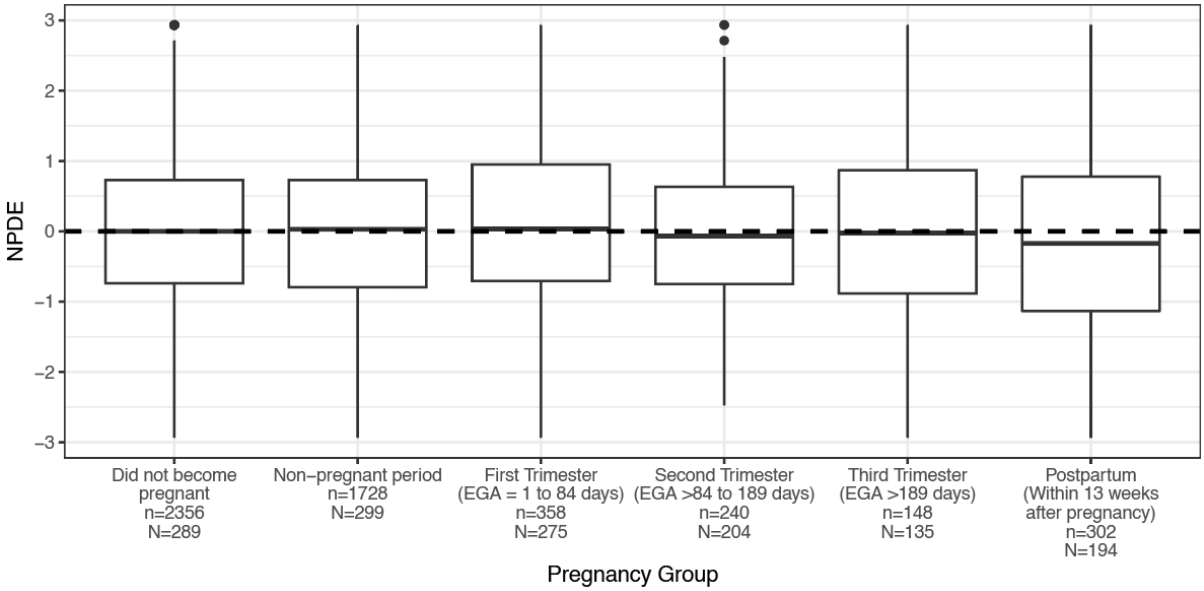
*C_{trough} is trough concentration at Weeks 26, 52, 78.

C_{max}=maximum concentration. C_{trough}=trough concentration. CV=coefficient of variation; EGA=estimated gestational age.

Population pharmacokinetic model evaluation

In the final population pharmacokinetic model, baseline body weight (implemented using fixed allometric scaling), sex assigned at birth, and fed status were retained as covariates on lenacapavir pharmacokinetic parameters. After accounting for these factors, pregnancy-related variables, including gestational age, pregnancy trimester, and postpartum status, were not identified as statistically significant covariates ($p>0{\cdot}01$). Normalised prediction distribution error diagnostics and visual predictive checks stratified by pregnancy trimester and postpartum status did not indicate systematic bias or meaningful deviation between observed and model-predicted lenacapavir plasma concentrations. These diagnostics supported the conclusion that pregnancy-related physiological changes, as captured by the evaluated variables, did not meaningfully affect lenacapavir pharmacokinetics (**Figure S3** and **Figure S4**).

***Figure S3*. Box plots of normalised prediction distribution errors (NPDEs) stratified by pregnancy group for the population pharmacokinetic model**

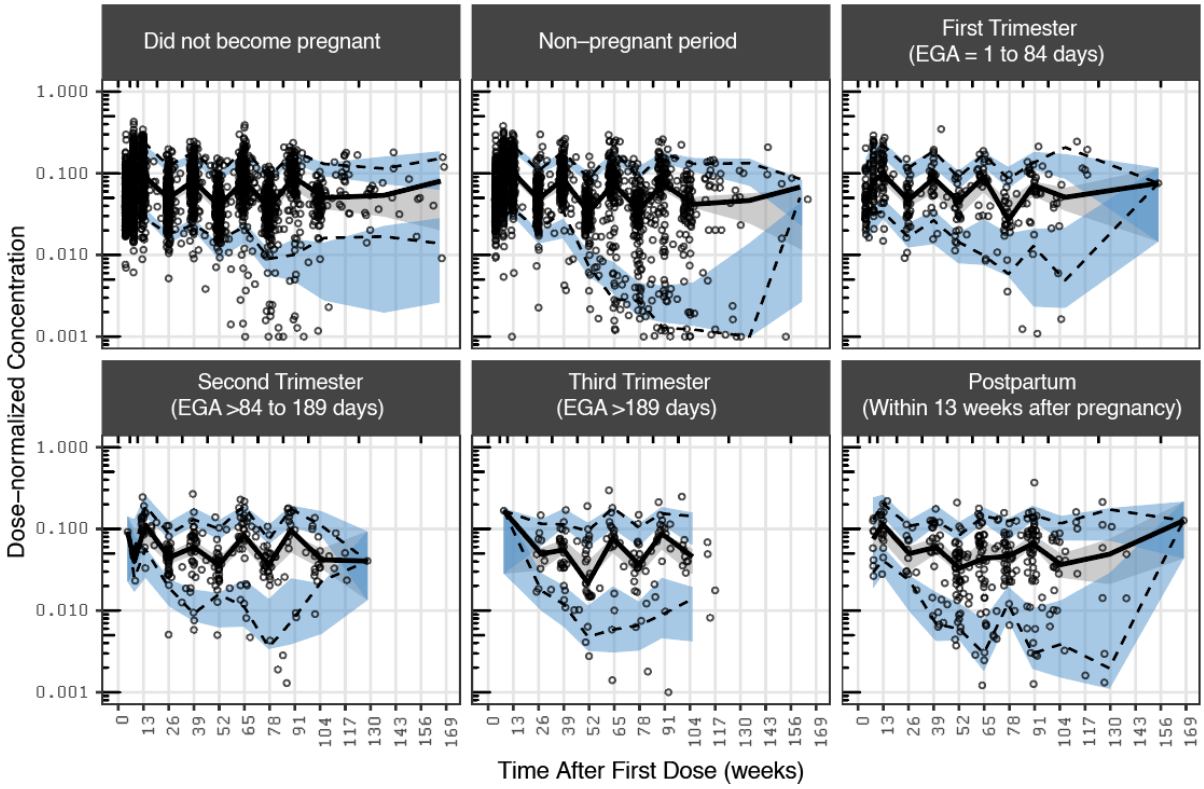


N is the number of participants and n is the number of samples in the box plots.

Boxes represent the interquartile range (Q1–Q3) with the median indicated by the horizontal line. Whiskers extend to the most extreme values within 1.5 x the interquartile range, and observations beyond the whiskers are shown individually. The dashed horizontal line at 0 indicates the expected NPDE under the model.

EGA=estimated gestational age. NPDE=normalised prediction distribution errors.

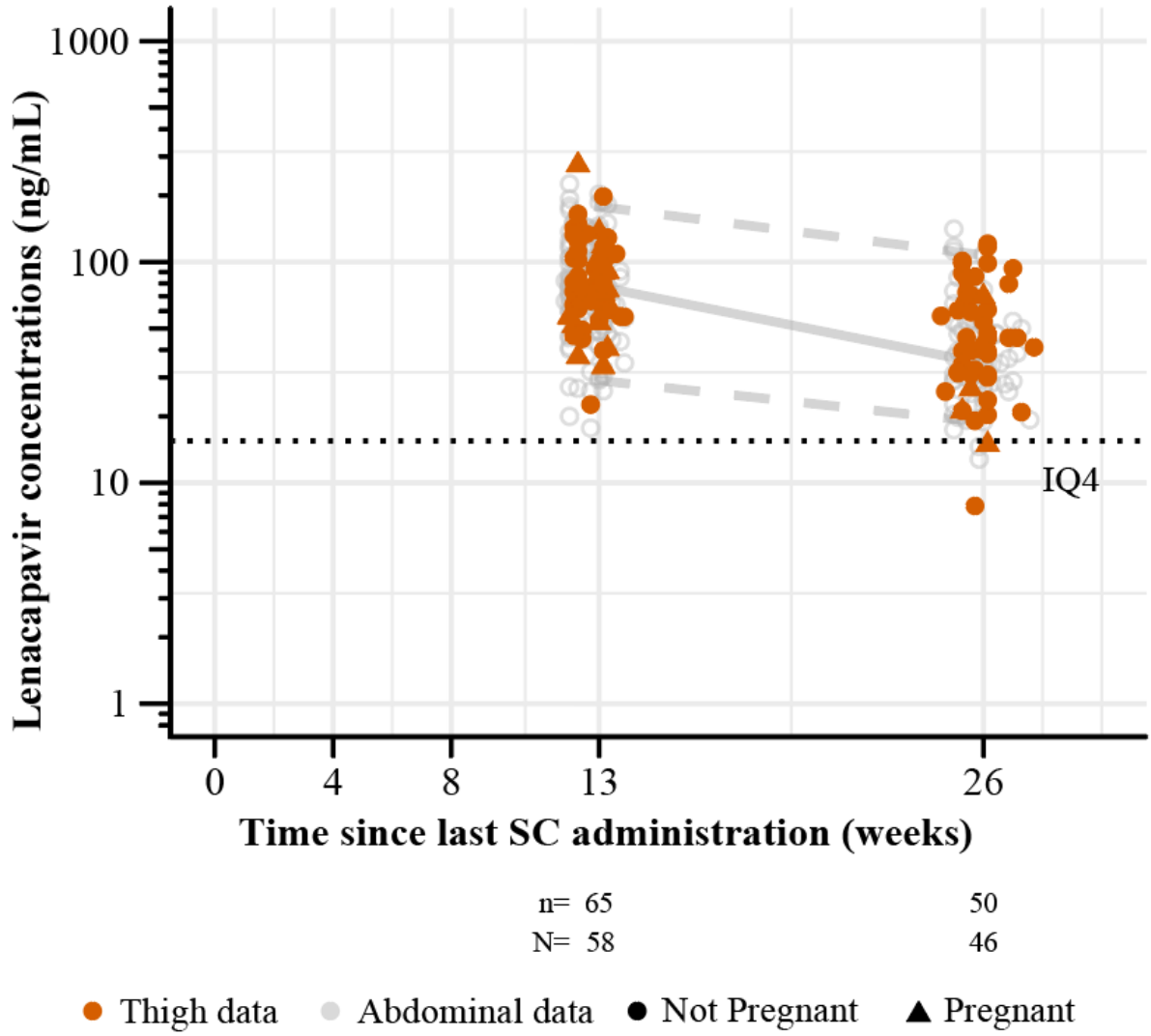
***Figure S4*. Visual predictive checks for the population pharmacokinetic model stratified by pregnancy group**



Points are observed lenacapavir concentrations. Solid and dashed lines are the median and 5th and 95th percentiles of observed lenacapavir concentrations, respectively. Shaded areas are the 90% confidence intervals of the median (grey), 5th (blue), and 95th (blue) percentiles of model simulations.

EGA=estimated gestational age.

***Figure S5*. Plasma lenacapavir concentrations following subcutaneous administration into abdomen and thigh**



Thigh data (orange points) includes 115 samples from 62 unique participants. Not all participants contributed data at all time points; therefore, sample size (N) varies across time points. Sample sizes (N and n) in the plot represent the thigh data. Abdominal data (grey points) includes 137 samples from 201 unique participants following the second SC lenacapavir administration in the randomised blinded phase; this accounted for drug accumulation as thigh injections were administered after the first dosing cycle. One data point (Week 39) that was below the limit of quantification following abdominal injections was excluded.

IQ4=inhibitory quotient 4. SC=subcutaneous.