IMPAACT P1026s

Primary Statistical Analysis Plan

Version 1.0

13 September 2021

PHARMACOKINETIC PROPERTIES OF ANTIRETROVIRAL AND RELATED DRUGS DURING PREGNANCY AND POSTPARTUM

ClinicalTrials.gov Identifier: NCT00042289

Protocol Version 10.0 (LOA #1 and LOA #2)

This is IMPAACT P1026s SAP Version 1.0 with names of authors, names of publication writing team members, and analysis timeline redacted.

Statistical and Data Analysis Center
Harvard T.H. Chan School of Public Health

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Version History

Version	Changes Made	Date Finalized
1.0	Original Version	*13 September 2021*

Note: From the beginning of the study in 2003, statisticians have followed the analysis plan in the Statistical Considerations section of the protocol, and this separate Primary Statistical Analysis Plan is created for submission to ClinicalTrials.gov.

1 Introduction

1.1 Purpose

This Primary Statistical Analysis Plan (SAP) describes the primary and key secondary outcome measures and other secondary outcome measures that will address specific study objectives of IMPAACT P1026s. The Primary SAP includes general analytic approaches for all primary outcome measures, key secondary outcome measures, and other outcome measures in the primary manuscript(s) or submitted to ClinicalTrials.gov (regardless of the reporting timeline). The Primary SAP facilitates discussion of the statistical analysis components among the lead study investigators and statisticians, helping them agree on the statistical analyses to be performed and presented in the primary analysis report.

If needed, a separate SAP will provide outlines of analyses for other objectives and outcome measures not included in the Primary SAP.

1.2 Version History

Not applicable; original version.

2 Study Overview

2.1 Overview of Study Design

P1026s is a Phase IV, prospective pharmacokinetic (PK) study to evaluate the pharmacokinetics of selected currently prescribed antiretroviral (ARV) medicines when used alone or with tuberculosis (TB) medicines during pregnancy or with postpartum hormonal contraceptives in HIV-infected pregnant and postpartum women. The washout PK of transplacentally acquired ARV and TB drugs will be studied in the infants born to study mothers who enter the study during pregnancy. HIV-uninfected pregnant women receiving TB treatment (first line or second line) will also be enrolled to serve as a control group to evaluate the interaction between TB and ARV drugs in pregnant women.

Study Arm Groups (see arm details in Section 7 Attachment 1 and Protocol Schema):

- ARVs without TB Treatment (10 Arms)
- ARVs with First Line TB Treatment (2 Arms - one for each ARV drug)
- No ARVs with First Line Tuberculosis Treatment (1 Arm)
- Second Line TB Treatment for Drug-Resistant TB with or without ARVs (1 Arm)
- ARVs with Postpartum Contraceptives (4 Arms)

There is no randomization and stratification. Each study arm will enroll a minimum of 12 women and have a target enrollment of 25 women with evaluable 3rd trimester PK data in the ARV and TB arms, or evaluable postpartum PK data in the contraception arms. Enrollment may be restricted or adjusted so that 2nd trimester PK data are collected from at least 12 women. When combined enrollment in the increased dose darunavir arms reach 25 participants, the PK data will be evaluated to determine if the data for these arms can be combined and whether enrollment of additional participants in these arms should continue. Sample size for an ARV or TB drug arm may be increased if additional infants need to be enrolled to collect infant washout PK data. The

sample size of the second line TB drug arm may be increased above 25 to obtain additional data for less commonly used second line TB drugs.

Eligible women can enroll between 20 0/7 -37 6/7 weeks gestation or 2-12 weeks postpartum, depending on study arm. All infants of mothers enrolled during pregnancy will be enrolled in utero, after maternal enrollment. Enrolled infants who meet the requirements for washout PK sampling will undergo PK sampling. HIV-infected pregnant women without TB will be followed for 6 – 12 weeks after delivery with the exception of women on DRV/r who will be followed for 2 – 3 weeks after delivery. HIV-infected and non-infected women receiving TB treatment (first or second line) will be followed for 2 – 8 weeks after delivery. Postpartum women will be followed until 6 – 7 weeks after the initiation of hormonal contraceptives. Infants will be followed for 16 – 24 weeks of life.

Analyses for the Final Analysis Report for each study arm will be done once the last participant in that arm has completed all study visits, laboratory assays have been completed, and all queries have been resolved.

2.2 Hypotheses

There is no hypothesis specified in the protocol.

2.3 Study Objectives and Outcome Measures

This Primary SAP addresses the following primary and secondary objectives in the study protocol. Some secondary objectives were intended to be exploratory, and will be considered as "Other study objectives". Therefore, this SAP will not include the outcome measures/analysis contents related to these exploratory objectives (see Section 2.3.2).

The study objectives below will be analyzed under an equivalence framework, including the comparisons between antepartum and postpartum PK parameters. The final analysis for each study arm will be done once the last participant for that arm has completed the last study visit and all queries have been resolved.

2.3.1 Primary Objectives and Outcome Measures

Arms for which the following objectives and outcome measures apply are shown in **bold**.

2.3.1.1 **(For arms in the ARVs without TB Treatment study arm group)**

Objective:

To describe the pharmacokinetic parameters during pregnancy of selected ARV drugs currently used in the clinical care of HIV-infected pregnant women, and to compare these parameters to a) historical pharmacokinetic data from non-pregnant women and b) postpartum pharmacokinetic data from the same women in the study cohorts.

Outcome measure: a) # of women who met area under the curve (AUC) target of ARVs in 2nd trimester (2T), 3rd trimester (3T) and postpartum (PP) (see PK target in Protocol Appendix V); b) AUC, C_{max} and C_{trough} of ARVs in 2T, 3T and PP

2.3.1.2 **(For arms in the ARVs with First Line TB Treatment arms or No ARVs with First Line TB Treatment study arm groups)**

Objective:

To describe the pharmacokinetic parameters during pregnancy and postpartum of selected ARV drugs (efavirenz, lopinavir/ritonavir) and first line TB drugs when co-administered as part of clinical care of HIV-infected pregnant women and of first line TB drugs when used in HIV-uninfected pregnant women.

Outcome measure: a) # of women who met the AUC target for ARVs and the C_{max} target for first line TB drugs in 2T, 3T and PP (see PK targets in Protocol Appendix V); b) ARV and first line TB drug AUC, C_{max} and C_{trough} in 2T, 3T and PP

2.3.1.3 **(For arms in the Second Line TB Treatment for Drug-Resistant TB with or without ARVs study arm group**)

Objective:

To describe the pharmacokinetic parameters during pregnancy and postpartum of second line TB drugs administered as part of clinical care to HIV-infected and HIV-uninfected pregnant women.

Outcome measure: Second line TB drug AUC, C_{max} and C_{trough} in 2T, 3T and PP

2.3.1.4 **(For arms in the ARVs with Postpartum Contraceptives study arm group)**

Objective:

To describe the pharmacokinetic parameters of ARV drugs in postpartum women before and after starting hormonal contraceptives.

Outcome measure: AUC of ARVs before and after initiation of contraceptives (2-12 weeks postpartum and 6-7 weeks after contraceptive initiation, respectively).

2.3.1.5 **(For arms in the ARVs with Postpartum Contraceptives study arm group)**

Objective:

To describe the concentrations of ethinyl estradiol, etonogestrel and other progestins in women using hormonal contraceptives and selected ARV drugs as compared to historical controls not using those ARV drugs.

Outcome measure: Plasma concentrations of contraceptives.

Note: No historical controls were provided by team pharmacologists and no analysis was done for this comparison. This will be noted in the ClinicalTrials.gov description of the analysis.

2.3.2 Secondary Objectives and Outcome Measures

2.3.2.1 **(For arms in the ARVs without TB Treatment, ARVs with First Line TB Treatment, or Second Line TB Treatment for Drug-Resistant TB with or without ARVs study arm groups).**

Objective:

To compare ARV and TB drug concentrations in plasma from cord blood with those in maternal plasma at the time of delivery.

Outcome measure: ARV and TB drug cord blood/maternal plasma concentration ratio at delivery

2.3.2.2 To assess plasma protein binding of highly bound ARV drugs during pregnancy and postpartum*****.

2.3.2.3 To assess ARV concentrations and HIV RNA/DNA concentrations in vaginal secretions among pregnant and postpartum and compare to simultaneous plasma concentrations*****.

2.3.2.4 To explore genetic sources for variability in ARV and TB drug exposure in pregnant women and their infants*****.

2.3.2.5 **(For all study arms)**.

Objective:

To describe maternal and infant safety and clinical outcomes.*

Outcome measure: N (%) of:

1) Adverse events (AE) of grade 3 or higher;

2) Infant neurological events of grade 1 or higher;

3) Adverse pregnancy outcomes including preterm birth, low birth weight, and fetal demise;

4) Congenital anomalies;

5) Infant HIV infection status as defined according to the most current IMPAACT definition

2.3.2.6 **(For arms in the ARVs without TB Treatment, ARVs with First Line TB Treatment, or Second Line TB Treatment for Drug-Resistant TB with or without ARVs study arm groups).**

Objective:

To describe the neonatal elimination of selected ARV and TB drugs acquired across the placenta after maternal dosing during pregnancy.

Outcome measure: Infant washout half-life and concentration of drug after birth (if infants meet the criteria specified in Protocol Section 4.4 for washout PK sampling)

2.3.2.7 To describe pharmacokinetics of ARV drug combinations in HIV-infected women on second line TB treatment*****.

***** Note: These 5 secondary objectives were intended to be exploratory, and will be considered as "Other study objectives". Therefore, this SAP will not include the outcome measures/analysis contents related to these objectives.

2.4 Overview of Sample Size Considerations

The sample size calculations will be based on assessing whether therapeutic dosing regimens for the study drugs produce adequate drug exposure during pregnancy as compared to historical data from non-pregnant adults.

Each study arm will enroll a minimum of 12 women and have a target enrollment of 25 women with evaluable 3rd trimester PK data for ARV and TB arms, or evaluable postpartum PK data for oral contraceptive arms. Women who do not have evaluable data will be replaced. Enrollment may be restricted or adjusted so that evaluable 2nd trimester PK data are obtained from at least 12 women. The sample size for an arm may also be increased if additional mothers and infants need to be enrolled to obtain evaluable infant washout PK data. Thus, the total number of participants enrolled per arm may be larger than 25 if women need to be replaced due to non-evaluable data, if additional women need to be enrolled to obtain 2nd trimester PK data, if additional mothers and infants need to be enrolled to obtain infant washout PK data and/or if additional women need to be enrolled to obtain enough second line TB drug data. When combined enrollment in the increased dose darunavir arms reaches 25 participants, the PK data will be evaluated to determine if the data for these arms can be combined and whether enrollment of additional participants in these arms should continue. The actual sample size will be based on the determination of PK exposure parameters, and whether or not the current dose provides adequate drug exposure to pregnant women.

2.5 Overview of Formal Interim Monitoring

Interim PK exposure monitoring during pregnancy (for ARVs only):

Based on data regarding the ARV drugs presently under study, the major concern is that the ARV drug exposure in pregnant women will be lower than that of the non-pregnant population, and the interim PK exposure monitoring rules are designed to detect this scenario quickly. In each study arm, at any time after a minimum of 12 participants have been enrolled, if six or more pregnant women have the third- trimester PK exposure parameter below the 10th percentile for the non-pregnant population, the study team will evaluate the adequacy of drug exposure in that arm, and with the agreement of the medical officers, determine whether enrollment to that arm should continue. The exact 80% confidence limits for 6 participants with low drug exposure out of a total of 25 are (0.13, 0.38), which exclude 10% and indicate strong evidence that the distribution of the PK parameter of interest for the pregnant women is different from that for the non-pregnant women. If a statistical difference appears to be clinically important, then enrollment into that study arm will stop and a new study arm at a higher dose will be developed, if feasible.

3 General Considerations

Each analysis will include all participants with evaluable data for that analysis. A participant's data will be deemed unevaluable by a protocol pharmacologist for a specific analysis if they do not have adequate pharmacokinetic evaluations to determine the pharmacokinetic exposure parameter of interest for that analysis. For safety analyses, a participant's data will be deemed unevaluable only if the participant did not receive any amount of the drug under study.

Visit windows are defined in Protocol Appendix IA-ID and Appendix II for mothers and infants, respectively. Specifically, maternal HIV RNA and CD4 tests for each PK visit allow a window of ± 8 weeks around the PK evaluation date. For mothers of non-contraceptive arms, entry visit (which may occur at the 2P or 3P visit) is the baseline; for mothers of contraceptive arms, entry visit (2-12 weeks postpartum) is the baseline. For infants, day of birth is defined as Day 0; birth visit is the baseline.

Continuous variables (e.g. gestational age) will be summarized with descriptive statistics (mean, median, standard deviation, interquartile range, and range). Categorical data (e.g. sex) will be summarized with frequencies and percentages for each category. Reports may also include listings with details related to safety events.

In this study, each arm will enroll independently. Final analysis for each arm will be done when that arm completes follow-up and PK assays have been run. Each study arm will have a separate Final Analysis Report (unless the team decides to combine analyses across certain arms).

4 Analysis Approaches

For the PK related objectives, this SAP defines the descriptive PK data summaries and PK comparisons to be done by the protocol statisticians. The pharmacokinetic parameters will be calculated by the protocol pharmacologists, and their derivation/analysis will be defined in a separate Pharmacokinetic SAP.

If PK concentration values are missing due to below the level of quantification (BLQ), they will be set to ½ the lower limit of quantitation (LLOQ) for calculations. If an entire concentration-time profile is BLQ, the profile will be reported separately and excluded from the summary PK analysis. The comparisons to be performed to address the primary objectives for certain arms will vary according to type of drug and timing of sampling. Table 1 summarizes these comparisons for each type of drug.

Table 1: Pharmacokinetic Parameter Comparisons to be Performed for Each Type of Drug for the Primary Objectives

Type of Drug	**Analysis Stage**	**Description of Pharmacokinetic Parameter Comparison**	**Type of Comparison**
ARV without TB Drugs	Stage 1	Antepartum PK parameter vs. 10th percentile from external, non-pregnant population	Between-participant
	Stage 2	Antepartum vs. postpartum PK parameters	Within-participant
ARV with First Line TB Drugs	Stage 1	Antepartum ARV PK parameter vs. 10th percentile from non-pregnant population	Between-participant
	Stage 2	Antepartum vs. postpartum ARV PK parameters	Within-participant
	Stage 3	ARV PK parameter with TB drugs vs. ARV PK parameter without TB drugs in previous P1026s arm	Between-participant
First Line TB drugs with ARVs (HIV+) and First Line TB drugs without ARVs (HIV-)	Stage 1*	Antepartum TB drug PK parameter vs. 10th percentile from non-pregnant population	Between-participant
	Stage 2	Antepartum vs. postpartum TB drug PK parameter, separately for HIV+ and HIV-	Within-participant**
	Stage 3	TB drug PK parameter with ARV (HIV+) vs. TB drug PK parameter without ARV (HIV-)	Between-participant
Second Line TB drugs with or without ARVs	Stage 2	Antepartum vs. postpartum TB drug PK parameters	Within-participant**
ARV with Contraceptives#	Stage 2	Postpartum ARV PK parameter before vs. after starting contraceptives	Within-participant
Contraceptives with ARVs	Stage 3	Postpartum contraceptive concentration with ARV vs. external historical controls not receiving ARV	Between-participant

*Stage 1 analysis for the TB drugs will not be done in real time and will only be done when batched PK data are available (unlike the Stage I analysis for ARVs).
**This comparison may have a smaller sample size than 25 because some women may complete their TB treatment before the postpartum PK visit.
#Interim AUC monitoring (Stage 1 analysis) is not done for these arms (only done during pregnancy).

In Stage I, an individual woman's PK exposure parameter during pregnancy will be compared in real time to the 10th percentile for that PK exposure parameter for that drug from a non-pregnant population, and the woman and her clinical provider will be notified if the woman's value is below the 10th percentile. If there is strong evidence that the true percentage of pregnant women having the PK exposure parameter below the 10th percentile for the non-pregnant population is greater than 10%, the study team will evaluate whether to stop enrollment early and whether to recommend that additional study of the drug/drug combination is necessary.

Stage 2 for the ARV drugs taken during pregnancy will implement a repeated measures design, in which antepartum and postpartum measurements from each woman will be compared. This stage requires a minimum of 12 participants in each arm to have postpartum PK measurements. If less than 12 participants out of the 25 in any arm have postpartum measurements, more participants will be enrolled in that arm until the minimum postpartum measurement number of 12

is reached. The comparisons will be made at the within-participant level, using the Wilcoxon signed rank test to assess whether there is a statistically significant difference in the PK exposure parameter in the pregnant versus non-pregnant conditions. 90% confidence limits for the geometric mean ratio of the PK exposure parameter in the pregnant versus non- pregnant conditions will also be calculated to describe the range of values that are consistent with the observed data and help the Protocol Team assess whether there is a clinically important difference in exposure in the pregnant versus non-pregnant conditions.

For the ARV drugs being taken with first line TB drugs, in addition to Stages 1 and 2, a third stage of analysis (Stage 3) will be performed which will compare the ARV PK exposure with first line TB drugs to the PK exposure for these ARVs without first line TB drugs in previously evaluated arms of the P1026s study. This analysis will involve a between-participants comparison of PK exposure parameters using the two-sample Wilcoxon rank sum test. 90% confidence limits for the geometric mean ratio of the PK exposure parameters in the two groups of women will also be calculated.

For the first line TB drugs, a Stage 1 analysis similar to that described above will be performed but only after completion of the concentration assays following batch shipment. Since the TB drug samples will not be shipped and assayed in real time and the duration of TB drug treatment is time limited, the participant may no longer be receiving the TB drug being studied when the TB drug assay results become available. The PK analysis for the first line TB drugs will also include the Stage 2 approach described above, in which a within-participants comparison of TB drug PK exposure during pregnancy and postpartum will be performed, separately for HIV-infected and HIV-uninfected women in each study arm. Note that the sample size for this comparison may be less than the desired 25 evaluable women if some women complete their TB medication course before the postpartum visit where the PK sampling will be done. In addition, a Stage 3 between-participants comparison of first line TB drug PK exposure in HIV-infected versus HIV-uninfected women will be performed (using the two-sample Wilcoxon rank sum test) to assess whether exposure to first line TB drugs differs with vs. without concomitant ARV drug use and presence of HIV infection. Note that if a significant difference is observed in the between-participants analysis, it will not be possible to determine whether this difference is due to ARV use or HIV infection.

For the second line TB drugs, the PK analysis will use the Stage 2 approach described above, in which a within-participants comparison of TB drug PK exposure during pregnancy and postpartum will be performed. Note that the sample size for these comparisons may be less than the desired 25 evaluable women per drug because the inclusion criteria for this study arm only require that women are receiving at least two of the specified second line TB drugs, and because some women may complete their TB medication course before the postpartum visit where the PK sampling will be done. It may or may not be possible to perform a Stage 3 between-participants comparison of second line TB drug PK exposure in HIV-infected versus HIV-uninfected women; depending on the number of women receiving each TB drug, it may be necessary to combine data from HIV-infected and HIV-uninfected women.

For the ARV drugs being taken with postpartum contraceptives, the Stage 1 analysis above will not be performed (the Stage 1 analysis will only be done for ARV during pregnancy). A within-participants analysis similar to the Stage 2 analysis described above will be performed, except

that the ARV PK exposure parameters before versus after starting contraceptives will be compared (instead of during pregnancy versus postpartum).

For the contraceptives, a Stage 3 analysis will be performed that will compare the contraceptive concentrations observed in P1026s women receiving a protease inhibitor to data from historical controls not receiving ARVs, as reported in the available literature.

For analyses of PK secondary objectives, descriptive statistics (mean, median, standard deviation, interquartile range, and range) will be tabulated for PK outcome measures. If most infant washout PK data are below the BLQ for a given study arm, the pharmacologist may determine that the analysis report should not include an infant washout PK data summary (this will be noted in the report).

For the secondary objectives related to safety and clinical outcomes, adverse events and other safety outcome measures will be tabulated, and descriptive statistics (mean, median, standard deviation, interquartile range, and range) will be calculated for CD4 and HIV-1 RNA measurements (for HIV-infected participants only).

4.1 Statistical Tests

Within-participant comparisons (e.g., between pregnant versus non-pregnant conditions or second versus third trimester) will be performed for continuous outcome measures using the Wilcoxon signed rank test and for dichotomous outcome measures using McNemar's test. If the statisticians determine there are not enough women with PK data available at both time points for a meaningful comparison, then no p-value will be calculated. In addition, if there are no discordant pairs for McNemar's test, then the test will not be performed. Between-participant comparisons will be performed for continuous outcome measures using the Wilcoxon rank sum test and for dichotomous outcome measures using the chi-square or Fisher exact test.

90% confidence limits for the geometric mean ratio of the PK exposure parameter in the pregnant versus non-pregnant conditions will also be calculated to describe the range of values that are consistent with the observed data and help the Protocol Team assess whether there is a clinically important difference in exposure in the two conditions. The confidence coefficient will be 90% rather than 95% to match the usual practice in the pharmacokinetic literature to show that two conditions are sufficiently similar (e.g, bioequivalence testing).

4.2 Limitation

The P1026s study design is opportunistic in that it enrolls women who are already receiving the study drugs as part of clinical care, but this design has one major limitation, namely that the results may be overly optimistic. Since participants must have been stable on the ARV drug/drug combination and/or TB drug combination for at least two weeks prior to PK sampling in order to enroll in the study, the study population will not include women who started the study drug and discontinued it within two weeks due to toxicity, intolerance, virologic failure, or any other reason. Thus, this study will not be able to identify pharmacokinetic, safety, or tolerance issues that occur very soon after drug initiation and estimates of the frequency of outcomes such as virologic response and mother-to-child HIV transmission may be overly optimistic. The fact that the results

of this study will generalize only to the population of women who are able to stay on the study drugs for at least two weeks will be discussed as a limitation of the study in presentations and publications of results. The Protocol Team acknowledges this limitation, but feels that the PK data obtained using the current design will still be quite valuable nonetheless.

5 Report Contents

Detailed descriptions of the content of each of the following sections are given in the AIP.

1) Maternal demographics summary
    - Race, ethnicity, and country
    - Age, gestational age, weeks after delivery and weight at PK visits
    - ARV/TB medication duration before antepartum visits; concomitant ARV/TB medications at antepartum visits
    - Viral load and CD4 at antepartum, delivery and postpartum visits

2) Maternal safety
    - List of AEs reported to Division of AIDS (DAIDS) as Expedited Adverse Events (EAEs)
    - List of Grade 3 or greater AEs
    - List of AEs that are possibly/probably/definitely related to study drugs, as determined by the Core Protocol Team and/or site investigators.

3) Maternal PK analysis (as applicable to study arm being analyzed)
    - PK descriptive summaries by parameter and study visit
    - PK parameter comparisons of antepartum vs. postpartum
    - Summary of mothers with AUC above 10^{th} percentile in antepartum vs. postpartum
    - Summary of cord blood/maternal plasma ratio

4) Infant demographics
    - Birth weight, length, gestational age and most definitive HIV status

5) Infant safety
    - List of congenital anomalies
    - List of AEs reported to DAIDS as EAEs
    - List of Grade 3 or greater AEs
    - List of AEs that are possibly/probably/definitely related to study drugs, as determined by the Core Protocol Team and/or site investigators.

6) Infant PK analysis
    - Washout concentration and half-life summary