







STUDY PROTOCOL

**REVISED** **Pharmacokinetics of drugs used to treat drug sensitive-tuberculosis in breastfeeding mother-infant pairs: An observational pharmacokinetic study [version 3; peer review: 1 approved, 2 approved with reservations]**

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**V3** **First published:** 12 May 2023, 8:216  
<https://doi.org/10.12688/wellcomeopenres.19113.1>  
**Second version:** 28 Feb 2024, 8:216  
<https://doi.org/10.12688/wellcomeopenres.19113.2>  
**Latest published:** 21 May 2024, 8:216  
<https://doi.org/10.12688/wellcomeopenres.19113.3>










## Abstract


### Background

Globally, more than half of women take medicines whilst breastfeeding. Data concerning the exposure of the breastfed infant to drugs and any related risks are sparse. Lactation studies are only rarely performed close to licensing for medicines anticipated to be widely used in women of childbearing age. Medicines taken by breastfeeding mothers on tuberculosis (TB) treatment can be transferred to the breastfed infant. Potential effects of anti-tuberculosis medicines on nursing infants are not well understood. Similarly, women face mental health challenges while taking medications, including postpartum depression, hence the need to assess the psychological behavior of a breastfeeding woman. Potential risks are the development of adverse drug effects in the breastfed infant and selection for resistance, whereas potential benefits might include exposure to potentially prophylactic concentrations of the drug. Pharmacokinetic studies are therefore

## Open Peer Review

Approval Status   

	1	2	3
<b>version 3</b> (revision) 21 May 2024	 <a href="#">view</a>	 <a href="#">view</a>	 <a href="#">view</a>
<b>version 2</b> (revision) 28 Feb 2024		  <a href="#">view</a>	
<b>version 1</b> 12 May 2023	 <a href="#">view</a>	 <a href="#">view</a>	

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necessary to understand this situation fully.

## Methods

This study will enroll 20 mothers receiving first-line anti-tuberculosis medicines, together with their breastfed infants, with the aim of characterizing the breastmilk transfer of the medicines from the mother to the infants. Samples of maternal blood, breastmilk, and breastfeeding infant's blood will be obtained at specific time points for bioanalysis of drug concentrations. Pharmacokinetic data will be analyzed using a population pharmacokinetic approach. Additionally, the study will assess the psychological status of breastfeeding women and the well-being of their infants. Maternal depression is linked to long-term negative consequences for the infant's physiological regulation, poor growth-promoting setting for the infants, and inappropriate interactive conduct, characterized by low compassion, constrained range of emotional expression, and varying provision of the infant's budding engagement.

## Conclusions

This study will provide the first systematic characterization of mother-to-infant transfer of first-line anti-tuberculosis medicines through breast milk. A mathematical pharmacokinetics model characterizing plasma-to-breastmilk transfer of rifampicin, isoniazid, ethambutol, and pyrazinamide will be developed and used to characterize infant exposure through breast milk. Our findings will contribute towards treatment optimization in breastfeeding and provide a framework to foster other lactation pharmacokinetic studies.

## Keywords

Tuberculosis, breastmilk pharmacokinetics, Africa, lactation

Any reports and responses or comments on the article can be found at the end of the article.

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**Author roles:** **Nakijoba R:** Investigation, Methodology, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing; **Nakayiwa Kawuma A:** Formal Analysis, Investigation, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Ojara FW:** Formal Analysis, Investigation, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Tabwenda JC:** Methodology, Project Administration; **Kyeyune J:** Project Administration; **Turyamureba C:** Methodology, Project Administration; **Peter Asiimwe S:** Methodology, Project Administration, Writing – Review & Editing; **Magoola J:** Project Administration; **Castelnuovo B:** Investigation, Writing – Original Draft Preparation, Writing – Review & Editing; **Buzibye A:** Formal Analysis, Investigation, Validation; **Waitt C:** Conceptualization, Funding Acquisition, Investigation, Methodology, Resources, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

**Grant information:** This work was supported by Wellcome [222075], a Wellcome Trust Clinical Research Career Development Fellowship awarded to Catriona Waitt.

*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

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**How to cite this article:** Nakijoba R, Nakayiwa Kawuma A, Ojara FW *et al.* **Pharmacokinetics of drugs used to treat drug sensitive-tuberculosis in breastfeeding mother-infant pairs: An observational pharmacokinetic study [version 3; peer review: 1 approved, 2 approved with reservations]** Wellcome Open Research 2024, 8:216 <https://doi.org/10.12688/wellcomeopenres.19113.3>

**First published:** 12 May 2023, 8:216 <https://doi.org/10.12688/wellcomeopenres.19113.1>

**REVISED Amendments from Version 2****Summary of changes made to the manuscript.**

1. The rationale for doing the behavior test needs was addressed in the background of the abstract, the statement below was added; ***“Similarly, women face mental health challenges whilst taking medications, including postpartum depression, hence the need to assess the psychological behavior of a breastfeeding woman”***
2. The sample size was clarified for all study drugs, ***as 20 pregnant or breastfeeding women in the*** study design section of the protocol.
3. Following the national protocol, infants of mothers diagnosed with ***DS-TB and treated*** will receive isoniazid preventive therapy for six months (the duration of the mothers' tuberculosis treatment). The statement was rephrased to include DS-TB and treated and ***“tuberculosis treatment”*** was deleted.
4. ***“The sample size will cater to all the drug categories. First-line tuberculosis treatment consists of rifampicin, isoniazid, ethambutol, and pyrazinamide administered as a fixed-dose combination pill”***. Added in the section of Treatments of participants, drug storage, and drug accountability
5. ***“The intercurrent of HIV and concomitant antiretroviral medication will be considered a covariate in the pharmacokinetic analysis”***. The statement has been added to the protocol section; Disease setting and patient population
6. ***“The commonly used Antiretroviral therapy (ART) could affect the pharmacokinetic exposure of the TB drugs. The information on the type and dose of ART drugs co-administered to a TB patient will be recorded and integrated into the analysis of the pharmacokinetics of TB drugs to characterise the differences in pharmacokinetic exposure between patients on concomitant TB / ART treatment and those receiving only TB drug”***, this has been added to the disease setting and patient population of the protocol.

**Any further responses from the reviewers can be found at the end of the article**

## Introduction

Approximately 50% of women take medication during breastfeeding<sup>1</sup>. Data regarding the exposure of the breastfed infant to drugs and any associated risks are sparse<sup>2</sup>. Drugs taken by the breastfeeding mother on tuberculosis (TB) treatment can be passed from the maternal circulation through her breastmilk to the breastfed infant<sup>3</sup>. This may cause effects on the infant<sup>4</sup>. Most TB drugs are metabolized by the liver, triggering a potential risk of drug accumulation in infants due to their immature liver function, particularly in premature infants<sup>5,6</sup>.

Most drugs are transferred to breastmilk in small quantities, and many have been used without obvious infant toxicity for many years<sup>3</sup>. Whilst data on TB drug penetration into breast milk is limited, information on clinically relevant infant exposure to these drugs is even more limited. This is an important information gap both for safety, and because therapeutic

concentrations could be 1) protective in exposed infants, obviating the need for TB preventive therapy or 2) sub-therapeutic concentrations could select for resistance in those infants infected with *Mycobacterium tuberculosis*.

Anxiety in mothers, especially those with very young children is not uncommon. Caring for an infant can cause apprehension. This often comes from the pressure to get everything right for the baby. In extreme cases, mothers might suffer postpartum depression. Therefore, the need to take medication during this period (for whatever condition) can aggravate an already fragile mental health state. The association between tuberculosis and depression is well established in non-pregnant adults, including in east African populations<sup>7,8</sup>. Depression can be associated with poorer adherence to medication, and therefore with worse treatment outcomes<sup>9</sup>, hence the need to explore the mental health status of a breastfeeding women.

In this observational study, we aim to characterize the breastmilk transfer of first-line antituberculosis drugs (rifampicin, isoniazid, ethambutol, and pyrazinamide) to the breastfed infant. In addition, we will evaluate the mental health of the breastfeeding mothers enrolled using the Generalised Anxiety Disorder Questionnaire (GAD-7), and Patient Health Questionnaire (PHQ-9), and the well-being of their infants will be assessed using the infant Gross Motor Development (GMD) checklist. Data from this protocol will be pooled and analyzed with data from two other protocols which share standardized methods, and are being conducted within the overarching MILK (Mother-to-Infant Lactation pharmacokinetic) program. Of the two protocols from which we will pool data, one is investigating mothers receiving treatment for uncomplicated malaria in Uganda<sup>10</sup> while another is investigating those on drug-resistant TB treatment in South Africa. This collective analysis will allow the exploration of beliefs and attitudes surrounding a range of medication use in breastfeeding mothers.

## Protocol

### Disease setting/patient population

Breastfeeding women receiving first-line antituberculosis treatment and their breastfed infants, including those living with HIV, will be recruited prospectively from the Infectious Diseases Institute (IDI) clinic, and IDI-affiliated Kampala City Council Authority (KCCA) clinics, all in Kampala, Uganda. KCCA clinics are government-funded health facilities that provide a wide range of services, including tuberculosis diagnosis and treatment to the general population, including pregnant and breastfeeding women. We anticipate that 60–80% of women requiring TB treatment will be living with HIV. Drug-drug interactions with antiretroviral therapy (ART) could affect the exposure of TB drugs. The intercurrent of HIV and concomitant antiretroviral medication will be considered as a covariate in the pharmacokinetic analysis. Data on concomitant medication will be recorded and included as a covariate in the pharmacokinetic analysis. The commonly used Antiretroviral therapy (ART) could affect the pharmacokinetic exposure

of the TB drugs. The information on the type and dose of ART drugs co-administered to a TB patient will be recorded and integrated into the analysis of the pharmacokinetics of TB drugs to characterise the differences in pharmacokinetic exposure between patients on concomitant TB / ART treatment and those receiving only TB drug. Following the national protocol, infants of mothers diagnosed with DS-TB and treated will receive isoniazid preventive therapy for six months (the duration of the mothers' tuberculosis treatment).

## Study objectives

### Primary

1. To characterise the transfer of rifampicin, isoniazid, ethambutol and pyrazinamide to the breastfed infant.
2. To determine the area under the concentration-time curve (AUC), clearance and volume of distribution of these drugs.

### Secondary

1. To describe covariates influencing drug exposure in maternal plasma, breastmilk and infant plasma
2. To develop a population pharmacokinetic model including the breastmilk and the infant as compartments, which will both enable optimal use of sparse data from future studies, and also enable simulations of different doses or combinations.
3. To assess depression and anxiety levels among breastfeeding mothers on first-line anti-TB drugs.
4. To assess beliefs about medicines in breastfeeding mothers receiving TB treatment.

## Study endpoints

### Primary endpoints

1. Concentrations of TB drugs in maternal plasma and breastmilk at pre-dose, 2, 4, 6, 8 and, in some cases, 24 hours post-dose.
2. Concentrations of drugs in infant blood at maternal pre-dose, and up to 8 hours post-maternal dose.
3. Area under the concentration-time curve (AUC) of TB drugs in maternal plasma and breastmilk.
4. Breastmilk-to-maternal plasma (M:P) ratio of TB drugs.

### Secondary endpoints

1. Maximum concentration (C<sub>max</sub>) and time to maximum concentration (T<sub>max</sub>) of TB drugs in maternal plasma and breastmilk.
2. Infant development (using Gross Motor Development score).
3. Depression and anxiety assessments for breastfeeding mothers.
4. Beliefs about medicines in breastfeeding mothers receiving TB treatment.

## Study design

20 Pregnant or lactating women requiring, or those who started, treatment for drug-sensitive tuberculosis will be identified and invited for sampling. If they are pregnant when identified, they will be invited for sampling after delivery. Participants will be identified from recruitment sites by the healthcare unit's study contact personnel. Plasma and breastmilk samples will be obtained pre-dose and at 2, 4, 6, and 8 hours post-dose. If logistics permit (for example living close to the research unit), participants will be invited for a further sample 24 hours post-dose. A heel prick sample will also be obtained from their breastfed infants at the maternal trough (prior to maternal dose) and at a random time point (once per infant) over the 8-hour pharmacokinetic sampling visit to characterize concentrations of these drugs over an 8-hour dosing interval. The total plasma and breastmilk concentrations of rifampicin, isoniazid, ethambutol, and pyrazinamide will be quantified using liquid chromatography mass spectrometry (LCMS). If a participant has her first pharmacokinetic profile in the intensive phase of TB treatment, (the intensive phase is two months of TB treatment where individuals take all four drugs, namely; rifampicin, isoniazid, ethambutol, and pyrazinamide), she will be invited for a subsequent sampling day with the same time points when she is on the continuation phase of therapy (rifampicin and isoniazid).

## Participant selection

### Inclusion criteria

Participants must meet all the following inclusion criteria to be eligible for enrollment in the study.

1. Participants who are willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other study procedures.
2. A woman is aged 18 years or older.
3. Receiving treatment for drug-sensitive tuberculosis.
4. Pregnant or breastfeeding at enrolment.
5. A personally signed and dated informed consent document indicating that the participant has been informed of all pertinent aspects of the study.

### Exclusion criteria

Participants presenting with any of the following will not be included in the study:

1. Severe maternal or infant illness which in the opinion of the patient's clinician would interfere with her participation in the study.
2. Breastfed infant is aged over 12 months.

## Treatments of participants, drug storage and drug accountability

All women (and infants) will continue to receive their tuberculosis treatment as prescribed by the physician from the respective TB clinical care sites. Drugs will be stored and dispensed from the relevant TB clinic pharmacy, with no special procedures relating to this observational protocol. The

sample size will cater for all the drug categories. First-line tuberculosis treatment consists of rifampicin, isoniazid, ethambutol and pyrazinamide administered as a fixed-dose combination pill. Concomitant medications will be documented on the (case report form) at every study visit.

**Study procedures**

**Informed consent**

Potential participants will be identified from the tuberculosis clinic at the IDI and KCCA clinics in Uganda. Informed consent will be obtained for women who express interest in study participation.

All eligible participants must sign an informed consent form before the conduct of any screening procedures. Participants will be encouraged to ask any inquiries concerning the study at this stage.

An impartial witness will be present during the informed consent discussion for participants that are unable to read or write. The witness should be able to read the consent form and participant information leaflet in the participant’s chosen language. After the written, informed consent form is read and explained to the participant, and after they have orally consented to their participation in the study and have either signed the consent form or provided their fingerprint, the witness will sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the

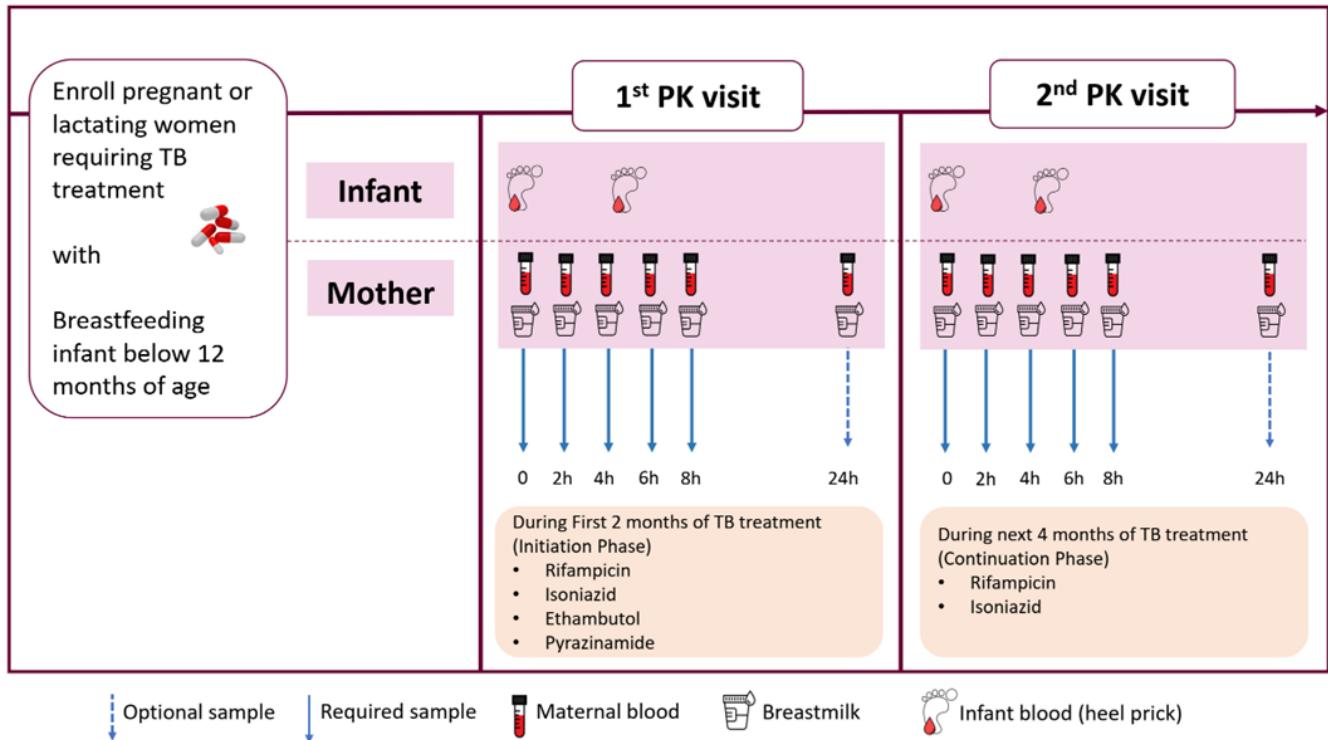
participant and that informed consent was freely given by the participant.

**Screening**

Participants will be assessed against the eligibility criteria. Some women who intend to breastfeed will be identified in late pregnancy but will still be on TB treatment during breastfeeding. In this situation, consent will be sought, and details of how to contact her will be recorded with the aim to bring her for the pharmacokinetic sampling detailed in Figure 1, at approximately 6 weeks postpartum. The exception to this will be when a mother switches from the four-drug intensive to the two-drug continuation phase of TB treatment before 6 weeks postpartum, in which case attempts to schedule the sampling visit whilst still on the intensive phase will be made.

**Pharmacokinetic study day**

An intravenous cannula will be inserted into her antecubital fossa, and samples taken for trough (pre-dose) drug measurement. After a standardized breakfast, she will be administered her medication. Blood samples will be collected at 2, 4, 6, 8 and ideally\* 24 hours post-dose. She will be advised to freely breastfeed her baby. She will be asked to provide a 2–5 ml sample of expressed breastmilk pre-dose, and at 2, 4, 6- and 8-hours post-dosing. A blood sample from the infant will be collected at maternal trough (pre-dose) and at a 3–8 hours post-maternal dose (the second time point will be allocated sequentially to ensure a spread of data points). The mother will be administered a standard lunch as detailed in Table 1.



**Figure 1.** Graphic representation of Pharmacokinetics procedures during the study period.

**Table 1. Schema for Pharmacokinetic sampling.**

		Study Period (8hr) <sup>^</sup> , time relative to maternal dose						
Subject	Study Procedure	On arrival	0h	2h	4h	6h	8h	24h <sup>^</sup>
Mother	Confirm willingness to proceed	x						
	Clinical assessment	x						
	BMI	x						
	Blood for creatinine and Albumin			x				
	Blood PK sample	x		x	x	x	x	x
	BM PK sample	x		x	x	x	x	x
	Standard meal		x			x		
	Observed dosing		x					
	PHQ9, GAD7, BMQ questionnaires				x <sup>~</sup>			
	Infant	Clinical assessment	x					
Weight		x						
PK sample		x				X <sup>+</sup>		

<sup>\*</sup>Time of second infant DBS to be recorded, between 3h to 8h

<sup>^</sup>Due to logistic constraints, the 24h may not be collected in every participant

<sup>~</sup>Can be any time during PK Day

\*Due to the logistic considerations of sampling a postpartum mother and her infant who may have travelled a long distance to the clinic, the 24-hour sample may not be collected in all cases.

Maternal albumin and creatinine will be sampled as they are important for isoniazid exposure. Maternal questionnaires will be filled out on each visit to assess depression and anxiety; Generalised Anxiety Disorder questionnaire (GAD-7), Patient Health Questionnaire (PHQ-9), and the Beliefs about Medicines Questionnaire (BMQ). Infant clinical assessment will include the use of the Gross Motor Development (GMD) checklist (Appendix 1,2, and 3 in the protocol).

#### End of TB treatment outcomes

End of TB treatment outcomes (cured, treatment completed, died, defaulted or transferred out) will be collected from the TB registry.

#### Participant withdrawal

Participants who may not comply with the protocol's required schedule of study visits or procedures will be withdrawn from the study. Also, participants may withdraw from the study at any time at their request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety or behavioral reasons.

For participants who may miss any study visits (for example, has given consent but then does not return for the pharmacokinetic sampling day detailed in [Figure 1](#)), every effort will be made to contact them using telephone numbers documented at enrollment. The investigator will inquire about the reason for withdrawal and follow up with the participant regarding any unresolved adverse events (AEs).

No further evaluations will be performed, and no additional data should be collected if a participant withdraws from the study, and also withdraws consent for disclosure of future information.

#### Assessments

##### Safety

We do not have concerns about the safety of the medication received since this is an observational study. However, adverse events related to the study procedures will be recorded in case report forms and responded to until the event has resolved. Any events that do occur will be listed and analyzed according to Division of Acquired Immune Deficiency Syndrome (DAIDS) criteria that provides an adverse event (AE) severity grading scale. There is no formal requirement for notification of adverse events to the regulatory authorities given that anti-tuberculosis drugs given are standard of care treatment and not an investigational product.

### **Pharmacokinetic assessments**

#### **Blood for pharmacokinetic analysis**

Pharmacokinetic samples will be obtained at the scheduled time relative to dosing and missed sample time will be re-scheduled.

#### **Sample handling**

Maternal plasma and breast milk will initially be processed and stored in a  $-80^{\circ}\text{C}$  at the IDI core laboratory before analysis. The quantification of drug concentrations in maternal and infant plasma and maternal breastmilk will be performed at the IDI core laboratory, or at the pharmacology specialty laboratory at the Division of Clinical Pharmacology, University of Cape Town (UCT) using liquid chromatography-tandem mass spectrometry.

#### **Validation of breastmilk assay/donor breastmilk**

Bioanalysis will employ a liquid chromatography-tandem mass spectrometry (LC-MS/MS) approach which must be validated separately in plasma and breastmilk. It is essential that the standards made to form the standard curve and the different levels of quality control (QC) are spiked into the matrix (plasma, milk, urine etc.) which will be used for the clinical samples. Given there is not yet a fully functioning donor human milk bank in Uganda, we will invite breastfeeding women who are not taking any medication to donate a small amount of breast milk for scientific reasons. These are likely to be staff and students working within IDI, and this approach is frequently used in other centres for the donation of 'blank' blood for assay validation. Given that these donors will be drawn from staff and students within the organization, the consent form has not been translated from English.

#### **Data analysis/statistical methods**

##### **Sample size determination**

This study is exploratory, as no prior study has characterized the exposure of these drugs in maternal plasma, breastmilk and infant plasma. There are no prior data upon which to build a sample size calculation, and there is no comparison between groups which requires statistical analysis with a pre-specified certainty. Since no information is available about the penetration of these drugs into breastmilk, we used the following approach, described in detail for rifampicin.

We modified a previously published population-pharmacokinetic model of rifampicin in plasma, adding a compartment to describe breastmilk concentrations. This was characterised using an approach similar to an effect compartment<sup>11</sup> described by a time delay and an accumulation ratio between breastmilk and plasma. The half-life of the delay was fixed to 1 hour and the accumulation ratio to 1.5, with 30% between-subject variability in both parameters. These were chosen to mimic a pharmacokinetic profile similar to Waitt *et al.*<sup>12</sup>. We assumed 15 individuals (considering a mother-infant dyad as a single unit) with an intensive pharmacokinetic sampling at 0, 1, 2, 4, 6 and 8 hours post-dose of paired plasma and breastmilk

(30% error in the breastmilk measurements was assumed) and performed Stochastic Simulations and Estimations (SSEs)<sup>13</sup> to evaluate the trial design. Our design can characterise all the typical values of the plasma pharmacokinetic parameters with precision of better than 11% RSE, and all the breastmilk parameters are well characterised with a precision of 1.14% and 0.591% RSE on delay and accumulation ratio, respectively.

An interim analysis will be performed based on the first five participants recruited in this study to assess the adequacy of the sampling design and the bioanalytical methodology in characterising drug concentrations across time.

#### **Analysis of endpoints**

Pharmacokinetic data will be analyzed using a population pharmacokinetic approach to estimate pharmacokinetic parameters and produce modelled fits to exposure data<sup>14,15</sup>. Inter-individual variability will be quantified and different covariates tested.

Non-compartmental methods will be used to assess correlations between maternal breast milk drug concentrations and measures of drug exposure in the infant (e.g. AUC) and pharmacodynamic factors.

#### **Analysis of primary endpoint**

##### **Non-compartmental analysis**

Plasma pharmacokinetic parameters including the maximum plasma concentration ( $C_{\text{max}}$ ), time to maximum plasma concentration ( $T_{\text{max}}$ ), and area under the plasma concentration versus time curve ( $AUC_{\text{last}}$ ,  $AUC_{\infty}$ ) for (drugs) will be estimated using non-compartmental analysis. If data permit or if considered appropriate, area under the plasma concentration versus time curve to infinity ( $AUC_{\text{inf}}$ ), terminal elimination half-life ( $t_{1/2}$ ), plasma clearance (CL or CL/F), apparent volume of distribution ( $V_d$  or  $V_d/F$ ) will be also estimated, as data allow. The single dose and/or steady-state PK parameters will be summarized descriptively by dose, cycle and day.

##### **Population pharmacokinetic (pop-PK) analysis**

The population pharmacokinetic models will be applied to evaluate whether the drug concentrations are consistent with the expected exposures previously reported. If discrepancies between the observed exposure and the model-expected levels are observed, the models will be adjusted by re-estimating the values of the pharmacokinetic parameter values, with the use of Bayesian priors.

Drug concentrations in breastmilk will be linked in the maternal population pharmacokinetic models using an effect compartment strategy, which will allow accurate estimation of the accumulation in breastmilk compared to plasma. Exposure to isoniazid and rifampicin in breastfed infants will be compared with the range of concentrations achieved in adults given therapeutic doses, assuming a minimal duration

of exposure from delivery and a maximal duration from the time the mother started the drug (to account for placental transfer).

### **Analysis of secondary endpoints**

The data derived from the Generalised Anxiety Disorder questionnaire (GAD-7), Patient Health Questionnaire (PHQ-9), the Beliefs about Medicines Questionnaire (BMQ) and the Infant Gross Motor Development (GMD) checklist will be analysed together with data from the other protocols within the overarching MILK fellowship (mothers on first line malaria treatment in Uganda and on drug-resistant TB treatment in South Africa) and a broader analysis of medication use in breastfeeding. This combined analysis will allow the exploration of beliefs and attitudes surrounding a range of medications used in breastfeeding mothers.

### **Safety analysis**

Adverse events relating to the study procedures will be categorized and analyzed.

### **Quality control and quality assurance**

During study conduct, periodic monitoring may be conducted to ensure that the protocol and good clinical practices (GCPs) are being followed. The monitors may review source documents to confirm that the data recorded on CRFs is accurate. Additionally, the study site may be subject to review by the institutional review board (IRB) and/or to inspection by appropriate regulatory authorities.

### **Data handling/record retention**

#### ***Case report forms (CRF)***

A CRF is required and should be completed for each included participant.

The investigator has ultimate responsibility for the collection and reporting of all clinical, safety and laboratory data entered on the CRFs and any other data collection forms (source documents) and ensuring that they are accurate, authentic/original, attributable, complete, consistent, legible, timely (contemporaneous), enduring and available when required. The CRFs must be signed by the investigator or by an authorized staff member to attest that the data contained on the CRFs is true. Any corrections to entries made in the CRFs in REDCap (Research Electronic Data Capture) database, source documents must be dated, initialed and explained (if necessary) and should not obscure the original entry.

#### ***Record retention and archiving***

To enable evaluations and/or audits, the investigator agrees to keep records, including the identity of all participating patients (sufficient information to link records, e.g., CRFs and clinic records), all original signed informed consent documents, soft copies of all CRFs, safety reporting forms, source documents, and detailed records of treatment disposition, and adequate

documentation of relevant correspondence (e.g., letters, meeting minutes, telephone calls reports).

Investigator records must be kept for as long as required by applicable local regulations (10 years in Uganda).

### **Confidentiality and insurance**

Clinical data will be entered into a study-specific database by designated staff on a regular basis from completed case record forms (CRF). Case record forms and other source documents will be kept in locked cabinets. Data will be entered on a regular basis to ensure that it is up to date. The database will be entered on regular basis on a secure computer, as will the pharmacokinetic data that will be received by the laboratories. Access to database will be given to authorized personnel only (members of the immediate study team) and a log of authorized personnel will be stored in the trial master file. CRF and trial documents will be kept in locked cabinets. No participant identifying information will be disclosed in any publication or in any conference activities arising from the study.

### **Institutional Review Board (IRB)**

The protocol, protocol amendments, informed consent documents, and other relevant documents must be approved by the IRB. All correspondence with the IRB should be retained in the regulatory or trial master file. Copies of IRB approvals should be filed with other study documents. The study was reviewed and approved by the Infectious Diseases Institute (IDI) Regulatory Ethics Committee (REC) REF: IDI REC 039/2021 on 06 December 2021, and the Uganda National Council of Science and Technology (UNCST): HS1948ES on 18 January 2023.

### **Ethical conduct of the study**

The study will be conducted in accordance with legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Participants and the Declaration of Helsinki. In addition, the study will be conducted in accordance with the protocol, GCP guidelines, and applicable local regulatory requirements and laws

### **Participant information and consent**

All parties will ensure protection of participant personal data and will not include participant names on any forms, reports, publications, or in any other disclosures, except where required by law. The informed consent document used in this study, and any changes made during the course of the study, must be prospectively approved by the IRB. The investigator, or a person designated by the investigator, will obtain written informed consent from each participant or the participant's legal representative before any study-specific activity is performed. The investigator will retain the original of each participant's signed consent document.

### Definition of end of study

This will occur when the study enrolls 20 mother infant pairs, and data analysis is complete.

### Publication and dissemination of study results

Study findings will be disseminated to researchers at scientific conferences and peer-reviewed infectious disease and pharmacology journals. Specifically, for Uganda, study results will be communicated to the National Tuberculosis and Leprosy programme in the National Department of Health through meetings and reports. The Community Engagement Plan will detail the engagement with communities at all stages of the protocol from inception through dissemination, and activities under the related Wellcome Public Engagement Enrichment Award Attaining Equity of Access to Research (At The EQUATOR) will ensure meaningful two-way dialogue throughout. Specifically, through At The EQUATOR, the following activities are proposed with regard to the MILK protocols.

#### During study set-up

Early consultation with community advisory board (CAB) to determine which community members to approach for dialogue. Initial community meeting with religious leaders, women's local representative and community members, involving oral discussion and written flyers with pictures and simplified, translated text. Identification of key community members who are interested in co-creation of materials and resources.

#### At site initiation

Community meetings will be held at site for Q&A and more specific information about recruitment, and radio broadcast or presentation of drama and song relating to activities will be conducted. Tailor-made flyers and posters including photos with key, simplified text will be shared.

#### Dissemination meetings/events

Study progress and results will be conducted and shared with stakeholders.

### Discussion and conclusions

This protocol has been designed with the aim to determine the pharmacokinetics of drugs used to treat drug-sensitive tuberculosis in breastmilk and describe their transfer to a breastfed infant. The current situation where there are no data describing this transfer for rifampicin, ethambutol and pyrazinamide is unacceptable.

Understanding infant exposure to anti-tuberculosis drugs is important to inform on potential risks and benefits to the infant. Measuring drug concentrations directly in the infant is the best way to fully understand of infant exposure and enable informed prediction of infant exposure. Many existing lactation pharmacokinetic studies have estimated maternal-to-infant transfer of drugs (infant exposure) from only maternal plasma and breastmilk drug concentrations.

Many authors cite ethical and logistical considerations as reasons for not performing infant blood sampling. However, over the past eight years at the IDI, we have sampled more than 350 mother-infant pairs without encountering such difficulties<sup>12,16,17</sup>). Examples of drugs samples for at IDI include: Tenofovir, Lamivudine, Efavirenz, and Delutegravir.

We have proposed a model-based population pharmacokinetics analysis approach for characterization of changes in drug concentrations across time. Population analysis will enable joint analysis of data from multiple participants, describing both the typical population- and individual-specific profiles. Model-based population analysis offers several advantages over the more commonly used non-compartmental analysis: it can be used for predictions and simulations and for intensive and sparse sampling. We anticipate that findings from this research will enable a clear description of optimal use of drug-sensitive tuberculosis drugs in breastfeeding mothers, will enable development of clear patient information materials and will provide a framework to foster other pharmacokinetic lactation studies in different disease areas.

### Study status

The study has attained 50% recruitment of the target study sample size.

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### Data availability

#### Underlying data

No underlying data are associated with this protocol.

#### Extended data

Zenodo: Pharmacokinetics of drugs used to treat drug sensitive-tuberculosis in breastfeeding mother-infant pairs: An observational pharmacokinetic study. <https://doi.org/10.5281/zenodo.7798348><sup>18</sup>

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

#### Author contributions

ANK and FWO will perform analysis of the data. RN, CT, AB SPA and CGB were involved in writing the protocol. RN, JCT, JK, and JM and are involved in collecting the data. CW and BC were responsible for the overall concept and design of the study protocol. All listed authors meet the criteria for authorship set forth by the International Committee for Medical Journal Editors and have contributed to, seen, and approved the final submitted version of the manuscript.

#### Acknowledgements

We wish to thank the MILK Study team members and study participants. In addition, special thanks go to members of the MILK Community advisory board and members of the IDI research office for their insightful feedback during the development of this protocol.

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# Open Peer Review

Current Peer Review Status:   

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## Version 3

Reviewer Report 03 July 2024

<https://doi.org/10.21956/wellcomeopenres.23911.r84001>

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**Belen P. Solans**

University of California, Los Angeles, USA

No further comments, previous comments should be revised

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Pharmacokinetics and pharmacodynamics model development in the context of paediatric infectious diseases

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 18 June 2024

<https://doi.org/10.21956/wellcomeopenres.23911.r85075>

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**Eric Decloedt** 

Stellenbosch University, Stellenbosch, South Africa

This is a succinct well-written protocol and the study should make a meaningful contribution to science.

The protocol will benefit from clarifications of the following points:

- The current inclusion criteria states “Pregnant or breastfeeding at enrolment”. Maternal

participants will therefore be at various stages of pregnancy and time post-partum. Some may be 2<sup>nd</sup> trimester, or 3<sup>rd</sup> trimester with varying durations post-delivery. However, the study objectives do not include pregnancy as a population of interest, but rather focus on breastfeeding mothers (IMPAACT P1026s recently published the PK of RHZE during pregnancy and post-partum [Van Schalkwyk M, et.al., 2023 (Ref 1)]). Will mothers be enrolled during pregnancy but only sampled post-partum? This is also not clear from Figure 1. My impression is that the focus is exclusively on breastfeeding mothers.

- What is the justification for the repeat PK sampling of continuation phase given that similar drugs (RIF & INH) will be sampled? Or do the investigators expect further maternal physiological changes beyond the first 6 weeks post-partum to take place? Explain the justification.
- The NCA analysis plan states that the PK parameter will be summarised descriptively by dose, cycle and day. What cycles and days are referred to? Intensive and continuation phase? Kindly clarify.
- A comment: The sample size for the mental health secondary objective is small and may suffer from recruitment bias. However, the data collected are potentially important for further studies and really only explorative. I am also interested in whether INH exposure (slow acetylator status) is associated with poorer mental health outcomes – beyond the scope of this project.
- To be addressed: Of importance would be a statement/data that the questionnaires GAD-7, PHQ-9 etc have been validated in this population.
- There is little detail on how the outcome “Beliefs about medicines in breastfeeding mothers receiving TB treatment” will be assessed.

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## Is the rationale for, and objectives of, the study clearly described?

Yes

## Is the study design appropriate for the research question?

Yes

## Are sufficient details of the methods provided to allow replication by others?

Partly

## Are the datasets clearly presented in a useable and accessible format?

Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Clinical pharmacology; Pharmacokinetics; HIV-associated comorbidity

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 28 May 2024

<https://doi.org/10.21956/wellcomeopenres.23911.r84002>

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**Fredrick Rosario-Joseph**

Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Colorado Denver, Denver, Colorado, USA

I accept the revised version of the manuscript.

**Is the rationale for, and objectives of, the study clearly described?**

Not applicable

**Is the study design appropriate for the research question?**

Not applicable

**Are sufficient details of the methods provided to allow replication by others?**

Not applicable

**Are the datasets clearly presented in a useable and accessible format?**

Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Placental -Fetal Programming

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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**Version 2**

Reviewer Report 13 March 2024

<https://doi.org/10.21956/wellcomeopenres.23359.r75637>

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**Belen P. Solans**

University of California, Los Angeles, USA

The authors have not modified the manuscript based on previous comments, I have no further comments than those previously made

**Is the rationale for, and objectives of, the study clearly described?**

Not applicable

**Is the study design appropriate for the research question?**

Not applicable

**Are sufficient details of the methods provided to allow replication by others?**

Not applicable

**Are the datasets clearly presented in a useable and accessible format?**

Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Pharmacokinetics and pharmacodynamics model development in the context of paediatric infectious diseases

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.**

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**Version 1**

Reviewer Report 27 February 2024

<https://doi.org/10.21956/wellcomeopenres.21189.r71785>

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**Belen P. Solans**

University of California, Los Angeles, USA

This article provides a study protocol for the characterization of the extent of transfer of drug

levels into the breast milk.

This protocol will benefit from language revision, as some sentences are disconnected and short, and the appropriate use of connectors would help to better understand the problem the researchers are trying to address.

**Introduction:**

- Second paragraph: references are lacking for the first sentence.
- Third paragraph: is depression consequence of taking the medication? It would seem so for the way its written, but I don't think that is the message the authors want to give.
- Four paragraph: clearly state here that you will be looking at DS-TB. What other protocols are the researchers referring to here? This seems vague, similar as with "the other data". If this is a protocol, detail should be given in order to appropriately understand what it is to be done.

**Protocol:**

- There is a high number of women expected to have concomitant infection with HIV. How would be ART handled or managed? Is there any recommended drugs that investigators expect women will be taking? Are there any known DDIs with those drugs and the first line anti-TB drugs?
- There is a sentence saying: "diagnosed with tuberculosis treatment", this should be diagnosed with DS-TB and treated.

**Objectives:**

Primary:

1. Here the authors say "characterize the transfer" - I think this would mean something more like characterizing the mechanism of action, whereas what the researchers are going to do here is to characterize the **extent** of the transfer.
2. Why do your primary objectives only include AUC, CL and V? Probably better to state that the secondary objective would be to determine the PK characteristics of the drugs. Also, why is V especially relevant here?

Secondary:

1. Describe or characterize?
2. How is primary objective 2 accomplished without this one? Also, for this part, I would suggest removing all referring to compartments etc, just to develop a model

**Study endpoints:**

- Pre-dose and up to 8h for children is not very well explained. I would suggest to better explain this as done with the mother sampling schedule, especially when later in the protocol it seems that is not "up to 8h" but rather between 3 and 8 h post maternal dose.

**Study design:**

- Would the authors require a minimum of mothers in a intensive phase to be able to carry on the study?

**Inclusion criteria:**

- Revise language of this, especially in point 1 (which I would move to 5). Should be: participant provides a signed and dated...

**General comments:**

- The administration of 3 doses prior the one in study should be observed somehow, even by directly reporting of the mother - this will help the pop PK analysis (knowing if you can assume steady state or not, etc).
- Weight of the mother and malnutrition markers for the children should be removed and analyzed in the PK model.
- If you did an SSE you should be able to provide the power you have to characterize the addition of that effect compartment as opposed to not having it to characterize the breast milk transfer. With respect to power, you should say you will not enroll more than 20 and that is the number it should be used as well.
- "inter-individual variability will be quantified in relation to the covariates". This is not correct, IIV can and should be characterize irrespective of the covariates included.
- The use of Non-Compartmental Analysis to characterize correlations or AUC is totally inappropriate if you are doing a popPK compartment model, even more so if you say it on the discussion.

**Is the rationale for, and objectives of, the study clearly described?**

Partly

**Is the study design appropriate for the research question?**

Yes

**Are sufficient details of the methods provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Pharmacokinetics and pharmacodynamics model development in the context of paediatric infectious diseases

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 15 Apr 2024

## RITAH NAKIJOBA

### Comment Response:

This protocol will benefit from language revision, as some sentences are disconnected and short, and the appropriate use of connectors would help to better understand the problem the researchers are trying to address. Thank you so much for that observation, the team has corrected the grammar in the sentences to make the statements clear, however, you could also specify the exact sentences that are not clear.

### Introduction:

**Second paragraph:** references are lacking for the first sentence.

-Reference has been added in the second part of the introduction. Also, the other studies have been mentioned in the last paragraph of the introduction and referenced (7)

**Third paragraph:** is depression a consequence of taking the medication? It would seem so for the way it's written, but I don't think that is the message the authors want to give.

-Anxiety in mothers, especially those with very young children is not uncommon. Caring for an infant can cause apprehension. This often comes from the pressure to get everything right for the baby. In extreme cases, mothers might suffer postpartum depression. Therefore, the need to take medication during this period (for whatever condition) can aggravate an already fragile mental health state. Similarly, taking medication in breastfeeding women may lead to mental health challenges such as depression, which is regularly comorbid with anxiety. Depression can be associated with poorer adherence to medication, and therefore with worse treatment outcomes<sup>6</sup>, hence the need to explore the mental health status of a breastfeeding women as mentioned in the introduction section of the manuscript.

**Four paragraphs:** clearly state here that you will be looking at DS-TB. What other protocols are the researchers referring to here? This seems vague, similar to "the other data". If this is a protocol, detail should be given to appropriately understand what is to be done.

-The other protocols that will collect data on the infant's gross motor development have been included as references in the last paragraph of the introduction and referenced (7)

**Protocol:** There is a high number of women expected to have a concomitant infection with HIV. How would be ART handled or managed? Is there any recommended drugs that investigators expect women will be taking? Are there any known DDIs with those drugs and the first-line anti-TB drugs?

-The Uganda Ministry of Health guidelines recommend the use of triple therapy; Tenofovir, lamivudine, and Dolutegravir as first-line ART drugs. The drugs taken by persons living with HIV will be documented. Studies have used population pharmacokinetic modeling to compare the pharmacokinetics of dolutegravir when co-administered with standard- versus high-dose rifampicin in adults with tuberculosis and HIV, Simulations of trough dolutegravir concentrations show that the 50 mg twice-daily regimen attains both the primary and secondary therapeutic targets of 0.064 and 0.3 mg/L, respectively, regardless of the dose of co-administered rifampicin, unlike the once-daily regimen. We anticipate that 60–80% of women requiring TB treatment will be living with HIV. Drug-drug interactions with antiretroviral therapy (ART) could affect the exposure of TB drugs. The intercurrentence of HIV

and concomitant antiretroviral medication will be considered as a covariate in the pharmacokinetic analysis, As stated in disease setting and population section. There is a sentence saying: "diagnosed with tuberculosis treatment", this should be diagnosed with DS-TB and treated. The sentence has been corrected under the disease setting and study population section.

**Objectives:** Primary: Here the authors say "characterize the transfer"- I think this would mean something more like characterizing the mechanism of action, whereas what the researchers are going to do here is to characterize the extent of the transfer. We thank the author for highlighting this possible source of confusion. We will characterize the pharmacokinetic processes associated with the transfer of the four drugs to the breastfed infant.

-An adjustment has been made "To characterize the pharmacokinetic transfer of rifampicin, isoniazid, ethambutol and pyrazinamide to the breastfed infant" Why do your primary objectives only include AUC, CL and V? Probably better to state that the secondary objective would be to determine the PK characteristics of the drugs. Also, why is V especially relevant here? We thank the reviewer for raising this comment. We acknowledge that there are several additional PK parameters (C<sub>max</sub>, T<sub>max</sub>..) that could be estimated from this data and indeed will be evaluated in the analysis. AUC in particular provides an overall measure of drug exposure and in lactation pharmacokinetic studies it is widely employed in comparing plasma-to-breast milk transfer of the drug, whereas CL and V are two parameters that closely affect the drug elimination. We have not made changes to the manuscript

**Secondary:** Describe or characterize?

-We thank the reviewer for this comment. More appropriately we will evaluate. We have changed secondary objective #1 How is primary objective 2 accomplished without this one? Also, for this part, I would suggest removing all referring to compartments etc, just to develop a model The primary objectives will be accomplished using non-compartmental analysis. A population pharmacokinetic model will be developed secondarily. The details of the model provides the rationale for developing the model, as such it is important to highlight the components of the model. We have edited this objective To develop a population pharmacokinetic model characterizing the transfer of drugs from maternal plasma to breast milk and resultant infant exposure

**Study endpoints:** Pre-dose and up to 8 hours for children are not very well explained. I would suggest better explaining this as done with the mother sampling schedule, especially when later in the protocol it seems that is not "up to 8h" but rather between 3 and 8 h post maternal dose.

-Thank you for this comment, however as stated in the protocol; A blood sample from the infant will be collected at the maternal trough (pre-dose) and at a 3– 8 hours post-maternal dose (the second time point will be allocated sequentially to ensure a spread of data points). The second time point will be taken at any hour between 3-8 hours post maternal dose, if the mother fails to complete the 8 hours, then the infant will be sampled earlier than the last time point.

**Study design:** Would the authors require a minimum of mothers in an intensive phase to be able to carry on the study?

-We need not specify a minimum number of mothers enrolled in the intensive phase however we shall ensure that more than 50% of the mothers are enrolled during the intensive phase.

**Inclusion criteria:** Revise language of this, especially in point 1 (which I would move to 5). Should be: participant provides a signed and dated...

-Thank you for this observation, we agree that signing the ICF comes after it has been confirmed that the study participant is eligible for the study. Having it as #5 does not change the fact that it is important criteria .

**General comments:** The administration of 3 doses before the one in the study should be observed somehow, even by directly reporting to the mother - this will help the pop PK analysis (knowing if you can assume a steady state or not, etc).

-You raise a very important point, unfortunately, the study will depend on patient adherence reports and pill count for the last three days before intensive PK sampling day. However, in order to capture this with the greatest degree of accuracy, the study nurses make phone calls to the study participants during the five days prior to the PK sampling day to remind them of the need for good adherence to the medication, and to capture the dosing time. Other procedures such as video DOTS are not possible in our setting because the majority of the study participants do not have access to smartphones. Weight of the mother and malnutrition markers for the children should be removed and analyzed in the PK model.

The study captures both weight and nutrition status of the mother and infant. Both variables will be removed and analysed in the model. As stated in analysis of endpoints section;" Inter-individual variability will be quantified and different covariates tested". If you did an SSE you should be able to provide the power you have to characterize the addition of that effect compartment as opposed to not having it to characterize the breast milk transfer. With respect to power, you should say you will not enroll more than 20 and that is the number it should be used as well.

-The half-life of the delay in maternal plasma-to-breast milk transfer and the accumulation rate constant were both fixed for the SSE. "inter-individual variability will be quantified in relation to the covariates". This is not correct, IIV can and should be characterize irrespective of the covariates included.

-We thank the reviewer for this point – We acknowledge our wording is confusing and have made amends: "The impact of covariates on Interindividual variability in pharmacokinetic parameters will be evaluated" The use of Non-Compartmental Analysis to characterize correlations or AUC is totally inappropriate if you are doing a popPK compartment model, even more so if you say it on the discussion.

-We thank the reviewer for this point – We acknowledge our wording is confusing. Comparison of exposure in maternal plasma and breast milk and infant exposure will be based on standard statistical tests and not non-compartmental pharmacokinetic analysis

**Competing Interests:** I declare no competing interests

<https://doi.org/10.21956/wellcomeopenres.21189.r59255>

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### **Fredrick Rosario-Joseph**

Division of Reproductive Sciences, Department of Obstetrics and Gynecology, University of Colorado Denver, Denver, Colorado, USA

The rationale for doing the behavior test needs to be addressed in the background of the abstract.

The total study population is n=20; how many n for each drug category (rifampicin, isoniazid, ethambutol, and pyrazinamide)?

What are the current studies dealing with TB drugs and its breastfeeding content?

Does the ethnicity have any impact on it?

Secondary metabolite levels are not measured? Any impact on the infant?

It is confusing and unclear suddenly introduce uncomplicated malaria data with TB resistance. What outcome was added to the current data? Be clear in explaining the rationale.

Again introducing the HIV-infected group (60-80%) and only the remaining 20% would be noninfected HIV group taking antituberculosis treatment? No possibility of getting the statistical test comparison?

What is the Km value of each drug, and what is the half-life?

"Antiretroviral therapy (ART) could affect exposure of TB drugs. Data on concomitant medication will be recorded and included as a covariate in the pharmacokinetic analysis" - Elaborate on it.

How would you separate the indirect absorption rate among each drug group? What analysis will eliminate the cross-over reaction among the drugs?

"Pregnant or lactating women requiring, or those who started, treatment for drug-sensitive tuberculosis will be identified and invited for sampling". How many n from each group? It isn't very clear with the study design.

What time points for the predose?

Will the weight be normalized to the maternal and infant plasma/breast milk concentration?

Is any sex-specific effect on absorption included in the analysis?

**Is the rationale for, and objectives of, the study clearly described?**

No

**Is the study design appropriate for the research question?**

Partly

**Are sufficient details of the methods provided to allow replication by others?**

No

**Are the datasets clearly presented in a useable and accessible format?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Placental -Fetal Programming

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.**

Author Response 15 Feb 2024

**RITAH NAKIJOBA**

**Reviewer 1 Comment Response** The rationale for doing the behavior test needs to be addressed in the background of the abstract.

The statement added on rationale for the behavior test in the background section of the abstract. Similarly, women face mental health challenges while taking medications, including postpartum depression. Specifically, it is recognized that rates of depression are high during treatment for tuberculosis. This may have an impact on their motivation to take their medicine, hence the need to assess the psychological behavior of a breastfeeding woman. [The total study population is n=20; how many n for each drug category \(rifampicin, isoniazid, ethambutol, and pyrazinamide\)?](#) The study will recruit 20 breastfeeding women overall. [The sample size will cater to all the drug categories. First-line tuberculosis treatment consists of rifampicin, isoniazid, ethambutol, and pyrazinamide administered as a fixed-dose combination pill.](#) This has been specified in the abstract. More detail is given further in the protocol. women will be recruited regardless of the phase of TB treatment, intensive and continuous phases. This is well documented in the study procedures under the screening section. There has been no strong data upon which to build a sample size calculation. We have discussed the interim analysis to refine some of our assumptions – adding a line to the abstract: “Pharmacokinetic data will be analyzed using a population pharmacokinetic approach, and an interim analysis of rifampicin concentrations will be conducted after the first 5 participants have had a pharmacokinetic sampling.” What are the current studies dealing with TB drugs and it is breastfeeding content?

No recent studies have investigated TB drugs in breastfeeding women. However, old studies from 1965-1990 explored TB drugs among breastfeeding women including; Ricci and Copaitich reported peak levels of INH in breast milk of 6 mcg/mL and 9 to 11 mcg/mL after the administration of 5 mg/kg and 10 mg/kg, respectively, given to three women, Knowles measured milk INH concentrations taken 1–10 hours after the administration of

5–10 mg/kg, Holdiness reported a maximum pyrazinamide breast milk concentration of 1.5 mcg/mL measured 3 hours after maternal ingestion of a single 1 gram dose as compared to a maternal plasma concentration of 42 mcg/mL 2 hours after the dose, Does the ethnicity have any impact on it?

Secondary metabolite levels are not measured? Any impact on the infant?

In previous studies in non-pregnant, non-breastfeeding adults, ethnicity has not had a significant impact on drug concentrations. For isoniazid, metaboliser status according to the polymorphic NAT enzyme plays a role. A full investigation of the impact of ethnicity and pharmacogenomic analysis is beyond the scope of this study, although our results may inform the design of a future, larger, multinational investigation. In this protocol, we will not measure any secondary metabolites in either plasma or breast milk. However, if our study finds that appreciable amounts of the drug end up in breastmilk/infant we can then assume that this might translate to particular metabolites as well. It is confusing and unclear to suddenly introduce uncomplicated malaria data with TB resistance. What outcome was added to the current data? Be clear in explaining the rationale.

This has been clarified to explain that these other studies are sister protocols taking place under the MILK fellowship. The drugs and diseases are different, but we are using the same approach to the mental health aspects of the protocols and therefore, those specific data will be analysed together with the other work. [Again, introducing the HIV-infected group \(60-80%\) and only the remaining 20% would be noninfected HIV group taking anti-tuberculosis treatment? Any possibility of getting the statistical test comparison?](#)

[The intercurrency of HIV and concomitant antiretroviral medication will be considered as a covariate in the pharmacokinetic analysis.](#) The statement has been added to the protocol section; Disease setting and patient population What is the Km value of each drug, and what is the half-life? Generally, high inter-patient variability in pharmacokinetics has been reported for the four first-line anti-tuberculosis drugs. Rifampicin has a time-to-peak plasma concentration of approximately two hours after oral dosing and a half-life of approximately 2.5 hours (doi: 10.2165/00003088-197803020-00002). The plasma half-life of isoniazid is closely related to the patient's genetics (doi: [10.1128/AAC.02599-12](#)), ranging from approximately one hour in fast acetylators to more than 3 hours in slow acetylators. The peak plasma concentrations of pyrazinamide range from 0.8 - 8 hours after dose, whereas its half-life ranges from 4.5 - 11 hours (doi.org/10.1007/s13318-018-00540-w; <https://doi.org/10.1128/AAC.02625-16>). The peak plasma concentrations of ethambutol occur 2 - 4 hours after administration and has a plasma half-life between 3-4 hours (doi: <https://doi.org/10.1128/aac.01583-19>). ["Antiretroviral therapy \(ART\) could affect exposure of TB drugs. Data on concomitant medication will be recorded and included as a covariate in the pharmacokinetic analysis" - Elaborate on it. "The commonly used Antiretroviral therapy \(ART\) could affect the pharmacokinetic exposure of the TB drugs. The information on the type and dose of ART drugs co-administered to a TB patient will be recorded and integrated into the analysis of the pharmacokinetics of TB drugs to characterise the differences in pharmacokinetic exposure between patients on concomitant TB / ART treatment and those receiving only TB drug.](#) Statement added How would you separate the indirect absorption rate among each drug group? What analysis will eliminate the cross-over reaction among the drugs?

Thank you for the question: For clarification, Although TB medication is administered as a

fixed dose combination, different assays will be applied to the pharmacokinetic samples to quantify the concentration of each drug for each patient at a given time point. From these concentration-time data, we will develop drug-specific population pharmacokinetic models, one for rifampicin, isoniazid, ethambutol and pyrazinamide. These mathematical models will describe the concentration-time profiles for each drug accounting for the dose administered and estimate different pharmacokinetic parameters. As such the rate and extent of drug absorption will be individually characterised for each of the four drugs basing on the collected pharmacokinetic data. "Pregnant or lactating women requiring, or those who started, treatment for drug-sensitive tuberculosis will be identified and invited for sampling". How many n from each group? It isn't very clear with the study design.

The target recruitment number of 20 has been added in the study design section to reflect the required sample size. What time points for the predose?

The pre-dose sample will be obtained at different time points depending on what time the participant has reported to the clinic. The participant will come to the clinic first thing in the morning before taking their dose of treatment. A pre-dose blood sample will be drawn within 30 minutes of the observed dosing. Will the weight be normalized to the maternal and infant plasma/breast milk concentration?

Weight and BMI will both be included as covariates in the pharmacokinetic model. Is any sex-specific effect on absorption included in the analysis? This population is breastfeeding mothers only. It is known that pregnancy may slow drug absorption due to delayed gastric emptying and reduced gastric pH – and some of this change may persist into the postpartum period. The intensive sampling after dose aims to ensure we characterize absorption well, and can then compare with similar work from non-pregnant adult populations, including both males and females.

**Competing Interests:** No competing interests