Official Title:
A Maternal Short Course of Tenofovir Disoproxil Fumarate and Infant Vaccine to Prevent Mother-to-child Transmission of Hepatitis B Virus

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iTAP-2

Primary Statistical Analysis Plan

Version 1.0

A Maternal Short Course of Tenofovir Disoproxil Fumarate and Infant Vaccine to Prevent Mother-to-Child Transmission of Hepatitis B Virus

Short Title:

Antiviral prophylaxis and infant vaccination to prevent perinatal hepatitis B infection

Based on Protocol Version 1.2

ClinicalTrials.gov Identifier: NCT03343431,

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Version History

Version	Changes Made	Date Finalized
1.0	Original Version based on protocol version 1.2 (dated July 19, 2019).	November 30, 2023

1 Introduction

1.1 Purpose

This Primary Statistical Analysis Plan (SAP) describes the primary and secondary outcome measures of the iTAP-2 study addressing primary and major secondary objectives of the study. The Primary SAP outlines the general statistical approaches that will be used in the analysis of the study. It has been developed to facilitate discussion of the statistical analysis components among the study team, and to provide agreement between the study team and statisticians regarding the statistical analyses to be performed and presented in the primary analysis report.

1.2 Version History

Not applicable; original version.

2 Study Overview

2.1 Overview of Study Design

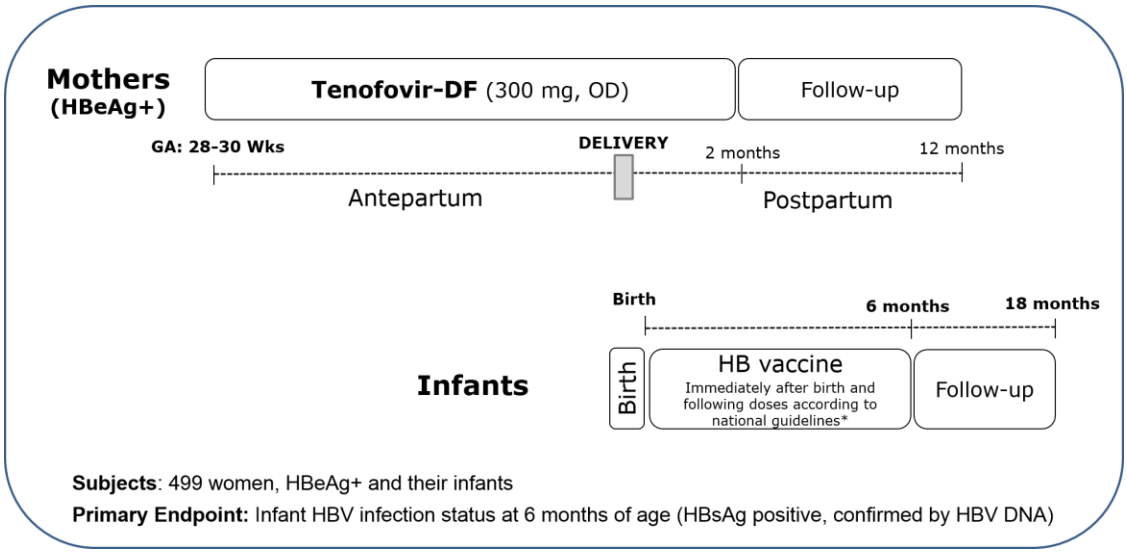
iTAP-2 is a "prospective, multicenter, international (Thailand and Lao PDR), open-label, single arm clinical trial in hepatitis B surface-antigen (HBsAg) and hepatitis B e-antigen (HBeAg) positive pregnant women (from 28 weeks until one year postpartum) and their infants (until 18 months of age)" (*Protocol Version 1.2, Section 3*). The goal of the study is to assess the risk of hepatitis B virus (HBV) perinatal transmission in infants who receive active immunization but no Hepatitis B immune globulin (HBIg), born to mothers with satisfactory virological response on tenofovir disoproxil fumarate (TDF) 300 mg per day from 28 weeks gestation to 2 months postpartum (*Protocol Version 1.2, Section 5.1*).

From *Protocol Version 1.2, Section 3:*

> Study visits for mothers… take place at 28, 32 and 36 weeks' gestation, at delivery, and 1, 2, 4, 6, and 12 months postpartum. Infant visits are scheduled at birth, 1, 2, 4, 6, 9, 12, and 18 months.
>
> After the beginning of treatment and at the latest at 36 weeks' gestation, virological response to study treatment will be assessed using HBV DNA load measurement. If the level of HBV DNA load has not significantly decreased (at least by 10 fold in IU/mL) and is above 200,000 IU/mL, the site study team will contact the participant to reinforce adherence to study treatment and possibly recommend HBIg administration to the newborn if available.
>
> Infants will receive HB vaccine immediately after birth and per national guidelines and their HBV infection status will be assessed at 6 months of age.

Maternal study treatment will be discontinued two months after delivery. However, women with baseline ALT >60 U/L will be assessed for [chronic] HBV treatment indications [after delivery and] before discontinuation during the postpartum period.

Diagram of Study Design (*Figure 2* from *Protocol Version 1.2):*



HB vaccine schedule in Thailand: at birth, 1, 2, 4, and 6 months
HB vaccine schedule in Lao PDR: at birth, at 6, 10, and 14 weeks

2.2 Study Eligibility

Study inclusion criteria at time of enrollment for consenting pregnant women consist of age ≥18 years old at 28 weeks (+/- 7 days), gestational age with positive HBsAg, positive HBeAg, and negative HIV antibody tests during the pregnancy (*Protocol Version 1.2, Sections 7.1.1 and 7.1.2*). Additionally, women must not display clinical symptoms of liver disease (*Protocol Version 1.2, Section 7.1.1*). All infants born alive to participating mothers can be enrolled in the study (*Protocol Version 1.2, Section 7.1.2*).

Study exclusion criteria include receipt of anti-HBV antivirals at any time during the 9 months before the enrollment, known liver cirrhosis or evidence of hepatocellular carcinoma, Cockcroft-Gault creatinine clearance <50 mL/min, confirmed dipstick proteinuria >1+ (>30 mg/dL) or normoglycemic glycosuria, evidence of pre-existing fetal anomalies incompatible with life, and any concomitant condition or treatment that, in the view of the clinical site investigator, would contraindicate participation or compromise adherence to treatment and satisfactory follow up in the study (*Protocol Version 1.2, Section 7.1.3*).

2.3 Hypothesis

From *Protocol Version 1.2, Section 4.2 and Section 8.1:*

> It is hypothesized that the HB viral load reduction at the time of delivery will ensure a low viral exposure at the time of delivery and HB vaccine given immediately at birth will provide an efficacious post-exposure prophylaxis, even in the absence of HBIg… We hypothesize that the risk of HBV mother-to-child transmission in this study will be lower than 2% (i.e., the upper limit of the 95% confidence interval of the observed rate is less than 2%) because the mothers receive tenofovir disoproxil fumarate during the last trimester of pregnancy and for two months following delivery and the infants will receive vaccine, starting with the birth dose.

2.4 Study Objectives

This Primary SAP addresses the primary and secondary objectives listed in the study protocol. From *Protocol Version 1.2, Section 5*:

2.4.1 Primary Objective

To demonstrate that the risk of HBV infection at 6 months of age is lower than 2% in infants who receive active immunization but no HBIg, born to HBeAg positive mothers with satisfactory virological response on TDF 300 mg per day from 28 weeks gestation to 2 months postpartum.

2.4.2 Secondary Objectives

1. To assess pregnancy outcomes, and infant outcomes until 18 months of age.
2. To retrospectively determine the risk of transient infection in infants not chronically infected at 18 months of age using anti-HBc antibodies.
3. To describe infants' response to HB vaccination in the absence of confounding from HBIg administration, assessed by anti-HBs antibody levels at 1, 2, 4, 6, 12 and 18 months.
4. To describe the changes in maternal HBV DNA level from initiation of TDF to delivery.
5. To compare the risk of infant HBV infection at 6 months of age with that observed in infants who received HBIg at birth plus HBV vaccine in one of the 2 arms of a previous study conducted in a similar setting in Thailand (iTAP, NCT01745822).
6. To assess the risk of transmission in all infants born in the study, including those born to women with unsatisfactory virological response to study treatment.

2.5 Study Visit Schedule

2.5.1 Maternal Follow-Up

From Protocol Version 1.2, Section 7.2.1:

Eligibility and pre-enrollment tests should be performed prior to enrollment. After enrollment at 28 weeks, maternal visits will be at 32, [and] 36 weeks gestation, delivery, and at 1, 2, 4, 6, and 12 months postpartum... All efforts should be made to strictly follow the maternal and infant visit schedule but occasional departures of no more than one week will be considered acceptable.

Schedule of visit and study procedures/assessments for mothers (*Table 2* from *Protocol Version 1.2):*

Pregnant Women / Mothers	*Antepartum*					*Postpartum*				
		Study treatment								
	Pre-enrollment	Enrollment* 28 w.	32 w.	36 w.	Delivery	1	2	4	6	12
						months after delivery				
Information about the study, consent process	X	X								
Counseling, Medical exam	X	X	X	X	X	X	X	X		X
Documented telephone contact with the participant									X	
Study treatment dispensation and/or adherence assessment (self-report and pill counts), return of unused study treatment		X	X	X	X	X	X			
Record results of previous HBsAg, HBeAg, HIV and HCV serology and sonogram results		X								
ALT (SGPT), AST (SGOT)[1]		X			X		X	X		
Complete Blood Count		X			X			X		
Serum creatinine[2]		X	X				X			
Dipstick glycosuria and proteinuria		X					X			
HBeAg										X
HBV DNA load		X		X[3]	X					
Tenofovir plasma level[4] (retrospective)				X	X					
Plasma storage		X	X	X	X	X	X	X		X
Cell pellets storage		X								
Total volume blood (mL)		16	10	6	11	4	13	10		8

[1] **APRI score** will be calculated at 4 months postpartum using the formula (AST/ULN) x 100) / platelet count (10^9/L) and **FIB-4 =** (age (yr) x AST (IU/L)) / (platelet count (10^9/L) x [ALT (IU/L)$^{1/2}$]).
[2]Creatinine clearance will be calculated using the Cockcroft-Gault formula.
[3]To be measured real-time
[4]To assess adherence in relation to virological response.
* Enrollment can be completed only after transmission to the study coordination center of all relevant information and approval.

From *Protocol Version 1.2, Section 7.2.1:*

> Women with ALT ≤ 60 U/L at enrollment will discontinue treatment two months after delivery. Those with ALT > 60 U/L will be assessed during the postpartum period for

potential long-term treatment indications before discontinuation of study treatment to prevent flares…

After the beginning of treatment and at the latest at 36 weeks' gestation, virological response to study treatment will be assessed using HBV DNA load measurement (the date of this assessment should be determined taking into account the risk of premature delivery). If the level of HBV DNA load has not significantly decreased (at least by 10-fold in IU/mL) and is above 200,000 IU/mL, the site study team will contact the participant to reinforce adherence to study treatment.

2.5.2 Infant Follow-Up

From *Protocol Version 1.2, Section 7.2.1:*

Each infant will have 8 [scheduled] study visits from birth to 18 months of age… Infant peripheral blood will be drawn for HBsAg test at 1, 2, 4, 6, 9, 12, [and] 18 months (before vaccine administration if at the same date). All infants will receive HB vaccine according to national guidelines. In Thailand infants will receive vaccine immediately after birth, then at 1, 2, 4, and 6 months. In Lao PDR infants will receive vaccine immediately after birth, then at 6, 10, and 14 weeks.

Following the assessment of maternal virological response to study treatment, HBIg administration to the newborn may be considered if the level of maternal HBV DNA load has not significantly decreased (at least by 10 fold in IU/mL) and is above 200,000 IU/mL.

If anti-HBs < 10 IU/L (and [available] HBsAg/HBV DNA negative) at 6, 12, and 18 months of age, the pediatrician in charge will consider revaccination.

Schedule of visits and study procedures/assessments for infants (*Table 3* from *Protocol Version 1.2):*

Infant assessments	**Birth**	**1 month**	**2 months**	**4 months**	**6 months**	**9 months**	**12 months**	**18 months**
Counseling and physical examination	X	X	X	X	X	X	X	X
Record date, time of HBV immunization	X	X	X	X	X			
HBsAg	X[2]	X[1]	X[1]	X[1]	X[1]	X	X	X
HBV DNA PCR	X[2]	X[2]	X[2]	X[2]	X[2]	X	X[2]	X[2]
Anti-HBs antibodies	X	X	X	X	X	X	X	X
Anti-HBc Antibodies								X

Plasma storage (including cord blood)	X	X	X	X	X	X	X	X
Cell pellets storage	X							
Total volume (mL)	4	5	5	5	5	5	5	5

[1] Blood must be drawn before injection of HB vaccine

[2] If HBsAg positive at 6 months

In case of planned early withdrawal from the study, the investigator will ask for [mother's] authorization to perform a last blood draw from her and her infant for safety evaluations and for documentation of the infant's HBV status (see [protocol] Tables 2 and 3).

2.6 Overview of Sample Size Considerations

A total of 499 pregnant women were targeted to be enrolled.

From *Protocol Version 1.2, Section 3 and Section 7.5.3:*

> A sample size of 386 evaluable cases is needed to provide 87% power to demonstrate that the upper 95% [Clopper-Pearson] confidence interval [(CI)] limit [(one-sided, exact based on the binomial distribution)] of the risk of transmission is lower than 2% (i.e., the observed risk would be lower than 0.5%). Accounting for 12% unsatisfactory virological response to study treatment at 36 weeks' gestation, and 12% unevaluable cases, 499 is the final sample size.

2.7 Interim Monitoring

A Data and Safety Monitoring Board (DSMB) monitors the study at least annually (see Protocol Version 1.2, Section 7.6 for more information). During the COVID-19 pandemic, monthly updates are sent to the DSMB summarizing accrual, study events related to the pandemic, COVID-19 cases, and public health interventions in Thailand in Lao PDR.

3 Outcome Measures

3.1 Primary Outcome Measure

For this study, the primary outcome measure is infant HBV infection at 6 months. Infant HBV infection is defined as a positive HBsAg test confirmed by HBV DNA detection by PCR at 6 months of age.

3.2 Secondary Outcome Measures

Secondary outcome measures for Secondary Objectives:

- For Objective 1:
    - Occurrence of pregnancy outcomes: full term or preterm live births, stillbirths, spontaneous abortions, induced abortions

- Occurrence of maternal and infant International Conference on harmonization (ICH) serious adverse events (SAEs) or grade 4 adverse events per DAIDS Grading able*, regardless of their relatedness to the study treatment

  Note: language for this outcome measure was modified from the protocol to clarify the outcome definition, per discussion with study chair

- For Objective 2: Detectable anti-HBc antibodies in children at 18 months of age
- For Objective 3: Levels of anti-HBs antibodies in infants at 1, 2, 4, 6, 12, and 18 months
- For Objective 4: HBV DNA levels in women at treatment initiation and time of delivery.
- For Objective 6: HBV infection status as defined for the primary outcome measure, among all infants

* U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. [July 2017]. [Internet].
Available from: https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf

4 Statistical Principles

4.1 Definitions

Gestational age (GA) at entry will be based on site reported ("Pre-Enrollment" CRF) GA based on the best estimate from obstetrician judgment guided by the review of the date of last menstruation period, physical exam and sonogram (see *protocol section 5.2*).

The date of enrollment will be taken from the enrollment date on the "Enrollment Visit" CRF. The date of delivery and date of birth will be taken from the date recorded on the "Delivery Visit" and "Declaration of Delivery" CRFs, respectively.

Baseline is defined as the measure taken during the enrollment visit, or the latest measure taken before enrollment if the enrollment measure is not available. Baseline measurements taken during the enrollment visit on the same date as treatment initiation are assumed to be taken before treatment initiation.

Unsatisfactory virological response to study treatment is defined as an HBV DNA load measurement between 20 days after the beginning of treatment and at the latest at 36 weeks gestation (taking into account the risk of premature delivery) that has not decreased by at least by 10-fold in IU/mL since baseline and is above 200,000 IU/mL. If there are multiple measurements, use the first measurement in the interval of ≥20 days from treatment initiation to the earlier of 36 weeks gestation and delivery.

Preterm birth is defined as a birth occurring at <37 weeks gestational age based on the Ballard Maturational Assessment of Gestational Age recorded on the "Newborn Visit" CRF.

Infant HBV infection at 6 months of age will be defined as a positive HBsAg test at 6 months of age confirmed by PCR detection of plasma HBV DNA.

A sample with an HBV DNA not detected will denote a lack of infection at time of sampling, regardless of HBsAg result. HBV DNA detectable will be defined as a quantifiable HBV DNA level above the limit of detection of the assay or a detected but unquantifiable HBV DNA level below the limit of detection. The limit of detection will be calculated as the standard detection limit of the assay (15 IU/mL) multiplied by the dilution factor.

According to the protocol, samples with a HbsAg positive test are to be assayed by PCR for HBV DNA detection. In case the HBV DNA PCR result is not available for any reasons, such HbsAg positive samples are considered HBV positive. In case the HBsAg test result is missing but HBV DNA PCR result is available, the PCR result is used. Infants with missing data for both HBsAg and HBV DNA at a given time point will be excluded from the analysis at the time point. However, an infant with no HbsAg and no HBV DNA PCR result at the 6-month visit will be deemed as having a 6-month positive HBV status for the primary analysis if the infant has two positive HbsAg and/or HBV DNA PCR results at either (1) two or more previous consecutive time points before the 6-month visit performed after 3 weeks of age and at least 3 weeks apart, or (2) at one time point before the 6 months window period and one after.

4.2 Visit Schedule and Analysis Windows

The abbreviations and visit week time windows for this analysis are shown in the tables below. Time visit windows are inclusive at the lower end of the range and exclusive at the upper (e.g., date of delivery + 2 months - 14 days ≤ MPP2 < date of delivery + 2 months + 14 days for Thailand). A month is defined as 365.25/12 days. Women delivering prior to 37 weeks will not be expected to have a 36 weeks gestational age (GA 36) visit.

Study Visit	Analysis Window	Window on CRFs
Pre-Enrollment		
Enrollment	≤3 months prior to date of enrollment to 1 day after (estimated GA 28 weeks -7 days/+7 days)	GA 28 weeks ±7 days
GA32	Estimated GA 32 weeks ±14 days	GA 32 weeks ±7 days
GA36	Estimated GA 36 weeks ±14 days	GA 36 weeks ±7 days
LD: Delivery	(-5/+14)	*No range on CRF or Manual of Operating Procedures (MOP).*
MPP1	For Thailand: 1 month (+/- 14 days) after delivery For Lao PDR: 6 weeks (+/- 14 days) after delivery	For Thailand: 1 month (+/- 7 days) after delivery For Lao PDR: 6 weeks (+/- 7 days) after delivery *Visit schedules differ because vaccine schedules differ by country*

MPP2	For Thailand: 2 months (+/- 14 days) after delivery For Lao PDR: 10 weeks (+/- 14 days) after delivery	For Thailand: 2 months (+/- 7 days) after delivery For Lao PDR: 10 weeks (+/- 7 days) after delivery
MPP4	For Thailand: 4 months (+/- 28 days) after delivery For Lao PDR: 14 weeks (-14/+28 days) after delivery *Include windows for both countries but have them not overlap with MPP2 and MPP6 visits*	For Thailand: 4 months (+/- 7 days) after delivery For Lao PDR: 14 weeks (+/- 7 days) after delivery
MPP6	For Thailand and Lao PDR: 6 months (-28/+42 days) after delivery	For Thailand and Lao PDR: 6 months (+/- 7 days) after delivery
MPP12	12 months (+/- 42 days) after delivery	12 months (+/- 7 days) after delivery
Study Visit	**Analysis Window**	**CRF Window on CRFs**
Birth	Birth date (+ 7 days)	Immediately after birth
C1	For Thailand: 1 month (+/- 14 days) after birth For Lao PDR: 6 weeks (+/- 14 days) after birth	For Thailand: 1 month (+/- 7 days) after birth For Lao PDR: 6 weeks (+/- 7 days) after birth
C2	For Thailand: 2 months (+/- 14 days) after birth For Lao PDR: 10 weeks (+/- 14 days) after birth	For Thailand: 2 months (+/- 7 days) after birth For Lao PDR: 10 weeks (+/- 7 days) after birth
C4	For Thailand: 4 months (+/- 28 days) after birth For Lao PDR: 14 weeks (-14/+28 days) after birth	For Thailand: 4 months (+/- 7 days) after birth For Lao PDR: 14 weeks (+/- 7 days) after birth
C6	For Thailand and for Lao PDR: 6 months (-28/+42 days) after birth *For HBsAg and HBV DNA measurements, (-30/+90), i.e. <9 months*	For Thailand and Lao PDR: 6 months (+/- 7 days) after birth

C9	9 months (+/- 42 days) after birth	9 months (+/- 7 days) after birth
C12	12 months (+/- 42 days) after birth	12 months (+/- 7 days) after birth
C18	18 months (+/- 42 days) after birth	18 months (+/- 7 days) after birth

If multiple evaluations in one visit window occur, clinic visits will be prioritized over telephone contacts for analysis, regardless of the proximity to the target visit date (i.e., if a telephone visit happened closer to the target visit date than a clinic visit, the clinic visit will still be used). If there are multiple records of a visit type (e.g., two clinic visits within the window), prior to enrollment the evaluation closest to enrollment will be used, and after enrollment (baseline) the evaluation closest in absolute value to the target visit time will be used. If two evaluations are equidistant from the target visit time, the earlier evaluation will be used.

4.3 Premature Study and Treatment Discontinuation

Premature study discontinuation will be summarized with categories death, unable to continue (detrimental to participant's health or well-being), lost to follow-up (or unreachable (protocol 7.1.4)), unable to get to clinic, unexpected closure of site, or withdrawn consent from the "Study Discontinuation" CRF.

Permanent study treatment discontinuation (before completion) will be defined any primary reason provided as other than "Completed as defined by the protocol" in the "Study Treatment Discontinuation" CRF.

4.4 Study Visit Compliance

For study visit compliance, the target number of women/infants at each scheduled visit will be calculated as the number of women/infants on-study that have passed the expected visit date given above by 7 days before 4 months post-partum/of age and by 14 days thereafter. For women, the LD visit will be expected if 40 weeks gestation has passed by 7 days before the woman discontinues the study.

4.5 Analysis Approaches

Adverse pregnancy outcomes and HBV transmission will be analyzed at the pregnancy level in that if there are one or more events in a multiple pregnancy they will count as one event for the pregnancy. Descriptions of the infant characteristics, study conduct, and infant safety will analyze infants from a multiple pregnancy separately. Unless otherwise stated, binary outcome measures will be summarized by estimating proportions and associated Clopper-Pearson confidence intervals (CIs): 90% CI for the primary objective analysis and analysis of objective 6, 95% CI otherwise. The Kaplan-Meier (KM) method and Greenwood's formula for the standard error will be used to calculate probability estimates and 95% CIs for time-to-event analyses.

HBV DNA related analyses will be carried out based on measurements from Chiang Mai laboratory in Thailand, as laboratory results from the local Laos laboratory were found to be systematically elevated when below 7 log10 HBV DNA, leading to recommendations for HBIg

administration when the protocol criteria for HBIg administration had, in fact, not been met. This was due to the Laos laboratory's use of an in-house method to measure HBV DNA rather than the benchmark Abbott method used in the Thai laboratory.

4.5.1 Analysis Sets

The following analysis sets will be flagged for analysis, and are referenced in analysis descriptions below.

Maternal

- *Maternal Full set*: all enrolled mothers not later found ineligible as indicated on the "Protocol Deviation Log" case report form (CRF)
- *Maternal Treated set*: Maternal Full Set who initiated study treatment (took at least one dose of TDF 300 mg/day)

Infant

- *Infant Full set*: live born infants of mothers in the Maternal Full Set who enrolled into the study
- *Infant Exposed set*: live born infants of mothers in Maternal Treated Set
- *Infant Exposed noHBIg set:* live born infants of mothers in the Maternal Treated Set who did not receive HBIg
- *Infant Exposed noHBIg and Satisfactory Maternal Virologic Response Set (Infant Exposed noHBIg-SMVR):* infants of Maternal Treated Set who did not receive HBIg, and whose mothers had a satisfactory virologic response to TDF.

4.5.2 Primary Objective

Infant HBV Infection

Objective: To demonstrate that the risk of HBV infection at 6 months of age is lower than 2% in infants who receive active immunization but no HBIg, born to HBeAg positive mothers with satisfactory virological response on tenofovir disoproxil fumarate (TDF) 300 mg per day from 28 weeks gestation to 2 months postpartum.

This analysis will be carried out on the Infant Exposed noHBIg-SMVR Set.

The proportion of infants with HBV infection at 6 months of age will be estimated and the exact (two-sided) 90% CI around this estimate will be calculated based on the Clopper-Pearson method with a one-sided significance level of 0.05. Pregnancies resulting in multiple births (e.g., twins) will be counted as one mother-infant pair, and will be counted as HBV-infected if at least one infant was infected. A complete case analysis approach will be applied, which includes only infants whose 6month endpoint is available (assuming infant data are missing completely at random).

Three supplementary/sensitivity analyses will be conducted: 1) supplementary: considering infants from multiple pregnancies separately, 2) sensitivity: imputing the last available HBV

infection status before 6 months for infants without 6-month status data, and 3) sensitivity: considering infants with missing data as HBV-infected. These protocol-specified analyses will be conducted at the: 1) infant level, 2) maternal level, and 3) maternal level, respectively.

An additional sensitivity analysis will be conducted including infants who missed the 6-month visit window due to the COVID-19 pandemic, but had available endpoint data later

4.5.3 Secondary Objectives

Pregnancy and Infant Outcomes

Objective 1: To assess pregnancy outcomes and infant outcomes until 18 months of age.

This analysis will be carried out on (1) the Maternal Treated Set and (2) Infant Exposed Set.

The proportions of women with full term live births, preterm live births (<37 weeks gestational age at birth), stillbirths, spontaneous abortions, and induced abortions, along with the two-sided Clopper-Pearson 95% CIs, will be provided separately, for each outcome. If any multiple gestation pregnancies result in different outcomes (e.g., twins with one full term live birth and one stillbirth), a “discordant birth outcome” category will also be included. A complete case analysis approach will be applied, which includes mothers with pregnancy outcome data available (assuming data are missing completely at random). A supplementary analysis will be conducted using a composite adverse pregnancy outcome (preterm live birth, stillbirth, spontaneous or induced abortions). An additional supplementary analysis using the composite adverse pregnancy outcome and including early neonatal death (i.e., death of a live newborn before (<) 7 days of age) will be conducted. The “Delivery Outcome” CRF will provide still birth information, and the “Adverse Events” CRF abortion information. Gestational age estimated by Ballard score will come from the “Newborn Visit” CRF.

The proportion of women with any serious adverse events (SAE: ICH SAE) or DAIDS grade 4 adverse events from enrollment until the latest study visit date will be provided, along with the two-sided Clopper-Pearson 95% CI; the proportion of infants with any adverse events from birth until the latest study visit date and two-sided Clopper-Pearson 95% CI will also be provided. Mothers (infants) who discontinue follow-up prior to the 12 months (18 months) study visit will have their outcome determined based on data available until the time of discontinuation (i.e., a mother who discontinued follow-up without a prior AE is assumed not to have an AE had they been observed for the intended duration [12/18 months, best case scenario]).

Time to first ICH SAE or DAIDS grade 4 adverse event will be illustrated with a Kaplan-Meier plot, censoring event time at the latest study visit date if no ICH SAE or DAIDS grade 4 adverse event occurs. Graded categories for event severity will be based on the NIAID Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1, July 2017.

The occurrence of ICH SAEs or DAIDS grade 4 adverse events and time to first ICH SAE or DAIDS grade 4 adverse event in infants will be analyzed in a similar way, with time starting at birth.

Infant Anti-HBc Antibodies

Objective 2: To retrospectively determine the risk of transient infection in infants not chronically infected at 18 months of age using anti-HBc antibodies.

This analysis will be carried out on the Infant Exposed Set who are HBsAg- at 18 months (not chronically infected, per protocol section 5.2).

The proportion of HBsAg- children with detectable anti-HBc antibodies at 18 months of age will be provided with the corresponding two-sided Clopper-Pearson 95% CI. Similarly, the proportion and 95% CI of HBsAg- children with detectable anti-HBc antibodies and undetectable HBV DNA will be provided. A complete case analysis approach will be applied, including HBsAg- infants with 18 months anti-HBc antibody data available (assuming data are missing completely at random).

Infant Anti-HBs Antibodies

Objective 3: To describe infants' response to HB vaccination in the absence of confounding from HBIg administration, assessed by anti-HBs antibody levels at 1, 2, 4, 6, 12 and 18 months.

This analysis will be carried out on the Infant Exposed noHBIg Set.

Infant anti-HBs antibody geometric mean concentrations along with their 95% CI (log (ln) result exponentiated, from t-statistic) will be tabulated and presented graphically by study visit (1, 2, 4, 6, 12, and 18 months). Proportions of children with anti-HBs antibody concentrations > 10 mIU/mL at each of these time points, along with the two-sided Clopper-Pearson 95% CIs, will be provided. A complete case analysis approach will be applied, which includes infants with antiHBs antibody data available at each timepoint (assuming data are missing completely at random).

Maternal HBV DNA Changes

Objective 4: To describe the changes in maternal HBV DNA level from initiation of TDF to delivery.

This analysis will be carried out on the Maternal Treated Set.

Descriptive distribution statistics, including median and quartiles, of change in $\log_{10}$ HBV DNA levels between treatment initiation and delivery will be calculated. The proportion of pregnant women with HBV DNA levels >200,000 IU/mL at the first antepartum visit after enrollment will be estimated with the corresponding two-sided Clopper-Pearson 95% CI. A complete case analysis approach will be applied, which includes women with HBV DNA levels available at each timepoint (assuming data are missing completely at random). An HBV DNA measurement from within (≤) 1 days prior to treatment initiation will be considered for HBV DNA level at treatment initiation. Similar analyses will be carried out for changes in maternal HBV DNA level from initiation of TDF to delivery.

Comparison to iTAP

Objective 5: To compare the risk of infant HBV infection at 6 months of age with that observed in infants who received HBIg at birth plus HBV vaccine in one of the 2 arms of a previous study conducted in a similar setting in Thailand (iTAP, NCT01745822).

This analysis will be carried out on the Infant Exposed Set.

The proportion of infants in this study with HBV infection at 6 months of age and Clopper-Pearson 95% CI will be estimated among infants in Thailand. Infants from the tenofovir arm of the iTAP study (NCT01745822) will be used as a comparison group (the statistic will be used, rather than participant-level data). Pregnancies resulting in multiple births will be counted as one mother-infant pair, and will be counted as HBV-infected if at least one infant was infected. HBV positive at 6 months of age will be defined as stated in 4.1. Infants who mistakenly received HBIg will be excluded (supplementary analyses will include them). A complete case analysis approach will be applied, which includes infants whose 6 months endpoint is available (assuming data are missing completely at random).

A two-sided Fisher exact test with mid p-values and a significance level of 0.05 will compare the proportions of infants with HBV infection at 6 months of age.

HBV Infection Status for All Infants

Objective 6: To assess the risk of transmission in all infants born in the study, including those born to women with unsatisfactory virological response to study treatment.

This analysis will be carried out on the Infant Full Set.

The proportion of infants with HBV infection at 6 months of age will be estimated, and the exact 90% CI around this estimate will be calculated based on the Clopper-Pearson method with a one-sided significance level of 0.05. Pregnancies resulting in multiple births will be counted as one mother-infant pair, and will be counted as HBV-infected if at least one infant was infected. A complete case analysis approach will be applied, which includes infants whose 6 months' endpoint is available (assuming data are missing completely at random).

5 Report Contents

Summaries will be included for both mothers and infants. Unless otherwise mentioned, summaries will be in table format and summarized in text.

1. Study Entry
    a. Accrual of Pregnant Women
        i. Number of pregnant women screened
        ii. Number of pregnant women enrolled by month.
        iii. Number of pregnant women enrolled by country and site.
    b. Eligibility Violations
        i. Description of women (and their infants) who were enrolled and later found ineligible as indicated on the "Protocol Deviation Log" CRF. Depending on frequency, by text or table.

2. Maternal Baseline Characteristics
    a. Median, 25th and 75th percentiles, min, max, categories if given: Age at enrollment (years) (categories: >=18-<25, ≥25-<35, ≥35), sonogram information available (categories: results available, not available, not performed), best estimate of gestational age at enrollment by site clinician based on last menstrual period (LMP) and sonogram (weeks), height (cm), weight (kg) pre-pregnancy and at enrollment, BMI pre-pregnancy (<18.5, Underweight; ≥18.5-<25, Normal; ≥25-<30, Overweight ; ≥30, Obese), HBV DNA load (log_{10} IU) (below limit of detection (defined as the standard detection limit of the assay multiplied by the dilution factor), limit of detection to ≤5, >5 - ≤7, >7, and by <=/>200,000 IU/ml), creatinine (mg/dL), creatinine clearance (mL/min) by Cockcroft-Gault (<50 ml/min, >=50 ml/min), ALT(SGPT) (unit) (<=200,000,>200,00), AST(SGOT) (unit), APRI score (10^9/L), FIB-4, hemoglobin (g/dL), white blood cell count (10^3 cells/mL), platelets (10^5 cell/mL), proteinuria (<=1+,>1+ (>30mg/dL)), glycosuria (<1+, >+1+ (>250mg/dl)).
3. Maternal Study Status and Extent of Follow-up
    a. Premature Study Discontinuation of Women
        i. Number (%): Study status for women by visit time points before delivery, between delivery and 2 month postpartum visit, between 2 and 6 month postpartum visit, between 6 and 12 month postpartum visit, and reached 12 month postpartum visit (completed study). Subcategories still on study, death, lost to follow-up, and withdrew consent.
        ii. Listing: Reasons for early discontinuation (e.g., death, loss to follow-up, or withdrawn consent).
    b. Median, 10th, 25th, 75th, 90th percentiles, min, max: Weeks from enrollment visit to last study visit for all and for women on study.
    c. Compliance with Study Follow-Up
        i. Target number (%) of women at each scheduled follow-up visits, actual number (%) of women with scheduled follow-up visit strictly within the visit window, Median (min-max, Q1-Q3) of actual gestational age at scheduled follow-up visits.
        ii. Missed visits during COVID-19 outbreak
4. Study Treatment
    a. Number (%): Days from enrollment to study first treatment dispensation (0, 1, 2, 3, >3 with reasons.
    b. Median, 25th and 75th percentiles, min, max, categories of weeks on study treatment: Dispensed study treatment (weeks) (<4, 4-<8, 8-<12, 12-<16, 16-<20, ≥20), dispensed study treatment during pregnancy for those who delivered (weeks) (<4, 4-<8, 8-<12, ≥12), duration of study treatment postpartum for those completed (weeks) (<4, 4-<8, 8-<12, ≥12), duration of study treatment at completion (weeks) (<4, 4-<8, 8-<12, 12-<16, 16-<20, ≥20).

c. Number (%): Women who prematurely discontinued study treatment, completed study treatment, and on study treatment; with information on ALT measurements after treatment discontinuation.
d. Median, 25th and 75th percentiles, min, max, categories: duration of study treatment for women who completed the planned treatment through 60 days post-partum, from initiation and from postpartum: <=12, 12-<16, 16-<20, >=20 weeks of study treatment; and 8-<12, 12-<=16 weeks study treatment postpartum.
e. Description of women who do not complete treatment within 60 days postpartum (ALT, timing of discontinuation)
f. Number (%) adherence by pill count (<80%, ≥80%-95%, ≥95%) at scheduled visits and overall, and by self-report (Has participant taken study drugs as prescribed since the last study visit? Yes, No)

5. Maternal HBV DNA
   a. Median, 25th and 75th percentiles, min, max on IU/ml and $\log_{10}$ (IU/ml), change in log10 (IU/ml); categories: HBV DNA load at initiation of TDF ($\log_{10}$ IU) (below limit of detection, limit of detection to ≤5, >5 - ≤7, >7), HBV DNA load at delivery ($\log_{10}$ IU) (below limit of detection, limit of detection to ≤5, >5 - ≤7, >7)
   b. Mean and standard deviation for change in log10 HBV DNA load between initiation of TDF to delivery, geometric mean and 95% CI
   c. Number (%), estimated proportion of maternal viral load at 36 weeks gestational age and at delivery (≤200,000 IU/mL, >200,000 IU/mL) with 95% CI.
   d. Number (%) with baseline/36 weeks gestational age maternal viral load ≤/> 200,000 IU/ml (2x2 shift tables for status change between baseline and first antepartum measurement) and at delivery
   e. Number (%) of mothers, satisfactory/unsatisfactory virological response.
6. Maternal Hematology and Biochemistry
   a. Jitter plots with median,5th, 25th and 75th, 95th percentiles and grade 4 (if specified) indicated: hemoglobin (g/dL) (graded), hematocrit (%), white blood cells (cells/mm^3) (graded), platelets (per mm^3) (graded), serum creatinine (mg/dL) (graded), Cockcroft-Gault creatinine clearance (mL/min), ALT/SGPT (U/L) (graded), AST/SGOT (U/L) (graded), APRI score, FIB-4 score.
   b. Categories with number (%) indicated: glycosuria (≤1+, 2-3+, 4+), proteinuria (graded).
   c. Additional ALT/SGPT summaries (proportion >60 IU/ml, >300 at study visit), management for women who ever had >60 IU/l
7. Maternal Safety
   a. Number (%): Maternal ICH SAE or grade 4 AE, by grade (1-5) and total, by MedDRA primary system organ class with subcategorization by preferred term (PT level)
   b. Maternal ICH SAE or grade 4 AE with text description, grade, outcome, study week, relatedness to study treatment if available

c. Estimated proportion of women with any ICH SAE or grade 4 AE with 95% CI.
d. Kaplan-Meier curve of time to first ICH SAE or grade 4 AE from enrollment.

8. Delivery Characteristics
    a. Median, 25th and 75th percentiles, min, max, categories: Best estimate of gestational age at delivery by the site clinician based on last menstrual period (LMP) and sonogram (<32, ≥32-<35, ≥35-<37, ≥37) (from the "Pre-Enrollment" CRF), time from onset of labor to delivery (hours) (<3, 3-<6, 6-<9, 9-<12, ≥12) overall and by mode of delivery, time from rupture of membranes to delivery (hours) (<3, 3-<6, 6-<9, 9-<12, ≥12).
    b. Number (%): Births (single, multiple), delivery outcome (full term live births, premature births, stillbirths, spontaneous abortions, induced abortions), mode of delivery (vaginal, C-section), women with labor before delivery, C-section before labor (yes, no).
    c. Premature Deliveries by WHO definition: Gestational age at delivery by LMP" continuous, >=32-<35, >=35-<37
    d. Vaginal C-section by type (Elective Cesarean, Emergency Cesarean, Assisted Vaginal) overall and by gestational age (<32, 32-<37 and >=37 weeks)
    e. Median 25th, 75th percentiles, min, max, categories. Duration of TDF at the time of delivery (<=4 weeks, >4weeks)
    f. Estimated proportions of women with live births, preterm live births, stillbirths, spontaneous abortions, and induced abortions, as well as composite outcome measures of preterm/stillbirth/abortion (and early neonatal death) with 95% CIs.
9. Infant Characteristics at Birth
    a. Number (%): Enrollments, Sex (male/female), Apgar score at 1, 5, and 10 minutes (0-3, 4-6, 7-10), any congenital malformation (yes/no).
    b. Median, 25th and 75th percentiles, min, max, categories: Gestational age at birth according to Ballard exam (weeks) (<32, 32-<37, ≥37).
    c. Birth weight (g), length (cm), head circumference (cm)
    d. WHO weight-for-age, length-for-age and head circumference for age Z-scores (by who.int)
10. Infant Study Status and Extent of Follow-Up
    a. Premature Study Discontinuation of Infants
        i. Number (%): Total births (multiple birth details), infants before 6 months of age, between 6 and 12 months of age, between 12 and 18 months of age, reached 18 months of age (completed study). Subcategories still on study, death, lost to follow-up, and withdrew consent.
        ii. Listing: Reasons for early discontinuation (e.g., death, loss to follow-up, or withdrawn consent).

    b. Median, 10th, 25th, 75th, 90th percentile, min max of actual age at last scheduled follow-up visit.

c. Telephone visits (see maternal)
d. Compliance with Study Follow-up
    i. Target number (%) of infants at each scheduled follow-up visits, actual number (%) of infants with scheduled follow-up visit strictly within the visit window.

11. Infant Vaccine Administration
    a. Hepatitis B Immune Globulin (HBIg)
        i. Number (%) of infants who were administered HBIg at birth by maternal viral load during pregnancy (≤200,000 IU/mL, >200,000 IU/mL, not available). During pregnancy defined as first measurement after enrollment.
        ii. Number (%) of infants who were administered HBIg at birth by maternal virological response to TDF during pregnancy (satisfactory, unsatisfactory, not available (missing data or delivered before 36 weeks)).
        iii. Median, 25th and 75th percentiles, min, max, categories: Duration from birth to HBIg dosing (hours) (<1, ≥1-<2, ≥2-<3, ≥3-<4, ≥4).
    b. Hepatitis B Vaccine
        i. Median, 25th and 75th percentiles, min, max, categories: Duration from birth to first HB vaccine dosing (minutes continuous, hours categorized) (<1, ≥1-<2, ≥2-<3, ≥3-<4, >=4-<24,>=24).
        ii. Compliance to HB vaccine by country, target number of infants at each visit, actual number (%) of infants at scheduled visits, number (%) with (<3, 3, 4 and for Thailand only: 5) HBV vaccines for infants who have reached 6 months of age. Note that vaccine schedules are different for Thailand and Lao PDR.
12. Breast Feeding categories: infant ever breastfed by visit week interval (e.g., between C1 and C2)
13. Infant Growth
    a. Jitter plots with median, 5^{th}, 25th and 75^{th}, 95^{th} percentiles: length (cm), weight (grams), and head circumference (cm)
    b. Jitter plots with mean and 95% confidence interval using the t-distribution: WHO Z-scores-for-age of weight, length, and head circumference at scheduled visits
14. Infant Safety
    a. Number (%): Infant ICH SEAs or grade 4 adverse events by MedDRA primary system organ class with subcategorization by preferred term.
    b. Infant ICH SAEs with text description, grade, outcome, study week, relatedness to study treatment if available
    c. Estimated proportion of infants with any ICH SAE or grade 4 AE with 95% CI.
    d. Kaplan-Meier curve of time to first ICH SAE or grade 4 AE from birth.
15. Hepatitis B Infection Status and Sero-Protection
    a. Primary Outcome
        i. Number (%) and description of multiple pregnancies counted as one mother-infant pair, number (%) with HBsAg test at 6 months of age,

number (%) HBsAg (positive/negative), number with HBV DNA test at 6 months of age, number (%) HBV DNA confirmation (detectable/undetectable), number (%) limit of HBV DNA detection (e.g. 15, 15x2, 15x3 IU/mL).

ii. Number with HBV infection at 6 months of age for primary analysis (complete case), estimated proportion with 90% CI.

iii. Number with HBV infection at 6 months of age for sensitivity analyses (infants from multiple pregnancies separate, impute last available HBV infection status, and missing as HBV-infected), estimated proportions of infections for each analysis with 90% CIs.

iv. Frequency of visit week timepoint for imputed last available infection status. Reasons for not having infection status available (e.g., missed visit, loss to follow-up, consent withdrawn, death)

b. Infant anti-HBs antibodies

i. Number (%) with anti-HBs antibody test at 1, 2, 4, 6, 12 and 18 months; anti-HBs geometric mean concentrations at 1, 2, 4, 6, 12 and 18 months with 95% CI; number (%) with sero-protection (anti-HBs antibody concentration > 10 mIU/mL) at 1, 2, 4, 6, 12 and 18 months with 95% CI.

c. HBV infection status for all infants

i. Number (%) with HBV infection at 6 months of age for secondary analysis (complete case) including infants born to mothers with an unsatisfactory virological response, estimated proportion with 90% CI.

ii. Listing of timing and results for all HBsAg, HBV DNA level (with target detectable/undetectable, if below assay quantification limit), anti HBs antibody level for infants with HBV infant infection at 6 months of age, with indicator for whether included in primary analysis.

iii. HBsAg and HBV DNA level by visit week for all infant measurements (months 9, 12 and 18)

d. Maternal HBeAg status at 12 months postpartum

16. Comparison to iTAP

a. Number (%) and description of multiple pregnancies counted as one mother-infant pair, number (%) with HBsAg test at 6 months of age, number (%) HBsAg (positive/negative), number with HBV DNA test at 6 months of age, number (%) HBV DNA (detectable/undetectable), number (%) limit of HBV DNA detection (e.g., 15, 15x2, 15x3 IU/mL), by study (iTAP or iTAP-2).

b. Number with HBV infection at 6 months of age and estimated proportion with 95% CI by study (iTAP or iTAP-2), p-value for the comparison between studies.

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