CLINICAL STUDY PROTOCOL V98_06E1 Version 1

A Phase 2, Non-Randomized, Controlled, Open-Label, Parallel-Group, Extension Study to Evaluate the Immunogenicity and Safety of the Second Dose of GBS Trivalent Vaccine in Healthy Non-pregnant Subjects

Evaluation of a Second Dose of GBS Trivalent Vaccine in Healthy Non-Pregnant Subjects

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PROTOCOL SYNOPSIS V98_06E1 VERSION 1

Name of Sponsor:	**Protocol number:**	**Generic name of study vaccine:**
GlaxoSmithKline Biologicals S.A.	V98_06E1	GBS Trivalent Vaccine

Title of Study:

A Phase 2, Non-Randomized, Controlled, Open-Label, Parallel-Group Extension Study to Evaluate the Immunogenicity and Safety of the Second Dose of GBS Trivalent Vaccine in Healthy Non-Pregnant Subjects.

Study Period: Approximately 6 months	**Clinical Phase:** 2

Background and Rationale:

Group B Streptococcus (GBS) is a major cause of neonatal disease in developed and developing countries. Newborns are very susceptible to GBS disease which is known to manifest with bacteremia, sepsis, meningitis or pneumonia typically during the first three months of life.

The infants may acquire the infection either *in utero* by the ascending route through the ruptured or intact membranes (Melin & Efstratiou 2013), by contact with the bacteria in the birth canal during parturition or after birth through contact with caregivers. Studies have shown that 25-35% of pregnant women are colonized with GBS in the lower genital and/or gastrointestinal tract and about 50% of colonized women transmit GBS vertically to the gastrointestinal tract or upper respiratory tract of neonates. One to two % of colonized infants develop invasive GBS disease (Baker 2013).

Current prevention strategies of GBS disease in infants, implemented mainly in developed countries, focus on maternal screening for recto-vaginal GBS colonization in late pregnancy (35-37 weeks of gestation), with subsequent use of intrapartum antibiotic prophylaxis (IAP) during delivery in GBS colonized women (www.cdc.gov/mmwr /pdf/rr/rr5910.pdf November 19 2010). Limitations of the current standard culture for GBS carriage detection include the need for prolonged incubation, which require antenatal screening culture resulting in poor predictive value for colonization status in labor and in missing of GBS carriage screening in some preterm labor cases. In the future, advances in diagnostic molecular technologies are expected to offer rapid GBS intrapartum screening test with higher sensitivity, specificity and predictive values (Di Renzo et al. 2014). The use of IAP however has little impact on GBS infections that occur after the first week of life (www.cdc.gov/abcs/reportsindings/survreports/

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gbs08.html Accessed January 27, 2014), thus alternative strategies for prevention of neonatal GBS disease remain to be an unmet medical need.

One alternative strategy is maternal vaccination against GBS. Maternal serotype-specific anti-capsular antibodies to GBS are transferred to the fetus trans-placentally, mainly in the third trimester of pregnancy, and persist in the infant throughout the first months of life, which is the period of high vulnerability to GBS disease (Englund et al. 1995; Glezon & Alpers 1999; Baker & Kasper 1977; Boyer et al. 1984). Case-control studies have linked higher serotype-specific maternal anti-capsular polysaccharide antibody concentrations to protection against neonatal GBS disease during the first week of life (Baker, Carey & Rench et al. 2013, Baker, Rench & McInnes 2003, Lin et al. 2004, Lin at el. 2001). These findings support the approach of maternal vaccination as a potential strategy for preventing GBS infection in the newborns and infants.

GSK has developed a trivalent glycoconjugate vaccine against GBS that contains the capsular polysaccharides (CPS) of GBS serotypes Ia, Ib and III, conjugated to a protein carrier, CRM_{197}, which enhances their immunogenicity.

Single 5 µg doses of adjuvanted and unadjuvanted GBS Trivalent Vaccine demonstrated immunogenicity and a favorable safety profile in approximately 400 pregnant and 320 non-pregnant subjects in Phase 1 and 2 studies (V98_06, V98_04, V98_05, V98_08). Single 20 µg doses of adjuvanted and unadjuvanted GBS Trivalent Vaccine were assessed in Phase 1and 2 (V98_06, V98_08) studies in approximately 200 non-pregnant subjects. The immunogenicity and safety profile of single dose of 5 µg and 20 µg doses were comparable.

In the Phase 1b study (V98_06) two-injection regimens at the same dose range of 5 µg and 20 µg (adjuvanted and unadjuvanted) with one month interval between injections were evaluated in approximately 160 non-pregnant subjects per dose. The immunogenicity of 5 µg and 20 µg doses administered as single or two injections courses were comparable. Two injection regimens at the same dose range did not impact the immune response and no clear dose-response relationship was observed for either GMC or percentage of subjects reaching pre-defined antibody concentrations at day 60 (which is approximately the mean time of delivery after vaccination in Phase 2 studies with pregnant subjects). The series of these concentrations was based on putative protective serotype-specific thresholds which corresponded with reduced risk of infant

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infection in the earlier case-control studies (Lin 2001, Lin 2004).

The candidate vaccine is intended to be administered to the pregnant woman in the third trimester of pregnancy to optimize antibody transfer to the fetus. The GBS antibody levels induced by vaccination are expected to decrease over time and re-vaccination during the subsequent pregnancy is likely to be required to ensure that protective levels of antibodies are achieved again. The repeat administration of conjugate vaccines with the same carrier protein (CRM_{197}) has the potential to affect vaccine immunogenicity through antigen competition (Pichichero ME. Hum Vaccin Immunother. 2013). Considering that CRM_{197} is a nontoxic variant of diphtheria toxin, the impact on existing anti-diphtheria antibody levels has to be considered and evaluated.

However, safety and immunogenicity of the second dose of GBS Trivalent Vaccine when administered after longer than one month has not been evaluated to date. The objective of this extension study is the initial assessment of safety and immunogenicity of the second dose of GBS Trivalent Vaccine following the longer time interval that is closer to the inter-pregnancy interval observed in the general population (Gemmill A, Lindberg LD. Short interpregnancy intervals in the United States. Obstet Gynecol. 122(1):64-71, 2013).

To address this objective, the subjects who received a single 5µg dose of GBS Trivalent Vaccine or placebo as part of the V98_06 study will be vaccinated and receive a second dose of 5µg approximately 4-6 years after the first vaccination. As the 20 µg dose formulation of GBS Trivalent Vaccine is not currently available, it will not be included in the current re-vaccination study. The placebo group from V98_06 study is included as a control that provides longitudinal data both for the immunogenicity and anti-diphtheria antibody levels and has the advantage over naive subjects of being a group that was randomized as part of the original study. Naive subjects who have never received GBS Trivalent Vaccine will also be enrolled to ensure adequate number of controls in case of significant loss to follow up of the V98_06 study placebo treated subjects.

While high levels of antibodies to GBS in a pregnant woman are predictive of lower incidence of infant GBS disease, (Lin 2001, Lin 2004, Baker CJ, Kasper DL NEJM, 1976), the levels of serotype-specific IgG antibodies needed in a mother in order to protect her infant from GBS have not yet been established. Until these levels have been established, a set of increasing serotype-specific ELISA IgG cut-off levels will be used to attempt to bracket where the assumed protective levels may be. The study objectives

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are non-specific as to the exact level needed to establish protection.

It is acknowledged that due to loss to follow up the study sample size may be limited. Also, due to baseline heterogeneity of subjects who received different adjuvants or no adjuvant with first vaccination, the study will be exploratory by nature and the results will be interpreted as such.

Study Objectives:

Primary Objectives:

Immunogenicity Objective

To evaluate the immunogenicity of a second dose of GBS Trivalent Vaccine (5 µg without adjuvant) administered approximately 4-6 years after the initial GBS vaccination, measured by ELISA.

Safety Objective

To assess the safety and tolerability of a second dose of GBS Trivalent Vaccine (5 µg without adjuvant) administered approximately 4-6 years after the initial dose.

Secondary Objectives:

To further characterize the immune response following a dose of GBS Trivalent Vaccine (5 µg without adjuvant) in study subjects and in subgroups of subjects defined by pre-vaccination serotype-specific GBS antibody LLQ status in the V98_06 study or V98_06E1 for the naïve subjects..

To assess the impact of a dose of the GBS Trivalent Vaccine (5 µg without adjuvant) on the serum level of anti-diphtheria antibodies in subjects who received study vaccination (active or placebo) as part of the V98_06 and naïve subjects from V98_06E1

Exploratory Objective:

To assess the immunogenicity of a dose of GBS Trivalent Vaccine (5 µg without adjuvant) in study subjects as measured by OPK and in subgroups of subjects defined by pre-vaccination serotype-specific GBS ELISA antibody LLQ status in the V98_06 or

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V98_06E1 for the naïve subjects.

Study Design:

This is a Phase 2, non-randomized, controlled, open-label, parallel-group, extension of the V98_06 Study. All eligible subjects will receive a single injection of 5 µg dose (of each conjugate polysaccharide - serotypes Ia, Ib, and III) of unadjuvanted GBS Trivalent Vaccine (Table 1).

Table 1 Treatment Groups and Number of Subjects

Treatment Groups	**Vaccine Dose (µg Ia/Ib/III Glycoconjugate, no adjuvant)**	**Number of Subjects**
V98_06: Single vaccination 5/5/5 no adjuvant	5/5/5	Up to 40
V98_06: Single vaccination 5/5/5 Alum	5/5/5	Up to 40
V98_06: Single vaccination 5/5/5 MF59 full dose	5/5/5	Up to 40
V98_06: Single vaccination 5/5/5 MF59 half dose	5/5/5	Up to 40
V98_06: Placebo (Enrollment Group 1 and Enrollment Group 2)	5/5/5	Up to 40
Naive subjects (not previously enrolled in V98_06)	5/5/5	20
Total – up to 220 subjects		

Following screening, eligible subjects will provide a blood sample for pre-vaccination measurement of serotype-specific GBS antibodies and will receive a single dose of unadjuvanted GBS Trivalent Vaccine containing 5 µg of each polysaccharide (serotypes Ia, Ib, and III). On day 31 and day 61 additional blood samples will be obtained to

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assess serotype-specific GBS antibody responses.

The following data will be collected for safety analysis:

- Day 1 (vaccination): A 30 minute post-injection assessment for signs or symptoms of anaphylaxis.
- Day 1 (vaccination) to day 7: Solicited local and systemic adverse events (AEs), body temperature and other indicators of solicited adverse events recorded daily using a Subject Diary.
- Day 1 (vaccination) to day 31: All unsolicited AEs.
- Day 1 to day 181: SAEs, Medically-attended AEs and AEs leading to discontinuation from the trial only.
- Day 1 to day 181: All concomitant medications administered in relation to the reported AEs, any vaccines administered and all antibiotics and any other prescription or over the counter medicine(s) deemed clinically significant by the investigator.

The study will include, 3 clinic visits (day 1 – vaccination, day 31 and day 61) and 3 safety calls (day15, 121 and 181).

Number of Subjects planned:

The total number of subjects to be enrolled into the trial will not exceed 220, which includes the total number of subjects who received the 5 µg single dose of GBS Trivalent Vaccine or placebo in the V98_06 study and up to 20 naive subjects.

Study Population and Subject Characteristics:

Healthy, non-pregnant subjects who have received a single 5 µg dose of GBS Trivalent Vaccine or placebo in the V98_06 study and healthy non-pregnant female subjects aged 22-46 years inclusive on the day of informed consent who have not received any GBS vaccine in the past.

The list of inclusion and exclusion criteria is included in protocol section 4, Selection of Study Population.

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Study Procedures:

After signing the informed consent form (ICF), all subjects will undergo review of medical history and concomitant medications/vaccinations. They will have a full physical examination and, if required, undergo urine pregnancy test to confirm they are not pregnant prior to vaccination.

Once eligibility is confirmed, subjects will receive 5 µg of unadjuvanted GBS Trivalent Vaccine.

Each subject will have:

- 3 Clinic Visits: at day 1 (day of vaccination), at day 31 and day 61
- 2 Subject Diary Reminder calls: at day 3 and day 5 (To remind the subject to complete the Subject Diary)
- 3 Safety Follow-up calls at day 15, 121 and 181

Blood for serology assessment of serotype-specific (Ia, Ib and III) GBS IgG antibody concentrations will be collected as follows:

- Approximately 20 mL of blood will be collected on day 1 (prior to vaccination), on day 31 and day 61.

Safety will be evaluated in all subjects as follows:

- Solicited local and systemic AEs occurring at the day of study vaccination (day 1) and the following 6 days (day 2 to day 7) will be recorded daily using a Subject Diary.
- Unsolicited AEs occurring within 30 days (day 1 to day 31) after study vaccination will be collected by interviewing the subject during the day 15 safety calls and day 31 clinic visits, and by review of available medical records.
- Medically-attended AEs, AEs leading to study withdrawal and SAEs will be collected during the entire study period. These data will be captured by interviewing the subject during the site visits and phone calls and by review of available medical records. Subjects will be encouraged to call the site in case of any medically-attended AEs or any AEs which they perceive as being of concern during the entire

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study period.

Study procedures at each visit are displayed in Table 2.

Table 2 Study Procedures

Vaccine Group	Visit for Vaccination	Subject Diary Reminder calls (To remind the subject to fill out the Subject Diary)	Visits for Blood Sampling for Immunogenicity	Safety Follow-up calls
Lyophilized GBS Trivalent Vaccine, 5µg of each glycoconjugate (serotype Ia/Ib/III), unadjuvanted	Day 1	Day 3 Day 5	Day 1 (pre-vaccination), Day 31 and Day 61	Day 15 Day 121 Day 181

Study Vaccines:

Lyophilized Formulation of GBS Trivalent Vaccine will be supplied by GSK.

Vial: Each vial contains 6 µg of each capsular polysaccharides (serotype Ia, Ib and III) conjugated to the *Corynebacterium diphtheriae* CRM_{197} carrier protein. At the time of injection, the vaccine will be reconstituted at the site with 0.6 mL sterile saline. The sterile saline will be supplied by GSK as 2.0 mL sterile saline for IM injection at a concentration of 0.9% of sodium chloride.

Dose: A dose of 5 µg of each glycoconjugate antigen administered with the single vaccine injection of 0.5 mL delivered by the IM route, preferably the deltoid muscle in the non-dominant arm.

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Primary Endpoints:

Immunogenicity Endpoints

Percentage and frequency of subjects who reach pre-defined sequential serotype-specific serum antibody levels for serotypes Ia, Ib and III at day 61 post vaccination, as measured by ELISA.

Safety Endpoints:

The frequency and percentage of subjects with solicited local and systemic AEs from vaccination to day 7 in study V98_06E1. Time intervals after vaccination that will be summarized are: the first 30 minutes after vaccination, days 1-3 (excluding the first 30 min), days 4-7 and days 1-7 (excluding the first 30 min)..

The frequency and percentage of subjects with any unsolicited AEs from the day of vaccination (day 1 in study V98_06E1) to day 31.

The frequency and percentage of subjects with SAEs, medically attended AEs, and AEs leading to study withdrawal from vaccination in study V98_06E1 to day 181

Secondary Endpoints:

The serotype-specific (Ia, Ib, and III) geometric mean antibody as measured by ELISA at days 31 and 61 post-vaccination in study V98_06E1 in all subjects and by pre-vaccination serotype-specific GBS antibody LLQ status in the V98_06 study or V98_06E1 study for the naive group.

The percentage of subjects reaching pre-defined sequential serotype-specific serum antibody levels for serotypes Ia, Ib and III at day 31 post vaccination as measured by ELISA in all subjects

The percentage of subjects reaching pre-defined sequential serotype-specific serum antibody levels for serotypes Ia, Ib and III at day 31 and 61 post vaccination as measured by ELISA in subjects according to pre-vaccination serotype-specific GBS antibody LLQ status in the V98_06 study or V98_06E1 study for the naive group.

The within subject geometric mean ratio (GMR) of serotype-specific (Ia, Ib & III) serum

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antibody levels as measured by ELISA at day 31 and 61. The GMRs will be determined relative to pre-vaccination in V98_06E1 and separately relative to pre-vaccination in V98_06 for groups except the naive group. The GMRs will be determined for all subjects and by pre-vaccination serotype-specific GBS antibody LLQ status in the V98_06 study or V98_06E1 study for the naive group.

Reverse cumulative distribution function (RCDF) curves of serotype-specific (Ia, Ib and III) serum antibody levels, as measured by ELISA, at pre-vaccination in V98_06 study or V98_06E1 study for the naive group, vaccination and day 31 and 61 post-vaccination in all subjects and by pre-vaccination serotype-specific GBS antibody LLQ status in the V98_06 study or V98_06E1 study for the naive group.

The anti-diphtheria geometric mean antibody concentrations measured by ELISA for samples collected before the first vaccination with GBS Trivalent Vaccine in the V98_06 study or V98_06E1 study for the naive group and on day 31 and day 61 post vaccination in V98_06E1.

Exploratory Endpoints:

The exploratory immunogenicity endpoints are:

The serotype-specific (Ia, Ib, and III) geometric mean antibody titer as measured by OPK at days 31 and 61 post-vaccination in study V98_06E1 in all subjects and by pre-vaccination serotype-specific GBS antibody LLQ status in the V98_06 study or V98_06E1 study for the naive group.

The serotype-specific (Ia, Ib, and III) geometric mean antibody titer as measured by OPK at day 1 pre-vaccination in study V98_06E1 in all subjects and by pre-vaccination serotype-specific GBS antibody LLQ status in the V98_06 study or V98_06E1 study for the naive group.

Exploratory OPK objectives will be addressed in a randomly selected subset of up to 60 subjects (10 subjects per arm).

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Statistical Analyses: Statistical Analyses:

The percentages of subjects with serotype-specific concentrations above a set of pre-defined sequential serotype-specific serum antibody levels and associated two-sided 95% CIs will be computed for each study group on day 1 before vaccination in V98_06E1, and on day 31 and day 61 post-vaccination (primary endpoint). These percentages will be graphically displayed as RCDFs.

Additionally, the data will be logarithmically transformed (base 10) before estimating the serotype-specific GMCs at day 1 before vaccination and at day 31 and day 61 post-vaccination. The GMRs, relative to the original baseline and separately relative to the pre-vaccination baseline in V98_06E1 and their associated 95% confidence intervals (CIs) will be computed for each study group.

Analyses will be performed for the following groups of subjects:

- All subjects
- Subjects determined by their serotype-specific LLQ status prior to initial vaccination in study V98_06 or V98_06E1 for the naive group
- Subjects who received no adjuvant in study V98_06
- Subjects who received alum adjuvant in study V98_06
- Subjects who received half dose of MF59 in study V98_06
- Subjects who received full dose of MF59 in study V98_06
- Subjects who received placebo in study V98_06
- Subjects who are naive and were not vaccinated as a part of V98_06

Safety analyses will be performed on the appropriate safety sets.

Interim Analysis:

There will be no interim analysis included in this study.

Data Monitoring Committee:

No DMC will be established for this study.

Table 3 Time and Events Table

Events	Visit Type / Day / Visit Window (Days) / Visit Number	Clinic Visit	Clinic Visit	Subject Diary Reminder Call	Safety Follow-up Call	Clinic Visit	Clinic Visit	Safety Follow-up Call	Safety Follow-up Call
	Visit Type	Clinic Visit	Clinic Visit	Subject Diary Reminder Call	Safety Follow-up Call	Clinic Visit	Clinic Visit	Safety Follow-up Call	Safety Follow-up Call
	Day		1	3, 5	15	31	61	121	181
	Visit Window (Days)	-5 to 1	n/a	(-1/+1)/(-1/+2)	-3 to +3	-7 to +7	-7 to +7	-14 to +14	-14 to +14
	Visit Number	Pre-vaccination	1		2	3	4	5	6
Study Event	**References**								
Screening									
Informed Consent [a]	Section 5.1.1	X							
Medical History	Section 5.1.2	X	X [b]						
Physical Exam	Sections 5.1.2 and 5.3.1		X [b]						
Pregnancy Test[c]	Sections 3.5 and 5.1.2		X [b]						
Exclusion/Inclusion Criteria	Section 4	X	X [b]						
30 Minutes Post Injection Assessment	Section 5.2.1		X						
Subject Diary Dispensed with Training	Section 5.2.1		X						
Immunogenicity									

Events	Visit Number	Visit Type	Clinic Visit	Clinic Visit	Subject Diary Reminder Call	Safety Follow-up Call	Clinic Visit	Clinic Visit	Safety Follow-up Call	Safety Follow-up Call
		Day		1	3, 5	15	31	61	121	181
		Visit Window (Days)	-5 to 1	n/a	(-1/+1)/(-1/+2)	-3 to +3	-7 to +7	-7 to +7	-14 to +14	-14 to +14
			Pre-vaccination	1		2	3	4	5	6
Study Event	**References**									
Serology Blood Draw	Section 3.5			X [b]			X	X		
Study Treatment										
Vaccination	Section 5.2			X						
Safety										
Subject Diary Reminder Call	Section 5.2.2				X					
Symptom Directed Physical Exam	Sections 5.1.2 and 5.3.1						X	X		
Subject Diary Reviewed and Collected	Section 5.3.1						X			
Assess all AEs	Section 7.1			X		X	X			
Assess SAEs	Section 7.1.4			X		X	X	X	X	X
Assess for medically attended AEs and AEs leading to withdrawal	Sections 7.1.4.1 and 7.1.3			X		X	X	X	X	X

Events	Visit Type	Clinic Visit	Clinic Visit	Subject Diary Reminder Call	Safety Follow-up Call	Clinic Visit	Clinic Visit	Safety Follow-up Call	Safety Follow-up Call
	Day		**1**	**3, 5**	**15**	**31**	**61**	**121**	**181**
	Visit Window (Days)	**-5 to 1**	**n/a**	**(-1/+1)/(-1/+2)**	**-3 to +3**	**-7 to +7**	**-7 to +7**	**-14 to +14**	**-14 to +14**
	Visit Number	**Pre-vaccination**	**1**		**2**	**3**	**4**	**5**	**6**
Study Event	**References**								
Assess relevant medications	Sections 5.1.2 and 6.5	X	X		X	X	X	X	X
Study Completion Procedures									
Study Termination	Section 5.5								X
	Notes: [a] Confirm consent form(s) signed prior to any procedures. [b] Procedure to be performed prior to vaccination [c] Only for women of child bearing potential								

LIST OF ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
BCDM	Biostatistics and Clinical Data Management
CFR	Code of Federal Regulations
CI	Confidence Interval
CMI	Cell Mediated Immunity
CPS	Capsular Polysaccharides
CRF	Case Report Form
CRM	Cross Reactive material
CRO	Contract Research Organization
DCF	Data Clarification Form
EC	Ethics Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EDT	Electronic Data Transfer
ELISA	Enzyme-linked Immunosorbent Assay
EMA	European Medicines Agency
FAS	Full Analysis Set
FDA	Food and Drug Administration
GBS	Group B Streptococcus
GCP	Good Clinical Practices
GMC	Geometric Mean Concentration
IAP	Intrapartum Antibiotic Prophylaxis
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
PCR	Polymerase Chain Reaction
PPS	Per Protocol Set
SAE	Serious Adverse Event
SDA	Source Data Agreement
SOC	System Organ Class

1. BACKGROUND AND RATIONALE

1.1 Background

Group B Streptococcus (GBS) is a major cause of neonatal disease in developed and developing countries. Newborns are very susceptible to GBS disease which is known to manifest with bacteremia, sepsis, meningitis, or pneumonia, typically during the first three months of life.

Infants may acquire the infection either *in utero* by the ascending route through the ruptured or intact membranes (Melin & Efstratiou 2013), by contact with the bacteria in the birth canal during parturition, or after birth through contact with caregivers. Studies have shown that 25-35% of pregnant women are colonized with GBS in the lower genital and/or gastrointestinal tract and about 50% of colonized women transmit GBS vertically to the gastrointestinal tract or upper respiratory tract of neonates. One to two % of colonized infants develop invasive GBS disease (Baker 2013).

Current prevention strategies of GBS disease in infants, implemented mainly in developed countries, focus on maternal screening for recto-vaginal GBS colonization in late pregnancy (35-37 weeks of gestation), with subsequent use of intrapartum antibiotic prophylaxis (IAP) during delivery in GBS colonized women (www.cdc.gov/mmwr /pdf/rr/rr5910.pdf November 19 2010). Limitations of the current standard culture for GBS carriage detection include the need for prolonged incubation, which require antenatal screening culture resulting in poor predictive value for colonization status in labor and in missing of GBS carriage screening in some preterm labor cases. In the future, advances in diagnostic molecular technologies are expected to offer rapid GBS intrapartum screening test with higher sensitivity, specificity and predictive values (Di Renzo et al. 2014). The use of IAP however has little impact on GBS infections that occur after the first week of life (www.cdc.gov/abcs/reportsindings/survreports/ gbs08.html Accessed January 27, 2014), thus alternative strategies for prevention of neonatal GBS disease remain to be an unmet medical need.

One alternative strategy is maternal vaccination against GBS. Maternal serotype-specific anti-capsular antibodies to GBS are transferred to the fetus trans-placentally, mainly in the third trimester of pregnancy, and persist in the infant throughout the first months of life, which is the period of high vulnerability to GBS disease (Englund et al. 1995; Glezon & Alpers 1999; Baker & Kasper 1977; Boyer et al. 1984). Case-control studies have linked higher serotype-specific maternal anti-capsular polysaccharide antibody concentrations to protection against neonatal GBS disease during the first week of life (Baker, Carey & Rench et al. 2013, Baker, Rench & McInnes 2003, Lin et al. 2004, Lin at el. 2001). These findings support the approach of maternal vaccination as a potential strategy for preventing GBS infection in the newborns and infants.

GSK has developed a trivalent glycoconjugate vaccine against GBS that contains the capsular polysaccharides (CPS) of GBS serotypes Ia, Ib and III, conjugated to a protein carrier, CRM_{197}, which enhances their immunogenicity.

Single 5 µg doses of adjuvanted and unadjuvanted GBS Trivalent Vaccine demonstrated immunogenicity and a favorable safety profile in approximately 400 pregnant and 320 non-pregnant subjects in Phase 1 and 2 studies (V98_06, V98_04, V98_05, V98_08). Single 20 µg doses of adjuvanted and unadjuvanted GBS Trivalent Vaccine were assessed in Phase 1and 2 (V98_06, V98_08) studies in approximately 200 non-pregnant subjects. The immunogenicity and safety profile of single dose of 5 µg and 20 µg doses were comparable.

In the Phase 1b study (V98_06) two-injection regimens at the same dose range of 5 µg and 20 µg (adjuvanted and unadjuvanted) with one month interval between injections were evaluated in approximately 160 non-pregnant subjects per dose. The immunogenicity of 5 µg and 20 µg doses administered as single or two injections courses were comparable. Two injection regimens at the same dose range did not impact the immune response and no clear dose-response relationship was observed for either GMC or percentage of subjects reaching pre-defined antibody concentrations at day 60 (which is approximately the mean time of delivery after vaccination in Phase 2 studies with pregnant subjects). The series of these concentrations was based on putative protective serotype-specific thresholds which corresponded with reduced risk of infant infection in the earlier case-control studies (Lin 2001, Lin 2004).

The candidate vaccine is intended to be administered to the pregnant woman in the third trimester of pregnancy to optimize antibody transfer to the fetus. The GBS antibody levels induced by vaccination are expected to decrease over time and re-vaccination during the subsequent pregnancy is likelyto be required to ensure that protective levels of antibodies are achieved again. The repeat administration of conjugate vaccines with the same carrier protein (CRM_{197}) has the potential to affect vaccine immunogenicity through antigen competition (Pichichero ME. Hum Vaccin Immunother. 2013). Considering that CRM_{197} is a nontoxic variant of diphtheria toxin, the impact on existing anti-diphtheria antibody levels has to be considered and evaluated. However, the safety and immunogenicity of the second dose of GBS Trivalent Vaccine when administered after longer than one month has not been evaluated to date.

1.2 Rationale

The objective of this extension study is the initial assessment of safety and immunogenicity of the second dose of GBS Trivalent Vaccine following the time interval that is close to the inter-pregnancy interval observed in the general population (Gemmill

A, Lindberg LD. Short interpregnancy intervals in the United States. Obstet Gynecol. 122(1):64-71, 2013).

To address this objective, the subjects who received a single 5µg dose of GBS Trivalent Vaccine or placebo as part of the V98_06 study, will be vaccinated and receive a second dose of 5µg approximately 4-6 years after the first vaccination. The placebo group from V98_06 study is included as a control that provides longitudinal data both for the immunogenicity and anti-diphtheria antibodies levels and has the advantage over naive subjects of being a group that was randomized as part of the original study. Naive subjects who have never received GBS Trivalent vaccine will also be enrolled to ensure adequate number of controls in case of significant loss to follow up of the V98_06 study placebo treated subjects.

While high levels of antibodies to GBS in a pregnant woman are predictive of lower incidence of infant GBS disease, (Lin 2001, Lin 2004, Baker CJ, Kasper DL NEJM, 1976), the levels of serotype-specific IgG antibodies needed in a mother in order to protect her infant from GBS have not yet been established. Until these levels have been established, a set of increasing serotype-specific ELISA IgG cut-off levels will be used to attempt to bracket where the assumed protective levels may be. The study objectives are non-specific as to the exact level needed to establish protection.

It is acknowledged that due to loss to follow up the study sample size may be limited. Also, due to baseline heterogeneity of subjects who received different adjuvants or no adjuvant with first vaccination, the study will be exploratory by nature and the results will be interpreted as such.

2. OBJECTIVES

2.1 Primary Objective(s)

Immunogenicity Objective

To evaluate the immunogenicity of a second dose of GBS Trivalent Vaccine (5 µg without adjuvant) administered approximately 4-6 years after the initial GBS vaccination, measured by ELISA.

Safety Objective

To assess the safety and tolerability of a second dose of GBS Trivalent Vaccine (5 µg without adjuvant) administered approximately 4-6 years after the initial dose.

2.2 Secondary Objective(s)

Immunogenicity Objectives

To further characterize the immune response following a dose of GBS Trivalent Vaccine (5 µg without adjuvant) in study subjects and in subgroups of subjects defined by pre-vaccination serotype-specific GBS antibody LLQ status in the V98_06 study or V98_06E1 for the naïve subjects.

To assess the impact of a dose of the GBS Trivalent Vaccine (5 µg without adjuvant) on the serum level of anti-diphtheria antibodies in subjects who received study vaccination (active or placebo) as part of the V98_06 and naïve subjects from V98_06E1.

2.3 Exploratory Objective(s)

To assess the immunogenicity of a dose of GBS Trivalent Vaccine (5 µg without adjuvant) in study subjects as measured by OPK and in subgroups of subjects defined by pre-vaccination serotype-specific GBS ELISA antibody LLQ status in the V98_06 or V98_06E1 for the naïve subjects.

3. STUDY DESIGN

3.1 Overview of Study Design

This is a Phase 2, non-randomized, controlled, open-label, parallel-group, extension of the V98_06 Study. All eligible subjects will receive a single injection of 5 µg dose (of each glycoconjugated polysaccharide - serotypes Ia, Ib, and III) of unadjuvanted GBS Trivalent Vaccine (Table 3-1).

Table 3-1 Treatment Groups and Number of Subjects

Treatment Groups	Vaccine Dose (µg Ia/Ib/III Glycoconjugate, no adjuvant)	Number of Subjects
V98_06: Single vaccination 5/5/5 no adjuvant	5/5/5	Up to 40
V98_06: Single vaccination 5/5/5 Alum	5/5/5	Up to 40
V98_06: Single vaccination 5/5/5 MF59 full dose	5/5/5	Up to 40
V98_06: Single vaccination 5/5/5 MF59 half dose	5/5/5	Up to 40
V98_06: Placebo (Enrollment Group 1 and Enrollment Group 2)	5/5/5	Up to 40
Naive* subjects (not previously enrolled in V98_06)	5/5/5	20
Total – up to 220 subjects		

*The naive group will be healthy non-pregnant female subjects aged 22-46 years inclusive on the day of informed consent who did not participate in the V98_06 study, who have not received any GBS vaccine in the past and who meet all of the inclusion/exclusion criteria for the current V98_06E1 study.

Following screening and the giving of consent, eligible subjects will provide a blood sample for pre-vaccination measurement of serotype-specific GBS antibodies and will receive a single dose of unadjuvanted GBS Trivalent Vaccine containing 5 µg of each polysaccharide (serotypes Ia, Ib, and III). On day 31 and day 61 additional blood samples will be obtained to assess serotype-specific GBS antibody responses.

The following data will be collected for safety analysis:

- Day 1 (vaccination): A 30 minute post-injection assessment for signs or symptoms of anaphylaxis.
- Day 1 (vaccination) to day 7: Solicited local and systemic adverse events (AEs), body temperature and other indicators of solicited adverse events recorded daily using a Subject Diary.
- Day 1 (vaccination) to day 31: All unsolicited AEs.
- Day 1 to day 181: SAEs, Medically-attended AEs and AEs leading to discontinuation from the trial only.
- Day 1 to day 181: All concomitant medications administered in relation to the reported AEs, any vaccines administered and all antibiotics and any other prescription or over the counter medicine(s) deemed clinically significant by the investigator.

The study will include 2 subject diary reminder calls (day 3 and day 5), 3 clinic visits (day 1 – vaccination, day 31 and day 61) and 3 safety calls (day15, day 121 and day 181).

All site staff will be properly trained on the study protocol and procedures.

3.2 Study Period

Each subject should expect to participate in the study for approximately 6 months, from the time of enrolment through the last study visit.

3.3 Blinding Procedures

This study is an Open-Label study. There is no blinding of the study treatment either to the Investigator, subject and site or sponsor staff. All persons involved in the conduct of the study will be aware of the vaccine allocation to each subject which will be a 5 µg dose of unadjuvanted GBS Trivalent Vaccine.

3.4 Data Collection

3.4.1 Data Collected from Subjects

The following data will be collected from each subject over the duration of their study participation:

- Demographic Information
- Adverse Events

- Medical History
- Concomitant Medications

All data collected must only be identified using the GSK Subject ID, as described in section 5.1.4, Randomization.

3.4.2 Tools Used for Data Collection

Data will be recorded in the Subject Diary and collected on Case Report Forms (CRFs).

Subject Diary

Paper Diaries (pDiaries) hereafter referred to as Subject Diaries will be the only source document allowed for solicited local and systemic adverse events (including body temperature measurements (preferably orally)), starting after the initial 30 minute post-vaccination period at the clinic. The following additional rules apply to documentation of safety information collected in the Subject Diary.

1. No corrections or additions to the information recorded by the subject within the Subject Diary will be allowed after it is delivered to the site.
2. Any blank or illegible fields on the Subject Diary must be described as missing in the CRF.

Case Report Forms

This study utilizes Case Report Forms (CRFs) to collect study-related data from each subject. A qualified site staff member(s) is required to enter subject data in the CRFs in English based on the medical information available in each subject's source record.

Data should be entered into the CRF in a timely fashion following each subject's clinic visit, study procedure, or phone call. Each subject's CRF casebook will be compared with the subject's source records by a GSK-approved study monitor (or designee) over the duration of the study in order to ensure data collection accuracy.

The following additional rules apply to documentation of Subject Diary information collected in the CRFs:

1. The site must enter all readable entries in the Subject Diary into the CRF, including those values that may be biologically implausible (e.g. body temperature: 400°C).
2. Any illegible or implausible data should be reviewed with the subject. If an underlying solicited or unsolicited adverse event is described on review with the

subject, this should be described in the source document and reported as an unsolicited adverse event in the Adverse Event CRF (e.g., if the subject above confirms body temperature of 40°C on the day in which body temperature: 400°C was written into her Subject Diary, this fever of 40°C should be recorded in the Adverse Event CRF).

3. Any newly described safety information (including a solicited adverse event) must not be written into the Subject Diary and must be described in the study file as a verbally reported adverse event. Any adverse event reported in this fashion must be described as an unsolicited adverse event and therefore entered on the Adverse Event CRF.

3.5 Collection of Clinical Specimens

The following clinical specimens are required to be collected from each subject (urine will only be collected for women of child bearing potential and those of non-child bearing potential who do not have available medical records to support non-child bearing potential status) in this study:

- Blood
- Urine

Processing of each specimen should be completed by a qualified site member and in accordance with the study-specific Clinical Specimen Laboratory Manual. Testing of clinical blood specimens will be performed by a GSK or designated laboratory. Refer to the study-specific Clinical Specimen Laboratory Manual for additional details.

Blood Specimens

Approximately of 20 mL sample of blood will be drawn from all subjects at visit 1 before vaccination, and at visit 3 and 4. The blood volume will not exceed 25 mL at each time point in order to provide the necessary serum volume (approximately half of the blood draw volume) for the serology assays.

The blood will be used for immunological assays. See section 7, Assessments for additional details.

The total amount of blood collected over the study period per subject will not exceed 75 mL.

Urine Specimens

Urine will be collected for pregnancy testing in females of child bearing potential (and those of non-child bearing potential who do not have available medical records to support

non-child bearing potential status). Urine will be collected at visit 1 before vaccination and the results recorded in the source document and CRF.

3.6 Stopping/Pausing Guidelines

There are no predetermined stopping rules in this study. Subjects may be withdrawn from the study according to investigator discretion as described in section 3.8, Premature Withdrawal from Study.

3.7 Data Monitoring Committee

No DMC will be established for this study.

3.8 Premature Withdrawal from Study

Subjects may withdraw at any time, or be dropped from the study at the discretion of the investigator should any untoward effects occur and/or for safety reasons. In addition, a subject may be withdrawn by the investigator or the Sponsor if she violates the study plan or for administrative reasons. The investigator or study coordinator must notify the Sponsor immediately when a subject has been withdrawn due to an adverse event.

The circumstances above are referred to as premature withdrawal from the study, and the reason for premature withdrawal should be clearly documented and detailed in the source documentation. The investigator should make every attempt to evaluate the subject's safety, including resolution of ongoing AEs, at the time of premature withdrawal. If a subject wants to withdraw from the study before all doses are administered or prior to the last planned study visit, the subject will be asked to be followed for safety for the duration of the study. When a subject withdraws, or is withdrawn, from the study, the procedures described in section 5.5.1, Early Termination Visit should be completed if possible.

The reasons for premature withdrawal from the study include: Adverse event, death, withdrawal of consent, lost to follow-up, administrative reason, and protocol deviation. These reasons are described in greater detail below.

Adverse Event

For any subject withdrawn from study participation prior to the planned Study Termination Visit, it is important to determine if an AE was associated with the reason for discontinuing the study. This AE must be identified on the AE CRF page by indicating "Withdrawn from study due to AE". Any ongoing AEs at the time of study withdrawal must be followed until resolution or stabilization.

Death

For any subject withdrawn from study participation due to death, this should be noted on the Study Termination CRF page and the associated SAE that led to the death must be reported.

Withdrawal of consent

The subject can withdraw consent for participation in the study at any time without penalty or loss of benefit to which the subject is otherwise entitled. Reason for early termination should be deemed as "withdrawal of consent" if the subject withdraws from participation due to a non-medical reason (i.e., reason other than AE). If the subject intends to withdraw consent from the study, the investigator should clarify if the subject will withdraw completely from the study or if the subject will continue study participation for safety, or a subset of other study procedures. If the subject requests complete withdrawal from the study, no further study interventions will be performed with the subject.

Lost to Follow-Up

For subjects who fail to show up for scheduled visits (clinic or telephone contacts), or for three consecutive visits, study staff are encouraged to make at least three documented attempts to contact the subject by telephone and at least one documented written attempt to contact the subject to encourage the completion of study termination procedures. These efforts to contact the subject should be recorded in the source document. The termination date for the subject to be captured on the Study Termination CRF page is the date of the last successful contact (clinic visit or telephone) with the subject.

Administrative Reason

Examples for subjects withdrawn from the study due to administrative reason can include: Sponsor decision to terminate the study, subject meeting a pre-specified withdrawal criterion, subject discontinuation for insurance issues, moving, no time, etc. This reason should be noted in the Study Termination CRF page and any ongoing AEs at the time of study withdrawal must be followed until resolution/stabilization.

If the clinical study is prematurely terminated by the Sponsor, the investigator is to promptly inform the study subjects and local EC and should assure appropriate therapy and follow up for the subjects. All procedures and requirements pertaining to the archiving of study documents should be followed. All other study materials (study medication/vaccines, etc.) must be returned to the Sponsor.

For subjects who are withdrawn from the study due to receipt of an excluded medication/vaccination or due to significant protocol non-compliance, this reason should be noted in the Study Termination CRF page.

Protocol Deviation

A protocol deviation is any change, divergence, or departure from the study design or procedures of a study protocol. In general, subjects associated with protocol deviations may remain in the study unless continuation in the study jeopardizes the subject's health, safety, or rights.

Investigators will apply due diligence to avoid protocol deviations. Under no circumstances should the investigator contact GSK or its agents, if any, monitoring the study to request approval of a protocol deviation, as no authorized deviations are permitted. If the investigator feels a change to the protocol would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by GSK and approved by the EC and health authorities it cannot be implemented.

Any subject who becomes pregnant during the study, despite the protocol requirement for adequate contraception, should be encouraged to continue participating in the study for safety follow-up. The site must complete a Pregnancy Report CRF (initial report) as soon as possible after learning of pregnancy occurrence (see section 7.1.6, Pregnancies for further details). If the subject withdraws from the study for any of the above categories except death, the site will obtain permission from the subject to continue to remain in contact with her until the outcome of the pregnancy is known, even if the outcome is not known until after the subject reaches the end of follow-up period.

3.9 End of Study

For the purpose of this protocol, end of study is defined as the completion of the Last Subject Last Visit (LSLV).

4. SELECTION OF STUDY POPULATION

4.1 Inclusion Criteria

In order to participate in this study, all subjects must meet ALL of the inclusion criteria described.

1. Healthy, non-pregnant subjects who have received a single 5 µg dose of GBS Trivalent Vaccine or placebo in the V98_06 study and healthy non-pregnant female subjects aged 22-46 years inclusive on the day of informed consent who have not received any GBS vaccine in the past.
2. Individuals who have voluntarily given written informed consent after the nature of the study has been explained according to local regulatory requirements, prior to study entry.
3. Individuals in good health as determined by the outcome of medical history, physical examination and clinical judgment of the investigator.
4. Individuals who can comply with study procedures including follow-up[1].
5. Females of childbearing potential who are using an effective birth control method[2] which they intend to use until the end of the study (day 181 visit) or females of non-childbearing potential[3].

4.2 Exclusion Criteria

1. Progressive, unstable or uncontrolled clinical conditions.

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1 A subject is considered to be compliant if the Investigator judges that the subject will complete the Subject Diary/ return for all the follow-up visits/ be available for telephone calls as scheduled in the study.

[2] The following birth control methods are considered effective:

- Abstinence
- Hormonal contraceptive (such as oral, injection, transdermal patch, implant) if used for at least 30 days prior to informed consent
- Diaphragm with spermicide, tubal occlusion device
- Intrauterine device (IUD)
- Tubal ligation
- Male partner using condom with spermicide
- Male partner having been vasectomized at least six months prior to informed consent

[3] A female is considered to be of non-childbearing potential only after hysterectomy, bilateral oophorectomy, or iatrogenic ablation of ovarian function.

2. Clinical conditions representing a contraindication to intramuscular vaccination and blood draws.
3. Abnormal function of the immune system resulting from:
    a. Clinical conditions, including but not limited to known or suspected HIV infection or HIV-related disease, a history of or an active autoimmune disorder (as per the judgment of the Investigator)
    b. Systemic administration of corticosteroids (PO/IV/IM) for more than 14 consecutive days within 90 days prior to informed consent.
    c. Administration of antineoplastic and immunomodulating agents or radiotherapy within 90 days prior to informed consent.
    d. Receipt of immunosuppressive therapy within 90 days prior to informed consent
4. Received immunoglobulins or any blood products within 180 days prior to informed consent.
5. Received an investigational or non-registered medicinal product within 30 days prior to informed consent.
6. Study personnel as an immediate family or household member.
7. Any other clinical condition that, in the opinion of the investigator, might interfere with the results of the study or pose additional risk to the subject due to participation in the study.
8. Individuals who received any other vaccines within 14 days for inactivated vaccines or 28 days for live vaccines prior to enrolment in this study or who are planning to receive any vaccine within 28 days from the study vaccination. Exception - an inactivated influenza vaccine may be administered up to 7 days prior to study vaccination or 7 days after study vaccination.
9. Individuals who anticipate becoming pregnant prior to the end of the study, Day 181 Visit.
10. Individuals who are nursing (breastfeeding).
11. Individuals who have had a previous immunization with a vaccine containing Group B Streptococcus antigens that was not part of V98_06 study.
12. Individuals with a fever (oral temperature $\geq$ 38°C) within 3 days prior to day 1 or use of antipyretics and/or analgesic medications within 24 hours prior to day 1.
13. Individuals with acute or chronic infection(s) that require systemic antibiotic treatment or antiviral therapy, within 7 days prior to day 1.

14. Individuals with a history of severe allergic reactions after previous vaccinations or medications, such as anaphylactic shock, asthma, urticaria, or other allergic reaction or hypersensitivity to any vaccine component or medical equipment including latex used in this study.
15. Individuals with any progressive or severe neurologic disorder, seizure disorder, epilepsy or Guillain-Barré syndrome.
16. Individuals with history of substance or alcohol abuse within the past 2 years.

4.3 Criteria for Delay of Vaccination

There may be instances when individuals meet all eligibility criteria for vaccination yet have a transient clinical circumstance which may warrant delay of vaccination: body temperature elevation (≥38.0° C) within 3 days prior to intended study vaccination, or use of antipyretics and/or analgesic medications within 24 hours prior to vaccination. Under such circumstances, a subject may be considered eligible for study enrolment after the appropriate window for delay has passed and inclusion/exclusion criteria have been rechecked, and if the subject is confirmed to be eligible.

5. STUDY PROCEDURES

The sections that follow provide an overview of the procedures that are to be followed in enrolling, evaluating, and following subjects who participate in this clinical study. Visits can be either clinic visits or safety follow-up telephone calls, as specified in the Table below and in the Time and Events Table 3

Table 5-1 Study Procedures

Visit Category	Procedures
Pre-vaccination Clinic Visit(s)	Section 5.1 describes procedures to be followed prior to study vaccination: informed consent, screening, enrolment, and randomization
Vaccination Clinic Visit(s)	Section 5.2 describes procedures to be followed during each clinic visit involving vaccination: vaccination, post-vaccination procedures, and post-vaccination reminders
Post-vaccination Visit(s)	Section 5.3 describes follow-up clinic visits and safety follow-up calls
Unscheduled Visit(s)	Section 5.4 describes possible procedures to be followed at unscheduled clinic visit
Study Termination Visit	Section 5.5 describes procedures to be followed at the last study visit for a subject (may include early termination visit)

5.1 Pre-vaccination Clinic Visit(s)

This section describes the procedures that must be performed for each potential subject prior to vaccination, including obtaining informed consent, screening and enrolment.

5.1.1 Informed Consent

"Informed consent" is the voluntary agreement of an individual or her legal guardian(s) to participate in research. Consent must be given with free will of choice, and without undue inducement. The individual must have sufficient knowledge and understanding of the nature of the proposed research, the anticipated risks and potential benefits, and the requirements of the research to be able to make an informed decision.

Informed consent following local EC guidance **must** be obtained before conducting any study-specific procedure (i.e., all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the subject source document in addition to maintaining a copy of the signed and dated informed consent.

If a subject is unable to read, an impartial witness should be present during the entire informed consent discussion. An impartial witness is defined as a person who is independent from study conduct, who cannot be unfairly influenced by those involved with the study, who attends the informed consent process if the subject or the subject's legally acceptable representative cannot read, and who reads the informed consent form and any other written information supplied to the subject. After the written informed consent form and any other written information to be provided to subjects, is read and explained to the subject and after the subject has verbally consented to the subject's participation in the study and, if capable of doing so, has signed and personally dated the informed consent form, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject and that informed consent was freely given by the subject.

5.1.2 Screening

After an individual has consented to participate in the study and informed consent is signed, that individual will be given a unique Screening Number manually created by the investigator. The subject's unique Screening Number will be documented in the Screening and Enrolment log. The eligibility of the subject will be determined based on the inclusion and exclusion criteria listed in section 4, Selection of Study Population and evaluated during this screening procedure.

Prior to study enrolment, demographic data will be collected from the subject, including: race, receipt of diphtheria containing vaccine up to 5 years prior to vaccination in the V98_06 study (if applicable), receipt of diphtheria containing vaccine between vaccination in the V98_06 study and enrolment into the V98_06E1 study (if applicable), receipt of diphtheria containing vaccine up to 5 years prior to the V98_06E1 study (naive subjects only), whether or not they participated in the parent V98_06 study and if applicable the Subject ID they were assigned in the parent V98_06 study.

Medical history will also be collected, including but not limited to any medical history that may be relevant to subject eligibility for study participation such as prior vaccinations, concomitant medications, and previous and ongoing illnesses or injuries. Relevant medical history can also include any medical history that contributes to the understanding of an adverse event that occurs during study participation, if it represents an exacerbation of an underlying disease/pre-existing problem.

If a subject reports they are of non-childbearing potential, the medical history in the form of medical records (verbal medical history only is not sufficient in this case) must be collected prior to vaccination or the subject will have to undergo a urine pregnancy test.

If applicable, prior and concomitant medications or vaccinations taken prior to start of study should be collected (refer to section 6.5, Prior and Concomitant Medications and Vaccines for further details).

Collect vital signs. Measure height and weight.

Perform pregnancy testing in women of childbearing potential (refer to section 3.5, Collection of Clinical Specimens for guidance regarding the procedure).

A general physical examination is to be performed by a qualified health care practitioner. "Qualified health care practitioner" refers to any licensed health care professional who is permitted by institutional policy to perform physical examinations and who is identified within the Study Staff Signature Log.

These data will be written in the source document (see section 9.1, Source Documentation). Should the physical assessment reveal any abnormal values or events, these must be documented in the CRF Adverse Events Form.

Prior to vaccination, approximately 20 mL of blood will be drawn from all subjects for the following testing: serology testing. Refer to section 3.5, Collection of Clinical Specimens.

In the event that the individual is determined ineligible for study participation, she is considered a screen failure. The reason for screen failure must be documented in the Screening and Enrolment log. If the individual is determined to be eligible for the study, she will be enrolled into the study.

5.1.3 Enrolment

After signing the informed consent form, if an individual is determined to be eligible for study participation, the investigator will enroll the subject and enter the manually created Subject ID and Subject Code into the EDC system. The Screening Number ceases to be used and remains in the Screening and Enrolment Log only.

5.1.4 Randomization

This is a single treatment open label study therefore no randomization will be carried out. The Subject ID will be the subject's unique identification number for all CRFs and associated study documentation that will be used for duration of the study.

If for any reason, after enrolment the subject fails to undergo treatment this is an Early Termination and the reason should be recorded in source document as specified in the Source Data Agreement (SDA). The information on these Early Termination subjects should be kept distinct in the source documentation from subjects who are screen failures, as described in section 5.1.2, Screening.

5.2 Vaccination Clinic Visit

Vaccination will be performed on day 1.

Ensure all serology samples are taken **prior** to each vaccination.

After completing the pre-vaccination procedures on day 1, administer the vaccine to the subject according to the procedures described in section 6.3, Vaccine Preparation and Administration.

5.2.1 Post-vaccination Procedures

The following post-vaccination procedures will be performed on day 1.

After vaccination, the subject will be observed for at least 30 minutes including observation for unsolicited adverse events, solicited adverse events, and body temperature measurement. Record all safety data collected during this time in the subject's source document.

A Subject Diary will be used in this study to document solicited adverse events. The Subject Diary is the only source for collection of these data; therefore, it is critical that the subject completes the Subject Diary correctly. The subject should be trained on how and when to complete each field of the Subject Diary.

The subject should be trained on how to self-measure local solicited adverse events and body temperature (preferably orally). The measurement of solicited local adverse events is to be performed using the ruler provided by the site.

The subject should be instructed how to perform body temperature measurement using the thermometer provided by the site. If the subject feels unusually hot or cold during the day, the subject should check body temperature. If the subject has fever, the highest body temperature observed that day should be recorded in the Subject Diary.

Subject Diary training should be directed at the individual(s) who will perform the measurements of adverse events and who will enter the information into the Subject Diary. This individual may not be the subject, but if a person other than the subject enters information into the Subject Diary, this person's identity must be documented in the

Subject Diary and/or subject's source record. Any individual that makes entries into the Subject Diary must receive training on completion of the Subject Diary at the time of the visit. This training must be documented in the subject's source record.

The same individual should complete the Subject Diary throughout the course of the study.

The site should schedule the subject diary reminder calls and the next study safety follow-up call with the subject.

The subject should be reminded of the next planned study activity. The subject will be reminded to complete the Subject Diary and to contact the site if there are any questions, and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit or to a visit to/by a doctor or is of concern.

5.2.2 Post-vaccination Reminders

Reminder calls or alerts are not intended to be an interview for collection of safety data. If the subject wishes to describe safety information, this information should only be collected by a healthcare professional at the site, and the safety data described must be written down in the subject's medical chart.

Subject Diary Reminder Calls

Subject Diary reminder calls will be performed on day 3 and day 5. The purpose of these calls is to remind the subject about completion of the Subject Diary. The call follows the Subject Diary Reminder Telephone Call Script provided to the site. The subject should be reminded to contact the site via the telephone number provided in the informed

5.3 Post-vaccination Visits

Post-vaccination visits will be performed on day 15, day 31, day 61, day 121 and day 181.

5.3.1 Follow-up Clinic Visits

Safety follow-up clinic visits will be performed on day 31 and day 61.

During the follow-up clinic visit at day 31, the Subject Diary will be reviewed. No changes to the information recorded within the Subject Diary are permissible. For details on the Subject Diary see sections 3.4.2, Tools Used for Data Collection and 5.2.1, Post-vaccination Procedures.

The subject will be interviewed to determine if unsolicited adverse events occurred (all unsolicited AEs up to day 31 and SAEs, medically attended AEs and AEs leading to withdrawal only up to day 61) and if any concomitant medications in relation to the reported AEs, and/or vaccines and/or antibiotics/other prescription or over the counter medicine(s) deemed clinically significant by the investigator were taken/received in the time since the last clinic visit.

This interview will follow a script which will facilitate the collection of relevant safety information. The healthcare professional reviewing these data will discuss the symptoms (if any) reported by the subject and will determine if any additional diagnoses and/or adverse events are present. Adverse events reported by the subject at this follow-up clinic visit must be recorded in the subject's source document and on an Adverse Events CRF, as specified in section 7.1, Safety Assessment, and not written on the script used for the interview.

Perform a brief symptom-directed physical examination, according to symptoms the subject has reported. This physical examination will include an examination of organ systems that are relevant to the investigator based on review of the subject's concurrent medical conditions, reported adverse events, concomitant medication use. The physical assessment must be performed by the investigator or designee of the investigator, who is qualified to perform a physical assessment in accordance with their institutional policy. Corresponding information is documented in the subject's source document and CRF(s).

Approximately 20 mL of blood will be drawn from all subjects for the following testing: serology testing. Refer to section 3.5, Collection of Clinical Specimens.

The site should schedule the next study clinic visit or safety call with the subject.

The subject will be reminded of the next planned study activity. The subject will be reminded to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit.

5.3.2 Safety Follow-up Calls

Safety follow-up calls will be performed on day 15, day 121 and day 181.

Safety follow-up calls are calls made to the subject by a healthcare professional designated on the site log. These calls will follow a script which will facilitate the collection of relevant safety information. The subject will be interviewed according to the script.

At day 15 information relating to all unsolicited adverse events and all concomitant medications administered in relation to them, any vaccines administered and all antibiotics and any other prescription or over the counter medicine(s) deemed clinically significant by the investigator will be collected.

At days 121 and 181 information relating to only SAEs, medically attended adverse events, AEs leading to withdrawal and all concomitant medications administered in relation to them, any vaccines administered and all antibiotics and any other prescription or over the counter medicine(s) deemed clinically significant by the investigator will be collected.

All safety information described by the subject must be written down in a designated location within the source document and not written on the script used for the telephone call.

The site should schedule the next study activity clinic visit or safety call with the subject.

The subject will be reminded to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit.

5.4 Unscheduled Visits

Not applicable for this study.

5.5 Study Termination Visit

The study termination visit will occur on day 181. The termination visit will be a telephone call. The date of termination is the day 181 visit or the last contact (clinic visit or telephone call) in which the subject's health status was assessed or, in cases where the subject does not agree to any further safety follow-up, it is the date consent is withdrawn. This date should be recorded on the termination CRF page. For visit procedures to be performed for a subject whose planned study participation ends prematurely, please see section 5.5.1, Early Termination Visit.

During the telephone call, the following procedures will be performed: interview of subject to collect medically attended adverse events, AEs leading to withdrawal, SAEs and concomitant medications/ vaccinations including concomitant medications administered in relation to the reported AEs, all antibiotics and any other prescription or over the counter medicine(s) deemed clinically significant by the investigator.

The site will review with the subject the plan of when information relating to the subject's participation in the study may be available (e.g., study results). It will also be discussed

how information relating to the subject's participation in the study will be shared with the subject's healthcare provider, if the subject chooses to share this information.

The site will complete the termination CRF page and this will mark the completion of the subject's participation in the study.

5.5.1 Early Termination Visit

When a subject is withdrawn from treatment or withdraws from the study, the investigator will notify the Sponsor and, when possible, will perform the procedures as outlined below. The reason(s) for the early termination will be included in the subject's source documentation. If the Early Termination Visit is a telephone call, collect as much information as possible. Early Termination Visits include subjects who were enrolled but not treated.

At the clinic visit or during the telephone call, procedures should be carried out according to the next closest scheduled visit. I.e., if the subject terminates early at clinic visit day 31 then all procedures outlined for the day 31 visit should be carried out. Similarly if they terminate early between day 121 and day 181 safety telephone calls all procedures as according to the day 181 safety call should be carried out.

The site will review with the subject the plan of when information relating to the subject's participation in the study may be available (e.g., study results). It will also be discussed how information relating to the subject's participation in the study will be shared with the subject's healthcare provider, if the subject chooses to share this information.

The site will complete the termination CRF page and this will mark the completion of the subject's participation in the study.

6. TREATMENT OF SUBJECTS

All vaccines associated with this study are to be stored separately from other vaccines and medications in a secure location under appropriate storage conditions with temperature monitoring. **All vaccines associated with this study must be checked for expiration date prior to use. Expired vaccines must not be administered to subjects.**

6.1 Study Vaccine

The term 'study vaccine' refers to those vaccines provided by the Sponsor, which will be evaluated as part of the study objectives. The study vaccine specific to this study is described below.

GroupB streptococcus (GBS) TrivalentVaccine

Lyophilized Formulation of GBS trivalent vaccine will be supplied by GSK.

- Vial

  Each vial contains 6 µg of each glycoconjugate (i.e. serotype Ia, Ib and III). Each of the three capsular polysaccharides is conjugated to the *Corynebacterium diptheriae* CRM_{197} carrier protein. At the time of injection, the vaccine will be reconstituted at the site with 0.6 mL sterile saline. The sterile saline will be supplied by GSK as 2.0 mL sterile saline for intramuscular (IM) injection at a concentration of 0.9% of sodium chloride.

- Dose

  A dose of 5 µg of each antigen with the single vaccine injection of 0.5 mL delivered by the IM route, preferably the deltoid muscle in the non-dominant-arm.

Vaccine lots to be used in this study have been produced via Good Manufacturing Practices. Different lots of Group B streptococcus glycoconjugate vaccine that may be used in this study are comparable based on product and process specifications.

The vaccine handling and preparation, including reconstitution, instructions will be developed and provided to the site prior to study start, placed in the investigator site file and the investigator should be referred here to review these materials prior to study start. The vaccine must be stored between 2°C to 8°C (in a refrigerator) and protected from light. DO NOT FREEZE.

Table 6.1-1 Group B Streptococcus Trivalent Vaccine Composition – Lyophilized Supplied as lyophilized product with 6 µg of each antigen per vial, to be reconstituted with 0.6mL of sterile normal saline (NaCl 9 mg/ml) to provide for a single dose as 0.5 mL of injectable suspension.

Active substances (active ingredient / antigen carrier protein)	Unit per dose (1 dose = 0.5mL)
GBS. CPS-Ia CRM_{197}	5 µg
GBS. CPS-Ib CRM_{197}	5 µg
GBS.CPS-III CRM_{197}	5 µg
Sodium chloride (tonicity modifying agent)	4.5 mg
Potassium dihydrogen phosphate (pH buffering agent)	0.34 mg
Mannitol (lyophilization bulking and tonicity modifying agent	7.5 mg
Water for injection (solvent)	Qs to 0.5 mL
Vaccine presentation	Vial

Saline solution (diluent)

GSK will provide commercially available sterile saline solution, supplied as 0.9% Sodium Chloride in 2.0 mL glass ampoules, for use at the site to reconstitute the lyophilized formulation of GBS Trivalent Vaccine. Only the saline solution provided by GSK is to be used for study vaccine preparation of the lyophilized formulation.

The saline solution may be stored at room temperature with temperatures at < 25°C or in a refrigerator. DO NOT FREEZE.

The package insert (or the summary of product characteristics) for the commercial saline solution will be included in the investigator site file and the investigator will be instructed to refer to these materials prior to study start.

6.2 Non-Study Vaccines

Not applicable for this study.

6.3 Vaccine Preparation and Administration

The investigator or designee will be responsible for oversight of the administration of vaccine to subjects enrolled in the study according to the procedures stipulated in this

study protocol. All vaccines will be administered only by personnel who are qualified to perform that function under applicable local laws and regulations for the specific study site.

Detailed vaccine preparation and administration instructions will be provided to investigators in the Protocol Ancillary Document prior to study start. The lyophilized vaccine must be used immediately after reconstitution, in accordance with the vaccine instructions.

PRECAUTIONS TO BE OBSERVED IN ADMINISTERING STUDY VACCINE:

Prior to vaccination, subjects must be determined to be eligible for study vaccination and it must be clinically appropriate in the judgment of the investigator to vaccinate. Eligibility for vaccination prior to first study vaccine administration is determined by evaluating the entry criteria outlined in protocol sections 4.1, Inclusion Criteria and 4.2, Exclusion Criteria.

Study vaccines should not be administered to individuals with known hypersensitivity to any component of the vaccines.

Standard immunization practices are to be observed and care should be taken to administer the injection intramuscularly. Before administering vaccine, the vaccination site is to be disinfected with a skin disinfectant (e.g., 70% alcohol). Allow the skin to dry. **DO NOT inject intravascularly or intragluteally**.

As with all injectable vaccines, trained medical personnel and appropriate medical treatment should be readily available in case of anaphylactic reactions following vaccine administration. For example, epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis should be available.

6.4 Vaccine Administration Error or Overdose of Vaccine

Vaccine administration error is defined as receiving a dose of study vaccine that was not reconstituted as instructed or administered by a different route from the intended route of administration. An overdose of study vaccine (whether accidental or intentional) is defined when a dosage higher than the recommended dosage is administered in one dose of study vaccine.

Any vaccine administration error or overdose of study vaccine detailed in this protocol must be reported as an adverse event, and if the vaccine administration error or overdose is associated with a serious adverse event, it must be reported as such within 24 hours to the Sponsor.

6.5 Prior and Concomitant Medications and Vaccines

All medications (excluding vitamins, minerals and nutritional supplements), vaccines and blood products taken or received by the subject within 30 days prior to administration of study vaccine are to be recorded on the Prior and Concomitant Medications CRF.

Medications taken for prophylaxis are those intended to prevent the onset of symptoms. Medications taken for treatment are intended to reduce or eliminate the presence of symptoms that are present.

When recording concomitant medications/vaccines, they should be checked against the study entry and continuation criteria in section 4, Selection of Study Population to ensure that the subject should be enrolled in the study.

The use of the following concomitant medications may interfere with the interpretation of the study objectives and therefore, are prohibited:

- use of antipyretics and/or analgesic medications within 24 hours prior to day 1
- any live vaccine 28 days prior to study vaccination
- any inactivated vaccines 14 days prior to study vaccination
- any vaccines within 28 days after study vaccination
- *exception*: an inactivated influenza vaccine may be administered up to 7 days prior to study vaccination or 7 days after study vaccination
- blood, blood products or a parenteral immunoglobulin preparation (except Rho(D) Immunoglobulin);
- immunoglobulins or any blood products within 180 days prior to informed consent
- antineoplastic and immunomodulating agents or radiotherapy within 90 days prior to informed consent.
- immunosuppressive therapy within 90 days prior to informed consent
- investigational or non-registered medicinal product within 30 days prior to informed consent
- Systemic administration of corticosteroids (PO/IV/IM) for more than 14 consecutive days within 90 days prior to informed consent

Concomitant medications that include all medications administered in relation to reported AEs, any vaccines administered and all antibiotics and any other prescription or over the counter medicine(s) deemed clinically significant by the Investigator taken

by/administered to the subject at and after enrolment and must be documented on the Concomitant Medications CRF.

6.6 Vaccine Supply, Labeling, Storage and Tracking

The Sponsor will ensure the following:

- Supply of the study vaccine
- Appropriate labeling of all study vaccine provided that complies with the legal requirements of each country where the study is to be performed.

The investigator must ensure the following:

- Acknowledge receipt of the study vaccines by a designated staff member at the site, including:
  - Confirmation that the vaccines were received in good condition
  - Confirmation to the Sponsor of the temperature range during shipment from the Sponsor to the investigator's designated storage location
  - Confirmation by the Sponsor that the vaccines are authorized for use.
- Proper storage of the study vaccines, including:
  - Storage in a secure, locked, temperature-controlled location.
  - Proper storage according to the instructions specified on the labels.
  - Appropriate record keeping and inventory of the study vaccines, including regular documentation of adequate storage temperature.
- Appropriate use of the study vaccines, including:
  - Not use of vaccines prior to receipt of authorization for use from the Sponsor.
  - Use only in accordance with the approved protocol.
  - Proper handling, including confirmation that the vaccine has not expired prior to administration.
  - Appropriate documentation of administration of vaccines to study subjects including:
    - Date, dosage, batch/lot numbers, expiration dates, unique identifying numbers assigned to subjects and study vaccines, and time of vaccine administration. This information will be maintained in an administration log that will be reviewed by the site monitor.

  - Reconciliation of all vaccines received from the Sponsor. Reconciliation is defined as maintaining records of which and how many vaccines were received, which vaccines were administered to subjects, which vaccines were destroyed at the site, and which vaccines were returned to the Sponsor, as applicable.

- Proper adherence to the local institutional policy with respect to destruction of study vaccines.
- Complete record keeping of vaccine use, wastage, return or destruction, including documentation of:
  - Copy of the site's procedure for destruction of hazardous material.
  - Number of doses destroyed, date of destruction, destruction code (if available), method of destruction, and name of individual performing destruction.

Vaccines that have been stored differently from the manufacturer's indications **must not** be used unless the Sponsor provides written authorization for use. In the event that the use cannot be authorized, the Sponsor will make every effort to replace the vaccine supply. All vaccines used in conjunction with this protocol must be stored separately from normal hospital/practice stocks to prevent unintentional use of study vaccines outside of the clinical study setting.

Monitoring of vaccine accountability will be performed by the study monitor during site visits and at the completion of the study.

At the conclusion of the study, and as appropriate during the course of the study, the investigator must ensure that all unused study vaccines, packaging and supplementary labels are destroyed locally (upon approval from Sponsor) or returned to the Sponsor.

7. ASSESSMENTS

7.1 Safety Assessment

The measures of safety used in this study are routine clinical procedures. They include a close vigilance for, and stringent reporting of, selected local and systemic adverse events routinely monitored in vaccine clinical studies as indicators of reactogenicity.

An adverse event (AE) is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product at any dose that does not necessarily have to have a causal relationship with this treatment. Therefore, an AE can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product. This definition includes intercurrent illnesses or injuries and exacerbation of pre-existing conditions.

The period of observation for AEs (or sub-set of AEs as according to the Time and Events Table 3) extends from the time the subject signs informed consent until he or she completes the specified safety follow-up period (until day 181 visit) or terminates the study early (whichever comes first). AEs occurring after the informed consent form is signed but prior to receiving study vaccine/product will be documented as an adverse event and recorded within source document. However, any AEs occurring prior to receipt of any study vaccine will be analyzed separately from “treatment emergent” AEs (AEs occurring after administration of the first study vaccine).

Adverse events are collected as either solicited or unsolicited adverse events. Solicited events are derived from organized data collection systems, such as Subject Diaries.

7.1.1 Solicited Adverse Events

The term “reactogenicity” refers to solicited signs and symptoms (“solicited adverse events”) occurring in the hours and days following a vaccination, to be collected by the subject for 7 consecutive days, using a pre-defined Subject Diary.

The following solicited adverse events are included in the Subject Diary. Each adverse event is to be assessed using the scoring system reported in parentheses below:

Solicited local adverse events:

The list of solicited local (injection site) adverse events is:

- Pain

- Erythema (mm)
- Swelling (mm)
- Warmth
- Induration (mm)
- Ecchymosis (mm)

Injection site erythema, swelling, induration and ecchymosis will be categorized based on the linear measurement of these events.

Injection site pain and warmth will be summarized according to none, mild (transient with no interference in normal daily activity), moderate (some interference in normal daily activity) or severe (unable to perform normal daily activity).

Solicited systemic adverse events:

The list of solicited systemic adverse events is:

- Chills
- Generalized myalgia
- Malaise
- Nausea
- Headache
- Fatigue
- Body rash
- Generalized arthralgia

Systemic events (except body rash) occurring up to day 7 after vaccination will be summarized according to: none, mild (transient with no interference in normal daily activity), moderate (some interference in normal daily activity) or severe (unable to perform normal daily activity). Body rash occurring up to day 7 after vaccination will be summarized according to: none (no rash), mild (localized area of the skin, or only one extremity), moderate (moderate area of the skin, or two or more body regions, without whole body involvement), or severe (most of the skin).

Other solicited reactions:

- Body temperature for fever (preferably oral, captured daily as measurements in degrees Celsius)
- Use of analgesics/antipyretics

Body temperature will be summarized based on the measurement and grade.

Use of analgesics/antipyretics will be summarized as "for treatment" or "for prophylaxis". Each local and systemic reaction will also be categorized as none vs. present.

The study staff must review the data entered into the Subject Diary as described in section 3.4.2, Tools Used for Data Collection and section 5.3.1, Follow-up Clinic Visit(s).

Note: Any solicited adverse event that meets any of the following criteria must be entered into subjects' source document (see section 9.1, Source Documentation) and also as an adverse event on the Adverse Event CRF:

- Solicited local or systemic adverse event that continues beyond day 7 after vaccination.
- Solicited local or systemic adverse event that leads to a visit to a healthcare provider (medically attended adverse event, see section 7.1.3, Evaluation of Adverse Events).
- Solicited local or systemic adverse event leading to the subject withdrawing from the study or the subject being withdrawn from the study by the investigator (adverse event leading to withdrawal, see section 7.1.3, Evaluation of Adverse Events).
- Solicited local or systemic adverse event that otherwise meets the definition of a serious adverse event (see section 7.1.4, Serious Adverse Events).

7.1.2 Unsolicited Adverse Events

An unsolicited adverse event is an adverse event that was not solicited using a Subject Diary and that was spontaneously communicated by a subject who has signed the informed consent.

Potential unsolicited AEs may be medically attended (defined as symptoms or illnesses requiring hospitalization, or emergency room visit, or visit to/by a health care provider), or were of concern to the subject. In case of such events, subjects will be instructed to contact the site as soon as possible to report the event(s). The detailed information about the reported unsolicited AEs will be collected by the qualified site personnel during the interview and will be documented in the subject's records.

Unsolicited AEs that are not medically attended nor perceived as a concern by subjects will be collected during interview with the subject and by review of available medical records at the next visit (see section 5.3, Post-vaccination Visit(s)).

7.1.3 Evaluation of Adverse Events

Every effort should be made by the investigator to evaluate safety information reported by a subject for an underlying diagnosis and to capture this diagnosis as the event in the AE page. In other words, the practice of reporting only symptoms (e.g., “cough” or “ear pain”) are better reported according to the underlying cause (e.g., “asthma exacerbation” or “otitis media”).

The severity of events reported on the Adverse Events CRF will be determined by the investigator as:

Mild:	transient with no limitation in normal daily activity.
Moderate:	some limitation in normal daily activity.
Severe:	unable to perform normal daily activity.

The relationship of the study treatment to an AE will be determined by the investigator based on the following definitions:

1. Not Related

The AE is not related to an investigational vaccine if there is evidence that clearly indicates an alternative explanation. If the subject has not received the vaccine, the timing of the exposure to the vaccine and the onset of the AE are not reasonably related in time, or other facts, evidence or arguments exist that reasonably suggest an alternative explanation, then the AE is not related.

2. Possibly Related

The administration of the investigational vaccine and AE are considered reasonably related in time and the AE could be explained by exposure to the investigational vaccine or by other causes.

3. Probably Related

Exposure to the investigational vaccine and AE are reasonably related in time and no alternative explanation has been identified.

The relationship of the study treatment to an unsolicited AE will be determined by the investigator.

Note: solicited AEs will not be evaluated for relationship to study treatment. Grading for severity of solicited local and systemic AEs is described in section 7.1.1, Solicited Adverse Events.

Adverse events will also be evaluated by the investigator for the co-existence of any of the other following conditions:

- "Medically attended adverse event": an adverse event that leads to a visit to a healthcare provider.
- AEs leading to withdrawal: adverse events leading to study or vaccine withdrawal.

All AEs, regardless of severity, will be monitored until resolution or until the investigator assesses them as chronic or stable. All subjects experiencing AEs - whether considered associated with the use of the study vaccine or not - must be monitored until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report should be supplied, if possible. The investigator's assessment of ongoing Adverse Events at the time of each subject's last visit should be documented in the subject's medical chart.

7.1.4 Serious Adverse Events

A serious adverse event (SAE) is defined as any untoward medical occurrence that at any dose results in one or more of the following:

- Death.
- Is life-threatening (i.e., the subject was, in the opinion of the investigator, at immediate risk of death from the event as it occurred); it does not refer to an event which hypothetically might have caused death if it were more severe.
- Required or prolonged hospitalization.
- Persistent or significant disability/incapacity (i.e., the event causes a substantial disruption of a person's ability to conduct normal life functions).
- Congenital anomaly/or birth defect.
- An important and significant medical event that may not be immediately life threatening or resulting in death or hospitalization but, based upon appropriate medical judgment, may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Adverse events which do not fall into these categories are defined as non-serious.

It should be noted that a severe adverse event need not be serious in nature and that a serious adverse event need not, by definition, be severe.

Serious adverse events will be captured both on the Vaccines Serious Adverse Event (VSAE) form as well as on the AE CRF. All SAEs will be evaluated by the investigator for relationship of the event to study vaccine. SAEs that are judged to be possibly or probably related to the study vaccine should be reported to the Sponsor as related/suspected events.

The relationship of the study treatment to an SAE will be determined by the investigator based on the following definitions:

1. Related/suspected

The SAE is judged by the investigator to be possibly or probably related to the study vaccine on the AE CRF page (see section 7.1.3, Evaluation of Adverse Events).

2. Not Related

The SAE is not related if exposure to the study vaccine has not occurred, **or** the occurrence of the SAE is not reasonably related in time, **or** the SAE is considered unlikely to be related to use of the study vaccine, i.e., there are no facts (evidence) or arguments to suggest a causal relationship.

The relationship of the study vaccine to an SAE will be determined by the investigator.

In addition, SAEs will be evaluated by the Sponsor or designee for "expectedness." An unexpected AE is one that is not listed in the current Summary of Product Characteristics or the Investigator's Brochure or an event that is by nature more specific or more severe than a listed event.

In addition, a pre-existing event or condition that results in hospitalization should be recorded on the Medical History CRF. If the onset of an event occurred before the subject entered the study (e.g., any pre-planned hospitalization for conditions like cosmetic treatments or for non-emergency routine visits for a pre-existing condition), the hospitalization would not lead to an AE being classified as serious unless, in the view of the investigator, hospitalization was prolonged as a result of participation in the clinical study or was necessary due to a worsening of the pre-existing condition.

7.1.4.1 Adverse Events of Special Interest

AESIs are not assessed during the study.

7.1.5 Methods for Recording Adverse Events and Serious Adverse Events

Findings regarding Adverse Events must be reported on an Adverse Events CRF, as specified in section 7.1.1, Solicited Adverse Events, and on the VSAE form, if applicable, which is part of the Investigator Site File. All findings in subjects experiencing AEs must be reported also in the subject's source document.

All SAEs which occur during the course of the study, whether considered to be associated with the study vaccination or not, must be reported **within 24 hours of the site becoming aware of the event** to GSK or its designee. Specific instructions and contact details for collecting and reporting SAEs to GSK will be provided to the investigator.

All SAEs are also to be documented on the Adverse Events CRF. Any medication or other therapeutic measures used to treat the AE will be recorded on the appropriate CRF(s) in addition to the outcome of the AE.

After receipt of the initial report, representatives of GSK or its designee will contact the investigator if it is necessary to obtain further information for assessment of the event.

All SAEs must be reported by the investigator to her corresponding EC and applicable regulatory authority in accordance with institutional policy/regulatory requirements and adequate documentation of this notification must be provided to the Sponsor.

GSK or its designee must also comply with the applicable regulatory requirement(s) related to the reporting of suspected unexpected serious adverse vaccine reactions (also known as SUSARs) to the regulatory authority and the EC. If a SUSAR or other safety signal relating to use of one of the study vaccines is reported to GSK or its designee, the Sponsor will communicate the information to the investigator and the investigator will be responsible for submitting this information to the EC and other relevant authorities.

7.1.5.1 Post-Study Events

Any SAE that occurs outside of the protocol-specified follow-up period and considered to be caused by the study vaccine must be reported to GSK or its designee. These SAEs will be processed by GSK or its designee as during the course of the study, until 6 months post LSLV. Instructions and contact details for collecting and reporting these suspected SAEs will be provided to the investigator.

7.1.6 Pregnancies

To ensure subjects' safety, each pregnancy in a subject after study vaccination must be reported to GSK or delegate within 72 hours of the site learning of its occurrence. If the subject agrees to submit this information, the pregnancy must be followed to determine

outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. This follow-up should occur even if intended duration of safety follow-up for the study has ended.

Pregnancy data must be recorded on a Pregnancy Report CRF (initial report) and Pregnancy Follow-Up CRF (outcome report) and reported to GSK or delegate. Instructions and contact details for submitting the Pregnancy CRFs will be provided to the investigator.

Any pregnancy outcome meeting the definition of a SAE (see section 7.1.4, Serious Adverse Events) must also be reported on the VSAE Report Form.

7.1.7 Safety Laboratory Measurements

This study has no safety laboratory measurements.

7.2 Efficacy Assessment

This study has no efficacy measurements.

7.3 Immunogenicity Assessment

For the primary and secondary immunogenicity objectives, the GBS serotype–specific (Ia, Ib, III) IgG antibody responses in the serum will be determined by ELISA.

Original samples from subjects who participated in the V98_06 study who are also participating in this V98_06E1 study may be re-tested for the purposes of consistency in the ELISA assay being used to analyze the samples collected in this extension study.

The GBS serotype-specific IgG ELISAs in sera will be carried out by Clinical Laboratory Sciences, GlaxoSmithKline, Marburg, Germany.

The diphtheria-specific IgG antibody levels will be measured by ELISA. Testing will be conducted by a designated, qualified external laboratory.

Functional activities of anti-GBS antibodies will be measured by opsonophagocytic killing assay (OPK). Testing will be conducted by a designated qualified external laboratory.

Technical details (or instructions) for specimen collection, processing and shipment will be described in Clinical Specimen Lab Manual which will be made available to site

personnel after site initiation (e.g. refer to the Clinical Specimen Lab Manual in the investigator site file).

8. STATISTICAL CONSIDERATIONS

8.1 Endpoints

8.1.1 Primary Endpoint(s)

8.1.1.1 Primary Safety Endpoint(s)

The frequency and percentage of subjects with solicited local and systemic AEs from vaccination to day 7 in study V98_06E1. Time intervals after vaccination that will be summarized are: the first 30 minutes after vaccination, days 1-3 (excluding the first 30 min), days 4-7 and days 1-7 (excluding the first 30 min)..

The frequency and percentage of subjects with any unsolicited AEs from the day of vaccination (day 1 in study V98_06E1) to day 31.

The frequency and percentage of subjects with SAEs, medically attended AEs, and AEs leading to study withdrawal from vaccination in study V98_06E1 to day 181.

8.1.1.2 Primary Efficacy Endpoint(s)

This study has no efficacy endpoints.

8.1.1.3 Primary Immunogenicity Endpoint(s)

The primary immunogenicity endpoints are the percentages of subjects who reach pre-defined sequential serotype-specific (Ia, Ib and III) serum antibody levels at day 61 post vaccination, as measured by ELISA.

8.1.2 Secondary Endpoint(s)

8.1.2.1 Secondary Safety Endpoint(s)

This study has no secondary safety endpoints.

8.1.2.2 Secondary Efficacy Endpoint(s)

This study has no efficacy endpoints.

8.1.2.3 Secondary Immunogenicity Endpoint(s)

The secondary immunogenicity endpoints are:

The percentage of subjects reaching pre-defined sequential serotype-specific (Ia, Ib and III) serum antibody levels as measured by ELISA at other time points and in subgroups of subjects defined by their pre-vaccination serotype-specific GBS antibody LLQ status in the V98_06 study (or the V98_06E1 study for the naive group).

The serotype-specific (Ia, Ib, and III) geometric mean concentrations (GMCs) as measured by ELISA at days 31 and 61 post-vaccination in study V98_06E1 and in the subgroups of subjects defined according to their pre-vaccination serotype-specific GBS antibody LLQ status in the V98_06 study (or V98_06E1 study for the naive group).

The within subject geometric mean ratio (GMR) of serotype-specific (Ia, Ib & III) serum antibody levels as measured by ELISA at day 31 and 61. The GMRs will be determined relative to pre-vaccination in V98_06E1 and separately relative to pre-vaccination in V98_06 for all groups except the naive group. The GMRs will also be determined for subgroups of subjects defined according to their pre-vaccination serotype-specific GBS antibody LLQ status in the V98_06 study or V98_06E1 study for the naive group.

Reverse cumulative distribution function (RCDF) curves of serotype-specific (Ia, Ib and III) serum antibody levels, as measured by ELISA, at pre-vaccination in V98_06 study or V98_06E1 study for the naive group, vaccination, day 31 and day 61 post-vaccination in all subjects and by pre-vaccination serotype-specific GBS antibody LLQ status in the V98_06 study or V98_06E1 study for the naive group.

The anti-diphtheria geometric mean antibody concentrations measured by ELISA for samples collected before the first vaccination with GBS Trivalent Vaccine in the V98_06 study or V98_06E1 study for the naive group and on day 61 post-vaccination in V98_06E1.

8.1.3 Exploratory Endpoint(s)

8.1.3.1 Exploratory Safety Endpoint(s)

This study has no exploratory safety endpoints.

8.1.3.2 Exploratory Efficacy Endpoint(s)

This study has no efficacy endpoints.

8.1.3.3 Exploratory Immunogenicity Endpoint(s)

The exploratory immunogenicity endpoints are:

The serotype-specific (Ia, Ib, and III) geometric mean titer (GMT) as measured by OPK at day 1 pre-vaccination and at days 31 and 61 post-vaccination in study V98_06E1 in study subjects and in subgroups of subjects defined by pre-vaccination ELISA serotype-specific GBS antibody LLQ status in the V98_06 study or V98_06E1 study for the naive group.

The within subject GMR of serotype-specific (Ia, Ib & III) serum titers as measured by OPK at day 31 and 61. The GMRs will be determined relative to pre-vaccination in V98_06E1. The GMRs will also be determined for subgroups of subjects defined according to their pre-vaccination ELISA serotype-specific GBS antibody LLQ status in the V98_06 study, or V98_06E1 study for the naive group.

Exploratory OPK objectives will be addressed in a randomly selected subset of up to 60 subjects (10 subjects per arm).

8.2 Success Criteria

There is no pre-defined success criterion for this study.

8.2.1 Success Criteria for Primary Objective(s)

8.2.1.1 Success Criteria for Primary Safety Objective(s)

There is no pre-defined success criterion for this study.

8.2.1.2 Success Criteria for Primary Efficacy Objective(s)

There is no pre-defined success criterion for this study.

8.2.1.3 Success Criteria for Primary Immunogenicity Objective(s)

There is no pre-defined success criterion for this study.

8.2.2 Success Criteria for Secondary Objective(s)

There is no pre-defined success criterion for this study.

8.2.2.1 Success Criteria for Secondary Safety Objective(s)

There is no pre-defined success criterion for this study.

8.2.2.2 Success Criteria for Secondary Efficacy Objective(s)

There is no pre-defined success criterion for this study.

8.2.2.3 Success Criteria for Secondary Immunogenicity Objective(s)

There is no pre-defined success criterion for this study.

8.3 Analysis Sets

The analysis sets described below are specific to study V98_06E1. Similar analysis sets have already been defined in study V98_06 for subjects receiving a second vaccination in this study.

8.3.1 All Enrolled Set

All screened subjects who provide informed consent and provide demographic and/or baseline screening assessments, regardless of the subject's treatment status in this study.

8.3.2 All Exposed Set

All subjects in the enrolled set who receive a study vaccination in study V98_06E1.

8.3.3 Safety Set

Solicited Safety Set (solicited local and systemic adverse events and other solicited adverse events)

All subjects in the Exposed Set with any solicited adverse event data and/or indicators of solicited adverse events (e.g., use of analgesics/antipyretics) reported in this study.

Unsolicited Safety Set (unsolicited adverse events)

All subjects in the Exposed Set with unsolicited adverse event data reported in this study.

Overall Safety Set

All subjects who are in the Solicited Safety Set and/or Unsolicited Safety Set.

Subjects will be analyzed as "treated" (i.e., according to the vaccine a subject received).

8.3.4 Full Analysis Set (FAS) Immunogenicity Set

Full Analysis Set Immunogenicity

All subjects in the All Enrolled Set who receive at least one study vaccination in study V98_06E1 and provide immunogenicity data at day 1 (pre-vaccination), day 31 or day 61 in V98_06E1.

8.3.5 Per Protocol (PP) Set Immunogenicity Set

All subjects in the FAS Immunogenicity set who:

- Correctly receive the vaccine (i.e., receive the vaccine to which the subject was randomized and at the scheduled time points) in V98_06 (for non-naïve subjects).
- Have no protocol deviations leading to exclusion (see section 8.3.8, Protocol Deviations) as defined prior to analysis.
- Are not excluded due to other reasons defined prior to analysis (see section 8.3.8, Protocol Deviations)

Examples for subjects excluded due to reasons other than protocol deviations are:

- Subjects who withdrew informed consent
- Premature withdrawal due to an adverse event.

8.3.6 Other Analysis Sets

There are no additional analysis sets.

8.3.7 Subgroups

Subgroups of subjects will be created based on the ELISA serotype-specific (Ia, Ib, III) antibody LLQ status prior to initial vaccination in study V98_06 (or V98_06E1 for the naive group).

8.3.8 Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures of a study protocol. A protocol deviation may be a reason to remove data from an analysis set at the time of analysis. CSR-reportable protocol deviations will be defined as exclusionary from the analysis according to protocol objectives and endpoints, which will be specified in the statistical analysis plan. In some cases exclusion of data may be due to a reason other than a protocol deviation, e.g. early termination.

8.4 Statistical Analysis Plan

8.4.1 Analysis of Demographic and Baseline Characteristics

Descriptive statistics (mean, standard deviation, median, minimum and maximum) for age, height and weight at enrolment will be calculated overall and by vaccine group.

Distributions of subjects by sex, race, ethnic origin, V98_06 vaccine formulation group (or naïve) and pre-vaccination LLQ status will be summarized overall and by vaccine group.

8.4.2 Analysis of Primary Objective(s)

8.4.2.1 Analysis of Primary Safety Objective(s)

8.4.2.1.1 Analysis of Extent of Exposure

The frequency and percentage of subjects with vaccinations in V98_06E1 will be summarized by vaccine group and overall for the Enrolled Set.

8.4.2.1.2 Analysis of Solicited Local, Systemic and Other Adverse Events

All solicited adverse events will be summarized according to defined severity grading scales. Use of medication to prevent/treat fever will be summarized according to frequencies and percentages reporting “Yes” and “No”.

Frequencies and percentages of subjects experiencing each adverse event will be presented for each symptom severity. Summary tables showing the occurrence of any local or systemic adverse event overall and at each time point will also be presented.

Post-vaccination solicited adverse events reported from day 1 to day 7 will be summarized for the intervals day 1-3, day 4-7, day 1-7 by maximal severity and by vaccine group, excluding the 30 minute measurement, which will be summarized separately. The severity of solicited local adverse events, including injection-site erythema, swelling and induration will be summarized according to categories based on linear measurement: 0 mm, <25 mm, 25 to 50 mm, 51-100 mm, > 100 mm.

Injection site pain/tenderness and systemic adverse events (except fever) occurring up to day 7 after each vaccination will be summarized according to “mild”, “moderate” or “severe”.

Each solicited local and systemic adverse event will also be further summarized as “none” versus “any”.

Implausible measurements (for further definition see statistical analysis plan) will be left out of the analysis.

Use of antipyretics and analgesics will be summarized by frequency, by type of use (prophylactic versus treatment), and percentage of subjects reporting use.

Body temperature will be summarized by 0.5 °C and 1.0 °C increments from 36.0 °C up to ≥40 °C and will be broken down according by route of measurement.

8.4.2.1.3 Analysis of Unsolicited Adverse Events

This analysis applies to all adverse events occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, recorded in AE CRF, with a start date on or after the date of first vaccination. AE starting prior to the first vaccination will only be listed. The original verbatim terms used by investigators to identify adverse events in the CRFs will be mapped to preferred terms using the MedDRA dictionary. The adverse events will then be grouped by MedDRA preferred terms into frequency tables according to system organ class.

All reported adverse events, as well as adverse events judged by the investigator as at least possibly related to study vaccine, will be summarized according to system organ class and preferred term within system organ class. These summaries will be presented by vaccination group. When an adverse event occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Separate summaries will be produced for the following categories:

- Serious adverse events.
- Adverse events that are possibly or probably related to vaccine.
- Adverse event leading to withdrawal.
- Adverse events leading to a medically attended visit.

Data listings of all adverse events will be provided by subject. In addition, adverse events in the categories above will be provided as listed data.

8.4.2.1.4 Analysis of Safety Laboratory Values

This study has no safety laboratory values.

8.4.2.2 Analysis of Primary Efficacy Objective(s)

This study has no efficacy objectives.

8.4.2.2.1 Statistical Hypotheses

Not applicable

8.4.2.2.2 Analysis Sets

Not applicable.

8.4.2.2.3 Statistical Methods

Not applicable.

8.4.2.3 Analysis of Primary Immunogenicity Objective(s)

The frequency and percentages of subjects with serotype-specific (Ia, Ib, and III) antibody concentrations above a set of pre-defined sequential serotype-specific serum antibody levels and associated two-sided 95% Clopper-Pearson CIs will be computed for each study group, for the MF59 study groups combined, for the unadjuvanted and Alum adjuvanted study groups combined, and the placebo and naive groups combined for concentrations at day 61 post-vaccination.

Differences in percentages between study groups will be computed and two-sided 95% confidence intervals for the difference constructed using the method of Miettinen and Nurminen. The following treatment group comparisons will be examined: each 5 µg group compared to the placebo group from study V98_06 and separately to the naïve group; the combined MF59 groups compared to the combined placebo and naïve groups; and, the combined unadjuvanted and Alum groups compared to the combined placebo and naïve groups.

8.4.2.3.1 Statistical Hypotheses

There are no statistical hypotheses. All analyses are descriptive.

8.4.2.3.2 Analysis Sets

The primary immunogenicity analyses will be based on the per-protocol set (PPS) at day 61. The primary analyses will be repeated using the Full Analysis Set (FAS) as a measure of the sensitivity/robustness of the results (further details are given in section 8.3).

8.4.2.3.3 Statistical Methods

8.4.3 Analysis of Secondary Objective(s)

8.4.3.1 Analysis of Secondary Safety Objective(s)

There are no secondary safety objectives.

8.4.3.1.1 Statistical Methods

8.4.3.2 Analysis of Secondary Efficacy Objective(s)

This study has no efficacy objectives.

8.4.3.3 Analysis of Secondary Immunogenicity Objective(s)

8.4.3.3.1 Statistical Hypotheses

There are no statistical hypotheses. All analyses are descriptive.

8.4.3.3.2 Analysis Sets

The secondary immunogenicity analyses will be based on the per-protocol set (PPS).

8.4.3.3.3 Statistical Methods

GBS

Percentages at Day 31 and RCDFs

The frequency and percentages of subjects with serotype-specific concentrations above a set of pre-defined sequential serotype-specific (Ia, Ib and III) serum antibody levels and associated two-sided 95% Clopper-Pearson CIs will be computed for each study group, for the MF59 study groups combined, for the unadjuvanted and Alum adjuvanted study groups combined, and the placebo and naive groups combined for concentrations at day 31 post-vaccination. Differences in percentages between study groups will computed and two-sided 95% confidence intervals for the difference constructed using the method of Miettinen and Nurminen. Differences in percentages between study groups will be computed and two-sided 95% confidence intervals for the difference constructed using the method of Miettinen and Nurminen. The following treatment group comparisons will be examined: each 5µg group compared to the placebo group from study V98_06 and separately to the naïve group; the combined MF59 groups compared to the combined

placebo and naïve groups; and, the combined unadjuvanted and Alum groups compared to the combined placebo and naïve groups.

Similar analyses will be performed for the subset of subjects defined by the pre vaccination serotype-specific GBS antibody LLQ status in the V98_06 study or V98_06E1 study for the naive group.

The percentages of subjects with serotype-specific concentrations above a set of pre-defined sequential serotype-specific serum antibody levels will be graphically displayed as RCDFs.

Geometric Mean Concentrations

Before any statistical analysis that assumes normally distributed observations, antibody concentrations will be $\log_{10}$-transformed. Individual concentrations below the limit of quantitation (LLQ) will be set to half that limit.

The logarithmically (base 10) transformed antibody concentrations will be modeled using an analysis of covariance (ANCOVA) model with a qualitative factor for vaccine group and log (base 10) pre-vaccination concentration in study V98_06, or V98_06E1 for the naive group, as a covariate. As this is a single-center study, site is not included as a covariate. Response in the V98_06 study was different in those with a pre-vaccination concentration below the LLQ, so pre-vaccination concentration is included. The adjusted GMC and the two-sided, 95%, confidence intervals (CIs) of the GMC will be calculated based on this model. The ratio of GMCs and the corresponding two-sided, 95% CI will also be calculated based on this model. The adjusted GMC and two-sided 95% CIs will be constructed by exponentiation (base 10) of the least square means of the logarithmically transformed (base 10) antibody concentration. The model can be written as:

$$\log_{10}(y_{ik}) = \mu + \alpha_i + \beta x_{ik} + \varepsilon_{ik}$$

where μ represents a common overall mean, α_i represents the vaccine group i effect (where vaccine group is either the V98_06 vaccine group, or naïve), β represents the common slope for the log10 prevaccination concentration, x_{ik} for subject k in vaccine group i, and ε_{ik} represents random error for subject k in vaccine group i. A similar approach will be used in the subgroups based on pre-vaccination LLQ status.

Geometric Mean Ratios

The logarithmically (base 10) transformed within subject ratio of antibody concentrations (post vaccination / baseline) will be modeled using an analysis of variance model with a qualitative factor for vaccine group (vaccine received in study V98_06 or naïve). The

adjusted GMR and the two-sided, 95% CIs of the GMR will be calculated based on this model. The model can be written as:

$\log_{10}(y_{ik}) = \mu + \alpha_i + \varepsilon_{ik}$

where μ represents a common overall mean, α_i represents the vaccine group i effect and ε_{ik} represents random error for subject k in vaccine group i. The baseline value is either the pre-vaccination concentration in study V98_06 or the pre-vaccination concentration in study V98_06E1 as both are to be used in separate analyses. A similar approach will be used in the subgroups based on pre-vaccination LLQ status.

Anti-diphtheria

Geometric Mean Concentrations

The logarithmically (base 10) transformed antibody concentrations will be modeled using an analysis of covariance (ANCOVA) model with a qualitative factor for vaccine group and log (base 10) pre-vaccination concentration in study V98_06, or V98_06E1 for the naive group, as a covariate. The adjusted GMC and the two-sided, 95%, confidence intervals (CIs) of the GMC will be calculated based on this model. The ratio of GMCs and the corresponding two-sided, 95% CI will also be calculated based on this model. The adjusted GMC and two-sided 95% CIs will be constructed by exponentiation (base 10) of the least square means of the logarithmically transformed (base 10) antibody concentration. The model can be written as:

$\log_{10}(y_{ik}) = \mu + \alpha_i + \beta x_{ik} + \varepsilon_{ik}$

where μ represents a common overall mean, α_i represents the vaccine group i effect (where vaccine group is either the V98_06 vaccine group, or naïve), β represents the common slope for the log10 prevaccination concentration, x_{ik} for subject k in vaccine group i, and ε_{ik} represents random error for subject k in vaccine group i.

Geometric Mean Ratios

The logarithmically (base 10) transformed within subject ratio of antibody concentrations (post vaccination / baseline) will be modeled using an analysis of variance model with a qualitative factor for vaccine group (vaccine received in study V98_06 or naïve). The adjusted GMR and the two-sided, 95% CIs of the GMR will be calculated based on this model. The model can be written as:

$\log_{10}(y_{ik}) = \mu + \alpha_i + \varepsilon_{ik}$

where μ represents a common overall mean, α_i represents the vaccine group i effect and ε_{ik} represents random error for subject k in vaccine group i. The baseline value is either

the pre-vaccination concentration in study V98_06 or the pre-vaccination concentration in study V98_06E1 for the naïve subjects.

Handling of missing values

For immunogenicity data, it may be reasonable to consider missing immunogenicity values as missing completely at random (MCAR), i.e., not informative. Therefore, the analysis will comprise a complete case analysis only, without introducing any bias. Imputation methods will not be used.

8.4.4 Analysis of Exploratory Objectives

8.4.4.1 Analysis of Exploratory Safety Objective(s)

This study has no exploratory safety objectives.

8.4.4.2 Analysis of Exploratory Efficacy Objective(s)

This study has no efficacy objectives.

8.4.4.3 Analysis of Exploratory Immunogenicity Objective(s)

Testing and analyses of exploratory endpoints may be performed after the Clinical Study Report (CSR) has been completed, in which case these data will be submitted as an addendum to the CSR.

Geometric Mean OPK Titers

The logarithmically (base 10) transformed antibody titers will be modeled using an analysis of covariance (ANCOVA) model with a qualitative factor for vaccine group and log (base 10) pre-vaccination titer in study V98_06E1 as a covariate. The adjusted GMT and the two-sided, 95%, confidence intervals (CIs) of the GMT will be calculated based on this model. The adjusted GMT and two-sided 95% CIs will be constructed by exponentiation (base 10) of the least square means of the logarithmically transformed (base 10) antibody titer. The ratio of GMTs and the corresponding two-sided, 95% CI will also be calculated based on this model. The model can be written as:

$\log_{10}(y_{ik}) = \mu + \alpha_i + \beta x_{ik} + \varepsilon_{ik}$

where μ represents a common overall mean, α_i represents the vaccine group i effect, β represents the common slope for the log10 pre-vaccination titer, x_{ik} for subject k in vaccine group i, and ε_{ik} represents random error for subject k in vaccine group i.

8.5 Sample Size and Power Considerations

Sample size for this study is not based on power considerations. The sample size is determined by the number of subjects from study V98_06 agreeing to participate in this study, plus 20 subjects not previously participating in V98_06.

8.6 Interim Analysis

There will be no interim analysis included in this study.

9. SOURCE DOCUMENTATION, STUDY MONITORING AND AUDITING

Study monitoring and auditing will be standardized and performed in accordance with the Sponsor's or delegated contract research organization's (CRO) standard operating procedures and applicable regulatory requirements (e.g., FDA, EMA, and ICH guidelines).

Prior to enrolment of the first study subject, GSK or delegate will train investigators and/or their study staff on the study protocol, all applicable study procedures, documentation practices and all electronic systems. CRFs supplied by the Sponsor must be completed for each enrolled subject (see section 8.3.1, All Enrolled Set for definition of enrolled subject). Documentation of screened but not enrolled subjects must be maintained at the site and made available for review by the site monitor. Data and documents will be checked by the Sponsor and/or monitor.

9.1 Source Documentation

Prior to the start of the study, the site staff participating in the study conduct will be instructed on what documents will be required for review as source documents. The kinds of documents that will serve as source documents will be agreed between Sponsor or delegate and investigator and designees and specified in the SDA prior to subject enrolment.

In addition, source documentation **must** include all of the following: subject identification (on each page), eligibility and participation, proper informed consent procedures, medical records to support non child bearing potential if applicable dates of visits, adherence to protocol procedures, adequate reporting and follow-up of adverse events, documentation of prior/concomitant medication/vaccines, study vaccine receipt/dispensing/return records, study vaccine administration information, any data collected by a telephone conversation with the subject and date of completion and reason.

The subject must also allow access to the subject's medical records. Each subject must be informed of this prior to the start of the study and consent for access to medical records may be required in accordance with local regulations.

All safety data reported by subjects must be written down in source documents prior to entry of the data into CRFs. If there are multiple sources of information (e.g., Subject Diary, verbal report of the subject, telephone contact details, medical chart) supporting the diagnosis of an adverse event, these sources must be identified in the source documents, discrepancies between sources clarified, the ultimate diagnosis must be justified and written in the source documents, and this diagnosis must be captured in the Adverse Event CRF (AE CRF).

9.2 Study Monitoring, Auditing and Source Data Verification

Prior to enrolment of the first study subject, GSK or its designee (e.g., a CRO) will develop a Clinical Monitoring Plan to specify how centralized and/or on-site monitoring, including clinical specimens reconciliation, will be performed for the study. Study progress will be monitored by GSK or its designee as frequently as necessary to ensure:

- that the rights and well-being of human subjects are protected,
- the reported study data are accurate, complete, and verifiable from the source documents and
- the conduct of the study is in compliance with the current approved protocol/amendment(s), GCP and applicable regulatory requirements.

Contact details for the GSK team or its designee involved in study monitoring will be provided to the investigator. Study data recorded on CRFs will be verified by checking the CRF entries against source documents in order to ensure data completeness and accuracy as required by study protocol.

Data verification may also be performed through a centralized review of data (e.g., checking for outliers or other anomalies). Additional documents such as the investigator site file, pharmacy records, and informed consent documentation must also be available for review if requested. Arrangements for monitoring visits will be made in advance in accordance with the monitoring plan, except in case of emergency.

The investigator and/or site staff must make source documents of subjects enrolled in this study available for inspection by GSK or its representative at the time of each monitoring visit and Sponsor audits, when applicable. These documents must also be available for inspection, verification and copying, as required by regulations, by officials of the regulatory health authorities (e.g., FDA, EMA and others) and/or ECs. The investigator and study site staff must comply with applicable privacy, data protection and medical confidentiality laws for use and disclosure of information related to the study and enrolled subjects.

10. DATA MANAGEMENT

10.1 Data Entry and Management

In this study, all clinical data (including, but not limited to, AE/SAEs, concomitant medications, medical history, and physical assessments), safety data, and immunogenicity data will be entered onto case report forms (CRFs) in a timely fashion by the investigator and/or the investigator's dedicated site staff. Data entered onto CRFs are stored on a secure website. The data collected on this secure website are assimilated into an electronic data capture (EDC) system, which is compliant with Title 21 Part 11 policies of the Code of Federal Regulations (FDA 1997). The data system includes password protection and internal quality checks. The EDC system will be designed and validated by the Sponsor prior to activation for data entry by sites. The investigator or designated delegate must review data entered and electronically sign the CRFs to verify their accuracy.

Access to the EDC system for data entry or review will require training and distinct individual access code assignments to those site staff members who will be entering study data and those involved in study oversight who may review study data. Data are collected within the EDC system, to which the Sponsor and site monitors have exclusively "read only" access.

10.2 Data Clarification

As part of the conduct of the trial, the Sponsor may have questions about the data entered by the site, referred to as queries. The monitors and the Sponsor are the only parties that can generate a query. All corrections and clarifications will be entered into the EDC system and will be identified by the person entering the information, the reason for the change, as well as the time of the changes made. If changes are made to a previously and electronically signed CRF, the investigator must confirm and endorse the changes.

10.3 Data Protection

GSK respects the subjects' rights to privacy and will ensure the confidentiality of their medical information in accordance with all applicable laws and regulations.

The Sponsor as Data Controller according to the European Directive on the protection of individuals with regard to the processing of personal data and on the free movement of such data (95/46/EC) confirms herewith compliance to Directive 95/46/EC in all stages of Data Management.

11. RECORD RETENTION

Investigators must retain all study records required by GSK and by the applicable regulations in a secure and safe facility. The investigator must consult a GSK representative before disposal of any study records, and must notify the Sponsor of any change in the location, disposition, or custody of the study files. Essential documents must be retained for 15 years. “Essential documents” are defined as documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced. These documents should be retained for a longer period, however, if required by the applicable national regulatory or institutional requirements.

These principles of record retention will also be applied to the storage of laboratory samples, provided that the integrity of the stored sample permits testing.

12. USE OF INFORMATION AND PUBLICATION

GSK assures that the key design elements of this protocol will be posted in a publicly accessible database such as clinicaltrials.gov, and in compliance with current regulations.

GSK also assures that key results of this clinical study will be posted in a publicly accessible database within the required time-frame from the end of study as defined in section 3.9, End of Study.

In accordance with standard editorial, ethical practices and current guidelines of Good Publication Practice (Graf 2009), GSK will generally support publication of multicenter studies only in their entirety and not as individual center data. In this case, a coordinating investigator will be designated by mutual agreement prior to the start of the study. The coordinating investigator will also sign the clinical study report on behalf of the principal investigators (CPMP/EWP/2747/00). Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements. Any formal publication of the study in which contribution of GSK personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate GSK personnel.

GSK must be notified of any intent to publish data collected from the study and prior approval from GSK must be obtained prior to submission for publication.

13. ETHICAL CONSIDERATIONS

13.1 Regulatory and Ethical Compliance

The study will be conducted in compliance with the protocol, GCP and applicable regulatory requirements.

This clinical study was designed and shall be implemented and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations including European Directive 2001/20/EC, US Code of Federal Regulations Title 21, and Japanese Ministry of Health, Labor, and Welfare, GSK codes on protection of human rights, and with the ethical principles laid down in the Declaration of Helsinki (European Council 2001, US Code of Federal Regulations, ICH 1997).

13.2 Informed Consent Procedures

Eligible subjects may only be included in the study after providing written informed consent, as described in section 5.1.1, Informed Consent/Assent. Before the start of the study, the investigator will have the informed consent and any other materials that will be provided to the subjects reviewed and approved by the EC. This review and approval will be documented and stored with other study documents. The investigator or designee must fully inform the subject of all pertinent aspects of the study. A copy of the written informed consent will be given to the subject or the designee. The subject/designee must be allowed ample time to ask about the details of the study and to make a decision as to whether or not to participate in the study. The subject **must** sign the consent form indicating their agreement to participate in the study before any study-related procedures are conducted. The informed consent process may be conducted up to 5 days prior to vaccination on day 1. If the subject is unable to read and write, a witness must be present during the informed consent discussion and at the time of informed consent signature.

Prior to the start of the study, GSK will provide to investigators a proposed informed consent form that complies with the ICH GCP guideline and regulatory requirements and is considered appropriate for this study. Any changes to the proposed consent form suggested by the investigator must be agreed to by GSK before submission to the EC and a copy of the approved version must be provided to the GSK monitor after EC approval.

Women of childbearing potential (and women of non-childbearing potential who do not provide medical records as evidence of the procedures) should be informed that taking the study medication may involve unknown risks to the fetus if pregnancy were to occur during the study and agree that in order to participate in the study they must adhere to the contraception requirements indicated in the protocol for the duration of the study.

13.3 Responsibilities of the Investigator and EC

The protocol and the proposed informed consent form must be reviewed and approved by a properly constituted EC before study start. Properly constituted EC is defined in ICH Guideline for Good Clinical Practice E6 (R1), Section 3 (ICH 1997). A signed and dated statement that the protocol and informed consent have been approved by the EC must be given to GSK before study initiation. Prior to study start and at any time the protocol is amended during study conduct, the investigator is required to sign a protocol signature page confirming her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to GSK monitors, auditors, GSK Clinical Quality Assurance representatives, designated agents of GSK, ECs, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulatory authority, the investigator must inform GSK immediately that this request has been made.

The investigator also responsible for the following:

- Maintaining a list of appropriately qualified persons to whom the investigator has delegated significant study-related duties.
- Demonstrating the capability of recruiting the required number of suitable subjects within the recruitment period.
- Demonstrating sufficient time and staffing to properly conduct and complete the study within the agreed study period.
- Ensuring that all persons assisting with the study are adequately informed about the protocol, the investigational products, and their study-related duties and functions
- Ensuring that appropriately trained health care professionals are responsible for all study-related medical decisions and for ensuring appropriate medical care of subjects experiencing any adverse event related to the study.
- If permission to do so is given by the subject ensuring that the subject's primary healthcare provider is informed of the subject's participation in the study.

The investigator should not implement any deviation from, or changes of the protocol without agreement by the Sponsor and prior review and documented approval/favorable opinion from the EC of an amendment, except where necessary to eliminate an immediate hazard(s) to study subjects, or when the change(s) involves only logistical or administrative aspects of the study (e.g., change in monitor(s), change of telephone number(s)). In addition, the investigator, or person designated by the investigator, should document and explain any deviation from the approved protocol.

The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to study subjects without prior EC approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted:

(a) to the EC for review and approval/favorable opinion,

(b) to the Sponsor for agreement and, if required,

(c) to the regulatory authority(ies).

13.4 Protocol Amendments

An amendment is a written description of change(s) to or formal clarification of a study protocol which may impact on the conduct of the clinical study, potential benefit of the clinical study, or may affect subject safety, including changes of study objectives, study design, subject population, sample sizes, study procedures, or significant administrative aspects. An administrative change of a study protocol is a minor correction or clarification that has no significant impact on the way the clinical study is to be conducted and no effect on subject safety (e.g., change of telephone number(s), logistical changes). Protocol amendments must be approved by GSK, health authorities where required, and the EC. In cases when the amendment is required in order to protect the subject safety, the amendment can be implemented prior to EC approval. Notwithstanding, the need for formal approval of a protocol amendment, the investigator is expected to take any immediate action required for the safety of any subject included in this study, even if this action represents a deviation from the protocol. In such cases, GSK should be notified of this action, the EC at the study site, and, if required by local regulations, the relevant health authority) should be informed within 10 working days.

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CLINICAL STUDY PROTOCOL AMENDMENT

Study Number: V98_06E1

Protocol Title: A Phase 2, Non-Randomized, Controlled, Open-Label, Parallel-Group Extension Study to Evaluate the Immunogenicity and Safety of the Second Dose of GBS Trivalent Vaccine in Healthy Non-Pregnant Subjects.

Amendment Number 1

Revised Protocol version 2 issued on 24 SEP 15

The present amendment reflects changes to the Protocol version 1 issued on 02 JUL 15

DESCRIPTION OF CHANGE(S) AND RATIONALE:

CHANGE 1 *(Page 16, Paragraph 3):*

Previously read: The anti-diphtheria geometric mean antibody concentrations measured by ELISA for samples collected before the first vaccination with GBS Trivalent Vaccine in the V98_06 study or V98_06E1 study for the naive group and on day 31 and day 61 post vaccination in V98_06E1.

Now reads: The anti-diphtheria geometric mean antibody concentrations measured by ELISA for samples collected before the first vaccination with GBS Trivalent Vaccine in the V98_06 study or V98_06E1 study for the naive group and on day 61 post vaccination in V98_06E1.

Rationale for Change: This is being removed due to an inconsistency error. When the body of the protocol was changed prior to finalization, the synopsis was not updated.

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