ASSIGN DMB

Statistical Analysis Plan

Part A and B

SAFETY AND IMMUNOGENICITY STUDY OF VLA15, A MULTIVALENT RECOMBINANT OSPA BASED VACCINE CANDIDATE AGAINST LYME BORRELIOSIS: A RANDOMIZED, CONTROLLED, OBSERVER-BLIND PHASE 2 STUDY IN A HEALTHY PEDIATRIC AND ADULT STUDY POPULATION

Protocol: VLA15-221

Confidential

Study Sponsor: Pfizer

Study Conducted By: Valneva Austria GmbH

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Study	VLA15-221
Document	Statistical Analysis Plan (SAP)
Version	FINAL 4.0
Date	28-Apr-2025

Revision History

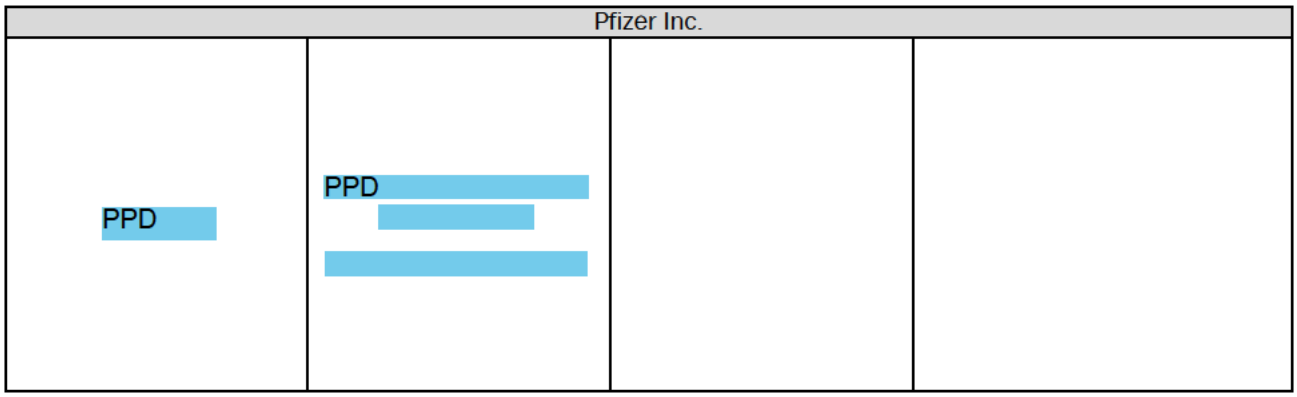
Version	Date	Reason for Revision
FINAL 1.0	03-Jan-2022	First version;
FINAL 2.0	03-Mar-2022	Clinical Study Protocol amendment 7.0 considered; handling of vaccinations (erroneously documented in the concomitant medication section) added; several definitions enhanced to avoid any possibility of ambiguity (programming of data cut-off, time window violation programming for immunogenicity samples, handling of missing assessments for AE, vaccination period assignment rules for AE); rule for actual treatment group programming added, as specified in Blind Data Review Meeting Report; tables for GMFRs as compared to Month 12 added; specific figures by age cohort added; Minor changes/corrections in layout, spelling, headers, and listing columns; present confidence intervals for GMTs in figures, instead of standard deviation;
FINAL 3.0	11-May-2023	CSP update 10.0 and pooling of Analysis 3.1 and 3.2;
FINAL 4.0	29-Apr-2025	CSP update 11.0;

Approval

Name	Role	Date	Signature
Author			
PPD	PPD		

Assign DMB			
PPD	PPD		
Valneva Austria GmbH			
PPD	PPD		
PPD	PPD		
PPD	PPD		



List of Abbreviations

AE	Adverse Event
AESI	Adverse Event of Special Interest
ANOVA	Analysis of Variance
approx.	Approximately
B.b.s.l.	*Borrelia burgdorferi* sensu lato
BDRM	Blinded Data Review Meeting
BFAS	Booster Full Analysis Set
BSAS	Booster Safety Analysis Set
CRA	Clinical Research Associates
CRO	Clinical Research Organization
DMC	Data Monitoring Committee
DRM	Data Review Meeting
e.g.	exempli gratia
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
EP	Endpoint
ET	Early Termination
FAS	Full Analysis Set
GMT	Geometric Mean Titer
GMFR	Geometric Mean Fold Rise
HIV	Human Immunodeficiency Virus
i.e.	id est
IgG	Immunoglobulin G
I.M.	Intramuscular
IMP	Investigational Medicinal Product
IRC	Internal Review Committee
mL	Milliliter(s)
OspA	Outer surface protein A
PBS	Polymerase Chain Reaction
PCR	Phosphate Buffered Saline
PP	Per-Protocol
PPAS	Per-Protocol Analysis Set
PT	Preferred Term

SAS	Statistical Analysis Set
SAE	Serious Adverse Event
SBA	Serum Bactericidal Assay
SCR	Seroconversion Rate
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
ST	Serotype
TLF	Table, Listing and Figure
Tukey's HSD	Tukey's Honestly Significant Difference
Yrs	Years

1. OVERVIEW

1.1 Study Objectives

1.1.1 Primary Objective

Safety:

- To assess the safety and tolerability profile of VLA15, given in a three- or two dose primary immunization schedule (Month 0-2-6 or Month 0-6), in a healthy study population aged 5 to 65 years up to Day 208 (Month 7).

Immunogenicity:

- To assess the immune response to VLA15, given in a three- or two dose primary immunization schedule (Month 0-2-6 or Month 0-6), in a healthy study population aged 5 to 65 years at Day 208 (Month 7).

1.1.2 Secondary Objectives

Safety:

- To assess the safety and tolerability profile of VLA15, given in a three- or two dose primary immunization schedule (Month 0-2-6 or Month 0-6), in a healthy study population aged 5 to 65 years, up to one year after the last primary vaccination (Month 18);
- To assess the safety and tolerability profile of VLA15 booster vaccinations (i.e., Booster 1 at Month 18, one year after completion of the primary vaccination schedule, Booster 2 at Month 30 and Booster 3 at Month 42) until study end.

Immunogenicity:

- To assess the immune response to VLA15, applied in a three- or two dose primary immunization schedule (Month 0-2-6 or Month 0-6), in a healthy study population aged 5 to 65 years, up to one year after the last primary vaccination (Month 18);
- To assess the immune response of VLA15 booster vaccinations (i.e., Booster 1 at Month 18, one year after completion of the primary vaccination schedule, Booster 2 at Month 30 and Booster 3 at Month 42) until study end.

Exploratory objective:

- To assess the functionality of Outer surface protein A (OspA) immune response at selected time points after primary and booster immunizations.

1.2 Study Design

VLA15-221 is a randomized, observer-blind (subject, sponsor and investigator/ site staff involved in clinical evaluation of subjects are blinded), placebo controlled, multicenter Phase 2 study in healthy subjects aged 5 to 65 years. The study is being conducted in two study parts (Part A: Main Study Phase, Part B: Booster Phase, see Figure 1). VLA15-221 was initiated with an age de-escalation of sentinel cohorts. Subject enrollment into Part A started with the adult cohort to allow the generation and review of appropriate safety data before pediatric cohorts were initiated.

In **Part A (Main Study Phase)** a total of approximately 600 subjects aged 5 to 65 years were randomized 1:1:1 into three study groups: Group 1 (approximately 200 subjects) received three intramuscular (I.M.) vaccinations of VLA15 at Month 0-2-6. Group 2 (approximately 200 subjects) received two I.M. VLA15 vaccinations at Month 0-6 and a placebo injection at Month 2 in order to keep the blind. Group 3 (approximately 200 subjects) received three I.M. placebo injections at Month 0-2-6. Within each study group subjects were enrolled 2:1:1 in three age cohorts (18-65 years, 12-17 years and 5-11 years)..

In Part A, all subjects received three I.M. vaccinations at Month 0-2-6 (i.e. Day 1-57-180). On Day 8/Visit 1A (i.e. 7 days after the first vaccination) a safety visit was performed (phone call for subjects aged 18-65, in-person visit for subjects aged 5-17 years). In-person visits were scheduled for all age cohorts one month after each vaccination. Blood samples for immunogenicity assessments were collected at the screening visit, Day 85, Day 180, Day 194 (in a subset of adult subjects), Day 208, Day 365/Month 12 and at Month 18.

In **Part B (Booster Phase)** all eligible subjects from Group 1 and 2 will receive a booster dose of VLA15 at Month 18, Month 30 and Month 42. Placebo injections will be administered to Group 3 subjects at Month 18, Month 30 and Month 42. Subjects will be followed-up until Month 48 (i.e., 2.5 years after start of Part B and six months after booster vaccination at Month 42) with blood samples obtained at 1, 5, 8 and 12 months following the Month 18 booster dose (study months 19, 23, 26 and 30), at 1, 9 and 12 months following the Month 30 booster dose (study months 31, 39 and 42) and at 1 and 6 months following the Month 42 booster dose (study months 43 and 48).

Table 1: Study Groups and Vaccinations

	Study Group	Subjects	Age Cohort (Age in years at Screening)	Treatment	Vaccination Schedule
Part A: Main Study Phase	Group 1	Total: 200 100 50 50	18-65 12-17 5-11	VLA15 180 µg	Month 0-2-6
	Group 2	Total: 200 100 50 50	18-65 12-17 5-11	VLA15 180 µg Placebo	Month 0-6 Month 2*
	Group 3	Total: 200 100 50 50	18-65 12-17 5-11	Placebo	Month 0-2-6
Part B: Booster Phase	Group 1	Total: 200 100 50 50	18-65 12-17 5-11	VLA15 180 µg	Month 18, 30 and 42
	Group 2	Total: 200 100 50 50	18-65 12-17 5-11	VLA15 180 µg	Month 18, 30 and 42
	Group 3	Total: 200 100 50 50	18-65 12-17 5-11	Placebo	Month 18, 30 and 42

*In order to keep the blind, subjects assigned to Group 2 will receive a sham injection of placebo at Month 2.

1.3 Endpoints

1.3.1 Primary Endpoint

Safety:

- Frequency of solicited local and solicited systemic Adverse Events (AEs) within 7 days after each and any vaccination of the primary vaccination series (Part A).

Immunogenicity:

- Geometric Mean Titers (GMTs) for Immunoglobulin G (IgG) against each OspA serotype ST1 to ST6, determined by an IgG binding assay, at Day 208/Month 7 (Part A).

1.3.2 Secondary Endpoints

Safety:

- Frequency of solicited local and solicited systemic AEs within 7 days after each booster dose administration (Part B);
- Frequency of Serious Adverse Events (SAEs) during the entire study;
- Frequency of Adverse Events of Special Interest (AESIs) during the entire study;
- Frequency of unsolicited AEs within 28 days after each vaccination;
- Frequency of SAEs, AESIs, unsolicited and solicited AEs stratified by age cohort.

Immunogenicity:

- Part A - Main Study Phase:
    - GMTs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at baseline (screening visit) and at Day 85, 180, 194[1], 365 and Month 18;
    - Seroconversion Rates (SCRs, definition see Section 5.2.1) for each OspA serotype specific IgG (ST1 to ST6), determined by an IgG binding assay, at Day 85, 180, 194[1], 208, 365 and Month 18;
    - Geometric Mean of the fold rise (GMFRs as compared to baseline) for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at Day 85 and 208;
    - GMTs, SCRs and GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at specified time-points, stratified by age cohort.
- Part B - Booster Phase:
    - GMTs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at Months 18, 19, 23, 26, 30, 31, 39, 42, 43 and 48;
    - SCRs for each OspA serotype specific IgG (ST1 to ST6), determined by an IgG binding assay, at Months 18, 19, 23, 26, 30, 31, 39, 42, 43 and 48;
    - GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at Months 19, 31 and 43;

[1] Visit 5A/Day194 data will be available for a subset of approximately 150 adult subjects.

- GMTs, SCRs and GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at specified time points, stratified by age cohort.

1.3.3 Exploratory endpoints

- GMTs for functional antibodies against each OspA serotype (ST1 to ST6), determined e.g. by Serum Bactericidal Assay (SBA), at selected time points, (at baseline (i.e. Visit 0), Day 208/Months 7, 18, 19, 3031 and e.g., 42, 43 and 48, if deemed meaningful).
- SCRs (definition see Section 5.2.2) for functional antibodies against each OspA serotype (ST1 to ST6), determined by SBA, at selected time points, (e.g., at Day 208/Months 7, 18, 19, 30, 31, 42, 43 and 48, if deemed meaningful).

1.4 Sample Size Calculation

The sample size has been chosen to allow detection of common AEs. A total of 400 subjects in the VLA15 groups (Group 1 and Group 2) will provide 95% confidence that an AE not seen in the study would have a true incidence of not more than 0.75% across all age cohorts. In addition, the overall group size for the two VLA15 study groups has been selected to provide a sufficient safety database and for determining the optimal vaccination schedule before advancing the vaccine candidate into Phase 3. Upon completion of the study, the total number of subjects exposed to the final dose as used for upcoming Phase 3 studies would be a minimum of approximately N=710. The database would, thus, allow 95% confidence that a given reaction would not be observed at a higher rate than 1:(710/3) rate, i.e. 0.4%, if it is not observed in the studies preceding Phase 3.

With respect to the primary endpoint, GMTs for ST1-6 specific IgGs on Day 208: In the absence of an established protective titer, sample size calculation is based on somewhat arbitrary differences in GMTs between VLA15 study groups to demonstrate which titer levels could be distinguished with the proposed sample size.

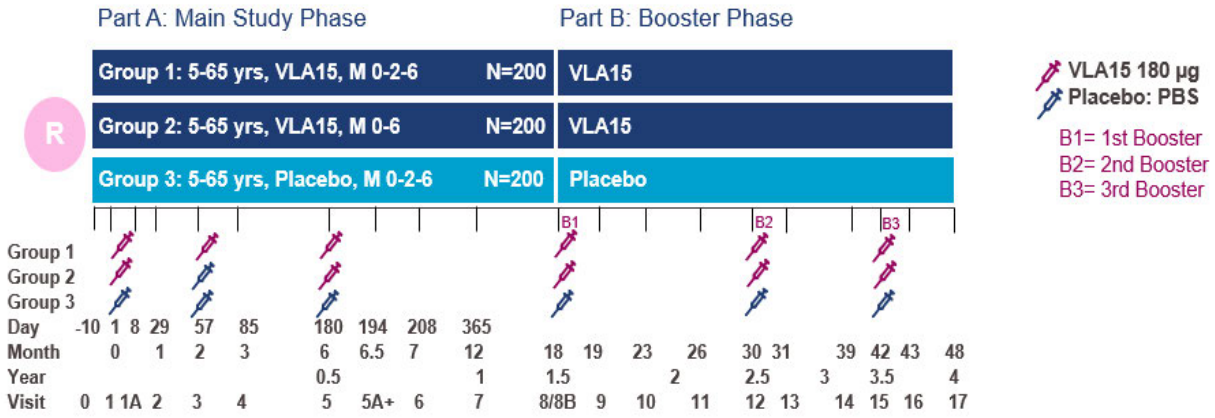
Titers observed in the primary endpoint immunogenicity analysis (i.e. Day 208; one month after third vaccination) of 86 subjects receiving 180 µg of VLA15 in ongoing Phase 2 study VLA15-202 were used as basis: In the 180 µg group (i.e. the dose chosen for further development of VLA15), an Enzyme-Linked Immunosorbent Assay (ELISA) GMT of 274.7 was observed for ST1 (i.e. the serotype with lowest titers) with a Standard Deviation (LOG10) of 0.53. A total of 180 subjects per group (assuming 10% of the 200 subjects per study group are excluded from the primary Per-Protocol (PP) analysis) will provide 80% power at a two-sided alpha level of 5% to distinguish a GMT of 274.7 in one study group from a putative higher GMT of 394.3 in the other study group.

The overall sample size of 200 subjects in the placebo group has been selected to allow for the internal validation of both safety and immunogenicity results.

1.5 Flowchart

1.5.1 Study Design

Figure 1: Study Design



1.5.2 Study Schedule

Table 2: Table of Events – Part A: Main Study Phase

Visit	V0 (1)	V1 (2)	V1A (3)	V2 (4)	V3 (2)	V4 (4)	V5 (2)	V5A (5)	V6 (4)	V7 (4)	V8 (4)	Early Termination (4) (6)
Timing Day (D) Month (M)	**D-10**	**D1**	**D8**	**D29 M1**	**D57 M2**	**D85 M3 V3 + 28d**	**D180 M6**	**D194 M6.5 V5 + 14d**	**D208 M7 V5 + 28d**	**D365 M12**	**D545 M18**	**< V8**
Time windows (D)	**-10 to 0**	**0**	**+2**	**+2/- 4**	**+/- 4**	**+/- 4**	**+4/-28**	**+/-2**	**+2/- 4**	**+/- 14**	**+/- 14**	**n/a**
Visit type	In-person	In-person	In-person or remotely	In-person or remotely	In-person	In-person or remotely	In-person	In-person or remotely	In-person or remotely	In-person or remotely	In-person or remotely	In-person or remotely
Informed consent/assent (7)	X											
Inclusion/exclusion criteria	X	X (Review)										
Vaccination delay criteria		X			X		X					
Demographic data	X											
Medical history incl. vaccinations	X	X (8)										
Concomitant medications/ treatments incl. vaccinations	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination, ECG (9)	X											
Vital signs (10)	X	X			X		X					
Evaluation of oral body temperature	X	X (11)			X (11)		X (11)					
HIV test [3.0 mL] (12)	X											
B.b. s.l. screening test [5.0 mL] (13)	X										X	X (14)
Serum Pregnancy test [2.0 mL] (15)	X											
Urine Pregnancy test (15)		X (16)		X	X (16)	X	X (16)		X	X	X	X
Immunogenicity blood sample [12.0 mL] (17)	X					X	X	X	X	X	X	
Assay development sample [75.0 mL] (18)		X				X			X			
Randomization (19)		X										
VACCINATION (20)		X			X		X					
Check for AEs following vaccination		X (21)			X (21)		X (21)					
Symptom-driven physical exam (22)		X	X (23)	X	X	X	X	X	X	X	X	X
Inspection of injection site of previous vaccinations			X (23)	X		X		X	X			X (25)
Explain eDiary (24)		X										
Review eDiary			X	X		X		X	X			X (26)
Check eDiary for SAEs/ AESIs			X	X	X	X	X	X	X	X	X	X
Scripted Safety Assessment			X	X	X	X	X	X	X	X	X	X
AE/ SAE/ AESI Assessment (27)			X	X	X	X	X	X	X	X	X	X
Blood Volume [mL]	17.0 (28); 19.0 (29); 20.0 (30); 22.0 (31)	75.0 (32)	0.0	0.0	0.0	12.0 (33); 87.0 (34)	12.0	12.0	12.0 (33); 87.0 (34)	12.0	17.0	5.0 (14)

Table 3: Table of Events – Part B. Booster Phase

Visit	V8B [35]	V9	V10	V11	V12	V13	V14	V15	V16	V17	Early Termination [36]
Month (M)	**M18**	**M19 V8B + 28d**	**M23**	**M26**	**M30**	**M31 V12 + 28d**	**M39**	**M42**	**M43 V15 + 28d**	**M48**	**< V17**
Time windows (D)	**V8 +14**	**+/-4**	**+/-14**	**+28/-14**	**+/-28**	**+/-4**	**+/-14**	**+/-28**	**+/-4**	**+/-14**	**n/a**
Visit type	In-person	In-person	In-person	In-person	In-person	In-person	In-person	In-person	In-person	In-person	In-person
Inclusion/Exclusion criteria (Booster Phase)	X (7)				X (7)			X (7)			
Vaccination delay criteria	X				X			X			
Concomitant medications/ treatments incl. vaccinations	X (37)	X	X	X	X	X	X	X	X	X	X
Physical examination (9)	X				X			X			
Vital signs (10)	X				X			X			
Evaluation of oral body temperature	X (11)				X (11)			X (11)			
B.b. s.l. screening test [5.0 mL] (13) (38)					X			X		X	X
Urine Pregnancy test (15)	X (16)	X	X		X (16)	X		X (16)	X	X	X
Immunogenicity blood sample [12.0 mL] (17)		X	X	X	X	X	X	X	X	X	
Assay development sample [75.0 mL] (18)		X				X			X		
VACCINATION (20)	X				X			X			
Check for AEs following vaccination	X (21)				X (21)			X (21)			
Symptom-driven physical exam (22)		X	X	X		X	X		X	X	X
Inspection of injection site of previous vaccinations		X				X			X		X (25)
Explain eDiary (24)	X				X			X			
Review eDiary		X				X			X		X (26)
Check eDiary for SAEs/ AESIs	X	X	X	X	X	X	X	X	X	X	X
Scripted Safety Assessment	X	X	X	X	X	X	X	X	X	X	X
AE/ SAE/ AESI Assessment (27)	X	X	X	X	X	X	X	X	X	X	X
Blood Volume [mL]	0.0	12.0 (33); 87.0 (34)	12.0	12.0	17.0	12.0 (33); 87.0 (34)	12.0	17.0	12.0 (33); 87.0 (34)	17.0	5.0

(1) Re-screening of subjects is allowed once. Assessments, which have been performed during the first screening visit, remain valid for 14 days. In case re-screening occurs outside this time frame, already performed measures have to be repeated.

(2) At on-site Visits, subjects will be supplied with urine pregnancy test kits to ensure that subjects can test for pregnancy at home in case the COVID-19 pandemic prohibits in-person visits at subsequent scheduled visits (Visit 2, 4, 6, 7 and 8). Pregnancy test handling has to be explained to subjects by trained study site staff during Visit 1.

(3) Visit 1A is a safety follow-up visit to be performed 7 days after the first vaccination (i.e., on Day 8). It is intended to perform Visit 1A remotely via a phone/video call in adult subjects (aged 18-65 years) and preferably as an in-person visit in adolescent subjects (aged 12-17 years) and children (5-11 years). If the COVID-19 pandemic prohibits in-person visits, Visit 1A may also be conducted remotely via a phone/video call in the younger age groups. For details on differences in respective study event procedures during in-person or remote visits, please refer to Section 4.2.1.

(4) Visit should preferably be conducted as an in-person visit. If an in-person is not feasible due to COVID-19, e.g., travel restrictions, local recommendations, circumstances at the study site's location that prohibit an in-person visit, or if the PI believes that the subject's safety and well-being might be jeopardized with an in-person visit at the study site due to COVID-19, the visit should be conducted remotely. In case subject visit has to be performed remotely, a mobile nurse professional will come to the subject's home and take the immunogenicity sample within the specified time window. Review of subject eDiary safety data will be performed by the study site via phone/video call. For details on differences in respective study event procedures during in-person or remote visits, please refer to Section 4.2.4. In order to perform urine pregnancy test at home, subjects will be supplied with additional test kits at the last on-site Visit.

(5) Visit 5A will be performed in a subset of approximately 150 adult subjects only.

(6) Every effort should be made to have discontinued subjects complete the early termination visit. If the subject is unwilling to perform an ET visit, a phone-call should be made to follow-up on Adverse Events and Concomitant Medications/ Vaccinations. Note: If a subject presents at a regular study visit and informs the site that it will discontinue the study after this visit, the study visit will not be performed as an ET visit, but as a regular study visit including all events that are described for the respective study and ET visit.

(7) Informed consent/assent may be obtained within 10 days before Visit 1. For the Booster Phase, all subjects are asked to reconfirm the consent/assent latest at the respective booster vaccination visit.

(8) Symptoms noted at Visit 1 (prior to first vaccination) are not considered AEs but will be recorded as medical history.

(9) Physical examination on the following body systems: general appearance (including assessment of body weight and height), skin, head/ eyes /ears/ nose/ throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, and neurological system. An ECG will be performed at Visit 0 only.

(10) Vital signs (Systolic and diastolic blood pressure and pulse rate while seated and at rest) to be measured prior to vaccination and in addition prior to discharge in case subject reports any complaints.

(11) To be performed prior to vaccination.

(12) The results of negative HIV tests that were performed up to 4 weeks before Visit 0 are acceptable (blood: HIV test 3.0 mL). Positive HIV test obtained by ELISA will have to be confirmed by a second method (e.g., Western Blot or PCR). HIV tests only in subjects aged ≥ 12 years.

(13) A commercially available Lyme borreliosis screening test will be performed (blood: 5.0 mL). Serum samples that are tested positive will have to be verified by a confirmatory immunoblot. Test result does not need to be available before randomization and remains valid for 4 weeks.

(14) In case an ET Visit is performed remotely, the collection of the Lyme borreliosis screening sample will be omitted.

(15) Female subjects aged ≥12 years and woman of childbearing potential. A woman is considered of childbearing potential if fertile and until becoming post-menopausal unless permanently sterile. A woman that is considered of non-childbearing potential must be e.g., surgically sterilized for at least 3 months prior to Visit 1 (e.g., by hysterectomy, bilateral salpingectomy, bilateral oophorectomy, transcervical sterilization), or postmenopausal for at least one year prior to Visit 1. For serum pregnancy test: tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable. Testing will only be performed in the 5 to 11 age cohort in female subjects after onset of menarche.

(16) At vaccination visits, all samples have to be obtained before vaccination. Pregnancy results must be available before vaccination.

(17) Blood will be collected for immunogenicity testing by an IgG binding assay and for supportive functional antibody testing by e.g., serum bactericidal assay.

(18) Assay development sera will be collected in adult subjects (age cohort 18 to 65 years) only and will be used for further development of clinical assays.

(19) To be performed by study staff otherwise not involved with study conduct to keep the study observer-blinded (i.e., un-blinded study staff)

(20) Preparation of vaccination must be done by designated unblinded staff members (4.2.12) only. Administration of the vaccine can be performed by blinded OR unblinded staff. Vaccination with the assigned treatment has to be administered into the deltoid of the non-dominant arm. Subjects should be observed for at least 30 minutes for treatment of any immediate reactions.
(21) If subject has any complaints after vaccination, a symptom-driven physical examination will be performed by the investigator prior to discharge.
(22) Except for Visit 1: Body systems for which the subject reports any symptoms should be evaluated and relevant abnormal findings documented as AEs. At vaccination days the symptom-driven physical exam is to be performed before administration of the vaccination.
(23) At in-person visits for subjects aged 5-17 years.
(24) At Visit 1, the subjects will be provided with thermometer and measuring tapes. The subjects will assess solicited local and systemic AEs themselves over a period of seven consecutive days after each vaccination. Subjects/ legal representative(s) will also be instructed to immediately inform the site in case of symptoms suggesting Lyme borreliosis, or any severe solicited AEs or other severe symptoms.
(25) For Early Termination prior to Visit 6 in Part A or Visit 9/ Visit 17 in Part B, the previous injection site should be inspected.
(26) Subject eDiary entries should be reviewed at the ET Visit if not done at previous visit.
(27) Unsolicited AEs will be collected within 28 days after each vaccination. SAEs and AESIs will be collected throughout the entire study conduct and documented in the eCRF. Symptoms noted at Visit 1 prior to vaccination are not considered adverse events but will be recorded as medical history.
(28) In females of non-childbearing potential (before onset of menarche) and male subjects aged <12 years i.e., without serum pregnancy and HIV test
(29) In females aged <12 years and after onset of menarche i.e., with serum pregnancy test and without HIV test
(30) In females of non-childbearing potential (surgically sterile or postmenopausal) and in males aged ≥12 years i.e., without serum pregnancy test and with HIV test
(31) In females aged ≥12 years and of childbearing potential i.e., with serum pregnancy test and HIV test
(32) A blood draw of in total 75.0 mL will be taken in subjects aged 18-65 years.
(33) A blood draw of in total 12.0 mL will be taken in subjects from following age cohorts: 5-11 years and 12-17 years.
(34) A blood draw of in total 87.0 mL (12.0 mL for immunogenicity and 75.0 mL for assay development) will be taken in subjects from age cohort 18-65 years.
(35) Visit 8B should preferably be performed on the same day as Visit 8.
(36) If the subject is unwilling to perform an ET visit, a phone call should be made to follow-up on Adverse Events and Concomitant Medications/Vaccinations.
(37) Only to be performed if V8B is performed more than 14 days after V8, otherwise the assessment is already captured at Visit 8.
(38) Test results do not need to be available before vaccination.

2. GENERAL CONSIDERATIONS

2.1 Conduct of Analysis

The following analyses are planned:

- Analysis 1 included all safety and available ELISA immunogenicity data from selected time points from Part A up to Day 208/ Month 7 including the primary endpoint.
    - Analysis 1.1: Cohort 1, aged 18-65 years
    - Analysis 1.2: Cohort 2, aged 12-17 years
    - Analysis 1.3: Cohort 3, aged 5-11 years

  Every age cohort was analyzed separately and were pooled with age cohorts of previous analyses as soon as data from each cohort was available.

  Pooled data from all three cohorts concluded Analysis 1;
- Analysis 2 included all safety data and available ELISA data of Part A up to Month 12 (i.e., 6 months after the third immunization) and was performed once all subjects had completed Visit 7;
- Analysis 3 included all safety data and immunogenicity data from selected time points from Part A up to Month 18 and safety and immunogenicity data from Part B up to Month 19.
- Analysis 4 included all safety and ELISA immunogenicity data of Part B up to Month 31. The analysis was performed once all subjects had completed Visit 13;
- Analysis 5 will include all safety and ELISA immunogenicity data of Part B up to Month 43. The analysis will be performed once all subjects have completed Visit 16;
- Analysis 6 will include all safety and ELISA immunogenicity data of Part B up to Month 48. The analysis will be performed after the last subjects completed the last study visit (Visit 17).

In case analyses fall close in timing, they may be merged.

In total, 5 Clinical Study Reports (CSRs) with following content will be compiled:

- CSR 1 compiles the data from Analysis 1;
- CSR 2 compiles the data from Analysis 2 and 3;
- CSR 3 compiles the data from Analysis 4;
- CSR 4 will include the data compiled in Analysis 5;
- CSR 5 will include the data compiled in Analysis 6.

2.2 Statistical Software and Quality Control

All statistical analyses will be performed using SAS® version 9.3 or higher. Tables, figures and data listings (TLFs) are generated in Microsoft® Word® as well as PDF® format.

Quality control of SAS® programs will include a review of the whole process of result generation:

- Review of all analysis SAS® programs;
- Review of SAS® log for errors, warnings and other notes that could indicate mistakes in the programs;
- Review of all tables, listings and figures for completeness and correctness.

As additional quality control measure, independent re-programming will be performed as described in Standard Operating Procedure (SOP) SAS04.

2.3 Randomization and Blinding

Subjects were allocated to study groups via the randomization module of the EDC system. Subjects were randomized 1:1:1 to the three study groups, randomization was stratified by age in a 2:1:1 (adults: 18-65 years; adolescents: 12-17; children: 5-11 years) ratio. Date and time of enrollment are defined as the time point at which the subject was randomized.

The study is an observer-blinded study, which is conducted in a blinded manner for the study investigators, the sponsor including laboratory personnel, and the subjects. An overview of persons who will be unblinded and blinded is provided below:

Unblinded:

- Designated study site staff who randomize subjects to study groups and are concerned with Investigational Medicinal Product (IMP) handling (i.e., perform preparation and maintain drug dispensing log). These unblinded study staff members will not conduct any other study procedures;
- Clinical Research Associates (CRAs) responsible for monitoring of IMP handling and related data for verifying drug accountability during the study and performing overall drug accountability;
- Dedicated statistical team at the Clinical Research Organization (CRO) performing statistical analyses for generation of safety data tables for the Data Monitoring Committee (DMC) and (Internal Review Committee (IRC);
- DMC members;
- IRC members.

Blinded:

- Study participants and legal representative(s);
- Investigators and other study staff involved in general study conduct and safety assessments;
- All other CRAs (responsible for monitoring study data apart from IMP handling/drug accountability);
- All other sponsor/ collaboration partner and CRO staff including laboratory personnel at the sponsor's labs for immunogenicity assessments.

Unblinding during the study:

The study sponsor/ collaboration partner and study statisticians were unblinded at the time of the primary endpoint analysis, i.e. Day 208 analysis, in each age cohort (i.e., Analysis 1.1, Analysis 1.2 and Analysis 1.3) after the respective database snapshot had been performed. All other study personnel including the investigators and other study staff involved in general study conduct and safety assessments as well as laboratory personnel who are performing analytical assays will remain blinded until study end.

2.4 Descriptive Analyses

Descriptive analyses of continuous variables (summary statistics) will be described with the number of non-missing observations, arithmetic mean, standard deviation (±SD), median, quartiles (Q1 and Q3) and range (minimum and maximum).

Descriptive analyses of continuous immunogenicity variables (i.e. tables for the GMT and GMFR) will be described with the number of non-missing observations, geometric mean, confidence intervals for the geometric mean, standard deviation of logarithmic values, median, quartiles (Q1 and Q3) and range (minimum and maximum).

Categorical variables (frequency statistics) will be described with the number of non-missing observations and percentages (%). Percentages will be calculated on the total number of non-missing observations, if not stated otherwise.

2.5 Center and Country Effect and Stratification

No country effect will be considered since all centers are located in the United States. The center effect will be taken into account and estimated by adding study site in ANOVA models (sensitivity analyses). Also other covariates will be used as described in Section 5.3.

Furthermore, selected tables will be presented by stratification factor age cohort. If not stated otherwise, the stratification will be implemented by showing the overall data in a first section followed by separate sections for each age cohort separately starting with the oldest age cohort. A separate column in the List of TLFs (Section 7) defines which tables are stratified by age cohort.

2.6 Handling Missing Data

Generally, missing values of immunogenicity variables will not be imputed, and the analyses will be limited to observed values. For missing data in AE evaluation (i.e. missing information if serious, medication taken, severity and causality) a worst case approach will be applied. In case of missing assignment to solicited or unsolicited, this AE will be neither counted in tables for solicited AEs nor in tables for unsolicited AEs but in tables for all AEs. For more details see Section 6.1.3.

2.7 Protocol Deviations

In general, Data Review Meetings (DRM) will be conducted on all available data to review protocol deviations, to discuss specific unforeseeable data issues, and to allocate the subjects to the analysis sets. In particular, the respective Per-Protocol Analysis Sets will be defined in the DRMs. In case analyses fall close in timing, DRMs may be merged. DRMs will be conducted at least prior Analyses 1.1, 1.2 and 1.3 in Part A to define the Per-Protocol Analysis Set and prior Analyses 3 and 4 to classify protocol deviations. Protocol deviations affecting time points after Day 365 will not lead to an exclusion of the subjects but only time points affected by the respective relevant protocol deviation will be excluded. Further DRMs might be conducted prior the respective database snapshots or database closure. Protocol deviations will be classified as “not relevant” or “relevant” deviations on a case by case decision, based on the potential impact on the immunogenicity analysis. Protocol deviations identified by the monitor will be classified as “major” or “minor” by the CRA prior the meetings.

Associated decisions of the DRMs will be documented and approved in the Data Review Meeting Report.

Protocol violations classified as “relevant” during the DRMs and / or classified as “major” by CRA will be described in the CSR (Clinical Study Report).

2.7.1 Part A: Main Study Phase

“Relevant” protocol violations that lead to exclusion from the Per-Protocol Analysis Set in the Main Study Phase (see Section 2.9.3) will include the following but are not limited to:

- Subjects with less than three primary vaccinations (Day 1, 57 and 180);
- Subjects who received the wrong IMP;
- Subjects who fulfilled exclusion criteria 2, 8, 9, 13;
- Subjects with substantial time window violations on vaccination visits (Visits 3 and 5)
    - Target Date of Visit 3 (Day 57) +/-10 days;
    - Target Date of Visit 5 (Day 180) +14/-28 days;
- Subjects with other deviations that may affect immune response.

2.7.2 Part B: Booster Phase

“Relevant” protocol violations that lead to exclusion from the Booster Per-Protocol Analysis Set (see Section 2.9.3) will include the following but are not limited to:

- Subjects enrolled despite exclusion from the PPAS of the Main Study Phase;
- Subjects who received a wrong booster IMP;
- Subjects who fulfilled the Booster Phase exclusion criteria 5 and 8;
- Subjects with other deviations that may affect immune response.

2.8 Exclusion of Time Points in the Per-Protocol Analysis

In the Per-Protocol Analysis immunogenicity samples that are outside the predefined time windows described will be excluded at respective visits (exclusion of subjects from the Per-Protocol Analysis Set is described in Section 2.9.3). Time windows for the exclusion of immunogenicity samples differ from the time windows for study visits (defined in CSP Section 4.2.4) because the time window instruction for study sites that define the time point of the subjects' return to subsequent visits is different from the time window for an immunogenicity sample to be eligible for the per-protocol immunogenicity analysis.

	Per-Protocol Window	
	Start date	End date
Visit 4	Visit 3 plus 28 days minus 10 days	Visit 3 plus 28 days plus 10 days
Visit 5	Visit 1 plus 179 days minus 28 days	Visit 1 plus 179 days plus 14 days
Visit 5a	Visit 5 plus 14 days minus 5 days	Visit 5 plus 14 days plus 5 days
Visit 6	Visit 5 plus 28 days minus 14 days	Visit 5 plus 28 days plus 14 days
Visit 7	Visit 1 plus 364 days minus 21 days	Visit 1 plus 364 days plus 21 days
Visit 8	Visit 1 plus 544 days minus 28 days	Visit 1 plus 544 plus 28 days

In the Booster Per-Protocol Analysis immunogenicity samples that are outside the predefined time windows described below will be excluded at the respective visit (exclusion of subjects from the Booster Per-Protocol Analysis Set is described in Section 2.7):

	Per-Protocol Window		Comments
	Start date	End date	
Visit 9 Month 19	Date of Visit 8B booster vaccination plus 28 days minus 14 days	Date of Visit 8B booster vaccination plus 28 days plus 14 days	
Visit 10 Month 23	Date of Visit 8B booster vaccination plus 5 months minus 28 days	Date of Visit 8B booster vaccination plus 5 months plus 28 days	5 months = 152 days
Visit 11 Month 26	Date of Visit 8B booster vaccination plus 8 months minus 28 days	Date of Visit 8B booster vaccination plus 8 months plus 28 days	8 months = 243 days
Visit 12 Month 30	Date of Visit 8B booster vaccination plus 12 months minus 36 days	Date of Visit 8B booster vaccination plus 12 months plus 36 days	12 months = 365 days
Visit 13 Month 31	Date of Visit 12 booster vaccination plus 28 days minus 14 days	Date of Visit 12 booster vaccination plus 28 days plus 14 days	
Visit 14 Month 39	Date of Visit 12 booster vaccination plus 9 months minus 28 days	Date of Visit 12 booster vaccination plus 9 months plus 28 days	9 months = 274 days
Visit 15 Month 42	Date of Visit 12 booster vaccination plus 12 months minus 36 days	Date of Visit 12 booster vaccination plus 12 months plus 36 days	12 months = 365 days
Visit 16 Month 43	Date of Visit 15 booster vaccination plus 28 days minus 14 days	Date of Visit 15 booster vaccination plus 28 days plus 14 days	
Visit 17 Month 48	Date of Visit 15 booster vaccination plus 6 months minus 28 days	Visit 15 booster vaccination plus 6 months plus 28 days	6 months = 183 days

2.9 Analysis Populations

In general, all Part B analyses are based on the analysis sets that were defined for Analysis 2 except AE analysis by vaccination period (details see Section 2.9.1).

2.9.1 Safety Analysis Set and Booster Safety Analysis Set

The Safety Analysis Set (SAS) includes all subjects who entered into the study and received at least one vaccination. The SAS will be used for all baseline, safety and tolerability analyses including demographic data, local/systemic tolerability, laboratory data, (S)AEs and AESIs.

All safety analyses for AEs table type after any vaccinations will be based on the SAS. Summary analyses for AEs by vaccination period will be limited to subjects that received the respective vaccination, i.e. in Analysis 3, certain type of AEs after first booster vaccination will be based on subjects that received the first booster vaccination at Month 18 and certain type of AEs after second booster will be limited to subjects that received the second booster vaccination. Other safety analysis (e.g. vital signs, physical examination) will be based on the SAS limited to subjects that performed the respective assessments.

The Booster Safety Analysis Set (BSAS) comprises as all subjects enrolled in Part B who received all respective booster vaccinations and will be defined prior each analysis separately. The BSAS will be used for demographic data.

All analyses based on the SAS will be carried out using the actual treatment received. Details for the derivation of the actual treatment are described in Section 2.11.

2.9.2 Full Analysis Set and Booster Full Analysis Set

The Full Analysis Set (FAS) is defined to include all subjects enrolled who received at least one vaccination. For Part A and B, selected immunogenicity analysis will be repeated for the FAS. The Booster Full Analysis Set (BFAS) is defined as all subjects who received all respective booster vaccinations and will be defined prior each Part B analysis separately. Demographic information will be repeated for the BFAS.

Subjects will be analyzed according to the study group they had been allocated to, rather than by the actual treatment they received.

If the SAS and FAS Population are identical (i.e. no mistreated) the analysis will be performed for the SAS and the FAS together, labeled with “Safety / Full Analysis Set”. If the BSAS and BFAS Population are identical (i.e. no mistreated) the analysis will be performed for the BSAS and the BFAS together, labeled with “Booster Safety / Booster Full Analysis Set”.

2.9.3 Per-Protocol Analysis Set

The Per-Protocol Analysis Set (PPAS) consists of the FAS population excluding subjects that meet one of the exclusion criteria described in Section 2.7.1). For immunogenicity analysis, the PPAS will be the primary analysis population. The PPAS will be defined for each age cohort in a BDRM prior the respective analysis (i.e. Analysis 1 and Analysis 2).

For Analysis 3 and ongoing the following approach will be performed: Immunogenicity analysis will be performed on the PPAS as defined in the DRM for Analysis 2 (i.e. data up to Month 12). Protocol deviations collected for the

remaining time points will also be classified in the respective DRM. In case of a relevant PD, the subjects will in general not be excluded from the entire PPAS of the respective analysis, but rather only affected time point(s) after a relevant PD had occurred. For example, if a subject received the wrong vaccine, the subject will be excluded from all subsequent time points in the per-protocol analysis, and all of this subject samples will remain in the FAS.

2.10 Subject Data Listings

All vaccinated subjects will be included in the listings, if not stated otherwise. Data listings will include the subject number as identifier (and parameter and/or visit if available) and are sorted by subject ID (and parameter and/or visit if available), if not stated otherwise. The planned study groups will be displayed in listings of the Overall Study Information, Baseline Evaluation (see Sections 3 and 4) and in the immunogenicity listings (see Section 5). Additionally, a column indicating if a subject is in the respective (B)PPAS is shown in all immunogenicity listings. The actual treatment will be displayed in the safety listings (see section 6)..

2.11 Columns in Tables and Derivation of Actual Treatment

- Tables in the overall study information (Section 3), baseline evaluation (Section 4) and unsolicited AEs or other safety results (Section 6) will show one column per study group (i.e. Group 1, Group 2, Group 3), a column for the two pooled VLA15 study groups, pooled together with subjects receiving VLA15 at least once but that cannot be assigned to Group 1 or Group 2 and a column showing all vaccinated subjects[2].
- Tables defined for immunogenicity analysis (Section 5) will show one column for each study group as randomized.
- Tables defined for safety analysis (Section 6) will be presented by following columns:
  - Tables showing Adverse Events after any vaccination will be presented by columns Group 1, Group 2, Group 3, a column for the two VLA15 study groups pooled including subjects that received VLA15 at least once but cannot be assigned to one of the existing study groups (label “Received any vaccination”) and a column for all subjects pooled.

    Columns Group 1, Group 2 and Group 3 include
    - All subjects that were randomized to the respective study group and received no wrong IMP at any vaccination;
    - All subjects that were randomized to a study group but erroneously received the vaccination schedule of another study group. E.g. If a subject was randomized to Group 2 but erroneously received VLA15 instead of placebo at the second vaccination and all other vaccinations were performed as randomized, the subject fulfills the vaccination schedule of Group 1 and is analyzed in Group 1.

    Column “Received any VLA15” includes
    - All subjects with actual study treatment of Group 1 and Group 2 as described above;

[2] Pooled VLA15 group column may also contain subjects that received wrong treatment that could not explicitly be assigned to Group 1 or 2

- All subjects that were mistreated and cannot be allocated to any existing study group as described above, i.e. subjects randomized to Group 2 but that received Placebo instead of VLA15 at the first vaccination and the remaining vaccinations as randomized and thus, cannot be allocated to the vaccination schedules of Group 1, Group 2 or Group 3 and will be analyzed in column “Received any VLA15;

- Adverse Events by vaccination period will be presented by separate tables for each vaccination presented by the columns “VLA15” and “Placebo”. Subjects will be allocated to those columns based on the actual treatment they received at the respective vaccination;
- Subjects in Groups 1 and 2 that missed a VLA15 vaccination will only be shown in columns "any VLA15" and "all subjects". Subjects in Group 1 that only missed second vaccination will be shown in column for Group 2 (and "any VLA15" and "all subjects"). Subjects from Group 3 that erroneously received VLA15 instead of Placebo will be shown in columns "any VLA15" and "all subjects".

In case of a statistical test an additional column showing the p-value will be displayed.

2.12 Medical Coding

Adverse events, medical history and concomitant procedures will be coded using Medical Dictionary for Regulatory Activities (MedDRA) and concomitant medications and vaccination history will be coded using WHO Drug Reference List and Anatomical Therapeutic Chemical (ATC) Classification System as described in the Coding Guideline.

Events reported in the adverse events log will be combined with solicited symptoms from the diary section. Solicited symptoms will be coded as described in Section 6.1.3.2.

2.13 Labelling of Visits

The following labels for visits are used in the analysis:

Visit	Label	Visit	Label
Visit 0	V0 (M0/Screening)	Visit 9	V9 (M19)
Visit 1	V1 (D1)	Visit 10	V10 (M23)
Visit 2	V2 (M1/D29)	Visit 11	V11 (M26)
Visit 3	V3 (M2/D57)	Visit 12	V12 (M30)
Visit 4	V4 (M3/D85)	Visit 13	V13 (M31)
Visit 5	V5 (M6/D180)[3]	Visit 14	V14 (M39)
Visit 6	V6 (M7/D208)	Visit 15	V15 (M42)
Visit 7	V7 (M12/D365)	Visit 16	V16 (M43)
Visit 8	V8 (M18/D545)	Visit 17	V17 (M48)

[3] Visit 5A (M6.5/D194) was performed by a subset of approximately 150 adult subjects only.

2.14 Changes in the Conduct of the Study or Planned Analysis

Statistical analyses are performed according to CSP. In case any statistical analysis deviates from SAP, all changes are described and justified in the CSR.

3. OVERALL STUDY INFORMATION

Analyses will be performed for the respective SAS, FAS and PPAS.

3.1 Data Points

The following information will be analyzed descriptively and corresponding details on the subject level will be provided in data listings:

- Subject overview
- Screening failures and reasons
- Randomization
- Violated inclusion/exclusion criteria (will only be listed)
- Study vaccination details
- Medication error (will only be listed, only applicable for Part B analyses)
- Visits details
- Attendance status and early termination details
- Protocol deviations
- Visits performed remotely

Specifications on tables and listings are provided in the List of TLFs (see Section 7).

3.2 Definitions

- Screening failures will be identified by checking if the “Visit attendance status of subject” is answered with “screening failure” on the eCRF page “End of Study”;
- For Analysis 1.1/1.2/1.3 and Analysis 1 the cut-off for early termination data is defined by Day 208/ Month 7;
- For Analysis 2 the cut-off for early termination data is defined by Day 365/ Month 12;
- For Analysis 3 the cut-off for early termination data is defined by Month 19;
- For Analysis 4 the cut-off for early termination data is defined by Month 31;
- For Analysis 5 the cut-off for early termination data is defined by Month 43;
- For Analysis 6 all available early termination data are analyzed;
- Early terminations up to data cut-off will be detected by calculating the last attended scheduled visit for subjects with visit attendance status “early termination” on the End of Study page in the eCRF.

4. BASELINE EVALUATION

Analyses will be performed for the SAS, FAS and PPAS for Part A and Part B. Demographic information will be repeated for the respective BSAS, BFAS and PPAS.

4.1 Data Points

The following information will be analyzed descriptively and corresponding details on the subject level will be provided in data listings:

- Demographic Information (gender, childbearing potential, age [years], age cohort, race, ethnicity, body height [cm], body weight [kg] and body mass index [kg/m^2])
- Physical examination (is only listed)
- ECG
- Vaccination history
- Medical History
- Prior/Concomitant Procedures
- Prior/Concomitant Medication
- HIV (is only listed)

4.2 Definitions

- Baseline analyses is based on data of Visit 0;
- The data point 'Demographic Information' will be repeated for boostered vs. non-boostered subjects by age cohort (i.e., columns Group 1 boostered, Group 1 non-boostered, Group 2 boostered, Group 2 non-boostered, Group 3 boostered, Group 3 non-boostered, Received any VLA15 boostered, Received any VLA15 non-boostered, Total boostered and Total non-boostered) for SAS and will be repeated by study site. 'Received any VLA15 boostered' will include subjects that received any VLA15 during main phase and all respective booster vaccinations. 'Received any VLA15 non-boostered' will include subjects that received any VLA15 during main phase but none of the respective booster vaccinations.
- Body height will be analyzed in centimeters (cm). Body height documented in inch (in) will be converted to cm using the following rule: height [cm] = height [in] × 2.54;
- Body weight will be analyzed in kilogram (kg). Body weight documented in pounds (lbs) will be converted to kg using the following rule: weight [kg] = weight [lbs] × 0.45359237;
- The Body Mass Index [kg/m_2] will be calculated as (kg/cm_2) × 10,000
- Medications / Procedures stopped prior (<) to Day 1 (Visit 1) will be considered prior medications /procedures, all other medications procedures will be considered to be concomitant. Medications / Procedures with a missing or incomplete end date where it cannot clearly be decided if the end date was before or after Day 1 (Visit 1) will be considered concomitant:
- For Analysis 1.1/1.2/1.3 and Analysis 1 the cut-off for medications / procedures data is defined by Day 208/ Month 7;

- For Analysis 2 the cut-off for medications / procedures is defined by Day 365;
- For Analysis 3 the cut-off for medications / procedures is defined by Month 19;
- For Analysis 4 the cut-off for medications / procedures is defined by Month 31;
- For Analysis 5 the cut-off for medications / procedures is defined by Month 43;
- For Analysis 6 all available medications / procedures are analyzed;
- Medications/procedures will be considered to have started up to data cut-off if the start date of the medication/procedure is before or on data cut-off (e.g. Day 208/ Month 7 for Analysis 1). In case of an incomplete start date where it cannot clearly be decided if the medication/procedure started up to data cut-off or not, the medication/procedure will be considered to have started up to data cut-off. For derivation of cut-off, the date of the respective Visit is used (e.g. date of Visit 6 for Day 208, date of Visit 7 for Day 365). In case no respective visit was performed, the cut-off is determined as date of Visit 1 plus number of days minus 1 (e.g. date of Visit 1 +207 if no Visit 6 date is available). For distances between visits defined as month, see Section 2.8 (e.g. date of Visit 1 + 544 + 28 if no Month 19 date is available);
- Medical history not stopped prior (<) to informed consent will be considered ongoing at study entry. Entries with a missing or incomplete stop date where it cannot clearly be decided if the stop date was before or after informed consent will be considered ongoing at study entry.
- Prior vaccinations erroneously reported in the concomitant medication section of the eCRF instead of the section “Vaccination history” will be excluded from the tables showing prior medications. Those entries will be detected by using the ATC level 2 “Vaccines” and will be added to the summary count in the vaccination history table.

5. IMMUNOGENICITY ANALYSIS

All immunogenicity analyses will be performed on the PPAS and will be repeated for selected analyses on the FAS. Selected TLFs will be repeated for the different age cohorts. Specifications are given in Section 7. The primary immunogenicity analysis will be based on the PPAS.

All immunogenicity analyses will contain all available time points, i.e. time points which were already analyzed in previous analyses will be repeated in the current analysis. All immunogenicity time points of Part A will further be repeated in Part B, if not stated otherwise.

For the evaluation of immunogenicity, human sera will be analyzed for IgG against each OspA serotype (ST1 to ST6) separately by a quantitative ELISA. ELISA immunogenicity data are available in Part A at Visit 0 and Visit 4 to Visit 8 as well as in Part B for Visits 9 to Visit 17. In addition to the IgG binding assay, a serum bactericidal assay (SBA) for each OspA serotype will assess functional antibodies in a subset of subjects to support the immunogenicity assessment. SBA is tested for all subjects in Group 1 and 2 and will be available at Visit 0 (Month 0), Visit 6 (Month 7), Visit 8 (Month 18), Visit 9 (Month 19), Visit 12 (Month 30), Visit 13 (Month 31), as well as Visit 15 (Month 42), Visit 16 (Month 43) and Visit 17 (Month 48), if deemed meaningful.

All available time points will be analyzed in the respective analysis, i.e. time points already analyzed in previous visits are repeated, if not stated otherwise.

5.1 Data Points

5.1.1 Tables and Listings

The following information will be analyzed separately for ELISA and SBA and for both study parts A and B, if not stated otherwise. Corresponding details on subject level will be provided in data listings.

Summary tables for categorical immunogenicity variables:

- Immunogenicity blood sample availability by time point
- ELISA:
    - Immunogenicity results/serostatus (report concentration/negative;) by OspA specific serotype by time point (including baseline serology status)
    - Subjects by OspA specific IgG serostatus by visit
    - SCRs for OspA specific IgG antibodies by visit
        - each OspA serotype (separate tables for ST1 to ST6)
        - all six OspA serotypes combined
        - ST1 and ST2 combined
- SBA:
    - Immunogenicity results/serostatus (SBA: positive/negative/pre-dilution) by OspA serotype by time point (including baseline serology status)
    - Subjects by SBA serostatus by time point
    - SCRs for SBA titer by visit
        - each OspA serotype (separate tables for ST1 to ST6)

- all six OspA serotypes combined
- ST1 and ST2 combined

Summary tables for continuous immunogenicity variables are provided:

- ELISA:
  - GMTs for IgG against each OspA specific serotype (ST1 to ST6) by visit (separate table for each OspA serotype).
  - GMFRs as compared to Visit 0 for IgG against each OspA serotype by visit (separate tables for each OspA serotype)
  - GMFRs as compared to Day 208 for IgG against each OspA serotype by visit (separate tables for each OspA serotype, only applicable for Part B)
  - GMFRs as compared to Month 12 for IgG against each OspA serotype by visit (separate tables for each OspA serotype, only applicable for Part B)
  - GMFRs as compared to Month 18 for IgG against each OspA serotype by visit (separate tables for each OspA serotype, only applicable for Part B)
  - GMFRs as compared to Month 30 for IgG against each OspA serotype by visit (separate tables for each OspA serotype, only applicable for Part B)
  - GMFRs as compared to Month 42 for IgG against each OspA serotype by visit (separate tables for each OspA serotype, only applicable for Part B)
- SBA:
  - GMTs for SBA titer against each OspA serotype (ST1 to ST6) by visit (separate table for each OspA serotype).
  - GMFRs as compared to Visit 0 for each OspA serotype by visit (separate tables for each OspA serotype)
  - GMFRs as compared to Day 208 for each OspA serotype by visit (separate tables for each OspA serotype, only applicable for Part B)
  - GMFRs as compared to Month 18 for each OspA serotype by visit (separate tables for each OspA serotype, only applicable for Part B)
  - GMFRs as compared to Month 30 for each OspA serotype (separate tables for each OspA serotype, only applicable for Part B)
  - GMFRs as compared to Month 42 for each OspA serotype (separate tables for each OspA serotype, only applicable for Part B, only if SBA testing for M42, M43 and M48 is deemed meaningful)

Correlation Analysis:

- ELISA:
  - Correlation analyses for each OspA Specific IgG titer with each other OspA specific IgG titer, respectively, at Day 208, Month 19, Month 31 and Month 43 (15 comparisons in total for each

time point; pooled VLA15 study groups and for each VLA15 study group separately; IgG ELISA seropositive samples at Screening will be excluded from the correlation analysis)

- SBA:
  - Correlation analyses for each OspA Specific SBA titer with each other SBA titer, respectively, at Day 208, Month 19 and Month 31, as well as Month 43 and Month 48, if deemed meaningful (15 comparisons in total for each time point; pooled VLA15 study groups and for each VLA15 study group separately; SBA seropositive samples at Screening will be excluded from the correlation analysis)
- Correlation analysis ELISA vs. SBA:
  - For each serotype separately, correlation analysis for ELISA titers vs. respective SBA titers at Day 208, Month 19 and Month 31, as well as Month 43 and Month 48, if deemed meaningful (pooled VLA15 study groups and for each VLA15 study group separately; IgG ELISA and/ or SBA seropositive samples at Screening will be excluded from the correlation analysis).

Inferential analysis will be performed as described in Section 5.3. Details are specified in Section 7.

5.1.2 Figures for ELISA and SBA

- ELISA:
  - Bar chart: OspA-specific IgG antibodies (GMT) including 95% confidence intervals for the GMT vs OspA specific serotypes by study group for each time point separately (y-axis: GMT; x-axis: OspA specific serotypes per study group)
  - Bar chart: OspA-specific IgG antibodies (GMT) including 95% confidence intervals for the GMT by serotype over time for study group 1 and 2 separately (y-axis: GMT; x-axis: serotypes)
  - Bar charts: OspA-specific IgG antibodies (GMT) including 95% confidence intervals for the GMT by serotype and age cohort at Day 208, Month 19, Month 31 and Month 43 for study groups 1 and 2 separately (y-axis: GMT; x-axis: serotypes)
  - Bar charts Seroconversion Rate:
    - Seroconversion Rate by OspA-specific IgG and study group (for each time point separately, y-axis: percentage of subjects, x-axis: OspA STs per study groups)
    - Seroconversion Rate for all OspA-specific IgG serotypes combined over time vs. study group (y axis: percentage of subjects, x axis: visits per study groups; Time points of Part A will not be repeated in Part B)
    - Seroconversion Rate for OspA-specific IgG serotypes ST1 and ST2 combined over time vs. study group (y axis: percentage of subjects, x axis: visits per study groups)
    - Seroconversion Rate in booster phase over time in study group 1 and 2 by serotype (y-axis: percentage of subjects, x-axis: visits of booster phase by serotype)
  - Line charts (y-axis: GMT, x-axis: study days for Part A and study months for Part B): OspA specific IgG antibodies (GMT) over time vs. study group for each ST1-6 separately

- Line charts (y-axis: GMT, x-axis: study days for Part A and study months for Part B): OspA specific IgG antibodies (GMT) over time vs. serotype for each study group separately
- For each OspA serotype and study group: Reverse cumulative distribution curves for percentage of subjects reaching certain OspA specific IgG titer over time
- For each OspA serotype and study group: Reverse cumulative distribution curves at Day 208, Day 365, Month 19, Month 31 and Month 43 for percentage of subjects reaching certain OspA specific IgG titer by age cohort
- Scatter plots representing the correlation between OspA specific IgG antibodies GMTs for each combination of two different OspA specific serotypes for all study groups pooled.

- SBA:
  - Bar charts: SBA titer (GMT) including 95% confidence intervals for the GMT vs OspA serotypes by study group for each time point separately (y-axis: GMT; x-axis: OspA STs per study groups)
  - Bar charts: SBA titer (GMT) including 95% confidence intervals for the GMT by serotype over time for study groups 1 and 2 separately (y-axis: GMT; x-axis: serotypes)
  - Bar charts: SBA titer (GMT) including 95% confidence intervals for the GMT by serotype and age cohort at Day 208, Month 19 and Month 31, as well as Month 43 and Month 48, if deemed meaningful, for study groups 1 and 2 separately (y-axis: GMT; x-axis: serotypes)
  - Bar Charts: SBA Seroconversion Rate by OspA serotype and study group (for each time point separately, y-axis: percentage of subjects, x-axis: OspA STs per study groups) as well as for ST1-ST6 combined and ST1+ST2 combined
  - Line charts (y-axis: GMT, x-axis: study days for Part A and study months for Part B): SBA titer (GMT) over time vs. study group for each ST1-6 separately
  - Line charts (y-axis: GMT, x-axis: study days for Part A and study months for Part B): SBA titer (GMT) over time vs. OspA specific serotype for each study group separately
  - For each OspA serotype and study group: Reverse cumulative distribution curves: percentage of subjects vs. SBA titer
  - For each OspA serotype and study group: Reverse cumulative distribution curves at Day 208, Month 19 and Month 31, as well as Month 43 and Month 48, if deemed meaningful: percentage of subjects vs. SBA titer by age cohort
  - Scatter plots representing the correlation between SBA titer for each combination of two different OspA specific serotypes at Day 208, Month 19 and Month 31, as well as Month 43 and Month 48, if deemed meaningful, for all study groups pooled
- ELISA and SBA:
  - Scatter plots representing the correlation between ELISA and SBA titer for each OspA serotype at Day 208 among IgG ELISA /SBA seronegative subjects at Screening (Part A only);
  - Scatter plots representing the correlation between ELISA and SBA titer for each OspA serotype at Month 19 among IgG ELISA/ SBA seronegative subjects at Screening (Part B only)

- Scatter plots representing the correlation between ELISA and SBA titer for each OspA serotype at Month 31 among IgG ELISA/ SBA seronegative subjects at Screening (Part B only)
- Scatter plots representing the correlation between ELISA and SBA titer for each OspA serotype at Month 43 among IgG ELISA/ SBA seronegative subjects at Screening (Part B only, only if deemed meaningful)
- Scatter plots representing the correlation between ELISA and SBA titer for each OspA serotype at Month 48 among IgG ELISA/ SBA seronegative subjects at Screening (Part B only, only if deemed meaningful)

5.2 Derivations and Definitions

- Baseline for immunogenicity ELISA and SBA analysis is defined as Visit 0.

5.2.1 ELISA

- Baseline OspA IgG seropositive/seronegative for ELISA is defined as follows:
  - ELISA samples scored as "negative" or below the quantitation limit of the ELISA (40 U/mL) will be replaced by 20 U/mL;
  - Subjects with screening values below the quantitation limit of the ELISA (40 U/mL) and samples scored as "negative" (i.e. replaced by 20 U/mL) will be considered "baseline OspA IgG seronegative" for each serotype.
  - Subjects with screening values of 40 U/mL and above will be considered "baseline OspA IgG seropositive" for each serotype.
- Seroconversion for ELISA is defined as:
  - For subjects that are seronegative at screening: a change from seronegative at screening to seropositive (i.e. antibody titer of ≥40 U/mL) at a certain time point.
  - For subjects that are seropositive at screening: a ≥ 4-fold rise in OspA IgG antibody titer from screening. A subject reaches a 4-fold increase in ELISA if the value at a certain visit after screening is at least 4-times higher than the value at screening. In case of missing values (missing at baseline or current time point), seroconversion will not be calculated.

5.2.2 SBA

- SBA samples scored as "negative" or below the quantitation limit of the SBA (for Serotype 1, 2, 4, 5 and 6: SBA CCI and Serotype 3: SBA CCI) will be replaced with half of the quantitation limit (ST1, 2, 4, 5, 6 by SBA titer of CCI and ST3 by SBA titer of CCI).
- SBA results for

CCI

- Definition of SBA seroconversion:
  - For subjects that are seronegative at screening the seroconversion is defined as a change from seronegative to seropositive CCI [redacted] at a certain time point.
  - CCI [redacted]
  - In case of missing values (missing at baseline or current time point), SBA seroconversion will not be calculated

5.3 Statistical Tests

- In general, all statistical tests comparing study groups in the immunogenicity analysis will include all study groups (i.e. Study Group 1, Study Group 2 and Study Group 3 for ELISA and Study Group 1 and Study Group 2 for SBA).
- ANOVAs with factors study group and age cohort will be performed for the comparison between Study Group 1, Study Group 2 and Study Group 3 for ELISA and between Study Group 1 and Study Group 2 for SBA for each OspA ST1 to ST6 respectively. For Interim Analysis A1.1, no factor age cohort will be used. The primary immunogenicity analysis of the Main Study Phase is the ANOVA for ELISA GMTs at Day 208 in the PPAS.
- This will be done using log10 transformed data and taking the anti-log of the resulting point estimates for the least squares means, least squares means differences and the corresponding 95 % CIs. Tukey's HSD test will be applied for pair-wise comparisons.
- The primary immunogenicity analysis will provide 95% Confidence Intervals for GMTs.
- ELISA:
    - ANOVAs will be performed for GMTs as well as GMFRs compared to screening at all available time points.
    - For Part B, ANOVAs will be additionally performed for GMFRs compared to Day 208, up to Month 18, then compared to Month 18 for all available time points from Month 18 to Month 30, compared to Month 30 for all available time points from Month 30 to Month 42 and compared to Month 42 for all available time points after Month 42.
    - Part A: Sensitivity analyses for GMTs (by visit) and GMFRs (at Day 208) will be performed for ANOVAs with factors study site, study group, age cohort, age cohort*study group and baseline *B.b.* s.l serostatus at screening.
    - Part B: Sensitivity analyses for GMTs (by visit) and GMFRs at Month 19, Month 31, Month 43 and Month 48 will be performed for ANOVAs with factors study site, study group, age cohort, age cohort*study group and *B.b.* s.l serostatus at screening/Month 18/Month 30/Month 42, respectively.
    - Summary tables for the OspA specific IgG titer against each OspA serotype and summary tables for the geometric mean fold-rise against each OspA serotype will be amended by an overall test (Kruskal-Wallis).
    - SCRs will be compared using Fisher-Freeman-Halton tests, a significant overall test will be amended by pair-wise tests (Fisher's exact test).
    - A non-parametric correlation analysis (Spearman) between OspA IgG antibodies GMTs for each combination of two different OspA specific IgG types will be performed for Day 208, Month 19, Month 31 and Month 43, Study Group 1 and Group 2 separately as well as for all VLA15 study groups together (pooled analysis).

- SBA:
  - ANOVAs (factors study group and age cohort) will be performed for GMTs at all available time points.
  - Part A: Sensitivity analyses for GMTs by visit will be performed for ANOVAs with factors study site, study group (Group 1 and Group 2), age cohort, age cohort*study group and *B.b.* s.l serostatus at screening.
  - Part B: Sensitivity analyses for GMTs by visit will be performed for ANOVAs with factors study site, study site, study group (Group 1 and Group 2), age cohort, age cohort*study group and *B.b.* s.l serostatus at Month 18 (for time point Month 19) and *B.b.* s.l serostatus at Month 30 (for time point Month 31), as well as *B.b*. s.l serostatus at Month 42 (for time point Month 43 and Month 48), if deemed meaningful.
  - Summary tables for the SBA titer against each OspA specific serotype and summary tables for the geometric mean fold-rise against each OspA serotype will be amended by an overall test (Kruskal-Wallis).
  - SCRs will be compared using Fisher-Freeman-Halton tests, a significant overall test is amended by pair-wise tests (Fisher's exact test).
  - A non-parametric correlation analysis (Spearman) between SBA GMTs for each combination of two different OspA serotypes will be performed for Day 208, Month 19 and Month 31, as well as for Month 43 and Month 48, if deemed meaningful, Study Group 1 and Study Group 2 separately as well as for all VLA15 study groups together (pooled analysis).
- ELISA and SBA correlation:
  - A non-parametric correlation analysis (Spearman between ELISA and SBA titer for each OspA serotype at Day 208, Month 19 and Month 31, as well as Month 43 and Month 48, if deemed meaningful) will be performed. SBA seropositive subjects at baseline will be excluded from the ELISA and SBA correlation analysis.

6. SAFETY ANALYSIS

Safety Analysis will be performed on SAS. For safety AE analysis in Part B, the SAS will be the analysis set for events after any vaccination. AE analysis by vaccination period will be performed on the subjects that received the respective vaccination (i.e. after first booster vaccination will be performed on subjects that received the first booster vaccination, after second booster vaccination will be performed on subjects that received the second booster vaccination). Data from early termination and unscheduled visits will be listed only, if not states otherwise.

6.1 Adverse Events

6.1.1 Documentation of Adverse Events

In general, there are four sources in the eCRF for AEs: Assessment after vaccination, AE Log, eDiary and Reviewed eDiary.

- The subjects should be kept under medical supervision for at least 30 min after vaccination. Any findings on solicited AEs have to be documented in eCRF section “Assessment after vaccination”.
- All subject record solicited AEs for the first seven days after each vaccination (starting on the day of vaccination) in eCRF Section “eDiaries”.
- The eDiary entries will be reviewed by a clinician together with the subject and the investigator performs an assessment by vaccination period (source “Reviewed eDiary” in eCRF). Severity categories of investigator’s assessment are not necessarily identical to the categories provided in the eDiary.
- A) Unsolicited AEs will be reported in the eCRF section “AE Log” up to 28 days after each vaccination. Thereafter, only SAEs and AESIs will be documented in the eCRF.

  B) Serious and/ or medically attended or solicited systemic Grade 3/4 reactions will also be documented in the eCRF “AE Log”. For solicited local and systemic AEs persisting beyond Day 6 after vaccination, the stop date will also be documented in the “AE Log”.

6.1.2 Data Points

The following information will be analyzed descriptively and corresponding details on the subject level will be provided in data listings:

<u>General (solicited and unsolicited AEs):</u>

- Adverse Events Overview (solicited and unsolicited, including stratification by age cohort; will be repeated for boostered vs. non-boostered subjects by age cohort (i.e., columns Group 1 boostered, Group 1 non-boostered, Group 2 boostered, Group 2 non-boostered, Group 3 boostered, Group 3 non-boostered, Received any VLA15 boostered, Received any VLA15 non-boostered, Total boostered and Total non-boostered; will be repeated by study site)
- Adverse Events Overview (solicited and unsolicited, including stratification by age cohort) by vaccination period
- Serious and related Serious Adverse Events (solicited and unsolicited) by SOC and PT

- Medically attended and related medically attended Adverse Events (solicited and unsolicited) by SOC and PT
- Adverse Events (solicited and unsolicited) leading to withdrawal from further vaccination by SOC and PT (Only applicable for Analysis 1 and it's sub-analyses)
- Adverse Events (solicited and unsolicited) leading to withdrawal from study by SOC and PT
- Non-Serious solicited and / or unsolicited AE by SOC and PT for PTs with Frequency >5 % in any study group
- Non-Serious solicited and / or unsolicited AE by SOC and PT for PTs with Frequency >10 % in any study group

Solicited AE Tables (eCRF Section "Subject Diary" combined with "Reviewed eDiary" and "Assessment after vaccination")

- The following tables will be provided after any vaccination
    - Solicited Adverse Events (by symptom, by maximum severity)
    - Severe solicited Adverse Events (by symptom)
    - Solicited local Adverse Events (by symptom, by maximum severity, separately for symptoms meeting FDA grading scale only)
    - Solicited systemic Adverse Events (by symptom, by maximum severity)
- The following tables will be provided by vaccination period
    - Solicited Adverse Events (by symptom, by maximum severity)
    - Severe solicited Adverse Events (by symptom)
    - Solicited local Adverse Events (by symptom, by maximum severity, separately for symptoms meeting FDA grading scale only)
    - Solicited systemic Adverse Events (by symptom, by maximum severity,)
- The following tables will be provided by diary day
    - Solicited Adverse Events (overall and by symptom)
    - Mean duration (in days) of solicited Adverse Events by symptom
    - Greatest single diameter (in cm) for present Erythema, Swelling and Induration
    - Body Temperature (in °C) for case of fever
- The following figures will be provided:
    - Bar chart: The number and percentage of subjects with solicited local Adverse Events by symptom and overall, by study group and grade for the diary period after each vaccination and for the whole treatment period.
    - Bar chart: The number and percentage of subjects with solicited local Adverse Events reaching FDA Grading Scale by symptom and overall, by study group and grade for the diary period after each vaccination and for the whole treatment period
    - Bar chart: The number and percentage of subjects with solicited systemic Adverse Events by symptom and overall, by study group and grade for the diary period after each vaccination and for the whole treatment period.

Unsolicited AE Tables (eCRF Section "Adverse Event Log) for specific types of AEs (e.g. any unsolicited AE, any unsolicited SAE)

- Unsolicited Adverse Events by SOC and PT (overall and additionally for types: related, related serious, severe, related severe and AESI)
- Unsolicited Adverse Event by Maximum Severity (overall and additionally for types medically attended AE and AESI)
- Unsolicited Adverse Event by Causality (overall and additionally for types medically attended and AESI)
- Adverse Events of Special Interest by SOC and PT

Confidence intervals and statistical tests will be performed as described in Section 6.1.3. Details are specified in Section 7.

6.1.3 Derivations and Definitions

6.1.3.1 General Principles for Analysis of Adverse Event

- In general the unsolicited AEs will be taken from the eCRF section “AE-log” and the solicited AEs will be derived from the eCRF sections “Assessment after Vaccination”, “eDiary” and “Review of eDiary”. For summaries and listings of solicited and unsolicited medically attended AEs and/or serious AEs, the eCRF section “AE-log” will be used as basis rather than taking those AEs from the diary. More details of the derivation of the solicited AEs are given in section 6.1.3.2.
- Tables showing “severe” events will include events with grade 3 or 4 or missing grade.
- For tables by maximum severity and worst causality, subjects will be only counted once in highest grading category and events are counted in each reported grading category.
- Percentages in tables that do not present data by time periods will be based on N (study group totals), if not stated otherwise.
- For Analysis 1.1/1.2.2/1.3 and Analysis 1 the cut-off for AEs is defined by Day 208
- For Analysis 2 the cut-off for AEs is defined by Day 365
- In general, for Analysis 3 the cut-off for AEs is defined by Month 19 (i.e., 28 days after first booster vaccination). A summary of AEs will also be presented up to Month 18 (i.e. Day 548).
- For Analysis 4 the cut-off for AEs is defined by Month 31 (i.e., 28 days after second booster vaccination)
- For Analysis 5 the cut-off for AEs is defined by Month 43 (i.e., 28 days after third booster vaccination)
- For Analysis 6 all available AEs are analyzed (up to Month 48)
- For Part A, AEs in the “AE log” will be considered to have started up to cut-off (e.g. Day 208) if the start date of the AE is before or on study day of the cut-off (e.g. date of Visit 6 (Day 208)). In case of an incomplete start date where it cannot clearly be decided if the AE started up to cut-off day or not, the AE will be considered to have started up to the cut-off. For derivation of cut-off, the date of the respective Visit is used (e.g. date of Visit 6 for Day 208, date of Visit 7 for Day 365). In case no respective visit was performed, the cut-off is determined as date of Visit 1 plus number of days minus 1 (e.g. date of Visit 1 +207 if no Visit 6

date is available). For distances between visits defined as month, see Section 2.8 (e.g. date of Visit 1 + 544 + 28 if no Month 19 date is available);

- For the analysis of non-serious solicited and unsolicited AE the following applies: Solicited AEs (eCRF section "Subject Diary" considering "Reviewed eDiary" and "Assessment after Vaccination") will be considered as non-serious AEs if "no" is ticked for question "Symptom fulfilling any SAE criteria" and unsolicited AEs (eCRF section "Adverse Event log") will be considered as non-serious if "Serious" is ticked with "no". Adverse Events will be only included for this analysis if their occurrence by PT in at least one study group in the Safety Population is 5 % or over 10 %, respectively.

6.1.3.2 Principles for Solicited Adverse Events

- In general, for tables summarizing solicited AEs, only the Subject Diary considering the Investigator's review and the "Assessment after vaccination" will be used.
- AE tables presented after any or after each vaccination will be based on the eCRF section "reviewed eDiary" and "Assessment after vaccination". Only available events in these eCRF section will be considered. If a subject has documented its symptoms in the eDiary section but no corresponding event in the eCRF section "reviewed eDiary" is available, the event will not be included.
- The overall maximum severity for each subject is derived using the sources "reviewed eDiary" and "Assessment after Vaccination":
    - The overall maximum severity by subject and each symptom is defined as the maximum of the documented Investigator assessment in the eCRF section "reviewed eDiary" and the severity entered in the eCRF section "Assessment after Vaccination". In case investigator assessment in the eCRF section "reviewed Diary" is not available, the maximum severity which was automatically populated from eCRF source "eDiary" into the eCRF Section "reviewed eDiary" will be used.
    - For symptoms Erythema/Redness and Fever, no automatically populated maximum severity from eCRF source "eDiary" is available. This will be calculated as described in the CSP up to Grade 3:

Table 4: Grading of Erythema and Fever according to CSP (Table 8)

Symptom	Age	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe
Injection Site Erythema or Redness[1]	5-11 years [B]	1.0 - ≤ 2.5 cm (0.39-0,98 inch) in diameter	> 2.5 - 5.0 cm [2] in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	> 5 cm [2] OR ≥ 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
	12-65 years [A]	2.5–5 cm (0.98–1.96 inch)	5.1–10 cm 1.97–3.94 inch	>10 cm >3.94 inch
Fever[4] (°C)/(°F)	5-65 years [A]	38.0–38.4/ 100.4–101.1	38.5–38.9/ 101.2–102.0	39.0–40/ 102.1–104

In case of no available severity investigator assessment in eCRF section "Reviewed eDiary", the grading will be based on the subject's grading in source "eDiary" as described above. For symptom Erythema/Redness for subject aged 5-11 years, this will be done by considering the diameter only but not taking aspects "with < 50% surface area of the extremity segment involved" for Grade 2 and "OR ≥ 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" for Grade 3 in account because this information is not available in the eDiary source.

- The presence of symptoms for tables presented by diary day will be based on the eCRF section "reviewed Diary" considering presence information which was populated by the eCRF from Section "eDiary" into "reviewed eDiary". In case the information about the presence of a symptom was revised by the Investigator, the Investigator information will be analyzed (section "reviewed Diary").
- Solicited AEs comprise reactions at the injection site or systemic reactions that are typical for vaccinations.
    - Solicited local AEs are the following injection site reactions: pain, tenderness, induration/ hardening, swelling and erythema/ redness.
    - Solicited systemic AEs are: headache, myalgia (muscle pain), arthralgia (joint pain), fever (oral body temperature), nausea, vomiting and fatigue.
- Solicited AEs are per definition regarded as related to IMP.
- For tables that summarize solicited AEs over several diary days / diary periods, the worst severity of all diary days / diary periods will be taken as the events severity.
- Percentages in tables for solicited AEs by diary period /diary day will be based on the number of subjects with available information (diary completed or symptom present on at least one day). In particular, if a symptom was not assessed at a certain diary day and the symptom is reported as not present on the other days, the subject is not included in the table of the respective symptom and diary period.

Adapted from:

- For the derivation of the duration of solicited Adverse Events by symptom and vaccination period the difference of the first occurrence and the last occurrence of the concerning event in the respective vaccination period will be taken, no matter if the event is continuous or not. Therefore, also the end-date if ongoing after day 6 will be considered. If the AE was ongoing after day 6 but has a missing or incomplete end date, the date of last attended visit in relation to the AE will be used as the end date.
- For swelling, redness and induration, the maximum diameter per diary period in cm will be derived as taking the maximum of all diameters reported in that period for that symptom and converting via [cm] = [in] × 2.54.
- The maximum body temperature per diary period for present symptom fever will be derived as taking the maximum temperature of all temperatures reported in that period and converting via [°C] = ([°F] – 32) × 5/9.
- Adverse Events from the Subject Diary will be coded according to the table below:

Event	SOC name	SOC code	PT name	PT code
Fatigue	General disorders and administration site conditions	10018065	Fatigue	10016256
Fever	General disorders and administration site conditions	10018065	Pyrexia	10037660
Headache	Nervous system disorders	10029205	Headache	10019211
Nausea	Gastrointestinal disorders	10017947	Nausea	10028813
Vomiting	Gastrointestinal disorders	10017947	Vomiting	10047700
Erythema/Redness	General disorders and administration site conditions	10018065	Injection site erythema	10022061
Induration/Hardening	General disorders and administration site conditions	10018065	Injection site induration	10022075
Muscle pain	Musculoskeletal and connective tissue disorders	10028395	Myalgia	10028411
Pain (pain without touching)	General disorders and administration site conditions	10018065	Injection site pain	10022086
Swelling	General disorders and administration site conditions	10018065	Injection site swelling	10053425
Joint pain	Musculoskeletal and connective tissue disorders	10028395	Arthralgia	10003239
Tenderness (pain upon touching)	General disorders and administration site conditions	10018065	Injection site pain	10022086

6.1.3.3 Principles for Unsolicited Adverse Events

- AEs in the AE log will be coded using the MedDRA version that is current at time point of the respective data snapshots and database closure. The version used will be indicated in the respective tables and listings and is documented in the CSR.
- Adverse events in the AE log will be considered related if the causality to IMP is reported as “probable” or “possible” (or missing causality)
- An AE in the AE-log will be considered as “leading to withdrawal from further vaccination” if for “action taken on IMP”, “second dose not administered” or “third dose not administered” is ticked.
- An AE in the AE-log will be considered as “leading to withdrawal from study” if in section “other action taken” the question “Withdrawn from study”, is answered with “yes”.
- Percentages in tables for unsolicited events or all events (solicited and unsolicited) over the whole study will be based on N (study group totals).
- For presentation of unsolicited AEs by vaccination period, a vaccination period is defined as the period 28 days after each vaccination.
    - 1st vaccination period: AE with start date/time at or after date/time of 1st vaccination and within 28 days of 1st vaccination. If no AE start time is given, the AE will be included if the start date is at or after the date of 1st vaccination and within 28 days of 1st vaccination.
    - Vaccination periods for 2nd and 3rd primary vaccinations and 1st, 2nd and 3rd booster vaccination periods are defined analogously
    - If a subject did not receive a certain vaccination, the respective vaccination period will not be defined.
    - In case of incomplete start dates where it cannot be clearly decided to which vaccination period the AE can be assigned, the AE will be assigned to the last received vaccination.

6.1.4 Confidence Intervals and Statistical Tests

95 % confidence intervals according to Altman will generally be provided for all AE rates. Differences between the three study groups will be assessed for significance using Fisher’s exact (Fisher-Freeman-Halton) test, whereby a significant overall test will be amended by pair-wise tests.

It is stated in detail in Section 7 for which tables such comparisons are made.

6.2 Lyme Borreliosis Screening

6.2.1 Data Points

Borrelia burgdorferi s.l. screening tests will be analyzed descriptively at scheduled or early termination visits. All results (i.e. including unscheduled visits, early termination visit and scheduled visits) are listed on subject-level.

- Lyme Borreliosis Screening results by visit (frequency statistics);
- Seroconversion rate of Lyme Borreliosis Screening from Visit 0 to Visit 8/ET (frequency statistics);
- Seroconversion rate of Lyme Borreliosis Screening from Visit 8 to Visit 12/ET (frequency statistics);

- Seroconversion rate of Lyme Borreliosis Screening from Visit 12 to Visit 15/ET (frequency statistics);
- Seroconversion rate of Lyme Borreliosis Screening from Visit 15 to Visit 17/ET (frequency statistics).

6.2.2 Derivations and Definitions

- In case no result is available at Visit 8 because the subject terminated early, the result from the Early Termination Visit will be considered instead. Same applies for Visit 12, 15 and 17;
- Seroconversion is defined as change from *B.b.* s.l. serostatus "negative" at Visit 0 to *B.b.* s.l. serostatus "positive" at Visit 8/ET for Main Study Phase. Seroconversion rate from Visit 8 to Visit 12, Visit 12 to visit 15 and Visit 15 to Visit 17 will be determined in the same manner. In general, seroconversions will only be determined in case both results (i.e. Visit 0 and Visit 8/ET for Main Study Phase, Visit 8 and Visit 12/ET for Analysis 4, Visit 12 and 15/ET for Analysis 5 as well as Visit 15 and Visit 17/ET for Analysis 6) are available.
- Seroconversion rate will be based on baseline seronegative subjects.

6.3 Other Safety Parameters

The following information will be analyzed descriptively from scheduled visits and corresponding details on the subject level of all visits will be provided in data listings:

- Systolic blood pressure by time point incl. measurements after vaccinations (summary statistics)
- Diastolic blood pressure by time point incl. measurements after vaccinations (summary statistics)
- Pulse rate by time point incl. measurements after vaccinations (summary statistics)
- Oral body temperature by time point (summary statistics)

The following information will only be listed:

- Physical Examination
- Pregnancy Test
- Injection Site Inspection
- Assessment after Vaccination (except solicited Adverse Events documented during post-vaccination assessment, see Section 6)
- Vaccination delay criteria

7. LIST OF TABLES, DATA LISTINGS AND FIGURES

In general, the numbering consists of the following components in the given order:

Description	Values
CSR Section	14 = Tables and figures 16.2 = Listings
Analysis number	1.1 = Analysis 1.1 1.2 = Analysis 1.2 1.3 = Analysis 1.3 1 = Analysis 1 2 = Analysis 2 3 = Analysis 3 4 = Analysis 4 5 = Analysis 5 6 = Analysis 6
Analysis Section	1 = Overall study information tables / listings 2 = Baseline evaluation tables / listings 3 = Immunogenicity analysis tables / listings 4 = Adverse events tables / listings 5 = Other safety and *B.b. s.l.* information tables / listings 6 = Immunogenicity figures 7 = Safety figures
Analysis Set and label for TLF headers	[Booster] Safety Analysis Set [Booster] Full Analysis Set [Booster] Per-Protocol Analysis Set Note: The analysis set which will be first produced within the statistical output will be numbered by 1, the number of the further analysis sets continuous depending on the order of appearance.
TLF ID	1 2 ...

The population digit is used only in case TLFs are produced for more than one Analysis Set. Further digits can be introduced during the programming of the analysis, if necessary (e.g. introducing sub-continuous numbering in case Listing 16.2.1.1 has to be divided in 16.2.1.1.1 and 16.2.1.1.2).

TLFs showing data up to a certain time point are marked as "[Time Points]". This will be replaced by the following:

Part A:

Analysis	A1.1	A1.2	A1.3	A1	A2
up to	Day 208	Day 208	Day 208	Day 208	Month 12

Part B:

Analysis	A3	A4	A5	A6
up to	Month 18/Month 19	Month 31	Month 43	Month 48

A List of Tables, Listings and Figures (TLFs) will be provided in an appendix to this SAP.

8. SHELLS OF TABLES, DATA LISTINGS AND FIGURES

For analysis preparation, no table shells or mock tables will be produced, but analysis drafts of TLFs might be generated based on dummy group allocation (dummy randomization list) and dummy immunogenicity data. These drafts are reviewed by the sponsor prior to SAP finalization and database snapshot.