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ABSTRACT:

The purpose of this study was to evaluate the pharmacokinetics of ritonavir-boosted fosamprenavir during pregnancy and postpartum. Amprenavir (the active moiety of fosamprenavir) and ritonavir intensive pharmacokinetic evaluations were performed at steady-state during the second and third trimesters of pregnancy and postpartum. Plasma concentrations of amprenavir and ritonavir were measured using high-performance liquid chromatography. Target amprenavir area under the concentration time curve (AUC) was >10th percentile (27.7 µg*hr/mL) of median area under the curve for ritonavir-boosted fosamprenavir in adults receiving twice-daily fosamprenavir/ritonavir, 700mg/100 mg. Twenty-nine women were included in the analysis. Amprenavir AUC_{0-12} was lower [(geometric mean ratio (GMR) 0.60 (CI 0.49-0.72; p<0.001)] while its apparent oral clearance was higher [(GMR 1.68 (CI 1.38-2.03; p<0.001)] in the third trimester compared to postpartum. Similarly, ritonavir AUC_{0-12} was lower in the second [GMR 0.51 (CI 0.28-0.91; p=0.09)] and third trimesters [(GMR 0.72 (CI 0.55-0.95; p=0.005)] compared to postpartum, while its apparent oral clearance was higher in the second [GMR 1.98 (CI 1.10-3.56; p=0.06)] and third trimesters [(GMR 1.38 (CI 1.05-1.82; p=0.009)] compared to postpartum. Amprenavir area under the curve exceeded the target for 6/8 (75%) in the 2nd trimester; 18/28 (64%) in the 3rd trimester; and 19/22 (86.4%) postpartum, and the trough concentrations (C_{min}) of amprevavir were 4-16 fold above the mean amprenavir-protein-adjusted IC_{50} of 0.146µg/mL. Although amprenavir

plasma concentrations in women receiving ritonavir-boosted fosamprenavir were lower during pregnancy compared to postpartum, the reduced amprenavir concentrations were still above the exposures needed for viral suppression.

Word count: 243/250 max.

INTRODUCTION:

Fosamprenavir (FPV), a calcium phosphoester prodrug of amprenavir (APV), in combination with low-dose ritonavir (RTV), is a protease inhibitor (PIs) that is not recommended for use in pregnant women living with HIV, but may be an option in certain circumstances. FPV is available as 700 mg tablets, and is currently dosed as FPV/RTV 700mg/100mg twice daily (1). Although FPV/RTV is not routinely used in preventing perinatal transmission, it is still of benefit in people living with HIV in countries where novel PIs are currently unavailable, or in FPV treatment-experienced adults living with HIV (2). APV has also been shown to be efficacious against breast cancer by inhibiting the activity of extracellular signal-regulated kinase 2 (ERK2), inhibiting tumor growth in human MCF-7 cancer cells, and inducing apoptosis both in-vitro and in-vivo, making APV a promising drug for future anti-cancer therapeutics (3).

Fosamprenavir, upon oral administration, is rapidly and extensively converted to the active drug APV in the intestinal mucosa (4-6). APV is

subsequently metabolized in the liver by cytochrome P450 3A4 (CYP3A4), primarily by oxidation to two major metabolites – M2 and M3 (7). APV is an inhibitor of the HIV-1 protease enzyme – it binds to the HIV protease active site, and blocks replication by inhibiting the cleavage of HIV-1 55 gag precursor protein into p17 and p24 core proteins, which are necessary for viral maturation (8). APV and its metabolites are excreted mainly in feces (75%) and urine (14%) (9). Due to physiological and immunological changes that occur during pregnancy (increased CYP3A activity,(10) increased volume of distribution, and increased renal clearance), there is decreased exposure of many antiretrovirals (ARVs), particularly the PIs during the second and third trimesters of pregnancy (11, 12).

The pharmacokinetics (PK) of FPV/RTV have been studied previously in pregnant and postpartum women attending HIV pregnancy clinics in New York, United States by Cespedes et al (13). Amprenavir exposure decreased by 35% during the 2nd trimester of pregnancy and by 25% during the 3rd trimester with 700mg/100mg FPV/RTV twice daily dosing when compared to postpartum (13). Similarly, APV trough plasma concentrations (C_{min}) decreased by 36% during the 2nd trimester and by 38% in the 3rd trimester of pregnancy with 700mg/100mg FPV/RTV twice daily dosing when compared to postpartum. However, the PK analysis of the Cespedes et al study was limited to six patients in the second trimester and nine patients in the third trimester and postpartum (13). A larger sample size is critically important in

PK studies as it provides a better understanding of intra and inter-individual variability needed for robust PK predictions (14). Therefore, the goal of the current study was to evaluate the PK of FPV/RTV (700/100 twice daily) during pregnancy using a larger and diverse sample size of women living with HIV from multiple countries.

RESULTS:

Demographic characteristics and clinical outcomes for the 29 participants are shown in ***Table 1***. Of the 29 participants, 8 were sampled in the second trimester, 28 in the third trimester, and 22 postpartum. The median age at delivery of the mothers participating in this study was 31 years (IQR 25.4, 34.1). Twelve (41%) women were non-Hispanic Black, 15 were Hispanic (52%), 1 participant (3%) was Asian, and 1 (3%) was White non-Hispanic. The median gestational age at the time of sampling was 24.6 weeks (IQR: 21.2, 25.6 weeks) in the 2nd trimester, 32.7 weeks (IQR 31.6, 35.0 weeks) in the 3rd trimester, and median postpartum sampling time was 6.7 weeks after delivery (IQR 6.0, 9.9) - ***Table 1***.

Plasma HIV-1 RNA was $\leq$75 copies/mL in 38% (3/8) of participants in the second trimester, 70% (19/27) in the third trimester, and 76% (13/17) postpartum. The median CD4 count (cells/mL) was 485 (IQR, 418, 571) in the second trimester, 491 (IQR 356, 635) in the third trimester, and 590 (IQR 394, 794) postpartum. The median gestational age at the time of delivery

was 38.7 (IQR 37.9, 39.4) weeks, and the median neonatal birth weight was 3238 grams (IQR 2935, 3478) – ***Table 1***.

Amprenavir and ritonavir PK parameters with standard adult dosing (FPV 700mg/ RTV 100mg twice daily) during the second trimester (n=8), third trimester (n=28), and two weeks postpartum (n=22) are presented in ***Table 2*** and ***Table 3***. Since FPV is the prodrug for APV, APV exposure was measured. APV AUC_{0-12} was lower in the 3rd trimester (geometric mean ratio, GMR 0.60 (CI 0.49-0.72; p<0.001) compared to postpartum – ***Figure 1***. The median and interquartile range of APV AUC was 43.5 µg*hr/mL (IQR 38.5, 50.4 µg*hr/mL) during the second trimester, 32.2 µg*hr/L (IQR 21.5, 39.7 µg*hr/mL) during the third trimester, and 51.6 µg*hr/mL (IQR 45.2, 59.6 µg*hr/mL) postpartum – ***Table 2***. APV AUC exceeded the target for 6/8 (75%) in the 2nd trimester, 18/28 (64%) in the 3rd trimester, and 19/22 (86.4%) postpartum – ***Figure 1***.

Amprenavir apparent oral clearance (CL/F) was higher in the 3rd trimester (GMR 1.68 (CI 1.38-2.03; p<0.001) compared to postpartum (P<0.001). Amprenavir minimum plasma concentration (C_{min}) [(GMR 0.97 (CI 0.55-1.71); p=0.01), APV initial serum concentration (C_0) [GMR 0.91(0.50-1.65)], and APV last observable quantifiable plasma concentration (C_{last}) [(GMR 0.60 (CI 0.45-0.81); p=0.004) were lower in the 3rd trimester compared to postpartum. Similarly, APV maximum plasma concentration (C_{max}) [(GMR 0.74

(CI 0.58-0.93); p=0.03) and trough serum concentrations at 12 hours (C_{12}) [GMR 0.56 (0.43-0.72)] were lower in the 3rd trimester compared to postpartum – ***Figure 2***. The minimum APV target trough concentrations for wild type virus of 0.4 µg/mL(15) was exceeded by 87.5% (7/8) women in the 2nd trimester, 96.4% (27/28) in the 3rd trimester, and 95.5% (21/22) postpartum – ***Figure 2***.

Ritonavir PK data are shown in ***Table 3***. Ritonavir AUC_{0-12} was lower in the 2nd [GMR 0.51 (CI 0.28-0.91; p=0.09)] and 3rd trimesters [(GMR 0.73 (CI 0.55-0.95; p=0.005)] compared to postpartum. Ritonavir apparent oral clearance (CL/F) was higher in the 2nd [GMR 1.98 (CI 1.10-3.56; p=0.06)] and 3rd trimesters [(GMR 1.38 (CI 1.05-1.82; p=0.005)] compared to postpartum. Ritonavir last observed quantifiable plasma concentration (C_{last}) [(GMR 0.45 (CI 0.20-1.03); p=0.08)] and minimum serum concentrations (C_{min}) [(GMR 0.49 (CI 0.24-0.99); p=0.08)] were lower in the 2nd trimester compared to postpartum. Ritonavir trough serum concentration at 12 hours (C_{12}) [GMR 0.70 (0.53-0.91); p=0.03] was lower in the 3rd trimester compared to postpartum.

Third trimester APV and RTV PK parameters by viral load (≤ 75 copies/mL versus >75 copies/mL) are shown in ***Table 4.*** No statistically significant associations between drug exposure and viral load suppression were detected. (***Table 4***). Four women (13.8 %) experienced adverse events that

were possibly treatment-related, including moderate to severe elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). All antiretrovirals received, and the number of mothers taking each at the time of PK evaluations, are summarized in ***Table 5*****.**

DISCUSSION:

Pregnancy is known to modify the actions of some drug metabolizing enzymes, impacting drug exposure (16-18). Previous PK data from the IMPAACT P1026s and the Pediatric AIDS Clinical Trials Group (PACTG) 353 studies demonstrated decreases in exposure of PIs during pregnancy, including lopinavir, atazanavir, saquinavir, indinavir, darunavir and nelfinavir (19-26). The largest decreases are notable in the third trimester, while second trimester concentrations were generally decreased to a lesser extent (27, 28). However, boosting with RTV improves the PK and pharmacodynamic (PD) profiles of most PIs (29, 30). For example, when APV is used without RTV, C_{min} values (0.280 µg/mL) were found to be very close to the EC_{90} (concentration producing 90% of the maximal antiviral effect) value of 0.228 µg/mL (5). However, with RTV boosting, C_{min} values were 8-9 fold higher (1.92 µg/mL) (31). These have direct implications for perinatal transmission and HIV viral resistance.

Physiologic changes during pregnancy can explain the decreased drug exposures of APV and RTV. FPV is rapidly and almost entirely hydrolyzed to APV and inorganic phosphate as it is absorbed from the gastrointestinal tract after oral administration (5, 6). APV is transported by P-glycoprotein (P-gp), and has a large apparent volume of distribution of over 430 liters (8). APV has a $T_{1/2}$ of 7.7 hours when unboosted, but increases to 15-23 hours when boosted with RTV. APV is a substrate of cytochrome P450 (CYP3A) enzymes; inhibitor of CYP3A4,(32) BRCP,(32) P-gp,(33) and OATS,(34) and is almost exclusively metabolized by CYP3A isoforms (2, 8). Therefore, the large volume of distribution, increased clearance, and increases in CYP3A activity during pregnancy (35% to 38%),(35) especially during the third trimester, likely contribute to the lower drug exposures and enhanced clearance of APV from the maternal plasma. APV is highly protein bound, with 90% of circulating plasma APV levels bound to plasma proteins (mainly alpha-1-acid glycoprotein) (36).

An understanding of known pharmacokinetic-pharmacodynamic (PKPD) relationships of APV (AUC, viral response and protein-adjusted IC_{50}/IC_{90}) in the context of lower exposures is needed to evaluate whether the decrease in APV exposure is clinically relevant during pregnancy. To effectively and consistently suppress HIV replication in people living with HIV, antiretroviral drug concentrations must achieve certain concentrations, and be maintained at concentrations that exceed the susceptibility of the virus to that

medication (15). This requires that the minimum drug concentrations exceed the inhibitory concentrations for particular strains of HIV virus (wild type, resistant type) (37). Steady state PKPD and efficacy relationships show that trough concentrations (C_{min}) of APV are good predictors of a decrease in viral load (5). In this study, the minimum APV target trough concentration for wild type virus of 0.4 µg/mL was exceeded by 87.5% (7/8) in the 2nd trimester, 96.4% (27/28) in the 3rd trimester, and 95.5% (21/22) postpartum – ***Figure 2***. Also, the trough concentrations (C_{min}) of APV were 4-16 fold above the mean APV protein-adjusted IC_{50} of 0.146µg/mL(5, 31) for wild-type HIV-1 virus – ***Table 2***. The 10th percentile median AUC for RTV-boosted FPV in adults on twice daily FPV/RTV, 700/100 mg (27.7 µg*hr/mL) was exceeded by 100% (8/8) in the second trimester, 92.9% (26/28) in the third trimester, and 100% (22/22) postpartum – ***Table 2***. In addition, using a cut off value of ≤75 copies/mL versus >75 copies/mL for undetectable viral load, we were not able to identify any statistically significant associations between drug exposure and viral load suppression (***Table 4***), although this was most likely due to the small sample size and lack of statistical power. Many women met the minimum trough concentrations during the second and third trimesters of pregnancy, as well as postpartum, suggesting that reductions in RTV-boosted APV exposures were not clinically significant.

While our current findings suggest that use of FPV/RTV 700mg/100mg twice daily in pregnant women do not provide comparable exposure to that of non-

pregnant adults, a dose adjustment may not be necessary as the majority of women fell above the 10th percentile AUC and had trough levels greater than 0.4 µg/mL. Those pregnant women whose APV troughs fell below this target may have an inadequate virologic response, so close monitoring of viral load in pregnant women receiving FPV is warranted. No participant in our study received an increased dose of FPV, so our data provide no information on the impact of dose adjustment on APV exposures during pregnancy. APV exposure may also be increased by increasing the RTV dose. Increased plasma RTV could provide a higher exposure to the boosted PI, slowing down metabolism of APV and increasing minimum trough concentrations, half-life and AUC values while minimizing adverse effects by concurrently decreasing the time to maximum plasma concentration (T_{max}) and C_{max}. However, RTV is often not well-tolerated due to gastrointestinal side effects, possibly limiting enthusiasm for using an increased dose in pregnant women.

Our study has several strengths. First, pregnant patients in the FPV arm of the IMPAACT 1026s study were followed in a longitudinal pattern throughout pregnancy and postpartum, during which evaluation of clinical findings related to FPV exposure occurred at regular time intervals. Second, because this was a prospective cohort study, confounding, recall and selection biases were minimized. Third, within-participant comparisons (second or third trimester versus postpartum) reduced concerns about heterogeneity during this PK study. Fourth, another strength of this study is the sample size - 29

participants. Fifth, complete PK data were available for 96.5% (28/29) participants evaluated in the third trimester of pregnancy and for 76% (22/29) evaluated postpartum.

This study had its limitations. First, this is an observational PK/safety study of a heterogeneous group of pregnant women receiving FPV/RTV for clinical care. There was variation in their background characteristics, and pregnant women who began FPV/RTV but did not tolerate it or demonstrated inadequate initial efficacy would have been taken off drug and not be eligible for the study. Second, we did not assess the pharmacogenomic relationship between FPV/RTV dosing and genetic resistance to HIV in pregnancy.

In conclusion, our findings confirm that RTV-boosted FPV exposure is decreased during pregnancy. Although exposure was lower during pregnancy, few women were found to have a trough level below the recommended trough level of 0.4 µg/mL, and the majority of women met the 10th percentile AUC of 27.7µg*hr/L during the second and third trimesters of pregnancy and postpartum. Most participants achieved a viral load <75 copies/mL, further suggesting adequate viral suppression despite decreased ARV exposure. However, our sample size was small, and further investigation of methods to achieve APV exposure during pregnancy equivalent to that in non-pregnant adults, such as increasing the ritonavir dose, is warranted.

MATERIALS & METHODS:

The study protocol, the informed consent documents, and all subsequent modifications were reviewed and approved by the local institutional review board (IRB)/Ethics Committee responsible for oversight of the study. The study followed all relevant human subject research guidelines. All participants provided signed informed consent before participation, and the study was registered in ClinicalTrials.gov [NCT00042289]. This study was done as part of the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) network P1026s, "*Pharmacokinetic Properties of Antiretroviral and Related Drugs during Pregnancy and Postpartum*" (ClinicalTrials.gov NCT00042289), an ongoing, multicenter, non-blinded, prospective Phase IV study of the PK and safety of selected ARVs in women living with HIV. The study included an arm for pregnant women receiving FPV 700 mg with ritonavir 100 mg twice daily.

Pregnant women living with HIV were eligible for enrolment if they were receiving 700mg/100mg FPV/RTV as part of clinical care for at least two weeks, and planned to continue the regimen. Exclusion criteria were concurrent use of medications known to interfere with the absorption, metabolism, or clearance of FPV or RTV, including multiple gestation and clinical or laboratory toxicity that, in the opinion of the site investigator, would likely require a change in the medication regimen during the study. Medications were prescribed by the participant's healthcare provider who

remained responsible for her clinical management throughout the study. Participants continued on study until the completion of postpartum PK sampling. For women enrolling during the second trimester of pregnancy, APV PK were determined in real time between 20 and 26 weeks gestation and repeated between 30 and 36 weeks gestation. Women enrolling in the third trimester had PK sampling performed between 30 and 36 weeks gestation. PK sampling was repeated between 6 and 12 weeks postpartum. Infants were enrolled at the same time as their mothers with maternal consent. An infant was considered HIV-negative if at least two nucleic acid tests were negative with one after 1 month and the other after 4 months of age. An infant was considered HIV-infection indeterminate if nucleic acid tests were negative but were not sufficient to meet the definitively negative criterion (i.e. two negative nucleic acid tests with one after 1 month and the other after 4 months of age), often because of withdrawal from the study before age 4 months.

Clinical and Laboratory Monitoring:

Maternal data obtained for this analysis were maternal age, ethnicity, weight, concomitant medications, CD4 and plasma viral load assay results. Plasma viral load assays were done locally and had lower limits of detection as high as 75 copies per milliliter, so all viral load measurements of 75 copies per milliliter or less were set to 75 copies per milliliter for data analyses. Maternal clinical and laboratory toxicities were assessed through clinical

evaluations (history and physical examination) and laboratory assays (alanine aminotransferase, aspartate aminotransferase, creatinine, BUN, albumin, bilirubin, hemoglobin) on each PK sampling day and at delivery. Infant data included birth weight, gestational age at birth, and HIV status, if available. The study team reviewed toxicity reports on monthly conference calls, although the participant's provider was responsible for clinical management. The Division of AIDS (DAIDS)/National Institute of Allergy and Infectious Diseases Toxicity Table for Grading Severity of Adult Adverse Experiences were used to report adverse events for study participants (38). All toxicities were followed through resolution.

Sample Collection and drug assays:

Participants were stable on their ARV regimen for at least 2 weeks before PK sampling. Seven plasma samples were drawn at the second trimester, third trimester, and postpartum PK evaluation visits, starting immediately before an oral FPV/RTV dose and at 1, 2, 4, 6, 8, 12 hours post-dose. Fosamprenavir/ritonavir were given as an observed dose. Other information collected included the time of the two prior doses, the two most recent meals, maternal height and weight. A single maternal plasma sample and an umbilical cord sample after the cord was clamped were collected at delivery. The University of California, San Diego (UCSD) Pediatric Clinical Pharmacology Laboratory, using a validated, reversed-phase multiplex high-performance liquid chromatography method, measured APV and RTV. The

lower limit of quantitation was 0.047 mcg/mL for APV and 0.094 mcg/mL for RTV. The University of California, San Diego, laboratory has been enrolled in the AIDS Clinical Trials Group Quality Assurance/Quality Control Proficiency Testing Program since 2001, which performs standardized inter-laboratory testing twice a year.

Pharmacokinetic and statistical analyses:

The maximum plasma concentration (C_{max}), minimum plasma concentration (C_{min}), and 12-hour post-dose concentration (C_{12}) were determined by direct inspection. For concentrations below the assay limit of detection, a value of one-half of the detection limit (0.024 mcg/mL for amprenavir and 0.047 mcg/mL for ritonavir) was used in summary calculations. AUC_{0-12} during the dosing interval (from time 0 to 12 hours post-dose) for APV and RTV were estimated using the trapezoidal rule. Apparent oral clearance (CL/F) from plasma was calculated as dose divided by AUC_{0-12}. The terminal slope of the curve (λ_z) was estimated from the last two measurable and declining concentrations between 6 and 12 hours post-dose. Half-life was calculated as dose divided by λ_z, and apparent volume of distribution (Vd/F) was determined by CL/F divided by λ_z. Amprenavir AUC_{0-12} was calculated for each woman, and compared with APV AUC_{0-12} in non-pregnant adults. Each participant's provider was notified of the participant's plasma concentrations and AUC_{0-12} within 2 weeks. If the APV AUC_{0-12} was below the target of 27.7 µg*hr/mL (the 10th percentile in non-pregnant adult populations), the provider was offered

the option of discussing the results and possible dose modifications with a study team pharmacologist.

Within-participant comparisons (second or third trimester versus postpartum) were performed for continuous outcome measures using the Wilcoxon signed-rank test and for dichotomous outcome measures using the McNemar's test, with $p<0.1$ considered statistically significant. 90% confidence limits for the geometric mean ratio of the PK exposure parameters were calculated to describe the range of values that were consistent with the observed data to assess whether there was a clinically significant difference in exposure. Data analysis was done using SAS (version 9.4, SAS Institute, Cary NC).

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Table 1: Demographic Characteristics and Outcomes (n=29)

Maternal characteristics	N(%) or median (Interquartile Range)
Age at delivery (years)	30.8 (25.4, 35.1)
Weight at delivery (kg)	83.0 (77.7, 99.0)
Race/Ethnicity Black Non-Hispanic Hispanic (Regardless of Race) White Non-Hispanic Asian, Pacific Islander Country Argentina Brazil USA	 12 (41%) 15 (52%) 1 (3%) 1 (3%) 2 (7%) 6 (21%) 21 (72%)
Second Trimester PK Evaluation	
Gestational age (weeks)	24.6 (21.2, 25.6)
Duration of FPV before PK evaluations (weeks)	7.2 (5.1, 63.1)
HIV-1 RNA in copies/mL (median)	159.0 (44.0, 627.5)
Number of mothers with viral load ≤75 copies/mL	3 (38%)
CD4 (cells/mm^3)	484.5 (417.5, 571.0)
Third Trimester PK Evaluation	
Gestational age (weeks)	32.7 (31.6, 35.0)
Duration of FPV before PK evaluations (weeks)	19.2 (10.7, 101.7)
HIV-1 RNA in copies/mL (median)	50.0 (48.0, 120.0)
Number of mothers with viral load ≤75	19 (70%)

copies/mL	
CD4 (cells/mm^3)	491.0 (356.0, 635.0)
At Delivery	
HIV-1 RNA in copies/mL (median)	50.0 (48.0, 77.5)
Gestational age (weeks)	38.7 (37.9, 39.4)
Number of mothers with viral load ≤75 copies/mL	21 (75%)
CD4 (cells/mm^3)	491.0 (358.0, 699.0)
Postpartum PK Evaluation	
Weeks post-delivery (weeks)	6.7 (6.0, 9.9)
HIV-1 RNA in 50 copies/mL (median)	50.0 (48.0, 56.0)
Number of mothers with viral load ≤75 copies/mL	13 (76%)
CD4 (cells/mm^3)	590.0 (394.0, 794.0)
Pregnancy outcomes	
Birth weight (grams)	3237.5 (2935.0, 3477.9)
Infant infection status	26 (90%) uninfected; 2 (7%) indeterminate[#]; 1 (3%) pending based on available data.

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473*[#]An infant was considered HIV-infection indeterminate if nucleic acid tests*

474*were negative but were not sufficient to meet the definitively negative*

475*criterion (i.e. two negative nucleic acid tests with one after 1 month and the*

476*other after 4 months of age), often because of withdrawal from the study*

477*before age 4 months.*

Table 2: Amprenavir (APV) Pharmacokinetics Comparison of 2nd Trimester (N=8) versus Postpartum (N=22); and 3rd Trimester (N=28) versus Postpartum (N=22)

PK Parameter	Second trimester (2T) median (IQR) (n=8)	Third trimester (3T) median (IQR) (n=28)	Postpartum (PP) median (IQR) (n=22)	Geometric mean Ratio, GMR [90% CI]; 2T/PP	p-value	Geometric mean Ratio, GMR [90% CI]; 3T/PP	p-value
APV AUC_{0-12} (µg*hr/mL)	43.50 (38.50, 50.40)	32.15 (21.45, 39.70)	51.60 (45.20, 59.60)	0.68 [0.44, 1.04]	0.22	0.60 [0.49, 0.72]	**<0.001**
APV CL/F (L/hr)	13.79 (11.91, 15.58)	18.66 (15.11, 28.23)	11.63 (10.07, 13.27)	1.48 [0.96, 2.27]	0.22	1.68 [1.38, 2.03]	**<0.001**
APV $T_{1/2}$ (hours)	8.67 (5.90, 13.57)	12.98 (8.50, 31.62)	14.26 (8.22, 28.25)	0.41 [0.009, 17.97]	1.00	1.02 [0.42, 2.49]	0.637
APV C_{min} (µg/mL)	1.91 (0.34, 2.39)	1.48 (0.86, 1.80)	2.42 (1.36, 3.08)	0.37 [0.10, 1.40]	0.16	0.97 [0.55, 1.71]	**0.01**
APV C_{last} (µg/mL)	2.05 (1.56, 2.65)	1.67 (1.13, 2.24)	2.80 (1.93, 3.82)	0.39 [0.10, 1.54]	0.22	0.60 [0.45, 0.81]	**0.004**
APV C_{max} (µg/mL)	5.61 (4.47, 6.64)	5.12 (3.60, 6.26)	6.75 (4.31, 9.24)	0.83 [0.56, 1.22]	0.16	0.74 [0.58, 0.93]	**0.03**
APV C_0 (µg/mL)	2.19 (1.05, 3.13)	1.70 (1.34, 2.28)	3.14 (1.56, 4.94)	0.71 [0.40, 1.27]	0.47	0.91 [0.50, 1.65]	**<0.001**
APV C_{12} (µg/mL)	2.12 (1.39, 2.67)	1.64 (1.16, 2.21)	2.87 (2.34, 3.41)	0.48 [0.14, 1.65]	0.44	0.56 [0.43, 0.72]	**<0.001**

*p-value for Wilcoxon rank-sum test; IQR = interquartile range, AUC_{0-12} = area under concentration (AUC) vs time curve (0 to 12 hours post-dose); Cl/F = apparent oral clearance; C_{min}= minimum concentration; C_{max}= maximum concentration; $T_{1/2}$ = elimination half-life; Clast = last observed quantifiable concentration; C_0 = initial concentration at time zero; C_{12} = concentration at 12 hours post-dose.

Table 3: Ritonavir (RTV) Pharmacokinetics Comparison of 2nd Trimester (N=8) versus Postpartum (N=22); and 3rd Trimester (N=28) versus Postpartum (N=22)

PK Parameter	Second trimester (2T) Median (IQR) (n=8)	Third trimester (3T) Median (IQR) (n=28)	Postpartum (PP) Median (IQR) (n=22)	Geometric mean Ratio, GMR [90% CI]; 2T/PP	p-value	Geometric mean Ratio, GMR [90% CI]; 3T/PP	p-value
RTV AUC_{0-12} (µg*hr/mL)	2.52 (1.35, 4.10)	3.68 (2.76, 5.64)	4.86 (2.73, 6.60)	0.51 [0.28, 0.91]	**0.09**	0.72 [0.55, 0.95]	**0.005**
RTV CL/F (L/hr)	47.16 (24.61, 74.15)	27.20 (17.74, 36.22)	20.63 (15.15, 36.72)	1.98 [1.10, 3.56]	**0.06**	1.38 [1.05, 1.82]	**0.009**
RTV $T_{1/2}$ (hours)	3.71 (3.06, 10.92)	4.08 (3.47, 6.65)	4.92 (3.03, 6.64)	1.05 [0.18, 6.14]	1.00	1.45 [0.57, 3.69]	0.520
RTV C_{min} (µg/mL)	0.07 (0.05, 0.12)	0.12 (0.05, 0.15)	0.10 (0.05, 0.20)	0.49 [0.24, 0.99]	**0.08**	1.08 [0.87,	0.720

						1.33]	
RTV C_{last} (µg/mL)	0.09 (0.06, 0.16)	0.13 (0.06, 0.24)	0.18 (0.08, 0.26)	0.45 [0.20, 1.03]	**0.08**	0.83 [0.63, 1.10]	0.250
RTV C_{max} (µg/mL)	0.41 (0.25, 0.73)	0.64 (0.51, 1.07)	0.77 (0.51, 1.08)	0.65 [0.37, 1.12]	0.30	0.83 [0.61, 1.11]	0.475
RTV C_0 (µg/mL)	0.13 (0.06, 0.24)	0.16 (0.11, 0.31)	0.19 (0.09, 0.41)	0.59 [0.31, 1.10]	0.30	0.87 [0.62, 1.22]	0.134
RTV C_{12} (µg/mL)	0.12 (0.05, 0.29)	0.16 (0.05, 0.20)	0.19 (0.08, 0.26)	0.71 [0.24, 2.14]	0.69	0.70 [0.53, 0.91]	**0.029**

*p-value for Wilcoxon rank-sum test; IQR = interquartile range, AUC_{0-12} = area under concentration (AUC) vs time curve (0 to 12 hours post-dose); Cl/F = apparent oral clearance; C_{min}= minimum concentration; C_{max}= maximum concentration; $T_{1/2}$ = elimination half-life; Clast = last observed quantifiable concentration; C_0 = initial concentration at time zero; C_{12} = concentration at 12 hours post-dose.

Table 4: Third Trimester APV and RTV PK Parameters by Viral Load Category

PK parameter	**Amprenavir (APV)**				**Ritonavir (RTV)**			
	Viral load ≤ 75 (N=19)	**Viral load >75 (N=8)**	**Total (N=27)**	**P - value ***	**Viral load ≤ 75 (N=19)**	**Viral load >75 (N=8)**	**Total (N=27)**	**p-value***
AUC_{0-12} (µg*hr/mL)**	30.4 (17.3, 39.6)	33.3 (31.4, 38.9)	32.1 (19.4, 39.6)	0.159	3.36 (1.85, 6.19)	3.68 (3.37, 5.38)	3.61 (2.76, 5.64)	0.580

>AUC_{0-12} median Yes No	 7 (54%) 12 (86%)	 6 (46%) 2 (14%)	 13 14	0.103	 8 (67%) 11 (73%)	 4 (33%) 4 (27%)	 12 15	1.000
C_0 (µg/mL)**	1.84 (1.03, 2.51)	1.58 (1.41, 2.16)	1.76 (1.26, 2.28)	0.577	0.18 (0.10, 0.34)	0.13 (0.12, 0.18)	0.16 (0.11, 0.31)	0.441
>C_0 median Yes No	 11 (79%) 8 (62%)	 3 (21%) 5 (38%)	 14 13	0.420	 11 (79%) 8 (62%)	 3 (21%) 5 (38%)	 14 13	0.420
C_{min} (µg/mL)**	1.29 (0.74, 2.20)	1.48 (1.32, 1.72)	1.42 (0.85, 1.83)	0.958	0.13 (0.05, 0.16)	0.12 (0.09, 0.15)	0.12 (0.05, 0.16)	0.872
>C_{min} median Yes No	 9 (69%) 10 (71%)	 4 (31%) 4 (29%)	 13 14	1.000	 10 (71%) 9 (69%)	 4 (29%) 4 (31%)	 14 13	1.000

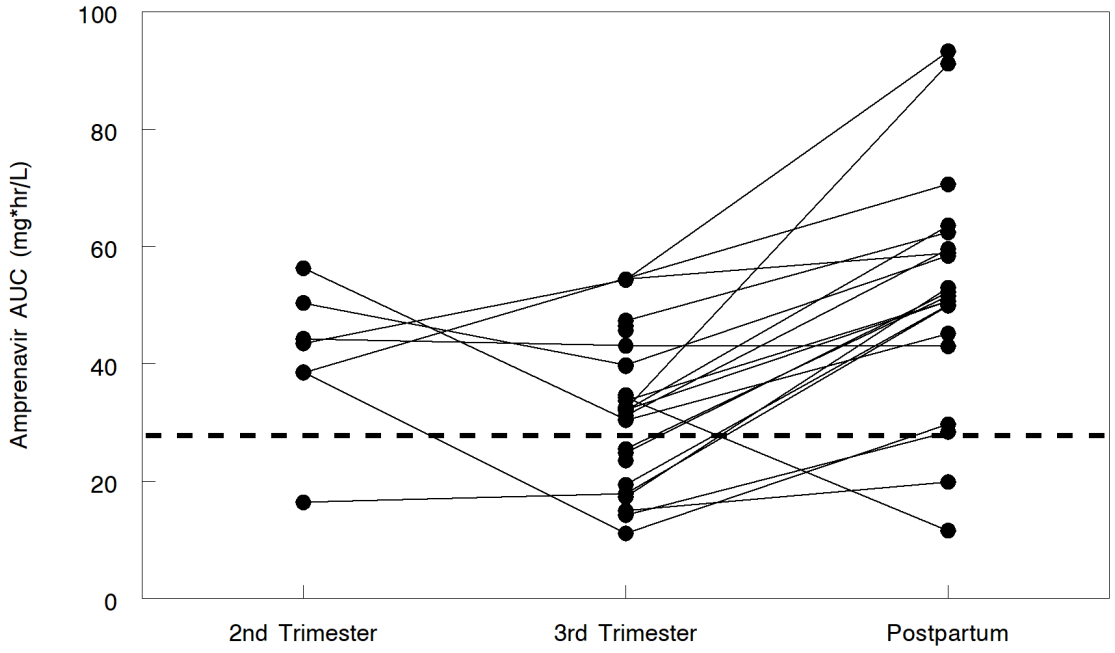
*Fishers exact test for categorized PK parameters, and Wilcoxon rank sum test for continuous PK parameters;

**AUC_{0-12} = area under concentration (AUC) vs time curve (0 to 12 hours post-dose); C_{min}= minimum (trough) drug concentration; C_0 = initial drug concentration at time zero. Median (IQR) is shown in the table.

<u>Table 5:</u> Number of Mothers Taking Each ARV at the Time of PK Evaluations.

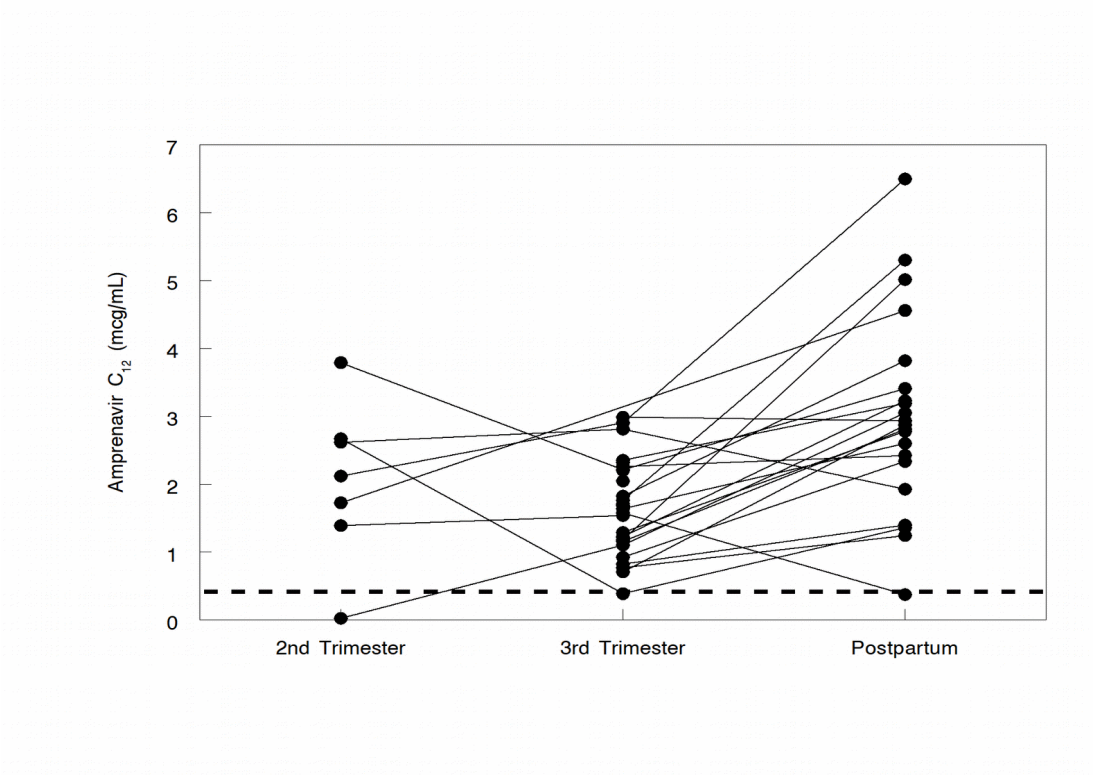
Drug	Number of mothers taking ARVs at the 2nd trimester PK visit	Number of mothers taking ARVs at the 3rd trimester PK visit
3TC (Lamivudine)	4	15
ABC (Abacavir)	0	2
DDI (Didanosine)	0	1
FPV (Fosamprenavir)	8	28
FTC (Emtricitabine)	4	12
NVP (Nevirapine)	0	1
RTV (Ritonavir)	8	27
TDF (Tenofovir disoproxil fumarate)	4	15
ZDV (Zidovudine)	4	14

Figure 1: Amprenavir AUC in women during the 2nd and 3rd trimester and postpartum.



*The estimated 10th percentile for the AUC of amprenavir after FPV/RTV 700/100 mg twice daily dosing is 27.7 mcg*hr/mL (represented by dashed line). One, ten, and two women fell below 10th percentile line in the second and third trimesters and postpartum states respectively.*

Figure 2: Amprenavir trough concentration at 12 hours in women during the 2nd and 3rd trimester and postpartum.



Dashed line represents 0.4mcg/mL, the minimum target trough concentration for wild type virus. One woman had a trough below this level at each evaluation period (2nd trimester, 3rd trimester, and postpartum).