Title: The pharmacokinetic parameters of tenofovir (TFV) in maternal blood and breast milk in women treated with daily Tenofovir Disoproxil Fumarate (TDF; 300mg) for prevention of mother to child transmission (PMTCT) of hepatitis B virus (HBV) mono-infection

Short Title: Tenofovir in pregnancy to prevent mother to child transmission of Hepatitis B.

Date and Version No: V.3.0, dated 28 October 2019

Trial registration number: NCT04114890

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OxTREC reference: 39-19

Protocol no.: VIR19001

Date and Version No: V.3.0, dated 28 October 2019

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Name	Department	Role & Function in this project	Email
Rose McGready	Professor tropical maternal and child health (Deputy Director SMRU)	Principal Investigator	rose@shoklo-unit.com
Marieke Bierhoff	Department of Internal Medicine and Tropical Diseases, Academic Medical Center Amsterdam, The Netherlands	Co-Principal Investigator	mariekebierhoff@yahoo.com
Podjanee Jittmala	Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University Thailand	Faculty Tropical Medicine Study Representative	Podjanee@tropmedres.ac
Joel Tarning	Department of Pharmacology, Mahidol Oxford Tropical Medicine Research unit, Mahidol University	Co-investigator	joel@tropmedres.ac
Clare Ling	Department of Microbiology, SMRU	Co-investigator	clare@tropmedres.ac
Francois Nosten	Director SMRU	Co-investigator	francois@tropmedres.ac
David Burger	Department of Pharmacy, Radboud University Medical Center, Nijmegen, The Netherlands	Co-investigator	David.Burger@radboudumc.nl

Conflict of Interest

The investigators declare no conflict of interest.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team and members of the Research Ethics Committee. This information cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the principal investigator.

Signature…… Date ……..

Name ……Rose McGready……

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Table of Abbreviations

ALT	Alanine transaminase
ARVs	Antiretrovirals
AUC	Area Under Curve
CRF	Case Report Form
DNA	DeoxyriboNucleic Acid
DOT	Directly Observed Treatment
EGA	Estimated Gestational Age
GCP	Good Clinical Practice
HB	Hepatitis B
HBeAg	Hepatitis B e antigen
HBIG	Hepatitis B immunoglobulin
HBsAg	Hepatitis B s antigen
HBV	Hepatitis B virus
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
MTCT	Mother to child transmission
OXTREC	Oxford Tropical Research Ethics Committee
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
PK	Pharmacokinetics
PMTCT	Prevention Mother to child transmission
REC	Research Ethics Committee
SOP	Standard Operating Procedure
SMRU	Shoklo Malaria Research Unit
TDF	Tenofovir disoproxil fumarate
TFV	Tenofovir

1. Synopsis

Study Title	The pharmacokinetic parameters of tenofovir (TFV) in maternal blood and breast milk in women treated with daily Tenofovir Disoproxil Fumarate (TDF; 300mg) for prevention of mother to child transmission (PMTCT) of hepatitis B virus (HBV) mono-infection.	
Internal ref.no.	VIR19001	
Study Design	Prospective descriptive dense pharmacokinetics study of Tenofovir (TFV) in 24 migrant pregnant women treated with Tenofovir disoproxil fumarate (TDF) to prevent mother to child transmission of Hepatitis B.	
Study Participants	Women with viable pregnancy with HBV mono-infection	
Planned Sample Size	24 pregnant women – 12 will enroll in 2nd trimester (T2) and 12 in 3rd trimester (T3)	
Planned Study Period	August 2019 - August 2021	
	Objectives	Outcome Measures
Primary	To measure the dense PK parameters of TFV in a steady-state during three gestational periods in TDF treated HBV mono infection	PK parameter profiles in maternal blood
Secondary	1. To investigate the TFV concentration , breastmilk and infants	- Maternal breastmilk TFV concentrations - Milk to maternal plasma concentration ratios (M/P) - Infant plasma drug concentration
	2. To investigate if/when HBV DNA is detected in blood and breastmilk after cessation of TDF.	HBV DNA in blood and breastmilk

2. Introduction – Background and Prior Research

Hepatitis B virus (HBV) can be asymptomatic for years but can also lead to chronic disease, cancer and hepatitis, hepatocellular carcinoma, and liver failure be eradicated with the current therapy. (1-3) Chronic maternal Hepatitis B virus (HBV) infection is an important source of perinatal transmission in regions of high HBV prevalence. In antenatal clinics at Shoklo Malaria Research Unit (SMRU), the Hepatitis B (HB) surface antigen (sAg) prevalence is 8.3% with a HB e-antigen (HBeAg) prevalence of 32.7% in those positive for HBsAg in 2012-2014.(4) Perinatal infection occurs in 70–90% of women with HBeAg positive chronic HBV compared with 0–30% in those with HBeAg negative chronic HBV (inactive carriers).(1) These infection rates reflect, in part, the failure of maternal and child health programs to prevent perinatal transmission with hepatitis B immunoglobulin (HBIG) and HB vaccines. Prevention of mother to child transmission (PMTCT) fails in an estimated 8–32% of cases with adequate preventive techniques.(5, 6) Antiretrovirals (ARVs), like Tenofovir Disoproxil Fumarate (TDF) are active against HBV and may reduce the risk of HBV transmission at birth if offered at the right time in pregnancy.(7-9)

One of the major gaps in implementing this strategy is adequate pharmacokinetic (PK) data in pregnant women that informs correct dosing. One recently published population PK study in 154 women who provided single maternal blood samples (32 and 36 weeks of pregnancy, at delivery, and at 1 and 2 months postpartum) reported a tenofovir area under curve (AUC) 0–24 that was estimated to be 20% (95% CI, 19-21%) lower during pregnancy than during postpartum suggesting no dose adjustments are needed in 3rd trimester.(10) Most PK studies for TDF in pregnancy have been for Human Immunodeficiency Virus type 1 (HIV) infections. However, these patients often receive additional antiretroviral medications, preventing conclusions on PK parameters of Tenofovir (TFV) alone (TFV results following absorption after conversion of the oral prodrug TDF).(11) Doses that are optimal for Human Immunodeficiency Virus (HIV) may not be appropriate for HBV.

When TDF is administered during pregnancy and potentially during lactation, it is important to establish the infant drug exposure. Previous human studies have shown that antiretrovirals administered to lactating mothers are present in the breast milk and have detected a low TDF breast milk concentration representing 0.03% or less of the proposed infant dosage.(13)((14) However, there is no data on this subject in therapeutic treatment of HBV infected women. In resource poor settings TDF administration will be ceased after 1 month post-partum and while

there is some data to understand what happens to viral load post cessation in non-pregnant, post-partum TDF cessation is less well understood but where immunity may differ critically.(12, 15) With breastmilk as the primary source of nutrition for babies in resource limited settings it is important to know the viral exposure from breastmilk, if any, as these settings may also have problems achieving birth dose, HBIG and the 3 dose vaccine completion.

3. Study rationale & justification

We propose a dense PK study of once daily TDF 300 mg during pregnancy given for PMTCT of HBV mono-infection. Tenofovir PK will be measured in maternal blood samples in steady-state, in the 2nd and 3rd trimesters and post-partum. The presence of HBV DNA in blood and breast milk will also be explored while women after cessation of treatment until 6 months post-partum.

4. Study Objectives and Outcome Measures

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure (if applicable)
Primary Objective To measure the dense PK parameters of TFV in a steady-state during three gestational periods in TDF treated HBV mono infection	PK parameter profiles s in maternal blood	Blood sampling every month and dense sampling every gestational period
Secondary Objectives To investigate the TFV concentration , breastmilk and infants	- Maternal breastmilk TFV concentrations - Milk to maternal plasma concentration ratios (M/P) - Infant plasma drug concentration	At delivery and 1 month post-partum
To investigate if/when HBV DNA is detected in blood and breastmilk after cessation of TDF.	HBV DNA in blood and breastmilk	Post-partum monthly maternal blood and breastmilk samples up to 6 months.

5. Study Design

Participants with HBV mono infection and a measurable HBV DNA viral load attending SMRU antenatal clinics (ANC) on the Myanmar-Thailand border will be invited if they have a gestational age of at least 20 weeks. A total of 24 women: 12 participants enrolled in 2nd trimester (EGA ≥20-<24 weeks) and 12 enrolled in 3rd trimester (EGA ≥28-<36) with complete samples are required.

Women, identified by routine antenatal care screening for HBV will be invited to participate if they are HBsAg positive and meet the inclusion and exclusion criteria. Participants will be provided TDF 300mg daily and will continue TDF treatment until one month after delivery. The TFV concentrations will be measured monthly before delivery, at delivery, in cord blood and 1 and 2 months postpartum. Additional dense PK blood sampling will be done in the second and third trimester and postpartum. Breast milk samples timed with mother blood and infant blood samples will be included to measure drug concentrations in breastmilk as possible presence of HBV DNA viral in breastmilk following cessation of TDF.

The dense sampling for pharmacokinetics assessments will occur at least after 2 weeks of TDF treatment in second trimester between 22–26 weeks gestation, in third trimester between 30–38 weeks gestation, and 4 weeks postpartum. The dense sampling will entail twelve blood samples in a 24 hour period (24ml of blood in total). Women will be counseled about the importance of adherence. Direct Observed Treatment (DOT) in the 3 days prior to the dense blood sampling will be performed. The woman can choose to be admitted for these days or if a home visitor lives close enough be supervised at home.

The follow-up for women will be until 6 months post-partum, to check for flares. TFV concentrations will be collected by venous blood testing but an indwelling catheter can be used during the rich sampling 24 hour period. Infants will be followed up from delivery, and at 1, 2, 4 and 6 months of age for vaccination and growth. Infant drug exposure is measured at month 1 and HBV DNA will be measured at birth from cord blood and HBsAg by venepuncture at 2 months of age. The flow chart is available in Appendix B.

Study duration

We will enroll volunteers into this study after ethical approval. Because we will enroll subjects in gestational windows we expect the recruitment and follow up to last at least 2 years.

6 Participant identification and recruitment

6.1 Study Population

Pregnant women aged 16-49 years with confirmed HBV mono infection (identifiable from the routine care provided at SMRU clinics) will be included.

6.2 Inclusion criteria

All of the inclusion criteria must be answered as 'yes' to consider the participant eligible:

- Single viable pregnancy at enrollment day
- Estimated Gestational Age (EGA) ≥20-<24 weeks for 2nd trimester or EGA ≥28-<34 for 3rd trimester
- Willing and able to give informed consent for participation in the study
- Hepatitis B infected (HBsAg and HBeAg confirmed positive or HBsAg confirmed positive and HBV DNA detected in HBeAg negative)
- Burmese and Karen female, 16-49 years (inclusive)
- Willing to take TDF daily during pregnancy
- Plans to deliver at SMRU clinics

6.3 Exclusion criteria

If any exclusion criteria are answered as 'yes' the subject may not enter the study:

- Undetectable HBV DNA in HBeAg negative women
- HIV infected or other chronic illness incompatible with the study requirements or receiving Immunosuppressive therapy
- Creatinine at screening >1 mg/dL
- Serum phosphate <2.4 mg/dL
- History of chronic kidney disease

6.4 Co-Enrolment Guidelines

Subjects can be enrolled into any other study while participating in this study as long as it doesn't interfere with the inclusion criteria or study procedures.

6.5 Study site

The study will take place in the Shoklo Malaria Research unit (SMRU) clinics, Mae sot.

7. Study Procedures

7.1 Pre-study training

Pre-study training is an essential part of this study to ensure this study is conducted according to Good Clinical Practice (GCP) and the approved version of protocol.

7.2 Subject screening and enrolment

Records of currently pregnant women attending SMRU antenatal care clinics (ANC) will be reviewed by the doctor and those who are HBV infected will be approached. For new pregnant women attending ANC, a routine part of antenatal care (not study related) is screening at the first visit for HBV and HIV. Samples collected from women identified as HBsAg positive by routine rapid detection kit Pacific Biotech Co Ltd. (2 drops of capillary blood) at the SMRU clinics will be sent to a certified laboratory in Thailand for confirmation of HBsAg and HBeAg status (routine). When the rapid test is found positive women will be invited to learn more about the study by discussion with a trained counsellor.

After signing the informed consent form, all HBV infected subjects will be given a screening number at the time of screening. If they pass screening and are enrolled into the study, they will be given a subject number.

7.3 Study Evaluation

7.3.1 Screening Visit, Day -7

At screening visits, the subjects who are tested HBV infected and have a viable singleton pregnancy will be required to undergo the following tests and procedures:

- Obtain informed consent

- Obtain blood samples for laboratory tests; biochemistry (creatinine, phosphate, ALT), HBsAg, and HBeAg tests (and HBV DNA qualitative test if the mother if HBeAg negative)

7.3.2 Study enrollment, Day 0 (Baseline)

- The participant comes to get an ultrasound to make sure there is a single viable pregnancy. The blood tests will be checked and if the participant is eligible, they can enroll in the study. If the criteria are not met, the participant can not enroll.
- Obtain demographic information
- Perform physical examination including vital signs, height and weight
- Provide medical history including history of all medications, vitamins, and allergies
- Provide obstetric data (Symphysis fundal height, fetal gestational age and heartbeat, details of any past pregnancies, complications, place of delivery and outcome will be recorded)

7.3.3 TDF administration

Generic GPO-TDF 300mg tablet will be used for this study. TDF 300mg will be administered daily and continue until 28 days post-partum.

The subject will be counseled about the importance of adherence. For the dense PK sampling it is of great importance to be in a steady state (see 7.3.5.1 “Pharmacokinetic Evaluation). To ensure this, we will perform DOT in the 3 days prior to the dense blood sampling. The subject can choose to be admitted for DOT administration (see 8.2.4 “Blood tests”)

Adherence survey and pill count will be performed monthly when the subject returns to the clinic to ensure compliance of TDF.

7.3.4 Evaluation of TDF regimen

Subjects will have creatinine and ALT concentrations checked every month. They will be asked about other side effects like nausea, vomiting or diarrhea.

7.3.5 Follow up

7.3.5.1 Monthly follow up during pregnancy

From the time TDF is started through the end of the study, the participants will return for monthly study visits. At each visit pregnancy will be checked for maternal and fetal well-being. Obstetric follow-up at each monthly visit will include the following:

- physical examination
- vital signs and weight
- symphysis fundal height measurement
- fetal heart beat
- concomitant medications
- blood sample for biochemistry (creatinine, ALT), and HBV DNA (See details in Appendix C).

7.3.5.1.1 Pharmacokinetic Evaluation

The dense PK sampling will occur after 2 weeks of TDF treatment in second trimester between 22–26 weeks gestation and in third trimester between 30–38 weeks gestation. The blood collections (approximately 2 ml) will be taken at the following times relative to the dose of TDF: pre-dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 hours post-dose.

7.3.5.2 Delivery

The following will be performed at delivery:

- Physical examination, vital signs, co-medication
- Pregnancy complications and mode of delivery will be recorded.
- Anthropometric measurements of the newborn will be done in the first hour of life and will include birth weight, length, head and arm circumference, by trained staff and according to work instructions and standard operating procedures.
- Vaccination standard HB vaccine at delivery (birth dose) and at 2, 4 and 6 months post-partum will be provided to newborns of mothers with chronic HBV infection.

- HBIG will be provided to all children born to mothers in the study according to the Thai National Guidelines.
- Mother blood sample will be obtained for clinical laboratory assessments; biochemistry (creatinine, Phosphate, ALT), TDF concentration and HBV DNA.
- Cord blood sample will be obtained after the birth of the baby and before delivery of the placenta for HBV DNA and TDF concentrations, and for HBsAg and HBeAg testing.
- Breast milk collection (until 2 days post partum).
- Pill count and adherence survey
- AE and concomitant medications

7.3.5.3 Post-partum follow up of mothers

The subject will be followed up monthly until 6 month post-partum. The following procedure will be performed on the mothers:

- Physical examination and vital signs at each post-partum visit.
- TDF will be continued until one month post-partum to theoretically decrease the risk of flares as their immune system returns to baseline.
- The last dense PK sampling will occur at 4 weeks post-partum.
- At the 1 month post-partum follow up, the woman will again have dense PK sampling of her blood. A single breastmilk sample will be taken 4 hours after drug dosing.
- At the 1 month post-partum visit, Creatinine will be measured, if Creatinine is increased (≥ 1.5 times from the baseline) this will be repeated according to routine care.
- The last blood sampling on PK (single blood sample) will be at the 2 month post-partum.
- At the 1 and 4 months post-partum visits, ALT will be measured (from 5ml and 2 ml respectively). If ALT is increased with a Grade 3 or higher (described in 13.3.1) but there is not a true flare, the ALT will be repeated at 6 months post partum.

- At each post-partum visit month 1 to month 6, breastmilk samples will be collectedand blood samples will be collected for HBV DNA tests

7.3.5.4 Post partum follow up of mothers with flare

The majority (95%) of the women will have the study endpoint at 6 months. However, if flare is detected, these women will be followed for a further 6-9 months and provided with drug therapy where monitoring shows it is required.

7.3.5.5 Post-partum follow up of infants

The following will be performed:

- Evaluation of growth at 1, 2, 4 and 6 months
- Vaccinations at 2, 4, and 6 months
- TDF drug concentration at 1 month
- Test for HBsAg at 2 months with a 3 mL venepuncture sample.

7.3.6 Subjects transferred to another hospital

If a subject needs to be referred to another hospital for an adverse event, such woman is still considered to be in the study and attempts will be made to obtain relevant data and follow them up after hospital discharge in accordance with the study schedule.

8. Notes on study procedures / investigations

8.1 Clinical

On the days of the dense PK sampling, vital signs will be done just before the blood sample to obtain an accurate blood pressure measurement in the absence of pain/stress associated with taking the blood sample.

A more detailed physical examination will be conducted if clinically indicated by the reported symptoms and the vital sign data. Further investigations will be done as clinically indicated. See on Adverse Events.

8.2 Blood sample collections

The total blood volume of blood over the course of the study for the mother depends on the timing of enrollment and the details are provided in the sample collection table below. On average a woman who commences participation in trimester 2 will give 144 mL total while participation commencing in trimester 3 will provide 96 mL total.

8.2.1. Mother blood sample collections

A 6 ml venipuncture sample of whole blood will be collected at baseline and during monthly follow up (Table 1). Three mL will be placed in EDTA, plasma separated, and stored in 2 separate Eppendorf tubes for later testing of HBV DNA concentration. Another 3 ml will be placed in a plain tube, serum separated and (HBsAg, HBeAg at baseline) ALT, creatinine, and serum phosphate will be measured. HBV DNA and TDF concentrations will be determined. Any leftover serum will be stored frozen at -80°C.

8.2.2. Cord blood sample collections

Cord blood samples of 12 mL will be collected after the birth of the baby and before delivery of the placenta. The cord will be cleaned with a swab and a 23 g needle inserted into the umbilical cord to aspirate 12 mL with 6 mL into EDTA and 6 mL into a plain tube. The tubes will be centrifuged and resulting plasma will be aliquoted in two separate Eppendorf tubes and tested for HBV DNA and TDF concentrations. Plain tubes will be centrifuged after clot formation and serum aliquoted, and eppendorf tubes sent for HBsAg and HBeAg testing at Mae Sot, or if leftover sample, frozen at -80°C.

8.2.3. Infant blood sample collections

Infant blood sample of 3 mL at the 1 and 2 month visit will be drawn up and 1.5 mL placed into EDTA and 1.5 mL into a plain tube. Resulting plasma from the 1 month EDTA blood sample will be aliquoted in two separate Eppendorf tubes and tested for HBV DNA and TDF concentrations. Plain tubes will be centrifuged after clot formation and serum aliquoted, and Eppendorf tubes sent for HBsAg testing at Mae Sot, or if leftover sample, frozen at -80°C.

8.2.4 Breastmilk sample collection

At each sample time, staff and the participant will assess if she has adequate breast milk to collect a sample without adverse effect to the baby (eg. insufficient nutrition) or herself (eg. excessive squeezing). Participants will have the option to obtain breast milk by manual expression or using a (electronic) pump. If the woman has a cracked or bleeding nipples, this will be noted and appropriate care will be provided for the woman. If possible, milk for measurement of viral load will be collected from an intact nipple.

A. Colostrum (Viral load):

When the woman begins to have adequate colostrum production postpartum (day 1-2 on average), she will be asked to express 3 ml of colostrum into a sterile container. The woman may express from either or both breasts. Use of a breast pump will be allowed but hand expression is expected to be most effective.

B. 1 month breast milk (PK and viral load):

At hour 4 post-dose the mother will be asked to express the entire contents of one breast (choose the more full breast) into one clean container. The breast milk will be measured, mixed and a minimum of 10 ml aliquot taken. Remaining milk will be given to the mother to feed back to her infant with spoon, cup, or syringe, according to her preference. Infant feeding will be allowed to continue on demand (as is the cultural norm and recommended by the WHO) between the drug dose and the breast milk sample, and exposure to the infant will be considered essentially continuous.

C. 2-6 month breast milk (viral load):

At a convenient time during the visit, the mother will be asked to express a minimum of 5 ml into one clean container.

Table 1. Sampling schedule

Type of sample	Timing of sample									
	EGA 20	EGA 24	EGA 28	EGA 32	EGA 36	delivery	PP 1 month	PP 2 month	PP 3,4,5,6 month	Total
Baseline										

Type of sample	Timing of sample									
	EGA 20	EGA 24	EGA 28	EGA 32	EGA 36	delivery	PP 1 month	PP 2 month	PP 3,4,5,6 month	Total
Mother blood	6ml	6ml	6ml	6ml	6ml	6ml	6ml	6ml	6ml	72ml
Dense PK		24ml			24ml		24ml			72ml
Delivery cord						12ml				12ml
Baby							3ml	3ml		6ml
Breastmilk						colostrum 3ml	10ml mature milk	5ml mature milk	5ml mature milk	38ml
Total mother blood (if enrolled at EGA 20 weeks)										144ml
Total mother blood (if enrolled at EGA 36 weeks)										96ml

8.2.4 Blood tests

Pharmacokinetic measures: We will measure TFV (and HBV DNA) concentrations every month but dense sampling will occur at three periods: in second trimester between 22–26 weeks gestation (if enrolled), in third trimester between 30–38 weeks gestation and 4 weeks post-partum. During dense PK sampling, blood will be drawn via an intravenous catheter inserted before the first sample. The exact time frames are shown in Table 2, where 0 is the sample before the first dose of TDF.

Table 2. PK sampling times

	Blood samples Dense PK timing, at baseline and hours after drug intake											
	0 (pre-dose)	**0.5**	**1**	**1.5**	**2**	**3**	**4**	**6**	**8**	**12**	**16**	**24**
EGA 22-26	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml
EGA 30-38	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml
1 month post-partum	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml	2.0ml

PK: Whole blood into EDTA tubes will be taken and spun down at 1500-2000 ×g for 10 minutes to obtain plasma. The plasma will then be aliquoted in two separate cryovials, labelled and stored at minus 80ºC until analysis in the laboratory of Prof. David Burger in Nijmegen, the Netherlands. This laboratory is accredited (NEN-EN-ISO 15189) and uses an in house validated Liquid chromatography–mass or tandem-mass spectrometry (LC-MS/MS).

Biochemistry: creatinine, ALT and phosphorus and HBV DNA (1st and delivery cycle): Another aliquot of whole blood 6 mL of blood will be drawn up and 3 ml put into EDTA and 3 ml into a plain tube. EDTA will be processed immediately. Blood will be centrifuged to separate plasma and serum for 5 minutes.

Plasma will be used for HBV DNA quantification using the HBV DNA assay Fast Track Diagnostics (http://www.fast-trackdiagnostics.com/human-line/products/ftd-hepatitis-b-dna/). Remaining serum or plasma will be stored at -80°C in case any test result need clarification.

8.3 Sample labelling

Study samples, including stored samples, will be labelled with:

- Subject number
- Date of specimen collection
- Time specimen collection
- Type of specimen

8.5 Safety net

The net will include:

- Pre-study training will be given to the study team with an emphasis on:
  - taking informed consent
  - performing monthly clinical assessments
  - making it easy to ask for a consult by senior colleagues
  - explaining how to use the TDF stopping rule as a guide (eg. kidney failure or ALT rise due to TDF treatment, see 13.3.1)
- check for hepatic flare (see 13.3.1)

9. Drug Management

9.1 Supply Storage and Handling

All TDF tablets in this study should be kept in a controlled room temperature (25°C or 77°F); excursions permitted between 15°C to 30°C (59°F to 86°F).

9.2 Drug Accountability

SMRU clinics will maintain a record of the numbers of all TDF tablets obtained, used by the subjects and returned to the pharmacy. These information will be recorded in the drug accountability record during the study. On study completion, a copy of the drug accountability record will be filed in the study folder. Unused TDF will be returned to the SMRU pharmacy.

9.3 Concomitant Medications

Subjects may develop minor illnesses during follow up. These will be treated by the research team.

Drugs that may cause kidney failure or interfere with the metabolism of TDF must is prohibited throughout the study participation. (e.g. aminoglycosides (e.g., gentamicin), and high-dose or multiple NSAIDs.

10. Discontinuation/Withdrawal of Participants from Study

Each subject has the right to withdraw from the study at any time. In addition, the Investigator may discontinue a subject from the study at any time if the Investigator considers it necessary for any reason.

10.1 Voluntary withdrawal of consent

Subjects may withdraw from the study at any time and for any reason.

10.2 PI withdrawal

A subject will be withdrawn by the PI from the study if:

- Significant protocol deviation (for example not taking medication for >2 months)
- Subjects experience adverse drug reaction that may affect their safety during the study as judged by the investigator
- Significant non-compliance with treatment regimen or study requirements
- Loss to follow up (>3 visits)

10.3 Management of withdrawn subjects

The care of pregnant women that have been withdrawn from the study will continue through the delivery and post-partum period irrelevant of the reason for withdrawal. It is important for pregnant women to receive proper antenatal, delivery and post-partum care. The data collected until the point of withdrawal will be included in the study and this is stated in the participant information sheet. The reason for withdrawal will be recorded in the CRF. If a subject withdraws after receiving TDF, Alanine transaminase (ALT) testing will be done (when possible) to monitor for hepatic flare. Every effort will be made to make sure that the subject understands this. Participants who withdraw will be replaced as complete sample sets are necessary for the analysis.

10.4 Study Discontinuation

The study may be discontinued by the ethics committee/s and/or the study sponsor. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of AEs indicating a potential health hazard to participants
- Investigators do not adhere to the protocol

11. Subject Safety monitoring & adverse event reporting

This study will use the Common Toxicity Criteria v 5.0 for grading adverse events. It is available from this website:

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf

11.1 Adverse Events

From the time TDF is started through the end of the study, the subjects will return for monthly study visits (timed with routine ANC visits) where a case report form, an adverse events (AE) form, and an adherence form will be completed. Pre-specified AEs include headache, nausea, vomiting, and ALT flares after discontinuation of TDF will not be recorded as an AE. Any unexpected AEs will be documented according to standard definitions and procedures.

11.2 Grading of an Adverse Event

We will use the CTCAE as a reference for grading adverse events

11.3 Relatedness of Adverse Event to TDF

In this study, the relationship between an AE and TDF will be reported as one of the following:

- Not related
- Unlikely related
- Possibly related
- Probably related or
- Definitely related.

11.4 Serious Adverse Event Recording

All adverse events are recorded in the CRF. A serious adverse event (SAE) occurring to a subject should be reported to the EC that gave a favorable opinion of the study where in the opinion of the Chief Investigator the event was 'related' (resulted from administration of any of the research procedures) and 'unexpected' in relation to those procedures. Reports of related and unexpected SAEs should be submitted within 5 working days of the Chief Investigator becoming aware of the event, using the SAE form.

11.5 Subject Management of Adverse Events

All AEs will be treated as clinically indicated, and if concomitant treatment is given this will be recorded on the Concomitant Medication CRF. If necessary, participants will be referred for specialist care/advice.

The subject will be followed and treated by the research team until the clinical or laboratory AE has resolved or stabilised. The physician should perform any tests that are clinically indicated.

11.6 Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect.

Other 'important medical events' may also be considered serious if they jeopardize the subject or require an intervention to prevent one of the above consequences.

11.7 Reporting of Serious Adverse Events

In this protocol, reporting means that SAEs must be reported by email by the study physicians to the study co-PI (mariekebierhoff@yahoo.com, 0987520822) within 24 hours of awareness using the SAE report form. The study co-PI (Marieke Bierhoff) will be responsible for reporting the SAEs to the Ethics Committee per required timeline.

11.8 Safety Review of the Laboratory Report

All laboratory data will be reviewed by the team physicians to determine if kidney failure or a laboratory AE is present. The physicians or the co-PI will sign the laboratory report to confirm the report has been seen. Safety information will be reviewed on a regular basis.

12. Statistics

12.1 The Number of Participants

As this is a descriptive study of the PK, the sample size of 24 participants, who generate 60 dense PK curves, is assumed to describe the PK profile adequately and generate robust parameter estimates with reasonable precision.

12.2 Study endpoints

12.2.1 Primary endpoint

The primary endpoint is the Dense TDF concentration for 24 participants repeated at 3 gestational periods (2^{nd} and 3^{rd} trimester of pregnancy and 1 month post-partum). The end of the study is the date of the last visit to the clinic by a study subject which is anticipated as when the last infant involved in the study reaches 6 months of age.

12.2.2 Secondary endpoints

These are:

- Infant drug exposure comparing TDF concentrations in breastmilk samples, infant plasma and maternal plasma.
- Presence or absence of HBV DNA in maternal blood and breastmilk particularly after TDF cessation

- Proportion of infants that are positive for HBsAg and HBV DNA at birth (using cord blood), and HBsAg 2 month of age

12.3 Data Analysis

Data cleaning will be performed prior to analysis to identify incomplete and incorrect data. All women will be censored at delivery for the analysis. Detailed statistical methods are described below.

12.3.1 Continuous data

These data will be summarized by medians (ranges) and means (standard deviations), as appropriate, and will include the biochemical parameters.

12.3.2 Pharmacokinetic data

Densely collected TDF samples will be analysed using a model-independent approach (i.e. non-compartmental analysis) with the help of pharmacokineticist specialists (prof David Burger and prof Joel Tarning). All collected samples will be pooled and analysed using nonlinear mixed-effects modelling to develop a detailed PK model that describes the concentration-time relationship and possible impact of clinical covariates, such as age and body weight. Particular focus will be on describing and quantifying potential PK differences during pregnancy (i.e. increasing gestational age) and compared to post-partum.

12.3.3 Infant drug exposure

Infant exposure to TFV will be estimated using drug concentration in maternal plasma, maternal breast milk, and infant plasma. Milk/plasma ratio will be calculated using the paired breast milk and plasma concentrations at 4 hours after dosing. This may slightly overestimate the M/P ratio as determined by the ratio of the concentration AUC for breast milk and plasma, as hour 4 is expected to be the peak breast milk concentration, and plasma concentrations will be decreasing. This M/P ratio will be compared with published values from prior studies in women receiving using TDF in combination with other antivirals for treatment or prevention of HIV.

13 Human subjects considerations

13.1 Ethical Review

All projects are discussed with the Tak Community Advisory Board (T-CAB) for relevance, advice and acceptability. The protocol, participant information sheet, informed consent form will be submitted to OxTREC and local ethics committees for written approval. The Investigator will submit and, where necessary, obtain approval from the above parties for all amendments to the original approved documents.

13.2 Informed Consent

Informed consent will be administered by the ANC counsellor trained in the procedure and fluent in the language of the participant. The subject (and guardian for minors) must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed. Illiterate participants will be accompanied by an independent literate witness throughout the procedure.

Written version of the participant information and informed consent will be presented to the participants (and guardians for minors) detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. Moreover, it will inform about the total blood volume that will be taken throughout the study, the sampling procedure for the PK and the follow up of the child. It will be clearly stated that the subject is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal. If someone withdraws after starting on drug, we will monitor for flares for 3 months for the woman's own safety. This is clearly stated in the information sheet.

The subject (and guardian in minors) will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, midwife or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of subject (and guardian for minors) dated signature and dated signature of the person who presented and obtained the informed consent (or thumbprint in the case of non-literate participants). The person who obtained the consent will be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participants. The original signed form will be retained at the study site.

The informed consent and assent forms are available as separate documents which have been written in English until their review by the Ethics Committees. This approved document will be translated to Karen and Burmese language and submitted to the Ethics Committees before the study commencement. The Karen and Burmese version will be back translated to English for accuracy.

13.3 Risks

13.3.1 Risks of oral TDF

TDF is globally being used in pregnant women for prevention of mother to child transmission of HIV. It is generally well tolerated with the most common adverse reactions identified from any of the large controlled clinical trials include rash, diarrhea, headache, pain, depression, asthenia, and nausea.(16, 17)

Other side effects are:

- Kidney failure defined as:
    - A rise in serum creatinine ≥ 1.5 times from the baseline OR
    - An absolute rise in serum creatinine of ≥26.5 μml/L (≥ 0.3 mg/dl) within 48 hours
- ALT rise to Grade 3.

  Grade 3 or higher is >5x upper limit of normal (ULN) if baseline levels were below the ULN; or if baseline levels were >ULN and, then grade 3 or higher is >3.5x baseline value. The normal value of ALT will be defined as 0-31 U/L.(18)
- Hepatic flare

  A tangible adverse consequence that requires further study is the potential for hepatitis flare following discontinuation of therapy in the mother. All women will continue on study until the 4 month post-partum visit. At the 1 and 4 months post-partum visits ALT will be measured (from a 5ml and 2 ml blood sample respectively). If 3 months after stopping TDF, a flare is detected (ALT >5x baseline if this was increased at baseline or >10x the upper limit of normal with no alternative diagnosis) the woman will continue to be monitored until resolution of the flare. If the flare worsens or does not resolve they will be placed on anti-HBV therapy and be reviewed by a physician. If ALT is increased but there is not a true flare,

(ALT>3.5x baseline if this was increased at baseline or >5x the upper limit of normal), they will be followed for an additional 6-9 months and repeat ALT testing done at 6 months.

13.3.2 Risk of Phlebotomy

The primary risks of phlebotomy include local discomfort, occasional bleeding or bruising of the skin at the site of needle puncture, and rarely haematoma or infection. There is a small risk of infection with cannulation but these will be minimized by universal precautions and removing the cannula directly after the last sample.

13.4 Benefits

13.4.1 Benefits to the individuals

Potentially treatment with TDF of the mother during pregnancy and post-partum will decrease the number of children infected with HBV though MTCT.

13.4.2 Benefits to society

This study will provide information on the PK of TFV following oral TDF for prevention of MTCT of HBV mono-infection, which is relevant to provision of correct dosing.

13.4.3 Future research & drug policy implications

With the recent shift in WHO policy and new guidelines for the Elimination of Hepatitis B. The issue of cessation of TDF in breast feeding mothers requires clarity and this study will provide additional insight in this area. Since TDF is not merely used in HBV PMTCT but also in HIV PMTCT, results of this study could potentially inform us about alternate regimens in this patient group as well.

13.5 Alternatives to Study Participation

Subjects are free to decline participation in this study and may withdraw from it at any time.

13.6 Compensation

In this study participants who get the dense PK sampling done will receive 300 Baht for that day. The participants who are admitted for DOT will receive additionally 100 baht per day admitted. Transportation costs for study related visits will be reimbursed. If the subject is withdrawn from the study before the study ends, the subject will be compensated for the time that the subject is actually enrolled in the study. In addition, a small gift valued 100 Baht for the baby will be given at 2 months.

13.7 Participants Confidentiality

All study related documents will be stored securely at the study site in locked filing cabinets in areas with access limited only to study staff. All laboratory specimens, including stored specimens, reports, study data collection, process, and administrative forms will be identified by a subject number.

Forms, logbooks, appointment books, informed consent forms, and any other listings that link subject numbers to other identifying information will be stored in a separate, locked file in an area with limited access. All databases will be secured with password-protected access systems. Participant's study information will not be released without the written permission of the participant, except as necessary for the independent monitoring. Representatives of sponsor, monitors, regulatory authorities, the ECs can request to see the source documents. The results of the research study will be published with subject details de-identified.

Subject's clinical data and results from blood analyses stored in our database may be shared with other researchers to use in the future. However, the other researchers will not be given any information that could identify the subject.

13.8 Insurance Arrangement

The University of Oxford has a specialist insurance policy in place, Newline Underwriting Management Ltd, at Lloyd's of London, which would operate in the event of any subject suffering harm as a result of their involvement in the research.

14. Laboratory specimens & practice

14.1 Laboratory Specimens

The study site will follow the principles of good clinical laboratory practice (GCLP) for clinical trials and local standard operating procedures (SOPs) and SOPs or work instructions specifically for this study for the collection, processing, labelling, transport and storage of all specimens.

The following laboratory tests/procedures will be performed as part of this protocol:

- routine biochemistry
- HBV DNA qualitative testing

- HBV DNA quantitative testing
- HBV serology (HBsAg and HBeAg)
- TFV drug concentrations (in Nijmegen, the Netherlands)

14.2 Quality Control and Quality Assurance

The clinics have their own systems for quality assurance (QA) e.g. running internal quality control, machine calibrations, in accordance with the manufacturer's instructions and relevant international guidelines. The PK laboratory at Nijmegen, the Netherlands has ISO accreditation for quality and competency and safety (NEN-EN-ISO 15189) since 2016.

14.3 Specimen Storage

Study site staff will store specimens in appropriate, fully functioning freezers (e.g. DNA can be stored at -20 to 80°C) so that the protocol related analyses can be performed. After these tests have been performed, blood will be kept for long term storage for 10 years in the first instance, if subjects give signed informed consent. Informed consent will specify that the stored blood samples may be used in the future for the analyses of hepatitis B related fields of research including genetics without participants identifier.

14.4 Specimen transport

After the last subject gave the ;ast PK blood sample, the specimens for TDF drug concentrations will be transferred from Mae Sot to Nijmegen, the Netherlands by air. In-house SOPs or work instructions will be followed for packing and transferring samples. The necessary IATA Regulations will be followed. The signed Material Transfer Agreement will be arranged and submitted to the local Ethics Committee.

15. Administrative procedures

15.1 Data Management

Direct access will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with regulations. During the study, investigators from the above mentioned institutions, and their institutional review boards, may have access to study documents like informed consent forms to make sure that the study is properly conducted. After the study, data may be shared with other scientists only after identifiers (names, date of birth, residence, etc.) have been removed.

Only those staff with permission to review the files will enter data. All study data will be entered on a MACRO database at SMRU using SOP at SMRU. The MACRO database will be password protected and only people working on the study will have access to this database. The participants will be identified by a study specific participants number and/or code in any database. The name and any other identifying detail will NOT be included in any study data electronic file. Data will be stored securely at SMRU for 20 years. Records will be kept in locked cabinets unless they are in use by the study team.

15.2 Record Retention

The Investigator will maintain, and store in a secure manner, complete, accurate study records throughout the study. Study records include CRFs, consent forms, and all other essential documents including reports and correspondence relating to the study, minutes of team minutes, e-mail communication with the sponsor.
All pertinent study documentation will be kept in the Investigator site file (ISF).
According to the University of Oxford policy on retention period, subject records will be retained until the youngest child participating in the trial reaches 21 or five years following completion of the study, whichever is longer. The study database will be retained indefinitely.

15.3 Use of Information and Publications

The results of this study will be published following international guidelines and norms. Any data published in the peer-reviewed medical literature will protect the identity of the subjects. This trial will be registered in a web based protocol registration scheme. All those who have made a substantial contribution will be co-authors on publications.

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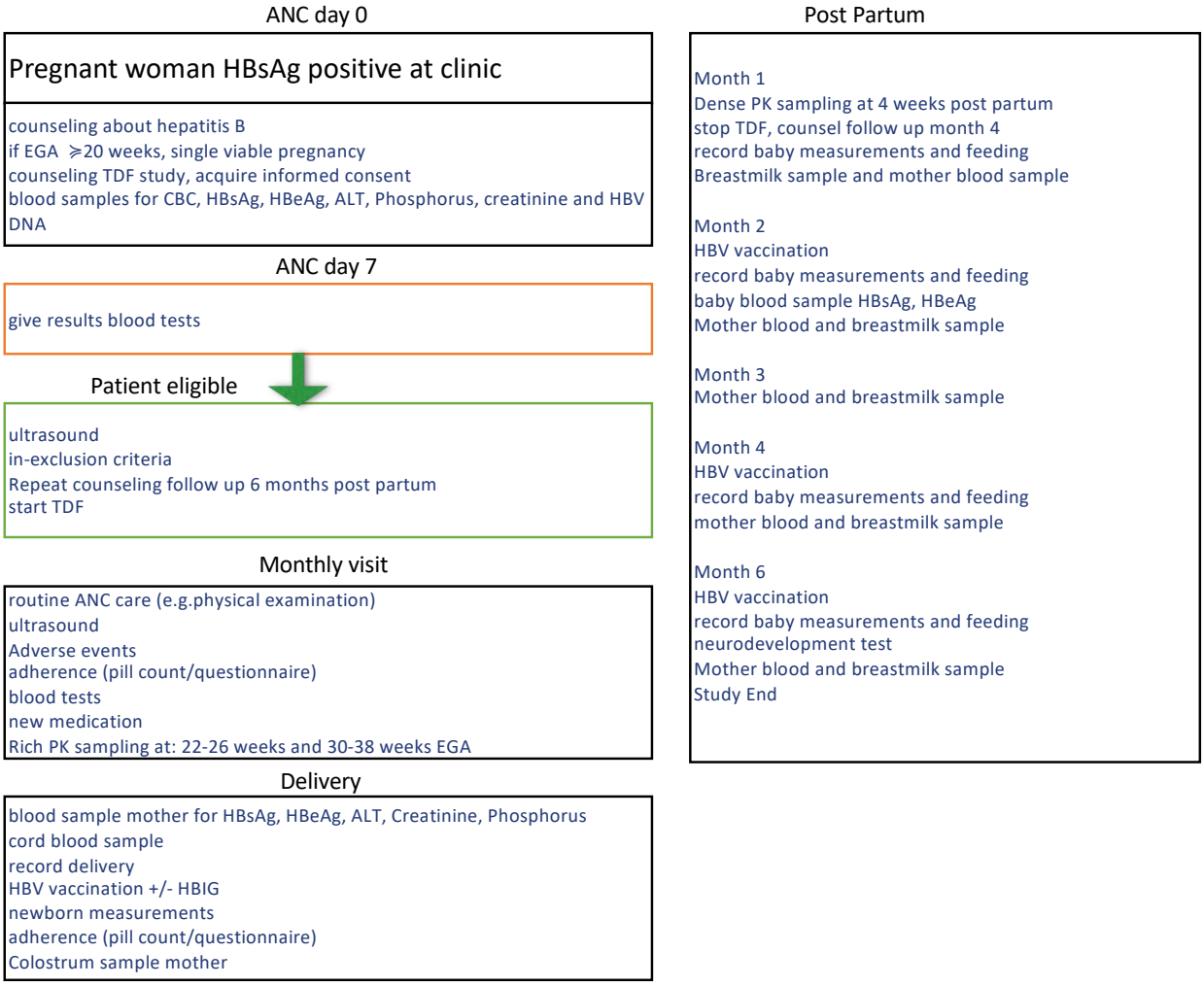
17. Appendix A: Thai National Guideline for prevention of maternal-to-child transmission of hepatitis B

(Pediatric Infectious Disease Society of Thailand)

18. Appendix B: Study flow



19. Appendix C: Schedule of study procedures

Activity		Visits timing									
		Day -7	**Day 0**	**Monthly pregnant visits**	**Delivery**	**1 mth post-partum**	**2 mth post-partum**	**3 mth post-partum**	**4 mth post-partum**	**5 mth post-partum**	**6 mth post-partum**
Mother											
Study Drug Dispensing			**X**	**X**	**X**	**X**					
Obtained Informed consent		X									
Medical History			X								
Physical Examination			X	X	X	X	X	X	X	X	X
Vital Signs			X	X	X	X	X	X	X	X	X
Measure Height and Weight			X	X							
Ultrasound			X	X							
Serology (HBsAg/HBeAg)		X			X/X[3]						
Eligibility checklist		X	X								
Enrollment			X								
HBV DNA		X		X	X	X	X	X	X	X	X
Blood Chemistry	Creatinine	X		X	X	X					
	Phosphate	X			X						
	ALT	X		X	X	X			X		X[1]
TDF concentration				X[2]	X	X[2]	X				
Adherence survey				X	X	X					
Pill count				X	X	X					
Directly Observed Therapy				X[4]		X					
AE and Concomitant Medication Assessment				X	X	X	X	X	X	X	X
Breastmilk sample collections					X	X	X	X	X	X	X
Infant											
Anthropometry: weight, length, head (and arm) circumference					X	X	X		X		X
Cord blood HBsAg/HBeAg/HBV DNA/TDF concentration					X						
HBV vaccination					X		X		X		X
Infant HBsAg							X				
Infant TDF concentration						X					

[1] Follow-up will continue if suspected or confirmed flare

[2] The dense PK blood sampling is further shown in table 2.

[3] in Cord blood

[4] Before dense PK sampling

20. Appendix D: Amendment History

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made

List details of all protocol amendments here whenever a new version of the protocol is produced. This is not necessary prior to initial REC submission.