CLINICAL STUDY PROTOCOL

A multi-Centre, randomised, placebo controlled, double-blind, parallel group study to evaluate the safety, tolerability and immunogenicity of two doses of Group B Streptococcus vaccine (GBS-NN/NN2) in women who are pregnant and living with HIV and women who are pregnant and do not have HIV.

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PROTOCOL SIGNATURE SHEET

Title: A multicentre, randomised, placebo controlled, double-blind, parallel group study to evaluate safety, tolerability and immunogenicity of two doses of Group B Streptococcus vaccine (GBS-NN/NN2) in women who are pregnant and living with HIV and women who are pregnant and do not have HIV.

Sponsors: Name and Address

MinervaX ApS
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DK-2200, Copenhagen N
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We, on behalf of MinervaX ApS, have read, understood and approve this protocol. We agree to comply with all requirements regarding the obligations of sponsor and all other pertinent requirements ICH-GCP, Guidance on South African Good Clinical Practice and Declaration of Helsinki (Fortaleza, Brazil 2013) and applicable regulatory requirements.

Sponsor Representatives Name (Print): _____

Sponsor Representative's Signature: _____ Date: _____

Principal Investigator:

I have read the following protocol and agree to conduct the study as outlined. I am aware of my responsibilities as an Investigator under the guidelines of Good Clinical Practice (ICH-GCP),[1] South African GCP, Declaration of Helsinki (Fortaleza, Brazil 2013), local regulations (as applicable), SOPs and the study protocol. I agree to conduct the study according to these guidelines and to appropriately direct and assist the staff under my control, who will be involved in the study.

Investigator's Name (print): _____

Investigator's Signature: _____Date: _____

[1] ICH Harmonised Tripartite Guideline E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95)

PROTOCOL OUTLINE

Title

A multicentre, randomised, placebo controlled, double-blind, parallel group study to evaluate the safety, tolerability and immunogenicity of two doses of Group B Streptococcus vaccine (GBS-NN/NN2) in women who are pregnant and living with HIV and women who are pregnant and do not have HIV.

Investigator(s), Study site(s)

Two sites study in South Africa and Uganda

University of the Witwatersrand, Johannesburg, South Africa (Respiratory and Meningeal Pathogens Research Unit based at Chris-Hani Baragwanath Academic Hospital and Rahima Moosa Mother and Child Hospital) and

MUJHU Research Collaboration/MUJHU Care Ltd at Kawempe National Referral hospital. Kawempe, UGANDA.

Phase: Phase II

Indication

Group B Streptococcus Vaccine (GBS-NN/NN2)

Objectives

Primary Objectives:

Safety: To evaluate the safety and tolerability of the GBS-NN/NN2 vaccine in women living with HIV and women without HIV and their new-born babies from vaccination up to delivery/birth.

Immunological:

To compare the transfer rate of vaccine- specific immunoglobulin G (IgG) antibody concentrations from the mother to the baby at birth in women living with HIV with the transfer rate in women without HIV. This endpoint will be used to determine the sample size calculation.

Secondary Objectives

Safety: The safety and tolerability of the GBS-NN/NN2 vaccine in the mother and baby over the first 12 months post-partum, as assessed at 6 and 12 months of age.

Immunological: The secondary immunological objectives are:

- To compare IgG antibody responses, specific to Rib, Alp1, Alp2, AlpC, GBS-NN and GBS-NN2, induced by the first and second vaccine doses over time in pregnant women living with HIV and pregnant women without HIV.
- To determine the concentrations of IgG antibodies specific to Rib, Alp1, Alp2, AlpC, GBS-NN and GBS-NN2 in cord blood at delivery in babies born to women with and without HIV.
- To evaluate the concentration of IgG antibodies specific to Rib, Alp1, Alp2, AlpC, GBS-NN and GBS-NN2 up to 6 months post-delivery in the mother and 3 months post-delivery in the baby in women with and without HIV.
- To evaluate the vaccine specific IgG concentration time profile over time, in the baby over the first 3 months of life and compare this between babies born of mothers living with and without HIV.

Exploratory Objectives

- [redacted]
- [redacted]

Endpoints

Primary Endpoints

Safety: The following endpoints in the mother will be evaluated to support this objective: local and systemic reactogenicity (headache, sore throat, "flu-like" symptoms, muscle aches, fever in the 72 hours post dose); adverse events; laboratory tests; dipstick urinalysis; vital signs; physical examination.

Admissions to hospital for a normal or following an elective Caesarean section leading to a healthy, term birth will not be deemed a serious adverse event, unless deemed an emergency caesarean section, in which case the reason the Emergency Lower Segment Caesarean Section (LSCS) was required will be reported as SAE.

In the new-born baby: gestational age at birth (based on ultrasound, date of last menstrual period and fundal height as per the Global Alignment of Immunization safety Assessment in pregnancy (GAIA) guidelines), weight, length, head circumference, Apgar score if available at 1, 5 and 10 minutes.

The data for the primary endpoint will be the data collected at the assessments conducted at delivery.

Immunological**:** The IgG antibody concentrations, specific to Rib, Alp1, Alp2, AlpC, GBS-NN and GBS-NN2, in individual maternal blood and cord blood at birth.

Secondary endpoints

Safety: Mothers and babies will be followed up for 12 months post-delivery, mothers will be assessed for significant adverse reactions with specific interest in potential immunotoxicity e.g. development of auto immune diseases.

Developmental milestones of the babies will be assessed at 6- and 12-months using age-appropriate ages and stages questionnaire, as per Appendices.

Immunological: The following endpoints will be evaluated, by group and time-point, to support these objectives:

- Geometric mean antibody concentration in µg/ml at 4 weeks post first (pre-second injection) and 4 weeks post second injection.
- Geometric mean antibody concentration in µg/ml of cord (or new-born blood within 48 hours of birth) and maternal blood at delivery.
- Geometric mean fold increase in antibody concentration in maternal blood at 4 weeks post first (pre-second injection) and 4 weeks post second injection.
- Geometric mean fold increase in antibody concentration in maternal blood from baseline to delivery.

- Proportion of mothers and new-born babies achieving vaccine specific IgG antibody concentrations, in maternal and cord blood samples respectively, above pre-specified arbitrary thresholds at delivery (these will be 0.5, 1, 2 µg/ml).

The primary immunological analysis will be the values of these endpoints at the time of delivery, which is anticipated to be approximately 10 to 14 weeks after the administration of the first dose of vaccine.

Study Design

This is a Phase II, randomised, placebo controlled, double-blind, parallel group, multi-centre study. There will be 4 groups, two groups of 80 participants to receive the potential vaccine and two groups of 20 participants to receive placebo.

Group 1: 80 women who are pregnant and living with HIV, who will receive two injections of GBS-NN/NN2. Each injection will contain 50 µg of GBS-NN and 50 µg of GBS-NN2.

Group 2: 80 women who are pregnant and do not have HIV who will receive two injections of GBS-NN/NN2. Each injection will contain 50 µg of GBS-NN and 50 µg of GBS-NN2.

Group 3: 20 women who are pregnant and living with HIV who will receive two injections of placebo.

Group 4: 20 women who are pregnant and do not have HIV who will receive two injections of placebo.

Participants will be screened at 24 to 28 weeks gestation, Day -14 to day -1. The groups will be dosed in parallel; the first dose of vaccine or placebo will be administered at Day 0 and the second dose will be administered Day 28±2 days after the first dose.

For the analysis of the immune responses the placebo groups will be combined. For safety the placebo groups will be analysed separately and combined for comparison with the potential vaccine groups.

Should any babies develop invasive GBS disease over the course of the study they will be treated as per normal standard of care. The number of babies in each group that develop invasive GBS disease will be compared although no cases are expected in this number of participants. The severity of the infection will also be assessed and compared. Blood samples will be obtained to assess the concentration of IgG antibodies specific for the vaccine from the babies as described in the schedule of events.

Study Duration

The study will take approximately 20 months to run from first participant first visit to last participant last visit. Each participant will be involved with the study for approximately 15 to 16 months including screening and follow-up

Inclusion and Exclusion criteria

Study participants fulfilling the following criteria are eligible to participate in the study:

1. Participants at least 18 years old and not older than 40 years of age.

2. Pregnant women who are between 26 weeks and 30 weeks (inclusive) gestation on the planned day of vaccination with a singleton, uncomplicated pregnancy. Gestational age to be determined on the following hierarchal basis with guidance to the Global Alignment of Immunization Safety Assessment in Pregnancy (GAIA) criteria in appendix 04:

    a. ultrasound estimate of gestational age,

    b. date of last menstrual period

    c. fundal height

3. HIV status to be based on rapid, confirmatory test, unless a documented test of the participant being sero-positive for HIV and history documented in the notes.

4. Women living with HIV, HIV viral load <1000, on antiretroviral therapy for at least 3 months prior to screening and clinically well.

5. Expected to be available for the scheduled clinic visits for the duration of the study, agree to be contacted by telephone during study participation, and is willing to give parental consent for her infant to participate in the study.

The exclusion criteria

Study participants who meet any of the following criteria are to be excluded from study participation:

1. Women who are HBSAg and/or HCV positive

2. Women who test positive for syphilis as per standard testing

3. Women knowingly carrying, at screening, a malformed or genetically abnormal foetus based on ultrasound

4. Women who have experienced a previous stillbirth prior to going into labour

5. Women with placenta previa

6. Women with documented chronic or pregnancy induced hypertension at screening

7. Women with 1+ protein in urine and hypertension defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) at ≥20 weeks of gestation in a woman with a previously normal blood pressure

8. Women with >1+ of protein in urine (regardless of blood pressure)

9. Women with gestational, type 1 or type 2 diabetes.

10. Women with glycosuria on dipstick

11. Women known to be allergic to any components of the vaccine, who are known to be allergic to aluminium or have had an allergic reaction to any previous vaccination.

12. Women with HIV viral load >1000 at screening.

13. Women living with HIV who have been on antiretroviral therapy for less than 3 months prior to screening. (Women who are diagnosed with HIV between screening and dosing will not be eligible.)

14. Women with history or presence of significant cardiovascular disease, pulmonary, hepatic, gallbladder or biliary tract, renal, haematological, gastrointestinal, endocrine, immunologic, dermatological, neurological, psychiatric, autoimmune disease, cognitive disorder or current infection and significant illness 4 weeks prior to randomization.

15. Women with current or history of drug or alcohol abuse within the last two years.

16. Women who have received a vaccine within 28 days of receiving the first dose of GBS NN/NN2 or placebo or who expect to require vaccination during the course of the study. (Vaccines recommended for administration during pregnancy e.g. tetanus toxoid, pertussis and influenza are permitted. Administration of concurrent vaccines must not be within 7 days of investigational vaccine.)

17. Women who have a fever (axillary temperature >37.9°C) on the day of dosing or have had an acute infection in the 7 days before dosing.

18. Women who have any bleeding disorders that prolong the bleeding time.

19. Women who are receiving immunosuppressive medication, including systemic steroids (inhaled and topical steroids are acceptable).

20. Women who have received blood or blood products and/or plasma derivatives or any immunoglobulin preparations in 12 weeks preceding screening.

21. Women with severe anaemia, haemoglobin < 9g/dL (90 g/L) as per Sheffield grading system (> Grade 1)

22. Women who are currently breast feeding

23. Women who are part of the study personnel or a close family member of study personnel.

24. Women who in the opinion of the investigator are not suitable to participate in the study.

25. Concurrent participation in another clinical trial during which the participant will be exposed to an investigational product

Investigational Medicinal Product and mode of administration

The GBS-NN/NN2 vaccine will be supplied in two components: 1) a sterile solution for injection containing "(NN+NN2)DP" protein 2.5 ml at 0.7 mg/ml (0.35 mg/ml of each protein component); and 2) vials

containing Alhydrogel 0.5ml at 1.4mg/ml Al^{3+}. A volume of 0.2 ml of (NN+NN2)DP will be added to 0.5 ml of Alhydrogel. A dose will be 0.5 ml of the resulting mixture.

Placebo will be 0.5 ml of 0.9% saline.

The dose will be a single injection on Days 0 and Day 28. Each injection will consist of either active vaccination with GBS-NN/NN2, which will contain 50 µg of GBS-NN and 50 µg of GBS-NN2, or placebo (saline).

Administration will be by intramuscular injection, preferably into the non-dominant arm. The dominant arm may be used if it is not possible to administer into the non-dominant arm e.g. due to an ongoing injection site reaction, or tattoo

Treatments and visit schedule

Participants will provide written informed consent and will be screened for eligibility within 14 days before first study vaccine administration (Day 0). Eligible participants will receive, either a dose of GBS-NN/NN2 or a dose of placebo on 2 occasions – the first dose will be administered on Day 0, and 4 weeks later the second dose of the primary course will be administered. Following vaccination participants will remain in the clinic for at least 30 minutes post vaccination.

During the study, participants will attend study visits as follows:

Screening up to 14 days before the first vaccination.

Visit 1 Day 0 vaccination: review baseline bloods, physical examination and vital signs, review eligibility and randomization, take blood for pre-dose antibody response, first vaccination administered, immediate post vaccination AEs, concomitant prescription medications.

Visit 2 Day 7 to10: Collect and review diary card, vital signs, AEs, concomitant medications. If the mother delivers before Visit 2, the assessment relating to the collection and review of the diary card, adverse events from first vaccination, scheduled for Visit 2 will be collected at delivery Visit 5.

Visit 3 Day 28: take bloods to assess immune response from first vaccination, safety bloods, adverse events from first vaccination, 2nd vaccination administered, immediate post second vaccination adverse events and concomitant prescription medications. If the mother delivers before visit 3 the assessments relating to safety bloods, adverse events from first vaccination, and concomitant prescription medications, scheduled for Visit 3 will be collected at delivery Visit 5.

Visit 4 Day 56: take bloods to assess immune response from second vaccination, safety bloods, collect and review diary card, adverse events from first and second vaccinations and concomitant prescription medications. If the mother delivers before Visit 4 the assessments relating to safety bloods, collection and review of the diary card, adverse events from first and second vaccinations, and concomitant prescription medications, scheduled for Visit 4 will be collected at delivery Visit 5.

Visit 5 Day of delivery/birth: collect bloods from mother and baby (cord blood or infant blood within 48 hours) for assessing antibody concentrations, adverse events and concomitant medications. Record, in the eCRF, result of routine standard of care HIV serology assessment for the mother, if it has been done at this time or is within

the standard of care testing window. If not, perform HIV rapid test. If mother delivers before V4, safety bloods will be done at delivery visit. For the baby the following information will be recorded:

> Gestational age, Apgar scores at 1, 5 and 10 minutes (If available), birth weight, length, head circumference and vital signs, a physical examination will be conducted.

Visit 6 28±4 days after birth: collect blood from baby for antibody concentrations, baby's assessment and maternal and baby adverse events and concomitant medications. Record, in the eCRF, result of routine standard of care HIV serology assessment for the baby, if it has been done at this time.

Visit 7 56±6 days after birth: collect blood from half the babies for antibody concentration, and maternal and baby adverse events and concomitant medications. Record, in the eCRF, result of routine standard of care HIV serology assessment for the baby, if it has been done at this time and not obtained previously.

Visit 8 84±6 days after birth: collect blood from the other half of the babies for antibody concentrations, baby's assessment and adverse events and concomitant medications. Record, in the eCRF, result of routine standard of care HIV serology assessment for the baby, if it has been done at this time and not obtained previously

Visit 9 182±8 day after birth: Follow-up study visit. Collect blood from mother for antibody concentrations, baby's age-appropriate developmental assessment and adverse events and concomitant medications.

Visit 10 365±8 day after birth: End of study follow up visit for Mother and Baby, baby's age-appropriate developmental assessment and adverse events and concomitant medications.

Participants will be provided with a 7-day diary card on Days 1 and 28 (days of vaccination) to record body temperature, adverse events and concomitant medications. An axillary thermometer and reactogenicity ruler will be supplied to participants. If appropriate, site staff can visit the participants at home to complete the diary card.

Sample size

The power calculation will be based on the paper by Heyderman 2016, in which they found an average standard deviation in the log transformed transfer rates between patients to be 0.96.

The sample sizes were calculated on the conservative assumption that only 80 participants per group would be enrolled, that there would be a 15% dropout rate, and that 80% of women would deliver infants that are at 37 weeks or more of gestation or weighed 2500 g or more at birth. Thus, a sample size of 54 per group would give approximately 90% power to detect a difference in placental transfer rate of 6% and if the dropout rate is such that only a sample size of only 40 per group completed the study this would give approximately 90% power to detect an 7% difference in placental transfer rates between any two study groups.

Statistical procedures

The safety data will be analysed using the two-way chi-square analysis and a pairwise comparison of the two treatment groups. The 95% confidence intervals will be calculated for all observed rates.

The immunological data will be analysed by means of ANOVAs on the log transformed data and pairwise comparisons performed on all the observed means. The results will be reported as geometric means and geometric confidence intervals.

Interim analysis (safety) will be performed on data collected up to Day 56 from the first 20 participants recruited on the study.

Study Centres

Up to a total of three centres will be selected from South Africa and Uganda

TABLE 1 SCHEDULE OF ASSESSMENTS

Schedule for Mothers

	Screening Period	Treatment and follow-up Period									
	Screening	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**	**Visit 10**
Assessment	Day -14 to Day -1 24 to 28 weeks GA	Day 0 26 to30 weeks GA	Day 7 to Day 10	Day 28±2	Day 56±2	Delivery	28±4 days post delivery	56±6 days post delivery	84±6 days post delivery	182±8 Days post delivery	365±8 Days post delivery
Informed Consent	X										
Inclusion/ Exclusion Criteria	X										
Demography	X										
Medical History	X										
Physical Examination	X	X		X			X			X	
Height, Weight, BMI, MUAC	X						X			X	
Vital signs[a]	X	X[b]	X	X[b]	X	X	X	X	X	X	
Urinalysis	X	X		X							
Ultrasound[h]	X										
Safety laboratory tests[D]	X			X	X	X[l]					
Hep B and Hep C	X										
Rapid HIV Test	X[f]					X[i]					
CD4 count	X[e]										
HIV viral load	X[e]										
Review of participant eligibility		X									
Randomisation		X									

	Screening Period	Treatment and follow-up Period									
	Screening	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**	**Visit 10**
Assessment	Day -14 to Day -1 24 to 28 weeks GA	Day 0 26 to30 weeks GA	Day 7 to Day 10	Day 28±2	Day 56±2	Delivery	28±4 days post delivery	56±6 days post delivery	84±6 days post delivery	182±8 Days post delivery	365±8 Days post delivery
Administration Investigational vaccine		X		X							
PD blood sample – antibody response		X		X	X	X				X	
Diary card		X	X	X	X	X					
Assessment of injection site[c]		X	X	X	X						
Urine pregnancy test											X[g]
AE check	X							X[k]			X[,k]
Prescription Concomitant medication check	X	X	X	X	X	X	X				X[,k]

Footnotes

a. Heart rate, blood pressure and axillary temperature.

b. Vital signs at dosing Visit will be recorded pre-dose and at 30min post-dose.

c. Photographs may be taken of injection site reactions as required.

d. Haematology (Red blood cell count, Haemoglobin, Haematocrit, Platelet count, White blood cell count with absolute differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils) counts) and Biochemistry (Sodium, Potassium, Chloride, Bicarbonate, Blood urea, Creatinine, Creatine Kinase, Glucose, Calcium, Albumin, Cholesterol, C-reactive protein, Triglycerides, Phosphorus (inorganic phosphate), Lactate dehydrogenase, Total protein, Globulin, Uric acid, Alkaline phosphatase, Aspartate aminotransferase, Alanine aminotransferase, Gamma-glutamyl-transferase, Total bilirubin and Direct bilirubin)

e. Only if participant is HIV positive

f. Only participant not known to be HIV positive

g. Any participant found to be pregnant at the end of the study will be followed up to delivery

h. Ultrasound for assessment of gestational age, confirmation of singleton pregnancy and no detectable congenital abnormalities

i. Standard of Care test

j Review of diary only

k. SAEs, AEs of special interest and related con-meds

l. Safety laboratory tests collected if delivery before V4

Schedule of assessments for Babies

	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10
Assessment	Delivery	28±4 days post delivery	56±6 days post delivery	84±6 days post delivery	182±8 Days post delivery	365±8 Days post delivery
Demography[a]	X					
Gestational History	X					
Physical Examination	X	X			X	
Head Circumference	X	X			X	
Length	X				X	
Weight	X	X	X	X	X	
Apgar Score[b]	X[b]					
Vital signs[c]	X	X	X	X	X	
HIV Status		X[g]	X[g]	X[g]	X[g]	
Developmental milestones					X[f]	X[f]
PD blood sample – antibody concentration	X[d]	X[e]	X[e,h]	X[e,h]		
AE check	X					
Con med check	X					

Footnotes

a. Gestational age and Gender
b. Apgar score at 1, 5 and 10 minutes if available (Available scores will be collected, Not recorded will be an option)
c. Heart rate.
d. Cord blood or Infant blood (heel prick or venous sample).
e. Heel prick or venous sample.
f. Age-appropriate developmental milestone scoring (Ages and Stages)
g. Record result of routine standard of care HIV serology assessment for the baby, if done as part of standard of care, in the eCRF if not recorded previously or if new results are available.
h 50% babies at 2 months and the other 50% at 3 months. Only 3 samples total per baby

LIST OF ABBREVIATIONS

AE	Adverse Event
ALP	Alkaline Phosphatase
Alp	Alpha Like Proteins
ATP	Adenosine Triphosphate
BHR	Bronchial Hyperactivity
CDC	Centers for Disease Control and Prevention
CRF	Case report form
CRO	Clinical Research Organisation
CSF	Cerebrospinal fluid
EOD	Early onset disease
FBC	Full Blood Count
g	Grams
g/dL	grams/decilitre
g/L	grams/litre
GAIA	Global Alignment of Immunization Safety Assessment in pregnancy
GBS	Group B Streptococcus or *Streptococcus agalactiae*
GBS-NN	Recombinant protein vaccine against GBS
GCP	Good Clinical Practice
ICH	International Council for Harmonization
ICS	Inhaled corticosteroids
IEC	Independent ethics committee
IFA	Incomplete Freund's Adjuvant
IgA	Immunoglobulin A

IgG	Immunoglobulin G
IM	Intramuscular
IMI	Innovative medicines initiative
IP	Investigational Product
IRB	Institutional Review Board
IV	Intravenous
L	Litre
LOD	Late onset disease
mg	milligrams
ml	millilitre
MUAC	Mid Upper Arm Circumference
NDA	National Drug Authority
[redacted]	[redacted]
PI	Principal Investigator
PMSH	Past Medical/ Surgical History
RBC	Red Blood Cell
Rib	A protein expressed on the surface of GBS strains
SAE	Serious Adverse Event
SAHPRA	South African Health Products Regulatory Authority
SUSAR	Suspected Unexpected Serious Adverse Reaction
TDaP	Vaccine against tetanus, diphtheria and pertussis
WHO	World Health Organization

1. INTRODUCTION AND STUDY RATIONALE

1.1 Background: Group B Streptococcus disease and vaccine

Streptococcus agalactiae, also referred to as Lancefield's group B Streptococcus (GBS), is a Gram-positive diplococcus representing one of the most common causes of life-threatening invasive bacterial infections in neonates and young infants. Infections can result in neonatal sepsis, pneumonia and meningitis, and is associated with significant mortality and morbidity, including long-term neuro-developmental [1,2] GBS is also associated with disease in adults like pregnant woman, elderly and adults with underlying condition such as HIV, cancer, cirrhosis and diabetes [1,3]. GBS had emerged as the predominant pathogen causing septicaemia and meningitis in neonates and infants living in diverse regions [4-6]. Women colonized with GBS have a significantly higher postpartum fever and endometritis. GBS infection in new-borns is usually classified as an early-onset disease (EOD) and late-onset disease (LOD), respectively, depending on the age of the infant at the time of disease manifestation. The EOD onset during the first 6 days of life and the LOD onset between day 7 to 89 of life and it is estimated that 60–90% of EOD occurs on the first day of life [7]. GBS colonizes the human gastrointestinal and genitourinary tracts, and throat, and vertical transmission from colonized mothers can lead to invasive disease in their offspring. Disease in neonates and young infants develops as a result of invasion of GBS across epithelial cells into the bloodstream [8]. HIV-exposed infants are at a higher risk of developing invasive GBS disease [9].

Advances in the diagnosis and treatment of GBS infections and global hygiene standards have significantly reduced the development of neonatal EOD. However, in the recent global meta-analysis, countries in the WHO Africa region had the highest pooled estimate of young infant (day 0–89) invasive GBS disease (1.21/1000 livebirths, 95% CI: 0.50–1.91) [10]. In another systematic review, focusing on data from sub-Saharan African countries, it was estimated that the incidence may be twice as high [11].

In an analysis of the global distribution of GBS, five serotypes (Ia, Ib, II, III, V) accounted for 94% of invasive disease in young infants [10]. These five serotypes have been most prevalent as causes of disease, with serotype III now accounting for 50% of all neonatal infections and 90% of cases of neonatal meningitis [12]. Serotype V is responsible for 10 to 15% of invasive GBS disease in young infants s in the United States, and in Sweden and has become the most common serotype isolated from nonpregnant adults with invasive disease [12].

The global incidence per 1 000 live births of invasive GBS disease in infants <90 days of age is estimated to be 0.53 (95% confidence interval (CI) 0.44 - 0.62) with a case fatality ratio of 9.6%.[13]. South Africa (SA) has registered the highest incidence rate at 2 - 3 per 1 000 live births over the last two and half decades [14] Of concern, the high incidence is also associated with a high case fatality ratio (13 - 18%) and there is significant neurological impairment among survivors, especially in those with meningitis (24%). In Uganda there was no information documented GBS anogenital prevalence. However, the study conducted by Namugongo *et al.*, presented the study with high incidence of Group B streptococcal anogenital colonization among pregnant women attending antenatal care at tertiary hospital, in Southwestern Uganda [15]. The high incidence in these two countries might be due to suboptimal implementation of intrapartum antibiotic prophylaxis (IAP) to women identified to

be at risk of having infants with EOD, coupled with maternal HIV infection being associated with a two-to-five-fold greater risk of invasive GBS disease in infants [15-17]. The increased susceptibility to invasive GBS disease in infants born to HIV-infected women is thought to be due to lower maternal protective GBS capsular antibody concentrations and the inefficient transplacental transfer thereof [18].

In the USA, the incidence of EOD has declined significantly (>80%) since the early 1990s through effective execution of secondary preventative strategies, i.e. antibiotics administered intravenously 4 hours before delivery to GBS-colonised women who were screened at 35 - 37 weeks' gestation. This universal screening approach (as recommended by the Centers for Disease Control and prevention (CDC) cannot be readily implemented in most low-to-middle-income countries, where a large proportion of deliveries occur outside health facilities, although this is not the case in SA. In addition, the high cost of screening pregnant women, providing IAP and creating the necessary infrastructure that enables the administration of intravenous IAP at least 4 hours prior to delivery has hampered preventive efforts in low-to-middle-income countries. Furthermore, the CDC recommendation has limitations such as false-negative cultures in colonised pregnant women, failure to institute IAP for sudden deliveries, and the limited effect of IAP on preventing preterm delivery, stillbirths and LOD [14].

Currently, South Africa, Uganda and other low-to-middle-income counties, practise the less successful risk-based approach to prevent invasive disease, i.e. IAP is only provided to mothers in labour with risk factors such as maternal fever, prolonged rupture of membranes or prematurity. This strategy is also failing in low-to-middle-income countries because risk factors do not identify all women who are at risk of transmitting GBS. Furthermore, healthcare workers may fail to recognise risk factors, and may not provide IAP in busy obstetric units. Penicillin is still drug of choice for infants who require treatment for invasive GBS disease [14].

GBS is the most common cause of neonatal sepsis and meningitis. SA has a very high incidence of invasive GBS disease and the provision of IAP would be costly and logistically difficult to implement; maternal GBS vaccination is a promising strategy [14].

1.2 GBS-NN/NN2

GBS is an encapsulated Gram-positive bacterium, divided into ten different serotypes (Ia, Ib, II-IX) classified based on their capsular polysaccharides. Serotypes Ia and III are the clinically most important, and serotypes Ia, Ib, II, III, IV and V together cover approximately 95% of all colonizing isolates recovered from pregnant women.

GBS expresses a family of surface proteins termed the Alpha-like proteins. The family consist predominantly of Rib, AlphaC, Alp1, Alp2 and Alp3 (Alp3 has an identical N-terminal domain to Alp2). The N-terminal domains of the Alp-proteins are involved in adhesion and invasion of epithelial cells, and antibodies directed against the N-terminal domains are highly protective against GBS infections in *in vitro* experiments and *in vivo* animal models. The Rib and AlphaC proteins are the most prevalent members of the Alp-protein family and antibodies against the N-terminal domains of these proteins also cross react with Alp1-3 N-terminal domains to some extent. MinervaX

generated an initial GBS vaccine candidate, GBS-NN, based on the N-terminal domains of Rib and AlphaC surface proteins of GBS, which was administered to 183 healthy women, in a two-part, 240 participants, placebo-controlled Phase I study (MVX13211). Detailed assessments of the functionality of the IgG antibodies generated in the study *in vitro* identified that the degree of cross reactivity with the other two N-terminal proteins Alp1 and Alp2/3 was not as robust as anticipated and therefore it was determined that, for a GBS protein vaccine to be effective, all four N-terminal protein domains need to be included. Hence, MinervaX modified the vaccine candidate to contain the 4 N-terminal proteins; RibN, AlpCN, Alp1N and Alp2/3N. The vaccine has been designated GBS-NN/NN2. The GBS-NN/NN2 vaccine candidate consists of two recombinant fusion proteins containing the two N-terminal regions of the Rib and AlphaC proteins (GBS-NN) and the two N-terminal regions of the Alp1 and Alp2/3 proteins (GBS-NN2).

The four proteins, Rib, AlphaC, and the Alpha-like proteins (Alp1, and Alp 2/3) are found on most isolates of serotypes Ia, Ib, II, III, IV, V, VI, VII, VIII and IX. Antibodies directed against the vaccine were found to recognize nearly 100% of 154 clinical isolates of serotypes Ia, Ib, II, III and V tested for antibody binding. Furthermore, the Rib protein is expressed on most serotype III strains, which cause almost all cases of meningitis, and by all strains of a hyper-virulent type III clone [19-21].

Naturally occurring antibodies against the full-length Rib, Alp 1, Alp2 and AlphaC proteins are found in pregnant women, likely due to exposure to GBS from colonization or infections such as GBS urinary tract infections, and such antibodies are efficiently transferred to their babies *in-utero*. Importantly, a correlation between high concentrations of such antibodies, and more importantly antibodies directed against the N-terminal domains, in the mothers and their new-borns are associated with a reduced incidence of invasive GBS disease in the new-borns [22]. Finally, Rib and AlphaC elicit protective immunity in animal models when administered with alum, an adjuvant accepted for human use [23-24], as a consequence, these proteins are of interest for vaccine development.

The N-terminal regions of the Rib and AlphaC have been found to elicit a protective immune response against GBS in mice, and a fusion protein derived from Rib and AlphaC was shown to be even more immunogenic than the repeat regions of the Rib and AlphaC proteins [24-26]. Furthermore, the GBS-NN fusion protein induces opsonic antibodies against prototype strains independent of the serotypes, protects adult mice against lethal challenges, with prototype strains of serotypes Ia, Ib, II, and III, following direct vaccination [24], and most importantly, protects new-born pups born to female mice vaccinated with GBS-NN when challenged with lethal doses of prototype strains of serotypes Ia and III, 12-48 hours after birth.

1.3 Alhydrogel

GBS-NN/NN2 will be administered bound to Alhydrogel as an adjuvant. Alhydrogel induces a Th2 response by improving the attraction and uptake of antigen by antigen-presenting cells (APCs). It can also activate innate immunity pathways triggered by pattern recognition receptors (PRRs).

1.4 Motivation for the study and justification

South Africa, Uganda and some European countries practise a risk-based approach to prevent invasive GBS disease, i.e. IAP is only provided to mothers in labour with risk factors such as maternal fever, prolonged rupture of membranes or prematurity [1,14]. This strategy is less effective than screening all pregnant women at 35 to 37-week gestation and providing IAP to all women with a positive culture from vaginal or rectal swabs. The strategy of providing IAP only to women at clinical risk is also failing in low-to-middle-income countries because risk factors do not identify all women who are at risk of transmitting GBS to their babies. Furthermore, healthcare workers may fail to recognise risk factors, and may not provide IAP in busy obstetric units. Other proposed preventive strategies, including chlorhexidine vaginal wipes, do not prevent EOD [1,14]. Rapid molecular diagnostic tests to detect maternal GBS colonisation during labour is promising but costly, and the struggle for most low-to-middle-income countries remains the timely and effective administration of IAP, thus rendering molecular tests futile in these settings.

These limitations, as well as the failure to implement these strategies in low-to-middle-income countries, calls for a paradigm shift regarding the future prevention of invasive GBS disease. Vaccinating pregnant women in the second trimester of pregnancy with the objective of increasing in maternal antibody concentrations, which will be transferred to the foetus in the last trimester, with the potential of providing protection to the new-born baby. Vaccination of pregnant woman in the second trimester has the potential to prevent over 90% of all invasive GBS disease. Maternal GBS vaccination is potentially cost effective and easy to implement in low-to-middle-income settings; furthermore, vaccination during pregnancy is becoming more acceptable to the public and health professionals. GBS is the most common cause of neonatal sepsis and meningitis. SA and Uganda have a very high incidence of invasive GBS disease and the provision of IAP is practically difficult; maternal GBS vaccination is a promising strategy.

MinervaX has developed a novel GBS vaccine candidate, based on the N-terminals domains of the alpha protein family of GBS surface proteins. The vaccine consists of two fusion proteins each containing two N-terminal domains: GBS-NN (containing the N-terminal domains of the Rib and Alpha C proteins – RibN and AlpCN) & GBS-NN2 (containing the N-terminal domains of the Alpha 1 and Alpha 2/3 proteins – Alp1N and Alp2/3N). These alpha proteins are found on most isolates of capsular polysaccharide serotypes Ia, Ib, II, III, IV, V, VI, VII and VIII. Therefore, it is anticipated that the GBS-NN/NN2 vaccine will confer protection against close to 100% of GBS clinical isolates. Antibodies directed against the vaccine were found to recognize the vast majority of 154 clinical isolates of serotypes Ia, Ib, II, III and V tested for antibody binding.

GBS-NN/NN2 demonstrated a good safety profile in non-pregnant women, with most AEs being mild and related to injection site reactions, no serious adverse events related to GBS-NN/NN2 have been reported. Based on this initial human study with GBS-NN/NN2 in healthy non-pregnant female volunteers, the proposed dosing regimen for pregnant women with the GBS-NN/NN2 vaccine is two doses administered 4 weeks apart at approximately 26 and 30 weeks of gestation. Each dose will contain 50µg of each fusion protein (GBS-NN and GBS-NN2) in combination with 500µg Alhydrogel).

2. STUDY OBJECTIVES AND STUDY END POINTS

2.1 Study Objectives

2.1.1 Primary objectives

There are two primary objectives, and they are assessing safety, tolerability and immunological effect of GBS-NN/NN2:

Safety:

To evaluate the safety and tolerability of the GBS-NN/NN2 vaccine in women living with HIV and mothers without HIV and their new-born babies from vaccination up to delivery/birth.

Immunological:

To compare the transfer rate of vaccine specific immunoglobulin G (IgG) antibody concentrations from the mother to the baby at birth in women living with HIV with the transfer rate in women without HIV. This endpoint will be used to determine the sample size calculation.

2.1.2 Secondary objectives

The secondary objectives are as follows:

Safety: The safety and tolerability of the GBS-NN/NN2 vaccine in the mother and baby over the first 12 months post-partum, as assessed at 6 and 12 months of age.

Immunological: The secondary immunological objectives are:

- To compare IgG antibody responses, specific to Rib, Alp1, Alp2, AlpC, GBS-NN and GBS-NN2, induced by the first and second vaccine doses over time in pregnant women living with HIV and pregnant women without HIV.
- To determine the concentrations of IgG antibodies specific to Rib, Alp1, Alp2, AlpC, GBS-NN and GBS-NN2 in cord blood at delivery in babies born to women with and without HIV.
- To evaluate the concentration of IgG antibodies specific to Rib, Alp1, Alp2, AlpC, GBS-NN and GBS-NN2 up to 6 months post-delivery in the mother and 3 months post-delivery in the baby in women with and without HIV.
- To evaluate the vaccine specific IgG concentration time profile over time, in the baby over the first 3 months of life and compare this between babies born of mothers living with and without HIV.

2.1.3 Exploratory Objectives

The exploratory objectives are as follows:

- [redacted]
- [redacted]

2.2 Study endpoints

2.2.1 Primary End points

Safety Endpoints

The main safety objective of the study will be to evaluate the safety and tolerability of the GBS-NN/NN2 vaccine in women living with HIV and mothers without HIV and their new-born babies from vaccination up to delivery/birth

The following safety endpoints in the mother will be evaluated to support this objective: local and systemic reactogenicity (headache, sore throat, "flu-like" symptoms, muscle aches, fever in the 72 hours post dose); adverse events; laboratory tests; dipstick urinalysis; vital signs; physical examination.

Admissions to hospital for a normal or following an elective Caesarean section leading to a healthy, term birth will not be deemed a serious adverse event, unless deemed an emergency caesarean section. Admissions to hospital for a normal or following an elective Caesarean section leading to a healthy, term birth will not be deemed a serious adverse event, unless deemed an emergency caesarean section, in which case the reason the Emergency Lower Segment Caesarean Section (LSCS) was required will be reported as a SAE.

In the new-born baby: gestational age at birth (based on ultrasound, date of last menstrual period, fundal height, as per the Global Alignment of Immunization safety Assessment in pregnancy (GAIA) guidelines), weight, length, head circumference, Apgar score at 1, 5 and 10 minutes (if available).

The data for the primary endpoint will be the data collected at the assessments conducted at delivery.

Immunological Endpoints

Immunological**:** The IgG antibody concentrations, specific to Rib, Alp1, Alp2, AlpC, GBS-NN and GBS-NN2, in individual maternal blood and cord blood at birth.

2.2.2 Secondary End points

Safety

Safety secondary data will be evaluated by following up mothers and babies for 12 months post-delivery, mothers will be assessed for significant adverse reactions with specific interest in potential immunotoxicity e.g. development of auto immune diseases.

Developmental milestones of the babies will be assessed at 6 and 12-months using age-appropriate ages and stages questionnaires (Appendix 3 (ASQ-3).

Immunological

The following endpoints will be evaluated, by group and time-point, to support immunological secondary objectives:

- Geometric mean antibody concentration in μg/ml at 4 weeks post first (pre-second injection) and 4 weeks post second injection.
- Geometric mean antibody concentration in μg/ml of cord (or new-born blood within 48 hours of birth) and maternal blood at delivery.
- Geometric mean fold increase in antibody concentration in maternal blood at 4 weeks post first (pre-second injection) and 4 weeks post second injection.
- Geometric mean fold increase in antibody concentration in maternal blood from baseline to delivery.
- Proportion of mothers and new-born babies achieving vaccine specific IgG antibody concentrations, in maternal and cord blood samples respectively, above pre-specified arbitrary thresholds at delivery (these will be 0.5, 1, 2 μg/ml).

The primary immunological analysis will be the values of these endpoints at the time of delivery, which is anticipated to be approximately 10 to 14 weeks after the administration of the first dose of vaccine.

3. STUDY DESIGN

This is a Phase II, randomised, placebo controlled, double-blind, parallel group, multi-centre study. The study is to assess the safety, tolerability and immunogenicity of two doses of Group B Streptococcus vaccine (GBS-NN/NN2) in women who are pregnant and living with HIV and women who are pregnant and do not have HIV and who are 18 to 40 years of age. The study will enrol approximately 200 study participants. The study will be separated into 4 groups, two groups of 80 participants to receive the potential vaccine and two groups of 20 participants to receive placebo.

Group 1:80 women who are pregnant and living with HIV, who will receive two injections of GBS-NN/NN2. Each injection will contain 50 µg of GBS-NN and 50 µg of GBS-NN2.

Group 2: 80 women who are pregnant and do not have HIV who will receive two injections of GBS-NN/NN2. Each injection will contain 50 µg of GBS-NN and 50 µg of GBS-NN2.

Group 3: 20 women who are pregnant and living with HIV who will receive two injections of placebo.

Group 4: 20 women who are pregnant and do not have HIV who will receive two injections of placebo.

Participants will be screened at 24 to 28 weeks gestation, Day -14 to day -1. The groups will be dosed in parallel; the first dose of vaccine or placebo will be administered at Day 0 and the second dose will be administered at Day 28±2 days after the first dose.

For the analysis of the immune responses the placebo groups will be combined. For safety, the placebo groups will be analysed separately and combined for comparison with the potential vaccine groups

3.1 Study visits for Mother

Study participants will have the following study visits:

Screening Period 24 to 28 Weeks of gestation:

Screening visit / Day -14 to Day -1 – To obtain informed consent and perform screening assessments

Treatment Period:

Visit 1/ Baseline visit / Day 0- To perform baseline assessments, blood draw for IgG/IgA antibody concentration pre-dose, randomization and Investigational Product (IP) administration

Visit 2/ Vitals visit/ Day 7 to10 – To perform assessments of vital signs, review diary card, AEs and concomitant medications.

Visit 3 / Treatment visit/ Day 28 – To perform visit 3 assessments, blood draw for IgG/IgA antibody concentrations pre-dose and safety; and IP administration.

Visit 4 /Follow Up / before delivery – Day 56 – Visit 4 follow-up assessments, review diary card, AEs, concomitant medications, blood draw for IgG/IgA antibody concentration and safety.

Visit 5 /Delivery/ Delivery assessments and blood draw for IgG/IgA antibody concentration, AEs and concomitant medications.

Post Delivery

Visit 6/ Day 28 - Post-delivery assessments and AE

Visit 7/ Day 56 - Post-delivery vital signs and AE

Visit 8/ Day 84 - Post-delivery vital signs and AE

Visit 9/ Day 182 - Post-delivery assessments, blood draw for IgG/IgA antibody concentration and AE

Visit 10/ Day 365 - Post-delivery, AEs of special interest

Window period +/-2 days for Visits 1 to 4, +/-4 days for Visit 6, +/-6 days for Visits 7 and 8, and+/-8 for Visits 9 and 10.

3.2 Study visit for Baby

Babies born in the study will have the following study visits:

Delivery

Visit 5 /Delivery/ Birth assessment, blood draw for IgG antibody concentration and visit assessments

Post Delivery

Visit 6/ Day 28 - Post-delivery assessments and blood draw for IgG/IgA antibody concentration for all babies and AEs

Visit 7/ Day 56 - Post-delivery assessments and blood draw for IgG/IgA antibody concentration from half of the babies and AEs

Visit 8/ Day 84 - Post-delivery vital signs, blood draw for IgG/IgA antibody concentration from the other half of the babies and AEs

Visit 9/ Day 182 - Post-delivery assessments, baby's age-appropriate developmental assessment, concomitant medication and AEs

Visit 10/ Day 365 - Post -delivery follow-up study visit: baby's age-appropriate developmental assessment, adverse events and concomitant medications

Window period +/-4 days for Visit 6, +/-6 days for Visits 7 and 8, and+/-8 for Visits 9 and 10.

Should any babies develop invasive GBS disease over the course of the study they will be treated as per normal standard of care. The number of babies in each group that develop invasive GBS disease will be compared although no cases are expected in this number of participants. The severity of the infection will also be assessed and compared. Blood samples will be obtained to assess the concentration of IgG antibodies specific for the vaccine from the babies as described in the schedule of events.

4. SELECTION OF PARTICIPANTS

4.1 Inclusion criteria

Study participants fulfilling the following criteria are eligible to participate in the study:

1. Participants at least 18 years old and not older than 40 years of age.
2. Pregnant women who are between 26 weeks and 30 weeks (inclusive) gestation on the planned day of vaccination with a singleton, uncomplicated pregnancy. Gestational age to be determined on the following hierarchal basis with guidance to the GAIA criteria in appendix 04:
    a. ultrasound estimate of gestational age,
    b. date of last menstrual period
    c. fundal height
3. HIV status to be based on rapid, confirmatory test, unless a documented test of the participant being sero-positive for HIV history documented in the notes.
4. Women living with HIV, HIV viral load <1000, on antiretroviral therapy for at least 3 months prior to screening and clinically well.
5. Expected to be available for the scheduled clinic visits for the duration of the study, agree to be contacted by telephone during study participation, and is willing to give parental consent for her infant to participate in the study

4.2 Exclusion criteria

Study participants who meet any of the following criteria are to be excluded from study participation:

1. Women who are HBSAg and/or HCV positive
2. Women who test positive for syphilis as per standard testing
3. Women knowingly carrying, at screening, a malformed or genetically abnormal foetus based on ultrasound
4. Women who have experienced a previous stillbirth prior to going into labour
5. Women with placenta previa
6. Women with documented chronic or pregnancy induced hypertension at screening

7. Women with 1+ protein in urine and hypertension, defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg) at ≥20 weeks of gestation in a woman with a previously normal blood pressure

8. Women with >1+ of protein in urine (regardless of blood pressure)

9. Women with gestational, type 1 or type 2 diabetes.

10. Women with glycosuria on dipstick

11. Women known to be allergic to any components of the vaccine, who are known to be allergic to aluminium or have had an allergic reaction to any previous vaccination.

12. Women with HIV viral load >1000 at screening.

13. Women living with HIV who have been on antiretroviral therapy for less than 3 months prior to screening. (Women who are diagnosed with HIV between screening and dosing will not be eligible.)

14. Women with history or presence of significant cardiovascular disease, pulmonary, hepatic, gallbladder or biliary tract, renal, haematological, gastrointestinal, endocrine, immunologic, dermatological, neurological, psychiatric, autoimmune disease, cognitive disorder or current infection and significant illness 4 weeks prior to randomization.

15. Women with current or history of drug or alcohol abuse within the last two years.

16. Women who have received a vaccine within 28 days of receiving the first dose of GBS NN/NN2 or placebo or who expect to require vaccination during the course of the study. (Vaccines recommended for administration during pregnancy e.g. tetanus toxoid, pertussis and influenza are permitted. Administration of concurrent vaccines must not be within 7 days of investigational vaccine.)

17. Women who have a fever (axillary temperature >37.9°C) on the day of dosing or have had an acute infection in the 7 days before dosing.

18. Women who have any bleeding disorders that prolong the bleeding time.

19. Women who are receiving immunosuppressive medication, including systemic steroids (inhaled and topical steroids are acceptable).

20. Women who have received blood or blood products and/or plasma derivatives or any immunoglobulin preparations in 12 weeks preceding screening.

21. Women with severe anaemia, haemoglobin < 9g/dL (90 g/L) as per Sheffield grading system (> Grade1)

22. Women who are currently breast feeding

23. Women who are part of the study personnel or a close family member of study personnel.

24. Women who in the opinion of the investigator are not suitable to participate in the study.

25. Concurrent participation in another clinical trial during which the participant will be exposed to an investigational product

5. STUDY TREATMENTS

5.1 Details of study treatments and Dosage

Participants will receive two doses of either GBS-NN/NN2 vaccine or Placebo, as a single injection on Days 0 and Day 28. The GBS-NN/NN2 vaccine will be supplied as a sterile solution for injection containing "(NN+NN2)DP" protein 2.5 ml at 0.7 mg/ml (0.35 mg/ml of each protein component) for dilution in Alhydrogel. A volume of 0.2 ml of (NN+NN2)DP will be added to 0.5 ml of Alhydrogel. A dose will be 0.5 ml of the resulting mixture.

Placebo will be 0.5 ml of 0.9% saline.

The dose will be a single injection on Days 0 and Day 28. Each injection will consist of either active vaccination with GBS-NN/NN2, which will contain 50 µg of GBS-NN and 50 µg of GBS-NN2, or placebo.

Administration will be by intramuscular injection, preferably into the non-dominant arm. The dominant arm may be used if it is not possible to administer into the non-dominant arm e.g. due to an ongoing injection site reaction, or tattoo

The treatment groups are:

Test Product 1: GBS-NN/NN2, 50 µg of GBS-NN and 50 µg of GBS-NN2, two single doses by injection at Day 0 and Day 28

Manufactured by: Biovian Ltd

Address: Tykistökatu 6B, Biocity FIN-20520 Turku, Finland.

Reference Product: 0.9% Normal saline 0.5 ml two single doses of injection at Day 0 and Day 28

Name and business address of the Holder Certificate of Registration:

The 0.9% saline for injection will be purchased by the sites and each site will use saline available in their respective countries.

The study medication will be stored as follows:

(NN+NN2)DP active protein vials will be stored frozen at approximately -20°C. Vials will be thawed at room temperature for 2 hours or overnight at 2-8°C prior to use in assembly steps.

Alhydrogel suspension vials are stored refrigerated at approximately 2°C to 8°C and must not be allowed to freeze. Vials will be taken out of the refrigerated storage prior to assembly steps. Further details are provided in the pharmacy manual.

5.2 Treatment assignment

The study medication will be administered only to participants included in this study and will follow the procedures set out in the latest (approved) version of the study protocol. Participants will be randomized to either receive two doses of either GBS-NN/NN2 vaccine or Placebo as a single injection on Day 0 and Day 28. The study will enrol approximately 200 women who are pregnant and living with HIV and women who are pregnant and do not have HIV and who are 18 to 40 years of age. On fulfilling the eligibility criteria, participants will be included in the study.

A sequence of participant screening numbers will be allocated to each study centre and these numbers will be assigned by the site to each participant screened. After signing the informed consent, participants will receive a 3-digit participant number that will be used to identify them throughout the screening process. In each centre, participant numbers will be assigned in sequential order. These numbers will be preceded by the 3-digit centre number to give the full 6- digit participant number.

An IBM central randomization schedule will be generated on a balanced basis with a randomization ratio of 4:4:1:1. (80 pregnant women living with HIV to receive GBS-NN/NN2; 80 pregnant women who do not have HIV to receive GBS-NN/NN2); 20 pregnant women who are living with HIV to receive placebo; 20 pregnant women who do not have HIV to receive placebo). Eligible participants will be randomised using a computerized IBM central randomisation system and 50% of babies will also be randomised at 2 months and the other 50% at 3 months.

Once the IBM database is set, it will generate a randomisation list in excel with password protection and will be emailed to the relevant unblinded personnel (Unblinded Pharmacist and the Unblinded Monitor). The unblinded team will store the randomisation away from the blinded study team.

During the screening visit, the site will enter the screening participant into the IBM system including the screening number. An automated alert email from IBM will generate to confirm the screening and the email will be sent to the Site, Unblinded Pharmacist, CRA, Unblinded CRA Project Manager and CTA. Once the screening is completed and the participant is ready to be randomised, the site will randomise the participant using the IBM randomisation system. The site will enter the participant information including HIV status to randomise a participant. Once the participant is randomised, the IBM system will generate a randomisation number and an email to the site. The unblinded pharmacist will take the email notification or printed version, match the number to the randomization list and prepare the vaccine/placebo accordingly.

The participants will use the same randomisation number throughout the study. The pharmacist will retain the lists of the allocation of unblinded medication prepared. The list must be kept in a safe place away from the blinded study team and will be monitored by the unblinded CRA.

5.3 Blinding, packaging, and labelling

Blinding will be managed by the IBM system and will be explained in section 10.2. The premature breaking of the code should be confined to emergency cases in which knowledge of the administered drug is necessary for adequate treatment. Whenever possible, the sponsor should be contacted before breaking the blinded emergency code. Should any code be broken, consideration must be made as to

whether the respective participant should be withdrawn from further participation in the study and a written explanation must be given by the investigator; the sponsor must be notified immediately.

5.4 Storage conditions

5.4.1 GBS (NN+NN2)DP

(NN+NN2)DP active protein vials will be stored frozen at approximately -20°C.

5.4.2 Alhydrogel

Alhydrogel suspension vials are stored refrigerated at approximately 2°C to 8°C and must not be allowed to freeze.

5.4.3 Shelf-Life

Component vials will have an expiry date as provided by the sponsor's contract manufacturer.

Assembled doses for administration to participants in this clinical trial will be assigned an expiry date of 24 hours from insertion of the needle in the GBS(NN+NN2)DP vial.

5.5 Supplies and accountability

The investigator or site staff will keep an inventory and acknowledge receipt of all shipments of study medication. All study medication must be kept in a locked area with access restricted to designated study personnel. The study medication must be stored in accordance with the manufacturer's instructions. The investigator or designee will also keep accurate records of the quantities of study medication dispensed, used, and unused. The unblinded site monitor will periodically check the supplies of study medication held by the investigator or pharmacist to ensure accountability of all study medication used.

During the study, the investigator will ensure drug accountability. At the end of the study all used and unused vials will be stored at the site. The site will request permission from the sponsor to return unused IP to LogicTrials for destruction. The site will be provided with proof of IP destruction from LogicTrials. Final report of drug accountability will be prepared and maintained by the investigator or designee.

5.6 Compliance

The date, time of dose (0.5 ml of GBS-NN/NN2 vaccine or placebo) and site (left or right arm, and route deep intramuscular) of administration of the IP will be documented on the source notes and this

information will be transcribed to the eCRF. The investigator should keep all study vials at the study site during the Treatment Period until the end of the study, when the sponsor will provide instructions regarding disposition of the IP. Study medication should be administered as per protocol. Study site personnel will document compliance.

The investigator or his/her designated and qualified representatives will dispense study medication only to participants enrolled in the study and in accordance with the protocol. The study medication will not be used for reasons other than that described in the protocol.

5.7 Contra-indications

GBS-NN/NN2 vaccination is contraindicated in participants known to suffer hypersensitivity reactions to vaccines or a known allergy to aluminium hydroxide. Potential participants, who have experienced a significant illness in the four weeks prior to randomisation will be excluded from the study and will be referred back for routine antenatal care.

The vaccine should not be given to participants with known or suspected, autoimmune diseases or who have an acute infection.

GBS-NN/NN2 should not currently be given to nursing mothers.

5.8 Special warnings and precautions for use

From the data gathered to date; administration of GBS-NN/NN2 has been associated with mild, self-limiting pain at the injection site. No systemic risks have been identified. There have been no reports of significant or persistent lesions at the injection site. The reported incidence of miscarriage, post vaccine exposure, is in line with the incidence seen in the general population and was comparable between the active and placebo groups with no dose relationship.

5.9 Interaction with other medicinal products and other forms of interaction

No drug / drug interaction studies have been conducted to date. GBS-NN/NN2 has not been administered at the same time as other vaccines. If other vaccines are required there should be a period of at least 1 week between the administration of GBS-NN/NN2 and other vaccines.

5.10 Concomitant Medication

All concomitant medications taken by participants must be recorded in the eCRF along with the reason the medication was required. Medication associated with routine deliveries will also be recorded. This includes medication used for analgesia, labour augmentation, anaesthetics - including pre-medication and spinal anaesthesia.

5.11 Prohibited medication

Participants should not receive immunosuppressive medication, including systemic steroids (inhaled and topical steroids are acceptable) during the course of the study.

5.12 Pregnancy and lactation

The study is enrolling women who are pregnant. The IP should not be given to lactating mothers or women who are not pregnant.

5.13 Side-effects and special precautions

Based on the results from the clinical studies to date and use of other protein vaccines adsorbed to Alhydrogel, or other aluminium containing adjuvants, transient local reactions at the injection site, including pain, oedema, bruising, erythema and induration, mainly of mild intensity, do occur. With GBS-NN/NN2 these reactions have been generally mild and self-limiting. In common with other vaccines participants may experience "flu like symptoms" following vaccination; such symptoms may include fatigue, malaise, myalgia and may be related to the induced immune response.

The normal adverse event profiles from Phase 1 studies suggests that a typical array of minor clinical study symptoms may be experienced by participants taking part in the study.

Unexpected adverse events may occur, including allergic reactions to the vaccine. At the time of vaccination, the investigator should have access to resuscitation equipment and medication, in case of a severe hypersensitivity reaction

It has been shown that naturally occurring antibodies against the N-terminal proteins, are present in the majority of non-pregnant women, and therefore, will be found in pregnant women. However, the possibility of, as yet, unknown side effects to antibodies should remain a consideration. All studies with GBS-NN/NN2 require that participants are monitored after vaccine administration (vital signs and general signs and symptoms), with regular follow-up visits for physician assessment and questioning regarding AEs will be conducted for all participants in the study.

GBS-NN/NN2, like other vaccines, may theoretically be associated with a transient febrile reaction following vaccination. Immune complex-mediated reactions are unlikely given the recombinant nature of GBS-NN/NN2; however, it is still possible that hypersensitivity may occur. None of these events have been seen to date with GBS-NN/NN2.

5.14 Know symptoms of overdose

A maximum tolerated dose in humans has not been determined; therefore, no data exist on the effects of overdose with GBS-NN/NN2. However, as the vaccine is administered by the study team under controlled conditions of a clinical trial, an overdose is unlikely. If an overdose does occur symptomatic treatment should be initiated along with general supportive care.

6. PRIOR AND CONCOMITANT ILLNESSES & MEDICATIONS

6.1 Prior and concomitant illnesses

The participant's medical history of illnesses or disorders that occurred prior to the time of informed consent will be documented in the CRF.

Additional illnesses that present from the time informed consent is obtained will be documented on the appropriate pages of the CRF. All maternal SAEs will be reported from the day of signing informed consent for study participation until 28 days post-delivery (Visit 6). Serious adverse reactions and AEs of special interest (e.g. autoimmune diseases) will be reported throughout the study period.

All AEs and SAEs in the baby from birth up to Day 365 (Visit 10) will be reported.

Illnesses first occurring or detected during the study, and worsening of a concomitant illness during the study, are to be regarded as adverse events and must be documented as such in the CRF.

6.2 Prior and concomitant medications

All additional medications being taken by the participants from the time consent is obtained until the end of the study are regarded as concomitant medications and must be documented on the appropriate pages of the case report form.. This will include all medications prescribed or purchased over the counter during pregnancy and labour, and all prophylactic medications.

7. STUDY PROCEDURES AND SCHEDULE

7.1 Overview of data collection

Participants will provide written informed consent and will be screened for eligibility within 14 days before first study vaccine administration (Day 0). Eligible participants will receive two doses of GBS-NN/NN2 or placebo – the first dose will be administered on Day 0, and 4 weeks later the second dose of the primary course will be administered. Following vaccination, participants will remain in the clinic for at least 30 minutes post vaccination.

For the procedures to be undertaken at each visit see Table 1 Schedule of Assessments

7.2 Description of study days

7.2.1 Screening visit (Day -14 to -1) Between 24- and 28-weeks of gestation

During this period potentially eligible participants will be identified and the study procedures will be explained to them. The investigator will provide all the information about the study to potential study participants. Any queries raised by the potential study participants will be satisfactorily answered by the investigator. During the informed consent process, complete information about the study, study procedures, potential risks and benefits, alternative treatments and study medication information will be provided to the potential participant.

If the potentially eligible participant wishes to proceed and enrol in the study, she will be asked to provide written informed consent and proceed to screening. Screening will be performed within 14 days before administration of the first dose of study vaccine (Day 0). The site will enter the screening participant into the IBM system including the screening number. An automated alert email from IBM will be generated to confirm the screening and the email will be sent to the site, Unblinded Pharmacist, CRA, Unblinded CRA Project Manager and CTA

Following the provision of consent, study participants will be screened for the eligibility. The following procedures will be performed in this screening visit.

- Informed consent process
- Inclusion and Exclusion criteria
- Demographic data, height, weight BMI, MUAC and race ethnicity
- Medical history including concomitant medications
- Obstetric history (including last menstrual period (LMP)
- Physical and obstetric examination (Including fundal height)
- Ultrasound assessment to determine the gestational age and to confirm singleton pregnancy with no detected abnormalities, unless a documented first trimester scan provides these data
- Vital Parameters: (Heart rate, blood pressure and axillary temperature).

- Foetal heart rate, foetal movement and symphysis fundi height
- Urinalysis (Dipstick)
- Safety laboratory tests (Full Blood Count and Blood Biochemistry (Haematology (Red blood cell count, Haemoglobin, Haematocrit, Platelet count, White blood cell count with absolute differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils) counts) and Biochemistry (Sodium, Potassium, Chloride, Bicarbonate, Blood urea, Creatinine, Creatine Kinase, Glucose, Calcium, Albumin, Cholesterol, C-reactive protein, Triglycerides, Phosphorus (inorganic phosphate), Lactate dehydrogenase, Total protein, Globulin, Uric acid, Alkaline phosphatase, Aspartate aminotransferase, Alanine aminotransferase, Gamma-glutamyl-transferase, Total bilirubin and Direct bilirubin): A blood sample of approximately 5 ml will be withdrawn for safety bloods tests. The blood samples will be sent to a local pathology laboratory to be assayed).
- Hepatitis B and C, and Rapid HIV test, viral load and CD4 count (A blood sample of approximately 12 ml)

All data will be entered into the screening visit section of the CRF by the staff at the investigator's site. Screening failures will also be entered in the CRF including demographics and the reason for failure.

7.2.2 Visit 1: (Day 0) Randomisation and Vaccination Visit

Patients will present at clinic at 26 to 30 weeks of gestation and eligibility will be checked. Those participants who remain eligible will be randomised to active or placebo. This will be Day 0 and can occur within 14 days of screening.

For randomisation, the site will randomise the participant using the IBM randomisation system. The site will enter the participant information including HIV status to randomise a participant. Once the participant is randomised, the IBM system will generate a randomisation number and an email to the site. The unblinded pharmacist will take the email notification or printed version, match the number to the randomization list and prepare the vaccine/placebo accordingly. At this time, the randomisation of the babies for blood sample collection at 2 and 3 months post-delivery will also be allocated by the IBM randomisation system.

The pharmacist will add the participant's number on the randomisation list to link a participant with the randomisation number given. The pharmacist will put the randomisation number on the label of the blinded vaccine for the study team to inject.

The following procedures will be performed in this visit:

- Review of participant eligibility
- Physical examination
- Vital Parameters: (Heart rate, blood pressure, maternal weight and axillary temperature, foetal heart rate and movements, and symphysis fundi height). Vital signs at the dosing visit will be recorded pre-dose and at 30min post-dose and when the participant leaves the site if the participant stays longer than 30 minutes after receiving the IP for medical reasons.

- Urinalysis (Dipstick)
- Document prior medications
- Randomisation
- Pharmacodynamic blood sampling for antibody response
- Administration of investigational vaccine
- Assessment of injection site (Photographs may be taken of injection site reactions as required)
- Prescription Concomitant medication and Adverse events check
- Dispense a diary, thermometer and reactogenicity ruler and train the study participants how to complete the diary card.
- Participant will be provided with the next visit date.
- eCRF shall be completed as soon as possible and within five days of the visit.

Important notes post vaccination:

The site should observe participants for 30 minutes post dose and record any adverse events. Thirty (30) minutes post vaccination an assessment of the injection site will be performed, and vital signs measured and recorded. If the site considers the participant to be fit enough, she can be discharged home. Vital signs will be assessed before the participant leaves the site if the participant remains at the site for longer than 30 minutes for medical reasons.

In the event there is an extreme injection site reaction, then consider photographing the injection site. Only the injection site should be photographed. Do not take a picture of the participant's face or any personal identifiers. Do not inject the vaccine on a distinctive tattoo as it will not be possible to assess the injection site.

7.2.3 Visit 2 (Day 7 to Day 10) – Treatment Visit

Study participants will return at Day 7 and the following assessments will be performed:

- Vital parameters. (Heart rate, blood pressure and axillary temperature, foetal heart rate, foetal movements maternal weight and symphysis fundi height measurement)
- Assessment of injection site (Photographs may be taken of injection site reactions if required)
- Investigator shall review use of concomitant medications and adverse events. All adverse events will be appropriately managed.
- The completed diary cards will be reviewed for satisfactory completion and relevant data transcribed to the eCRF.
- eCRF shall be completed as soon as possible and within five days of the visit.

- If the mother delivers before Visit 2, the assessment relating to the collection and review of the diary card, adverse events from first vaccination, scheduled for Visit 2 will be collected at the delivery Visit 5.

7.2.4 Visit 3 (Day 28 ±2) – Treatment visit

The site will take bloods to assess the immune response from first vaccination and, safety bloods, prior to administering the second dose of IP. Adverse events and concomitant prescription medications will be assessed and recorded.

Study participants will visit at Day 28 and the following assessments will be performed:

- Vital signs: (Heart rate, blood pressure and axillary temperature, foetal heart rate, foetal movements, maternal weight, and symphysis fundi height measurement). Vital signs at the dosing visit will be recorded pre-dose and at 30min post-dose and when the participant leaves the site if the participant stays longer than 30 minutes after receiving the IP for medical reasons.
- Urinalysis (Dipstick)
- Safety laboratory tests (Full Blood Count and Blood Biochemistry (Haematology (Red blood cell count, Haemoglobin, Haematocrit, Platelet count, White blood cell count with absolute differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils) counts) and Biochemistry (Sodium, Potassium, Chloride, Bicarbonate, Blood urea, Creatinine, Creatine Kinase, Glucose, Calcium, Albumin, Cholesterol, C-reactive protein, Triglycerides, Phosphorus (inorganic phosphate), Lactate dehydrogenase, Total protein, Globulin, Uric acid, Alkaline phosphatase, Aspartate aminotransferase, Alanine aminotransferase, Gamma-glutamyl-transferase, Total bilirubin and Direct bilirubin): A blood sample of approximately 5 ml will be withdrawn for safety bloods tests. The blood samples will be sent to a local pathology laboratory to be assayed.
- Pharmacodynamic blood sampling for antibody response (approximately 20 ml)
- Administration of investigational vaccine
- Assessment of injection site (Photographs may be taken of injection site reactions if required) pre-dose and 30 minutes post-dose.
- Investigator shall review use of concomitant medications and adverse events. All adverse events will be appropriately managed.
- Participant will be provided with the next visit date and the new diary.
- The filled-in diary cards will be reviewed for satisfactory completion and re-education on diary card completion if required.
- eCRF shall be completed as soon as possible and within five days of the visit.
- If the mother delivers before visit 3, the assessments relating to safety bloods, adverse events from first vaccination, and concomitant prescription medications, scheduled for Visit 3 will be collected at the delivery Visit 5.

7.2.5 Visit 4 (Day 56 ±2) – Treatment follow-up visit

Study participants will visit at Day 56 and the following assessments will be performed:

- Vital Parameters: (Heart rate, blood pressure and axillary temperature, foetal heart rate, foetal movements and measurement of symphysis fundi height and maternal weight).
- Safety laboratory tests (Full Blood Count and Blood Biochemistry(Haematology (Red blood cell count, Haemoglobin, Haematocrit, Platelet count, White blood cell count with absolute differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils) counts) and Biochemistry (Sodium, Potassium, Chloride, Bicarbonate, Blood urea, Creatinine, Creatine Kinase, Glucose, Calcium, Albumin, Cholesterol, C-reactive protein, Triglycerides, Phosphorus (inorganic phosphate), Lactate dehydrogenase, Total protein, Globulin, Uric acid, Alkaline phosphatase, Aspartate aminotransferase, Alanine aminotransferase, Gamma-glutamyl-transferase, Total bilirubin and Direct bilirubin): A blood sample of approximately 5 ml will be withdrawn for safety bloods tests. The blood samples will be sent to a local pathology laboratory to be assayed.
- Pharmacodynamic blood sampling for antibody response (approximately 20ml)
- Assessment of injection sites (Photographs may be taken of injection site reactions if required)
- Investigator shall review use of concomitant medications and adverse events. All adverse events will be appropriately managed.
- The completed diary cards will be collected and reviewed for satisfactory completion and relevant data transcribed to the eCRF.
- eCRF shall be completed as soon as possible and within five days of the visit.
- If the mother delivers before Visit 4, the assessments relating to safety bloods, collection and review of the diary card, adverse events from first and second vaccinations, and concomitant prescription medications, scheduled for Visit 4 will be collected at the delivery Visit 5.

7.2.6 Visit 5 – Day of delivery visit

- On the day of delivery, the following information shall be recorded for the mother: Vital Parameters: (Heart rate, blood pressure and axillary temperature).
- Pharmacodynamic blood sampling for antibody response (venous sample from mother of approximately 20ml).
- For women not living with HIV: Record delivery HIV test result for the mother. If a test result is not available or not required by local testing regime, a rapid HIV test will be performed.
- Investigator shall review concomitant medications and adverse events. All adverse events will be appropriately managed.
- If the participant goes into labour prior to Day 56, safety laboratory tests associated with Day 56 visit will be collected on the day of delivery. The site will collect relevant data which will

be transcribed to the eCRF. The diary card that was scheduled to be collected at Day 56, will also be collected from the participant.

- eCRF shall be completed as soon as possible and within five days of the visit.
- Participant will be provided with the next visit date. (In post-natal ward or at home depending on the local norms.)

The investigator site will collect the following information and samples for the baby:

- Demographic data (Length, Weight and Gender, Type of delivery (normal vaginal delivery, forceps, Ventouse (vacuum assisted delivery), caesarean section), Gestational history and age.
- Physical examination including head circumference. Any congenital abnormalities will be noted. Apgar Score - Apgar score will be done at 1, 5 and 10 minutes if available (Available scores will be collected)
- Vital Parameters: (Heart rate).
- Pharmacodynamic blood sampling for antibody concentration: cord blood sample at birth, venous blood or heel prick sample within 48 hours of birth if cord blood is not obtained.
- Investigator shall review use of concomitant medications and adverse events. All adverse events will be appropriately managed for the baby.
- eCRF shall be completed as soon as possible and within five days of the visit.

7.2.7 Visit 6 (Day 28 ±4) – Post-delivery visit

Study participants will visit at Day 28 post-delivery and the following assessments will be performed with regards to the mother:

- Physical Examination
- Weight, Height, MUAC and BMI
- Vital Parameters: (Heart rate, blood pressure and axillary temperature).
- Investigator shall review adverse events. All adverse events will be appropriately managed.
- eCRF shall be completed as soon as possible and within five days of the visit.

The baby will be assessed and the following assessments will be performed:

- Physical examination including head circumference
- Weight
- Vital Parameters: (Heart rate).
- Record, in the eCRF, result of routine standard of care HIV serology assessment for the baby, if it has been done at this time
- Pharmacodynamic blood sampling for antibody concentration (Heel prick or venous sample).

- Investigator shall review use of concomitant medications and adverse events. All adverse events will be appropriately managed. Routine vaccinations to be recorded.
- eCRF shall be completed as soon as possible and within five days of the visit.

7.2.8 Visit 7 (Day 56 ±6) – Post-delivery visit

Study participants will visit at Day 56 post-delivery and the following assessments will be performed with regards to the mother:

- Vital Parameters: (Heart rate, blood pressure and axillary temperature).
- Investigator shall review use of concomitant medication and adverse events. All adverse events will be appropriately managed.
- eCRF shall be completed as soon as possible and within five days of the visit.

The baby will be assessed and the following assessments will be performed:

- Weight
- Vital Parameters: (Heart rate).
- Pharmacodynamic blood sampling for antibody concentration on 50% of babies (Randomised by IBM system from data management (Heel prick or venous sample).
- Investigator shall review use of concomitant medications and adverse events. All adverse events will be appropriately managed.
- Record, in the eCRF, result of routine standard of care HIV serology assessment for the baby, if it has been done at this time, if not recorded previously.
- eCRF shall be completed as soon as possible and within five days of the visit.

7.2.9 Visit 8 (Day 84 ±6) – Post-delivery visit

Study participants will visit at Day 84 post-delivery and the following assessments will be performed with regards to the mother:

- Vital Parameters: (Heart rate, blood pressure and axillary temperature).
- Investigator shall review use of concomitant medication and adverse events. All adverse events will be appropriately managed.
- eCRF shall be completed as soon as possible and within five days of the visit.

The baby will be assessed and the following assessments will be performed:

- Weight
- Vital Parameters: (Heart rate).
- Pharmacodynamic blood sampling for antibody concentration on 50% of babies (randomised by IBM Randomisation system from data management) (Heel prick or venous sample).

- Investigator shall review use of concomitant medications and adverse events. All adverse events will be appropriately managed.
- Record, in the eCRF, result of routine standard of care HIV serology assessment for the baby, if it has been done at this time, if not recorded previously or if new data available.
- eCRF shall be completed as soon as possible and within five days of the visit.

7.2.10 Visit 9 (Day 182 ±8) – Post-delivery visit/ Follow-up

Study participants will visit at Day 182 post-delivery and the following assessments will be performed with regards to the mother:

- Physical Examination
- Weight, Height, MUAC and BMI
- Vital Parameters: (Heart rate, blood pressure and axillary temperature).
- Pharmacodynamic blood sampling for antibody response
- Investigator shall review use of concomitant medication and adverse events. All adverse events will be appropriately managed.
- eCRF shall be completed as soon as possible at least with five days.

The baby will be assessed and the following assessments will be performed:

- Physical examination including head circumference
- Length and Weight
- Vital Parameters: (Heart rate).
- Developmental milestones – Age-appropriate Ages and Stages at month 6
- Investigator shall review use of concomitant medications and adverse events. All adverse events will be appropriately managed.
- eCRF shall be completed as soon as possible and within five days of the visit.

7.2.11 Visit 10 (Day 365 ±8) – Post-delivery visit/ Safety and development Assessment Follow-up - End of Study

Study participants will visit at Day 365 post-delivery, which is the end of study visit. The baby will be assessed and the following assessments will be performed:

- The use of concomitant medication and adverse events. All adverse events will be appropriately managed. Per the investigator discretion, adverse events may be regarded as ongoing, however per definition SAE's are to be followed up to resolution, where possible.
- Assess the baby as per standard of care.
- Assess developmental milestones – Age-appropriate Ages and Stages at month 12.

The mother will be assessed, and the following assessments will be performed:

- Urine pregnancy test.
- The use of concomitant medications and adverse events.
- All adverse events will be appropriately managed.
- The mother will be assessed for AEs of special interest (development of Autoimmune Diseases since the administration of the vaccine) which will be recorded in the eCRF.

7.3 Methods of data collection

7.3.1 Specific data for selection of participants

The participants will be selected on the basis detailed in *section 4*

7.3.2 Immunogenicity data

Immunogenicity data will be assessed at the following time points:

For the mother, blood will be drawn (approximately 20ml) for the Immunogenicity assessments at Visit 1 (Day 0), Visit 3 (Day 28), Visit 4 (Day 56), at delivery visit and Visit 9 (Day 182).

The concentration of vaccine specific IgG and IgA antibodies will be measured by ELISA

For the baby, the assessments will be performed at visits 5 to 8 by confirming the concentration of vaccine specific IgG and IgA antibodies.

7.3.3 Safety data

7.3.3.1 Adverse events

Safety will be evaluated using adverse events spontaneously reported during the study, assessment of injection area, physical examinations/vital signs, urinalysis and clinical laboratory data. Solicited and unsolicited AEs will be collected at all visits and on the diary card. The diary card will be used to collect solicited injection site reactions as well as temperature and solicited adverse event data for the 7 days post each injection. Congenital abnormalities to be reported as per standard adverse event and serious adverse event criteria.

7.3.3.2 Clinical laboratory data

Clinical laboratory data will be gathered at screening, visit 3 and visit 4. The results of all tests will be assessed. Abnormal results will be classified as clinically significant (CS) or not clinically significant (NCS). CS results will be reported as AEs and as SAEs if any of the criteria for seriousness are met.

The total blood volume anticipated to be drawn from the pregnant women will be approximately 170 ml, the volume of each test is estimated in the following table. The total blood volumes to be drawn from the babies is estimated to be 20 ml over the first 84 days of life.

Table of blood volume to be drawn, per test, from the mother:

	Number of Tests	Blood Volume per test	Total Blood volume
Biochemistry	3	10	30
Haematology	3	5	15
Hep B and Hep C	1	10	10
Rapid HIV Test	2	1	2
CD4 count	1	2.5	2.5
HIV viral load	1	10	10
PD blood sample – antibody response	5	20	100
Total blood volume			169.5

Immunology samples will be processed and analysed by the Medical Research Council/Uganda Virus Research Institute Laboratory in Entebbe, Uganda and CERBA Laboratory, South Africa. The analysis of the IgG titres towards the GBS-NN and GBS-NN2 vaccine antigens will be done at the MRC Laboratory including for samples collected from South African study participants. Analysis of the IgG titres towards the 4 AlpN antigens will be done by CERBA. Left over specimens from the immunology samples will be stored at CERBA, South Africa until the end of the study and shipped to Lund University, Immunology Division, Sweden. Analysis of IgA (possibly IgA1 and IgA2), and IgM as well as functional opsonophagocytic killing assay and epithelial cell invasion inhibition assays will be done by Lund Immunology Laboratory. Appropriate biological sample import and export permits will be obtained as required to meet the respective country requirements. All participants will be offered the opportunity to give consent to the long-term storage and future testing of the left- over immunology specimens.

The MRC Laboratory, Entebbe and CERBA Laboratory follow internationally recognized research ethics, Good Clinical Practices (GCP), and Good Clinical Laboratory Practices (GCLP) guidelines; staff are International Air Transport Association (IATA) certified for specimen shipping.

7.3.3.3 Physical examinations/ vital parameters

As part of the safety evaluation, physical examinations and vital signs parameters will be performed.

Vitals signs:

- Temperature in degree Celsius (recorded to the nearest 0.1 degree) will be measured by axillary thermometer
- Blood Pressure in mmHg will be measured by automated device or manually
- Heart rate in beats per minute will be measured by automated device or manually

- MUAC, Height and Weight (BMI will be calculated)
- Height measured in m and recorded to the nearest 0.1 cm
- Weight measured in kg and recorded to the nearest 0.1kg

Full Physical Examination

Targeted Physical Examination (Cardiovascular, Respiratory and Abdomen)

Injection sites will be assessed for erythema, induration, itching and pain, see Appendix 2

For laboratory assessment – Urinalysis (Dipstick)

8. PARTICIPANT SAFETY

8.1 Definitions

8.1.1 Adverse event

The term **adverse event** refers to any sign, symptom, syndrome, or illness that appears or worsens in a participant during the clinical study and that may impair the well-being of the participant. The term also covers laboratory findings or results of other diagnostic procedures that are considered to be clinically relevant (e.g., that requires unscheduled diagnostic procedures or treatment measures, or result in withdrawal from the study).

The adverse event may be:

- Any unfavourable and unintended sign (including reactions from overdose, abuse, incorrect use of any treatment, or interaction)
- Any new disease or exacerbation of an existing disease (e.g. increase in frequency or worsening in nature)
- Any deterioration in measurements of laboratory values or other clinical tests (e.g. vital signs or X-ray) that results in symptoms, a change in treatment, or discontinuation from the IP
- Recurrence of an intermittent medical condition (e.g. headache) not present at baseline
- Other medical events regardless of their relationship to the IP, such as accidents, falls and any injuries resulting from them.

No causal relationship with the study medication or with the clinical study itself is implied by the use of the term 'adverse event'.

AEs will be recorded from the time informed consent is obtained until the end of study visit. All maternal SAEs will be reported from the day of signing informed consent for study participation to 28 days post-delivery (Visit 6). Serious adverse reactions and AEs of special interest (e.g. autoimmune diseases) will be reported throughout the study period. All AEs and SAEs in the baby from birth up to Day 365 (Visit 10) will be recorded

Adverse events fall into the categories 'non serious' and 'serious' (see *Section 8.1.2 Serious Adverse Event)*.

8.1.2 Serious Adverse Events

A serious adverse event is one that at any dose:

- Results in death

- Is life-threatening[1]
- Requires a participant's hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity[2]
- Is a congenital anomaly/birth defect
- Is medically important[3].

[1] 'Life-threatening' means that the participant was at immediate risk of death at the time of the serious adverse event; it does not refer to a serious adverse event that hypothetically might have caused death if it were more severe.

[2] 'Persistent or significant disability or incapacity' means that there is a substantial disruption of a person's ability to carry out normal life functions.

[3] Medical and scientific judgement should be exercised in deciding whether other adverse events may be considered serious because they jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation, and development of drug dependency or drug abuse.

Cases of overdose with an adverse event that meets one of the criteria given above should be reported as 'serious'.

The term 'severe' is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance. This is not the same as 'serious', which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Admissions to hospital for a normal or following an elective Caesarean section leading to a healthy, term birth will not be deemed a serious adverse event, unless deemed an emergency caesarean section, in which case the reason the Emergency Lower Segment Caesarean Section (LSCS) was required will be reported as SAE.

Categorization of Adverse Events

Intensity Classification

AEs will be classified as mild, moderate or severe according to the following criteria:

Mild: Awareness of sign, symptom, or event, but easily tolerated.

Moderate: Discomfort enough to cause interference with usual activity and may warrant intervention.

Severe: Incapacitating with inability to do usual activities or significantly affects clinical status and warrants intervention.

Causality Classification

The relationship of an AE to the study vaccine will be classified according to the following:

Related: An event that: follows a reasonable temporal sequence from administration of the vaccine, follows a known or expected response pattern to the suspected vaccine, could not be reasonably explained by the known characteristics of the participant's clinical state and, if necessary, the reaction returns on re-starting the vaccine (re-challenge).

Unrelated: An event that: does not follow a reasonable temporal sequence from administration of the vaccine and for which there is sufficient and conclusive information that the event is not related to the study vaccine.

8.1.3 Overdose

The vaccine is administered by the study team under controlled conditions of a clinical trial; therefore, an overdose is unlikely. If an overdose does occur symptomatic treatment should be initiated along with general supportive care. Cases of overdose with an adverse event that meets one of the criteria given in section 8.1.2 should be reported as 'serious'

8.2 Period of observation

For the purposes of this study, the period of observation extends from the time of signing informed consent until the end of study visit. All adverse events that occur must be documented and reported in accordance with the instructions for the completion of adverse event reports. Adverse events occurring between signing the consent form and the first dose of study medication will be classified as non-treatment emergent. All maternal SAEs will be reported from the day of signing informed consent for study participation to 28 days post-delivery (Visit 6). Serious adverse reactions and AEs of special interest (e.g. autoimmune diseases) will be reported throughout the study period. All AEs and SAEs in the baby from birth up to day 365 (Visit 10) will be reported.

The Investigator will review results from the physical examinations. All new and aggravated findings, as compared with baseline, must be identified, and recorded as AEs in the eCRF.

AEs already recorded and designated as 'continuing' should be reviewed at each subsequent assessment.

For all AEs, the Investigator must obtain adequate information to determine the outcome of the AE and to assess whether it meets the criteria for classification as an SAE. For all AEs, sufficient information should be obtained by the Investigator to determine the causality of the AE (i.e. study vaccine or other illness). The Investigator is required to assess causality and record that assessment on the eCRF. Follow-up of the AE, after the date of therapy discontinuation, is required if the AE or its

sequelae persist. Any AE that is still ongoing at the end of study visit will have an outcome of 'ongoing' recorded in the eCRF, however the Investigator will continue to follow up ongoing related AEs and record information in the source documents. Related SAEs will be followed until the event resolves or the event or sequelae stabilise, and this information will be reported to the Sponsor using the SAE reporting forms.

8.3 Documentation and reporting of adverse events by investigator

The following approach will be taken for documentation:

All adverse events (whether serious or non-serious) must be documented on the 'adverse event' page of the case report form.

If the adverse event is serious (see *Section 8.1.2 Serious Adverse Event*), the investigator must complete, in addition to the 'Adverse Event' page in the case report form, a 'Serious Adverse Event' form at the time the serious adverse event is detected. This form must be sent to Diamond Pharmacovigilance Services.

Contact Details for SAE / Pregnancy Reporting:

Diamond PV Services Ltd

T: +44 (0) 1279 406759

F: +44 (0) 1279 418964

Email: pvservices@diamondpharmaservices.com

The following mandatory information must be provided within 24 hours for each SAE/Pregnancy report:

- Protocol number
- Participant number
- AE
- IP
- Investigator's name and contact details

Causality assessment should be completed as soon as possible.

Every attempt should be made to describe the adverse event in terms of a diagnosis. If appropriate, concomitant symptoms should also be listed below the diagnosis. If only non-specific signs or symptoms are present, then these should be recorded as a diagnosis.

All participants who have adverse events, whether considered associated with the use of the study medication or not, must be monitored to determine the outcome. The clinical course of the adverse event will be followed up according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found or the investigator considers it

medically justifiable to terminate follow-up. Should the adverse event result in death, a full pathologist's report should be supplied, if possible.

All questions on the completion of adverse event report forms and any further forms issued to the investigator after the study is completed or after database lock or to clarify unresolved issues should be addressed to the sponsor.

8.4 Immediate reporting of SAEs and SUSARs

Serious adverse events and adverse events that fulfil a reason for expedited reporting must be reported to Diamond Pharmacovigilance Services as described below:

The investigator must report to Diamond Pharmacovigilance Services, all SAEs on the study specific SAE form, within 24 hours of learning about the event, regardless of relationship to study drug. All maternal SAEs will be reported from the day of signing informed consent for study participation to 28 days post-delivery (Visit 6). Serious adverse reactions and AEs of special interest (e.g. autoimmune diseases) will be reported throughout the study period. All AEs and SAEs in the baby from birth up to Day 365 (Visit 10) will be recorded.

All information about SAEs / occurrences of pregnancies will be collected and reported via the SAE / Pregnancy notification form and sent by email message or facsimile (contact information will be contained in the investigator site file). The investigator will send the initial report within 24 hours of becoming aware of the SAE. The initial report will include the minimum information as described *in* *Section 8.3.*

When reporting SAEs, all relevant information relating to investigations, laboratory results and clinical findings, including dates of such, should be included in the report. If it is easier, a photocopy of all such information in relation to the SAE may be included. The participant's identity must be protected, and all participant identifiers must be redacted. Laboratory results should be signed off and commented on by the investigator, and normal ranges should be included.

Any further data updates or additional information should be sent to the contact details above within 1 working day of knowledge of the additional information. This information should also be clearly recorded on the appropriate CRF pages. Diamond Pharmacovigilance Services will submit the SAEs to the South African Health Products Regulatory Authority (SAHPRA) in accordance with SAHPRA's safety reporting requirements. The site will report the SAEs directly to their Ethics Committee and copy the Diamond PV services and Sponsor.

Diamond Pharmacovigilance Services will expedite the reporting of all suspected unexpected serious adverse reactions (SUSARs) to all concerned, including investigator(s)/institutions(s), for onward transmission to the relevant research ethics committee(s), SAHPRA and Ugandan National Drug Authority (NDA). The expedited reporting should occur within the timeframe and format specified by the Ethics Committees, SAHPRA and the Ugandan NDA.

8.5 DSMB

The study will be overseen by a DSMB as described in the DSMB charter.

A Data Study Monitoring Board (DSMB) will be assembled and will be responsible for the periodic monitoring of safety. The DSMB is responsible for safeguarding the interests of trial participants by assessing the safety of the interventions during the trial. The DSMB members will review the protocol prior to the trial start and the plan for the collection of safety data before the first patient is enrolled. A multidisciplinary group with expertise in the fields of GBS, drug safety and statistics. The DSMB will consist of a minimum of three members who have no direct role in the conduct of this clinical trial, or any other conflict of interest, and they operate based on a study specific DSMB Charter. The DSMB will review interim and cumulative safety data. The DSMB may recommend discontinuation of the study if they find evidence of unacceptable risk to participants. MinervaX will have the responsibility for the final decision regarding the DSMB-recommended course of action. The composition and the DSMB meeting schedule will be included in the DSMB charter.

The DSMB will meet in person or by conference call prior to the start of recruitment, and at least yearly during the recruitment and follow-up phases of the trial. The Project management team (PMT) is responsible for coordinating the meeting date, time and venue for the DSMB meetings.

The first full review by the DSMB will be after the first 20 participants have been recruited or as decided by the DSMB chair. At this meeting, the following will be reviewed: Data related to safety and data relating to trial recruitment. Subsequent DSMB meetings will be decided at the previous DSMB meeting with a minimum of annual meetings. At the meetings, the following will be reviewed: Data related to safety since last meeting, data relating to trial recruitment, an interim analysis - if detailed in the trial protocol and stopping rules - if detailed in the trial protocol. Additional ad hoc meetings of the DSMB may be scheduled if requested by either the PI(s)/TPT/Trial Steering Committee (TSC) or the DSMB.

Following its review of the data, the DSMB will reach consensus on its list of recommendations. This may include: continuing the trial unchanged; continuing the trial with modifications; or terminating the trial. The DSMB may also make recommendations about other aspects of the trial such as the recruitment of participants and the conduct of the trial. All recommendations will be sent to the TSC.

9. WITHDRAWALS

9.1 Withdrawal of participants

Participants may be withdrawn from study for the following reasons:

- At their own request or at the request of their legally authorized representative
- If, in the investigator's opinion, continuation in the study would be detrimental to the participant's well-being

Participants must be withdrawn from receipt of study vaccination under the following circumstances, as agreed upon by the investigator and sponsor, on a case-by-case basis:

- Serious adverse reaction
- Intolerable adverse event as determined by the investigator and/or participant.
- Participant lost to follow-up.
- Development of an illness requiring a prohibited medication.
- Development of an interfering medical condition that will affect the participant's participation in the study.

Any participant, who, during the vaccination period, in the investigator's opinion, has clinically significant deviations from normal laboratory values, which would put the participant at risk or make interpretation of protocol results difficult, should be withdrawn from receipt of further vaccination but followed up according to the protocol visit schedule until the end of the study to collect safety information. For participants who are withdrawn from the study for other reasons e.g. personal circumstances, withdrawal of consent, early Discontinuation Visit and Study Termination information should be completed in the participant's CRF if possible. Additional clinical laboratory samples will be obtained if deemed appropriate by the investigator. **If possible, the investigator will perform visits 9 and 10 procedures as a final visit for discontinued participants.**

Note: When the participant has not met the inclusion criteria, she will not be randomised. Randomising participants without meeting inclusion criteria is a major protocol violation.

In all cases, the reason for withdrawal must be recorded in the case report form and in the participant's medical records. The participant must be followed up to establish whether the reason was an adverse event, and, if so, this must be reported in accordance with the procedures in *Section 8.3* As far as possible, all examinations scheduled for the final end of study visit must be performed on all participants who receive study medication but do not complete the study according to protocol. The investigator must make every effort to contact participants lost to follow-up.

9.2 Replacement of participants

Participants will not be replaced in this study.

10. EMERGENCY PROCEDURES

10.1 Emergency sponsor contact

In emergency situations, the investigator should contact the monitoring team by telephone at +27 11 431 0791 or +27 82 789 7445**.** Alternatively, the investigator can send an email to info@onqsa.co.za or catherinel@onqsa.co.za.

10.2 Emergency identification of study medication

The premature breaking of the code should be confined to emergency cases in which knowledge of the administered medicine is necessary for adequate treatment. Whenever possible, the sponsor should be contacted before breaking the blinded emergency code. Should any code be broken, consideration must be made as to whether the respective patient should be withdrawn from further participation in the study and a written explanation must be given by the investigator; the sponsor must be notified immediately.

IBM system will be used as the computerised code breaking system. The investigator will receive a special password to break the blind on IBM.

10.3 Emergency treatment

During and following a participant's involvement in the trial, the investigator/institution should ensure that adequate medical care is provided to a participant for any adverse events, including clinically significant laboratory values, related to the trial. The investigator/institution should inform a participant when medical care is needed for intercurrent illness(es) of which the investigator becomes aware.

11. STATISTICAL PROCEDURES

11.1 Database

All data collected in the study will be captured in an electronic database called IBM Merge® eCRF. Data capturing will be verified and validation checks will be conducted. All queries will be resolved with the investigators or delegate.

11.2 Treatment arms

The study design will consist of 4 groups, two groups of 80 participants to receive the potential vaccine and two groups of 20 participants to receive placebo.

Group 1 will be 80 women who are pregnant and living with HIV, who will receive two injections of GBS-NN/NN2. Each injection will contain 50 µg of GBS-NN and 50 µg of GBS-NN2.

Group 2 will be 80 women who are pregnant and do not have HIV who will receive two injections of GBS-NN/NN2. Each injection will contain 50 µg of GBS-NN and 50 µg of GBS-NN2.

Group 3 will be 20 women who are pregnant and living with HIV who will receive two injections of placebo.

Group 4 will be 20 women who are pregnant and do not have HIV who will receive two injections of placebo.

The groups will be dosed in parallel; the first dose of vaccine or placebo will be administered at 26 to 30 weeks of gestation and the second dose will be administered 28±2 days after the first dose.

11.3 Analysis population

The following populations will be taken into account for each study part:

- Intention to Treat Set (ITT): All recruited mothers who receive at least one dose of the study vaccine or placebo and their babies will be taken into account in the description of the population (disposition, demographic or baseline characteristics);
- Maternal Immunogenicity Set (MIG): The subset of pregnant women who receive at least one dose of the study vaccine or placebo with available pre- and post- vaccination titres

- Baby Immunogenicity Set (BIG): The Babies born to mothers who have received at least one dose of the study vaccine or placebo and whose cord blood or venous blood is collected within 48 hours of birth

- Maternal Per Protocol Set (MPP): All participants who receive both doses of the study vaccine and provide evaluable samples for analysis on the day of) of the primary immunological endpoint and do not violate the protocol.

- Baby Per Protocol Set (BPP): All babies born to mothers who receive two doses of vaccine and provide evaluable samples for analysis (cord or venous blood within 24 hrs of birth) and whose mothers did not violate the protocol.

- The Primary and Secondary Safety analyses will be performed on the ITT set. The Secondary Immunological analyses will be performed on the MIG and BIG sets. The "primary" immunological analysis (i.e. the results at delivery will also be performed on the PP set.

Sample size to be recruited:

A total of 200 pregnant women and their babies.

The 'per-protocol' group will be used for the statistical analysis of immunogenicity. The 'Intention to treat' group will be used for the safety analyses.

11.4 Analysis variables

For the analyses of the discrete (safety) data the numbers of the different levels adverse events would be used.

For the immune response analyses, the primary endpoints, the levels, most probably log transformed data, of the observed values of the IgG levels of the four AlpN proteins (Rib, Alp1, Alp2 AlpC) measured on the mothers and babies at the different time periods will be used.

For the secondary and exploratory endpoints, the other continuous variables like vital statistics' change over time and clinical laboratory data will be analysed.

11.5 Statistical methods

11.5.1 Listings and descriptive statistics

All observed and derived parameters, as well as population characteristics, will be listed and described using summary statistics. Descriptive statistics (n, mean, SD, median, minimum and maximum) will be calculated for each quantitative variable (unless otherwise stated). All listings will include repeated and unscheduled measurements.

11.5.2 Statistical analyses

The safety data will be analysed using the two-way chi-square analysis and a pairwise comparison of the two treatment groups. The 95% confidence intervals will be calculated for all observed rates.

The immunological data will be analysed by means of ANOVAs on the log transformed data and pairwise comparisons performed on all the observed means. The results will be reported a geometric means and geometric confidence intervals.

For the analysis of the immune responses and safety the placebo groups will be combined.

Safety and tolerability

- 80 mothers living with HIV treated with GBS-NN/NN2 and their babies compared to 80 mothers without HIV vaccinated with GBS-NN/NN2 and their babies
- 40 placebo vaccinated mothers and their babies compared to 80 mothers living with HIV vaccinated with GBS-NN/NN2 and their babies
- 40 placebo vaccinated mothers and their babies compared to 80 mothers without HIV vaccinated with GBS-NN/NN2 and their babies

Immunogenicity

- 80 mothers living with HIV vaccinated with GBS-NN/NN2 compared to 80 mothers without HIV vaccinated with GBS-NN/NN2; GMT at 4 weeks post each injection, at delivery and at 6 months after delivery.
- 40 placebo vaccinated mothers compared to 80 mothers living with HIV, vaccinated with GBS-NN/NN2; GMT at 4 weeks post each injection, at delivery and at 6 months after delivery.
- 40 placebo vaccinated mothers compared to 80 mothers living without HIV vaccinated with GBS-NN/NN2; GMT at 4 weeks post each injection, at delivery and at 6 months after delivery.

- Babies of 80 mothers living with HIV vaccinated with GBS-NN/NN2 compared to babies of 80 mothers without HIV vaccinated with GBS-NN/NN2; GMT at delivery and at 1 month after delivery.
- Babies of 40 placebo vaccinated mothers compared to babies of 80 mothers living with HIV, vaccinated with GBS-NN/NN2; GMT at delivery and at 1 month after delivery.
- Babies of 40 placebo vaccinated mothers compared to babies of 80 mothers living without HIV vaccinated with GBS-NN/NN2; GMT at delivery and at 1 month after delivery.80 mothers living with HIV vaccinated with GBS-NN/NN2 compared to 80 mothers without HIV vaccinated with GBS-NN/NN2; fold increase in IgG concentrations at delivery over baseline
- 80 mothers living with HIV vaccinated with GBS-NN/NN2 compared to 40 mothers vaccinated with placebo; fold increase in IgG concentrations at delivery over baseline.
- 80 mothers without HIV vaccinated with GBS-NN/NN2 compared to 40 mothers vaccinated with placebo; fold increase in IgG concentrations at delivery over baseline.

- 80 mothers living with HIV vaccinated with GBS-NN/NN2 compared to 80 mothers without HIV vaccinated with GBS-NN/NN2; the proportion that achieve IgG concentrations above the specified threshold concentrations of 0.5, 1 and 2 µg/ml at delivery.
- 80 mothers living with HIV vaccinated with GBS-NN/NN2 compared to 40 mothers vaccinated

with placebo; the proportion that achieve IgG concentrations above the specified threshold concentrations of 0.5, 1 and 2 µg/ml at delivery.

- 80 mothers without with HIV vaccinated with GBS-NN/NN2 compared to 40 mothers vaccinated with placebo; the proportion that achieve IgG concentrations above the specified threshold concentrations of 0.5, 1 and 2 µg/ml at delivery.

- Babies of 80 mothers living with HIV vaccinated with GBS-NN/NN2 compared to babies of 80 mothers without HIV vaccinated with GBS-NN/NN2; the proportion that achieve IgG concentrations above the specified threshold concentrations of 0.5, 1 and 2 µg/ml at delivery.
- Babies of 80 mothers living with HIV vaccinated with GBS-NN/NN2 compared to babies of 40 mothers vaccinated with placebo; the proportion that achieve IgG concentrations above the specified threshold concentrations of 0.5, 1 and 2 µg/ml at delivery
- Babies of 80 mothers without with HIV vaccinated with GBS-NN/NN2 compared to babies of 40 mothers vaccinated with placebo; the proportion that achieve IgG concentrations above the specified threshold concentrations of 0.5, 1 and 2 µg/ml at delivery

Details of the planned analyses will be described in the SAP and some (but not all) important features are listed below and presented in the protocol statistical part:

The following data (safety, immunogenicity and exploratory endpoints) will be described according to vaccine dose groups (and according to visits if relevant):

- Demographic and baseline data;
- Safety data including:
  - vital signs (heart rate, blood pressure and body temperature)
  - safety laboratory tests (haematology, biochemistry)
  - adverse events
  - physical examinations
  - assessment results of the injection site (local reactogenicity) and systemic reactogenicity using scale variables;
- Pharmacodynamic blood sample results (antibody response).

Baby

- Apgar scores at 1, 5 and 10 minutes
- Gestational age
- Head circumference, weight and length
- Type of delivery

Exploratory endpoints will be analysed and reported in a separate report.

Descriptive statistics

Descriptive statistics for quantitative parameters will be provided using number of observations (N), mean, standard deviation (SD), standard error of the mean (SEM), minimum, median and maximum. These data would be tested for Normality of the underlying distributions, but would, most probably have to be transformed to normality by performing log transformations and all the analyses performed on these transformed data. The results will be back-transformed and the Geometric means and confidence intervals reported. The ratio data (rates) would in any case have to be transformed.

Descriptive statistics for qualitative parameters will be provided using absolute frequencies (n) and relative frequencies (%).

Inferential statistics

Statistical tests will be carried out to compare results between treatment groups, depending on studied variable type. These analyses will be fully detailed in the SAP.

For categorical endpoints, such as local and systemic reactogenicity, a comparison of events intensity (scores) between vaccine dose groups can be performed with an adequate statistical test dealing with categorical variables (for example: Fisher exact test, Chi square test or generalized estimating equations).

11.5.3 Missing data

No imputation for missing data will be done.

11.5.4 Safety data

Safety variables, including adverse events and concomitant medication will be listed. Demographic and anthropometric variables will be listed and described for all participants who entered the study (safety population), as well as for the protocol population groups. A listing reflecting summary statistics will be provided for vital signs. Abnormal laboratory results will be flagged and listed.

11.6 Interim analysis

Interim analysis (safety) will be performed on data collected up to Day 56 from the first 20 participants recruited on the study. The interim analysis will focus on safety and tolerability of the GBS-NN/NN2 vaccine in the mother. The following safety endpoints in the mother will be evaluated during the interim safety analysis: local and systemic reactogenicity (headache, sore throat, “flu-like” symptoms, muscle aches, fever in the 72 hours post dose); adverse events; laboratory tests; dipstick urinalysis; vital signs; physical examination. Assessments of the in-utero growth of the babies will also be included.

The interim analyses will be performed without any unblinding for the sponsor and the statistician. An independent designee will be mandated by OnQ research and MinervaX to perform the analysis. The results will be sent directly to the DSMB and TSC members. The DSMB and TSC will send recommendations to the sponsor.

11.7 Sample size justification

The power calculation will be based on the paper by Heyderman 2016[27]: in which, the average standard deviation in the log transformed placental transfer ratios between patients to be 0.96.

The sample sizes were calculated on the conservative assumption that only 80 participants per group would be enrolled, that there would be a 15% dropout rate, and that 80% of women would deliver infants that are at 37 weeks or more of gestation or weighed 2500 g or more at birth. Thus, a sample size of 54 per group would give approximately 90% power to detect a difference in placental transfer rate of 6% and if the dropout rate is such that only a sample size of only 40 per group completed the study this would give approximately 90% power to detect a 7% difference in placental transfer rates between any two study groups.

12. ETHICAL AND LEGAL ASPECTS

12.1 Good clinical practice

The procedures set out in this study protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the sponsor and investigator abide by the ICH-E6 (R2) guidelines for Good Clinical Practice, *Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa, Second Edition 2006,* and the ethical principles laid down in the current revision of the *Declaration of Helsinki*. The study will also be carried out in keeping with local legal and regulatory requirements.

12.2 Delegation of investigator responsibilities

The investigator will ensure that all persons assisting with the trial are adequately informed about the protocol, any amendments to the protocol, the study treatments, and their trial-related duties and functions. All training concerning the study will be documented on the training sheet.

The investigator should maintain a list of sub investigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties.

12.3 Participant information and informed consent

The investigator will not undertake any measures *specifically* required for the clinical study until valid consent has been obtained.

It is recommended that the investigator inform the participant's primary physician about the participant's participation in the trial if the participant has a primary physician and if the participant agrees to the primary physician being informed.

12.4 Confidentiality

Participant names will not be supplied to the sponsor. Only the participant number will be recorded in the case report form, and if the participant name appears on any other document (e.g., pathologist report), it must be redacted before a copy of the document is supplied to the sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. The participants will be told that representatives of the sponsor, IEC/IRB, or regulatory authorities/ auditors may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

The investigator will maintain a personal participant identification list (participant numbers with the corresponding participant names) to enable records to be identified.

12.5 Protocol amendments

Neither the investigator nor the sponsor will alter this study protocol without obtaining the written agreement of the other. Once the study has started, amendments should be made only in exceptional cases. The changes then become part of the study protocol.

12.6 Approval of the study protocol and amendments

Before the start of the study, the study protocol, informed consent form, and any other appropriate documents will be submitted to the independent ethics committee (IEC)/institutional review board (IRB) with a cover letter or a form listing the documents submitted, their dates of issue, and the site (or region or area of jurisdiction, as applicable) for which approval is sought. If applicable, the documents will also be submitted to the authorities, in accordance with local legal requirements.

Study medication can only be supplied to the investigator after documentation on **all** ethical and legal requirements for starting the study has been received by the sponsor. This documentation must also include a list of the members of the IEC/IRB and their occupation and qualifications. If the IEC/IRB will not disclose the names of the committee members, it should be asked to issue a statement confirming that the composition of the committee is in accordance with GCP. Formal approval by the IEC/IRB should preferably mention the study title, study code, study site (or region or area of jurisdiction, as applicable), and any other documents reviewed. It must mention the date on which the decision was made and must be officially signed by a committee member.

Before the first participant is enrolled in the study, all ethical and legal requirements must be met.

The IEC/IRB and, if applicable, the authorities must be informed of all subsequent protocol amendments, in accordance with local legal requirements. Amendments must be evaluated to determine whether formal approval must be sought and whether the informed consent document should also be revised.

The investigator must keep a record of all communications with the IEC/IRB and, if applicable, between a coordinating investigator and the IEC/IRB. This also applies to any communication between the investigator (and national PI) and the authorities.

12.7 Ongoing information for independent ethics committee/ institutional review board

If required by legislation or the IEC/IRB, the investigator must submit to the IEC/IRB:

- Information on serious or unexpected adverse events as soon as possible
- Periodic reports on the progress of the study.

12.8 Premature closure of the study

The sponsor or the investigator has the right to close this study at any time. As far as possible, this should occur after mutual consultation. The IEC/IRB must be informed, if required by legislation.

Should the study be closed prematurely, all study materials (completed, partially completed, and blank case report forms, study medication, etc.) must be returned to the sponsor, as if the study had been completed.

12.9 Record retention

The following records must be retained by the investigator for a *minimum* of 15 years after the completion or termination of the study:

- Signed informed consent forms for all participants
- Participant identification code list, screening log, and enrolment log
- Record of all communications between the investigator and the IEC/IRB
- Composition of the IEC/IRB (or other applicable statement as described in *Section 12.6 Approval of the study protocol and amendments,*)
- Record of all communications between the investigator and sponsor (or CRO)
- List of sub-investigators and other appropriately qualified persons to whom the investigator has delegated significant trial-related duties, together with their roles in the study and their signatures
- Copies of case report forms and of documentation of corrections for all participants
- Drug accountability records
- Record of any body fluids or tissue samples retained
- All other source documents (participant records, hospital records, laboratory records, etc.)
- All other documents as listed in Section 8 of the ICH consolidated guideline on GCP (Essential Documents for the Conduct of a Clinical Trial)

However, because of international regulatory requirements, the sponsor may request retention for a longer period of time. The investigator must therefore obtain approval in writing from the sponsor prior to destruction of any records.

Normally, these records will be held in the investigator's archives. If the investigator is unable to meet this obligation, he or she must ask the sponsor for permission to make alternative arrangements. Details of these arrangements should be documented.

12.10 Liability and insurance

The sponsor and CRO has subscribed to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

12.11 COVID-19 pandemic

The study site will follow national and local hospital guidelines for the control and prevention of COVID-19 infections in the conduct of clinical trials.

13. STUDY MONITORING AND AUDITING

Monitoring and auditing procedures developed by the sponsor will be followed, in order to comply with GCP guidelines. On-site checking of the case report forms for completeness and clarity, verification with source documents, and clarification of administrative matters will be performed.

13.1 Study monitoring

The sponsor will monitor the study and there will be blinded and unblinded monitoring. Monitoring will be done by personal visits from a representative of the sponsor (site monitor) who will review the case report forms and source documents. The unblinded monitor will review the IP section of the study. By frequent communications (letter, telephone, and fax), the site monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements.

All unused study materials are to be returned to the sponsor after the clinical phase of the trial has been completed.

13.2 Source data verification and on-site audits

Regulatory authorities, the IEC/IRB, and/or the sponsor's clinical quality assurance group may request access to all source documents, case report forms, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

14. DOCUMENTATION AND USE OF STUDY FINDINGS

14.1 Documentation of study findings

An electronic case report form will be provided by the sponsor for each participant.

All protocol-required information collected during the study must be entered by the investigator, or designated representative, in the case report form. Details of case report form completion and correction will be explained to the investigator. If the investigator authorizes other persons to make entries in the case report form, the names, positions, signatures, and initials of these persons must be supplied to the sponsor.

The investigator, or designated representative, should complete the electronic case report form pages as soon as possible after information is collected, preferably on the same day that a study participant is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

A source data location list will be prepared and updated during the study. This list will be filed in both the trial master file and the investigator study file.

The completed case report form must be reviewed and signed by the investigator named in the study protocol or by a designated sub-investigator.

The sponsor will retain the data base of all electronic case report forms. The investigator will retain a copy of all completed case report forms on a CD ROM.

14.2 Use of study findings

All information concerning the product as well as any matter concerning the operations of MinervaX ApS , such as clinical indications for the drug, its formula, methods of manufacture and other scientific data relating to it, that have been provided by MinervaX ApS and are unpublished, are confidential and must remain the sole property of MinervaX ApS. The investigator will agree to use the information only for the purposes of carrying out this study and for no other purpose unless prior written permission from MinervaX ApS is obtained.

The sponsor has full ownership of the original case report forms completed as part of the study.

The investigator agrees that the results of the study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. If necessary, the authorities will be notified of the investigator's name, address, qualifications, and extent of involvement.

The sponsor will prepare a final report on the study.

The investigator will be required to sign a statement that he or she has read the report and confirms that, to the best of his or her knowledge, it accurately describes the conduct and results of the study.

The findings of the study may be published in a scientific journal or presented at a scientific meeting. Before submitting the results of the study for publication or presentation, the investigator will allow MinervaX ApS 30 days in which to review and comment on the manuscript. For any publication manuscript prepared by MinervaX ApS, MinervaX ApS reserves the right to select the investigators who will be authors and review the manuscript. MinervaX ApS will allow the selected investigators 30 days for full review of the manuscript before publication. It is generally preferable that the results of the study be published together.

15. STUDY DURATION AND DATES

The duration of this study in South Africa is expected to be about 20 months, with participant recruitment expected to commence in October 2020 and the study expected to complete in June 2022. The actual overall study duration or participant recruitment period may vary.

16. DECLARATION OF SPONSOR

This study protocol was subject to critical review and has been approved by the appropriate protocol review committee of the sponsor. The information it contains is consistent with:

- The current risk-benefit evaluation of the investigational product
- The moral, ethical, and scientific principles governing clinical research as set out in the *Declaration of Helsinki* , ICH E6 (R2) *Guidelines for Good Clinical Practice* and the *Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa*, Second Edition (2006)(SA-GCP applicable to only South African sites)

The investigator will be supplied with details of any significant or new findings, including adverse events, relating to treatment with the investigational product.

17. REFERENCES

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APPENDICES

The appendices will be managed as stand-alone documents. Any revisions made to the documents during study implementation will not impact the version of the Protocol and vice versa.

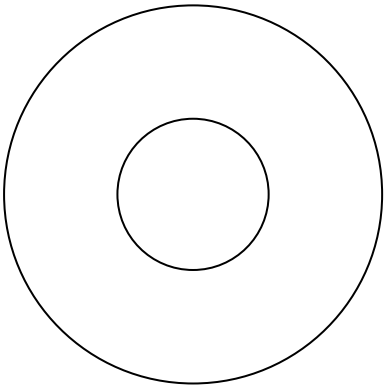
Appendix 1	**Participant Diary Card**
Appendix 2	**Injection Site Assessments**
Appendix 3	**ASQ-3, 4- 6-, 10- and 12-Month Questionnaires**
Appendix 4	**GAIA Criteria**

Appendix 1: Participant Diary Card

APPENDIX 2: INJECTION SITE TOLERABILITY ASSESSMENT

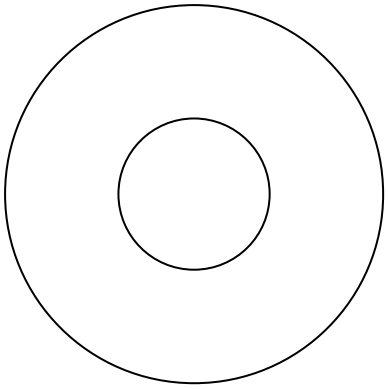
Tolerability assessments will be performed by a member of the clinical staff. An additional assessment by a physician will be made in the event of a local reaction being evaluated as moderate or severe. All measurements (in cm) refer to the longest dimension.

Erythema/Redness



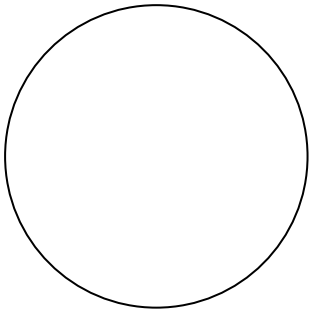
Grade	Description:	
0	NONE	No visible redness
1	MILD	Greater than 0 to 2 cm (incl.) redness
2	MODERATE	Greater than 2 to 5 cm (incl.) redness
3	SEVERE	Greater than 5 cm redness

Bruising



Grade	Description:	
0	NONE	No visible bruising
1	MILD	Greater than 0 to 2cm (incl.) bruising
2	MODERATE	Greater than 2 to 5cm (incl.) bruising
3	SEVERE	Greater than 5cm bruising

Induration



Grade	Description:	
0	NONE	No swelling detected
1	MILD	Palpable “firmness” only
2	MODERATE	Less than or equal to 4 cm swelling
3	SEVERE	Greater than 4 cm swelling

Itching

Grade	Description:
N:	ABSENT
Y:	PRESENT

Pain

Participants will be asked if they experience any pain. If the answer is yes, they will be asked to rate the level of pain on a scale of 0-10. Assessment of pain will commence after first injection.

Any other local reaction (for example: necrosis or ulceration)? Please specify

Appendix 3: ASQ-3, 4-, 6-, 10- and 12-Month Questionnaires

Please refer to the attached ASQ-3, 4-, 6-, 10- and 12-Month Questionnaires

Appendix 4: GAIA Criteria

Level 1	Level 2A	Level 2B	Level 3A	Level 3B
Certain LMP* *with* confirmatory 1st trimester scan (≤13 6/7 weeks).	**Certain LMP* with 2nd trimester scan (14 0/7 weeks to 27 6/7 weeks). If LMP and U/S do not correlate, default to U/S GA assessment.**	**Uncertain LMP with 2nd trimester scan (14 0/7 weeks to 27 6/7 weeks).**	**Certain LMP *with* 3rd trimester scan – 28 0/7 weeks +.**	**Uncertain LMP *with* FH.**
OR	OR		OR	OR
1st trimester scan (≤13 6/7 weeks).	**Certain LMP* *with* 1st trimester physical examination.**		**Certain LMP *with* confirmatory 2nd trimester FH.**	**Uncertain LMP *with* new-born physical assessment.**
AND			OR	OR
			Certain LMP with birth weight.	**Uncertain LMP *with* new-born physical assessment.**
AND			OR	
			Uncertain LMP with 1st trimester physical examination.	