CLINICAL TRIAL PROTOCOL

A Phase 2, Observer-blind, Randomized, Controlled Study to Evaluate the Safety and Immunogenicity of Two Formulations of Investigational Meningococcal Groups ACYWX Conjugate Vaccine, Administered to Healthy Malian Children 12-16 Months of Age

Protocol number	: ACYWX-02 / CVIA058
Version	: Final Version 2.0
Date	: 11 August 2017
Amendment	: 1
Investigational Product	:Meningococcal Groups ACYWX Conjugate Vaccine (NmCV-5)

Sponsored by:
Serum Institute of India Private Limited (SIIPL)
PATH

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LIST OF ABBREVIATIONS

AE	Adverse event
CDC	Centers for Disease Control and Prevention (US)
CI	Confidence interval
CPPT	1-cyano-4 pyrrolidinopyridiniumtetrafluoroborate
CRF	Case report form
CRM	Cross reactive material
CRO	Contract research organization
CVD	Centre for Vaccine Development, University of Maryland
CVD-Mali	Centre pour le Développement des Vaccins du Mali
DSMB	Data Safety Monitoring Board
EPI	Expanded Program on Immunization
FDA	Food and Drug Administration (US)
FIH	First in human
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
GMTs	Geometric mean titers
ICF	Informed consent form
IM	Intramuscular
IMD	Invasive meningococcal disease
IRB	Institutional review board
hSBA	Human complement serum bactericidal activity
MedDRA	Medical Dictionary for Regulatory Activities
NmCV-5	*Neisseria meningitidis* Groups ACYWX Conjugate Vaccine
PHE	Public Health England
PI	Principal Investigator
PT	Preferred term
q.s.	Quantum satis
rSBA	Rabbit complement serum bactericidal activity
SAE	Serious adverse event
SBA	Serum bactericidal activity
SIIPL	Serum Institute of India Private Limited.
SOC	System organ class
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TT	Tetanus toxoid
WHO	World Health Organization
WHO DD	World Health Organization Drug Dictionary

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PROTOCOL SIGNATURE PAGE

PATH and SIIPL, the study Sponsors, will keep a list of Investigator(s), who must provide a *Curriculum Vitae* (CV) and a copy of their medical licenses to the Sponsors or Sponsor representatives. The Sponsors will keep a list and qualification records of all relevant Sponsor study personnel.

PAREXEL will conduct site monitoring and provide medical monitoring, data management, statistical analysis, quality assurance, safety and pharmacovigilance services.

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"I have read the foregoing protocol and agree to conduct the study as outlined herein."

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STUDY SYNOPSIS

Title of the Study:

A Phase 2, Observer-blind, Randomized, Controlled Study to Evaluate the Safety and Immunogenicity of Two Formulations of Serum Institute of India Private Limited Investigational Meningococcal Groups ACYWX Conjugate Vaccine, Administered to Healthy Malian Children 12-16 Months of Age

Phase of Development: Phase 2

Name of the Study Vaccine:

Meningococcal Groups ACYWX Conjugate Vaccine (NmCV-5; Serum Institute of India Private Limited)

Indication:

Prophylaxis against *Neisseria meningitidis* infection caused by serogroups A,C,Y, W, & X.

Protocol No.: ACYWX-002 / CVIA058

Study Rationale:

Neisseria meningitidis is a leading cause of bacterial meningitis and sepsis worldwide, capable of causing outbreaks and epidemics of invasive disease. Meningococcal disease causes high rates of morbidity and mortality even among patients who receive early antibiotic treatment.

Based on antigenic differences in their capsular polysaccharide, 13 serogroups of *N. meningitidis* have been identified. Virtually all disease-associated isolates are encapsulated, with six serogroups (A, B, C, W, Y and X) being responsible for the large majority of invasive meningococcal infections worldwide.

The best option for the control of meningococcal disease is the use of effective vaccines that would include all six of the most common serogroups responsible for invasive disease.

Several combined meningococcal conjugate vaccines against serogroups A, C, W and Y (Menactra®, Menveo® and Nimenrix®) containing capsular oligosaccharides conjugated to a protein carrier have been developed and successfully licensed. They are immunogenic across all age groups, including young children, confer long lasting protection and are able to prime for immunological memory.

Recently, two meningococcal vaccines against invasive meningococcal disease caused by *Neisseria meningitidis* group B (Bexsero® and Trumenba®) were licensed and recommended in some countries; however, information about their effectiveness against diverse serogroup B strains and the duration of protection are limited.

Currently, there is no licensed vaccine that is protective against *Neisseria meningitidis* group X, which has caused multiple recent outbreaks in Africa and suggests a hyperendemicity and high capacity for localised epidemics.

Following the successful development of a meningococcal group A conjugate vaccine (MenAfriVac®) and its introduction via mass vaccination campaigns in 15 African countries with the highest burden of disease, Serum Institute of India Private Limited (SIIPL) has developed a candidate pentavalent conjugate vaccine composed of *Neisseria meningitidis* groups A, C, Y, W and X capsular polysaccharides (NmCV-5). The individual polysaccharides are conjugated to protein carriers, CRM_{197} or tetanus toxoid, and formulated with or without aluminum phosphate as an adjuvant.

The vaccine is intended for the prevention of invasive meningococcal diseases caused by vaccine serogroups in countries with high incidence of the disease, such as the countries in the African meningitis belt. The target population includes children and adults covering an age group of 9 months and above.

The first-in-human Phase 1 study was initiated among 60 healthy adults in the USA to evaluate safety and immunogenicity of both the adjuvanted and non-adjuvanted formulations of the NmCV-5 with respect to Menactra, a quadrivalent meningococcal conjugate vaccine (n=20 in each group). Following analysis of interim safety data at 28 days after vaccination, no safety issues were identified. The pattern of solicited reactions and adverse events among adjuvanted and non-adjuvanted NmCV-5 recipients were similar to the control group receiving Menactra. Immunogenicity responses in the first 20 subjects receiving NmCV-5 were also found promising with rising titers for all five serogroups in both adjuvanted and non-adjuvanted formulations (n=10 in each group). This satisfactory safety and immunogenicity profile paves the way to the Phase 2 study in Malian toddlers.

This study is designed to evaluate safety and immunogenicity of the non-adjuvanted and adjuvanted formulations of the investigational NmCV-5 vaccine in healthy children 12-16 months of age, in comparison with the licensed quadrivalent meningococcal conjugate vaccine (MenACWY-D; Menactra®). Menactra has been selected as an active control given the large safety database accumulated since the vaccine was introduced in the US in 2005, prequalified by WHO and progressively introduced in other countries. Both vaccines will be administered according to a 0, 3 month schedule to meningococcal vaccine-naïve healthy subjects.

The ability of NmCV-5 to elicit functional bactericidal antibodies against each serogroup will be measured with the serum bactericidal activity assay using rabbit complement as the exogenous complement source (rSBA).

Additional assays to further characterize the immune response to NmCV-5 or MenACWY-D vaccines (e.g. serum bactericidal activity assay with human complement [hSBA]) may be performed.

Study Hypothesis:

The non-adjuvanted and adjuvanted formulations of the investigational NmCV-5 vaccine are safe and well tolerated and induce robust immune responses against all of the five vaccine

serogroups at 1 month after a 2-dose vaccination series in healthy children 12-16 months of age.

Primary Objective:

1. To assess the reactogenicity of non-adjuvanted and adjuvanted formulations of NmCV-5 vaccine in comparison with the licensed MenACWY-D vaccine, as measured by the percentage of subjects with at least one severe solicited AE reported within 7 days after any study vaccination.

Secondary Immunogenicity Objectives:

1. To assess immunogenicity of non-adjuvanted formulation of NmCV-5 vaccine in comparison with MenACWY-D vaccine, as measured by rSBA against serogroups A, C, W, Y and X at 1 month after the second vaccination.
2. To assess immunogenicity of adjuvanted formulation of NmCV-5 vaccine in comparison with non-adjuvanted formulation of NmCV-5 vaccine, as measured by rSBA against serogroups A, C, W, Y and X, at 1 month after the second vaccination.
3. To assess immune response elicited by non-adjuvanted and adjuvanted formulations of NmCV-5 vaccine and MenACWY-D vaccine at 1 and 3 months after the first vaccination.

Secondary Safety Objective:

1. To evaluate the safety and reactogenicity of adjuvanted and non-adjuvanted formulations of NmCV-5 vaccine in healthy children, when compared to MenACWY-D.

Exploratory objective:

1. To assess immunogenicity of adjuvanted and non-adjuvanted formulations of NmCV-5 vaccine and MenACWY-D vaccine, as measured by hSBA against serogroups A, C, W, Y and X at baseline, 1 month after the first vaccination and 1 month after the second vaccination (in a subset of subjects)
2. To further assess immunogenicity of adjuvanted and non-adjuvanted formulations of NmCV-5 vaccine and MenACWY-D vaccine, as measured by rSBA against serogroups A, C, W, Y, and X at baseline, 1 month and 3 months after the first vaccination and 1 month after the second vaccination.

Study Design and Methodology:

Design: Phase 2, randomized (2:2:1), controlled, observer-blind, single-center study in healthy children 12 to 16 months of age with three study groups.

Duration of the study: The study duration is approximately 6 months for each subject.

Vaccination schedule: 0, 3 months.

Study groups:

- NmCV-5_non-adjuvanted group: approximately 150 subjects receiving the non-adjuvanted formulation of NmCV-5 vaccine at Visit Day 0 and Visit Day 84.
- NmCV-5_adjuvanted group: approximately 150 subjects receiving the adjuvanted formulation of NmCV-5 vaccine at Visit Day 0 and Visit Day 84.

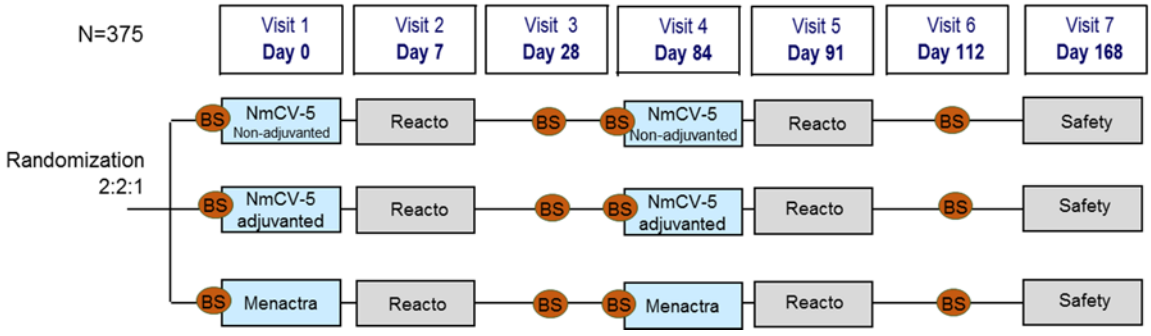
- ACWY-D group: approximately 75 subjects receiving the licensed MenACWY-D (Menactra®) vaccine at Visit Day 0 and Visit Day 84.

Randomization: At Visit Day 0, prior to the study vaccination, subjects will be randomized into the three study groups according to a 2:2:1 ratio.

Blinding: Observer-blind study.

Data collection: Electronic Case Reporting Form (eCRF).

Table A: Study design



Note: BS – blood sample; ACWY-D - Menactra®.

Blood sample schedule: Four blood samples (approximately 5 mL each) at Visit 1 (Day 0, first vaccination), Visit 3 (Day 28), Visit 4 (Day 84, second vaccination) and Visit 6 (28 days after second vaccination).

Study visits: Seven visits at Day 0, Day 7, Day 28, Day 84, Day 91 (7 days post Dose 2), Day 112 (28 days post Dose 2) and Day 168 (84 days post Dose 2).

Solicited AEs: occurring during 7 days following each study vaccination will be assessed daily by site staff and recorded in the home visit worksheets and the CRF at Visit 2 and Visit 5.

Unsolicited AEs: occurring within 28 days after each study vaccination will be collected weekly by site staff during home and site visits.

AEs leading to study withdrawal and SAEs: will be collected during the entire study period. These data will be captured through the home visit worksheets, by interviewing the subjects' parents / guardians during the home visits, the planned and unplanned site visits, and by review of available medical records.

Written agreement to delay the EPI MenAfriVac dose scheduled at 9 months of age: Prior to written informed consent, subject's parents/guardians may be asked to sign a pre-screening agreement as early as 9 months of age in order to delay the EPI MenAfriVac dose until they can be considered for enrolment in the trial (trial subjects must be at least 12 months of age, have not received any meningococcal vaccines and have not received any vaccinations in the past 28 days).

Written informed consent will be obtained from the subject's parents / guardians before conducting any study-specific procedures.

After signing informed consent, a review of medical history and prior medications/vaccination, a physical examination, and confirmation of subject eligibility, subjects will be enrolled into the study.

Number of Subjects:

A total of approximately 375 subjects are planned for enrolment into this study. This would yield 150 subjects for each of the NmCV-5 groups (non-adjuvanted and adjuvanted) and 75 subjects for the MenACWY-D group. Assuming a 10% drop-out rate, this should provide approximately 135 or 67 evaluable subjects per respective study group.

Study Population and Subject Characteristics: Subjects will be included in the study if they meet all specified eligibility criteria.

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

1. Male and female children between 12 months and 16 months old inclusive (minimum 365 days of age and maximum 16 months plus 29 days of age);
2. For whom parent(s)/legal guardian(s) have given written informed consent after the nature of the study has been explained according to local regulatory requirements;
3. Who the investigator believes that their parent(s)/ guardian(s) will be available for all the subject visits and would comply with the requirements of the protocol (e.g., timely reporting of adverse events, availability for study site visits and home visits);
4. Individuals in good health as determined by the outcome of medical history, physical examination and clinical judgment of the investigator.
5. Individuals who completed their local infant EPI schedule through 9 months of age (except MenAfriVac dose). A birth dose of OPV is not required.

Exclusion Criteria: In order to participate in this study, all subjects must meet NONE of the exclusion criteria described.

1. History of any meningococcal vaccine administration.
2. Current or previous, confirmed or suspected disease caused by *N. meningitidis*.
3. Household contact with and/or intimate exposure to an individual with any laboratory confirmed *N. meningitidis* infection within 60 days of enrolment.
4. History of severe allergic reactions after previous vaccinations or hypersensitivity to any study vaccine component including tetanus, diphtheria and diphtheria toxoid (CRM_{197}).
5. Acute or chronic, clinically significant pulmonary, cardiovascular, metabolic, neurological, hepatic, or renal functional abnormality, as determined by medical history or physical examination.
6. Any confirmed or suspected condition with impaired/altered function of immune system (immunodeficient or autoimmune conditions).

7. Have any bleeding disorder which is considered as a contraindication to intramuscular injection or blood draw.
8. Severe acute malnutrition.
9. A family history of congenital or hereditary immunodeficiency.
10. History of either hepatitis B or hepatitis C virus infection or human immunodeficiency virus infection.
11. Major congenital defects.
12. Chronic administration (defined as more than 14 days) of immunosuppressants or other immune-modifying drugs within three months prior to the study vaccination or planned use throughout the study period. (For corticosteroids, this means prednisone, or equivalent, ≥ 0.5 mg/kg per day. Inhaled, intranasal and topical steroids are allowed).
13. Administration of blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation in the past 3 months or planned use throughout the study period.
14. Administration of any vaccine within 28 days prior to enrolment in the study or planned administration of any vaccine within 14 days before or after any study vaccination.
15. Use of any investigational or non-registered drug or vaccine within 30 days prior to the administration of study vaccines or planned during the study.
16. Malaria infection as confirmed by a Rapid Diagnostic Test.

    Note: Subjects positive at screening may be treated for malaria as per national guidelines, and if subject remains eligible, vaccinated no earlier than five days after completing treatment.
17. Individuals who are close family members of individuals conducting this study.
18. Have experienced a moderate or severe acute infection and/or fever (defined as temperature ≥ 37.5°C) within 3 days prior to enrolment.
19. Have received systemic antibiotic treatment within 3 days prior to enrolment.
20. Non-residence in the study area or intent to move out within six months.
21. Any condition which, in the opinion of the investigator, might pose additional risk to the subject due to participation in the study.

Study Vaccines and Control Vaccine Composition:

The NmCV-5 investigational vaccine is a fixed-combination available as the lyophilized powder containing meningococcal groups A and X polysaccharides conjugated to tetanus toxoid and meningococcal groups C, W and Y polysaccharides conjugated to CRM_{197} protein. The vaccine will be reconstituted with either saline alone (non-adjuvanted formulation) or saline containing aluminum phosphate at 125 mcg Al^{3+} /dose (adjuvanted formulation) just prior to administration. After reconstitution, the NmCV-5 vaccine formulations to be used will have the following composition per 0.5 mL of injectable solution

Table B: Composition of reconstituted non-adjuvanted and adjuvanted formulations of NmCV-5 vaccine (per 0.5 mL dose)

Vaccine component	Non-adjuvanted NmCV-5 (per 0.5 mL dose)	Adjuvanted NmCV-5 (per 0.5 mL dose)
Meningococcal A polysaccharide[1]	5 µg	5 µg
Meningococcal C polysaccharide[2]	5 µg	5 µg
Meningococcal Y polysaccharide[2]	5 µg	5 µg
Meningococcal W polysaccharide[2]	5 µg	5 µg
Meningococcal X polysaccharide[1]	5 µg	5 µg
Sucrose	2.42 mg	2.42 mg
Sodium Citrate	0.40 mg	0.40 mg
TRIS (Trometamol)	0.098 mg	0.098 mg
Aluminum phosphate Al^{3+}		125 µg /dose
0.9% Sodium Chloride	q. s.	q. s.
Tetanus Toxoid	7.8 to 33.4 µg	7.8 to 33.4 µg.
CRM_{197}	11.7 to 50.1 µg	11.7 to 50.1 µg

Note: [1] each polysaccharide conjugated to Tetanus Toxoid; [2] each polysaccharide conjugated to CRM_{197}; q.s. -*Quantum satis*

The licensed MenACWY-D polysaccharide conjugate vaccine (Menactra®, manufactured by Sanofi Pasteur Inc.) is supplied as a single 0.5 mL dose formulated in sodium phosphate buffered isotonic sodium chloride solution to contain 4 µg each of meningococcal A, C, Y, and W polysaccharides conjugated to approximately 48 µg of diphtheria toxoid protein carrier (Table C). No preservative or adjuvant is added during manufacture. Each 0.5 mL dose may contain residual amounts of formaldehyde of less than 2.66 µg.

Table C: Composition of Menactra® (per 0.5 mL dose)

Vaccine component	Per Dose of 0.5 mL
Meningococcal group A polysaccharide	4 µg
Meningococcal group C polysaccharide	4 µg
Meningococcal group Y polysaccharide	4 µg
Meningococcal group W polysaccharide	4 µg
Diphtheria toxoid protein total	48 µg
Sodium phosphate	0.7 mg
Sodium chloride	4.35 mg

Endpoints:

Primary reactogenicity endpoint:

- Percentage of subjects with at least one severe solicited AE* within 7 days after any study vaccination (Days 0-6 and Days 84-90)

**Solicited AEs include tenderness, swelling/induration, irritability, drowsiness, decrease eating, vomiting, and fever.*

Secondary immunogenicity endpoints:

- Percentage of subjects with rSBA titer ≥ 8 against serogroups A, C, W, Y and X at Visits Day 0, Day 28, Day 84 and Day 112.
- Percentage of subjects with rSBA titer ≥ 128 against serogroups A, C, W, Y and X at Visits Day 0, Day 28, Day 84 and Day 112.
- Percentage of subjects with fourfold rise in rSBA titers against serogroups A, C, W, Y and X at Visits Day 28 and Day 112.
    - For subjects with a pre-vaccination rSBA titer < 8, a post-vaccination titer of ≥ 32;
    - For subjects with a pre-vaccination rSBA titer ≥ 8, an increase in rSBA titer of at least 4 times the pre-vaccination titer.
- rSBA GMT for serogroups A, C, W, Y and X at Visits Day 0, Day 28, Day 84 and Day 112.

Secondary safety endpoints:

- Solicited local and systemic AEs reported during the 7 days after each vaccination (Days 0-6 and Days 84-90);
- Unsolicited AEs reported during 28 days after each vaccination (Days 0-27 and Days 84-111);
- AEs leading to premature withdrawal during the entire study period;
- SAEs reported during the entire study period.

Exploratory immunogenicity endpoints:

- Percentage of subjects with hSBA titer ≥ 8 against serogroups A, C, W, Y and X at Visits Day 0, Day 28 and Day 112 (in a subset of subjects).
- Percentage of subjects with hSBA seroresponse against serogroups A, C, W, Y and X at Visits Day 28 and Day 112 (in a subset of subjects), defined as:
    - for subjects with baseline hSBA titer < 4, post vaccination hSBA titer ≥ 8;
    - for subjects with baseline hSBA titer ≥ 4, an increase of at least four times the pre-vaccination hSBA.
- hSBA GMTs for serogroups A, C, W, Y and X at Visits Day 0, Day 28 and Day 112 (in a subset of subjects).

- Percentage of subjects with rSBA titer ≥4, ≥16, ≥ 32, and ≥ 64 against serogroups A, C, W, Y and X at Visits Day 0, Day 28, Day 84 and Day 112
- Percentage of subjects with a fourfold increase in rSBA titers against serogroups A, C, W, Y and X at Visits Day 28 and Day 112 with the following criteria:
    - For subjects with a pre-vaccination rSBA titer < 4, a post-vaccination titer of ≥ 16;
    - For subjects with a pre-vaccination rSBA titer ≥ 4, a post-vaccination titer of at least 4 times the pre-vaccination titer.
- Among subjects with a pre-vaccination rSBA titer ≥4, ≥8, ≥16, ≥ 32, ≥ 64, and ≥ 128, percentage of subjects with a post-vaccination titer of at least 4 times the pre-vaccination titer at Visits Day 28 and Day 112

Statistical Consideration:

With 150 enrolled subjects per NmCV-5 arm and 75 subjects in the MenACWY-D arm, the study has a power of 80% to detect a 15.4% difference in proportion of subjects with severe solicited AEs reported within 7 days after any study vaccination between each NmCV-5 group and the MenACWY-D group, assuming a rate of severe reactions in the MenACWY-D group of 5%, a drop-out rate of 10%, and alpha of 0.05.

Power to show comparability of immune response in terms of the percentage of subjects with antibody titer greater than or equal to the cut-off level, based on various group sizes and equal underlying expected underlying percentages, was done using one-sided Miettinen and Nurminen test [21] and is provided in Table D.

Assuming the expected proportion of subjects with antibody response greater than or equal to the cut-off (e.g. rSBA titer ≥ 8) is at least 95% for each of the vaccine serogroups in the NmCV-5 non-adjuvanted and MenACWY-D groups, then with 135 / 67 evaluable subjects per group, the study has power of 86% to demonstrate that the lower limit (LL) of 95% confidence interval (CI) for difference between the non-adjuvanted NmCV-5 group and MenACWY-D group is above -10% (commonly accepted non-inferiority margin) for each serogroup. With the same assumptions, the overall power to demonstrate that the lower limit (LL) of 95% confidence interval (CI) for difference between the non-adjuvanted NmCV-5 group and MenACWY-D group is above -10% for all serogroups would be at 46%.

Table D: Power to show comparability of immune response in terms of the percentage of subjects with antibody titer greater than or equal to the cut-off level, based on various group sizes and equal true underlying percentages

Expected percentage of subjects who achieve titers ≥ cut-off	Number of evaluable subjects per group	Power to demonstrate that the LL of 95% CI of the difference between groups is > -10% for an individual serogroup	Overall Power to demonstrate that the LL of 95% CI of the difference between groups is > -10% for all serogroups
85%	120 vs. 60	47%	2%
	135 vs. 67	52%	4%

	150 vs. 75	56%	5%
90%	120 vs. 60	61%	8%
	135 vs. 67	65%	12%
	150 vs. 75	70%	17%
95%	120 vs. 60	81%	36%
	135 vs. 67	**86%**	**46%**
	150 vs. 75	89%	56%

Power to show that the ratio of rSBA GMT in the adjuvanted NmCV-5 group to that in the non-adjuvanted NmCV-5 group is at least 2 was calculated using a two-sample t-test according to different expected rSBA GMTs in the non-adjuvanted NmCV-5 group and assumed standard deviations (SD) of log2-transferred rSBA titers and is provided in Table E.

Table E: Power to detect at least a 2-fold increase of rSBA GMTs after a 2-dose series with the adjuvanted NmCV-5 vs. non-adjuvanted NmCV-5, based on various group sizes and different assumed SD of log 2 rSBA titers

Expected rSBA GMTs in non-adjuvanted NmCV-5 group at 1 month post-Dose 2	Assumed SD of log2 rSBA titers	Number of evaluable subjects per group	Power to detect at least a 2-fold rSBA GMTs in adjuvanted NmCV-5 vs non-adjuvanted NmCV-5 for an individual serogroup	Overall power to detect at least a 2-fold rSBA GMTs in adjuvanted NmCV-5 vs non-adjuvanted NmCV-5 for all serogroups
500 to 1500 for various serogroups	1.5	150 vs. 150	>99%	> 99%
		135 vs. 135	>99%	> 99%
		120 vs. 120	>99%	> 99%
	2.0	150 vs. 150	99%	95%
		135 vs. 135	98%	92%
		120 vs. 120	97%	86%
	2.5	150 vs. 150	93%	70%
		135 vs. 135	**91%**	**61%**
		120 vs. 120	87%	50%
	3.0	150 vs. 150	82%	37%
		135 vs. 135	78%	29%
		120 vs. 120	73%	21%
	3.5	150 vs. 150	69%	16%
		135 vs. 135	65%	11%
		120 vs. 120	60%	8%

Assuming the true standard deviation of log2 rSBA titers is below than or equal to 2.5 for each of the five vaccine serogroups, with 135 evaluable subjects per group, the study has a power of 91% to detect a 2-fold rSBA GMT increase in the adjuvanted NmCV-5 vs non-adjuvanted NmCV-5 for each serogroup. With the same assumptions, the overall power to detect a 2-fold rSBA GMT increase in the adjuvanted NmCV-5 vs non-adjuvanted NmCV-5 for all serogroups would be at least 61%.

Due to the hypothesis generating nature of this study, all statistical comparisons for reactogenicity, safety, and immunogenicity endpoints will be carried out without an

adjustment for multiple comparisons. All statistical tests except for non-inferiority will be two-sided with a type I error rate of 5%. The 95% confidence interval will be presented for estimations, as appropriate.

Analyses Sequence:

- An interim analysis including safety, reactogenicity and immunogenicity results (rSBA) at 1 month after the first vaccination will be performed. Individual listings will not be generated at this point and any access to subject-level information about study groups will be masked.
- The final safety, reactogenicity and immunogenicity analysis (rSBA) will be performed at 3 months after the second vaccination.

Data and Safety Monitoring Board:
An independent Data and Safety Monitoring Board (DSMB) will be set up for periodic review of safety data during the study conduct. The composition and responsibilities of DSMB members are presented in the DSMB charter.

1 INTRODUCTION& BACKGROUND INFORMATION

1.1 Pathogen

Among several different bacteria that can cause meningitis, *Neisseria meningitidis* (a gram-negative aerobic capsular bacterium, also referred as meningococcus), is known to cause large epidemics. *Neisseria meningitidis* otherwise a harmless commensal of human nasopharynx, under appropriate conditions is responsible for invasive meningococcal disease (IMD)–a spectrum of diseases that includes most commonly meningitis and fulminant septicaemia. Pneumonia, myocarditis, or pericarditis may also occur in IMD, albeit less commonly.[1, 2]

Meningococci are classified into serogroups based on their capsular polysaccharides. Six of these serogroups viz. A, B, C, W, X, and Y are known to cause the majority of IMD cases.[1]

These serogroups have the potential to cause large epidemics but this potential varies in each serogroup with respect to time and geographic location.[3] Serogroup A for example has been the most important cause of meningitis in sub-Saharan Africa and its outbreaks are commonest during December to June. On the other hand, most IMD cases in Europe and USA are caused by serogroup B, followed by C and Y (Figure 2). Most recently, cases of serogroup X are on the rise, especially in Africa.[1, 4]

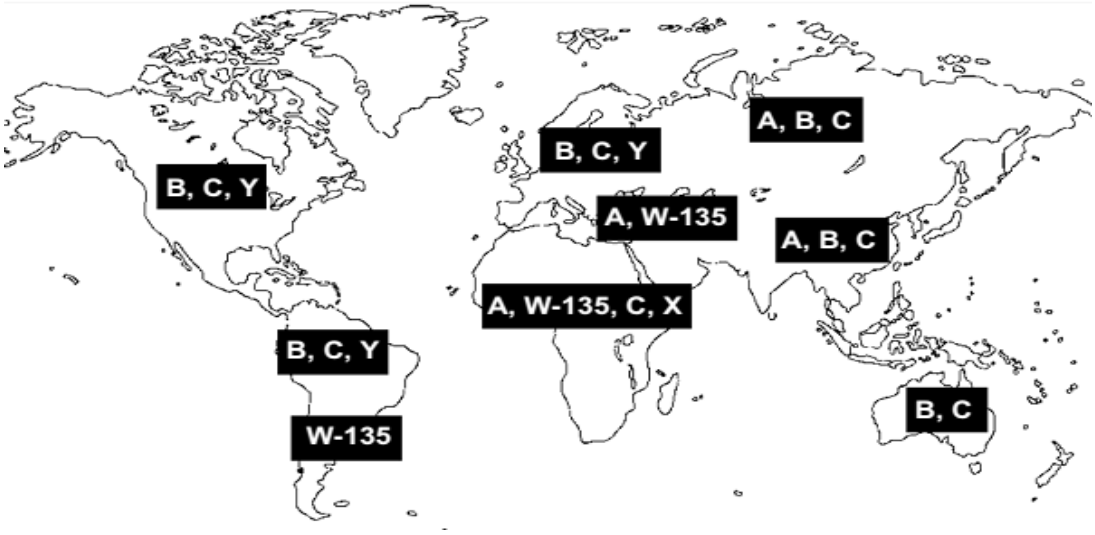


Figure 1: Global serogroup distribution of invasive meningococcal disease.[1]

Apart from serogroup variability, IMD also shows variation with respect to age groups, with peak incidence occuring among children less than 2 years of age and adolescents between 16 and 21 years. During epidemics, disease rates show a shift towards older age groups.[1]

1.2 Burden of Disease

Meningococcal disease commonly alternates between an endemic situation of few isolated cases and fatal unpredictable epidemics in the African meningitis belt (a large area that spans sub-Saharan Africa from Senegal in the west to Ethiopia in the east).[5] This region

has the highest annual incidence of IMD and is plagued with problem of recuring epidemics of large proportions. The incidence has been known to reach rates of up to 1000 cases per 100,000 people or 1% of the entire population.[3] This was seen during the large epidemics of 1996 and 2000 through 2001. In 1996 and 1997 there were > 250,000 cases, an estimated 25,000 deaths, and disability in 50,000 people. It is believed to be the largest epidemic in history. Similarly in the 2006 to 2007 epidemic season, there were 53,438 suspected cases and 3,816 deaths were reported to WHO from 15 African countries.[4]

Outside of the African meningitis belt, the incidence of IMD is significantly low, although there have been reports of outbreaks. In such areas any substantial increase in IMD cases above that which is expected at that place and time is considered as outbreak.[3, 4]

Respiratory droplets are the main source of meningococcal transmission and occurs most commonly due to close contact with an asymptomatic carrier.[6] Symptoms of IMD mainly include fever, loss of appetite, lethargy, vomiting, diarrhoea, photophobia and convulsions, and usually manifest 1 to 14 days after acquiring the pathogen.[2]

Meningococcal meningitis and fulminant septicemia—the two most serious presentations of IMD—may prove fatal in 50% of cases if untreated within 24 to 48 hours. Even when adequately treated, the mortality is close to 10%. Among the survivors of meningitis, it may cause in 10 to 20% of cases, severe permanent brain damage and sequelae such as mental retardation, deafness, epilepsy, or other neurological disorders.[1, 4]

1.3 Meningococcal vaccines

The majority of the meningococcal epidemics in the African meningitis belt over the years have been caused by serogroup A with incidence rates as high as 500 cases per 100,000 population. In an effort to overcome this problem in a cost-effective way, the Meningitis Vaccine Project (MVP), a partnership between the WHO and PATH, was established with funding from the Bill and Melinda Gates Foundation.[7] The resultant product was a monovalent vaccine against serogroup A, MenAfriVac®, manufactured by the Serum Institute of India Private Limited and developed specifically to tackle the meningitis A (MenA) epidemics in the African meningtis belt.[8]

MenAfriVac® is a lyophilized vaccine containing purified meningococcal A polysaccharide conjugated to tetanus toxoid.[1] The vaccine underwent an elaborate clinical development process that included a Phase 1 study in India, followed by seven Phase 2 and 3 studies conducted in India and the African meningitis belt.[9, 10]

The vaccine was subsequently licensed in 2009 and was prequlaified by WHO in 2010. Since then it has been widely used in over 294 million individuals of 1 to 29 years of age and has been found to be highly effective and safe.[3, 8, 11-14]

Use of MenAfriVac® has been highly effective at preventing IMD of serogroup A, reducing it by nearly 100%, eliminating outbreaks as well as decreasing carrier states.[11, 15] It also reduced the incidence in those too young or too old to have been vaccinated, demonstrating an additional indirect herd effect. In the data generated for two years since its use started in three African countries viz. Burkina Faso, Mali and Niger, there have been no cases of MenA disease reported in the vaccinated population.[8] As MenAfriVac has continued to be rolled out across the meningitis belt until 2017, Men A cases have continued to remain

extremely low with a handful of breakthrough cases reported in recent years (2015-2017).[16, 17]

The best option for the control of meningococcal disease globally is the use of effective vaccines that would include all six of the most common serogroups responsible for invasive disease.

Several combined meningococcal conjugate vaccines against serogroups A, C, W and Y (Menactra®, Menveo® and Nimenrix®) containing capsular oligosaccharides conjugated to a protein carrier have been developed and successfully licensed.[18-20] They are immunogenic across all age groups, including young children, confer long lasting protection and are able to prime for immunological memory.

Recently, two meningococcal vaccines against invasive meningococcal disease caused by *Neisseria meningitidis* group B (Bexsero® and Trumenba®) were licensed and recommended in some countries; however, information about their effectiveness against diverse serogroup B strains and the duration of protection are limited.

The widespread use of conjugate meningococcal vaccines, especially in higly endemic settings and at a population level has reduced the burden of IMD drastically since the pre-vaccination era. In spite of this, the use of conjugate vaccines is still a big hurdle in the developing world due to its high cost. Unlike the Men A vaccine, which was specifically developed for the meningitis belt in Sub-Saharan Africa and hence was very cost effective, the tetravalent vaccine still remains elusive for that kind of application in this region.[21, 22]

1.4 Study vaccine

Currently, there is no vaccine available against serogroup X of *N. meningitidis*. This bacterium has caused many outbreaks in Africa and Europe in recent past. The serogroup X has been responsible for outbreaks between 2006 and 2010 in Kenya, Niger, Togo, Uganda, and Burkina Faso, the latter with at least 1,300 cases of serogroup X meningitis among the 6,732 reported annual cases.[23]

As a result, SIIPL has developed the candidate vaccine NmCV-5, which is a polyvalent conjugate vaccine composed of serogroups A, C, Y, W, and X of *Neisseria meningitidis* capsular polysaccharides, conjugated to protein carriers, CRM and tetanus toxoid, with aluminum phosphate as an adjuvant. It is intended for the prevention of meningitis and/or septicemia caused by serogroups A, C, Y, W, and X *N. meningitidis* in countries where the disease is endemic and causes large epidemics such as the countries in the African meningitis belt. The target population consists of infants and children and adults aged 9 months and above.

1.4.1 Summary of Nonclinical Studies

The summary of completed non-clinical studies is presented in Table 1.4.1-1. The detailed description of the toxicology and immunogenicity results are included in the latest version of the Investigator Brochure.

Table 1.4.1-1: Summary of non-clinical studies

Study Title	Study Site	Study Type & Compliance	Animal Model
A 5-week subcutaneous dose-ranging immunogenicity study of monovalent meningococcal serogroup X polysaccharide conjugate (MenX-TT) with and without aluminum phosphate adjuvant in mice	SIIPL	Immunogenicity Non-GLP	Swiss albino mice
A 7-week subcutaneous immunogenicity study of the MenX-TT component in the NmCV-5 presentation with and without aluminum phosphate adjuvant in mice.	National Institute for Biological Standards and Control, London (NIBSC), United Kingdom	Immunogenicity Non-GLP	BALB/c mice
A 5-week intramuscular immunogenicity study of candidate SIIPL NmCV-5 vaccine formulations in comparison to commercial vaccine in rabbits	SIIPL	Immunogenicity Non-GLP	New Zealand white rabbits
A 5-week intramuscular dose-ranging immunogenicity study of pentavalent SIIPL NmCV-5 vaccine (tox formulation) with and without aluminum phosphate adjuvant in rabbits	SIIPL	Immunogenicity Non-GLP	New Zealand white rabbits
A 7-week intramuscular toxicity study of [pentavalent] meningococcal (A,C,Y,W,X) polysaccharide conjugate vaccine (freeze-dried) in New Zealand white rabbits with a 6-week recovery	MPI Research, Inc, 54943 North Main Street Mattawan, MI49071-8353, U.S.A.	Toxicity & immunogenicity study. GLP	New Zealand white rabbits
Series of toxicology studies of 4-Pyrrolidinopyridine (4-PP, a byproduct of CPPT conjugation chemistry)	MPI Research, Inc, 54943 North Main Street Mattawan, MI49071-8353, U.S.A. and BioReliance, Rockville, MD	Byproduct toxicology. Mutagenicity GLP	Rats, guinea pigs, prokaryotic & eukaryotic cells

There are no toxicological or other findings indicating any significant safety issues for the NmCV-5 vaccine formulations. The completed studies showed that the NmCV-5 pentavalent vaccine formulation produced a strong immune response against all five serogroups. The addition of adjuvant resulted in an increase in immune response for all five serogroups with a statistically significant increase for three serogroups.

The toxicology data with NmCV-5 formulations, and the nonclinical experience with the component vaccines, support the clinical testing of the proposed formulations.

1.4.2 Clinical Studies

A first-in-human (FIH) Phase 1 clinical study was designed and initiated in healthy US adults to evaluate the safety and immunogenicity of the study vaccine. Two formulations

of NmCV-5 vaccine (adjuvanted and non-adjuvanted) were tested in comparison with the licensed quadrivalent serogroups A, C, W and Y vaccine (Menactra®) in 60 volunteers aged 18 to 45 years.

The subjects were followed up for 6 months post vaccination which involved visits to study site at day 7, 28 & 84 and a phone call at six months. All 60 subjects had completed their day 28 follow up. An interim analysis with Day 28 safety data of the first 58 subjects and immunogenicity data of the first 30 subjects was performed.

All study vaccines were well-tolerated, and the rate of solicited local as well systemic reactions reported till day 7 post vaccination with NmCV-5 was comparable to Menactra. There were no NmCV-5 vaccine related unsolicited adverse events (AEs) during 28 day follow up. No serious AEs were reported during the study. Overall, no safety concerns around the use of NmCV-5 were raised from this study.

Immunogenicity was analysed via serum bactericidal activity assay using baby rabbit complement. The results for each 10 subjects receiving adjuvanted and non adjuvanted formulation of NmCV-5 showed that both the vaccines are immunogenic. The GMTs were comparable to those seen among subjects receiving Menactra.

More details of interim results can be found in the investigator's brochure.

There is no clinical data of NmCV-5 available in children 1-2 years of age, although it is anticipated that the vaccine will have a safety profile similar to that of currently licensed vaccines against meningococci including Menactra® (reference vaccine in present study) and MenAfriVac®.

Apart from local solicited reactions such as tenderness, erythema and swelling, other common (≥10%) solicited systemic reactions in children 9 to 12 months of age who received Menactra® included irritability, abnormal crying, drowsiness, appetite loss, vomiting, and fever.[18, 24] Solicited reactions were considered those occurring within seven days of vaccination.

1.5 Rationale for Study Design

Substantial safety data are available for currently licensed monovalent and polyvalent meningococcal conjugate vaccines (Menactra®, Menveo®, Nimenrix® and MenAfrivac®) across all age groups, including infants and toddlers.[3, 18-20] Safety data indicate a favorable risk/benefit profile with low rates of solicited and unsolicited AEs following vaccination. After completion of the Phase 1 clinical study in adults and following a positive outcome of safety data assessment by the Safety Review Team, clinical development of NmCV-5 vaccine will be continued in children 12-16 months of age (the target group for routine meningococcal vaccination in Africa).

The immunogenicity data of licensed polyvalent meningococcal vaccines indicate that a 2-dose vaccination series is required to induce a robust immune response against all vaccine serogroups in children 9 to 23 months of age. As such, a 2-dose vaccination schedule will be tested in this study. Immune responses will be evaluated at one month after each study vaccination and prior to administration of the second vaccine dose, to assess possible waning of antibody titers.

Immunogenicity of adjuvanted and non-adjuvanted formulations of NmCV-5 will be compared with the licensed quadrivalent meningococcal conjugate vaccine (MenACWY-D; Menactra®). Menactra® has been selected as an active control given the large safety database accumulated since the vaccine was introduced in the US in 2005, prequalified by WHO and progressively introduced into other countries.

Both vaccines will be administered to meningococcal vaccine-naïve healthy subjects to avoid possible enhancement of immune response due to a previous meningococcal vaccine administration.

Mali introduced MenAfriVac during a national campaign in 2010 and 2011 for the prevention of Group A *N.meningitidis* in the 1 to 29 years of age. This campaign was part of a broader effort to eliminate Group A meningococcal meningitis in Sub-Saharan Africa, with all countries located in the meningitis belt introducing the Men A conjugate vaccine between 2010 and 2016. In the same period, epidemic meningococcal disease caused by serogroup A has been observed to practically disappear from the immunized regions following a herd immunity effect. Mali's first Men A case since 2010 was reported in Kieneba district in March 2016. This case occurred in a 15 year old who had not been vaccinated as part of the campaign that took place in that district in 2011. Neighboring Guinea experienced an epidemic in 2014-15 secondary to disruptions in health care due to the Ebola outbreak and a MenAfriVac campaign that was started only in 2015. To maintain effort in the protection against Men A in Mali, an introduction into the EPI schedule for MenAfriVac-5 (MenAfriVac with a 5 µg concentration in Men A polysaccharide) at 9 months of age was completed in February 2017 and a national catch-up campaign to immunize the 1 to 5 years of age (born after the initial campaign) was implemented on 5-14 June, 2017.[16, 17, 25-29]

Because of the need for vaccine-naïve healthy subjects aged 12-16 months for this study, recruitment efforts will need to start as early as 9 months to identify potential subjects and postpone the administration of meningococcal vaccine.

Subjects will be enrolled as soon as possible once they meet all eligibility criteria. If found not eligible, they will be followed to receive the MenAfriVac.

Given the almost complete absence of invasive meningococcal disease caused by serogroup A in Mali following the national preventive vaccination campaign in 2010-11, the introduction of MenAfriVac in the EPI in February 2017 and the MenAfriVac National Catch-up Campaign completed in 2017 in the 1 to 5 years of age with reported coverage rates of 112% in the study area and 104.7% nationally, the herd immunity effect is expected to be reinforced for several years, preventing any new circulation of Men A in the study area.[30, 31] Under this context, the deferral of Men A vaccination constitutes an acceptable risk / benefit ratio as subjects would receive either two doses of polyvalent meningococcal vaccine during the clinical study or, alternatively, a dose of meningococcal group A vaccine by a delay of 3 months.

2 HYPOTHESIS, OBJECTIVES AND ENDPOINTS

2.1 Study Hypotheses

Both formulations of investigational NmCV-5 vaccine (adjuvanted and non-adjuvanted) administered intramuscularly to healthy children 12-16 months of age will be safe and well tolerated.

NmCV-5 (adjuvanted and non-adjuvanted formulations) administered according to a 0,3 month schedule to healthy children will elicit a measurable functional immune response against all vaccine serogroups at 1 month after the second vaccination.

2.2 Study Objectives

2.2.1 Primary objective

1. To assess the reactogenicity of non-adjuvanted and adjuvanted formulations of NmCV-5 vaccine in comparison with the licensed MenACWY-D vaccine, as measured by the percentage of subjects with at least one severe solicited AE reported within 7 days after any vaccination.

2.2.2 Secondary Immunogenicity Objectives

1. To assess immunogenicity of non-adjuvanted formulation of NmCV-5 vaccine in comparison with MenACWY-D vaccine, as measured by rSBA against serogroups A, C, W, Y and X at 1 month after the second vaccination.
2. To assess immunogenicity of adjuvanted formulation of NmCV-5 vaccine in comparison with non-adjuvanted formulation of NmCV-5 vaccine, as measured by rSBA against serogroups A, C, W, Y and X, at 1 month after the second vaccination.
3. To assess immune responses elicited by non-adjuvanted and adjuvanted formulations of NmCV-5 vaccine and MenACWY-D vaccine at 1 and 3 months after the first vaccination.

2.2.3 Secondary Safety Objective

1. To evaluate the safety and reactogenicity of adjuvanted and non-adjuvanted formulations of NmCV-5 vaccine in healthy children, when compared to MenACWY-D.

2.2.4 Exploratory Objective

1. To assess immunogenicity of adjuvanted and non-adjuvanted formulations of NmCV-5 vaccine and MenACWY-D vaccine, as measured by hSBA against serogroups A, C, W, Y and X at baseline, 1 month after the first vaccination and 1 month after the second vaccination (in a subset of subjects).
2. To further assess immunogenicity of adjuvanted and non-adjuvanted formulations of NmCV-5 vaccine and MenACWY-D vaccine, as measured by rSBA against serogroups A, C, W, Y, and X at baseline, 1, month and 3 months after the first vaccination and 1 month after the second vaccination.

2.3 Study Endpoints

2.3.1 Primary reactogenicity endpoint:

1. Percentage of subjects with at least one severe solicited AE* within 7 days after any study vaccination (Days 0-6 and Days 84-90).

   **Solicited AEs include tenderness, swelling/induration, irritability, drowsiness, decrease of eating, vomiting, and fever*

2.3.2 Secondary immunogenicity endpoints:

1. Percentage of subjects with rSBA titer ≥ 8 against serogroups A, C, W, Y and X at Visits Day 0, Day 28, Day 84 and Day 112.
2. Percentage of subjects with rSBA titer ≥ 128 against serogroups A, C, W, Y and X at Visits Day 0, Day 28, Day 84 and Day 112.
3. Percentage of subjects with fourfold rise in rSBA titers against serogroups A, C, W, Y and X at Visits Day 28 and Day 112.
   a. For subjects with a pre-vaccination rSBA titer < 8, a post-vaccination titer of ≥ 32;
   b. For subjects with a pre-vaccination rSBA titer ≥ 8, an increase in rSBA titer of at least 4 times the pre-vaccination titer.
4. rSBA GMT for serogroups A, C, W, Y and X at Visits Day 0, Day 28, Day 84 and Day 112.

2.3.3 Secondary safety endpoints:

1. Solicited local and systemic AEs reported within 7 days after each vaccination (Days 0-6 and Days 84-90);
2. Unsolicited AEs reported during 28 days after vaccination (Days 0-27 and Days 84-111);
3. AEs leading to premature withdrawal during the entire study period;
4. SAEs reported during the entire study period.

2.3.4 Exploratory immunogenicity endpoints:

1. Percentage of subjects with hSBA titer ≥ 8 against serogroups A, C, W, Y and X at Visits Day 0, Day 28 and Day 112 (in a subset of subjects)
2. Percentage of subjects with hSBA seroresponse against serogroups A, C, W, Y and X at Visits Day 28 and Day 112 (in a subset of subjects), defined as:
   - for subjects with baseline hSBA titer < 4, post vaccination hSBA titer ≥ 8;
   - for subjects with baseline hSBA titer ≥ 4, an increase of at least four times the pre-vaccination hSBA
3. hSBA GMTs for serogroups A, C, W, Y and X at Visits Day 0, Day 28 and Day 112 (in a subset of subjects).

4. Percentage of subjects with rSBA titer ≥ 4, ≥ 16, ≥ 32, and ≥ 64 against serogroups A, C, W, Y and X at Visits Day 0, Day 28, Day 84 and Day 112.

5. Percentage of subjects with a fourfold increase in rSBA titers against serogroups A, C, W, Y and X at Visits Day 28 and Day 112 with the following criteria:

   - For subjects with a pre-vaccination rSBA titer < 4, a post-vaccination titer of ≥ 16;
   - For subjects with a pre-vaccination rSBA titer ≥ 4, a post-vaccination titer of at least 4 times the pre-vaccination titer.

6. Among subjects with a pre-vaccination rSBA titer ≥4, ≥8, ≥16, ≥ 32, ≥ 64, and ≥ 128, percentage of subjects with a post-vaccination titer of at least 4 times the pre-vaccination titer at Visits Day 28 and Day 112.

3 STUDY DESIGN

3.1 Methodology

Design: Phase 2, randomized (2:2:1), controlled, observer-blind, single-center study in healthy children 12 to 16 months of age with three study groups.

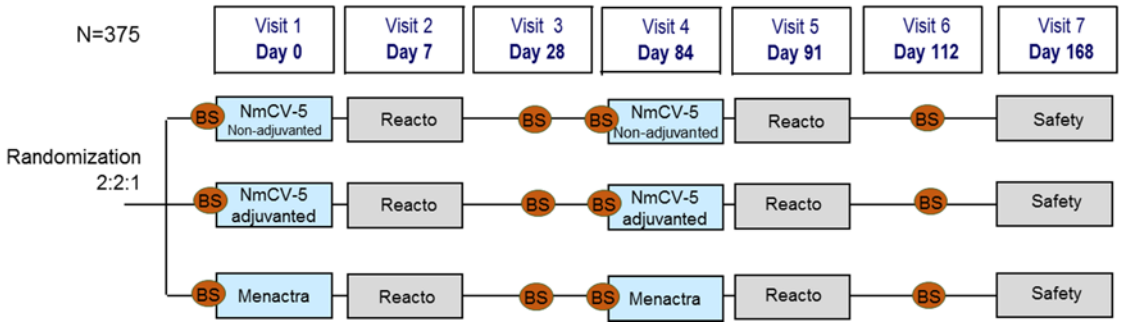
Duration of the study: The study duration is approximately 6 months for each subject.

Vaccination schedule: 0, 3 month.

Study groups:

- NmCV-5_non-adjuvanted group: approximately 150 subjects receiving the non-adjuvanted formulation of NmCV-5 vaccine at Visit Day 0 and Visit Day 84.
- NmCV-5_adjuvanted group: approximately 150 subjects receiving the adjuvanted formulation of NmCV-5 vaccine at Visit Day 0 and Visit Day 84.
- ACWY-D group: approximately 75 subjects receiving the licensed MenACWY-D (Menactra®) vaccine at Visit Day 0 and Visit Day 84.

Figure 3.1-1. Study design



Note: BS – blood sample; ACWY-D –Menactra®

Randomization: At Visit Day 0, prior to the study vaccination, subjects will be randomized into the three study groups according to a 2:2:1 ratio.

Blinding: Observer-blind study.

Data collection: Electronic Case Reporting Form (eCRF).

Blood sample schedule: Four blood samples (approximately 5 mL each) at Visit 1 (Day 0, first vaccination), Visit 3 (Day 28), Visit 4 (Day 84, second vaccination) and Visit 6 (28 days after second vaccination).

Study visits: Seven visits at Day 0, Day 7, Day 28, Day 84, Day 91 (7 days post Dose 2), Day 112 (28 days post Dose 2) and Day 168 (84 days post Dose 2).

Solicited AEs: occurring during 7 days following each study vaccination will be assessed daily by site staff and recorded in the home visit worksheets and the CRF at Visit 2 and Visit 5.

Unsolicited AEs: occurring within 28 days after each study vaccination will be collected weekly by site staff during home and site visits.

AEs leading to study withdrawal and SAEs: will be collected during the entire study period. These data will be captured through the home visit worksheets, by interviewing the subjects' parents / guardians during the home visits, the planned and unplanned site visits, and by review of available medical records.

Written agreement to delay the EPI MenAfriVac dose scheduled at 9 months of age: Prior to written informed consent, subject's parents/guardians may be asked to sign a pre-screening agreement as early as 9 months of age in order to delay the EPI MenAfriVac dose until they can be considered for enrolment in the trial (trial subjects must be at least 12 months of age, have not received any meningococcal vaccines and have not received any vaccinations in the past 28 days).

Written informed consent will be obtained from the subject's parents / guardians before conducting any study-specific procedures.

After signing informed consent, a review of medical history and prior medications/vaccination, a physical examination, and confirmation of subject eligibility, subjects will be enrolled into the study.

Table 3.1-2: Times and Events Table

Visit Number	-	1	2	3	4	5	6	7
Visit Type	-	**Clinic Visit**	**Home or Clinic Visit**	**Clinic Visit**	**Clinic Visit**	**Home or Clinic Visit**	**Clinic Visit**	**Clinic Visit**
Visit time and window	-	**Day 0**	**Day 7 (+3)**	**Day 28 (+14)**	**Day 84 (±14)**	**Day 91 (+3)**	**Day 112 (+14)**	**Day 168 (±14)**
Time After Last Vaccination	9-12 months of age		7 days post dose 1	28 days post dose 1	84 days post dose 1	7 days post dose 2	28 days post dose 2	84 days post dose 2
Agreement to delay MenA vaccination	X							
Delayof MenA vaccination	X							
Informed Consent		X[a]						
Demographic Data		X[a]						
Exclusion/Inclusion Criteria		X[a]						
Medical History		X[a]						
General Physical Examination [b]		X[a]	X	X	X[a]	X	X	X
Randomization		X[a]						
Eligibility for second vaccination/ delay [c]					X[a]			
Blood Collection [d]		X[a]		X	X[a]		X	
Study Vaccination		X			X			
30-Minute Post-Vaccination Assessment [e]		X			X			
Review of solicited and unsolicited AEs by medical staff		X	X	X	X	X	X	X
Recording of solicited AEs by field staff [f]		*Daily for 6 days*			*Daily for 6 days*			
Recording of unsolicited AEs by field staff [g]		*Daily for 6 days*		*Weekly from Day 7 to Day 28*	*Daily for 6 days*		*Weekly from Day 7 to Day 28 post dose 2*	
Reporting of SAEs		*from Day 0 to study completion*						
Recording of concomitant medications and vaccinations		*From Day 0 to study completion*						
Study Termination		*In case of termination before Visit 7: early termination procedures must be followed.*						X

a. Procedure to be performed prior to vaccination
b. Physical examination must be performed by a qualified health professional in accordance with local regulations and licensing requirements designated within the Site Responsibility Delegation Log.
c. Prior to the second vaccination investigator must check eligibility criteria for subsequent vaccination and criteria for vaccination delay as specified in sections 4.3 and 4.4.
d. Approx. 5 mL of blood drawn at each specified visit. See section 4.4 for criteria of blood draw delay.

e. At least 30 minutes post-vaccination, immediate reactions will be evaluated by the investigator. Assessment of solicited local and systemic AEs and post-vaccination temperature measurement will be performed by study staff. Solicited local and systemic AEs, any unsolicited AEs, vital signs and body temperature will be recorded in source documents and eCRF.
f. Beginning the day following study vaccination and subsequent 5 days, solicited local and systemic AEs and other indicators of reactogenicity (i.e. body temperature and use of analgesics/antipyretics) will be assessed daily by field staff and recorded in the home visit worksheets.
g. Unsolicited AEs will be collected by field staff during weekly contacts of subject's parents/guardians and recorded in the home visit worksheets.

4 STUDY POPULATION

This will be a single site study conducted at the Centre pour le Développement des Vaccins du Mali (CVD-Mali), Bamako, Mali.

4.1 Inclusion Criteria

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

1. Male and female children between 12 months and 16 months old inclusive (minimum 365 days of age and maximum 16 months plus 29 days of age);
2. For whom parent(s)/legal guardian(s) have given written informed consent after the nature of the study has been explained according to local regulatory requirements;
3. Who the investigator believes that their parent(s)/ guardian(s) will be available for all the subject visits and would comply with the requirements of the protocol (e.g., timely reporting of adverse events, availability for study site visits and home visits);
4. Individuals in good health as determined by the outcome of medical history, physical examination and clinical judgment of the investigator.
5. Individuals who completed their local infant EPI schedule through 9 months of age (except MenAfriVac dose). A birth dose of OPV is not required.

4.2 Exclusion criteria

In order to participate in this study, all subjects must meet NONE of the exclusion criteria described.

1. History of any meningococcal vaccine administration.
2. Current or previous, confirmed or suspected disease caused by N. meningitidis.
3. Household contact with and/or intimate exposure to an individual with any laboratory confirmed N. meningitidis infection within 60 days of enrolment.
4. History of severe allergic reactions after previous vaccinations or hypersensitivity to any study vaccine component including tetanus, diphtheria and diphtheria toxoid (CRM197).
5. Acute or chronic, clinically significant pulmonary, cardiovascular, metabolic, neurological, hepatic, or renal functional abnormality, as determined by medical history or physical examination.
6. Any confirmed or suspected condition with impaired/altered function of immune system (immunodeficient or autoimmune conditions).
7. Have any bleeding disorder which is considered as a contraindication to intramuscular injection or blood draw.
8. Severe acute malnutrition.
9. A family history of congenital or hereditary immunodeficiency.

10. History of either hepatitis B or hepatitis C virus infection or human immunodeficiency virus infection.
11. Major congenital defects.
12. Chronic administration (defined as more than 14 days) of immunosuppressants or other immune-modifying drugs within three months prior to the study vaccination or planned use throughout the study period. (For corticosteroids, this means prednisone, or equivalent, ≥ 0.5 mg/kg per day. Inhaled, intranasal and topical steroids are allowed).
13. Administration of blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation in the past 3 months or planned use throughout the study period.
14. Administration of any vaccine within 28 days prior to enrolment in the study or planned administration of any vaccine within 14 days before or after any study vaccination.
15. Use of any investigational or non-registered drug or vaccine within 30 days prior to the administration of study vaccines or planned during the study.
16. Malaria infection as confirmed by a Rapid Diagnostic Test.

    Note: Subjects positive at screening may be treated for malaria as per national guidelines, and if subject remains eligible, vaccinated no earlier than five days after completing treatment.
17. Individuals who are close family members of individuals conducting this study.
18. Have experienced a moderate or severe acute infection and/or fever (defined as temperature ≥ 37.5°C) within 3 days prior to enrolment.
19. Have received systemic antibiotic treatment within 3 days prior to enrolment.
20. Non-residence in the study area or intent to move out within six months.
21. Any condition which, in the opinion of the investigator, might pose additional risk to the subject due to participation in the study.

4.3 Criteria for Administration of Second Study Vaccination

Prior to receipt of the second dose of study vaccine, subjects must be evaluated to confirm that they are eligible for subsequent vaccination. If subjects meet any of the criteria listed below, they should not receive the second vaccination:

1. Subjects who experienced any immediate allergic reaction after the previous study vaccination.
2. Subjects who experience any serious adverse event judged to be related to study vaccination, including hypersensitivity reactions.
3. Subjects who develop any clinically significant medical condition which, in the opinion of the investigator, may pose additional risk to the subject if he/she receives the second vaccination.

Subjects who meet any of these criteria must not receive further study vaccinations. However, these subjects should be encouraged to continue study participation, as discussed in section 6.7.

4.4 Criteria for Delay of Vaccination and/or Blood Sampling

After enrolment, subjects may encounter clinical circumstances that warrant a delay in subsequent study vaccination. These situations are listed below. In the event that a subject meets a criterion for delay of vaccination, the subject may receive study vaccination once the window for delay has passed as long as the subject is otherwise eligible for study participation.

1. Acute moderate or severe infection with or without fever within 3 days of intended study vaccination.
2. Fever is defined as body temperature >37.5° C within 3 days of intended study vaccination.
3. Malaria infection as confirmed by a Rapid Diagnostic Test (subject may be vaccinated no earlier than five days after completing treatment).
4. Administration of any vaccine not foreseen by the study protocol within 14 days prior the intended study vaccination.

There are also circumstances under which repeat vaccination is a contraindication in this study. These circumstances are presented in section 4.3.

There is a clinical circumstance that warrants delay of blood collection for immunogenicity assessments in this study. This situation is listed below. In the event that a subject meets a criterion for delay of blood collection, blood collection may proceed once the window for delay has passed.

1. Subject has received a dose of systemic antibiotics within 3 days before the intended blood collection.

5 TREATMENT OF SUBJECTS

5.1 Description of study vaccines

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

Investigational vaccines

The NmCV-5 investigational vaccine is a fixed-combination available as the lyophilized powder containing meningococcal groups A and X polysaccharides conjugated to tetanus toxoid and meningococcal groups C, W and Y polysaccharides conjugated to CRM_{197} protein. The vaccine will be reconstituted with either saline alone (non-adjuvanted formulation) or saline containing aluminum phosphate at 125 mcg Al^{3+} /dose (adjuvanted formulation) just prior to administration.

N. meningitidis A, C, Y, W, and X polysaccharides are produced by cultivating cells in fed batch fermentation and purified after separation. Tetanus toxin is derived from *Clostridium tetani* grown in a modified medium and purified. CRM is expressed by *Pseudomonas fluorescens* strain and used after it is purified. Meningococcal polysaccharides are covalently conjugated to TT or CRM and purified to make the final formulated vaccine.

The NmCV-5 lyophilized powder component contains sucrose, sodium citrate and trometamol as excipients. This component is presented as a freeze-dried powder in a five-dose vial.

The adjuvanted diluent containing aluminum phosphate used to prepare the adjuvanted NmCV-5 is a sterile white homogeneous suspension presented in a five-dose glass ampoule containing an actual fill volume of 3.1 mL ± 0.1 mL.

0.9% Sodium Chloride used to prepare the non-adjuvanted NmCV-5 vaccine is a clear, colorless liquid presented in 10 mL ampoules. A volume of 3.1 mL will be used to reconstitute the lyophilized component of NmCV-5 vaccine.

After reconstitution the NmCV-5 vaccine formulations to be used will have the following composition per 0.5 mL of injectable solution:

Table 5.1-1: Composition of reconstituted non-adjuvanted and adjuvanted formulations of NmCV-5 vaccine (per 0.5 mL dose)

Vaccine component	Non-adjuvantedNmCV-5 (per 0.5 mL dose)	AdjuvantedNmCV-5 (per 0.5 mL dose)
Meningococcal A polysaccharide[1]	5 µg	5 µg
Meningococcal C polysaccharide[2]	5 µg	5 µg
Meningococcal Y polysaccharide[2]	5 µg	5 µg
Meningococcal W polysaccharide[2]	5 µg	5 µg
Meningococcal X polysaccharide[1]	5 µg	5 µg
Sucrose	2.42 mg	2.42 mg
Sodium Citrate	0.40 mg	0.40 mg
TRIS (Trometamol)	0.098 mg	0.098 mg
Aluminum phosphate Al^{3+}	-	125 µg /dose
0.9% Sodium Chloride	q. s.	q. s.
Tetanus Toxoid	7.8 to 33.4 µg	7.8 to 33.4 µg.
CRM_{197}	11.7 to 50.1 µg	11.7 to 50.1 µg

Note: [1] each polysaccharide conjugated to Tetanus Toxoid; [2] each polysaccharide conjugated to CRM_{197}; q.s. -*Quantum satis*

Active comparator

The licensed MenACWY-D polysaccharide conjugate vaccine (Menactra®, manufactured by Sanofi Pasteur Inc.) is supplied as a single 0.5 mL dose formulated in sodium phosphate buffered isotonic sodium chloride solution to contain 4 µg each of meningococcal A, C, Y, and W polysaccharides conjugated to approximately 48 µg of diphtheria toxoid protein carrier. No preservative or adjuvant is added during manufacture. Each 0.5 mL dose may contain residual amounts of formaldehyde of less than 2.66 µg.

Table 5.1-2: Composition of MenACWY-D (per 0.5 mL dose)

Vaccine component	Per Dose of 0.5 mL
Meningococcal group A polysaccharide	4 µg
Meningococcal group C polysaccharide	4 µg

Meningococcal group Y polysaccharide	4 µg
Meningococcal group W polysaccharide	4 µg
Diphtheria toxoid protein total	48 µg
Sodium phosphate	0.7 mg
Sodium chloride	4.35 mg

5.2 Precautions to be observed in administrating study vaccines

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 inclusion / exclusion criteria as specified in protocol sections 4.1 and 4.2.

Eligibility for subsequent study vaccination is determined by following the criteria outlined in sections 4.3 and 4.4.

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.

As with all vaccines, appropriate medical treatment (like adrenaline 1:1000, anti-histamine [diphenhydramine], corticosteroids [hydrocortisone] and resuscitation equipment etc.) must be available at the site, and staff and supervision must be readily available in case of rare anaphylactic or any severe allergic reactions following administration of the study vaccine.

Prompt use of resuscitation measure can be lifesaving and must be implemented at the first suspicion of anaphylaxis.

5.3 Preparation and administration of the study vaccine

The adjuvanted NmCV-5 is prepared by aseptically injecting the whole content of a five dose single ampoule of adjuvant containing diluent into the five dose vial containing the lyophilized MenACYWX component. This reconstitutes the lyophilized MenACYWX component with gentle agitation. The final reconstituted vaccine (total volume approximately 0.6 mL per dose; injection volume 0.5 ml per dose) is then ready for administration. For the purpose of this study, one five dose vial of reconstituted adjuvanted NmCV-5 will be used to draw a single vaccine dose for one subject only. After reconstitution the vaccine must be used as soon as possible but not later than two hours, during which time it may be kept at room temperature but must not be frozen.

The non-adjuvanted NmCV-5 is prepared by aseptically injecting approximately 3.1 mL of 0.9% Sodium Chloride diluent into the five dose vial containing the lyophilized MenACYWX component. This reconstitutes the lyophilized MenACYWX component with gentle agitation. The final reconstituted vaccine (total volume approximately 0.6 mL per dose; injection volume

0.5 ml per dose) is then ready for administration. For the purpose of this study, one five dose vial of reconstituted non-adjuvanted NmCV-5 will be used to draw a single vaccine dose for one subject only. After reconstitution the vaccine must be used as soon as possible but not later than two hours, during which time it may be kept at room temperature but must not be frozen.

MenACWY-D (Menactra®) vaccine is prepared by aseptically withdrawing all fluid from the single-dose vial using a sterile needle and syringe. Vaccine must be administered within two hours of preparation.

All study vaccines should be visually inspected after reconstitution. In the event of any foreign particulate matter and/or any unusual appearance of the study vaccine, please set the vial aside and inform the unblinded monitor.

All study vaccines (injected volume of 0.5 mL) will be administered as per randomization schedule via intramuscular injection in the anterolateral area of the thigh on Visit Day 0 and Visit Day 84.

The investigator or designee will be responsible for oversight of the administration of vaccine to subjects enrolled in the study according to the procedures presented in this study protocol. All vaccines will be prepared and administered only by designated unblinded personnel who are qualified to perform that function under applicable local laws and regulations for the specific study site.

All study vaccines to be administered to the subjects must be stored in a safe and locked place with no access by unauthorized personnel.

The study vaccines will be stored at the defined temperature range (i.e. +2 to +8 C). The storage temperature of the vaccines will be monitored daily with temperature monitoring devices and will be recorded.

Any temperature deviation, i.e. temperature outside the range (+2 to +8°C), must be reported to the sponsor as soon as detected. Following the exposure to such a temperature deviation, vaccines will not be used until written approval has been given by the sponsor. Expired vaccines must not be administered.

5.4 Vaccine supply, labelling, storage, accountability and disposal

The sponsor will ensure the following:

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

The investigator must ensure the following:

- Indication of appropriately trained unblinded site staff to manage vaccine supply, accountability, preparation and administration.
- Acknowledge receipt of the study vaccines by a designated staff member at the site, including confirmation that the vaccines:
    - were received in good condition;

  - remained within the appropriate temperature range during shipment from the sponsor to the investigator's designated storage location;
  - have been confirmed by the sponsor as 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:
  - 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 number, expiration date, unique identifying numbers assigned to subjects and study vaccines, and time of vaccine administration. This information will be maintained in an accountability log that will be reviewed by the unblinded site monitor;
    - proper reconciliation of all study vaccines received from the sponsor. Reconciliation is defined as maintaining records of which and how many vaccines were received, which vaccines (and volume thereof) were administered to subjects, and which vaccines were destroyed at the site.
- Proper adherence to the local institutional policy with respect to destruction of study vaccines.
- Complete record keeping of vaccine use, wastage, or destruction, including documentation of:
  - copy of the site's procedure for destruction of hazardous material;
  - number of doses destroyed, date of destruction, method of destruction, and name of individual performing destruction

Vaccines that have been stored differently from the manufacturer's instructions 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 practice stocks to prevent unintentional use of study vaccines outside of the clinical trial setting.

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

6 STUDY PROCEDURES

6.1 General considerations

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP), all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

The study will be initiated only after approvals from relevant IRB/IECs and the National Regulatory Authority have been obtained.

Freely given and written or witnessed/ thumb printed informed consent must be obtained from each subject's parent(s)/guardian(s) prior to participation in the study.

6.2 Community permission

Given the local social structure, once the ethical and regulatory approvals are in place, community meetings will be organized to present the study to the communities where the site team plans to conduct the study. These meetings will be organized following a specific site procedure:

1. The informed consent forms will be translated into the local language and recorded onto an audiotape through a certified process;
2. Community meetings will be scheduled with the elders, the religious representatives, the social leaders and village chiefs in each community where the study will be conducted. At these meetings, the study will be explained in detail and, when applicable, the audiotaped consent will be played;
3. The study will be discussed and the study team will answer any questions. The community leaders would review the information and confirm their permission for the conduct of the study.
4. The leaders or a local crier will announce the study and direct interested persons to the site. The study site team would then be allowed to start recruitment activities and approach potential subjects.

6.3 Recruitment

The site will develop a study specific recruitment plan and will use several approaches for recruitment:

- The list of children 8-15 months of age with their home addresses will be obtained from the demographic surveillance system. Community liaisons/field workers will visit each household and inform parents/guardians about the study. If interested, the parent/guardian will be directed to visit the site clinic for more information.
- The study team will collaborate with the community clinics to search potential subjects when they come for their EPI vaccinations. Study staff will be present at the community clinics during vaccination days. If interested, the parent/guardian will be referred to the site staff for information on the study. Further details are described in section 6.6.1.

6.4 Informed Consent

"Informed consent" is the voluntary agreement of an individual or his/her legal guardian to participate in research. Consent must be given with free will of choice, and without 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 of the parents/legal guardians following local IRB/EC guidance **must** be obtained before conducting any study-specific procedures.

The informed consent will have a two-step process.

- An initial informed consent process will be conducted as early as 9 months of age. At this stage, the parent(s)/guardian(s), based on their interest in the study would decide if they agree to delay the EPI MenAfriVac dose until their child is eligible to participate in the study (i.e. 12 months). If they agree, subject's parents/guardians will be asked to sign the pre-screening agreement (Delay of MenAfriVac agreement form) which is included in the informed consent document.
- A second informed consent process will be conducted as early as 12 months of age. If the parent(s)/guardian(s) agree for their child to participate in the study, subject's parents/guardians will be asked to sign the study consent form.

The child would be considered enrolled in the study as soon as the two-step process for informed consent was completed and the parent(s)/guardian(s) have agreed to participation in the study.

The process of obtaining agreement for delay of MenAfriVac and the process of obtaining informed consent for study participation should be documented in the source documents. Two originals of the informed consent form should be signed and dated for the delay of MenAfriVac. One original will be given to the parent(s)/guardian(s) and one original will be kept at the site. At the time of final informed consent, the same originals will be signed and dated for consent to participate in the study. One original will be returned to the parent(s)/guardian(s) and one original will be kept at the site. Additional specifics regarding the informed consent processes are located in section 11.3 of the protocol.

If subject's parent(s)/guardian(s) 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's legally acceptable representative cannot read, and who reads the informed consent form and any other written information supplied to the parent/legal guardian. After the written informed consent form and any other written information to be provided to subject's parent/guardian, is read and explained to the subject's guardian/parent and after the subject's parent/guardian has verbally consented to the subject's participation in the study and, if capable of doing so, has signed or fingerprinted 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's parent/guardian and that informed consent was freely given by the subject's parent/guardian.

6.5 Randomization and Blinding Procedures

6.5.1 Randomization

At Visit Day 0 each subject will be randomized according to a 2:2:1 ratio into one of the three study groups using a randomization list. Randomization will occur on the day subjects are to receive their first study injection, after confirmation of eligibility and immediately prior to injection.

The unblinded designated personnel will be provided with the randomization list that includes treatment assignment. Based on the assigned treatment the unblinded personnel will prepare the study vaccine to be given to each subject. The unblinded personnel will maintain the randomization list in a secure place.

If for any reason, after signing the informed consent form, the subject (who has passed screening) fails to be randomized, the reason for not being randomized should be recorded in source documents. The information on these eligible subjects, who are not randomized, should be kept distinct in the source documentation from screen failures.

6.5.2 Blinding

The study is designed as an observer-blind study. Observer-blind means that during the course of study, the parents/guardians of the subjects and the study personnel responsible for the evaluation of any study endpoint (e.g. safety and reactogenicity) will be unaware which vaccine was administered. The vaccine preparation and administration will be done by designated unblinded personnel who will not participate in any of the clinical study evaluations.

Unblinded study personnel will prepare the vaccine out of view of the subject, the subject's parents/guardians and the blinded study personnel, and will administer the vaccine in a separate room. Considering the fact that all the three vaccines are likely to have distinct appearances, even when drawn into syringes, the syringes will be masked with an opaque wrapping before administration.

The laboratories in charge of the serology testing will also be blinded in terms of subject identifier and study group.

The treatment and sample blinding will be maintained during the entire study period. For the planned interim analysis, individual listings will not be generated and any access to subject-level information about study groups will be masked.

The treatment code should only be broken if investigator/physician in charge of the subject feels that the case cannot be treated without knowing the identity of the study vaccine.

If emergency unblinding is deemed to be necessary, and time permitting, the investigator is encouraged to discuss with the sponsor prior to retrieving the subject's assigned vaccine code.

6.6 Delay of MenA vaccination

At the time of signing of the agreement for delay of vaccination, a sequential ID number will be assigned to the child on a pre-screening log.

The pre-screening agreement is not a consent or confirmation of the subject's participation in the study. After signing the agreement, investigator will schedule a recruitment visit (Visit 1) at 12

months of age and provide parents/guardians a site card with date and time of the planned visit and a confirmation of meningococcal vaccination deferral.

Every child who has delayed the MenAfriVac will be followed by the site until the child is either enrolled in the study or receives the MenAfriVac dose according to the EPI. This will need to be documented in the source and the CRF.

6.7 Study visits

The schedules of evaluations and procedures that must be performed at specific time points are described in the following sections. The Time and Event table (Table 3.1-2) summarizes the frequency and timings of various baseline, immunogenicity and safety evaluations.

6.7.1 Visit #1 (Day 0): screening, enrolment, blood draw and first vaccination

After the subject's parent/guardian has consented to participate in the study and informed consent is signed, screening procedures will be performed for all subjects and will include the following:

- Entry of the subject ID in the screening log
- Confirm that the individual meets ALL inclusion and NO exclusion criteria (refer to sections 4.1 and 4.2 for further details).
- Review/collect demographic data such as age, gender and ethnicity.
- Review/collect medical history.
- Review/collect prior/concomitant medications and evidence of prior vaccinations.
- Review of systems: an interview that queries the subject's parent/guardian as to any clinical findings the subject has experienced across each organ system. The questions tend to be grouped by system organ class and are intended to remind the parent/guardian of any forgotten or medical conditions that may be relevant.
- Perform a general physical examination including measurement of body temperature, height, weight, and resting vital signs: heart rate and respiratory rate. The height and weight need to be recorded in source documents.
- Note: If the subject's body temperature[1] is ≥37.5°C, vaccination must be postponed until three days after the fever has resolved. Vaccination is also postponed for any clinically significant acute infection and/or systemic antibiotic use within 3 days (see section 4.4 for further details).
- Perform Rapid Diagnostic Test for malaria. In case of a positive result, vaccination should be postponed until at least five days after completing treatment as per national guidelines.

[1]Axillary measurement of temperature with a digital thermometer is the preferred route for temperature measurement in this study; other routes of measurement are acceptable if axillary measurement is not possible.

Eligibility criteria must be reviewed on the day of vaccination. All screening procedures must be documented in the source documents.

In the event that the individual is determined ineligible for study participation, he/she is considered a "screen failure". The reason for screen failure must be documented in the Screening Log. The individual should receive meningococcal group A vaccination, if it was not administered previously.

Following procedures will be performed in case of eligible subjects

- Study personnel will take a photo of subject and his/her parent/guardian for the study identification card. This study identification card and a copy of consent form will be given to each subject's parent(s)/guardian(s). Both documents will have phone numbers with 24 hours access in case of emergency.
- Collect blood sample (approximately 5 mL) for serology testing. Refer to section 7.2 for further details regarding general handling of blood sample.
- Unblinded staff will perform subject randomization (refer to section 6.5.1 for further details) and prepare vaccine following assigned study group.

After confirming eligibility and enrolling subject into the study on Visit 1, study vaccination will be performed by unblinded study nurse according to the assigned study vaccine and according to the procedures described in section 6.5.2.

After vaccination, the subject will be observed for at least 30 minutes for any immediate post-vaccination reactions, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines. Solicited local and systemic AEs, any unsolicited AEs, vital signs and body temperature will be recorded. All safety data collected within 30 minutes post-vaccination should be recorded in the subject's source documents.

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After all procedures have been completed, the investigator will remind the subject's parents/guardians about safety evaluation during daily home visits within 6 days following study vaccination that will be performed by trained staff under investigator's supervision and documented on the home visit worksheets.

The investigator or a delegate should ensure that all information is recorded in the source documents and accurately transcribed in the eCRF as soon as possible after the subject's visit has been completed.

The subject's parent/guardian will be reminded to contact the site if there are any questions. In addition, the subject's parent/guardian will get an instruction to contact the site immediately if the subject has a medical condition that leads to a hospitalization or an emergency room visit.

Subject's parents/guardians will be reminded of the study Visit 2 (7 days after vaccination), which will be conducted by a designated medical staff at the site clinic or the subject's home.

6.7.2 Visit #2(Day 7[+3]): reactogenicity assessment

Study subjects will return for follow-up evaluations to the clinical study site 7 days following vaccination. Alternatively, a home visit may be performed by a medically qualified site

personnel. At this visit, investigator or delegate will perform the following procedures and evaluations in the following order:

1. Review of solicited and unsolicited AEs collected during days 1-6 after vaccination using home visit worksheets;
2. Assessment of any ongoing solicited AEs on Day 7 post-vaccination, including measurement of body temperature (note that all ongoing solicited AEs beyond day 7 must be followed up daily by site staff until resolution);
3. Medical interview of subject's parents/guardians to assess any unsolicited AEs, SAEs since previous study visit;
4. Collection of concomitant medications and vaccinations;
5. Physical examination including assessment of vital signs (as in Visit 1).

The investigator or a delegate should ensure that all information is recorded in the source documents and accurately transcribed in the eCRF as soon as possible after the subject's visit has been completed.

The subjects' parents/guardians will be reminded to contact the site in case of any questions and to contact the site immediately if the subject has a medical condition that leads to a hospitalization or an emergency room visit.

The investigator will remind the subject's parents/guardians about weekly safety evaluations that will be performed by trained site staff at subject's home or by phone. Information about unsolicited AEs will be documented on the home visit worksheets.

All subject's parents/guardians will be reminded to return to the clinic 28 days (0/+14 days) after vaccination for Visit 3.

6.7.3 Visit # 3 (Day 28 [+14]): blood draw

Study subjects will return for follow-up evaluations to the clinical study site 28 days following the first vaccination. At this visit, investigator or delegate will perform the following procedures and evaluations in the following order:

1. Review of any unsolicited AEs collected during weekly contacts using home visit worksheets.
2. Medical interview of subject's parents/guardians to determine if any unsolicited AEs or SAEs occurred and if any concomitant medications or vaccines were taken / received since the last study visit.
3. Check any ongoing AEs and concomitant medications since the last study visit (Visit 2) and record the resolution date (the end date), if available, in the source documents and CRF.
4. Physical examination including assessment of vital signs (as in Visit 1).
5. Check criteria for blood draw delay as specified in section 4.4. If criteria for blood draw delay are applied, re-schedule blood draw.
6. Collection of blood sample (approximately 5 mL) for serology testing. Details regarding the volume of blood and testing to be performed are in section 7.

The investigator or a delegate should ensure that all information is recorded in the source documents and accurately transcribed in the eCRF as soon as possible after the subject's visit has been completed.

The subjects' parents/guardians will be reminded to contact the site in case of any questions and to contact the site immediately if the subject has a medical condition that leads to a hospitalization or an emergency room visit.

All subject's parents/guardians will be reminded to return to the clinic 84 days (-14/+14 days) after vaccination for Visit 4.

6.7.4 Visit #4 (Day 84 [-14/+14]): blood draw and second vaccination

Study subjects will return for follow-up evaluations to the clinical study site 84 days following the first vaccination. At this visit, investigator or delegate will perform the following procedures and evaluations in the following order:

1. Medical interview of subject's parents/guardians to determine if any SAEs occurred and if any concomitant medications or vaccines were taken / received since the last study visit.
2. Check any ongoing AEs and concomitant medications since the last study visit (Visit 3) and record the resolution date (the end date), if available, in the source documents and CRF.
3. Physical examination including assessment of vital signs (as in Visit 1).
4. Check criteria for blood draw delay as specified in section 4.4. If criteria for blood draw delay are applied, re-schedule blood draw and second vaccination.
5. Perform Rapid Diagnostic Test for malaria. In case of a positive result, vaccination should be postponed until at least five days after completing treatment as per national guidelines.
6. Collection of blood sample (approximately 5 mL) for serology testing. Details regarding the volume of blood and testing to be performed are in section 7.
7. Check any contraindication for study vaccination or criteria for vaccination delay as specified in sections 4.3 and 4.4.
8. If subject eligible for study vaccination, study vaccine will be administered by unblinded study nurse according to the procedures described in sections 5.3 and 6.5.2.
9. After vaccination, the subject will be observed for at least 30 minutes for any immediate post-vaccination reactions, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines. Solicited local and systemic AEs, any unsolicited AEs, vital signs and body temperature will be recorded. All safety data collected within 30 minutes post-vaccination should be recorded in the subject's source documents.

After all procedures have been completed, the investigator will remind the subject's parents/guardians about safety evaluation during daily home visits within 6 days following

second vaccination that will be performed by trained staff under investigator's supervision and documented on the home visit worksheets.

The investigator or a delegate should ensure that all information is recorded in the source documents and accurately transcribed in the eCRF as soon as possible after the subject's visit has been completed.

The subjects' parents/guardians will be reminded to contact the site in case of any questions and to contact the site immediately if the subject has a medical condition that leads to a hospitalization or an emergency room visit.

All subject's parents/guardians will be reminded of study visit 7 days (+3 days) after the second vaccination (Visit 5), which will be conducted by a designated medical staff at the site clinic or the subject's home.

6.7.5 Visit #5 (7 days after Visit 4 [+3]): reactogenicity assessment

Study subjects will return for follow-up evaluations at study site 7 days following the second vaccination. Alternatively, a home visit may be performed by a medically qualified site personal. At this visit, investigator or delegate will perform the following procedures and evaluations in the following order:

1. Review of solicited and unsolicited AEs collected during days 1-6 after second vaccination using home visit worksheets.
2. Assessment of any ongoing solicited AEs 7 days post second dose, including measurement of body temperature (note that all ongoing solicited AEs beyond day 7 must be followed up daily by site staff until resolution).
3. Medical interview of subject's parents/guardians to assess any unsolicited AEs, SAEs since previous study visit.
4. Collection of concomitant medications and vaccinations.
5. Physical examination including assessment of vital signs (as in Visit 1).

The investigator or a delegate should ensure that all information is recorded in the source documents and accurately transcribed in the eCRF as soon as possible after the subject's visit has been completed.

The subjects' parent/guardian will be reminded to contact the site in case of any questions and to contact the site immediately if the subject has a medical condition that leads to a hospitalization or an emergency room visit.

The investigator will remind the subject's parents/guardians about two weekly safety evaluations that will be performed by trained site staff at subject's home or by phone. Information about unsolicited AEs will be documented on the home visit worksheets.

All subject's parent(s)/guardian(s) will be reminded to return to the clinic 28 days (0/+14 days) after the second vaccination for Visit 6.

6.7.6 Visit # 6 (28 days after Visit 4 [+14]): blood draw

Study subjects will return for follow-up evaluations to the clinical study site 28 days following the second vaccination. At this visit, investigator or delegate will perform the following procedures and evaluations in the following order:

1. Review of any unsolicited AEs collected during weekly contacts using home visit worksheets.
2. Medical interview of subject's parents/guardians to determine if any unsolicited AEs or SAEs occurred and if any concomitant medications or vaccines were taken / received since the last study visit.
3. Check any ongoing AEs and concomitant medications since the last study visit (Visit 5) and record the resolution date (the end date), if available, in the source documents and eCRF.
4. Physical examination including assessment of vital signs (as in Visit 1).
5. Check criteria for blood draw delay as specified in section 4.4. If criteria for blood draw delay are applied, re-schedule blood draw.
6. Collection of blood sample (approximately 5 mL) for serology testing. Details regarding the volume of blood and testing to be performed are in section 7.

The investigator or a delegate should ensure that all information is recorded in the source documents and accurately transcribed in the eCRF as soon as possible after the subject's visit has been completed.

The subjects' parents/guardians will be reminded to contact the site in case of any questions and to contact the site immediately if the subject has a medical condition that leads to a hospitalization or an emergency room visit.

All subject's parents/guardians will be reminded to return to the clinic 84 days (-14/+14 days) after the second vaccination for Visit 7.

6.7.7 Visit # 7 (84 days after Visit 4 [-14/+14]): study completion

Study subjects will return to the clinical study site 84 days following the second vaccination for the end of study visit. At this visit, investigator or delegate will perform the following procedures and evaluations in the following order:

1. Medical interview of subject's parents/guardians to determine if SAEs occurred and if any concomitant medications or vaccines were taken / received since the last study visit.
2. Check any ongoing AEs and concomitant medications since the last study visit (Visit 6) and record the resolution date (the end date), if available, in the source documents and eCRF.
3. Physical examination including assessment of vital signs (as in Visit 1).

The investigator or a delegate should ensure that all information is recorded in the source documents and accurately transcribed in the eCRF as soon as possible after the subject's visit has been completed.

After collection of safety information, the site staff will review the plan of when information relating to the subject's participation in the study may be available (e.g., study results and treatment assignments). 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's parent/guardian chooses to share this information.

After this visit, the subject's participation in the study will be completed. Source records will be completed and all information will be recorded in the eCRF (including "Study Termination" page).

6.8 Subject Withdrawal Criteria

A subject may discontinue study participation at any time prior to the last planned study visit. This is referred to as premature withdrawal from the study (see below for a description of withdrawal from study vaccine which refers to those subjects who do not receive the second vaccination but continue in the study for safety follow-up and/or other procedures).

From the analysis perspective, a 'premature withdrawal' from the study refers to any subject who was not available for the termination visit foreseen in the protocol (Visit 7). A subject is considered a 'premature withdrawal' from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected from this subject from the date of premature withdrawal / last contact.

The reasons for premature withdrawal from the study include:

- Adverse event
- Death
- Withdrawal of consent
- Lost to follow-up
- Administration reason
- Protocol deviation
- Other

NOTE: Before entering any alternate category as the reason for the subject's discontinuation from the study, the investigator should make every effort to investigate whether or not safety concerns (adverse event or death) may have been related to the subject's discontinuation from the study. If a safety concern has been associated with the subject's discontinuation, this must be described on the Termination eCRF page, even if it is not the primary reason for the subject's discontinuation.

Adverse event as reason for premature study withdrawal:

For any subject withdrawing from study participation prior to Visit 7 (study completion 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 CRF page by indicating "Withdrawn from study due to AE".

Death as reason for premature study withdrawal:

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

Withdrawal of consent as reason for premature study withdrawal:

The parents/guardians 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 parent/guardian 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 complete withdrawal from the study by the subject is specified, no further study interventions will be performed with the subject.

Date of Subject Termination:

The date of termination is the date of the last contact (clinic or home visit or telephone contact) 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.

Lost to follow-up as reason for premature study withdrawal:

For subjects who fail to show up for scheduled visits (clinic or home visit), study staff are encouraged to make at least three documented attempts to contact the subject's parents/guardians and encourage the completion of study termination procedures. These efforts to contact the subject's parents/guardians should be recorded in the source documents. The termination date for the subject to be captured on the Termination eCRF page is the date of the last successful visit (clinic or telephone) with the subject.

Administrative reason as reason for premature study withdrawal:

For subjects who are withdrawn from the study due to sponsor decision (e.g., meeting pre-specified withdrawal criteria or termination of study by the sponsor), this reason should be noted in the Termination eCRF page and any ongoing AEs at the time of study withdrawal must be followed until resolution/stabilization.

Protocol deviation as reason for premature study withdrawal:

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. 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 Termination eCRF page.

Any ongoing AEs at the time of study withdrawal must be followed until resolution/stabilization.

If a subject is withdrawn prematurely from the study for a reason other than those outlined above, this reason must be documented in the Termination eCRF page.

In addition, subject may be "withdrawal of study vaccination". Subjects may be withdrawn from the second study vaccination for several reasons including but not limited to: AE related to earlier vaccinations or failure to meet criteria for repeat vaccination (see section 4.3). Subjects who are

withdrawn from study vaccination should be encouraged to continue in the study for safety follow-up and other procedures as appropriate until the scheduled termination visit (Visit 7).

Withdrawn subjects will not be replaced.

The sponsor or the investigator (following consultation with the sponsor) has the right to discontinue this study at any time. If the clinical study is prematurely terminated, the investigator is to promptly inform the study subjects and local EC/IRB 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.

6.9 Prior and Concomitant therapy

6.9.1 Prior Medications and Vaccines

The following are considered prior medications for this protocol:

- any investigational or non-registered product (drug or vaccine) received prior to enrolment;
- any vaccine administered prior to enrolment;
- any immunosuppressant or other immune-modifying drug including systemic steroids, within 3 months prior to enrolment;
- any blood, blood product and/or plasma derivative or any parenteral immunoglobulin preparation within 3 months prior to enrolment;
- any systemic antibiotic within 3 days prior to enrolment;
- Any antipyretic/analgesic within 24 hours prior to first vaccination.

If these medications were administered to the subject within the specified window prior to the first study vaccination, they must be recorded on the Concomitant Medications page of eCRF.

6.9.2 Concomitant Medications and Vaccines

At each study visit, the investigator will ask the parents about any prescription or over-the-counter medication(s) taken since the last visit. Any medications taken at any time during the study period must be recorded on source documents and the case report form with trade and/or generic name, indication, dose, start and end dates.

Any treatments and/or medications specifically contraindicated, e.g., any investigational or non-registered product, any immunosuppressant and immune-modifying drug including systemic steroids, any immunoglobulin and blood product should be checked at each study visit subsequent to the study vaccination. If any became applicable during the study, it will not require withdrawal of the subject from the study but may determine a subject's evaluability in the per-protocol analysis. See section 9.4 for definition of study populations to be evaluated.

Any vaccine not foreseen in the study protocol in the period starting at Visit 1 (Day 0) and ending at Visit 7 must be recorded in the eCRF.

6.9.3 Prophylactic antipyretics / analgesics

The use of prophylactic medications for potential adverse reactions during the study period will be discouraged.

The use of antipyretics and/or analgesic medications within 24 hours prior to the study vaccination must be recorded in eCRF. The administration of antipyretics / analgesics within 7 days after study vaccination must be identified and the reason for their use (prophylaxis versus treatment) must be described in the source documents and Concomitant Medications eCRF.

Medications taken for prophylaxis are those administered in the absence of any symptom and intended to prevent the onset of post-vaccination symptoms. Medications taken for treatment are intended to reduce or eliminate the symptoms that are present.

7 ASSESSMENTS OF IMMUNOGENICITY

The functional measure of immunogenicity used in this study, Serum Bactericidal Activity (SBA) Assay, is a measure of the ability of antibodies, in concert with complement, to kill meningococci. Two serum bactericidal assays will be employed in this study: SBA with rabbit complement (rSBA) and SBA with exogenous human complement (hSBA) for *N. meningitidis* serogroups A, C, W, Y and X test strains.

The SBAs used to measure immunogenicity in this study are standard, i.e., widely used and generally recognized as reliable, accurate, and relevant (able to describe the quality and extent of the immune response).

However, the primary assay selected for this study will be the rSBA assay. A validated method will be used for groups A, C, Y and W. The assay for group X will be validated using samples from the Phase I clinical trial.

In recent years, Serum Bactericidal Activity using human complement (hSBA) is deemed to represent a more specific assay for measuring high avidity functional antibody, although a good correlation is shown with SBA using rabbit complement.[32] However, complement pooling and validation steps for hSBA imply increased complexity and costs. Hence hSBA in this study will be pursued only as an exploratory endpoint. The laboratory to conduct hSBA analysis has not been identified at this stage.

7.1 Specification of the immunogenicity parameters

The measures of immunogenicity using rSBA assay will be rSBA GMTs, the percentages of subjects who achieve rSBA titers ≥ 8 and ≥ 128 and the percentage of subjects with four-fold rises in rSBA titer against serogroups A, C, W, Y and X.

A post-vaccination rSBA titer ≥ 8 is used as an accepted correlate of protection against invasive meningococcal disease caused by serogroup C.[33]The rSBA titer ≥ 8 has been considered a surrogate level of short term protection and a titer ≥ 128 a surrogate of long term protection.[34]

Immunogenicity testing for meningococcal polysaccharide A, C, Y, W, and X specific antibodies using rSBA assay will be performed on sera collected at baseline (Visit Day 0), at one and three months after the first vaccination (Visits Day 28 and Day 84) and at one month after the second vaccination (Visit Day 112).

The measures of immunogenicity using hSBA assay will be hSBA GMTs, the percentages of subjects who achieve hSBA titers ≥ 8 and the percentages of subjects with hSBA seroresponse against serogroups A, C, W, Y and X.

A post-vaccination hSBA titer ≥ 8 is used as an accepted correlate of protection against invasive meningococcal disease.[35, 36]

Immunogenicity testing using hSBA assay will be conducted in a randomly selected subset of subjects (50 subjects per arm) at baseline (Visit Day 0), at one month after the first vaccination (Visit Day 28) and at one month after the second vaccination (Visit Day 112).

Table 7.1-1: Immunological readouts and priority testing

Study Visit	Sample time point	Group	Planned Number of subjects	Assay (serogroup)	Priority Rank
Visits Day 0, Day 28, Day 84 and Day 112	Baseline, 1 month post-Dose 1, Pre-Dose 2, 1 month post-Dose 2	All groups	375	rSBA (serogroup A)	1
			375	rSBA (serogroup Y)	2
			375	rSBA (serogroup X)	3
			375	rSBA (serogroup C)	4
			375	rSBA (serogroup W)	5
Visits Day 0, Day 28 and Day 112	Baseline, 1 month post-Dose 1, 1 month post-Dose 2	All groups	150	hSBA (serogroup A)	6
			150	hSBA (serogroup Y)	7
			150	hSBA (serogroup X)	8
			150	hSBA (serogroup C)	9
			150	hSBA (serogroup W)	10

Collected samples may be used in other assays, for test improvement or development of analytical methods related to the study vaccines or the disease under evaluation to allow a more reliable measurement of the vaccine response. Any sample testing will be done in line with the study protocol and with the consent of the individual subject's parent / guardian.

7.2 Methods for processing, labelling and storage of serum samples

An approximately 5 mL sample of blood will be drawn from all subjects at Visits Day 0, Day 28, Day 84 and Day 112. The blood samples should be collected before the study vaccination when applicable. The blood volume of 5 mL at each time point is needed in order to provide the necessary serum volume (approximately 40% of the blood draw volume) for the serology assays.

In order to minimize pain an anesthetic cream or patch (e.g. EMLA adhesives or cream) may be used at the site of blood sample draw, according to local practice. Not more than two attempts should be made to draw the required volume of blood.

The blood will be processed and aliquoted according to the Clinical Specimen Lab Manual. All aliquots will be stored at a temperature of -20°C or below. Each serum tube will be labeled with the labels provided by the Sponsor. Serum samples will be sent to the Sponsor or will be collected by a representative of the Sponsor.

Serum samples will be labeled by a code that only the study site can link to the subject name. All stored research samples will be logged into a secure database. Any use of the samples will be documented. Complete instructions for labeling and storage of serum samples are included in the Clinical Specimen Lab Manual, which is stored in the Investigator Site File.

Testing of samples will be performed at the Vaccine Evaluation Unit at Public Health England (PHE) in Manchester UK or a delegate laboratory. Samples may be stored at several different repositories and laboratories to complete the analyses required to meet study primary, secondary and exploratory analyses.

Samples will be retained in accordance with regulatory guidance for retention of essential study documents as described in section 12. Subjects will be informed and asked to agree to long-term storage of specimens for use in future research through a specific consent form. At study closure, PATH and SIIPL will designate a storage facility for the retention of the consented samples.

Should future research on the samples be initiated after study closure, a specific new study protocol will be developed and submitted to relevant Scientific and Ethics Committees following the informed consent requirements.

7.3 Biohazard Containment

As transmission of blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as recommended by the United States Centers for Disease Control and Prevention (CDC). All biological specimens will be transported using packaging mandated by 42 CFR Part 72. All dangerous goods materials, including diagnostic specimens and infectious substances, must be transported according to instructions detailed in the International Air Transport Association (IATA) Dangerous Goods Regulations.

Biohazardous waste will be contained according to institutional, transportation/carrier, and all other applicable regulations.

8 ASSESSMENT OF SAFETY

8.1 Specification of safety parameters

Safety assessment includes occurrence of solicited local and systemic adverse reactions within 7 days of each vaccine dose; unsolicited adverse events within 28 days after each vaccine dose, AEs leading to withdrawal and SAE throughout the entire study period.

8.2 Solicited adverse events

The term “reactogenicity” refers to selected signs and symptoms (“adverse events”) occurring in the hours and days following a vaccination, to be collected at the study site at 30 (+15) minutes

post vaccination and by site staff during home visits for 6 consecutive days, using pre-defined home visit worksheets (i.e., solicited adverse events).

The following adverse events are included in the home visit worksheet. Each adverse event is to be assessed using severity grading scale based on the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events provided in Appendix II. The grades are from Mild (Grade 1) to Life Threatening (Grade 4). In case a severe solicited AE is reported, the subject will be further assessed by the healthcare professional and the intensity score will be verified.

The presence of any ongoing solicited AEs will be assessed by healthcare professional at site or home visit 7 days after each study vaccination. In case of ongoing solicited AEs, the site staff will continue contacting the subject daily until AE resolution. The last day of solicited symptom presence and the maximum intensity beyond 7 days post-vaccination will be recorded.

Following are the local and systemic solicited AEs which are collected during study.

Solicited local reactions: tenderness, swelling/induration.

Solicited systemic reactions: irritability, drowsiness, decrease eating, vomiting, and fever.

8.3 Unsolicited Safety Measurements

8.3.1 Unsolicited Adverse Events

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.

As a consistent method for collecting unsolicited AEs, the subject's parents / guardians should be asked a non-leading question such as:

'Has your child acted differently or felt different in any way since receiving the vaccine or since the last study visit?'

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital records, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an unsolicited AE/SAE on the eCRF or SAE Report screens as applicable.

The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

The severity of AEs will be determined by the investigator. The AEs will be graded from Mild (Grade 1) to Death (Grade 5) based on the *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events* provided in Appendix II.

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. 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.

The relationship of the study treatment to an unsolicited AE will be determined by the investigator. Solicited AEs will not be evaluated for relationship to study vaccine – all solicited AEs will be considered to be causally related to vaccination.

The causality assessment is made on the basis of the available information at the reporting time point. Assessment of causality can change according to follow-up information.

The actions taken in response to an unsolicited AE will be coded as below. One or more of these actions may be selected:

0 - No action taken

1 - Study vaccine temporarily interrupted

2 - Study vaccine permanently discontinued due to this adverse event

3 - Concomitant medication taken

4 - Non-drug therapy given

5 - Physician visit

6 - Hospitalization/prolonged hospitalization

Outcome of any unsolicited AE reported during the study period will be assessed as:

- Recovered/resolved
- Recovering/resolving
- Not recovered/not resolved
- Recovered with sequelae/resolved with sequelae
- Fatal (SAEs only)

Please note: any solicited adverse event that meets any of the following criteria must also be entered as an adverse event on the Adverse Event eCRF:

- 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.
- Solicited local or systemic adverse event that otherwise meets the definition of a serious adverse event.

Treatment of any AE is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of an AE should be recorded in the subject's eCRF.

8.3.2 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 SAE form as well as on the AE eCRF. All SAEs will be evaluated by the investigator for relationship of the event to study vaccination.

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

- Related/suspected: the SAE is judged by the investigator to be related to the study vaccine on the AE eCRF page;
- 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.

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 eCRF. If the onset of an event occurred before the subject entered the trial (e.g., any pre-planned hospitalization for a chronic condition 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 trial or was necessary due to a worsening of the pre-existing condition.

8.3.3 Methods for Assessing and Recording AEs and SAEs

The period of observation for AEs extends from the time the subject signs informed consent until he or she completes the final study visit (Visit 7) or terminates the study early. AEs occurring after the informed consent form is signed but prior to receiving study vaccine will be documented as an adverse event and recorded on the Adverse Events eCRF and within source documents. However, 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).

All AEs meeting criteria for reporting, regardless of severity, will be monitored by the investigator until resolution or stabilization. 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. All findings must be reported on an Adverse Events eCRF and on the SAE form, if necessary, which is part of the Investigator Site File. All findings in subjects experiencing AEs must be reported also in the subject's medical records.

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** by telephone, email or fax to PAREXEL. Contact details for submitting SAEs to PAREXEL and instructions for completion of documentation will be provided in a handout located in the Investigator Site File.

The SAE form will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding an SAE, the SAE should still be submitted within 24 hours. Once additional relevant information is received, the SAE form should be updated **within 24 hours**. The investigator will always provide an assessment of causality at the time of the initial report.

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

After receipt of the initial report, representatives of sponsor 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 his/her corresponding EC/IRB or applicable regulatory authorities in accordance with institutional policy/regulatory requirements and adequate documentation of this notification must be provided to the sponsor.

The Sponsor and its designee must also comply with the applicable regulatory requirement(s) related to the reporting of Suspected Unexpected Serious Adverse Reactions (also referred to as "SUSARs") to the regulatory authority(ies) and the IRB/EC. If a SUSAR or other safety signal relating to use of one of the study vaccines is reported to the sponsor 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/IRB and other relevant authorities.

8.4 Safety Laboratory Measurements

This study has no safety laboratory measurements.

8.5 Data and Safety Monitoring Board

An independent Data and Safety Monitoring Board will be set up for periodic review of safety data during the study conduct. The composition and responsibilities of DSMB members are presented in the DSMB charter.

8.6 Study pause

The following study pause rules will pause or halt further vaccinations. However subjects already enrolled will continue to be followed for safety during the pause. These pause rules refer to suspected adverse reactions and will be triggered if any of the events described below are met during the conduct of the study:

- Rule 1: 1 or more subjects experience any vaccine-related Grade 4 AE or SAE.
- Rule 2: 1 or more subjects experience the following Grade 3 or greater local reaction classified as related to vaccination by the PI: ulceration, necrosis, or sterile abscess at the injection site requiring drainage or surgical intervention.
- Rule 3: ≥ 10.0% of unique subjects experience the same vaccine-related Grade 3 general solicited reactogenicity after vaccination, with severity and relatedness confirmed by the PI. In the case of fever, the episode must last longer than 24 hours and occur within 1 week post vaccination, and be confirmed by the PI without evidence of other medical causes (e.g., malaria, acute gastroenteritis). *Note: the 10.0% threshold will be calculated on an ongoing basis to adjust for a changing number of enrolled subjects.

8.6.1 Study pause procedure

If pause criteria have been met, the Sponsor Pharmacovigilance team (SIIPL, PATH & PAREXEL Medical Monitors) will be notified by the PAREXEL Pharmacovigilance Center and a meeting will be convened with the Principal Investigator for an *ad hoc* review.

- If the PI (or designee), the PAREXEL medical monitor, or any member of the Sponsor medical team identifies that a pause criterion may have been met or propose the study be paused on a discretionary basis, all vaccinations and enrollment will be suspended. The Sponsor Pharmacovigilance team will be notified and will expeditiously (within 48 hours) convene to review all available, relevant information with the PI.
- The Pharmacovigilance team and the PI reviews will be summarized with consensus decision whether the study should continue without change, be modified, or be stopped.
- The DSMB will be notified if a pause rule has been met and will be kept informed of sponsor decision. DSMB may be asked to review pause reasons and events.
- If at any time, a decision is made to discontinue administration of study product in all subjects, expeditious notification will be provided by the Sponsor to the NRA and by the PI to the IEC within 48 hours.
- If the Sponsor with the PI decides to re-start the study after review, enrollment and vaccination may resume. The IEC will be notified of this decision.

9 STATISTICAL CONSIDERATIONS

9.1 Overview and General Considerations

This study is a Phase 2 observer-blind randomized, controlled clinical trial to assess the safety and immunogenicity of the adjuvanted and non-adjuvanted formulations of polyvalent conjugate vaccine composed of serogroups A, C, Y, W, and X *Neisseria meningitidis* capsular polysaccharides (NmCV-5). The primary objective is to evaluate the reactogenicity of adjuvanted and non-adjuvanted formulations of NmCV-5 vaccine in comparison with the licensed comparator (Menactra®). The secondary objectives are to assess safety and the immune response of adjuvanted and non-adjuvanted formulations of NmCV-5 vaccine.

A detailed statistical analysis plan for preparation of the final study report will be created and made final prior to database lock and unblinding. All statistical analyses will be performed using SAS® software Version 9.3 or later.

Medical history and AEs will be coded using the current version of the Medical Dictionary for Regulatory Activities (MedDRA). The frequency count and percentage of subjects will be summarized according to the coded terms of system organ class and preferred term. Subject-wise data listing will be provided.

9.2 Randomization scheme

The randomization scheme will be maintained by the Statistics and Product Support Services (SPSS) at PAREXEL. Subjects will be enrolled into the study and randomized as per a pre-defined randomization list. The randomization list will include treatment assignment for subjects. It will be generated electronically by SPSS at PAREXEL and a print out of the same will be provided only to the designated unblinded site personnel.

9.3 Sample size and power

With 150 enrolled subjects per NmCV-5 arm and 75 subjects in the MenACWY-D arm, the study has a power of 80% to detect a 15.4% difference in proportion of subjects with severe solicited AEs reported within 7 days after any study vaccination between each NmCV-5 group and the MenACWY-D group, assuming the rate of severe reactions in the MenACWY-D group of 5%, a drop-out rate of 10%, and alpha of 0.05.

Power to show comparability of immune response in terms of the percentage of subjects with antibody titer greater than or equal to the cut-off level, based on various group sizes and equal expected underlying percentages, was done using one-sided Miettinen and Nurminentest [21] and is provided in Table 9.3-1.

Assuming the expected proportion of subjects with antibody response greater than or equal to the cut-off (e.g. rSBA titer ≥ 8) is at least 95% for each of the vaccine serogroups in the NmCV-5 non-adjuvanted and MenACWY-D groups, then with 135 / 67 evaluable subjects per group, the study has power of 86% to demonstrate that the lower limit (LL) of 95% confidence interval (CI) for difference between the non-adjuvanted NmCV-5 group and MenACWY-D group is above -10% (commonly accepted non-inferiority margin) for each serogroup. With the same assumptions, the overall power to demonstrate that the lower limit (LL) of 95% confidence interval (CI) for difference between the non-adjuvanted NmCV-5 group and MenACWY-D group is above -10% for all serogroups would be at least 46%.

Table 9.3-1: Power to show comparability of immune response in terms of the percentage of subjects with antibody titer greater than or equal to the cut-off level, based on various group sizes and equal true underlying percentages

Expected percentage of subjects who achieve titers ≥ cut-off	Number of evaluable subjects per group	Power to demonstrate that the LL of 95% CI of the difference between groups is > -10% for an individual serogroup	Overall power to demonstrate that the LL of 95% CI of the difference between groups is > -10% for all serogroups
85%	120 vs. 60	47%	2%
	135 vs. 67	52%	4%
	150 vs. 75	56%	5%
90%	120 vs. 60	61%	8%
	135 vs. 67	65%	12%
	150 vs. 75	70%	17%
95%	120 vs. 60	81%	36%
	135 vs. 67	**86%**	**46%**
	150 vs. 75	89%	56%

Power to show that the ratio of rSBA GMT in the adjuvanted NmCV-5 group to that in the non-adjuvanted NmCV-5 group is at least 2 was calculated using a two-sample t-test according to different expected rSBA GMTs in the non-adjuvanted NmCV-5 group and assumed standard deviations (SD) of log2-transferred rSBA titers and is provided in Table 9.3-2.

Table 9.3-2: Power to detect at least a 2-fold increase of rSBA GMTs after a 2-dose series with the adjuvanted NmCV-5 vs. non-adjuvantedNmCV-5, based on various group sizes and different assumed SD of log 2 rSBA titers

Expected rSBA GMTs in non-adjuvanted NmCV-5 group at 1 month post-Dose 2	Assumed SD of log2 rSBA titers	Number of evaluable subjects per group	Power to detect at least a 2-fold rSBA GMTs in adjuvanted NmCV-5 vs non-adjuvanted NmCV-5 for an individual serogroup	Overall power to detect at least a 2-fold rSBA GMTs in adjuvanted NmCV-5 vs non-adjuvanted NmCV-5 for all serogroups
500 to 1500 for various serogroups	1.5	150 vs. 150	>99%	> 99%
		135 vs. 135	>99%	> 99%
		120 vs. 120	>99%	> 99%
	2.0	150 vs. 150	99%	95%
		135 vs. 135	98%	92%
		120 vs. 120	97%	86%
	2.5	150 vs. 150	93%	70%
		135 vs. 135	**91%**	**61%**
		120 vs. 120	87%	50%
	3.0	150 vs. 150	82%	37%
		135 vs. 135	78%	29%
		120 vs. 120	73%	21%
	3.5	150 vs. 150	69%	16%
		135 vs. 135	65%	11%
		120 vs. 120	60%	8%

Assuming the true standard deviation of log2 rSBA titers is below than or equal to 2.5 for each of the five vaccine serogroups, with 135 evaluable subjects per group, the study has power of 91% to detect a 2-fold rSBA GMT increase in the adjuvanted NmCV-5 vs non-adjuvanted NmCV-5 for each serogroup. With the same assumptions, the overall power to detect a 2-fold rSBA GMT increase in the adjuvanted NmCV-5 vs non-adjuvanted NmCV-5 for all serogroups would be at least 61%.

9.4 Analysis Populations

9.4.1 Enrolled Population

All screened subjects who provide informed consent and received a subject ID, regardless of the subject's randomization and treatment status in the trial.

9.4.2 Exposed Population

All subjects in the enrolled population who receive a study vaccination.

9.4.3 Immunogenicity Populations

Immunogenicity analyses will be performed on both the Full Analysis (FA) Population and Per Protocol (PP) Population.

Full Analysis Population

All subjects in the enrolled population who were randomized, received a study vaccination, and provide an evaluable serum sample at least at one time point post-vaccination.

The analysis based on this population will serve as supportive results for all secondary immunogenicity objectives.

Subjects in the FA population will be analyzed “as randomized” (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received).

Per Protocol Population

All subjects in the FA Population who correctly received two doses of study vaccine per randomization with no major protocol violations that are determined to potentially interfere with the immunogenicity assessment of the study vaccines. This population will serve as the primary analysis population for all immunogenicity objectives.

Due to unpredictability of some irregularities, the criteria for exclusion of subjects from the Per Protocol Population will be determined before the database is locked and will be based on the blind review of protocol violations.

9.4.4 Safety Population

All subjects in the enrolled population who received a study vaccination and had any safety data available.

Subjects will be analyzed as "treated" (i.e., according to the actual vaccine received at the first dose). All safety analyses will be performed using this population. The denominators for different safety endpoints may vary according to the number of subjects with available data for the specific endpoint. For example, the solicited adverse event endpoints will be based only on those who have the corresponding CRF data regardless of other safety follow-up data.

9.5 Analysis Plan

9.5.1 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 by overall and by vaccine group.

Distributions of subjects by gender, race and ethnicity will be summarized overall and by vaccine group.

Using the World Health Organization Drug Dictionary (WHO DD), prior and concomitant medications will be tabulated by drug classification, preferred drug name and study group.

9.5.2 Statistical Methods for Primary Objective

Precision estimates of the percentages of subjects with at least one severe solicited AE reported within 7 days after any vaccination will be computed by vaccine group using the two-sided 95% Clopper-Pearson confidence intervals.[37]

The differences between groups (Group NmCV-5_non-adjuvanted - Group MenACWY-D and Group NmCV-5_adjuvanted - Group MenACWY-D) in the percentages of subjects with at least one severe solicited AE reported within 7 days after any vaccination will be provided along with their two-sided 95% CIs obtained by the Miettinen and Nurminen method.[38]

The analyses of primary endpoint will be descriptive.

9.5.3 Statistical Methods for Secondary Objectives

9.5.3.1 Secondary Safety Objectives

The design of the study allows a comparison among groups after each vaccination and after any vaccination.

9.5.3.1.1 Analysis of Extent of Exposure

The extent of exposure for subjects in each study group will be described.

9.5.3.1.2 Analysis of Solicited Adverse Events

All solicited adverse events will be summarized according to defined severity grading scales.

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. The difference between groups (Group NmCV-5_non-adjuvanted - Group MenACWY-D and Group NmCV-5_adjuvanted - Group MenACWY-D) in percentage of subjects with at least one local or systemic adverse event overall and at each time point will be calculated along with their two-sided 95% CIs obtained using the Miettinen and Nurminen method.

Post-vaccination solicited adverse events reported from Day 0 to Day 6 and from Day 84 to Day 90 will be summarized by maximal severity and by vaccine group. Separate analysis will be performed for solicited AEs reported 30 minutes after vaccination. All the solicited reactions occurring up to 7 days after each vaccination will be summarized according to severity grading based on the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events provided in Appendix II.

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

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 separately according to the 2 schemes described below and will be broken down according to route of measurement:

- by 0.5 °C increments from 36.0°C up to ≥40°C
- <36.0, ≥36.0-<37.0, ≥37.0-<38.0, ≥ 38.0-<39.0, ≥39.0-<40, ≥40°C

9.5.3.1.3 Analysis of Unsolicited Adverse Events

All the adverse events occurring during the study, judged either as related, or not related to vaccination by the investigator, will be recorded.

The original verbatim terms used by investigators to identify adverse events in the eCRFs 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 related to study vaccine, will be summarized according to system organ class and preferred term within system organ class. When an adverse event occurs more than once for a subject, it will be considered as multiple events and each event will be presented distinctly.

The summaries will be presented by period of onset and will include frequency distributions of the different adverse events:

- All unsolicited AEs reported within 28 days after each vaccination;
- AEs leading to premature withdrawal from the study during the entire study period;
- SAEs reported during the entire study period.

The analysis of unsolicited adverse events comprises the following categories:

- Any unsolicited AEs;
- Related unsolicited AEs;
- SAEs;
- Related SAEs;
- Unsolicited AEs leading to withdrawal from the study;
- Unsolicited AEs leading to withdrawal from study vaccination but remaining in the study;
- Unsolicited AEs leading to hospitalization;
- Any AEs leading to death.

Data listings of all adverse events will be provided by subject.

9.5.3.2 Analysis of Secondary Immunogenicity Objectives

For each *N. meningitidis* serogroup the percentages of subjects with rSBA titers ≥ 8 and rSBA titers ≥ 128 and the corresponding exact two-sided 95% CIs based on Clopper-Pearson method will be calculated for each study group at baseline, one and three months after the first vaccination and one month after the second vaccination.

The rSBA titers at each visit for each study group will be logarithmically transformed (base10) to fulfill the normal distribution assumption. For each *N. meningitidis* serogroup, the GMTs and GMRs (post vaccination / baseline) will be calculated with their associated two-sided 95% CIs, by exponentiating the corresponding log-transformed means and their 95% CIs.

Percentages of subjects with four-fold titer rise and the corresponding exact two-sided 95% CIs based on the Clopper-Pearson method against these serogroups will be calculated for each study group at one month after the first vaccination and one month after the second vaccination (Visit Day 28 and Visit Day 112). The ratio of GMTs and GMRs between the study groups and the corresponding CI will be constructed by exponentiating the mean difference and the confidence limits in log10 (titer), using ANOVA. The difference in percentages between groups will be provided along with the two-sided 95% CIs that will be constructed using the method of Miettinen and Nurminen. In addition, a reverse cumulative distribution plot of each measure will be created.

9.5.4 Statistical Methods for Exploratory Immunogenicity Objective

For each *N. meningitidis* serogroup the percentages of subjects with hSBA titers ≥ 8 and the corresponding exact two-sided 95% CIs based on Clopper-Pearson method will be calculated for each study group at baseline, one month after the first vaccination and one month after the second vaccination.

The hSBA titers at each visit for each study group will be logarithmically transformed (base10) to fulfill the normal distribution assumption. For each *N. meningitidis* serogroup, the GMTs and GMRs (post vaccination / baseline) will be calculated with their associated two-sided 95% CIs, by exponentiating the corresponding log-transformed means and their 95% CIs.

Percentages of subjects with hSBA seroresponse rise and the corresponding exact two-sided 95% CIs based on the Clopper-Pearson method against these serogroups will be calculated for each study group at one month after the first vaccination and one month after the second vaccination (Visit Day 28 and Visit Day 112). The ratio of GMTs and GMRs between the study groups and the corresponding CI will be constructed by exponentiating the mean difference and the confidence limits in log10 (titer), using ANOVA. The CIs for the rate difference will be constructed using the method of Miettinen and Nurminen.[38] In addition, a reverse cumulative distribution plot of each measure will be created.

For each N. meningitidis serogroup the percentages of subjects with rSBA ≥4, ≥16, ≥ 32, and ≥ 64 and the corresponding exact two-sided 95% CIs based on Clopper-Pearson method will be calculated for each study group at baseline, one and three months after the first vaccination and one month after the second vaccination.

Percentages of subjects with four-fold titer rise with criteria described in exploratory immunogenicity endpoints and the corresponding exact two-sided 95% CIs based on the Clopper-Pearson method against these serogroups will be calculated for each study group at one month after the first vaccination and one month after the second vaccination.

9.5.5 Handling of Dropouts and Missing Data

Missing data will not be imputed and will be analyzed as if they were randomly missing.

9.5.6 Multiplicity

Due to hypothesis generating nature of this study, all statistical comparisons for reactogenicity, safety, and immunogenicity endpoints will be carried out without an adjustment for multiple comparisons. All statistical tests except for non-inferiority will be two-sided with a type I error rate of 5%. The 95% confidence interval will be presented for estimations, as appropriate.

9.6 Analyses Sequence

Serology testing will be prioritized in the order of that rSBA testing of samples collected at baseline and 1 month after each study vaccination will be performed first and then rSBA testing of samples taken at 3 months after the first vaccination.

According to this order of testing, analyses will be performed stepwise as follows:

- An interim analysis including safety, reactogenicity and immunogenicity results (rSBA) at 1 month after the first vaccination will be performed. Individual listings will not be generated at this point and any access to subject-level information about study groups will be masked.

- The final safety, reactogenicity and immunogenicity analysis (rSBA) will be performed at 3 months after the second vaccination.

10 QUALITY CONTROL AND QUALITY ASSURANCE

10.1 Pre-study Documentation

Prior to enrolment of subjects at the study site, specific regulatory documents must be available, such as Institutional Ethics Committee (IECs) approvals; curriculum vitae for investigator and study staff; standard operating procedures (SOPs) and other essential documents. Sponsor/designee will inform the investigator which documents need to be provided according to the applicable regulatory requirements.

10.2 Monitoring

Sponsor monitoring responsibilities will be provided through qualified and appropriately trained individuals designated by PAREXEL to carefully monitor all aspects of the study. A site initiation visit will be conducted prior to the beginning of the study and monitoring will be conducted during and at closeout of the study by the study monitor.

During the course of the study, the monitors will visit the clinical sites at intervals in order to verify that:

- The data are authentic, accurate and complete
- The safety and rights of subjects are being protected
- The study is conducted in accordance with the approved protocol (and any subsequent amendment), GCP and all applicable regulatory requirements

Monitors will periodically contact the site and perform site visits. The extent, nature and frequency of site visits will be decided before the start of the study and will be based on considerations as study objectives, study design and complexity, and enrolment rate. During these contacts, the monitor will:

- Check and assess the progress of the study
- Review study data collected
- Perform source data verification, identify any issues and address their resolution

Monitoring will be conducted according to ICH-GCP. The individuals responsible for monitoring the study will have access to all records necessary to ensure the integrity/validity of the recorded data and will periodically review the progress of the study.

The investigator must agree to allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the monitor to discuss findings and any relevant issues.

The monitor must contact the site prior to the start of the study to discuss the protocol and data collection procedures with the site personnel.

The investigator should allow representatives of the Ethics Committee, Regulatory Authority and the sponsor to visit the study site.

10.3 Data Management and Processing

The site PI is responsible for ensuring the accuracy, completeness, and timelines of the data reported. Data collection is the responsibility of the clinical trial staff at the study site under the supervision of the site PI. PAREXEL is responsible for data management activities, including quality review, analysis and reporting of the study data according to SOPs.

Data Collection

Data will be entered electronically by site study staff over the Internet in eCRF. The data system includes password protection and internal quality checks, such as automatic range checks to identify data that appear inconsistent, incomplete or inaccurate. Instructions for use of the system are included in the eCRF User's guide.

Clinical data will be entered directly from the source documents. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. All the information required by the study protocol must be entered into eCRF. An explanation must be provided for any missing data. Source documentation supporting the eCRF data should document the dates and details of study procedures, AEs and subject status. The PI/site staff will maintain information in the eCRFs and all source documents that support the data collected from each subject.

The study monitor will check for completeneess and accuracy of eCRF during the monitoring visits.

Data Management Procedures

The site staff should complete the eCRFs as soon as possible after the information is collected. Completed eCRFs must be submitted for each screened subject who signs the study specific ICF. PAREXEL is responsible for data management activities, including quality review, analysis and reporting of the study data according to the SOPs.

Internal data quality checks such as automatic range checks, checks to identify data that appear inconsistent, incomplete or inaccurate are programmed into eCRF that does real time review of the data as and when clinical data is entered into the system by the site staff.

The Data Management team will review the data for quality and will provide several quality assurance reports to ensure that study data are clean and complete. Quality assurance reports will include, but are not limited to, the following: missing forms, missing values and out of range values, automated data queries, and manual review of study data. Data queries will be distributed to the sites at scheduled time period for the site staff to review and update the database.

Coding

All medical verbatim terms will be coded by a medical doctor according to MedDRA (adverse events, medical history and concomitant diseases) and the WHO Drug Dictionary enhanced version (concomitant medication).

Database Lock Procedures

Database will be locked upon completion of the following activities:

- All subjects have completed the follow up visits
- All the subjects data have been entered in the database
- All data anomalies have been resolved
- Study monitoring has been completed
- All the listings of the database have been reviewed and discussed for assessment of consistency and medical plausibility.

Procedures for Analysis

The data will be analyzed as per the **pre-specified Statistical Analysis Plan (SAP)** after the database lock**.** An audit trail will be kept of all subsequent changes to the data.

10.4 Study and Site Closure

Upon completion of the study, the monitor and the investigator will conduct the following activities:

- Data clarification and/or resolution
- Accounting, reconciliation and return to sponsor or destruction at sites of used and unused vaccines
- Review of site study records for completeness
- Return of all study data to Sponsors or designee.

Sponsors reserve the right to temporarily suspend or prematurely discontinue this study at any time for any other reason.

If the study is stopped or suspended prematurely, Sponsor will inform the investigator(s) as well as the regulatory authorities about the decision and the reasons for termination or suspension. If such action is taken, all effort must be made to ensure the safety of the subjects enrolled in the study. The investigator(s) will inform the responsible IECs/IRBs and provide the reason for the suspension or termination.

In case of premature study or study site closure, the monitor will conduct all activities as indicated above.

10.5 Audits and Inspections

For the purpose of compliance with ICH-GCP and regulatory guidelines, it may be possible that the sponsor/designee or a national regulatory authority may conduct a site audit/inspection. This may occur at any time from start to after conclusion of the study.

The investigator agrees to allow the auditor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor to discuss findings and any relevant issues.

If a regulatory authority requests an inspection, the investigator must inform the sponsor or its designee immediately about this request. The investigator(s) and the study coordinator(s) must make the relevant records available for inspection and must be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies. The sponsor will provide any needed assistance in responding to regulatory audits or correspondence.

11 REGULATORY AND ETHICAL REQUIREMENTS

11.1 Ethics committee review and communication

It is the investigator's responsibility to ensure that this protocol is reviewed and approved by the IECs responsible for the study sites. The IECs must also review and approve the Informed Consent Form and any other written information to be provided to the subject. Written IECs approval shall be obtained prior to study start.

No deviations from, or changes to, the protocol shall be initiated without prior written IEC approvals of an appropriate amendment, except when necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the study (e.g., change of monitor(s), telephone number(s)). The investigator shall provide to the sponsors a statement from the IEC confirming the IEC is organized and operates according to GCP and applicable laws and regulations.

11.2 Protocol Amendments

Any significant change in the study protocol shall be addressed in a written protocol amendment, which will be signed by the investigator(s) and the sponsors. It is the investigator's responsibility to submit protocol amendments to the IECs) and to obtain written approval where required.

A protocol amendment may be implemented after it has been approved by IECs. In the case of a protocol change intended to eliminate an apparent immediate hazard to subjects, the change may be implemented immediately. In this case, the change must be later documented in an amendment and reported to the IECs as soon as possible. Amendments affecting only logistical or administrative aspects of the study may not require formal IEC approval. Logistical and administrative amendments (e.g., concerning a change of telephone number) shall be submitted to the IECs for information purposes. However, the investigator must provide the sponsors with written verification that such logistical or administrative amendments are submitted to the relevant IECs.

11.3 Subject Information and Informed Consent

The investigator or his/her designee shall inform the parent of all pertinent aspects pertaining to the study. The process for obtaining the agreement of the parent for delay of vaccination and the informed consent of the parent for the child to participate in the study shall be in accordance with ICH GCP, the Declaration of Helsinki and all applicable regulatory requirements.

The investigator shall describe the study to the subject/parent. The investigator shall give the parent ample time and opportunity to inquire about details of the study and ask any questions.

In case of illiterate individuals, the study will be explained to them by the investigator or his/her designee and the Informed consent form(ICF) read for them in the presence of an impartial witness. The witness shall personally sign and date the consent form/agreement while a fingerprint will be requested from illiterate individuals.The process of agreement for delay of vaccination and informed consent for study participation should be described in the site procedures/source.

Original ICF must be kept on file by the investigator for possible inspection by IEC members, regulatory authorities and the sponsors (or their designees). The parent must receive a copy of the signed ICF, and any subsequent updates or amendments.

Prior to including any subject in the clinical study, his/her parent's/guardian's free and expressed informed consent must be obtained in writing. The written informed consent must be signed and dated by both investigator/designee and by the parent/guardian prior to any study related procedure. In case the parent/guardian is illiterate, he/she will affix a thumbprint on the consent form to confirm his/her consent. In this case, the impartial witness will also sign the informed consent to confirm the process has been soundly and transparently performed.

The study monitor shall check the documentation of the individual ICF during each monitoring visit.

11.4 Subject Confidentiality

The investigator(s) must ensure that subject confidentiality is maintained. Personal identifiers will not be included in any study reports. The subjects will be identified by the subject number and by subject initials. If a subject's name appears on any other document (e.g., pathologist report), it will be obliterated before the copy of the document is supplied to the sponsor. Study findings stored on a computer will be subject to local data protection laws. The parent will be informed that representatives of the sponsor, IEC, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in the strictest confidence.

11.5 Ethical conduct of the study

This study will be conducted in compliance with the approved clinical trial protocol, IEC and the following guidelines:

1. Declaration of Helsinki (Revised Fortaleza, 2013) (Appendix I).
2. ICH Harmonized Tripartite Guideline for Good Clinical Practice E6 (R2).
3. NRA guidelines.

11.6 Minimizing Risk

Delay of EPI MenAfriVac dose

This study requires a temporary delay in the MenAfriVac dose recommended to be received as part of the EPI in Mali at 9 months. This is necessary to ensure subjects enrolled at 12 months did not receive a Men A containing vaccine in order to evaluate the Men A component of the study vaccine.

Men A epidemics have practically disappeared in the region since the introduction of MenAfriVac in the 1 to 29 years age group in the West African Region. Mali's first Men A case since 2010 was reported in Kieneba district in March 2016. This case occurred in a 15 year old who had not been vaccinated as part of the campaign that took place in that district in 2011. However, pockets of disease have occurred, such as in neighbouring Guinea where the MenAfriVac campaign was delayed to 2015 due to the Ebola crisis.[16, 17, 25-28]

In February 2017, Mali introduced the MenAfriVac in the EPI schedule at 9 months for all children. In addition, from 5 to 14 June 2017, Mali conducted a National Catch-up Campaign for the unvaccinated cohorts 1 to 5 years of age. The reported coverage rates for the campaign were 104.7% nationally and 112% in the study area.[30, 31]

The recent implementation of these interventions lead to a new umbrella of protection (herd immunity effect) preventing the circulation of the Men A pathogen in the study area for years to come. In this context, the risk of Men A exposure falls dramatically close to zero for subjects deferring the MenAfriVac.

Nevertheless, all delayed subjects will be closely followed by the site with routine monthly checks to ensure that they are fine. Parents/guardian would be briefed on meningitis symptoms and any suspicion of meningitis would need to be reported to site for adequate treatment response following national guidelines. Delayed subjects would be scheduled to return to the clinic as soon as they will potentially meet the study criteria, hence delay will be limited to no more than 3 months.

In case a subject will not be enrolled in the study, the site will follow-up to ensure they are offered a dose of MenAfrivac to comply with the EPI schedule.

Safety Risks from Vaccine

As with all injectable vaccines, appropriate medical treatment and supervision should always be readily available in case of a rare anaphylactic event following the administration of the vaccine. The mainstay in the treatment of severe anaphylaxis is the prompt use of adrenaline, which can be lifesaving.

Post vaccination, the subject should remain under observation for no less than 30 minutes for occurrences of immediate or early allergic reactions. Corticosteroids and antihistaminics should also be available in addition to supportive measures such as oxygen inhalation and intravenous fluids (IV fluids).

Satisfactory safety and immunogenicity results were obtained after 3 months following a single dose in 40 healthy adults in the Phase I trial conducted in the USA (20 doses adjuvanted and 20 doses non-adjuvanted) allowing us to proceed to Phase II. However, this Phase II trial is the first time this vaccine will be administered to children. Specific pause criteria and a DSMB have been put in place to ensure adequate controls for early signal detection.

Risks from inadequate Men A protection

As previously discussed, the risk of Men A exposure has become extremely low. Nevertheless, the Menactra vaccine is a licensed vaccine conferring protection against Men A as does the MenAfriVac. However, there is no guarantee at this time that the NmCV-5 vaccine will provide adequate protection to immunized children against Men A. It is likely that immunogenicity results obtained after the second dose of this trial will be able to confirm the subjects' protection against Men A. In case, results are unsatisfactory, the site will ensure subjects who received NmCV-5 during the study will be offered a dose of MenAfriVac after the completion of the study.

Risk of inadequate protection against other Neisseria meningitidis strains
Currently, no multivalent conjugate meningitis vaccines are routinely available in Mali to prevent epidemic meningitis. It is worth noting that subjects participating in this study will receive a multivalent meningitis vaccine that could provide protection against other circulating strains of *Neisseria meningitidis.* In particular, strains that continue to cause epidemics in Sub-Saharan Africa, such as Men C, W, Y and X.
However, at this time, such benefit is guaranteed only for those in the control group receiving the licensed Menactra with established evidence of protection against Men C, W, and Y. The results from the Phase 1 study for NmCV-5 in adults are promising and include an additional strain: the Men X, but there is no guarantee that the results would replicate in the younger age group.

Risks from blood draw
Drawing peripheral blood can cause discomfort; it might cause minor bleeding and/or bruising where the needle enters the skin, and very rarely might cause infection. This risk will be mitigated by ensuring that only study staff members who are adequately trained in safe drawing of blood conduct this portion of the study. It is not anticipated that the risks in any one of the vaccine groups would be higher than others.

Risks from participating in a clinical trial
In addition to the risks described above, all clinical trial subjects are likely to experience increased inconvenience due to the visits as they may be of longer duration than standard treatment, which might cause more anxiety. They also incur additional risks to their privacy from participating in a clinical trial. This risk will be minimized by ensuring that subject confidentiality is maintained by enforcing appropriate data collection, storage, and analysis techniques and employing the use of unique identifiers with carefully stored linking files. There may be additional risks to study trial participation that we do not know about.

12 DATA HANDLING AND RECORD KEEPING

Investigators must retain all study records required by Sponsors and by the applicable regulations in a secure and safe facility. The investigator must consult a sponsor's 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 until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

"Essential documents" are defined as documents that individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced (e.g. signed protocol and all amendments; ethics committee approval for the study protocol and all amendments; all source documents; eCRF records; subjects' Informed Consent etc.). These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. The Committee for Human Medicinal Products for Human Use (CHMP) requires retention for the maximum period of time permitted by the institution, but not less than 15 years (ICH E6, 4.9.5). It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained (ICH E6, 5.5.12). The documents should not be destroyed without the written permission from the sponsor.

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.

13 INSURANCE OF STUDY SUBJECTS

Pending IRB approval, subjects will be compensated for their time in this study, and be reimbursed for travel to attend study visits. The study ICF will state the plan for reimbursement and health care treatment. Study subjects will not be charged for study vaccinations, research clinic visits, research-related examinations, or research-related laboratory tests.

All the subjects participating in this study are insured by Sponsor against any injury caused by any AE causally related to the study investigational product. The cost of medical care needed for treatment of vaccine related AEs (including SAEs) occurring among trial subjects will be borne by sponsor and as required by the Rules and Regulations passed by NRA.

All the subjects participating in this study will be covered by the Sponsor for any illness or condition diagnosed during trial participation. For these events, the study site medical team will attend the subject following the local standard of care and the national guidelines, free of charge to the subject for the duration of the study. At the end of the study, in case of further treatment required, the study team will provide adequate referrals in the health care system, but further treatment will not be covered.

14 PUBLICATION POLICY & CONFIDENTIALITY

PATH and SIIPL hold the exclusive rights to publish the study results. Due credit will be given to the investigators and their team in case the results of the study are published.

All proprietary or confidential information communicated to the investigator by or for PATH/SIIPL or communicated to the investigator during the course of and/or as a result of the clinical study is the exclusive property of Sponsors, and the investigator shall ensure that the same shall be kept strictly confidential by him/her and any other person connected with the clinical study and shall not be disclosed, either orally or in written form, by him/her or such person to any third party without the prior written consent of Sponsors.

The investigator shall communicate the results of the clinical study promptly to PATH/SIIPL.

All rights and interests worldwide in any inventions, know-how, or other intellectual or industrial property rights which arise during the course of and/or as a result of the clinical study which is the subject of this protocol or which otherwise arise from the information or materials supplied under this protocol, shall be assigned to, vest in and remain the property of Sponsors.

15 REFERENCES

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16 APPENDIX I: WORLD MEDICAL ASSOCIATION. DECLARATION OF HELSINKI: ETHICAL PRINCIPLES FOR MEDICAL RESEARCH INVOLVING HUMAN SUBJECTS (FORTALEZA, 2013)

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient’s best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician’s knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be

evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician

or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be

subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention. Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all subjects who still need an intervention identified as beneficial in the trial. This information must also be disclosed to subjects during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

17 APPENDIX II: DIVISION OF AIDS (DAIDS) TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS, VERSION 2.1. [MARCH 2017].

Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events

Version 2.1
March 2017

Division of AIDS
National Institute of Allergy and Infectious Diseases
National Institutes of Health
US Department of Health and Human Services

Table of Contents

Glossary and Acronyms

AE	Adverse event; Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure.
ALT (SGPT)	Alanine aminotransferase (serum glutamic pyruvic transaminase)
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate aminotransferase (serum glutamic-oxaloacetic transaminase)
AV	Atrioventricular
Basic Self-care Functions	Adult Activities such as bathing, dressing, toileting, transfer or movement, continence, and feeding. Young Children Activities that are age and culturally appropriate, such as feeding one's self with culturally appropriate eating implements.
BMI z-score	Body mass index z- score; A body reference norm. Specifically, the number of standard deviations a participant's BMI differs from the average BMI for their age, sex, and ethnicity.
BMD t-score	Bone mineral density t-score; The number of standard deviations above or below the mean bone mineral density of a healthy 30 year old adult of the same sex and ethnicity as the participant.
BMD z-score	Bone mineral density z-score; The number of standard deviations a participant's BMD differs from the average BMD for their age, sex, and ethnicity.
BPAP	Bilevel positive airway pressure; A mode used during noninvasive positive pressure ventilation.
Chemical Pregnancy	A pregnancy in which a positive pregnancy test is followed by a negative pregnancy test without evidence of a clinical pregnancy loss.
CNS	Central nervous system
CPAP	Continuous positive airway pressure
DAERS	DAIDS Adverse Experience Reporting System; An internet-based system developed for clinical research sites to report Expedited Adverse Events (EAEs) to DAIDS. It facilitates timely EAE report submission and serves as a centralized location for accessing and processing EAE information for reporting purposes.
Disability	A substantial disruption of a person's ability to conduct normal life functions.
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
Hospitalization	Does not include the following hospital admissions: under 24 hours, unrelated to an adverse event (e.g., for labor and delivery, cosmetic surgery, social or administrative for temporary placement [for lack of a place to sleep]), protocol-specified, and for diagnosis or therapy of a condition that existed before the receipt of a study agent and which has not increased in severity or frequency.
INR	International normalized ratio

Glossary and Acronyms

Intervention	Medical, surgical, or other procedures recommended or provided by a healthcare professional for the treatment of an adverse event.
IV	Intravenous
IVIG	Intravenous immune globulin
LDL	Low density lipoprotein
LLN	Lower limit of normal
Life-threatening AE	Any adverse event that places the participant, in the view of the investigator, at immediate risk of death from the reaction when it occurred (i.e., it does not include a reaction that would have caused death if it had occurred in a more severe form).
NA	Not applicable
Participant ID	The identification number assigned to a study participant which is used to track study-related documentation, including any reported AEs.
PR Interval	The interval between the beginning of the P wave and the beginning of the QRS complex of an electrocardiogram that represents the time between the beginning of the contraction of the atria and the beginning of the contraction of the ventricles.
PT	Prothrombin time
PTT	Partial thromboplastin time
QTc Interval	The measure of time between the onset of ventricular depolarization and completion of ventricular repolarization corrected for ventricular rate.
RBC	Red blood cell
SI	Standard international unit
ULN	Upper limit of normal
Usual Social & Functional Activities	Activities which adults and children perform on a routine basis and those which are part of regular activities of daily living, for example: <u>Adults</u> Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, or pursuing a hobby. <u>Young Children</u> Activities that are age and culturally appropriate, such as social interactions, play activities, or learning tasks.
WBC	White blood cell
WHO	World Health Organization
WNL	Within normal limits

Introduction

The Division of AIDS (DAIDS) oversees more than 300 clinical trials domestically and internationally, which evaluate the safety and efficacy of therapeutic products, vaccines, and other preventive modalities. Adverse event (AE) data collected during these clinical trials form the basis for subsequent safety and efficacy analyses of pharmaceutical products and medical devices. Incorrect and inconsistent AE severity grading can lead to inaccurate data analyses and interpretation, which in turn can impact the safety and well-being of clinical trial participants and future patients using pharmaceutical products.

Over the years, DAIDS scientific knowledge and experience have expanded necessitating a revision of the DAIDS grading table, which serves as a guide for assessing the severity of AEs (including clinical and laboratory abnormalities) in participants enrolled in DAIDS-sponsored and -supported clinical trials. The *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0* replaces the grading table published in 2004 and updated in 2009. In version 2.0, AEs not previously included in the DAIDS grading table, but which now are deemed medically important events, are included while other AEs have been removed. Some AE severity grading descriptions have been revised to more appropriately reflect the presentation of these events in clinical settings and their impact on clinical trials. For example, DAIDS performed an extensive literature search and reviews of select DAIDS clinical trial data in revising certain hematology parameters (i.e., hemoglobin, white cell counts, and absolute neutrophil counts). DAIDS also took into consideration the U.S. Food and Drug Administration's guidance regarding the use of local laboratory reference values and ethnic differences among certain healthy adolescent and adult populations in defining parameter limits. Finally, the revised DAIDS grading table also contains an updated glossary and acronyms section, an expanded instructions for use section, and an appendix that provides more age-specific information for an AE of concern to DAIDS.

DAIDS is grateful to the DAIDS Grading Table Working Group, numerous government and non-government affiliated medical subject matter experts and reviewers who were instrumental in the revision of the DAIDS grading table.

Instructions for Use

General Considerations

The *Division of AIDS* (*DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0* consists of parameters, or AEs, with severity grading guidance that are to be used in DAIDS clinical trials for safety data reporting to maintain accuracy and consistency in the evaluation of AEs. The term "severe" is not the same as the term "serious" in classifying AEs. The severity of a specific event describes its intensity, and it is the intensity which is graded. Seriousness, which is not graded, relates to an outcome of an AE and is a regulatory definition.

Clinical sites are encouraged to report parameters in the DAIDS grading table as they are written to maintain data consistency across clinical trials. However, since some parameters can be reported with more specificity, clinical sites are encouraged to report parameters that convey additional clinical information. For example, diarrhea could be reported as neonatal diarrhea; seizures, as febrile seizures; and pain, as jaw pain.

The DAIDS grading table provides an AE severity grading scale ranging from grades 1 to 5 with descriptions for each AE based on the following general guidelines:

- Grade 1 indicates a mild event
- Grade 2 indicates a moderate event
- Grade 3 indicates a severe event
- Grade 4 indicates a potentially life-threatening event
- Grade 5 indicates death (*Note*: This grade is not specifically listed on each page of the grading table).

Other points to consider include:

- Use age and sex values as applicable.
- Unless noted, laboratory values are for term neonates. Preterm neonates should be assessed using local laboratory normal ranges.
- Where applicable, Standard International (SI) units are included in italics.

Selecting and Reporting a Primary AE Term

When selecting a primary AE term to report, sites should select the term that best describes what occurred to the participant. For example, a participant may present with itching, urticaria, flushing, angioedema of the face, and dyspnea. If the underlying diagnosis is determined to be an acute allergic reaction, sites should report "Acute Allergic Reaction" as the primary AE term.

Primary AE terms should be reported using the DAIDS Adverse Experience Reporting System (DAERS) only if they meet expedited reporting criteria. However, all primary AE terms should be reported using protocol-specific case report forms (CRFs). Because the reported information is stored in different databases (i.e., safety and clinical), sites should report primary AE terms using the same terminology for data consistency.

Instructions for Use

When reporting using DAERS, other clinically significant events associated with a primary AE term that more fully describe the nature, severity, or complications of the primary AE term should be entered in the "Other Events" section. However, the severity grade for these events must be lower than or equal to the severity grade of the primary AE term. In the example above, dyspnea and angioedema of the face may be entered in the "Other Events" section, because they are more descriptive and provide additional information on the severity of the acute allergic reaction. However, their severity grades must be lower than or equal to the severity grade of the primary AE term of "Acute Allergic Reaction".

Differences exist in the reporting and recording of information (e.g., signs and symptoms, clinically significant events) in DAERS and CRFs. Therefore, sites should refer to their protocols and CRF requirements for further instructions.

Grading Adult and Pediatric AEs
When a single parameter is not appropriate for grading an AE in both adult and pediatric populations, separate parameters with specified age ranges are provided. If no distinction between adult and pediatric populations has been made, the listed parameter should be used for grading an AE in both populations.

Reporting Pregnancy Outcomes
In the *Pregnancy, Puerperium, and Perinatal* section, all parameters are pregnancy outcomes and should be reported using the mother's participant ID. If an infant is not enrolled in the same study as the mother, any identified birth defects should be reported using the mother's participant ID. However, if an infant is enrolled in the same study as the mother or in another study, any identified birth defects should be reported using the infant's participant ID. Sites should refer to the applicable network standards for reporting abnormal pregnancy outcomes on the CRFs.

Determining Severity Grade for Parameters between Grades
If the severity of an AE could fall in either one of two grades (i.e., the severity of an AE could be either grade 2 or grade 3), sites should select the higher of the two grades.

Laboratory Values
General. An asymptomatic, abnormal laboratory finding without an accompanying AE should not be reported to DAIDS in an expedited timeframe unless it meets protocol-specific reporting requirements. Sites should refer to the applicable network standards for reporting abnormal laboratory findings on the clinical case report forms.

Values below Grade 1. Any laboratory value that is between the ULN and grade 1 (for high values) or the LLN and grade 1 (for low values) should not be graded or reported as an AE. Sites should consult the *Manual for Expedited Reporting of Adverse Events to DAIDS, Version 2.0* and their protocol when making an assessment of the need to report an AE.

Overlap of Local Laboratory Normal Values with Grading Table Ranges. When local laboratory normal values fall within grading table laboratory ranges, the severity grading is based on the ranges in the grading table unless there is a protocol-specific grading criterion for the laboratory

Instructions for Use

value. For example, "Magnesium, Low" has a grade 1 range of 1.2 to < 1.4 mEq/L, while a particular laboratory's normal range for magnesium may be 1.3 to 2.8 mEq/L. If a study participant's magnesium laboratory value is 1.3 mEq/L, the laboratory value should be graded as grade 1.

Appendix Usage
Appendix A takes priority over the main grading table in all assessments of total bilirubin for term and preterm neonates.

Using Addenda 1-3: Grading Tables Used in Microbicide Studies
In protocols involving topical application of products to the female and male genital tracts or rectum, strong consideration should be given to using Addenda 1-3 (see below) as the primary grading tables for these areas. Although these grading tables are used specifically in microbicide studies, they may be used in other protocols as adjuncts to the main grading table (i.e., the *Division of AIDS (AIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0*). It should be clearly stated in a protocol which addendum is being used as the primary grading table (and thus takes precedence over the main grading table) and which addendum is being used in a complementary fashion.

- Addendum 1 – Female Genital Grading Table for Use in Microbicide Studies- *http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables*
- Addendum 2 – Male Genital Grading Table for Use in Microbicide Studies – *http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables*
- Addendum 3 – Rectal Grading Table for Use in Microbicide Studies – *http://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables*

Estimating Severity Grade for Parameters Not Identified in the Grading Table
The functional table below should be used to grade the severity of an AE that is not specifically identified in the grading table. In addition, all deaths related to an AE are to be classified as grade 5.

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Clinical adverse event **NOT** identified elsewhere in the grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

Major Clinical Conditions

Cardiovascular

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Arrhythmia (by ECG or physical examination) *Specify type, if applicable*	No symptoms AND No intervention indicated	No symptoms AND Non-urgent intervention indicated	Non-life-threatening symptoms AND Non-urgent intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Blood Pressure Abnormalities[1]				
Hypertension *(with the lowest reading taken after repeat testing during a visit)* *≥ 18 years of age*	140 to < 160 mmHg systolic OR 90 to < 100 mmHg diastolic	≥ 160 to < 180 mmHg systolic OR ≥ 100 to < 110 mmHg diastolic	≥ 180 mmHg systolic OR ≥ 110 mmHg diastolic	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
< 18 years of age	> 120/80 mmHg	≥ 95th to < 99th percentile + 5 mmHg adjusted for age, height, and gender (systolic and/or diastolic)	≥ 99th percentile + 5 mmHg adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
Hypotension	No symptoms	Symptoms corrected with oral fluid replacement	Symptoms AND IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Cardiac Ischemia or Infarction *Report only one*	NA	NA	New symptoms with ischemia (stable angina) OR New testing consistent with ischemia	Unstable angina OR Acute myocardial infarction
Heart Failure	No symptoms AND Laboratory or cardiac imaging abnormalities	Symptoms with mild to moderate activity or exertion	Symptoms at rest or with minimal activity or exertion (e.g., hypoxemia) OR Intervention indicated (e.g., oxygen)	Life-threatening consequences OR Urgent intervention indicated (e.g., vasoactive medications, ventricular assist device, heart transplant)

[1] Blood pressure norms for children < 18 years of age can be found in: Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents. *Pediatrics* 2011;128;S213; originally published online November 14, 2011; DOI: 10.1542/peds.2009-2107C.

Cardiovascular

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Hemorrhage (with significant acute blood loss)	NA	Symptoms AND No transfusion indicated	Symptoms AND Transfusion of ≤ 2 units packed RBCs indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs (for children, packed RBCs > 10 cc/kg) indicated
Prolonged PR Interval or AV Block *Report only one* *> 16 years of age*	PR interval 0.21 to < 0.25 seconds	PR interval ≥ 0.25 seconds OR Type I 2nd degree AV block	Type II 2nd degree AV block OR Ventricular pause ≥ 3.0 seconds	Complete AV block
≤ 16 years of age	1st degree AV block (PR interval > normal for age and rate)	Type I 2nd degree AV block	Type II 2nd degree AV block OR Ventricular pause ≥ 3.0 seconds	Complete AV block
Prolonged QTc Interval[2]	0.45 to 0.47 seconds	> 0.47 to 0.50 seconds	> 0.50 seconds OR ≥ 0.06 seconds above baseline	Life-threatening consequences (e.g., Torsade de pointes, other associated serious ventricular dysrhythmia)
Thrombosis or Embolism *Report only one*	NA	Symptoms AND No intervention indicated	Symptoms AND Intervention indicated	Life-threatening embolic event (e.g., pulmonary embolism, thrombus)

[2] As per Bazett's formula.

Dermatologic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Alopecia (scalp only)	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	NA	NA
Bruising	Localized to one area	Localized to more than one area	Generalized	NA
Cellulitis	NA	Non-parenteral treatment indicated (e.g., oral antibiotics, antifungals, antivirals)	IV treatment indicated (e.g., IV antibiotics, antifungals, antivirals)	Life-threatening consequences (e.g., sepsis, tissue necrosis)
Hyperpigmentation	Slight or localized causing no or minimal interference with usual social & functional activities	Marked or generalized causing greater than minimal interference with usual social & functional activities	NA	NA
Hypopigmentation	Slight or localized causing no or minimal interference with usual social & functional activities	Marked or generalized causing greater than minimal interference with usual social & functional activities	NA	NA
Petechiae	Localized to one area	Localized to more than one area	Generalized	NA
Pruritus[3] (without skin lesions)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA
Rash *Specify type, if applicable*	Localized rash	Diffuse rash OR Target lesions	Diffuse rash AND Vesicles or limited number of bullae or superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Stevens-Johnson syndrome OR Toxic epidermal necrolysis

[3] For pruritus associated with injections or infusions, see the *Site Reactions to Injections and Infusions* section (page 23).

Endocrine and Metabolic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Diabetes Mellitus	Controlled without medication	Controlled with medication OR Modification of current medication regimen	Uncontrolled despite treatment modification OR Hospitalization for immediate glucose control indicated	Life-threatening consequences (e.g., ketoacidosis, hyperosmolar non-ketotic coma, end organ failure)
Gynecomastia	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing pain with greater than minimal interference with usual social & functional activities	Disfiguring changes AND Symptoms requiring intervention or causing inability to perform usual social & functional activities	NA
Hyperthyroidism	No symptoms AND Abnormal laboratory value	Symptoms causing greater than minimal interference with usual social & functional activities OR Thyroid suppression therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., thyroid storm)
Hypothyroidism	No symptoms AND Abnormal laboratory value	Symptoms causing greater than minimal interference with usual social & functional activities OR Thyroid replacement therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., myxedema coma)
Lipoatrophy[4]	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	Disfiguring changes	NA

[4] Definition: A disorder characterized by fat loss in the face, extremities, and buttocks.

Endocrine and Metabolic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Lipohypertrophy[5]	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	Disfiguring changes	NA

[5] Definition: A disorder characterized by abnormal fat accumulation on the back of the neck, breasts, and abdomen.

Gastrointestinal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated (e.g., tube feeding, total parenteral nutrition)
Ascites	No symptoms	Symptoms AND Intervention indicated (e.g., diuretics, therapeutic paracentesis)	Symptoms recur or persist despite intervention	Life-threatening consequences
Bloating or Distension *Report only one*	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Cholecystitis	NA	Symptoms AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences (e.g., sepsis, perforation)
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)
Diarrhea *≥ 1 year of age*	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 to 6 stools over baseline per 24-hour period	Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
< 1 year of age	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Life-threatening consequences (e.g., liquid stools resulting in severe dehydration, hypotensive shock)
Dysphagia or Odynophagia *Report only one and specify location*	Symptoms but able to eat usual diet	Symptoms causing altered dietary intake with no intervention indicated	Symptoms causing severely altered dietary intake with intervention indicated	Life-threatening reduction in oral intake
Gastrointestinal Bleeding	Not requiring intervention other than iron supplement	Endoscopic intervention indicated	Transfusion indicated	Life-threatening consequences (e.g., hypotensive shock)

Gastrointestinal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Mucositis or Stomatitis *Report only one and specify location*	Mucosal erythema	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Life-threatening consequences (e.g., aspiration, choking) OR Tissue necrosis OR Diffuse spontaneous mucosal bleeding
Nausea	Transient (< 24 hours) or intermittent AND No or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 to 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Pancreatitis	NA	Symptoms with hospitalization not indicated	Symptoms with hospitalization indicated	Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis)
Perforation (colon or rectum)	NA	NA	Intervention indicated	Life-threatening consequences
Proctitis	Rectal discomfort with no intervention indicated	Symptoms causing greater than minimal interference with usual social & functional activities OR Medical intervention indicated	Symptoms causing inability to perform usual social & functional activities OR Operative intervention indicated	Life-threatening consequences (e.g., perforation)
Rectal Discharge	Visible discharge	Discharge requiring the use of pads	NA	NA
Vomiting	Transient or intermittent AND No or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)

Musculoskeletal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Arthralgia	Joint pain causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Myalgia (generalized)	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions
Osteonecrosis	NA	No symptoms but with radiographic findings AND No operative intervention indicated	Bone pain with radiographic findings OR Operative intervention indicated	Disabling bone pain with radiographic findings causing inability to perform basic self-care functions
Osteopenia[6] *≥ 30 years of age*	BMD t-score -2.5 to -1	NA	NA	NA
< 30 years of age	BMD z-score -2 to -1	NA	NA	NA
Osteoporosis[6] *≥ 30 years of age*	NA	BMD t-score < -2.5	Pathologic fracture (e.g., compression fracture causing loss of vertebral height)	Pathologic fracture causing life-threatening consequences
< 30 years of age	NA	BMD z-score < -2	Pathologic fracture (e.g., compression fracture causing loss of vertebral height)	Pathologic fracture causing life-threatening consequences

[6] BMD t and z scores can be found in: Kanis JA on behalf of the World Health Organization Scientific Group (2007). Assessment of osteoporosis at the primary health-care level. Technical Report. World Health Organization Collaborating Centre for Metabolic Bone Diseases, University of Sheffield, UK. 2007: Printed by the University of Sheffield.

Neurologic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acute CNS Ischemia	NA	NA	Transient ischemic attack	Cerebral vascular accident (e.g., stroke with neurological deficit)
Altered Mental Status (for Dementia, see *Cognitive, Behavioral, or Attentional Disturbance* below)	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR Obtundation OR Coma
Ataxia	Symptoms causing no or minimal interference with usual social & functional activities OR No symptoms with ataxia detected on examination	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Disabling symptoms causing inability to perform basic self-care functions
Cognitive, Behavioral, or Attentional Disturbance (includes dementia and attention deficit disorder) *Specify type, if applicable*	Disability causing no or minimal interference with usual social & functional activities OR Specialized resources not indicated	Disability causing greater than minimal interference with usual social & functional activities OR Specialized resources on part-time basis indicated	Disability causing inability to perform usual social & functional activities OR Specialized resources on a full-time basis indicated	Disability causing inability to perform basic self-care functions OR Institutionalization indicated
Developmental Delay *< 18 years of age* *Specify type, if applicable*	Mild developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Moderate developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Severe developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Developmental regression, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated OR Headache with significant impairment of alertness or other neurologic function

Neurologic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Neuromuscular Weakness (includes myopathy and neuropathy) *Specify type, if applicable*	Minimal muscle weakness causing no or minimal interference with usual social & functional activities OR No symptoms with decreased strength on examination	Muscle weakness causing greater than minimal interference with usual social & functional activities	Muscle weakness causing inability to perform usual social & functional activities	Disabling muscle weakness causing inability to perform basic self-care functions OR Respiratory muscle weakness impairing ventilation
Neurosensory Alteration (includes paresthesia and painful neuropathy) *Specify type, if applicable*	Minimal paresthesia causing no or minimal interference with usual social & functional activities OR No symptoms with sensory alteration on examination	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities	Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions
Seizures ***New Onset Seizure*** *≥ 18 years of age*	NA	NA	1 to 3 seizures	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
< 18 years of age (includes new or pre-existing febrile seizures)	Seizure lasting < 5 minutes with < 24 hours postictal state	Seizure lasting 5 to < 20 minutes with < 24 hours postictal state	Seizure lasting ≥ 20 minutes OR > 24 hours postictal state	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Pre-existing Seizure	NA	Increased frequency from previous level of control without change in seizure character	Change in seizure character either in duration or quality (e.g., severity or focality)	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Syncope	Near syncope without loss of consciousness (e.g., pre-syncope)	Loss of consciousness with no intervention indicated	Loss of consciousness AND Hospitalization or intervention required	NA

Pregnancy, Puerperium, and Perinatal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Stillbirth (report using mother's participant ID) *Report only one*	NA	NA	Fetal death occurring at ≥ 20 weeks gestation	NA
Preterm Birth (report using mother's participant ID)	Live birth at 34 to < 37 weeks gestational age	Live birth at 28 to < 34 weeks gestational age	Live birth at 24 to < 28 weeks gestational age	Live birth at < 24 weeks gestational age
Spontaneous Abortion or Miscarriage[7] (report using mother's participant ID) *Report only one*	Chemical pregnancy	Uncomplicated spontaneous abortion or miscarriage	Complicated spontaneous abortion or miscarriage	NA

[7] Definition: A pregnancy loss occurring at < 20 weeks gestational age.

Psychiatric

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Insomnia	Mild difficulty falling asleep, staying asleep, or waking up early causing no or minimal interference with usual social & functional activities	Moderate difficulty falling asleep, staying asleep, or waking up early causing more than minimal interference with usual social & functional activities	Severe difficulty falling asleep, staying asleep, or waking up early causing inability to perform usual social & functional activities requiring intervention or hospitalization	NA
Psychiatric Disorders (includes anxiety, depression, mania, and psychosis) *Specify disorder*	Symptoms with intervention not indicated OR Behavior causing no or minimal interference with usual social & functional activities	Symptoms with intervention indicated OR Behavior causing greater than minimal interference with usual social & functional activities	Symptoms with hospitalization indicated OR Behavior causing inability to perform usual social & functional activities	Threatens harm to self or others OR Acute psychosis OR Behavior causing inability to perform basic self-care functions
Suicidal Ideation or Attempt *Report only one*	Preoccupied with thoughts of death AND No wish to kill oneself	Preoccupied with thoughts of death AND Wish to kill oneself with no specific plan or intent	Thoughts of killing oneself with partial or complete plans but no attempt to do so OR Hospitalization indicated	Suicide attempted

Respiratory

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acute Bronchospasm	Forced expiratory volume in 1 second or peak flow reduced to ≥ 70 to < 80% OR Mild symptoms with intervention not indicated	Forced expiratory volume in 1 second or peak flow 50 to < 70% OR Symptoms with intervention indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Forced expiratory volume in 1 second or peak flow 25 to < 50% OR Symptoms causing inability to perform usual social & functional activities	Forced expiratory volume in 1 second or peak flow < 25% OR Life-threatening respiratory or hemodynamic compromise OR Intubation
Dyspnea or Respiratory Distress *Report only one*	Dyspnea on exertion with no or minimal interference with usual social & functional activities OR Wheezing OR Minimal increase in respiratory rate for age	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities OR Nasal flaring OR Intercostal retractions OR Pulse oximetry 90 to < 95%	Dyspnea at rest causing inability to perform usual social & functional activities OR Pulse oximetry < 90%	Respiratory failure with ventilator support indicated (e.g., CPAP, BPAP, intubation)

Sensory

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Hearing Loss *≥ 12 years of age*	NA	Hearing aid or intervention not indicated	Hearing aid or intervention indicated	Profound bilateral hearing loss (> 80 dB at 2 kHz and above) OR Non-serviceable hearing (i.e., >50 dB audiogram and <50% speech discrimination)
< 12 years of age (based on a 1, 2, 3, 4, 6 and 8 kHz audiogram)	> 20 dB hearing loss at ≤ 4 kHz	> 20 dB hearing loss at > 4 kHz	> 20 dB hearing loss at ≥ 3 kHz in one ear with additional speech language related services indicated (where available) OR Hearing loss sufficient to indicate therapeutic intervention, including hearing aids	Audiologic indication for cochlear implant and additional speech-language related services indicated (where available)
Tinnitus	Symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Symptoms causing inability to perform usual social & functional activities	NA
Uveitis	No symptoms AND Detectable on examination	Anterior uveitis with symptoms OR Medical intervention indicated	Posterior or pan-uveitis OR Operative intervention indicated	Disabling visual loss in affected eye(s)
Vertigo	Vertigo causing no or minimal interference with usual social & functional activities	Vertigo causing greater than minimal interference with usual social & functional activities	Vertigo causing inability to perform usual social & functional activities	Disabling vertigo causing inability to perform basic self-care functions
Visual Changes (assessed from baseline)	Visual changes causing no or minimal interference with usual social & functional activities	Visual changes causing greater than minimal interference with usual social & functional activities	Visual changes causing inability to perform usual social & functional activities	Disabling visual loss in affected eye(s)

Systemic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acute Allergic Reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with intervention indicated OR Mild angioedema with no intervention indicated	Generalized urticaria OR Angioedema with intervention indicated OR Symptoms of mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR Laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Cytokine Release Syndrome[8]	Mild signs and symptoms AND Therapy (i.e., antibody infusion) interruption not indicated	Therapy (i.e., antibody infusion) interruption indicated AND Responds promptly to symptomatic treatment OR Prophylactic medications indicated for ≤ 24 hours	Prolonged severe signs and symptoms OR Recurrence of symptoms following initial improvement	Life-threatening consequences (e.g., requiring pressor or ventilator support)
Fatigue or Malaise *Report only one*	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating symptoms of fatigue or malaise causing inability to perform basic self-care functions
Fever (non-axillary temperatures only)	38.0 to < 38.6°C or 100.4 to < 101.5°F	≥ 38.6 to < 39.3°C or ≥ 101.5 to < 102.7°F	≥ 39.3 to < 40.0°C or ≥ 102.7 to < 104.0°F	≥ 40.0°C or ≥ 104.0°F
Pain[9] (not associated with study agent injections and not specified elsewhere) *Specify location*	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization indicated

[8] Definition: A disorder characterized by nausea, headache, tachycardia, hypotension, rash, and/or shortness of breath.
[9] For pain associated with injections or infusions, see the *Site Reactions to Injections and Infusions* section (page 23).

Systemic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Serum Sickness[10]	Mild signs and symptoms	Moderate signs and symptoms AND Intervention indicated (e.g., antihistamines)	Severe signs and symptoms AND Higher level intervention indicated (e.g., steroids or IV fluids)	Life-threatening consequences (e.g., requiring pressor or ventilator support)
Underweight[11] *> 5 to 19 years of age*	WHO BMI z-score < -1 to -2	WHO BMI z-score < -2 to -3	WHO BMI z-score < -3	WHO BMI z-score < -3 with life-threatening consequences
2 to 5 years of age	WHO BMI z-score < -1 to -2	WHO Weight-for-height z-score < -2 to -3	WHO Weight-for-height z-score < -3	WHO Weight-for-height z-score < -3 with life-threatening consequences
< 2 years of age	WHO BMI z-score < -1 to -2	WHO Weight-for-length z-score < -2 to -3	WHO Weight-for-length z-score < -3	WHO Weight-for-length z-score < -3 with life-threatening consequences
Unintentional Weight Loss (excludes postpartum weight loss)	NA	5 to < 9% loss in body weight from baseline	≥ 9 to < 20% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated (e.g., tube feeding, total parenteral nutrition)

[10] Definition: A disorder characterized by fever, arthralgia, myalgia, skin eruptions, lymphadenopathy, marked discomfort, and/or dyspnea.
[11] WHO reference tables may be accessed by clicking the desired age range or by accessing the following URLs: http://www.who.int/growthref/who2007_bmi_for_age/en/ for participants > 5 to 19 years of age and http://www.who.int/childgrowth/standards/chart_catalogue/en/ for those ≤ 5 years of age.

Urinary

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Urinary Tract Obstruction	NA	Signs or symptoms of urinary tract obstruction without hydronephrosis or renal dysfunction	Signs or symptoms of urinary tract obstruction with hydronephrosis or renal dysfunction	Obstruction causing life-threatening consequences

Site Reactions to Injections and Infusions

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Injection Site Pain or Tenderness *Report only one*	Pain or tenderness causing no or minimal limitation of use of limb	Pain or tenderness causing greater than minimal limitation of use of limb	Pain or tenderness causing inability to perform usual social & functional activities	Pain or tenderness causing inability to perform basic self-care function OR Hospitalization indicated
Injection Site Erythema or Redness[12] *Report only one* *> 15 years of age*	2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area AND Symptoms causing no or minimal interference with usual social & functional activities	≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social & functional activities	≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Symptoms causing inability to perform usual social & functional activities	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
≤ 15 years of age	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection Site Induration or Swelling *Report only one* *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*
≤ 15 years of age	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*
Injection Site Pruritus	Itching localized to the injection site that is relieved spontaneously or in < 48 hours of treatment	Itching beyond the injection site that is not generalized OR Itching localized to the injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA

[12] Injection Site Erythema or Redness should be evaluated and graded using the greatest single diameter or measured surface area.

*Laboratory Values**
Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acidosis	NA	pH ≥ 7.3 to < LLN	pH < 7.3 without life-threatening consequences	pH < 7.3 with life-threatening consequences
Albumin, Low (g/dL; *g/L*)	3.0 to < LLN *30 to < LLN*	≥ 2.0 to < 3.0 *≥ 20 to < 30*	< 2.0 *< 20*	NA
Alkaline Phosphatase, High	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Alkalosis	NA	pH > ULN to ≤ 7.5	pH > 7.5 without life-threatening consequences	pH > 7.5 with life-threatening consequences
ALT or SGPT, High *Report only one*	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Amylase (Pancreatic) or Amylase (Total), High *Report only one*	1.1 to < 1.5 x ULN	1.5 to < 3.0 x ULN	3.0 to < 5.0 x ULN	≥ 5.0 x ULN
AST or SGOT, High *Report only one*	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Bicarbonate, Low (mEq/L; *mmol/L*)	16.0 to < LLN *16.0 to < LLN*	11.0 to < 16.0 *11.0 to < 16.0*	8.0 to < 11.0 *8.0 to < 11.0*	< 8.0 *< 8.0*
Bilirubin ***Direct Bilirubin[13], High*** *> 28 days of age*	NA	NA	> ULN with other signs and symptoms of hepatotoxicity.	> ULN with life-threatening consequences (e.g., signs and symptoms of liver failure)
≤ 28 days of age	ULN to ≤ 1 mg/dL	> 1 to ≤ 1.5 mg/dL	> 1.5 to ≤ 2 mg/dL	> 2 mg/dL
Total Bilirubin, High *> 28 days of age*	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN with other signs and symptoms of hepatotoxicity.	≥ 5.0 x ULN with life-threatening consequences (e.g., signs and symptoms of liver failure).
≤ 28 days of age	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates

*Reminder: An asymptomatic abnormal laboratory finding without an accompanying AE should not be reported to DAIDS in an expedited time frame unless it meets protocol-specific reporting requirements.

[13] Direct bilirubin > 1.5 mg/dL in a participant < 28 days of age should be graded as grade 2, if < 10% of the total bilirubin.

Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Calcium, High (mg/dL; *mmol/L*)				
≥ *7 days of age*	10.6 to < 11.5 *2.65 to < 2.88*	11.5 to < 12.5 *2.88 to < 3.13*	12.5 to < 13.5 *3.13 to < 3.38*	≥ 13.5 ≥ *3.38*
< *7 days of age*	11.5 to < 12.4 *2.88 to < 3.10*	12.4 to < 12.9 *3.10 to < 3.23*	12.9 to < 13.5 *3.23 to < 3.38*	≥ 13.5 ≥ *3.38*
Calcium (Ionized), High (mg/dL; *mmol/L*)	> ULN to < 6.0 *> ULN to < 1.5*	6.0 to < 6.4 *1.5 to < 1.6*	6.4 to < 7.2 *1.6 to < 1.8*	≥ 7.2 ≥ *1.8*
Calcium, Low (mg/dL; *mmol/L*)				
≥ *7 days of age*	7.8 to < 8.4 *1.95 to < 2.10*	7.0 to < 7.8 *1.75 to < 1.95*	6.1 to < 7.0 *1.53 to < 1.75*	< 6.1 *< 1.53*
< *7 days of age*	6.5 to < 7.5 *1.63 to < 1.88*	6.0 to < 6.5 *1.50 to < 1.63*	5.50 to < 6.0 *1.38 to < 1.50*	< 5.50 *< 1.38*
Calcium (Ionized), Low (mg/dL; *mmol/L*)	< LLN to 4.0 *< LLN to 1.0*	3.6 to < 4.0 *0.9 to < 1.0*	3.2 to < 3.6 *0.8 to < 0.9*	< 3.2 *< 0.8*
Cardiac Troponin I, High	NA	NA	NA	Levels consistent with myocardial infarction or unstable angina as defined by the local laboratory
Creatine Kinase, High	3 to < 6 x ULN	6 to < 10x ULN	10 to < 20 x ULN	≥ 20 x ULN
Creatinine, High **Report only one*	1.1 to 1.3 x ULN	> 1.3 to 1.8 x ULN OR Increase to 1.3 to < 1.5 x participant's baseline	> 1.8 to < 3.5 x ULN OR Increase to 1.5 to < 2.0 x participant's baseline	≥ 3.5 x ULN OR Increase of ≥ 2.0 x participant's baseline
Creatinine Clearance[14] or eGFR, Low **Report only one*	NA	< 90 to 60 ml/min or ml/min/1.73 m^2 OR 10 to < 30% decrease from participant's baseline	< 60 to 30 ml/min or ml/min/1.73 m^2 OR 30 to < 50% decrease from participant's baseline	< 30 ml/min or ml/min/1.73 m^2 OR ≥ 50% decrease from participant's baseline or dialysis needed
Glucose (mg/dL; *mmol/L*)				
Fasting, High	110 to 125 *6.11 to < 6.95*	> 125 to 250 *6.95 to < 13.89*	> 250 to 500 *13.89 to < 27.75*	≥ 500 ≥ *27.75*
Nonfasting, High	116 to 160 *6.44 to < 8.89*	> 160 to 250 *8.89 to < 13.89*	> 250 to 500 *13.89 to < 27.75*	≥ 500 ≥ *27.75*

[14] Use the applicable formula (i.e., Cockcroft-Gault in mL/min or Schwartz, MDRD, CKD-Epi in mL/min/1.73m2). Sites should choose the method defined in their study and when not specified, use the method most relevant to the study population.

*Reminder: Choose the method that selects for the higher grade.

Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Glucose, Low (mg/dL; *mmol/L*) *≥ 1 month of age*	55 to 64 *3.05 to <3.55*	40 to < 55 *2.22 to < 3.05*	30 to < 40 *1.67 to < 2.22*	< 30 *< 1.67*
< 1 month of age	50 to 54 *2.78 to < 3.00*	40 to < 50 *2.22 to < 2.78*	30 to < 40 *1.67 to < 2.22*	< 30 *< 1.67*
Lactate, High	ULN to < 2.0 x ULN without acidosis	≥ 2.0 x ULN without acidosis	Increased lactate with pH < 7.3 without life-threatening consequences	Increased lactate with pH < 7.3 with life-threatening consequences
Lipase, High	1.1 to < 1.5 x ULN	1.5 to < 3.0 x ULN	3.0 to < 5.0 x ULN	≥ 5.0 x ULN
Lipid Disorders (mg/dL; *mmol/L*) ***Cholesterol, Fasting, High*** *≥ 18 years of age*	200 to < 240 *5.18 to < 6.19*	240 to < 300 *6.19 to < 7.77*	≥ 300 *≥ 7.77*	NA
< 18 years of age	170 to < 200 *4.40 to < 5.15*	200 to < 300 *5.15 to < 7.77*	≥ 300 *≥ 7.77*	NA
LDL, Fasting, High *≥ 18 years of age*	130 to < 160 *3.37 to < 4.12*	160 to < 190 *4.12 to < 4.90*	≥ 190 *≥ 4.90*	NA
> 2 to < 18 years of age	110 to < 130 *2.85 to < 3.34*	130 to < 190 *3.34 to < 4.90*	≥ 190 *≥ 4.90*	NA
Triglycerides, Fasting, High	150 to 300 *1.71 to 3.42*	>300 to 500 *>3.42 to 5.7*	>500 to < 1,000 *>5.7 to 11.4*	> 1,000 *> 11.4*
Magnesium[15], Low (mEq/L; *mmol/L*)	1.2 to < 1.4 *0.60 to < 0.70*	0.9 to < 1.2 *0.45 to < 0.60*	0.6 to < 0.9 *0.30 to < 0.45*	< 0.6 *< 0.30*
Phosphate, Low (mg/dL; *mmol/L*) *> 14 years of age*	2.0 to < LLN *0.65 to < LLN*	1.4 to < 2.0 *0.45 to < 0.65*	1.0 to < 1.4 *0.32 to < 0.45*	< 1.0 *< 0.32*
1 to 14 years of age	3.0 to < 3.5 *0.97 to < 1.13*	2.5 to < 3.0 *0.81 to < 0.97*	1.5 to < 2.5 *0.48 to < 0.81*	< 1.5 *< 0.48*
< 1 year of age	3.5 to < 4.5 *1.13 to < 1.45*	2.5 to < 3.5 *0.81 to < 1.13*	1.5 to < 2.5 *0.48 to < 0.81*	< 1.5 *< 0.48*
Potassium, High (mEq/L; *mmol/L*)	5.6 to < 6.0 *5.6 to < 6.0*	6.0 to < 6.5 *6.0 to < 6.5*	6.5 to < 7.0 *6.5 to < 7.0*	≥ 7.0 *≥ 7.0*
Potassium, Low (mEq/L; *mmol/L*)	3.0 to < 3.4 *3.0 to < 3.4*	2.5 to < 3.0 *2.5 to < 3.0*	2.0 to < 2.5 *2.0 to < 2.5*	< 2.0 *< 2.0*

[15] To convert a magnesium value from mg/dL to mmol/L, laboratories should multiply by 0.4114.

Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Sodium, High (mEq/L; *mmol/L*)	146 to < 150 *146 to < 150*	150 to < 154 *150 to < 154*	154 to < 160 *154 to < 160*	≥ 160 *≥ 160*
Sodium, Low (mEq/L; *mmol/L*)	130 to < 135 *130 to < 135*	125 to < 130 *125 to < 130*	121 to < 125 *121 to < 125*	≤ 120 *≤ 120*
Uric Acid, High (mg/dL; *mmol/L*)	7.5 to < 10.0 *0.45 to < 0.59*	10.0 to < 12.0 *0.59 to < 0.71*	12.0 to < 15.0 *0.71 to < 0.89*	≥ 15.0 *≥ 0.89*

Hematology

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Absolute CD4+ Count, Low (cell/mm^3; *cells/L*) *> 5 years of age (not HIV infected)*	300 to < 400 *300 to < 400*	200 to < 300 *200 to < 300*	100 to < 200 *100 to < 200*	< 100 *< 100*
Absolute Lymphocyte Count, Low (cell/mm^3; *cells/L*) *> 5 years of age (not HIV infected)*	600 to < 650 *0.600 x 10^9 to < 0.650 x 10^9*	500 to < 600 *0.500 x 10^9 to < 0.600 x 10^9*	350 to < 500 *0.350 x 10^9 to < 0.500 x 10^9*	< 350 *< 0.350 x 10^9*
Absolute Neutrophil Count (ANC), Low (cells/mm^3; *cells/L*) *> 7 days of age*	800 to 1,000 *0.800 x 10^9 to 1.000 x 10^9*	600 to 799 *0.600 x 10^9 to 0.799 x 10^9*	400 to 599 *0.400 x 10^9 to 0.599 x 10^9*	< 400 *< 0.400 x 10^9*
2 to 7 days of age	1,250 to 1,500 *1.250 x 10^9 to 1.500 x 10^9*	1,000 to 1,249 *1.000 x 10^9 to 1.249 x 10^9*	750 to 999 *0.750 x 10^9 to 0.999 x 10^9*	< 750 *< 0.750 x 10^9*
≤ 1 day of age	4,000 to 5,000 *4.000 x 10^9 to 5.000 x 10^9*	3,000 to 3,999 *3.000 x 10^9 to 3.999 x 10^9*	1,500 to 2,999 *1.500 x 10^9 to 2.999 x 10^9*	< 1,500 *< 1.500 x 10^9*
Fibrinogen, Decreased (mg/dL; *g/L*)	100 to < 200 *1.00 to < 2.00* OR 0.75 to < 1.00 x LLN	75 to < 100 *0.75 to < 1.00* OR ≥ 0.50 to < 0.75 x LLN	50 to < 75 *0.50 to < 0.75* OR 0.25 to < 0.50 x LLN	< 50 *< 0.50* OR < 0.25 x LLN OR Associated with gross bleeding
Hemoglobin[16], Low (g/dL; *mmol/L*)[17] *≥ 13 years of age (male only)*	10.0 to 10.9 *6.19 to 6.76*	9.0 to < 10.0 *5.57 to < 6.19*	7.0 to < 9.0 *4.34 to < 5.57*	< 7.0 *< 4.34*
≥ 13 years of age (female only)	9.5 to 10.4 *5.88 to 6.48*	8.5 to < 9.5 *5.25 to < 5.88*	6.5 to < 8.5 *4.03 to < 5.25*	< 6.5 *< 4.03*

[16] Male and female sex are defined as sex at birth. For transgender participants ≥13 years of age who have been on hormone therapy for more than 6 consecutive months, grade hemoglobin based on the gender with which they identify (i.e., a transgender female should be graded using the female sex at birth hemoglobin laboratory values).

[17] The most commonly used conversion factor to convert g/dL to mmol/L is 0.6206. For grading hemoglobin results obtained by an analytic method with a conversion factor other than 0.6206, the result must be converted to g/dL using appropriate conversion factor for the particular laboratory.

Hematology

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
57 days of age to < 13 years of age (male and female)	9.5 to 10.4 *5.88 to 6.48*	8.5 to < 9.5 *5.25 to < 5.88*	6.5 to < 8.5 *4.03 to < 5.25*	< 6.5 *< 4.03*
36 to 56 days of age (male and female)	8.5 to 9.6 *5.26 to 5.99*	7.0 to < 8.5 *4.32 to < 5.26*	6.0 to < 7.0 *3.72 to < 4.32*	< 6.0 *< 3.72*
22 to 35 days of age (male and female)	9.5 to 11.0 *5.88 to 6.86*	8.0 to < 9.5 *4.94 to < 5.88*	6.7 to < 8.0 *4.15 to < 4.94*	< 6.7 *< 4.15*
8 to ≤ 21 days of age (male and female)	11.0 to 13.0 *6.81 to 8.10*	9.0 to < 11.0 *5.57 to < 6.81*	8.0 to < 9.0 *4.96 to < 5.57*	< 8.0 *< 4.96*
≤ 7 days of age (male and female)	13.0 to 14.0 *8.05 to 8.72*	10.0 to < 13.0 *6.19 to < 8.05*	9.0 to < 10.0 *5.59 to < 6.19*	< 9.0 *< 5.59*
INR, High (not on anticoagulation therapy)	1.1 to < 1.5 x ULN	1.5 to < 2.0 x ULN	2.0 to < 3.0 x ULN	≥ 3.0 x ULN
Methemoglobin (% hemoglobin)	5.0 to < 10.0%	10.0 to < 15.0%	15.0 to < 20.0%	≥ 20.0%
PTT, High (not on anticoagulation therapy)	1.1 to < 1.66 x ULN	1.66 to < 2.33 x ULN	2.33 to < 3.00 x ULN	≥ 3.00 x ULN
Platelets, Decreased (cells/mm^3; *cells/L*)	100,000 to < 125,000 *100.000 x 10^9 to < 125.000 x 10^9*	50,000 to < 100,000 *50.000 x 10^9 to < 100.000 x 10^9*	25,000 to < 50,000 *25.000 x 10^9 to < 50.000 x 10^9*	< 25,000 *< 25.000 x 10^9*
PT, High (not on anticoagulation therapy	1.1 to < 1.25 x ULN	1.25 to < 1.50 x ULN	1.50 to < 3.00 x ULN	≥ 3.00 x ULN
WBC, Decreased (cells/mm^3; *cells/L*)				
> 7 days of age	2,000 to 2,499 *2.000 x 10^9 to 2.499 x 10^9*	1,500 to 1,999 *1.500 x 10^9 to 1.999 x 10^9*	1,000 to 1,499 *1.000 x 10^9 to 1.499 x 10^9*	< 1,000 *< 1.000 x 10^9*
≤ 7 days of age	5,500 to 6,999 *5.500 x 10^9 to 6.999 x 10^9*	4,000 to 5,499 *4.000 x 10^9 to 5.499 x 10^9*	2,500 to 3,999 *2.500 x 10^9 to 3.999 x 10^9*	< 2,500 *< 2.500 x 10^9*

Urinalysis

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Glycosuria (random collection tested by dipstick)	Trace to 1+ or ≤ 250 mg	2+ or > 250 to ≤ 500 mg	> 2+ or > 500 mg	NA
Hematuria (not to be reported based on dipstick findings or on blood believed to be of menstrual origin)	6 to < 10 RBCs per high power field	≥ 10 RBCs per high power field	Gross, with or without clots OR With RBC casts OR Intervention indicated	Life-threatening consequences
Proteinuria (random collection tested by dipstick)	1+	2+	3+ or higher	NA

Appendix A.
Total Bilirubin Table for Term and Preterm Neonates

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Total Bilirubin[18], High (mg/dL; *µmol/L*)[19]				
Term Neonate[20] *< 24 hours of age*	4 to < 7 *68.4 to < 119.7*	7 to < 10 *119.7 to < 171*	10 to < 17 *171 to < 290.7*	≥ 17 *≥ 290.7*
24 to < 48 hours of age	5 to < 8 *85.5 to < 136.8*	8 to < 12 *136.8 to < 205.2*	12 to < 19 *205.2 to < 324.9*	≥ 19 *≥ 324.9*
48 to < 72 hours of age	8.5 to < 13 *145.35 to < 222.3*	13 to < 15 *222.3 to < 256.5*	15 to < 22 *256.5 to < 376.2*	≥ 22 *≥ 376.2*
72 hours to < 7 days of age	11 to < 16 *188.1 to < 273.6*	16 to < 18 *273.6 to < 307.8*	18 to < 24 *307.8 to < 410.4*	≥ 24 *≥ 410.4*
7 to 28 days of age (breast feeding)	5 to < 10 *85.5 to < 171*	10 to < 20 *171 to < 342*	20 to < 25 *342 to < 427.5*	≥ 25 *≥ 427.5*
7 to 28 days of age (not breast feeding)	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN	≥ 5.0 x ULN
Preterm Neonate[20] *35 to < 37 weeks gestational age*	Same as for ***Total Bilirubin, High, Term Neonate*** (based on days of age).	Same as for ***Total Bilirubin, High, Term Neonate*** (based on days of age).	Same as for ***Total Bilirubin, High, Term Neonate*** (based on days of age).	Same as for ***Total Bilirubin, High, Term Neonate*** (based on days of age).
32 to < 35 weeks gestational age and < 7 days of age	NA	NA	10 to < 14 *171 to < 239.4*	≥ 14 *≥ 239.4*
28 to < 32 weeks gestational age and < 7 days of age	NA	NA	6 to < 10 *102.6 to < 171*	≥ 10 *≥ 171*
< 28 weeks gestational age and < 7 days of age	NA	NA	5 to < 8 *85.5 to < 136.8*	≥ 8 *≥ 136.8*
7 to 28 days of age (breast feeding)	5 to < 10 *85.5 to < 171*	10 to < 20 *171 to < 342*	20 to < 25 *342 to < 427.5*	≥ 25 *≥ 427.5*
7 to 28 days of age (not breast feeding)	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN	≥ 5.0 x ULN

[18] Severity grading for total bilirubin in neonates is complex because of rapidly changing total bilirubin normal ranges in the first week of life followed by the benign phenomenon of breast milk jaundice after the first week of life. Severity grading in this appendix corresponds approximately to cut-offs for indications for phototherapy at grade 3 and for exchange transfusion at grade 4.

[19] A laboratory value of 1 mg/dL is equivalent to 17.1 µmol/L.

[20] Definitions: Term is defined as ≥ 37 weeks gestational age; near-term, as ≥ 35 weeks gestational age; preterm, as < 35 weeks gestational age; and neonate, as 0 to 28 days of age.