valneva

Clinical Study Protocol

DRUG SUBSTANCE(S)	VLA15
VERSION NO.	Final 4.0
STUDY CODE	VLA15-201
DATE	13-Jun-2019

IMMUNOGENICITY AND SAFETY STUDY OF VLA15, A MULTIVALENT RECOMBINANT OSPA BASED VACCINE CANDIDATE AGAINST LYME BORRELIOSIS, IN HEALTHY ADULTS AGED 18 TO 65 YEARS - A RANDOMIZED, CONTROLLED, OBSERVER-BLIND PHASE 2 STUDY.

Phase 2 study

Study Protocol VLA15-201

EudraCT number: 2018-003379-37

IND number: 17199

Responsible Clinical Project Manager [redacted] **Clinical Project Manager**

[redacted]

Sponsor **Valneva Austria GmbH**

[redacted]

Campus Vienna Biocenter 3
1030 Vienna, Austria

valneva

Clinical Study Protocol Synopsis

DRUG SUBSTANCE(S)	VLA15
VERSION NO.	Final 4.0
STUDY CODE	VLA15-201
DATE	13-Jun-2019

IMMUNOGENICITY AND SAFETY STUDY OF VLA15, A MULTIVALENT RECOMBINANT OSPA BASED VACCINE CANDIDATE AGAINST LYME BORRELIOSIS, IN HEALTHY ADULTS AGED 18 TO 65 YEARS - A RANDOMIZED, CONTROLLED, OBSERVER-BLIND PHASE 2 STUDY.

INVESTIGATIONAL PRODUCT, dosage and mode of administration

VLA15 is a multivalent Outer surface protein A (OspA) based vaccine candidate designed for the prevention of Lyme disease. The vaccine targets the majority of *Borrelia* strains expressing clinically relevant OspA serotypes (STs) present in Europe (ST1 to ST6) and the U.S. (ST1). The vaccine includes three proteins, each containing the C-terminal half of two OspA serotypes linked to form three fusion proteins of ~35 kDa (ST1 and ST2, ST4 and ST3, and ST5 and ST6).

Three VLA15 dose groups will be investigated in this Phase 2 study VLA15-201: 90 µg, 135 µg and 180 µg. Each dose will be applied in a formulation with aluminum hydroxide adjuvant and will be administered in three intramuscular (I.M.) vaccinations on Days 1, 29 and 57.

COMPARATOR PRODUCT, dosage and mode of administration

Placebo: Phosphate Buffered Saline (PBS) solution; three I.M. vaccinations, on Days 1, 29 and 57.

STUDY OBJECTIVES

Primary objective:

- To determine the optimal dose of VLA15 in healthy adults aged 18 - 65 years up to Day 85.

Secondary objectives:

Immunogenicity:

- To assess the immune response of VLA15 in healthy adults aged 18 – 65 years up to Month 12 (i.e. 10 months after the primary vaccination series.

Safety:

- To assess the safety profile of VLA15 in healthy adults aged 18 – 65 years up to Month 12.

STUDY DESIGN

This 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 (Figure 1).

In the Run-in phase, a total of 120 subjects aged 18 to 40 years were to be randomized stratified by study site 1:1:1:1 to receive VLA15 90 µg w/ alum, VLA15 135 µg w/ alum, VLA15 180 µg w/ alum, or placebo (30 subjects per treatment group) as I.M. vaccinations on Days 1, 29 and 57. Dosing was to be adjusted by injection volume (see Table 1). Two safety visits were to be performed after the first vaccination: a safety phone call at Day 4 (i.e. Visit 1a, three days after the first vaccination) and an in-person visit at Day 8 (i.e. Visit 1b, seven days after the first vaccination). After all subjects enrolled in the Run-in phase completed Visit 4 (Day 85, i.e. 28 days after the third vaccination) a DSMB reviewed all available safety data up to Day 85 in a scheduled DSMB meeting and gave the recommendation that all treatment groups were safe and well tolerated so far and could be continued in the Main Study phase based on available safety data. After the DSMB meeting, two dose groups (135 µg and 180 µg of VLA15 w/ alum) were selected for further investigation. The two higher dose groups were selected. No safety concerns associated with any of the VLA15 treatment groups were identified by the independent DSMB.

In the Main Study phase, a total of 450 subjects aged 18 to 65 years will be randomized stratified by study site, age group and baseline *B.b.* s.l. serostatus 2:2:1 to receive 135 µg or 180 µg VLA15 w/ alum (180 subjects each) or placebo (90 subjects), as I.M. vaccinations on Days 1, 29 and 57. Subjects will be enrolled in two age groups (18-49 years and 50-65 years) in a ratio of approx. 2:1. An interim analysis on safety and immunogenicity data will be performed after all subjects have completed Visit 6 (i.e. Day 236, six months after the last vaccination). This interim analysis will cover safety and immunogenicity data up to Visit 4 (i.e. Day 85, four weeks after the last vaccination) as well as safety data up to Visit 6 (i.e. Day 236, six months after the last vaccination). Final analysis of safety and immunogenicity data will be performed after all subjects have completed the follow-up period up to Visit 7 (i.e. Day 365/ Month 12).
In both study phases, target is to enroll approx. 10 % or more of subjects that are baseline seropositive for *Borrelia burgdorferi sensu latu* (*Bb s.l.*). This is aimed to be achieved through selection of endemic recruitment areas as well as database searches for *Bb s.l.* seropositive subjects.

Figure 1 Study Design

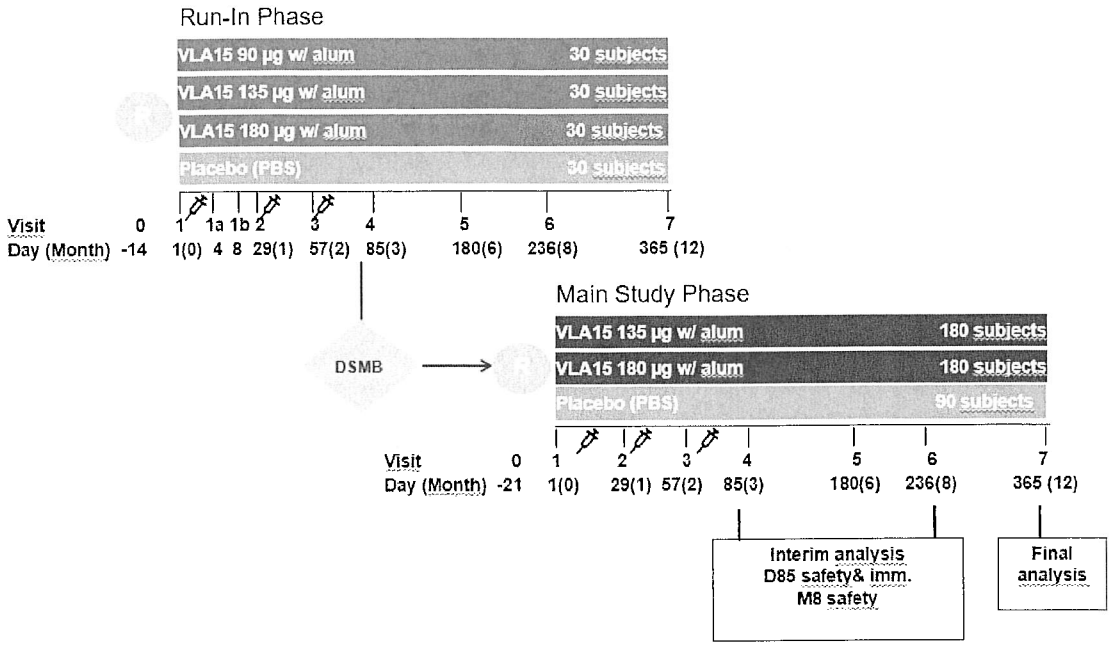

Table 1 Treatment Groups and Vaccinations

Group	Treatment	Injection Volume (mL)	Days of Vaccination
90 µg	VLA15 90 µg w/ alum	0.50	1, 29, 57
135 µg	VLA15 135 µg w/ alum	0.75	1, 29, 57
180 µg	VLA15 180 µg w/ alum	1.00	1, 29, 57
Placebo	PBS	1.00	1, 29, 57

SUBJECT ENROLMENT

Run-in phase: For safety reasons, in the first three weeks of the Run-in phase, recruitment was to be limited to 15 subjects per week. Thereafter recruitment was to be limited to 30 subjects per week.

Main Study phase: In the Main Study phase recruitment will be performed without recruitment restrictions.

INVESTIGATOR AND SITES

Multicenter study in Lyme borreliosis endemic areas in the U.S. and Europe, in total approx. 10 centers.

PROPOSED START DATE

Q4 2018

STUDY DURATION

Study duration per subject will be approximately 13 months.

Overall study duration is estimated to be 22 months. The end of the study is defined as the date of the last visit performed by the last subject.

STUDY PARTICIPANTS

A total of approx. 570 healthy subjects aged 18 to 65 years will be enrolled in this study.

Run-in phase:
In the Run-in phase subjects aged 18 to 40 years were to be enrolled. A total of 120 healthy subjects were to be enrolled into four treatment groups (30 subjects per treatment group), and were to receive 90 µg, 135 µg or 180 µg of VLA15 w/ alum or placebo. After DSMB review of safety data up to Day 85, the two higher dose groups (135 µg and 180 µg of VLA15 w/ alum) were selected to be further investigated in the Main Study phase.

Main Study phase:
Overall 450 healthy subjects aged 18 to 65 years will be enrolled in the Main Study phase and will receive 135 µg or 180 µg of VLA15 w/ alum (180 subjects each) or placebo (90 subjects). Subjects will be enrolled in two age groups (18-49 years and 50-65 years) in a ratio of approx. 2:1.

In both study phases, target is to enroll approx. 10 % or more of subjects that are baseline seropositive for *Borrelia burgdorferi sensu latu* (*Bb s.l.*). This is aimed to be achieved through selection of endemic recruitment areas as well as database searches for *Bb s.l.* seropositive subjects.

CRITERIA FOR INCLUSION/EXCLUSION

Approximately 570 male or female adults who satisfy the inclusion and exclusion criteria listed below will be enrolled in the study.

Inclusion criteria:

Subjects must meet **ALL** of the following criteria to be eligible for this study:

Run-in phase:

1. Subject is aged 18 to 40 years at the day of screening (Visit 0);

Main Study phase:

1. Subject is aged18 to 65 years at the day of screening (Visit 0);

Run-in phase and Main Study phase:

2. Subject is of good general health, including subjects with pharmacologically controlled chronic conditions;
3. Subject has an understanding of the study and its procedures, agrees to its provisions, and gives written informed consent prior to any study-related procedures;
4. If subject is of childbearing potential:
    a. Subject has a negative serum pregnancy test at screening (Visit 0);

b. Subject agrees to employ adequate birth control measures for the duration of the study (please refer to section 6.4).

Exclusion criteria (Run-in phase and Main Study phase):

Subjects who meet **ANY** of the following criteria are **NOT** eligible for this study:

1. Subject has a chronic illness related to Lyme borreliosis (LB), an active symptomatic LB as suspected or diagnosed by a physician, or received treatment for LB within the last 3 months prior to Visit 0;
2. Subject received previous vaccination against LB.;
3. Subject had a tick bite within 4 weeks prior to Visit 1;
4. Subject has a medical history of or currently has a clinically relevant disease (e.g. cardiovascular, respiratory, neurologic, psychiatric conditions) which poses a risk for participation in the study, based on investigators judgement, such as individuals with poorly controlled or unstable disease, ongoing suspected or active inflammation, or poor compliance with pharmacologic treatment. Subjects with pharmacologically controlled conditions like osteoarthritis, depression, or asthma are eligible;
5. Subject has a medical history of or currently has a neuroinflammatory or autoimmune disease, including Guillain Barré Syndrome;
6. Subject has a known thrombocytopenia, bleeding disorder, or received anticoagulants in the 3 weeks prior to first vaccination or until Day 57 (Visit 3), contraindicating I.M. vaccination as judged by the investigator;
7. Subject has received an active or passive immunization within 28 days before first vaccination at Visit 1 and until Day 85; except for influenza (seasonal or pandemic) and pneumococcal vaccines which may be administered outside a 7-days interval before or after any trial vaccination;
8. Subject has received any other non-registered medicinal product in another clinical trial within 28 days prior to VLA15 vaccination at Visit 1 (Day 1) and throughout the entire study period or has received a registered medicinal product in another clinical trial within 28 days prior to VLA15 vaccination at Visit 1 (Day 1) and up to Day 85;
9. Subject has a known or suspected defect of the immune system that would prevent an immune response to the vaccine, such as subjects with congenital or acquired immunodeficiency, including infection with human immunodeficiency virus (HIV), status post organ transplantation or immuno-suppressive therapy within 30 days prior to Visit 1. Immuno-suppressive therapy is defined as administration of chronic (longer than 14 days) prednisone or equivalent ≥0.05 mg/kg/day. Topical and inhaled steroids are allowed;
10. Subject has a history of anaphylaxis or severe allergic reactions or a known hypersensitivity or allergic reactions to one of the components of the vaccine;
11. Subject had any malignancy in the past 5 years. If treatment for cancer was successfully completed more than 5 years ago and the malignancy is considered to be cured, the subject may be enrolled;
12. Subject had acute febrile infections within 10 days prior to first vaccination;
13. Subject is pregnant (positive serum pregnancy test at screening), has plans to become pregnant during the course of the study or is lactating at the time of enrollment. Women of childbearing potential that are unwilling or unable to employ an adequate birth control measure for the duration of the study.
14. Subject has donated blood or blood-derived products (e.g. plasma) within 30 days or received blood or blood-derived products (e.g. plasma) within 90 days prior to first

vaccination in this study or plans to donate or use blood or blood products during the course of the study;

15. Subject has any condition that, in the opinion of the investigator, may compromise the subject's well-being, might interfere with evaluation of study endpoints, or would limit the subject's ability to complete the study;
16. Subject is committed to an institution (by virtue of an order issued either by the judicial or the administrative authorities);
17. Subject is in a dependent relationship with the sponsor, an investigator or other study team member, or the study center. Dependent relationships include close relatives and household members (i.e. children, partner/spouse, siblings, parents) as well as employees of the investigator or study center personnel.

Delay Criteria for Vaccination

Vaccination will be delayed if:

1. Subject has an acute illness with or without elevated body temperature (≥100.4 °F [38.0 °C]) within 3 days prior to the scheduled vaccination. Subjects may be rescheduled for vaccination at a later date provided that the illness has resolved (body temperature <100.4 °F [38.0 °C]);
2. Subject has received antipyretics within 4 hours prior to the scheduled time of vaccination. In this case the vaccination should be performed at a later date.

In addition, the following criteria must be met:

1. For a rescheduled **first** vaccination:
   a. All inclusion and none of the exclusion criteria are met; In case not all of these criteria are met, the subject will be excluded from the study.
   b. The rescheduled visit should be within the specified time window (i.e. within 21 days after the screening visit). In case a first vaccination cannot be rescheduled within the specified time window (i.e. within 21 days after the screening visit), the subject might be invited for a rescreening.

2. For a rescheduled **second or third** vaccination:

   The rescheduled visit should be within the specified time window.

STUDY ENDPOINTS

Primary Endpoint

+ GMTs (Geometric Mean Titers) for IgG against each OspA serotype ST1 to ST6, determined by ELISA at Day 85.

Secondary Endpoints:

Immunogenicity:

+ GMTs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1, 29, 57, 180, 236, and Month 12;
+ SCRs (Seroconversion Rate, defined as four-fold increase in IgG titer compared to baseline) for each OspA serotype specific IgG (ST1 to ST6), determined by ELISA, at Day 29, 57, 85, 180, 236, and Month 12;
+ GMFR (Geometric Mean of the fold rise as compared to baseline) for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 29, 57, 85, 180, 236 and Month 12;
+ GMTs, SCRs and GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1, 29, 57, 85, 180, 236, and Month 12, stratified by age group.

Safety:

+ Frequency of SAEs during the entire study;
+ Frequency of related SAEs during the entire study;
+ Frequency of AESIs during the entire study;
+ Frequency of related AESIs during the entire study;
+ Frequency of unsolicited AEs during the entire study (incl. clinically relevant laboratory parameters);
+ Frequency of related unsolicited AEs during the entire study (incl. clinically relevant laboratory parameters);
+ Frequency of solicited local and solicited systemic AEs within 7 days after each and after any vaccination.
+ Frequency of SAEs, AESIs, solicited and unsolicited AEs during the entire study stratified by age group.

SAMPLE SIZE JUSTIFICATION

The sample size for the Run-in phase has been chosen to allow detection of common AEs with the three initial dose levels. 30 Subjects will provide 95 % confidence that an AE not seen in the Run-in phase would have a true incidence of below 10 %.

The overall group size for the two doses (VLA15 w/ alum 135 µg and VLA15 w/ alum 180 µg) evaluated in the Main Study phase has been selected to provide a sufficient safety database and for determining the optimal dose before advancing the vaccine candidate into Phase 3. Upon completion of the study, the total number of subjects exposed to the dose used for Phase 3 trials would be a minimum of approximately N=210. The database would thus allow 95 % confidence that a given reaction would not be observed at a higher rate than 1:(210/3) rate, i.e. 1.4 %, if it is not observed in the trials preceding Phase 3.

With respect to the primary endpoint, GMTs for ST1-6 specific IgGs on Day 85: In the absence of an established protective titer, sample size calculation is based on somewhat arbitrary differences in GMTs between VLA15 treatment groups, in order to demonstrate which titer levels could be distinguished with the proposed sample size. Titers observed in Phase 1 were used as basis: In the 90 µg w/ alum group (i.e. the lowest dose group used in the present Phase 2 study), a GMT of 61.3 was observed for ST1 (i.e. the serotype with lowest titers in Phase 1) with a Standard Deviation (LOG10) of 0.51. A total of 189 subjects per group (assuming 10 % of the 210 subjects per treatment group are excluded from primary PP analysis) will provide 80 % power at a two-sided alpha level of 5 % to distinguish a GMT of 61.3 in one treatment group from a putative higher GMT of 86.1 in another dose group. An approximately 1.5 fold higher titer could thus be distinguished. A 1.5 fold difference in GMTs is often considered a relevant difference in vaccine studies, e.g. when setting non-inferiority boundaries.
The overall sample size of 120 subjects in the placebo group has been selected to allow for the internal validation of both safety and immunogenicity results.

STATISTICAL METHODS

The primary immunogenicity analysis will be an overall and group-wise comparison of the GMTs against each OspA ST1 to ST6 in the per-protocol (PP) populations between treatment groups at Day 85 (i.e. 28 days after the last immunization with VLA15) by ANOVA (factors treatment group, study site). In addition, GMTs and GMFR's against each OspA serotype ST1 to ST6 will be compared overall and pair-wise between treatment groups on all time points. SCRs will be compared overall and pair-wise between treatment groups by Fisher Freeman Halton test and Fisher exact test, respectively. Defined immunogenicity analyses will be repeated on the modified intent-to-treat (mITT) population, and will be repeated stratified by baseline *B.b.* s.l. serostatus, region and age.

All subjects entered into the study, who received at least one vaccination, will be included in the safety analysis. The number and percentage of subjects with solicited local and solicited systemic AEs up to 7 days after each vaccination, up to 7 days after any vaccination, and the number and percentage of subjects with unsolicited AEs, medically attended AEs, AESIs and SAEs will be presented for each treatment group overall and by body system/ preferred term. Differences between the treatment 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. Changes in laboratory values and the frequency of abnormal values will be analyzed descriptively. Defined safety analysis will also be repeated stratified by baseline *B.b.* s.l. serostatus, region and by age.

INTERIM/ FINAL ANALYSIS

An interim analysis on safety and immunogenicity data will be performed after all subjects have completed Visit 6 (i.e. Day 236, six months after the last vaccination) and testing of immunogenicity samples up to Visit 4 (i.e. Day 85, four weeks after the last vaccination) has been completed. Data from selected dose groups in the Main Study phase and data from respective dose groups from the Run-in phase will be pooled for this and all further analyses.

A final data analysis will be conducted once the last subject has completed the study, i.e. Visit 7 (Month 12).

STUDY REPORTS

An interim study report will be compiled and will contain all data from all subjects on safety and immunogenicity up to Day 85 (Visit 4) as well as all safety data from all subjects up to Day 236 (i.e. 6 months after the last vaccination). A final Clinical Study Report will be written after the final analysis, once all data from all subjects are analyzed.

SAFETY AND DATA MONITORING

All subjects will report all symptoms (solicited local and systemic AEs, unsolicited AEs) after each vaccination, as described below.

Solicited Adverse Events

Solicited local and systemic adverse events will be assessed for absence, presence, severity and duration by the subjects themselves. The assessments will be recorded once daily into a Subject Diary. Assessments by the subjects will occur for a total of seven consecutive days starting at the day of each vaccination. The subjects will be instructed to carefully observe the injection site until all symptoms resolve.

Solicited local Adverse Events (AEs)

Solicited local adverse events include the following: pain, tenderness, induration/hardening, swelling and erythema/redness.

Solicited systemic Adverse Events (AEs)

Solicited systemic adverse events include: headache, myalgia (muscle pain), arthralgia (joint pain), fever (oral body temperature), flu-like symptoms, nausea, vomiting and fatigue.

Unsolicited Adverse Events

Unsolicited AEs will be captured in the Subject Diary or Memory Aid throughout the study period. The Subject Diary will be verified by the study physician together with the subject at the subject's next visit to the study site, prior to these data being entered into the eCRF and prior to the next vaccination (when applicable).

Definition of Adverse Events of Special Interest (AESIs)

An adverse event of special interest (serious or non-serious) is one of scientific and medical concern specific to the sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate. Such an event might warrant further investigation in order to characterize and understand it.

Collection and evaluation of Adverse Events of Special Interest (AESIs)

Subjects will be carefully monitored for development of AESIs. Since a previous LB vaccine was accused of inducing auto-immune symptoms similar to those caused by disseminated LB infection, e.g. autoimmune arthritis, such events will constitute AESIs. In addition, the onset of any potentially autoimmune or neuro-inflammatory disorders will constitute AESIs. A subunit vaccine like VLA15 is not considered capable of inducing LB as such. Nevertheless, any potential LB cases are of relevance to development of the vaccine and will therefore receive particular attention and be captured as AESIs as well. Therefore, symptoms suggesting a LB-associated event and/ or onset of potentially autoimmune or neuro-inflammatory disorders will receive special attention. Identification of such events from a pre-defined list of AESIs and symptoms suggesting a Borrelia infection will be assessed in a guided approach as described below.

The following symptoms will receive particular consideration:

- Expanding red or bluish-red patch (≥5 cm in diameter) with or without central clearing;
- Symptoms suggesting an arthritis (e.g. recurrent attacks or persisting objective joint swelling (synovitis) in one or a few large joints);
- Neurological symptoms (e.g. meningo-radiculitis, meningitis, encephalitis, myelitis, cerebral vasculitis, facial palsy);
- Cardiac symptoms (e.g. atrio-ventricular conduction disturbances, rhythm distrurbances, myocarditis);
- Immune-mediated disorders as proposed by FDA for previous clinical programs (please refer to APPENDIX 1).

As part of unsolicited AE assessments, at study Visits 1-7 and ET, if applicable, the investigator will be guided through a scripted safety assessment (i.e. questionnaire) to enquire about symptoms that are consistent with Lyme borreliosis, allowing the investigator to assess whether there is a clinical suspicion for infection with Borrelia or a LB-associated event. In addition, presence of or symptoms suggesting one of the other AESIs from the pre-defined list will be determined by the investigator.

In case there is clinical suspicion for Lyme borreliosis or a LB-associated event, investigators are advised to perform a clinical workup as described in Appendix 2, including specialist referral as needed. Subjects with suspected other AESIs (i.e. immune-mediated disorders) should also be referred to a respective clinical expert for full diagnostic work-up as needed. Retrospective investigation of a pre-vaccination sample may be considered for clinical work-up. The investigator will request the medical records from the clinical expert, if applicable. In case an AESI is identified (by the investigator or a clinical specialist upon referral or without referral) the investigator will fill out the AESI Report Form with all available information, including information provided by the clinical expert, if applicable, and will provide the AESI Report Form together with the medical records to the DSMB through the study's Safety Desk. For cases of Lyme borreliosis or LB-associated events, the DSMB will confirm the diagnosis. In case an AESI (LB or immune-mediated disorders as depicted in the pre-defined list) has already been diagnosed by a healthcare specialist prior to identification of a potential AESI by the investigator at the study visit, the investigator will also provide the AESI Report Form together with available medical records to the DSMB through the study's Safety Desk. In addition, the DSMB will regularly review accruing AEs and can recommend to the investigator specialist work-up as needed for any case they consider potential AESIs or cases of LB. The DSMB will do a final adjudication of all AESIs and will assess whether

cases were new in onset and whether there is any relationship to application of the study vaccine. Narratives with detailed case descriptions will be provided for all AESIs.

DATA SAFETY MONITORING BOARD (DSMB)

An independent DSMB, which will include Lyme borreliosis experts (i.e. a general practitioner and expert in LB diagnosis, a rheumatologist and a neurologist who are LB experts), will be installed to review accruing safety information, and if necessary, to determine whether study or individual subject stopping rules have been met. The DSMB will ad hoc review all cases of SAEs. In addition, the DSMB will confirm diagnosis of potential LB cases based on medical records received from the investigator after clinical workup of potential LB cases according to a standardized procedure as described in [Appendix 2]. The DSMB will do an adjudication of all AESIs and will assess whether cases were new in onset and whether there is any relationship to application of the study vaccine. During vaccination periods (i.e. Day 1 to Day 57 from Run-in phase and Main Study phase), the DSMB will periodically review listings of SAEs, Deaths, AESIs, medically attended AEs, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination. In addition, DSMB meetings will be scheduled. Please refer to the written DSMB charter including a detailed description of DSMB set-up and processes.

Table 2 TABLE OF EVENTS - RUN-IN PHASE

Visit	V0	V1	V1a	V1b	V2	V3	V4	V5	V6	V7	Early Termination (1)
Timing: Day (D) Month (M)	**D-14**	**D1**	**D4**	**D8**	**D29 M1**	**D57 M2**	**D85 M3**	**D180 M6**	**D236 M8**	**D365 M12**	**before V7**
Time windows	**-14 to -1**	**0**	**+/- 1**	**+/- 1**	**+/- 4**	**+/- 4**	**+/- 4**	**+ 7/- 14**	**+ 7/ - 14**	**+/- 14**	**n/a**
Visit type	In-person	In-person	Phone call	In-person	In-person	In-person	In-person	In-person	In-person	In-person	In-person
Informed consent (2)	X										
Inclusion/exclusion criteria	X	X (Review)									
Vaccination delay criteria		X			X	X					
Demographic data	X										
Medical history incl. vaccinations	X	X (3)									
Concomitant medications/ treatments incl. vaccinations	X	X	X	X	X	X	X	X	X	X	X
Physical examination (4), ECG	X										
Vital signs (5)	X	X			X	X					
Evaluation of oral body temperature	X	X (6)			X (6)	X (6)					
HIV test [3.5 mL] (7)	X										
Bb s.l. screening test [4mL] (8)	X									X	X
Baseline serology sample [5.0 mL] (9)	X										
Serum Pregnancy test [3.5 mL] (10)	X										
Urine Pregnancy test (10)		X (11)			X (11)	X (11)	X	X	X	X	X
Clinical chemistry [8.5 mL] (12)	X			X	X (11)	X (11)	X			X	
Hematology [4 mL] (13)	X			X	X (11)	X (11)	X			X	
Coagulation blood sample [4.5 mL] (14)	X										
Urinalysis (15)	X			X	X (11)	X (11)	X			X	
Immunogenicity blood sample, (16)		X (11) [54 mL]			X (11) [27 mL]	X (11) [54 mL]	X [54 mL]	X [27 mL]	X [27 mL]	X [54 mL]	
Randomization (17)		X									
VACCINATION (18)		X			X	X					
Check for AEs following vaccination		X			X	X					
Symptom-driven physical exam (19)		X (20)		X	X (20)	X (20)	X	X	X	X	X
Inspection of injection site of previous vaccinations				X	X (21)	X (21)	X				X
Distribute and explain Subject Diary (22)		X			X	X					
Review and collect Subject Diary				X		X	X				X (23)
Distribute and explain Memory Aid				X			X	X	X		
Review and collect Memory Aid					X			X	X	X	X (23)
AE/ SAE/ AESI Assessment (24)		X	X	X	X	X	X	X	X	X	X
Blood Volume [mL]	33.0 (10); 29.5 (25)	54.0		12.5	39.5	66.5	66.5	27	27	70.5	4.0

Table 3 TABLE OF EVENTS – MAIN STUDY PHASE

Visit	V0	V1	V2	V3	V4	V5	V6	V7	Early Termination (1)
Timing Day (D) Month (M)	**D-21**	**D1**	**D29 M1**	**D57 M2**	**D85 M3**	**D180 M6**	**D236 M8**	**D365 M12**	**before V7**
Time windows	**-21 to -1**	**0**	**+/- 4**	**+/- 4**	**+/- 4**	**+ 7/- 14**	**+ 7/ - 14**	**+/- 14**	**n/a**
Visit type	In-person	In-person	In-person	In-person	In-person	In-person	In-person	In-person	In-person
Informed consent (2)	X								
Inclusion/exclusion criteria	X	X (Review)							
Vaccination delay criteria		X	X	X					
Demographic data	X								
Medical history incl. vaccinations	X	X (3)							
Concomitant medications/ treatments incl. vaccinations	X	X	X	X	X	X	X	X	X
Physical examination (4), ECG	X								
Vital signs (5)	X	X	X	X					
Evaluation of oral body temperature	X	X (6)	X (6)	X (6)					
HIV test [3.5 mL] (7)	X (26)								
Bb s.l. screening test [4mL] (8)	X (26)							X	X
Baseline serology Sample [5.0 mL] (9)	X (26)								
Serum Pregnancy test [3.5 mL] (10)	X (26)								
Urine Pregnancy test (10)		X (11)	X (11)	X (11)	X	X	X	X	X
Clinical chemistry [8.5 mL] (12)	X (26)		X (11)	X (11)	X			X	
Hematology [4 mL] (13)	X (26)		X (11)	X (11)	X			X	
Coagulation blood sample [4.5 mL] (14)	X (26)								
Urinalysis (15)	X		X (11)	X (11)	X			X	
Immunogenicity blood sample, (16)		X (11) [54 mL]	X (11) [27 mL]	X (11) [54 mL]	X [54 mL]	X [27 mL]	X [27 mL]	X [54 mL]	
Randomization (17)		X							
VACCINATION (18)		X	X	X					
Check for AEs following vaccination		X	X	X					
Symptom-driven physical exam (19)		X (20)	X (20)	X (20)	X	X	X	X	X
Inspection of injection site of previous vaccinations			X (21)	X (21)	X				X
Distribute and explain Subject Diary (22)		X	X	X					
Review and collect Subject Diary			X	X	X				X (23)
Distribute and explain Memory Aid					X	X	X		
Review and collect Memory Aid						X	X	X	X (23)
AE/ SAE/ AESI Assessment (24)		X	X	X	X	X	X	X	X
Blood Volume [mL]	33.0 (10); 29.5 (25)	54.0	39.5	66.5	66.5	27.0	27.0	70.5	4.0

(1) 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 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 visit; but in addition, a Lyme borreliosis screening test will be performed.
(2) Occurs before screening and prior to any study-related procedures.
(3) Symptoms noted at Visit 1 (prior to first vaccination) are not considered AEs but will be recorded as medical history.
(4) Physical examination on the following body systems: general appearance, skin, head/ eyes /ears/ nose/ throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, and neurological system. If applicable, physical examination as well as ECG performed within the study VLA15-202 is acceptable for study VLA15-201 if within the specified visit window.
(5) 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.
(6) To be performed prior to vaccination.
(7) The results of negative HIV tests that were performed up to 30 days before Visit 0 are acceptable (blood: HIV test 3.5 mL). Positive HIV test obtained by ELISA will have to be confirmed by a second method (e.g. Westernblot or PCR).
(8) A commercially available C6 ELISA assay (VlsE ELISA) will be performed (blood: 4 mL). Serum samples that are tested positive will have to be verified by a confirmatory immunoblot. Test results need to be available before randomization and remain valid for 4 weeks.
(9) A baseline serology sample will be taken at the screening visit and might be used for work-up of suspected LB-associated, autoimmune or neuroinflammatory events (e.g. analysis of Rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPA), etc, as appropriate), (blood: 5.0 mL). If applicable, a baseline serology sample collected at the study site within the study VLA15-202 is acceptable for study VLA15-201 if within the specified visit window.
(10) In women of childbearing potential. A woman is considered of childbearing potential if fertile, following menarche 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.
(11) At vaccination visits, all samples have to be obtained before vaccination. Pregnancy results and urinalysis must be available before vaccination.
(12) Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP (blood: 8.5 mL). Test results of current visit do not need to be available before vaccination. Tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable.
(13) Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets (Ethylenediaminetetraacetic acid [EDTA] blood: 4 mL). Test results of current visit do not need to be available before vaccination. Tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable.
(14) Prothrombin time, aPTT, fibrinogen (blood: 4.5 mL). Tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable.
(15) Standard urine dipstick: pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes. Tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable.
(16) Blood will be collected for immunogenicity testing by ELISA and for supportive functional antibody analysis by e.g. growth inhibition assay, surface binding assay or serum transfer experiments (passive protection animal model).
(17) To be performed by study staff otherwise not involved with study conduct to keep the study observer-blinded (i.e. un-blinded study staff)
(18) Study vaccine has to be administered by study staff otherwise not involved with study conduct to keep the study observer-blinded. Subjects enrolled in the Run-in phase should be observed for at least one hour, subjects enrolled in the Main Study phase should be observed for at least 30 min after vaccination for treatment of any immediate reactions.
(19) 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.
(20) If subject has any complaints after vaccination, a second symptom-driven physical examination will be performed by the investigator prior to discharge.
(21) The injection site of previous injections should be evaluated by study staff prior to the next vaccination.
(22) 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.

(23) Unreturned Subject Diaries/ Memory Aids should be collected at the Early Termination Visit. For Early Terminations prior to Visit 4, the previous injection site should be inspected.

(24) AEs, SAEs and AESIs will be collected throughout study conduct. Symptoms noted at Visit 1 (prior to vaccination) are not considered adverse events but will be recorded as medical history.

(25) Women of non-childbearing potential and male subjects.

(26) If applicable, HIV test, Lyme borreliosis screening test, serum pregnancy test, clinical chemistry tests, hematology tests, and coagulation tests performed at the study site within the study VLA15-202 are acceptable for study VLA15-201 if within the specified visit window. As such, if test results are available, respective blood samples do not need to be collected again for the present study. Similar, if applicable, a baseline serology sample collected at the study site within the study VLA15-202 is acceptable for study VLA15-201 if within the specified visit window.

SIGNATURE PAGE

Title of Clinical Trial: **IMMUNOGENICITY AND SAFETY STUDY OF VLA15, A MULTIVALENT RECOMBINANT OSPA BASED VACCINE CANDIDATE AGAINST LYME BORRELIOSIS, IN HEALTHY ADULTS AGED 18 TO 65 YEARS - A RANDOMIZED, CONTROLLED, OBSERVER-BLIND PHASE 2 STUDY.**

Study Code: VLA15-201

EudraCT no: 2018-003379-37

IND number: 17199

With their signature, investigators and sponsor agree to conduct this study in accordance with the protocol, International Conference on Harmonization (ICH) and Good Clinical Practice (GCP) guidelines and with the applicable local regulatory requirements. Moreover, the site will keep all information obtained from the participation in this study confidential unless otherwise agreed in writing.

Principal Investigator

Print Name	Signature	Date
[redacted] Clinical Project Manager Valneva Austria GmbH	Signature	Date
[redacted] Senior Scientist Clinical Strategy Valneva Austria GmbH	Signature	Date
[redacted] Chief Medical Officer Valneva Austria GmbH	Signature	Date

LIST OF RESPONSIBLE PERSONNEL

Sponsor's Responsible Medical / Safety Officer:

[redacted]

Monitoring:

[redacted]

Safety Desk:

[redacted]

Study Medical Monitor:

[redacted]

Serious Adverse Event (SAE) reporting by fax and/or phone or email within 24 hours after discovery:

[redacted]

Laboratories:

Immunogenicity Sample Logistics, Storage:

[redacted]

Immunological Assays:

Valneva Austria GmbH
Clinical Serology

[redacted]

IMP Logistics, Labelling and packaging:

[redacted]

Statistical Analysis:

[redacted]

Data Management:

[redacted]

TABLE OF CONTENTS

CLINICAL STUDY PROTOCOL **1**
CLINICAL STUDY PROTOCOL SYNOPSIS **2**
Definition of Adverse Events of Special Interest (AESIs) 10
LIST OF RESPONSIBLE PERSONNEL **18**
TABLE OF CONTENTS **20**
LIST OF TABLES **22**
LIST OF FIGURES **22**
LIST OF ABBREVIATIONS **23**
1. INTRODUCTION **25**
1.1 DISEASE BACKGROUND 25
1.2 LYME BORRELIOSIS VACCINES 25
1.3 VACCINE CANDIDATE VLA15 26
1.4 PREVIOUS RESULTS 27
1.4.1 Repeat dose toxicology study in rabbits 27
1.4.2 *In vivo* efficacy studies in mice 27
1.4.3 Clinical studies with VLA15 29
1.5 STUDY RATIONALE 31
1.6 RISK - BENEFIT ANALYSIS 32
2. STUDY OBJECTIVES **34**
2.1 PRIMARY OBJECTIVE 34
2.2 SECONDARY OBJECTIVES 34
3. SELECTION OF STUDY POPULATION **34**
4. INVESTIGATIONAL PLAN **37**
4.1 STUDY ENDPOINTS 37
4.2 OVERALL STUDY DESIGN AND PLAN 38
4.2.1 Overall study design 38
4.2.2 Discussion of study design 39
4.2.3 Study events description 41
4.2.4 Number of subjects and study centres 48
4.2.5 Timely conduct of the study 48
4.2.6 Study duration 48
4.2.7 Assignment of subjects to treatment groups 49
4.2.8 Subject Identification 49
4.2.9 Randomization 49
4.2.10 Blinding 49
5. INVESTIGATIONAL MEDICINAL PRODUCTS **50**
5.1 DESCRIPTION OF IMP 50
5.1.1 VLA15 Drug Product w/ Alum 50
5.1.2 VLA Placebo 51
5.2 PACKAGING & LABELLING OF IMP 51
5.3 CONDITION OF STORAGE OF IMP 51
6. TREATMENT OF SUBJECTS **52**
6.1 INVESTIGATIONAL TREATMENT 52
6.1.1 Dose and dosing schedule 52
6.1.2 Preparation and method of administration 52
6.1.3 Treatment duration 53
6.2 PRIOR AND CONCOMITANT THERAPY 53
6.2.1 Permitted prior and concomitant therapy 53
6.2.2 Forbidden prior and concomitant therapy 53
6.3 TREATMENT COMPLIANCE 54
6.3.1 Drug dispensing and accountability 54
6.4 PREGNANCY TESTING AND BIRTH CONTROL 54
7. ASSESSMENT OF IMMUNOGENICITY **55**
7.1 IMMUNOGENICITY MEASUREMENTS 55
7.1.1 OspA serotype specific IgG antibodies (ELISA) 55
7.1.2 Supportive functional assays 55
8. ASSESSMENT OF SAFETY **55**
8.1 PHYSICAL EXAMINATION 56
8.2 VITAL SIGNS, ECG AND ORAL BODY TEMPERATURE 56
8.3 SOLICITED AES AND SUBJECT DIARY 56
8.3.1 Collection, assessment and documentation of solicited AEs 56

8.3.2 Severity of solicited AEs 57
8.4 UNSOLICITED AEs 59
8.4.1 Collection, assessment and documentation of unsolicited AEs 59
8.4.2 Evaluation of unsolicited AEs....60
8.4.2.1 Definition of medically-attended AEs 60
8.4.2.2 Definition of Serious Adverse Events (SAEs) 60
8.4.2.3 Definition of Adverse Events of Special Interest (AESIs) 61
8.4.2.4 Collection and evaluation of Adverse Events of Special Interest (AESIs)...61
8.4.2.5 Severity of unsolicited AEs 63
8.4.2.6 Causality assessment....63
8.4.2.7 Outcome....63
8.4.2.8 Actions taken....64
8.4.3 Timeframe for reporting of unsolicited AEs 64
8.4.4 SAE reporting procedures 64
8.4.5 Pregnancy reporting procedures....65
8.5 LABORATORY PARAMETERS 66
8.6 SAFETY MONITORING.... 68
8.6.1 Data Safety Monitoring Board (DSMB) and initiation of the Main Study phase 68
8.6.2 Sponsor 69
8.6.3 Investigator 69
9. STATISTICAL METHODS AND SAMPLE SIZE....71
9.1 GENERAL ASPECTS.... 71
9.2 ANALYSIS POPULATIONS 71
9.3 IMMUNOGENICITY ANALYSIS 72
9.4 SAFETY ANALYSIS.... 72
9.5 GENERAL CONSIDERATIONS FOR THE DETERMINATION OF THE OPTIMAL DOSE 73
9.6 DETERMINATION OF SAMPLE SIZE.... 74
9.7 INTERIM ANALYSIS/ FINAL ANALYSIS 75
10. DEVIATIONS FROM THE PROTOCOL 75
10.1 RELEVANT PROTOCOL DEVIATIONS.... 75
10.2 PREMATURE SUBJECT WITHDRAWAL FROM STUDY OR TREATMENT.... 75
10.2.1 Individual stopping criteria 75
10.3 DOCUMENTATION OF PREMATURE WITHDRAWAL 76
10.4 SUBSEQUENT THERAPY 76
11. ETHICAL AND REGULATORY ASPECTS 76
11.1 ETHICAL/ REGULATORY FRAMEWORK 76
11.2 INSTITUTIONAL REVIEW BOARD/ INDEPENDENT ETHICS COMMITTEE 76
11.3 SUBJECT INFORMATION AND INFORMED CONSENT 77
12. QUALITY CONTROL AND QUALITY ASSURANCE 77
12.1 SOURCE DATA AND RECORDS 77
12.2 PERIODIC MONITORING 77
12.3 AUDIT AND INSPECTION.... 77
12.4 CONFIDENTIALITY OF SUBJECT'S DATA 78
13. DATA HANDLING AND RECORD KEEPING 78
13.1 INFORMATION OF INVESTIGATORS.... 78
13.2 ELECTRONIC CASE REPORT FORMS (ECRFS).... 78
13.2.1 eCRF entries....78
13.2.2 Changes to eCRF data 78
13.2.3 eCRF entry validation 78
13.2.4 Data collection 79
13.3 CODING OF ADVERSE EVENTS, DRUGS AND DISEASES 79
13.4 INVESTIGATOR FILE.... 79
13.4.1 Maintenance 79
13.4.2 Archiving and destruction 79
13.5 PROVISION OF ADDITIONAL INFORMATION 79
14. CHANGES IN THE CONDUCT OF THE STUDY....79
14.1 PROTOCOL AMENDMENTS 79
14.2 STUDY TERMINATION – STUDY STOPPING RULES 80
15. REPORTING AND PUBLICATION....81
15.1 CLINICAL STUDY REPORT.... 81
15.2 PUBLICATION POLICY.... 81
16. LIABILITIES AND INSURANCE....81

17. APPENDIX 1 82
18. APPENDIX 2 83
19. REFERENCES 86

LIST OF TABLES

Table 1 Treatment Groups and Vaccinations 4
Table 2 TABLE OF EVENTS - RUN-IN PHASE 13
Table 3 TABLE OF EVENTS – MAIN STUDY PHASE 14
Table 4: Efficacy of VLA15 in two mouse challenge models using either ticks infected with *B. burgdorferi* (ST1), *B. afzelii* (ST2) or *B. bavariensis* (ST4) (tick challenge) or *in vitro* grown *B. garinii* (ST5 or ST6) (needle challenge) for challenge 28
Table 5: Solicited Local Adverse Events After Any Vaccination by Symptom, Safety Population 30
Table 6: Solicited Systemic Adverse Events After Any Vaccination by Symptom, Safety Population 31
Table 7: Amount of alum and antigen per treatment group 32
Table 8 Treatment dose with protein content, injection volumes and alum amount 50
Table 9 Grading of Solicited Adverse Events 58
Table 10 Grading Scale for Abnormal Laboratory Assessments 67

LIST OF FIGURES

Figure 1 Study Design 4

LIST OF ABBREVIATIONS

ACPA	Anti-citrullinated protein antibodies
AE	Adverse Event
AESI	Adverse Event of Special Interest
Alum	$Al(OH)_3$, Aluminum Hydroxide
ANOVA	Analysis of variance
aPTT	Activated Partial Thromboplastin time
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
ATC	Anatomical Therapeutic Chemical
BSL2	Biological Safety Level 2
CA	Competent Authority
CR	Clinically Relevant
CRA	Clinical Research Associate
CMO	Contract Manufacturing Organization
eCRF	Electronic Case Report Form
CRO	Contract Research Organization
CRP	C-reactive protein
CSR	Clinical Study Report
DSMB	Data Safety Monitoring Board
e.g.	For Example
EC	Ethics Committee
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	European Medicines Agency
EudraCT	European Clinical Trials Database
ET	Early Termination
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
GLP	Good Laboratory Practice
GMFR	Geometric Mean Fold Rise
GMT	Geometric Mean Titer
HIV	Human Immunodeficiency Virus
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
i.e.	That Is
IEC	Independent Ethics Committees
I.M.	Intramuscular
IgG	Immunoglobulin G
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
kD	Kilo Dalton
ITT	Intent-to-Treat
mITT	Modified Intent-to-Treat
LB	Lyme borreliosis
MedDRA	Medical Dictionary for Regulatory Activities
µg	Microgram
mm	Millimeter(s)
mg	Milligram(s)
min	Minute(s)
mL	Milliliter(s)
N/A	Not Applicable
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse

	Events
No.	Number(s)
OspA	Outer surface protein A
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PP	Per Protocol
RF	Rheumatoid Factor
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCR	Seroconversion Rate
SOP	Standard Operating Procedures
ST	Serotype
ULN	Upper Limit of Normal
V	Visit
WBC	White blood cell
WHO	World Health Organization
w/	With
w/o	Without

1. INTRODUCTION

1.1 Disease Background

Lyme borreliosis (LB) is an emerging, tick-borne zoonotic disease caused by several genospecies of the spirochete *Borrelia burgdorferi* sensu lato (s.l.). It is recognized as the most common vector-borne disease in both Europe and North America [1]. In Europe, incidence based on notified cases report about 85,000 cases per year [2], however, due to inconsistent case reporting and the fact that LB is often undiagnosed, this number is largely underestimated [3,4]. In the US, the Center for Disease Control and Prevention (CDC) estimates about 300,000 cases annually which is almost a 10-fold increase to reported cases [5,6] . The incidence of LB has a bimodal distribution with respect to age. Two target populations are mainly affected: children aged 5-14 years and the adult population aged 50-64 years [3,7].

In Europe, most human infections are caused by four genospecies, presenting six serotypes (STs): *B. afzelii* (ST2), *B. garinii* (ST3, ST5 and ST6), *B. burgdorferi* sensu stricto (s.s.) (ST1) and *B. bavariensis* (ST4). In the US, *B. burgdorferi* s.s. (ST1) is found in almost 100 % of cases. Very recently, a new genospecies named *Borrelia mayonii* has been described, which was found in few clinical specimens isolated in the Upper Midwest of the US [8].

The most common clinical manifestation of LB is a gradually expanding erythematous skin rash called erythema migrans (EM), a distinct sign of early localized *Borrelia* infection. An EM appears within days to weeks at the location of the tick bite and is often accompanied by symptoms of fatigue, fever, headache, mild stiff neck, arthralgia, or myalgia [1]. In approximately 70 %-80 % of LB cases patients develop an EM [9,10].

If untreated or treated inadequately, the infection can disseminate to other parts of the body and can cause serious late stage manifestations affecting the nervous system (facial palsy, meningitis, myelitis, and encephalitis), joints (recurrent or persistent large joint synovitis), or heart (e.g. conduction abnormalities and carditis).

The most common late stage clinical manifestations of LB that develops in about 30 % of patients include musculoskeletal manifestations, such as Lyme arthritis. Lyme arthritis comprises recurrent attacks or long-lasting joint swelling (synovitis), usually in one or a few joints most commonly the knee, which develops months after a tick bite. Nervous system manifestations include Lyme neuroborreliosis, most commonly presented as cranial neuropathy with facial nerve palsy, possibly with bilateral involvement (bilateral Bell's palsy), within a few weeks of infection. In adults, the disease typically presents as painful meningoradiculoneuritis and facial palsy. In contrast, children most frequently develop headache due to meningitis, and facial palsy. In children there are shorter lasting symptoms and better outcomes. Cardiac manifestations in LB appear to be uncommon, and Lyme carditis usually presents within two months of infection as myocarditis with acute intermittent atrioventricular heart block. In Europe, more severe skin manifestations (e.g. acrodermatitis chronica atrophicans (ACA), borrelial lymphocytoma) can result from disseminated infection as late complications [9].

1.2 Lyme borreliosis vaccines

Two OspA based Lyme borreliosis vaccines have previously shown to be efficacious to prevent Lyme borreliosis in humans: LYMErix (Smith Kline Beecham) and ImuLyme (Pasteur Mérieux Connaught). Both vaccines contained Outer surface protein A (OspA) from *B. burgdorferi* (ST1) as antigen, a surface exposed lipoprotein of ~28.5 kD. OspA is one of the dominant antigens expressed by the spirochetes when present in an unfed tick. During tick feeding the incoming blood signals the downregulation of OspA expression, allowing the spirochetes to migrate to the salivary glands and further into the blood of the host. OspA-based LB vaccines act on spirochetes in the tick gut, where spirochetes are neutralized by

anti-OspA antibodies in a complement independent manner, before they can infect the human.

In LYMErix, OspA was absorbed to aluminum hydroxide and demonstrated vaccine efficacy of 49 % in the first tick season (i.e. after 2 vaccine doses) and of 76 % in the second tick season (i.e. after 3 doses) in clinical Phase III testing [11]. ImuLyme was tested in a non-adjuvanted formulation of OspA in clinical Phase III and conferred protection in 68 % and in 92 % of subjects in the first and second tick season, respectively (after 2 and 3 vaccine doses) [12].

LYMErix has been licensed and marketed in the US from 1998 to 2002, when it was voluntarily withdrawn from the market. A relationship between the Lyme borreliosis vaccine and joint reactions was hypothesized because of partial homology of OspA ST1 in the vaccine with hLFA-1 antigen (human leukocyte function-associated antigen-1) that was claimed to induce antibiotic-refractory Lyme arthritis in a subset of naturally infected patients. The hypothesis could not be proven. On the contrary, a retrospective study of joint complaints after vaccination reported to the Vaccine Adverse Event Reporting System showed no unusual number of such complaints. In the Phase III study of the vaccine, the incidence of transient arthralgia was non-significantly increased in vaccinees, but the incidence of arthritis was not increased as compared to the placebo group [13].

ImuLyme (Pasteur Mérieux Connaught) was never marketed based on a commercial decision.

In Europe, no Lyme borreliosis vaccine has been licensed until now.

More recently, a clinical Phase I/II study has been performed by Baxter BioScience. Similar to the vaccine candidate VLA15, the vaccine candidate is a multivalent OspA based LB vaccine, designed to provide protection against the most prevalent OspA ST1 to ST6 [14,15]. The study was conducted in two parts: In Part 1, 300 healthy adults aged 18 to 65 years who were seronegative for antibodies against *Borrelia* were included in the study and randomly assigned to six treatment groups. They received three doses of 30 µg, 60 µg or 90 µg OspA antigen w/ or w/o Alum (Day 0, 28,. 56) and a booster dose 9-12 months after the first immunization. In Part 2, further 350 subjects, either seronegative or seropositive for antibodies against *Borrelia* were enrolled and received either 30 or 60 µg OspA antigen w/ Alum on Days 0, 28, 56 and a booster dose at 6 or 9-12 months after the first immunization. Overall, it could be shown that the vaccine candidate was safe and well tolerated and induced substantial antibody responses against all six OspA serotypes.

1.3 Vaccine Candidate VLA15

Valneva's VLA15 vaccine candidate is composed of three ~35 kDa fusion proteins (designated as Lip-D1B2B, Lip-D4Bva3B and Lip-D5B6B), each containing the C-terminal part of two OspA serotypes representing the serotype dominating in the USA and the six serotypes that are prevalent in Europe. Each fusion protein is built of two subunits containing the C-terminal half of two OspA serotypes, fused together via a linker. The C-terminal half of OspA is the exposed part of OspA on the surface of spirochetes and therefore readily accessible for antibodies. In order to stabilize the OspA subunits at physiological temperatures and preserve the structure needed to induce protective immunity, one disulfide bond per subunit has been introduced. The 21 residues long linker used to fuse the two subunits is derived from two N-terminal loops from *B. burgdorferi* OspA (ST1) and designed to induce flexibility and distance between the subunits to keep epitopes accessible. In order to ensure high immunogenicity, each protein is expressed with a signal sequence for attachment of an N-terminal lipid moiety.

Further, the putative T cell epitope in OspA ST1 which presents homology to human leukocyte function-associated antigen-1 (hLFA-1) and previously claimed to induce antibiotic-refractory Lyme arthritis in a subset of naturally infected patients has been replaced with the corresponding sequence from OspA ST2. The design of the VLA15 vaccine has been

chosen in order to induce a strong serotype specific immunity needed for protection against infection by *Borrelia* expressing OspA ST1 to ST6.

1.4 Previous Results

1.4.1 Repeat dose toxicology study in rabbits

Safety and tolerability of VLA15 was tested in a repeated dose toxicology study in male and female New Zealand White rabbits. The study was performed under GLP conditions and according to respective guidelines from WHO and EMA. Animals were dosed with 4 intramuscular injections (each dose 90µg of antigen) administered at two weeks intervals. VLA15 was administered with or without an aluminium hydroxide adjuvant (alum). Results from this study were supportive for clinical use, and are summarized in the VLA15 Investigator's Brochure.

1.4.2 *In vivo* efficacy studies in mice

The efficacy of VLA15 was studied for five of the six OspA serotypes in two mouse challenge models: a tick challenge model for ST1, ST2 and ST4, where immunized mice were challenged with ticks harboring *B. burgdorferi* (ST1), *B. afzelii* (ST2) or *B. bavariensis* (ST4) and a needle challenge model, where immunized mice were challenged subcutaneously with *in vitro* grown *B. garinii* (ST5 or ST6). Groups of mice immunized with the corresponding full-length OspA; Lip-OspA1-His (ST1), Lip-OspA2-His (ST2), Lip-OspA4-His (ST4), Lip-OspA5-His (ST5) or Lip-OspA6-His (ST6) were included as positive control in the respective experiment.

VLA15 induced significant protection compared to placebo at a 3 µg dose against challenge with ticks harboring *B. burgdorferi* (ST1), *B. afzelii* (ST2) or *B. bavariensis* (ST4) which was equal or better than full length OspA with the corresponding serotype (Table 4). For the *B. burgdorferi* (ST1) challenge, two different strains (Pra1 and Pra4) were used. Immunizing with 0.03 µg or 0.003 µg VLA15 provided significant protection in mice, when *B. afzelii* (ST2) infected ticks were used for challenge (Table 4). When different doses (3 µg, 0.3 µg, 0.03 µg and 0.003 µg) of VLA15 were assessed for vaccine efficacy against a challenge with either *B. garinii* (ST5) or *B. garinii* (ST6), significant protection could be shown down to 0.03µg (Table 4).

Table 4: Efficacy of VLA15 in two mouse challenge models using either ticks infected with *B. burgdorferi* (ST1), *B. afzelii* (ST2) or *B. bavariensis* (ST4) (tick challenge) or *in vitro* grown *B. garinii* (ST5 or ST6) (needle challenge) for challenge

Immunization		Infected/Total								
Immunogen	Dose	*B. burgdorferi* (ST1)		*B. afzelii* (ST2)		*B. bavariensis* (ST4)		*B. garinii* (ST5)		*B. garinii* (ST6)
		Exp4185 (Pra4)	Exp4239 (Pra1)	Exp4175 (IS1)	Exp4176 (IS1)	Exp4322 (Marx1)	Exp4323 (Marx1)	Exp4276 (PHei)	Exp4277 (PHei)	Exp4235 (KL11)
Challenge		tick	tick	tick	tick	tick	tick	needle	needle	needle
Lip-OspA1-His	1 µg	0/4*	1/9*	-	-	-	-	-	-	-
Lip-OspA2-His	1 µg	-	-	0/8**	0/9***	-	-	-	-	-
Lip-OspA4-His	1 µg	-	-	-	-	0/6**	0/5^{ns}	-	-	-
Lip-OspA5-His	1 µg	-	-	-	-	-	-	0/10***	0/10***	-
Lip-OspA6-His	1 µg	-	-	-	-	-	-	-	-	5/10*
VLA15	3 µg	0/5*	0/7**	0/9***	0/7***	0/6**	0/5^{ns}	0/10***	1/10***	3/10**
	0.3 µg	-	-	-	-	0/7**	0/9**	0/10***	0/10***	-
	0.03 µg	-	-	0/9***	2/7**	0/3*	0/6*	2/10*	3/10**	-
	0.003 µg	-	-	7/10^{ns}	1/8***	2/4	2/7	7/10^{ns}	7/10^{ns}	-
Placebo	-	5/6	5/6	5/5	8/8	7/8	5/7	8/10	9/9	10/10

Ticks infected with *B. burgdorferi* OspA ST1 (strains Pra1 or Pra4), *B. afzelii* OspA ST2 (strain IS1) or *B. bavariensis* OspA ST4 (Strain Marx1) or *in vitro* grown *B. garinii* OspA ST5 (strain PHei) or *B. garinii* OspA ST6 (strain KL11) were used for challenge. For tick challenge, only mice with at least one fully or almost fully (≥48 hours feeding) fed tick were included in the readout. P-values were calculated with Fisher's exact test (two tailed); * <0.05, ** <0.01 and *** <0.001 and ^{ns} not significant.

In summary, it was shown that VLA15 is highly immunogenic and produces a long lasting immune response. Protective efficacy against four *Borrelia* species (*B. burgdorferi*, *B. afzelii*, *B. bavariensis* and *B. garinii*) including five clinically relevant OspA serotypes (1, 2, 4, 5 and 6) could be demonstrated in mouse models using either infected ticks or *in vitro* grown spirochetes for challenge.

For more details on immunogenicity and efficacy, please refer to VLA15 Investigator's Brochure.

1.4.3 Clinical studies with VLA15

First data from a Day 84 Interim Analysis of an observer-blind, partially randomized, multi-center dose escalation Phase 1 study (VLA15-101) are available. In this first-in-human study a total of 179 subjects aged 18 to < 40 years were enrolled in six treatment groups (approx. 30 subjects per treatment group) to receive three I.M. vaccinations of VLA15 12 µg w/ Alum, VLA15 12 µg w/o Alum, VLA15 48 µg w/ Alum, VLA15 48 µg w/o Alum, VLA15 90 µg w/ Alum or VLA15 90 µg w/o Alum on Days 0, 28 and 56. Primary objective of this study is to assess the safety and tolerability of VLA15 on Day 84 (i.e. one month after the third vaccination). Secondary objectives include safety and immunogenicity of VLA15 until one year after the first vaccination.

Interim data showed that VLA15 was generally safe and well tolerated in all treatment groups with no associated safety concerns. VLA15 was immunogenic in all doses and formulations tested, i.e. OspA-specific IgG antibody responses were seen in all treatment groups and against all OspA serotypes.

Overall, 166 of 179 subjects (92.7 %) reported any solicited or unsolicited AE up to Day 84. The rate of subjects that experienced solicited or unsolicited AEs were lower in the 12 µg groups (86.2 % each) compared with the higher treatment groups (93.5 % to 96.8 %). Differences between the treatment groups were not significant.

The majority of AEs were mild or moderate. A total of eight subjects (4.5 %) experienced severe related AEs; all of them were solicited AEs, as such counted by definition as related: one subject (3.4 %) in the 12 µg w/o alum group (headache), one subject (3.2 %) in the 48 µg w/ alum group (headache, excessive fatigue, myalgia), one subject (3.4 %) in the 48 µg w/o alum group (excessive fatigue), three subjects (9.7 %) in the 90 µg w/ alum group (two subjects with pain and tenderness, one subject with arthralgia and myalgia), and two subjects (6.7 %) in the 90 µg w/o alum group (nausea, headache). A total of 22 subjects (12.3 %) reported 29 related unsolicited AEs, all mild or moderate. The most frequent of these was dizziness, reported by four subjects (2.2 %) on six occasions. Dizziness was experienced by not more than one subject in any treatment group. Fatigue was the next most frequent unsolicited AE, reported by four subjects (2.2 %) on four occasions. Fatigue was experienced by not more than one subject in any treatment group. Related unsolicited AEs were most frequent in the 12 µg w/o alum treatment group (seven subjects [24.1 %], seven AEs) and least frequent in the 90 µg w/ alum treatment group (two subjects [6.5 %], three AEs).

Two unrelated Serious AEs were reported: one subject (3.4 %) in the 12 µg w/o Alum group (Fracture os sacrum and L2) and one subject (3.4 %) in the 12 µg w/ Alum group (Left extremities numbness). No cases of Arthritis or Rheumatoid Arthritis, AEs potentially associated with Lyme Borreliosis, were observed. Arthralgia was reported as solicited AE in 10.6 % of subjects and as unsolicited AE in 1.1 %. There were no neurological concerns (no facial paralysis, no GBS, no seizures/convulsions) and no vaccine-related hypersensitivity reactions.

Solicited local AEs were reported by 72.4 % (12 µg w/o alum group) to 96.7 % (90 µg w/o alum group) of subjects. Overall, solicited local AEs were significantly less common in the

12 µg groups compared with the 48 µg and 90 µg groups. No significant difference was observed between combined adjuvanted and combined non-adjuvanted treatment groups. The most common solicited local AEs were pain (67.0 %) and tenderness (84.4 %). Rates of solicited local AEs declined after the first vaccination (Table 5).

Table 5: Solicited Local Adverse Events After Any Vaccination by Symptom, Safety Population

	12µg w/ Alum N=29 n (%)	12µg w/o Alum N=29 n (%)]	48µg w/ Alum N=31 n (%)	48µg w/o Alum N=29 n (%)	90µg w/ Alum N=31 n (%)	90µg w/o Alum N=30 n (%)	Pooled VLA15 groups N=179 n (%)
Any AE	22 (75.9)	21 (72.4)	29 (93.5)	26 (89.7)	29 (93.5)	29 (96.7)	156 (87.2)*
Pain	13 (44.8)	13 (44.8)	22 (71.0)	23 (79.3)	26 (83.9)	23 (76.7)	120 (67.0)**
Tenderness	21 (72.4)	20 (69.0)	28 (90.3)	25 (86.2)	29 (93.5)	28 (93.3)	151 (84.4)***
Erythema	1 (3.4)	4 (13.8)	7 (22.6)	3 (10.3)	5 (16.1)	4 (13.3)	24 (13.4)
Swelling	4 (13.8)	4 (13.8)	6 (19.4)	3 (10.3)	8 (25.8)	2 (6.7)	27 (15.1)
Induration	4 (13.8)	7 (24.1)	7 (22.6)	3 (10.3)	6 (19.4)	8 (26.7)	35 (19.6)
Itching	0 (0.0)	2 (6.9)	2 (6.5)	3 (10.3)	1 (3.2)	1 (3.3)	9 (5.0)

n: number of subjects with event
N: number of subjects studied in treatment group
*Significant p-value overall; Significant pairwise comparisons: 12µg+ vs 90µg-, 12µg- vs 48µg+, 12µg- vs 90µg+, 12µg- vs 90 µg-
** Significant p-value overall; Significant pairwise comparisons: 12µg+ vs 48µg-, 12µg+ vs 90µg+, 12µg+ vs 90 µg-, 12µg- vs 48µg-, 12µg- vs 90µg+, 12µg- vs 90 µg-
*** Significant p-value overall; Significant pairwise comparisons: 12µg+ vs 90µg+, 12µg+ vs 90 µg-, 12µg- vs 90µg+, 12µg- vs 90 µg-

Solicited systemic AEs were reported by 58.1 % (48 µg w/ alum group, 90 µg w/ alum group) to 76.7 % (90 µg w/o alum group) of subjects, with no statistically significant differences. No statistically significant difference was observed between combined adjuvanted and combined non-adjuvanted treatment groups. The most common solicited systemic AEs were headache (44.7 %), excessive fatigue (25.1 %) and myalgia (25.1 %), with no statistically significant differences. Rates of solicited systemic AEs declined with the number of vaccinations.

Table 6: Solicited Systemic Adverse Events After Any Vaccination by Symptom, Safety Population

	12µg w/ Alum N=29 n (%)	12µg w/o Alum N=29 n (%)]	48µg w/ Alum N=31 n (%)	48µg w/o Alum N=29 n (%)	90µg w/ Alum N=31 n (%)	90µg w/o Alum N=30 n (%)	Pooled VLA15 groups N=179 n (%)
Any AE	19 (65.5)	19 (65.5)	18 (58.1)	18 (62.1)	18 (58.1)	23 (76.7)	115 (64.2)
Headache	11 (37.9)	15 (51.7)	13 (41.9)	15 (51.7)	10 (32.3)	16 (53.3)	80 (44.7)
Fever	0 (0.0)	0 (0.0)	2 (6.5)	0 (0.0)	1 (3.2)	0 (0.0)	3 (1.7)
Flu like symptoms	5 (17.2)	4 (13.8)	3 (9.7)	3 (10.3)	5 (16.1)	5 (16.7)	25 (14.0)
Nausea	7 (24.1)	8 (27.6)	4 (12.9)	5 (17.2)	4 (12.9)	6 (20.0)	34 (19.0)
Vomiting	2 (6.9)	2 (6.9)	1 (3.2)	1 (3.4)	0 (0.0)	1 (3.3)	7 (3.9)
Rash	1 (3.4)	1 (3.4)	0 (0.0)	1 (3.4)	0 (0.0)	1 (3.3)	4 (2.2)
Excessive fatigue	9 (31.0)	9 (31.0)	5 (16.1)	7 (24.1)	6 (19.4)	9 (30.0)	45 (25.1)
Arthralgia	3 (10.3)	3 (10.3)	4 (12.9)	1 (3.4)	3 (9.7)	5 (16.7)	19 (10.6)
Myalgia	7 (24.1)	6 (20.7)	8 (25.8)	4 (13.8)	9 (29.0)	11 (36.7)	45 (25.1)

n: number of subjects with event
N: number of subjects studied in treatment group
No significant differences

VLA15 was immunogenic in all doses and formulations tested. Adjuvanted formulations were more immunogenic compared to respective non-adjuvanted formulations of the same dose level. A statistically significant dose-response was observed between the 12 µg dose groups and higher dose groups, but not between the 48 and 90 µg groups. The 90 µg w/ Alum group induced SCRs against individual OspA serotypes ranging between 71.4% for ST1 and 96.4 % for ST2. There was a consistent pattern with regards to immune responses to VLA15 in the 6 serotypes, i.e., the 48 and 90 µg w/ Alum groups showed highest GMTs for all OspA serotypes. IgG antibody titers and SCRs were substantially higher after three immunizations (Day 84) compared to after two immunizations (D 56) for all 6 OspA serotypes.

Safety and Immunogenicity results of the Day 84 Interim Analysis of the Phase 1 study VLA15-101 are described in more detail in the Clinical Study Report, dated 06 April 2018.

1.5 Study Rationale

Interim data of a first-in-human study with VLA15 has shown a favorable safety profile of the vaccine. VLA15 was immunogenic in all doses and formulations tested, i.e. OspA-specific IgG antibody responses were seen in all treatment groups and against all OspA serotypes, with a clear dose response between the 12 µg dose groups and the higher 48 µg and 90 µg dose groups. Even with the limited sample size in the Phase 1 study, the results very clearly indicate that the adjuvanted groups induce a better immune response compared to non-adjuvanted groups at the same dose level. Safety does not appear to differ between the adjuvanted and the non-adjuvanted groups.

This Phase 2 study is conducted to further study different treatment groups in a larger sample size, and to generate a sufficient safety database for advancing the candidate

vaccine to Phase 3 testing. As Phase 1 data clearly indicate a better immune response with the adjuvanted treatment groups compared with the non-adjuvanted treatment groups, only adjuvanted treatment groups were selected for Phase 2. In terms of dose selection for Phase 2, as circulating antibody levels are of utmost importance for OspA-based vaccines, further dose increase aiming to induce earlier and higher immune response, is considered. As we have seen a good safety profile and no safety concern so far, two higher dose groups will be tested in this Phase 2 study in addition to the 90 µg w/ alum group: 135 µg w/ alum and 180 µg w/ alum. Therefore, as a safety precaution, a Run-in phase with 30 subjects per treatment group is planned for VLA15-201. Full randomization in the Main Study phase will be initiated after D85 safety data have been reviewed by an independent Data Safety Monitoring Board (DSMB) and a recommendation to initiate the Main Study phase has been obtained from the DSMB. Immunogenicity assessment will be done using a serotype specific full-length OspA-ELISA for each serotype. As this Phase 2 study is intended to define the dose for Phase 3, the assay will be validated and will be filed to our IND prior to testing. Additional supportive serological assays to assess functional antibodies will be developed and will also be employed for the Phase 2 study in a subset of subjects.

As in VLA15-101, the dose will be adjusted per volume. For this purpose, a new blending of VLA15 was prepared that contains the same amount of Alum per mL (i.e. 500 µg/ mL), but a higher protein concentration (i.e. 180 µg/ mL). Extractable volume from vial and maximum volume to be applied is 1.0 mL. The three doses will be applied using 0.5 mL, 0.75 mL and 1.0 mL to apply 90 µg, 135 µg and 180 µg respectively. Please refer to Table 7 for the amount of Alum and the injection volumes for the three dose groups.

This formulation is planned to be the final formulation to be used in Phase 3 and for product commercialization, hence, the safety profile that we obtain in about 200 subjects in this Phase 2 study will be the safety profile of the final formulation to be developed further. Therefore, potential confounding of the safety profile due to different volumes and/or amounts of Alum may be seen but is considered acceptable in this Phase 2 study.

Table 7: Amount of alum and antigen per treatment group

Group	Antigen Dose	Injection Volume (mL)	Alum Content
90 µg w/ alum	90 µg	0.50	250 µg
135 µg w/ alum	135 µg	0.75	375 µg
180 µg w/ alum	180 µg	1.00	500 µg
Placebo	PBS	1.00	-

1.6 Risk - Benefit Analysis

Risks:
Results from a GLP repeat dose toxicity and local tolerance study in rabbits with four bi-weekly intramuscular injections of 90 µg VLA15 w/ or w/o Alum were supportive for clinical use.

First safety data in humans are available from an interim analysis of an ongoing Phase 1 first-in-human study. Data revealed that VLA15 was generally safe and well tolerated and there were no safety concerns so far, as concluded by an independent Data Safety Monitoring Board that reviewed accruing safety information during treatment phase. Most AEs were reported to be mild or moderate and comprised typical vaccine reactions: solicited injection site reactions (mainly pain (67.0 %) and tenderness (84.4 %) at the injection site) or solicited systemic reactions (mainly headache (44.7 %), excessive fatigue (25.1 %) and

myalgia (25.1 %)). Reactogenicity after the second and third dose decreased compared to the first dose. Please refer to section 1.4.3 for a detailed description of the safety data of VLA15 available so far. Overall, the AE profile of VLA15 appeared comparable to other lipidated recombinant vaccines or lipid-containing formulations that might be associated with an increased inflammatory response through interaction with Toll-like receptors [16]. For VLA15, rates of pain in the 90 µg w/ alum dose group, the group that will be used in planned Phase 2 study, was 73.3 %, 44.4 % and 37.0 % after the first, second and third vaccination, respectively. In comparison, respective rates after each vaccination for Trumenba® (licensed Meningococcal Group B vaccine which contains lipidated proteins) were 84.2 %, 79.3 % and 80.4 %, after the first, second and third vaccination [17]. For Bexsero® (licensed Meningococcal Group B vaccine that includes lipid-containing formulations) rates were 90 % and 83 % after the first and after the second vaccination [18]. Rates for the previously licensed OspA vaccine LYMErix™ were 82 % after the first vaccination and 76 % after the second vaccination in a Month 0-1-12 schedule [19]. Rates for the lipidated chimeric OspA based vaccine candidate from Baxter were between 29 % and 44 % for the different treatment groups after the first vaccination [14]. Rates of fever appeared to be comparably low: 0 % after the first and second vaccination and 4.0 % after the third vaccination with VLA15 90 µg w/ alum, compared to from 1.2 % to 2.4 % for Trumenba®, 1 % to 5 % for Bexsero®, 1 % to 1.5 % for LYMErix™, and 0 % to 8 % for the Baxter Lyme vaccine candidate (depending on the treatment group, after the first vaccination only).

Current clinical safety data in humans is available for subjects aged 18 to 40 years. During the Main Study Phase of present study VLA15-201 subjects aged 40 to 65 years will also be enrolled. As common for clinical vaccine studies a similar safety profile in both age groups is expected.

In VLA15-201, VLA15 will also be tested in an adult population that was previously infected with *Borrelia Burgdorferi sensu lato (B.B. s.l.)*. The safety of OspA based vaccines has previously been shown with LYMErix [19,20] and a similar multivalent OspA based vaccine [15]. In addition, a theoretical risk put forward for a previously licensed OspA vaccine, although never proven, has been eliminated in the design of VLA15: the putative T cell epitope in OspA ST1 presenting homology to human leukocyte function-associated antigen-1 (hLFA-1) and claimed to induce antibiotic-refractory Lyme arthritis in a subset of naturally infected patients, has been eliminated through the replacement by corresponding sequence from OspA ST2.

As with any vaccine, the VLA15 vaccine might induce allergic and anaphylactic reactions, the process of vaccination may also trigger syncope. The needle pricks for blood sampling may also cause local reactions such as edema.

Overall, the safety profile for VLA15 to date is favorable. The safety data support further development for all doses and formulations and are also supportive for extending the dose in order to further improve the immunogenicity.

Benefits:

OspA ST1 based vaccines have been shown to be protective against LB in humans before and VLA15 was effective in animal models. However, as VLA15 is a new multivalent construct that has not yet been tested for clinical efficacy, the subjects might not directly benefit from vaccination with VLA15.

In view of the positive safety profile from the first-in-human study with VLA15 and the usually limited risks associated with vaccinations, the risk benefit ratio for VLA15 is assessed to be positive.

2. STUDY OBJECTIVES

2.1 Primary Objective

- To determine the optimal dose of VLA15 in healthy adults aged 18 - 65 years up to Day 85.

2.2 Secondary Objectives

Immunogenicity:

- To assess the immune response of VLA15 in healthy adults aged 18 – 65 years up to Month 12 (i.e. 10 months after the primary vaccination series).

Safety:

- To assess the safety profile of VLA15 in healthy adults aged 18 – 65 years up to Month 12.

3. SELECTION OF STUDY POPULATION

CRITERIA FOR INCLUSION/EXCLUSION

Approximately 570 male or female adults who satisfy the inclusion and exclusion criteria listed below will be enrolled in the study.

***Inclusion criteria*:**

Subjects must meet **ALL** of the following criteria to be eligible for this study:

Run-in phase:

1. Subject is aged 18 to 40 years at the day of screening (Visit 0);

Main Study phase:

1. Subjects aged18 to 65 years at the day of screening (Visit 0);

Run-in phase and Main Study phase:

2. Subject is of good general health, including subjects with pharmacologically controlled chronic conditions;
3. Subject has an understanding of the study and its procedures, agrees to its provisions, and gives written informed consent prior to any study-related procedures;
4. If subject is of childbearing potential:
    a. Subject has a negative serum pregnancy test at screening (Visit 0);
    b. Subject agrees to employ adequate birth control measures for the duration of the study (please refer to section 6.4).

Exclusion criteria (Run-in phase and Main Study phase):

Subjects who meet **ANY** of the following criteria are **NOT** eligible for this study:

1. Subject has a chronic illness related to Lyme borreliosis (LB), an active symptomatic LB as suspected or diagnosed by a physician, or received treatment for LB within the last 3 months prior to Visit 0;
2. Subject received previous vaccination against LB;
3. Subject had a tick bite within 4 weeks prior to Visit 1;
4. Subject has a medical history of or currently has a clinically relevant disease (eg cardiovascular, respiratory, neurologic, psychiatric conditions) which poses a risk for participation in the study, based on investigators judgement, such as individuals with poorly controlled or unstable disease, ongoing suspected or active inflammation, or poor compliance with pharmacologic treatment. Subjects with pharmacologically controlled conditions like osteoarthritis, depression, or asthma are eligible;
5. Subject has a medical history of or currently has a neuroinflammatory or autoimmune disease, including Guillain Barré Syndrome;
6. Subject has a known thrombocytopenia, bleeding disorder, or received anticoagulants in the 3 weeks prior to first vaccination or until Day 57 (Visit 3), contraindicating I.M. vaccination as judged by the investigator;
7. Subject has received an active or passive immunization within 28 days before first vaccination at Visit 1 and until Day 85; except for influenza (seasonal or pandemic) and pneumococcal vaccines which may be administered outside a 7-days interval before or after any trial vaccination;
8. Subject has received any other non-registered medicinal product in another clinical trial within 28 days prior to VLA15 vaccination at Visit 1 (Day 1) and throughout the entire study period or has received a registered medicinal product in another clinical trial within 28 days prior to VLA15 vaccination at Visit 1 (Day 1) and up to Day 85;
9. Subject has a known or suspected defect of the immune system that would prevent an immune response to the vaccine, such as subjects with congenital or acquired immunodeficiency, including infection with human immunodeficiency virus (HIV), status post organ transplantation or immuno-suppressive therapy within 30 days prior to Visit 1. Immuno-suppressive therapy is defined as administration of chronic (longer than 14 days) prednisone or equivalent ≥0.05 mg/kg/day. Topical and inhaled steroids are allowed;
10. Subject has a history of anaphylaxis or severe allergic reactions or a known hypersensitivity or allergic reactions to one of the components of the vaccine;
11. Subject had any malignancy in the past 5 years. If treatment for cancer was successfully completed more than 5 years ago and the malignancy is considered to be cured, the subject may be enrolled;
12. Subject had acute febrile infections within 10 days prior to first vaccination;
13. Subject is pregnant (positive serum pregnancy test at screening), has plans to become pregnant during the course of the study or is lactating at the time of enrollment. Women of childbearing potential that are unwilling or unable to employ an adequate birth control measure for the duration of the study;
14. Subject has donated blood or blood-derived products (e.g. plasma) within 30 days or received blood or blood-derived products (e.g. plasma) within 90 days prior to vaccination in this study or plans to donate or use blood or blood products during the course of the study;

15. Subject has any condition that, in the opinion of the investigator, may compromise the subject's well-being, might interfere with evaluation of study endpoints, or would limit the subject's ability to complete the study;
16. Subject is committed to an institution (by virtue of an order issued either by the judicial or the administrative authorities);
17. Subject is in a dependent relationship with the sponsor, an investigator or other study team member, or the study center. Dependent relationships include close relatives and household members (i.e. children, partner/spouse, siblings, parents) as well as employees of the investigator or study center personnel.

Delay Criteria for Vaccination

Vaccination will be delayed if:

1. Subject has an acute illness with or without elevated body temperature (≥100.4 °F [38.0 °C]) within 3 days prior to the scheduled vaccination. Subjects may be rescheduled for vaccination at a later date provided that the illness has resolved (body temperature <100.4 °F [38.0 °C]);
2. Subject has received antipyretics within 4 hours prior to the scheduled time of vaccination. In this case the vaccination should be performed at a later date.

In addition, the following criteria must be met:

1) For a rescheduled **first** vaccination:

1. All inclusion and none of the exclusion criteria are met; In case not all of these criteria are met, the subject will be excluded from the study.
2. The rescheduled visit should be within the specified time window (i.e. within 21 days after the screening visit). In case a first vaccination cannot be rescheduled within the specified time window (i.e. within 21 days after the screening visit), the subject might be invited for a rescreening.

2) For a rescheduled **second or third** vaccination:

The rescheduled visit should be within the specified time window.

4. INVESTIGATIONAL PLAN

4.1 Study Endpoints

Primary Endpoint:

+ GMTs (Geometric Mean Titers) for IgG against each OspA serotype ST1 to ST6, determined by ELISA at Day 85.

Secondary Endpoints:

Immunogenicity:

+ GMTs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1, 29, 57, 180, 236, and Month 12.
+ SCRs (Seroconversion Rate, defined as four-fold increase in IgG titer compared to baseline) for each OspA serotype specific IgG (ST1 to ST6), determined by ELISA, at Day 29, 57, 85, 180, 236, and Month 12.
+ GMFR (Geometric Mean of the fold rise as compared to baseline) for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 29, 57, 85, 180, 236 and Month 12.
+ GMTs, SCRs and GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1, 29, 57, 85, 180, 236, and Month 12, stratified by age group.

Safety:

+ Frequency of SAEs during the entire study;
+ Frequency of related SAEs during the entire study;
+ Frequency of AESIs during the entire study;
+ Frequency of related AESIs during the entire study;
+ Frequency of unsolicited AEs during the entire study (incl. clinically relevant laboratory parameters);
+ Frequency of related unsolicited AEs during the entire study (incl. clinically relevant laboratory parameters);
+ Frequency of solicited local and solicited systemic AEs within 7 days after each and after any vaccination.
+ Frequency of SAEs, AESIs, solicited and unsolicited AEs during the entire study stratified by age group.

4.2 Overall Study Design and Plan

4.2.1 Overall study design

This 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 (Figure 1).

In the Run-in phase, a total of 120 subjects aged 18 to 40 years were to be randomized stratified by study site 1:1:1:1 to receive VLA15 90 µg w/ alum, VLA15 135 µg w/ alum, or VLA15 180 µg w/ alum, or placebo (30 subjects per treatment group) as I.M. vaccinations on Days 1, 29 and 57. Dosing was to be adjusted by injection volume (see Table 1). Two safety visits were to be performed after the first vaccination: a safety phone call at Day 4 (i.e. Visit 1a, three days after the first vaccination) and an in-person visit at Day 8 (i.e. Visit 1b, seven days after the first vaccination). After all subjects enrolled in the Run-in phase completed Visit 4 (Day 85, i.e. 28 days after the third vaccination) a DSMB reviewed all safety data up to Day 85 in a scheduled DSMB meeting and gave the recommendation that all treatment groups were safe and well tolerated so far and could be continued in the Main Study phase based on available safety data.. After the DSMB meeting, two dose groups (135 µg and 180 µg of VLA15 w/ alum) were selected for further investigation. The two higher dose groups were selected based on DSMB recommendation obtained on June 11th in a scheduled DSMB meeting. No safety concerns associated with any of the VLA15 treatment groups were identified by the independent DSMB.

In the Main Study phase, a total of 450 subjects aged 18 to 65 years will be randomized stratified by study site, age group and baseline *B.b.* s.l. serostatus 2:2:1 to receive 135 µg or 180 µg VLA15 w/ alum (180 subjects each) or placebo (90 subjects), as I.M. vaccinations on Days 1, 29 and 57. Subjects will be enrolled in two age groups (18-49 years and 50-65 years) in a ratio of approx. 2:1. An interim analysis on safety and immunogenicity data will be performed after all subjects have completed Visit 6 (i.e. Day 236, six months after the last vaccination). This interim analysis will cover safety and immunogenicity data up to Visit 4 (i.e. Day 85, four weeks after the last vaccination) as well as safety data up to Visit 6 (i.e. Day 236, six months after the last vaccination). Final analysis of safety and immunogenicity data will be performed after all subjects have completed the follow-up period up to Visit 7 (i.e. Day 365/Month 12).
In both study phases, target is to enroll approx. 10 % or more of subjects that are baseline seropositive for *Borrelia burgdorferi sensu latu* (*Bb s.l.*). This is aimed to be achieved through selection of endemic recruitment areas as well as database searches for *Bb s.l.* seropositive subjects.

Figure 1 Study Design

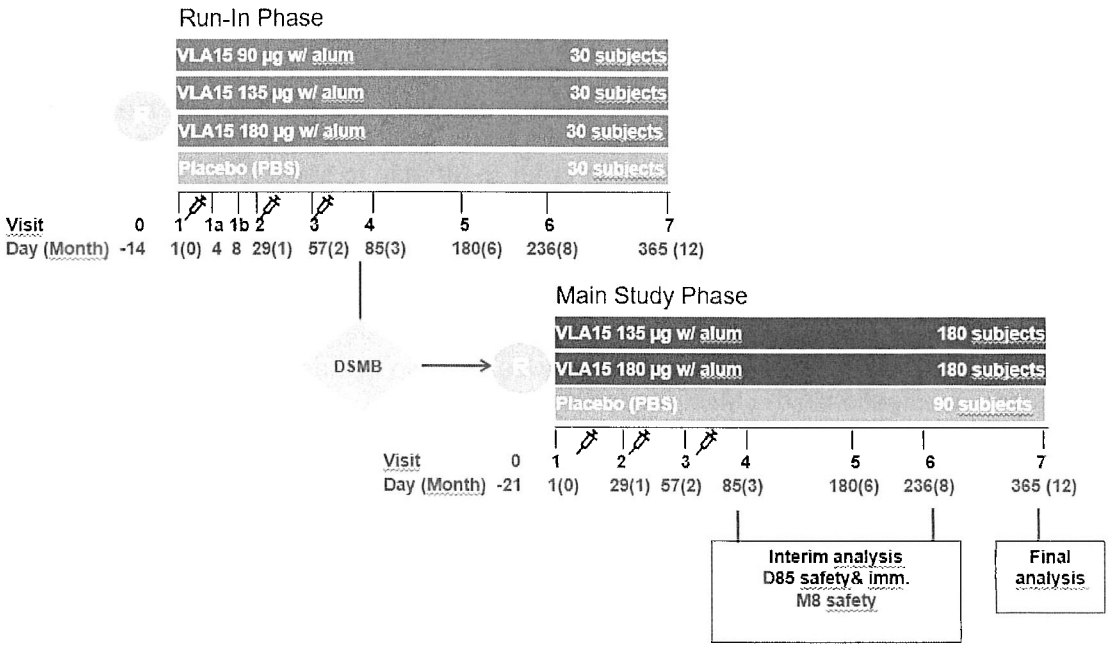

Table 1 Treatment Groups and Vaccinations

Group	Treatment	Injection Volume (mL)	Days of Vaccination
90 µg	VLA15 90 µg w/ alum	0.50	1, 29, 57
135 µg	VLA15 135 µg w/ alum	0.75	1, 29, 57
180 µg	VLA15 180 µg w/ alum	1.00	1, 29, 57
Placebo	PBS	1.00	1, 29, 57

SUBJECT ENROLMENT

Run-in phase: For safety reasons, in the first three weeks of the Run-in phase, recruitment was to be limited to 15 subjects per week. Thereafter recruitment was to be limited to 30 subjects per week.

Main Study phase: In the Main Study phase recruitment will be performed without recruitment restrictions.

4.2.2 Discussion of study design

Rationale for dose/formulation:

The first-in-human study VLA15-101, even with the limited sample size of 30 subjects per group, has clearly indicated that the adjuvanted groups induce a better immune response

compared to non-adjuvanted groups at the same dose level. Safety does not appear to differ between the adjuvanted and non-adjuvanted groups. Therefore, only adjuvanted VLA15 treatment groups will be further investigated in Phase 2. As circulating antibody levels are of utmost importance and a boosting effect upon natural infection cannot be expected for an OspA based vaccine, further dose increase aiming to induce earlier and higher immune response, is considered. For the first-in-human study, we decided to limit the dose at 90 µg as a safety precaution. As we have seen a good safety profile and no safety concern so far, we will increase the dose in Phase 2 to a maximum of 180 µg/ dose. This is well in the range of other lipidated recombinant proteins that have been tested in clinical development and that are now licensed (e.g. for Trumenba®, a 200 µg dose was tested down to the age of 18 months in clinical studies and was considered to be well tolerated by the authors [21]). A medium 135 µg dose will be investigated in order to be able to see a dose response and to have a fall back in case of safety signals with the 180 µg dose. Three dose levels will be evaluated initially in the Run-in phase. After DSMB review, the two highest safe doses will be further investigated in the Main Study phase. Given that OspA based vaccines induce antibodies which block tick-borne transmission of the spirochete to the host, high antibody titers are of utmost importance because no boosting effect upon infection can be expected. Therefore, unless safety signals suggest otherwise, the two highest safe dose groups will be selected for investgation in the Main Study phase.

Rationale for immunization schedule

The proposed primary immunization schedule for VLA15 will consist of three immunizations administered one month apart (Month 0-1-2). The same schedule has been applied in Phase 1. In this study IgG antibody titers and SCRs were substantially higher after three immunizations compared to after two immunizations for all OspA serotypes, hence a three-dose primary vaccination schedule will be considered for further development. These results confirm experiences from clinical studies with previous OspA based vaccines: LYMErix™ has been licensed with two I.M. immunizations one month apart in the first year (Month 0-1) and a booster dose 1 year later (Month 12). However, after licensure, a clinical study with LYMErix™ that investigated an alternative schedule of three immunizations one month apart (i.e. Month 0-1-2) revealed that the third immunization within the first year increased total IgG titers by around a factor of 3 and would therefore have increased efficacy in the first tick season [22]. Similarly, immunogenicity data from the Baxter vaccine candidate revealed that there was a substantial further increase of IgG titers after the third immunization [14].

Rationale for study population

The proposed study population consists of subjects aged ≥18 to ≤65 years of age of good general health, including subjects with pharmacologically controlled chronic conditions who live in LB endemic regions. As a safety precaution in the Run-in phase, subject aged ≥18 to ≤40 years will be enrolled in order to reduce the chance of observed adverse safety outcomes that might be due to medical conditions that are more likely to occur in older adults and were not identified prior to study entry. In the Main Study phase, subjects aged 18 to 65 years will be enrolled. Major exclusion criteria in both study parts include chronic illness related to Lyme borreliosis (LB), an active symptomatic LB as suspected or diagnosed by a physician, treatment for LB within the last three months prior to Visit 0, a tick bite within four weeks prior to Visit 1, history of a neuroinflammatory or autoimmune disease, history of immunodeficiency or ongoing immunosuppressive therapy, known history of anaphylaxis, pregnancy and lactation or any active or passive vaccination within 28 days prior to first study vaccination and during treatment phase. Subjects tested positive for HIV at screening are excluded from the study due to the possible impact of HIV infection on the immunogenicity of the study vaccine.

In contrast to Phase 1, subjects with a positive serology test result for *Borrelia burgdorferi sensu lato (B.b. s.l.)* antibodies (i.e. subjects that were previously infected with *B.b. s.l.)* are enrolled.

The rationale for selecting this study population is to evaluate the most appropriate dose in one of the anticipated target populations for a LB vaccine: adults from 18 to 65 years of age who live in Lyme disease endemic areas.

The results of this study will be used as basic data on safety, tolerability and dose finding of VLA15 for further immunogenicity, efficacy and safety studies in adults and children, who are, besides older adults, the target populations that are mainly affected by Lyme borreliosis.

This study was designed according to the Note for Guidance on Clinical Evaluation of New Vaccines (CHMP/VWP/164653/2005), where applicable. Feedback that was obtained from FDA in an End-of-Phase 1 meeting on May 29, 2018 and from a scientific advice procedure with EMA on 18 Oct 2018 has been taken into consideration in the design of the protocol.

4.2.3 Study events description

For an outline of procedures required at each visit, please refer to the Tables of Events (Table 2 for the Run-in phase and Table 3 for the Main Study phase).

The study consists of a Screening Visit within 14 days (run-in phase) or 21 days (main study phase) before the first administration of the investigational medicinal product (IMP) and an experimental part with eight in-person visits (Days 1, 8, 29, 57, 85, 180, 236, and 365) and one phone call visit (Day 4) during the Run-in phase, and seven in-person visits (Days 1, 29, 57, 85, 180, 236, and 365) during the Main Study phase. Vaccinations will be performed on Days 1, 29 and 57.

Subjects enrolled during the Run-in phase will be observed for one hour after each vaccination before discharge from study site. Subjects enrolled during the Main Study phase will be observed for 30 min after each vaccination before discharge from study site.

In the visit descriptions below, all tasks not explicitly mentioned to be performed by unblinded study staff will be performed by blinded study staff.

For all study visits subjects do not have to be in a fasted state.

The following procedures will be performed:

Visit 0, Screening Visit (1 to 14 days prior to Visit 1 in run-in phase, 1 to 21 days prior to Visit 1 in main study phase):

Signed and dated informed consent must be obtained before any study specific procedures are undertaken.

- Check of inclusion and exclusion criteria.
- Document demographic data, complete medical history, vaccination history covering the last three years prior to screening, concomitant medications/treatments.
- Perform physical examination (general appearance, skin, head/ eyes/ ears/ nose/ throat, cardiovascular system, respiratory system, abdominal and gastrointestinal system, musculoskeletal system, neurological system and lymph nodes), measure ECG and vital signs (systolic and diastolic blood pressure, pulse rate) in seated position and at rest, measure oral body temperature. If applicable, physical examination as well as ECG performed within the study VLA15-202 is acceptable for study VLA15-201 if within the specified visit window.

- Laboratory tests*:
  - HIV test: A positive HIV test obtained by ELISA will have to be confirmed by a second method [e.g. Western blotting or PCR]. No test for HIV must be performed, if HIV negativity has been established within the last 30 days prior to Visit 0.
  - Lyme borreliosis screening test: For serological screening on previous infection with Lyme borreliosis, a commercially available C6 ELISA assay (VlsE ELISA) will be performed. Serum samples that are tested positive will be verified by a confirmatory immunoblot. Test result need to be available before randomization and remain valid for 4 weeks.
  - Baseline Serology Sample: A baseline serology sample will be taken at the screening visit and might be used for work-up of suspected LD-associated, autoimmune or neuroinflammatory events (e.g. analysis of Rheumatoid factor (RF), anti citrullinated protein antibodies (ACPA) etc, as appropriate).
  - Serum pregnancy test: A serum pregnancy test has to be performed for each woman of childbearing potential†. If the test is positive, the subject must be excluded from the trial.
  - Clinical Chemistry: Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP.
  - Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
  - Coagulation: Prothrombin time, aPTT, fibrinogen.
  - Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.

Visit 1, Day 1:

- Review inclusion and exclusion criteria.
- Check vaccination delay criteria.
- Document any changes to medical history and concomitant medication/treatments including vaccination(s) since the previous study visit. Symptoms noted at Visit 1 prior to first vaccination are not considered AEs but will be recorded as medical history.
- Perform symptom-driven physical examination, measure vital signs (systolic and diastolic blood pressure, pulse rate) in seated position and at rest, measure oral body temperature (vaccination should be postponed in case of an acute febrile illness).
- Laboratory tests:
  - Urine pregnancy test: In women of childbearing potential. Pregnancy test result must be obtained prior to vaccination.
- Collect immunogenicity blood sample (before vaccination).

* If applicable, HIV test, Lyme borreliosis screening test, serum pregnancy test, clinical chemistry tests, hematology tests, and coagulation tests performed at the study site within the study VLA15-202 is acceptable for study VLA15-201 if within the specified visit window. As such, if test results are available, respective blood samples do not need to be collected again for the present study. Similar, if applicable, a baseline serology sample collected at the study site within the study VLA15-202 is acceptable for study VLA15-201 if within the specified visit window.

† 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.

- **Designated unblinded staff member only:** Randomize subject to treatment group as described in section 4.2.9. As this study is performed observer-blind, subjects must not be informed about the treatment group they have been allocated to.
- **Designated unblinded staff member only:** Prepare and administer first vaccination according to assigned treatment group into deltoid of the non-dominant arm.
- Blinded staff member takes over subject and:
  - *For subjects enrolled in Run-in phase:* Observe subject for 1 hour after vaccination for immediate treatment of possible AEs.
  - *For subjects enrolled in Main Study phase:* Observe subject for 30 min after vaccination for immediate treatment of possible AEs.
- Record any AEs and local and systemic tolerability following vaccination, if applicable.
- Distribute Subject Diary, thermometer and measuring device: Instruct subject how and when to complete the diary. Subject will also be instructed to immediately inform the site in case they experience any severe solicited AEs (see Section 8.3.2 for severity grading of AE) or other severe symptoms or have suspicion or diagnosis of Lyme borreliosis.
- If the subject has any complaints, perform a symptom-driven physical examination and record vital signs prior to discharge. Subject will only be discharged if in the opinion of the investigator no further concerns exist.

Visit 1a (for subjects that are enrolled during Run-in phase only), Day 4 (+/- 1 day):

- Visit 1a is performed as a safety phone call.
- Clarify with subject any questions related to completion of subject diary.
- Ask subject for adverse events, including solicited and unsolicited AEs, local and systemic tolerability, and changes in concomitant medications including vaccinations since Visit 1.
- In case of reported solicited or unsolicited AEs, assessments of AEs have to be done by an investigator.

Visit 1b (for subjects that are enrolled during Run-in phase only), Day 8 (+/- 1 day):

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Review and collect the Subject Diary, including verification of entries with the subject. Clinician to re-assess severity of reported solicited local and systemic AEs. In addition re-assess causality for any reported medically attended or severe solicited local and systemic AEs.
- Record any unsolicited AEs since the last study visit.
- Distribute and explain Memory Aid to document unsolicited Adverse Events. Subject will be instructed to immediately inform the site in case they experience any severe symptoms or have suspicion or diagnosis of Lyme borreliosis.
- Inspect vaccination site from 1st vaccination for (ongoing) Adverse Events.
- Laboratory tests:

- Clinical Chemistry Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP.
- Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
- Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.

Visit 2, Day 29/ Month 1 (+/- 4 days):

- Check vaccination delay criteria.
- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination, measure vital signs (systolic and diastolic blood pressure, pulse rate) in seated position and at rest, measure oral body temperature (vaccination should be postponed in case of an acute febrile illness).
- *For subjects enrolled in Run-in phase:*
  Review and collect Memory Aid, including verification of entries with the subject.
- *For subjects enrolled in Main Study phase:*
  Review and collect Subject Diary, including verification of entries with the subject. Clinician to re-assess causality for any reported medically attended or severe solicited local and systemic AEs.
- Record any unsolicited AEs since the last study visit.
- Inspect vaccination site from 1st vaccination for (ongoing) Adverse Events.
- Laboratory tests (all samples have to be obtained before vaccination, pregnancy results and urinalysis must be available before vaccination):
  - Urine pregnancy test: In women of childbearing potential. Pregnancy test result must be obtained prior to vaccination.
  - Clinical Chemistry: Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP.
  - Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
  - Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.
- Collect immunogenicity blood sample (before vaccination).
- **Designated unblinded staff member only:** Prepare and administer second vaccination according to assigned treatment group into deltoid of the non-dominant arm.
- Blinded staff member takes over subject and:
  - *For subjects enrolled in Run-in phase:* Observe subject for 1 hour after vaccination for immediate treatment of possible AEs.
  - *For subjects enrolled in Main Study phase:* Observe subject for 30 min after vaccination for immediate treatment of possible AEs.
- Record any AEs and local and systemic tolerability following vaccination, if applicable.
- Distribute Subject Diary, instruct subject how and when to complete the diary. Subject will also be instructed to immediately inform the site in case they experience any severe

solicited AEs (see section 8.3.2 for severity grading of AE) or other severe symptoms or have suspicion or diagnosis of Lyme borreliosis.

- If the subject has any complaints, perform a symptom-driven physical examination and record vital signs prior to discharge. Subject will only be discharged if in the opinion of the investigator no further concerns exist.

Visit 3, Day 57/ Month 2 (+/- 4 days):

- Check vaccination delay criteria.
- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination, measure vital signs (systolic and diastolic blood pressure, pulse rate) in seated position and at rest, measure oral body temperature (vaccination should be postponed in case of an acute febrile illness).
- Review and collect Subject Diary, including verification of entries with the subject. Clinician to re-assess causality for any reported medically attended or severe solicited local and systemic AEs.
- Record any unsolicited AEs since the last study visit.
- Inspect vaccination site from 2nd vaccination for (ongoing) Adverse Events.
- Laboratory tests (all samples have to be obtained before vaccination, pregnancy results and urinalysis must be available before vaccination):
  - Urine pregnancy test: In women of childbearing potential. Pregnancy test result must be obtained prior to vaccination.
  - Clinical Chemistry: Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP.
  - Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
  - Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.
- Collect immunogenicity blood sample (before vaccination).
- **Designated unblinded staff member only:** Prepare and administer third vaccination according to assigned treatment group into deltoid of the non-dominant arm.
- Blinded staff member takes subject over and:
  - *For subjects enrolled in Run-in phase:* Observe subject for 1 hour after vaccination for immediate treatment of possible AEs.
  - *For subjects enrolled in Main Study phase:* Observe subject for 30 min after vaccination for immediate treatment of possible AEs.
- Record any AEs and local and systemic tolerability following vaccination, if applicable.
- Distribute Subject Diary, instruct subject how and when to complete the diary. Subject will also be instructed to immediately inform the site in case they experience any severe solicited AEs (see section 8.3.2 for severity grading of AE) or other severe symptoms or have suspicion or diagnosis of Lyme borreliosis.
- If the subject has any complaints, perform a symptom-driven physical examination and record vital signs prior to discharge. Subject will only be discharged if in the opinion of the investigator no further concerns exist.

Visit 4, Day 85/ Month 3 (+/- 4 days):

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Review and collect Subject Diary, including verification of entries with the subject. Clinician to re-assess causality for any reported medically attended or severe solicited local and systemic AEs.
- Record any unsolicited AEs since the last study visit.
- Inspect vaccination site from 3rd vaccination for (ongoing) Adverse Events.
- Laboratory tests:
  - Urine pregnancy test: In women of childbearing potential. Pregnancy test result must be obtained prior to vaccination.
  - Clinical Chemistry: Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP.
  - Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
  - Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.
- Collect immunogenicity blood sample.
- Distribute and explain Memory Aid to document unsolicited Adverse Events. Subject will be instructed to immediately inform the site in case they experience any severe symptoms or have suspicion or diagnosis of Lyme borreliosis.

Visit 5, Day 180/ Month 6 (+7/- 14 days):

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Review and collect Memory Aid, including verification of entries with the subject.
- Record any unsolicited AEs since the last study visit.
- Laboratory tests:
  - Urine pregnancy test: In women of childbearing potential.
- Collect immunogenicity blood sample.
- Distribute and explain Memory Aid to document unsolicited Adverse Events. Subject will be instructed to immediately inform the site in case they experience any severe symptoms or have suspicion or diagnosis of Lyme borreliosis.

Visit 6, Day 236/ Month 8 (+7/- 14 days):

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Review and collect Memory Aid, including verification of entries with the subject.

- Record any unsolicited AEs since the last study visit.
- Laboratory tests:
  - Urine pregnancy test: In women of childbearing potential.
- Collect immunogenicity blood sample.
- Distribute and explain Memory Aid to document unsolicited Adverse Events. Subject will be instructed to immediately inform the site in case they experience any severe symptoms or have suspicion or diagnosis of Lyme borreliosis.

Visit 7, Day 365/ Month 12 (+/- 14 days):

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Review and collect Memory Aid, including verification of entries with the subject.
- Record any unsolicited AEs since the last study visit.
- Laboratory tests:
  - Urine pregnancy test: In women of childbearing potential.
  - Lyme borreliosis screening test: For serological screening on previous infection with Lyme borreliosis, a commercially available C6 ELISA assay (VlsE ELISA) will be performed. Serum samples that are tested positive will be verified by a confirmatory immunoblot.
  - Clinical Chemistry: Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP.
  - Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
  - Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.
- Collect immunogenicity blood sample.

Unscheduled Visit:

Unscheduled visits and any assessments performed during the visit (e.g. physical examination, laboratory test) should be documented in the source data and the eCRF.

Early Termination:

Subjects who terminate participation or who are withdrawn from the study prematurely will undergo the following investigations during an Early Termination Visit, if possible:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- If Early Termination visit is before Visit 4, inspect vaccination site from previous vaccination for (ongoing) Adverse Events.

- Review and collect unreturned subject diaries or Memory Aids, including verification of entries with the subject. Study physician to re-assess causality for any reported medically attended or severe solicited local and systemic AEs.
- Record any unsolicited AEs since the last study visit.
- Laboratory tests:
  - Urine pregnancy test: In women of childbearing potential.
  - Lyme borreliosis screening test: For serological screening on infection with Lyme borreliosis, a commercially available C6 ELISA assay (VlsE ELISA) will be performed. Serum samples that are tested positive will be verified by a confirmatory immunoblot.

The reason for early termination should be clarified in as much detail as possible. If an AE was the reason for early study termination details on that specific AE(s) should be captured.

If the subject is unwilling to perform an ET Visit, a phone call should be made as soon as possible after termination to follow-up on concomitant medication including vaccination(s) and AEs ongoing (including persisting injection site reactions, if applicable) and any new AEs since the previous study visit should be documented. The reason for early termination should be clarified in as much detail as possible. If an AE was the reason for early study termination details on that specific AE(s) should be captured. See also section 10.3 of the study protocol.

Note: If subjects present at a regular study visit within the acceptable time window and inform that they will discontinue the study after this visit, the study visit will not be performed as an ET visit, but will be performed and documented as a regular study visit including all events that are described for respective study visit. In this case a Lyme borreliosis screening test will be performed in addition for serological screening on infection with Lyme borreliosis.

4.2.4 Number of subjects and study centres

Overall, a total of approx. 570 healthy subjects aged ≥18 to ≤65 years will be enrolled.

In the Run-in phase, 120 subjects aged ≥18 to ≤40 years will be enrolled.

In the Main Study phase, a total of 450 subjects aged ≥18 to ≤65 years will be enrolled in two age groups (18-49 years and 50-65 years) in a ratio of approx. 2:1.

It is planned to have approx. 10 study centers in Lyme disease endemic areas in the US and Europe. The enrollment will be stopped as soon as the required number of subjects is reached.

4.2.5 Timely conduct of the study

Planned study start for the Run-in phase is December 2018.

An independent DSMB was to review all safety data up to Day 85 in a scheduled DSMB meeting and was to give a recommendation for selection of two dose groups for further enrolment in the Main Study phase. Based on the DSMB recommendation, the two higher dose groups (VLA15 w/ alum 135 µg and 180 µg) will be continued in the main study phase.

Planned study start for the Main Study phase is July 2019.

4.2.6 Study duration

Study duration per subject will be approximately13 months:

- Screening period: max. 21 days
- Treatment period: 57 days (+/- 4 days)

- Follow-up period: 10 months (+/- 14 days) after the third vaccination

Overall study duration is estimated to be 22 months. The end of the study is defined as the date of the last visit performed by the last subject.

4.2.7 Assignment of subjects to treatment groups

In the Run-in phase 120 subjects will be randomized into four treatment groups to receive one of the four treatments displayed in Table 1 with the procedure described in section 4.2.9.

In the Main Study phase 450 subjects will be randomized to receive 135 µg or 180 µg VLA15 w/ alum or placebo according to the procedure described in section 4.2.9.

4.2.8 Subject Identification

At Visit 0 a 10-digit subject number will be assigned to each subject. The first five digits are the study identifier (15201 for this study). The sixth and seventh digit is the site identification number (e.g. 01). The last three digits are assigned in ascending order as the subjects are screened.

4.2.9 Randomization

Subjects will be allocated to treatment groups via the EDC system. Eligible subjects will be randomized stratified by study site 1:1:1:1 in the Run-in Phase and stratified by study site, age group and baseline *B.b.* s.l. serostatus 2:2:1 in the Main Study Phase according to a randomization list created by a statistician. Date and time of enrolment will be defined as the time point at which the subject is registered in the system and the subject is allocated to a treatment group.

4.2.10 Blinding

The study will be an observer-blinded trial, which will be conducted in a blinded manner for the study investigators, the sponsor including laboratory personnel, and the subjects. Only designated study staff who randomize subjects into treatment groups and perform preparation and application of the vaccinations will be unblinded. These unblinded study staff members will not be involved with trial conduct otherwise. An overview of persons who will be unblinded is provided below:

Unblinded:

- Designated study site staff who randomize subjects to treatment groups and are concerned with IMP handling (i.e. perform preparation and application of the study vaccine, maintain drug dispensing log detailing the dates and quantities of IMP administered to each subject). These unblinded study staff members will not be involved with trial conduct otherwise.
- CRAs responsible for monitoring of IMP handling and related data for verifying drug accountability during the study and performing overall drug accountability.
- Statistical team at the CRO performing statistical analyses for generation of safety data tables for the DSMB.
- DSMB members.

Blinded:

- Study participants

- 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 and CRO staff including laboratory personnel at the sponsor's labs for immunogenicity assessments.

Blinding process:
To ensure that study participants cannot tell the group they have been allocated to from the physical appearance and the content of the syringe, preparation of IMP must be done by unblinded staff members only in a separate room, unobserved by blinded staff members and the subject. After drawing the respective injection volume into syringes and visual check, the syringe content will be masked by covering the syringe with a white, non-see-through adhesive label wrapped around the syringe.

Unblinding during the study:
The blind must not be broken for anyone involved in the trial conduct. Unblinding of individual cases can be performed by the investigator in case of emergency and if knowledge of the treatment assignment is mandatory for emergency treatment. Designated personnel at [redacted] and a sponsor representative otherwise not involved in the study conduct may be unblinded for individual cases of SAEs that are both unexpected and suspected to have a causal relationship to the trial vaccines (SUSARs), to fulfil safety reporting requirements. Procedures are described in a safety management plan.
The study sponsor and trial statisticians will be unblinded at the time of the Interim Analysis, after the respective database snapshot has been performed.

5. INVESTIGATIONAL MEDICINAL PRODUCTS

Valneva's VLA15 Lyme borreliosis vaccine candidate is composed of three ~35 kDa fusion proteins, each containing the C-terminal part of two OspA serotypes, fused together by a 21 residues long linker and stabilized each by one disulfide bond. The proteins, designated as Lip-D1B2B (ST1 and ST2), Lip-D4Bva3B (ST4 and ST3) and Lip-D5B6B (ST5 and ST6), are attached to a lipid moiety on their N-Terminus. The putative T-cell epitope in OspA ST1 presenting homology to human leukocyte function-associated antigen-1 (hLFA-1), that has previously be claimed to induce antibiotic-refractory Lyme arthritis in a subset of naturally infected patients, has been replaced with corresponding sequence from OspA ST2.

VLA15 is formulated with aluminum hydroxide as adjuvant (Alum, VLA15 w/ Alum). PBS will be used as placebo.

5.1 Description of IMP

5.1.1 VLA15 Drug Product w/ Alum

VLA15 Drug Product w/ Alum consists of the three proteins Lip-D1B2B, Lip-D4Bva3B and Lip-D5B6B that are formulated in 1:1:1 ratio in buffer (10 mM L-Methionine, 10 mM NaH_2PO_4 dihydrate, 150 mM NaCl, 5% (w/v) Sucrose, 0.05% (v/v) Tween®20 at pH 6.7) to a concentration of 180 µg/mL total protein (i.e. 60 µg/mL for each protein).

After sterilizing filtration of the VLA15 solution, sterile aluminum hydroxide is added aseptically to a target concentration of 0.5 mg/mL aluminum. The VLA15 Drug Product w/ Alum is filled into 2R Type I Plus® glass vials (1.2 mL filling volume per vial, resulting in 1.0

mL extractable volume) closed with 13 mm injection Flurotec® stoppers secured by crimp caps.

VLA15 w/ Alum is available as a white to off-white suspension of 180 µg/mL protein in a 2 mL glass vial. VLA15 w /Alum should be stored at +2-to +8°C and a retest date of 24 months from date of production will be assigned. This retest date will be extended to 36 month with data from ongoing stability program provided no significant trends in product quality are observed.

The VLA15 w/ Alum IMP is used for vaccinations in the 90 µg w/ Alum, 135 µg w/ Alum and the 180 µg w/ Alum groups.

The dose has to be adjusted by volume as described in Section 6.1.2.

Table 8 Treatment dose with protein content, injection volumes and alum amount

Treatment	Injection Volume (mL)	Amount of Alum per injection (µg)
VLA15 90 µg w/ Alum	0.5	250
VLA15 135 µg w/ Alum	0.75	375
VLA15 180 µg w/ Alum	1.00	500

5.1.2 VLA Placebo

The VLA Placebo is a PBS buffer based on Dulbecco's PBS media formulation without Calcium and Magnesium.

The VLA Placebo is filtered and filled in sterile 2R glass vials under constant stirring. The filling volume is 1.2 mL, ensuring an extractable volume of 1.0 mL. The vials are 2R Type I Plus® glass vials closed with 13 mm injection Flurotec® secured by aluminum crimp caps.

Only excipients of non-human and/or non-animal origin are used for VLA Placebo formulation. Storage of VLA Placebo should be done at +2-8°C.

For the time being, a retest date of 24 months will be assigned for VLA Placebo, based on stability data of previous VLA Placebo batches. This retest date will be extended to 36 month with data from ongoing stability program provided no significant trends in product quality are observed.

5.2 Packaging & labelling of IMP

Packaging and labeling of both IMPs (VLA15 w/ Alum and Placebo) is performed by [redacted]. Labels will be written in accordance to local law.

5.3 Condition of storage of IMP

VLA15 w/ Alum and Placebo will be stored in a refrigerator at **+2°C to +8°C (+35° to +46°F)** in a room not accessible to unauthorized persons. Temperature monitoring systems will be used.

DO NOT FREEZE THE VACCINE!

6. TREATMENT OF SUBJECTS

6.1 Investigational Treatment

6.1.1 Dose and dosing schedule

VLA15 will be administered at three different doses: 90 µg w/ Alum, 135 µg w/ Alum, and 180 µg w/ Alum. Placebo will be administered as a 1 mL injection. Each subject will receive three I.M. vaccinations on Day 1, 29 and 57.

Dose will be adjusted by volume (see Table 1).

Table 1 Treatment Groups and Vaccinations

Group	Treatment	Injection Volume (mL)	Days of Vaccination
90 µg	VLA15 90 µg w/ alum	0.50	1, 29, 57
135 µg	VLA15 135 µg w/ alum	0.75	1, 29, 57
180 µg	VLA15 180 µg w/ alum	1.00	1, 29, 57
Placebo	PBS	1.00	1, 29, 57

6.1.2 Preparation and method of administration

All IMPs are filled in glass vials with a minimum extractable volume of 1.0 mL.

Remove the vial from the refrigerator. Invert vial at least three times to provide a homogenous turbid suspension (VLA15 w/ Alum) before drawing the respective injection volume into the syringe. Refer to Table 1 for volumes to be drawn. DO NOT SHAKE!

Preparation of IMP needs to be done by designated unblinded staff members only in a separate room unobserved by the subject and blinded study staff. A second unblinded staff performs a check on volume and IMP/ Placebo (4 eye-principle). After drawing the respective injection volume into a syringe and visual check, the syringe will be masked by covering it with a non-see-through adhesive label so that subject and blinded study staff cannot detect content of the syringe. Identification of the syringe is guaranteed by placing a tear-off label containing Kit number and Subject number onto the label.

Administration should take place shortly after the preparation of the syringe, a maximum time period of one hour for removal of the vial from the fridge to administration has to be observed. Just prior to administration invert the syringe at least three times again to ensure a homogenous suspension (be aware that Alum will settle down within short time).

Subjects will receive the injections of VLA15 or Placebo I.M. in the deltoid region of the non-dominant arm. In case of ongoing local AEs from previous vaccination at the respective injection site, vaccination in the contra-lateral arm should be administered.

A study specific IMP manual with further details on IMP handling will be provided.

Dose will be adjusted by volume:

The allocated **dose** of the respective treatment of VLA15 **will be adjusted by volume** as follows:

- Subjects allocated to the **VLA15 w/ Alum 90 µg group** will receive **0.50 mL (500 µl)** of VLA15 w/ Alum I.M. in the deltoid region of the upper non-dominant arm.
- Subjects allocated to the **VLA15 w/ Alum 135 µg group** will receive **0.75 mL (750 µl)** of VLA15 w/ Alum I.M. in the deltoid region of the upper non-dominant arm.
- Subjects allocated to the **VLA15 w/ Alum 180 µg group** will receive **1.00 mL (1000 µl)** of VLA15 w/ Alum I.M. in the deltoid region of the upper non-dominant arm.
- Subjects allocated to the **Placebo group** receive **1.00 mL (1000 µl)** of Placebo (PBS) I.M. in the deltoid region of the upper non-dominant arm.

6.1.3 Treatment duration

Each subject will be vaccinated within a period of approximately 57 days (vaccinations on Days 1, 29 and 57).

6.2 Prior and Concomitant Therapy

6.2.1 Permitted prior and concomitant therapy

Any vaccination within the last three years prior to Visit 0, and any medication within three months prior to Visit 0 have to be documented in the respective section of the eCRF.

Any medication taken or vaccination received during the study has to be documented in the eCRF.

6.2.2 Forbidden prior and concomitant therapy

The following prior and concomitant therapy is not allowed:

- Treatment for Lyme borreliosis (LB) within the last three months prior to Visit 0.
- Previous vaccination against LB with any (investigational) vaccine.
- Administration of anticoagulants within the three weeks prior to the first vaccination (Visit 0, Day 1) or until Visit 3 (Day 57), contraindicating I.M. vaccination as judged by the investigator.
- Administration of any other non-registered medicinal product within 28 days prior to VLA15 vaccination at Visit 1 (Day 1) and throughout the entire study period or administration of any registered medicinal product in another clinical trial within 28 days prior to VLA15 vaccination at Visit 1 (Day 1) and up to Day 85.
- Administration of any active or passive immunization 28 days before first VLA15 vaccination at Visit 1 (Day 1) and up to Day 85 (i.e. 4 weeks after the last VLA15 immunization); except for influenza (seasonal or pandemic) and pneumococcal vaccines which may be administered outside a 7-days interval before or after any trial vaccination.
- Administration of chronic (longer than 14 consecutive days) prednisone or equivalent ≥0.05 mg/kg/day. Topical and inhaled steroids are allowed.

- Administration of any blood or blood-derived product within 90 days before Visit 0 (Screening Visit) and during the entire study period.

Subjects are to be asked about concomitant medication and vaccinations at each visit; any concomitant medication or vaccination has to be documented.

All forbidden concomitant medications are reflected in exclusion criteria.

6.3 Treatment Compliance

6.3.1 Drug dispensing and accountability

A drug shipment log will be kept current by the site, detailing the date and quantity of IMP received from and returned to the sponsor. Moreover a current drug dispensing log has to be maintained by designated unblinded staff, detailing the dates and injection volume of IMP administered to each subject. This documentation will be available to the designated unblinded CRA to verify drug accountability during the study and to perform overall drug accountability.

Any unused IMP and used vials will be accounted for and returned to the sponsor.

6.4 Pregnancy testing and birth control

Women of childbearing potential with a negative pregnancy test and the use of adequate birth control before (as defined below) and during conduct of the study are eligible for inclusion into the study. A woman is considered of childbearing potential if fertile, following menarche and until becoming post-menopausal unless permanently sterile. Women of childbearing potential must have a negative serum pregnancy test at Visit 0 (Screening Visit), a negative urine pregnancy test before each vaccination and should be practicing an acceptable method of birth control. Urine pregnancy tests will be conducted in women of childbearing potential at each study visit and at the ET Visit (if applicable).

Women of childbearing potential are required to practice birth control throughout the entire study period. An acceptable method of birth control is defined as those, which result in a low failure rate (i.e. less than 1 % per year) when used consistently and correctly. Such methods include combined (estrogen and progesterone containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal or transdermal), progesterone-only hormonal contraception associated with inhibition of ovulation (oral, injectable or implantable), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), vasectomized partner, sexual abstinence or same sex relationships. Hormonal contraception associated with inhibition of ovulation need to be in place at least for 30 days prior to Visit 1.

Women without childbearing potential do not need to perform any birth control. A women is considered of non-childbearing potential, if she is surgically sterilized for at least 3 months prior to Visit 1 (permanent sterilization methods include hysterectomy, bilateral salpingectomy or bilateral oophorectomy [23], transcervical sterilization (Essure and Adiana procedures), tubal ligation, or being postmenopausal for at least one year prior to the study start as confirmed by a gynecologist. For reporting of pregnancies refer to section 8.4.5.

7. ASSESSMENT OF IMMUNOGENICITY

7.1 Immunogenicity Measurements

Immunogenicity assessments measuring OspA serotype specific by IgG ELISA will be performed on samples collected at Visits 1-7.

All steps of the assays will be performed at Valneva Austria GmbH Vienna, Department of Clinical Serology according to Standard Operating Procedures (SOPs). Work will be performed in an environment that is certified to Biological Safety Level 2 (BSL2), internally audited, and conform to GCLP requirements. Raw data will be stored on a separate, secure server in a defined Information Technology environment complying with regulatory standards. Raw data print outs are stored in respective study binders.

Details on the analysis of immunogenicity assessments described below will be provided in the Statistical Analysis Plan (SAP).

7.1.1 OspA serotype specific IgG antibodies (ELISA)

For the evaluation of immunogenicity, human sera will be analyzed for IgG against each OspA serotype (ST1 to ST6) separately by ELISA. Dilution series of sera will be added to microtiter plates coated with full length OspA ST1/ST2/ST3/ST4/ST5 or ST6. Presence of binding IgGs will be detected with an anti-human IgG enzyme conjugate followed by addition of the substrate. The optical density of the colored end product is proportional to the amount of protein specific IgG present in the serum that can be quantified on the basis of the Reference Substance curve by four-parameter logistic fit and parallel line analysis. As this Phase 2 study is intended to define the dose for Phase 3, the assay will be validated and will be filed to the IND prior to testing.

7.1.2 Supportive functional assays

[redacted]

8. ASSESSMENT OF SAFETY

Please refer to Table 2 for the Run-in phase and Table 3 for the Main Study phase (Tables of Events) for exact time points.

AE occurrence rates/ frequencies will be used to evaluate the secondary study objective on safety.

Solicited AEs will be recorded as detailed in section 8.3. Unsolicited AEs, SAEs and AESIs will be recorded and reported as detailed in section 8.4.

The following safety measures will be taken:

8.1 Physical Examination

At Visit 0 all subjects will undergo a physical examination on the following body systems: general appearance, skin, head/ eyes /ears/ nose/ throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, and neurological system.

A symptom-driven physical examination will be performed at all study visits except the Screening Visit (Visit 0), i.e. only in case a symptom is reported by the subject, a system-based assessment will be performed for a detailed check of the affected body system(s). A symptom-driven examination should also be performed in case the subject has complaints within the observation time after vaccination.

Any symptom reported, including worsening of existing conditions, will be recorded as an AE unless it occurred prior to vaccination at Visit 1. Symptoms noted during the symptom-driven physical examination at Visit 1 (prior to vaccination) are not considered AEs but are recorded as medical history.

8.2 Vital Signs, ECG and Oral Body Temperature

Vital signs (i.e. systolic and diastolic blood pressure, pulse rate) and oral body temperature will be measured at Visits 0, 1, 2, and 3. Vital signs will be recorded in a seated position and at rest. At Visit 1, 2 and 3, vital signs and oral body temperature will be measured prior to vaccination. An ECG will be measured at Visit 0.

8.3 Solicited AEs and Subject Diary

Subject diaries will be distributed after each vaccination and cover the first seven days after each vaccination (starting on the day of vaccination).

Solicited AEs are listed (predefined) in the Subject Diaries and comprise reactions at the injection site or systemic reactions typical for vaccinations. Solicited AEs are per definition regarded as related to IMP.

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), flu-like symptoms, nausea, vomiting and fatigue.

In case of an emergency, the study participants will be given a 24 h telephone number they can call to receive instruction and information from study staff. In case of severe local and systemic reactions, the study physician should be contacted outside from scheduled study visits.

8.3.1 Collection, assessment and documentation of solicited AEs

At each vaccination visit, a Subject Diary will be distributed and explained to the subject. Subjects will be trained to assess presence of solicited AEs and impact on daily activities; to measure the size of the affected area with a measuring tape (for symptoms induration/ hardening, swelling and erythema/ redness), and instructed to take oral temperature once every day. Unsolicited AEs may be entered into a free text field in the Subject Diary to aid memorizing the AEs.

Assessments will start on the day of vaccination and take place once daily for a total of seven consecutive days. Assessments of oral temperature should occur preferably in the late afternoon; if a subject develops fever and oral temperature is measured more than once per day, the maximum temperature of the day should be noted. For other symptoms the maximum severity observed on a given day should be recorded. The subject will note down information about the symptom by ticking the appropriate description on a list. Additionally, the following information will be collected: symptoms present after Day 6 (yes/ no), last day of symptoms (date).

Subjects should report severe solicited AEs or other severe symptoms immediately by telephone as instructed in the Subject Diary.

The Subject Diary will be verified by a clinician together with the subject at the subject's next visit to the study site. The investigator should enquire whether any solicited AE required medical attention (i.e. subject was seeking medical care at a doctor's office, emergency service, or hospital, but not use of self-medication) or if an event was an SAE. The investigator must not make any changes or revisions to the Subject Diary at any time. The investigator should not suggest answers when performing the diary verification with the subject but can question and call the subject's attention to obviously wrong and misleading entries. If the subject comes to the conclusion that a Subject Diary question had been misunderstood or that an entry was made by mistake, the subject has the opportunity to make corrections to the best of his knowledge. Retrospective changes after the site visit or after the Subject Diary data have been entered into the eCRF are not permitted.

After Subject Diary verification, the clinician will perform a severity assessment on the basis of the information provided by the subject in the Subject Diary. The severity assessment will be based on the grading scale in Table 9 below. Severity categories in this table are NOT identical to the categories provided in the Subject Diary. The investigator or authorized delegate will enter diary data and his severity assessment into the eCRF.

For solicited AEs which are serious and/ or medically attended, the investigator will carry out more detailed assessments as performed for unsolicited AEs (causality, outcome, action taken) and will document them in the eCRF.

For solicited local and systemic AEs persisting beyond Day 6 after vaccination, stop date will also be documented in the eCRF.

Any unsolicited AE documented on the Subject Diary should be documented and assessed as outlined in section 8.4.

8.3.2 Severity of solicited AEs

Severity grading of solicited AEs by the investigator will be performed according to Table 9 below. Criteria are based on the FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Subjects Enrolled in Preventive Vaccine Clinical Trials (2007), where possible.

Table 9 Grading of Solicited Adverse Events

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life threatening (Grade 4)
Local Reactions				
Pain	Does not interfere with activity	Interferes with activity or repeated use of non-narcotic pain reliever >24 hours	Prevents daily activity or any use of narcotic pain reliever	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Induration/ Hardening[1/2]	2.5–5 cm (0.98–1.96 inch) and does not interfere with activity	5.1–10 cm (1.97 – 3.94 inch) or interfere with activity	>10 cm (>3.94 inch) or prevents daily activity	Necrosis
Swelling[1/2]	2.5–5 cm (0.98–1.96 inch) and does not interfere with activity	5.1–10 cm (1.97 – 3.94 inch) or interfere with activity	>10 cm (>3.94 inch) or prevents daily activity	Necrosis
Erythema/ Redness[1]	2.5–5 cm 0.98–1.96 inch	5.1–10 cm 1.97–3.94 inch	>10 cm >3.94 inch	Necrosis or exfoliate dermatitis

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life threatening (Grade 4)
Systemic Reactions				
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Arthralgia[3]	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Fever[4] **(°C)/ (°F)**	38.0–38.4/ 100.4–101.1	38.5–38.9/ 101.2–102.0	39.0–40/ 102.1–104	>40/ >104

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life threatening (Grade 4)
Flu-like symptoms[3]	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Nausea	No interference with activity or 1–2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Vomiting	No interference with activity or 1–2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

[1] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable;
[2] Induration/ hardening and swelling should be evaluated and graded using the functional scale as well as the actual measurement;
[3] Symptom not described in the FDA guidelines.
[4] Oral temperature; no recent hot or cold beverages or smoking.

8.4 Unsolicited AEs

An AE is any untoward medical occurrence in a subject administered an investigational product, whether or not related to this treatment. All new abnormalities or any exacerbation in intensity or frequency (worsening) of a pre-existing condition during or after the first vaccination have to be documented as AEs.

Study participants will be given a 24 h telephone number they can call to receive instruction and information from study staff in case of emergency. In the event of severe local and systemic reactions, the study physician should be contacted immediately, outside from scheduled study visits.

Unsolicited local and systemic AEs are defined as follows:

- Any solicited local or systemic AE if it has an onset date more than 6 days after vaccination.
- Any other symptom or untoward medical event.
- Any abnormal laboratory assessment that is clinically relevant (in the opinion of the investigator)

8.4.1 Collection, assessment and documentation of unsolicited AEs

The Subject Diary contains a section where subjects can enter unsolicited AEs. Additionally, the investigator should enquire about unsolicited AEs during each study visit.

Clinically relevant laboratory parameter changes constitute unsolicited AEs, too, unless they are considered a symptom of an underlying AE or part of a syndrome that is reported as AE (e.g. presence of blood cells in urine in a person diagnosed with urinary tract infection). In addition, symptoms noted during the symptom-driven physical exams (unless already

covered by respective solicited AE) constitute unsolicited AEs. At study Visits 1b, 4, 5 and 6, when no vaccination is given and hence no Subject Diary is issued, Memory Aids will be handed out to subjects to note down any either ongoing or new AEs and dates of onset/ resolution, whether medication was taken in response and whether medical advice was sought.

The investigator will follow-up each AE until it is resolved or until the medical condition of the subject is stable. All relevant follow-up information will be reported to the sponsor until the end of the study for the subject. SAEs ongoing at the time of Visit 7 (or ET Visit) will be followed until resolution or achievement of stable clinical conditions, latest until the global end of the study.

Unsolicited AEs need to be assessed for causality and graded for severity by the investigator, using a grading of mild, moderate, severe or life-threatening. For the severity grading of unsolicited AEs, see section 8.4.2.4.

All unsolicited AEs need to be documented in the respective AE section of the eCRF during every study visit (Visit 1-7, or ET visit if applicable), regardless of their source (e.g. open question to subject, lab parameters from Visit 2-7, symptom-driven physical examination, unsolicited AEs noted in the Subject Diary/ Memory Aid).

Any systemic symptom is regarded as separate AE. However, if the investigator considers several systemic symptoms to be in the context of one underlying diagnosis, he may merge them into one single appropriate AE. The AE term entered into the electronic case report form (eCRF) should contain all symptoms summarized to one event (e.g. "Influenza with flu-like-symptoms, fever and headache").

The following information will be documented for each unsolicited AE: Causality, Severity, Outcome, Seriousness, Medically-attended, Action Taken to Treat AE, Action Taken on IMP Start and Stop Dates.

8.4.2 Evaluation of unsolicited AEs

8.4.2.1 Definition of medically-attended AEs

All adverse events where subjects are seeking medical care (i.e. doctor's office, emergency service, hospital, but not use of self-medication).

8.4.2.2 Definition of Serious Adverse Events (SAEs)

A serious AE (SAE) is any untoward medical occurrence that at any dose:

- Results in death.
- Is life-threatening.
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Is a congenital anomaly/birth defect.
- Is another medically important condition.

This definition also applies to progression of disease leading to a serious outcome.

Neither the condition, leading to a hospitalization or prolonged hospitalization, nor the medical procedure itself need to be reported as a serious adverse event in the following circumstance: Hospitalization or prolonged hospitalization for diagnostic or elective medical procedures planned prior to first vaccination to treat a pre-existing condition which did not change in severity.

In this case the underlying diagnosis or condition should be reported in the medical history section of the eCRF. The corresponding medical procedure should be documented as a comment to the underlying diagnosis or condition in the medical history section of the eCRF.

The sponsor will classify the SAEs as either expected or unexpected.

Expected: An AE that is listed in the current Investigator's Brochure.

Unexpected: An AE that is not listed in the current Investigator's Brochure, or it differs because of greater severity or greater specificity.

8.4.2.3 Definition of Adverse Events of Special Interest (AESIs)

An adverse event of special interest (serious or non-serious) is one of scientific and medical concern specific to the sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate. Such an event might warrant further investigation in order to characterize and understand it [24].

8.4.2.4 Collection and evaluation of Adverse Events of Special Interest (AESIs)

Subjects will be carefully monitored for development of AESIs. Since a previous LB vaccine was accused of inducing auto-immune symptoms similar to those caused by disseminated LB infection, e.g. autoimmune arthritis, such events will constitute AESIs. In addition, the onset of any potentially autoimmune or neuro-inflammatory disorders will constitute AESIs. A subunit vaccine like VLA15 is not considered capable of inducing LB as such. Nevertheless, any potential LB cases are of relevance to development of the vaccine and will therefore receive particular attention and be captured as AESIs as well. Therefore, symptoms suggesting a LB-associated event and/ or onset of potentially autoimmune or neuro-inflammatory disorders will receive special attention. Identification of such events from a pre-defined list of AESIs and symptoms suggesting a Borrelia infection will be assessed in a guided approach as described below.

The following symptoms will receive particular consideration:

- Expanding red or bluish-red patch (≥5 cm in diameter) with or without central clearing;
- Symptoms suggesting an arthritis (e.g. recurrent attacks or persisting objective joint swelling (synovitis) in one or a few large joints);
- Neurological symptoms (e.g. meningo-radiculitis, meningitis, encephalitis, myelitis, cerebral vasculitis, facial palsy);
- Cardiac symptoms (e.g. atrio-ventricular conduction disturbances, rhythm distrurbances, myocarditis);
- Immune-mediated disorders as proposed by FDA for previous clinical programs (please refer to APPENDIX 1).

As part of unsolicited AE assessments, at study Visits 1-7 and ET, if applicable, the investigator will be guided through a scripted safety assessment (i.e. questionnaire) to enquire about symptoms that are consistent with Lyme borreliosis, allowing the investigator to assess whether there is a clinical suspicion for infection with Borrelia or a LB-associated event. In addition, presence of or symptoms suggesting one of the other AESIs from the pre-defined list will be determined by the investigator.

In case there is clinical suspicion for Lyme borreliosis or a LB-associated event, investigators are advised to perform a clinical workup as described in Appendix 2, including specialist referral as needed. Subjects with suspected other AESIs (i.e. immune-mediated disorders)

should also be referred to a respective clinical expert for full diagnostic work-up as needed. Retrospective investigation of a pre-vaccination sample may be considered for clinical work-up. The investigator will request the medical records from the clinical expert, if applicable. In case an AESI is identified (by the investigator or a clinical specialist upon referral or without referral) the investigator will fill out the AESI Report Form with all available information, including information provided by the clinical expert, if applicable, and will provide the AESI Report Form together with the medical records to the DSMB through the study's Safety Desk. For cases of Lyme borreliosis or LB-associated events, the DSMB will confirm the diagnosis. In case an AESI (LB or immune-mediated disorders as depicted in the pre-defined list) has already been diagnosed by a healthcare specialist prior to identification of a potential AESI by the investigator at the study visit, the investigator will also provide the AESI Report Form together with available medical records to the DSMB through the study's Safety Desk. In addition, the DSMB will regularly review accruing AEs and can recommend to the investigator specialist work-up as needed for any case they consider potential AESIs or cases of LB. The DSMB will do a final adjudication of all AESIs and will assess whether cases were new in onset and whether there is any relationship to application of the study vaccine. Narratives with detailed case descriptions will be provided for all AESIs.

The AESI Report Form should be reported by the investigator by fax or email to [redacted]

[redacted]

8.4.2.5 Severity of unsolicited AEs

In general, for AEs mild (Grade 1), moderate (Grade 2), severe (Grade 3) and potentially life threatening (Grade 4) are defined as follows:

Mild: Awareness of signs or symptoms, but easily tolerated, does not interfere with daily activities.

Moderate: Discomfort enough to interfere with usual activity but not requiring medical intervention.

Severe: Incapable of work/ usual activity and requiring medical intervention.

Potentially life threatening: Occurrence places the patient or subject at immediate risk of death.

For a standardized approach, the NCI-CTCAE v 4.03, 2010 using Grade 1 = mild, Grade 2 = moderate, Grade 3 = severe and Grade 4 = potentially life threatening will be used for AEs, which do not readily fall into the mild/ moderate/ severe/ potentially life threatening categories described above.

8.4.2.6 Causality assessment

Probable: A reaction that follows a reasonable temporal sequence from administration of the investigational medicinal product; or that follows a known or expected response pattern to the suspected treatment; or that is confirmed by stopping or reducing the dosage of the treatment; and that could not reasonably be explained by known characteristics of the subject's clinical state.

Possible: A reaction that follows a reasonable temporal sequence from administration of the investigational medicinal product; that follows a known or expected response pattern to the suspected treatment; but that could readily have been produced by a number of other factors.

Unlikely: Reports not following a reasonable temporal sequence from administration of the investigational medicinal product; an event, which may have been produced by the subject's clinical state or by other environmental factors.

Not related (unrelated): Events for which sufficient information exists to conclude that the etiology is unrelated to the investigational medicinal product.

AEs with a causality reported as probable or possible will be considered related to study drug. AEs with missing causality assessment will be regarded as related unless further specified. All other AEs will be considered as not related to study drug.

8.4.2.7 Outcome

- recovered/ resolved
- recovered/ resolved with sequelae
- not recovered/ not resolved
- fatal
- unknown

NOTE: A subject's death per se is not an event, but an outcome. The event which resulted in the subject's death must be fully documented and reported, regardless of being considered treatment-related or not.

8.4.2.8 Actions taken

AEs requiring therapy must be treated with recognized standards of medical care to protect the health and well-being of the subject. Appropriate resuscitation equipment and medicines must be available to ensure the best possible treatment of an emergency situation.

The investigator must be adequately trained in the treatment of allergic reactions including the proper use of rescue medication. The facility has to be equipped with an emergency set that is readily available.

The treatment of severe allergic reactions involves prompt treatment with oxygen, antihistamines, prednisolone, epinephrine and theophylline as required. An appropriately sized intravenous line will be available to ensure fast infusion of colloid volume substitution.

In the case of a severe anaphylactic reaction subjects will promptly be transferred to the intensive care unit of a nearby hospital.

The action taken by the investigator must be documented:

a) in general	**b) on the investigational product**
None	Not applicable
Drug therapy started	None
Diagnostic test performed (e.g. laboratory)	Delay of further vaccination
Medical procedure started (e.g. surgery)	Second dose not administered
Unknown	Third dose not administered
Withdrawn from study	

8.4.3 Timeframe for reporting of unsolicited AEs

All AEs occurring during the study after administration of the first vaccination must be recorded in the eCRF. SAEs must be reported to the sponsor in expedited manner as outlined below.

8.4.4 SAE reporting procedures

Correct SAE reporting will have to cite a diagnosis or a symptom. Any diagnosis and any symptom is regarded as separate SAE. However, if the investigator considers several symptoms to be in the context of one underlying diagnosis, he may specify the diagnosis as the reportable SAE and describe the attendant symptoms in one single appropriate SAE report.

Medical or diagnostic procedures due to an underlying disease or symptom are not considered an AE but a consequent measure following an AE. A correct SAE report will therefore have to specify the disease or symptom as the reportable AE and the medical or diagnostic procedure as action taken.

The investigator must report immediately after discovery all SAEs that are:

- Fatal
- Life-threatening
- Suspected to be related to study treatment

Regardless of the description above, any SAE should be reported by fax or email within 24 hours after the investigator has become aware of it to [redacted] Under certain circumstances the initial notification could be done by phone, but nevertheless a written SAE Report Form has to be submitted within 24 hours to:

[redacted]

In addition, expedited and periodic reporting to Competent Authorities and IRBs/ IECs will be performed in accordance with local requirements. Further reporting details can be found in the study-specific SAE procedure which is in accordance with respective US/ EU requirements, International Conference on harmonization (ICH) GCP, national laws and site-specific requirements. SAEs that are considered as probably or possibly related and additionally are unexpected need to be reported according to the requirements for suspected unexpected serious adverse reactions (SUSARs).

SAE reports will be reviewed by a study site's physician and by [redacted] the Medical Monitor, Valneva Austria GmbH and the independent DSMB.

8.4.5 Pregnancy reporting procedures

Women must not become pregnant during the entire clinical study period. If a subject becomes pregnant during the study, she must immediately inform the investigator. No further study vaccinations must be administered and the subject should attend all remaining visits as planned.

Reporting requirements start with the first vaccination until Visit 7 (or ET Visit, if applicable). All pregnancies will be followed up for three months after delivery or termination of the pregnancy. Any effect on either mother or fetus should be determined. A pregnancy which led to a congenital anomaly/ birth defect must be followed-up by the investigator longer or until resolution which will be decided on individual basis and in accordance with the sponsor.

The investigator should report pregnancies to [redacted] within 24 hours of being notified using the Pregnancy Report Form. Reporting procedures are similar to SAE reporting procedures (contacts and processing), although a pregnancy is not considered an SAE.

If a seriousness criterion applies in addition to the pregnancy (e.g. hospitalization, congenital anomaly/birth defect) the pregnancy qualifies as an SAE. In such case a Pregnancy Report Form and an SAE Report Form have to be filled out.

8.5 Laboratory Parameters

The following laboratory parameters will be assessed at time points specified in the Table of Events (Table 2 and Table 3). Parameters will be analyzed by local laboratories according to the applicable laboratory SOP:

- HIV test: A positive HIV test obtained by ELISA will have to be confirmed by a second method [e.g. Western blotting or PCR], at Visit 0 only.
- Baseline Serology: a baseline serology sample will be taken at the screening visit and might be used for work-up of suspected LB-associated, autoimmune or neuroinflammatory events (e.g. analysis of Rheumatoid factor (RF) and/or anti citrullinated protein antibodies (ACPA), as appropriate).
- Serum pregnancy test (in women of childbearing potential, refer to section 6.4 for consideration of women as being of childbearing potential).
- Urine pregnancy test (in women of childbearing potential, refer to section 6.4 for consideration of women as being of childbearing potential).
- Clinical Chemistry: Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, C-reactive protein (CRP).
- Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
- Coagulation: Prothrombin time, aPTT, fibrinogen.
- Urinalysis: a standard urine dipstick for determining pH-Value, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.

The following laboratory parameter will be assessed at time points specified in the Table of Events (Table 2 and Table 3) and will be analyzed by a local laboratory or a central laboratory as specified by the sponsor:

- *Borrelia burgdorferi* s.l. screening by commercially available C6 ELISA assay (VlsE ELISA), at Visit 0, Visit 7 and ET, if applicable, and in cases where subject informs at a regular visit that it will discontinue the study after this visit. Serum samples that are tested positive will be verified by a confirmatory immunoblot.

An amount of 3.5 mL will be withdrawn for the HIV screening sample and a blood sample of 4.0 mL will be taken for the *B.b.* s.l. screening test. For the baseline serology sample, 5.0 mL will be taken, and 3.5 mL will be taken for the serum pregnancy test. An amount of 8.5 mL will be taken for clinical chemistry testing, 4.0 mL for hematology testing and further 4.5 mL for testing of coagulation factors at screening. The maximum total blood volume including blood samples for immunogenicity testing is 70.5 mL (Visit 7) (as depicted in Table 2 and Table 3).

- All laboratory assessments and the clinical relevance of abnormal values will be documented in the eCRF.
- Abnormal laboratory assessments that are clinically relevant (in the opinion of the investigator) need to be documented as unsolicited AEs and assessed further for severity according to Table 10, for causality and other assessments done for unsolicited AE. Laboratory assessments, for which no grading is described in Table 10, are graded as described in section 8.4.2.4.

- Abnormal laboratory assessments that are considered a symptom of an underlying AE or part of a syndrome that is reported as AE (e.g. presence of blood cells in urine in a person diagnosed with urinary tract infection) do NOT additionally need to be documented as unsolicited AE, but a respective comment should be added to that AE.
- For statistical analysis, laboratory assessments will be graded according to the grading scale provided in Table 10. The grading scale is based on the FDA Guidance for Industry, Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (September 2007), where applicable.

Table 10 Grading Scale for Abnormal Laboratory Assessments

	Mild (Grade 1)[1]	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially life threatening (Grade 4)[2]**
Hematology Parameters				
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	<8.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	<8.5
Hematocrit	Outside normal range[3]			
Erythrocyte count	Outside normal range[3]			
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	>25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	<1,000
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	<500
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	<25,000

	Mild (Grade 1)[1]	Moderate (Grade 2)	Severe (Grade 3)	Potentially life threatening (Grade 4)[2]
Clinical Chemistry Parameters				
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	>2.5 or requires dialysis
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	<125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	>150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – /Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	<3.1
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	<7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	>12.0
AST – increase by factor	1.1 – 2.5 x ULN[4]	2.6 – 5.0 x ULN	5.1 – 10 x ULN	>10 x ULN
ALT – increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	>10 x ULN
Alkaline phosphatase – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	>10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	>1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
CRP	Outside normal range[3]			

[1] In case local laboratory normal ranges and absolute Grade 1 limits overlap, Grade 1 limits will prevail, i.e. the value will be classified as Grade 1 abnormality even if it is within local laboratory normal ranges. Values between the local laboratory normal ranges and absolute Grade 1 limits will be reported as no abnormality (Grade 0).
[2] The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia unsolicited AE if the subject had a new seizure associated with the low sodium value.
[3] As the FDA Scale does not provide any grading for Hematocrit, Erythrocyte count and CRP, these will only be analyzed as "outside normal range", as determined by local laboratory standards without further differentiation.
[4] "ULN" is the upper limit of the normal range

8.6 Safety Monitoring

8.6.1 Data Safety Monitoring Board (DSMB) and initiation of the Main Study phase

An independent DSMB, which will include Lyme borreliosis experts (i.e. a general practitioner and expert in LB diagnosis, a rheumatologist and a neurologist who are LB experts) will be

installed to review accruing safety information, and if necessary, to determine whether study or individual subject stopping rules are met. The DSMB will ad hoc review all cases of SAEs. In addition, the DSMB will confirm diagnosis of potential LB cases based on medical records received from the investigator after clinical workup of potential LB cases according to a standardized procedure as described in [Appendix 2]. The DSMB will do a final adjudication of all AESIs and will assess whether cases were new in onset and whether there is any relationship to application of the study vaccine. During vaccination periods (i.e. Day 1 to Day 57 in the Run-in phase and in the Main Study phase), the DSMB will periodically review listings of SAEs, deaths, AESIs, medically attended AEs, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination. In addition, DSMB meetings will be scheduled. Please refer to the written DSMB charter including a detailed description of DSMB set-up and processes.

After all subjects enrolled in the Run-in phase have completed Visit 4 (Day 85, i.e. 28 days after the third vaccination), the DSMB reviewed all available safety data during a scheduled DSMB meeting. The DSMBgave the recommendation that all treatment groups were safe and well tolerated so far and could be continued in the Main Study phase based on available safety data. . Based on this DSMB review, the two higher dose groups (135 µg and 180 µg w/ alum) will be advanced into the full study and enrolment of subjects into the Main Study phase will be initiated. The investigator, the IRB/EC and competent authority will receive the DSMB recommendation letter after dose decision has been taken.

The DSMB will review the following data:

- Any case reports of SAEs will be reviewed on an ad-hoc basis.
- During vaccination periods (i.e. Day 1 to Day 57 in the Run-in phase and in the Main Study phase), the DSMB will periodically review listings and summary tabulations of SAEs, AESIs, deaths, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccinations, e.g. bi-weekly, until the last subject reached Day 57. Schedules may be adjusted as described in the DSMB charter.
- At periodic scheduled meetings, the DSMB will review listings and summary tabulations of SAEs, AESIs, deaths, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination. Meetings will be scheduled e.g. monthly during vaccination periods until the last subject has reached Day 57. Schedules may be adjusted as described in the DSMB charter.
- Ad-hoc DSMB reviews will be initiated if at any time during study conduct enrolment is interrupted by a principal investigator, the sponsor or the medical monitor at the CRO for any safety reasons, or if a pre-specified study stopping rule applies, as described in section 14.2.

The DSMB will review available safety data and will make recommendations to the sponsor regarding further conduct of the study, further vaccinations in the study and/or protocol modifications to be installed for safety reasons.

8.6.2 Sponsor

Until the last subject reached Day 57, available safety data will be reviewed by the sponsor on a regular basis to identify any potential safety concerns and applicability of study stopping rule as described in section 14.2.

8.6.3 Investigator

To ensure information exchange on safety across sites during recruitment and treatment phases, investigators will be provided safety listings once a week until the last subject

reached Day 57. These listings present information on all grade 3 and 4 solicited and unsolicited adverse events reported in the safety database.

9. STATISTICAL METHODS AND SAMPLE SIZE

9.1 General Aspects

The data will be analyzed by Assign Data Management and Biostatistics GmbH. A SAP will be provided describing in more detail, how the study results will be evaluated.

Data will be summarized by treatment group and, where appropriate, by visit and age group. Descriptive statistics (number of observations, mean, standard deviation, minimum, median, and maximum) will be provided for continuous variables (e.g. age and weight). Frequency counts and percentages will be presented for categorical variables (e.g. gender).

All data exclusions, including premature terminations, will be detailed and tabulated. Data listings will include enrolled subjects.

The analyses of baseline characteristics including demographic variables, medical and vaccination history and concomitant medications will be subject to descriptive analyses.

AEs and medical history will be coded using the MedDRA coding dictionary. Concomitant medications (including vaccinations) will be coded using the WHO Drug Dictionary.

More detailed criteria to identify subjects in each analysis population, other research questions of interest not covered in this protocol, the definition of endpoints and details of their calculation, as well as how to deal with missing, unused and spurious data will be covered in the SAP. Generally, missing values of immunogenicity variables will not be imputed, and the analysis will be limited to observed values. For missing data in AE evaluation (e.g. severity information) a worst case approach will be applied. If a change of the planned analyses is considered necessary after protocol finalization, this will be described and justified in the SAP. If a change is made after the final statistical analysis has been performed, this will be described and justified in the CSR.

9.2 Analysis Populations

Statistical analyses will pool subjects from both run-in and main study phase.

Safety Population

The safety population includes all subjects who entered into the study and received at least one vaccination. The safety population will be used for all safety and tolerability analyses including demographic data, local/systemic tolerability, laboratory data, (S)AEs and AESIs. All analysis based on the safety population will be carried out using the actual treatment received.

Per-Protocol (PP) Population

The PP population will exclude an enrolled subject if one of the following criteria is met (further criteria may be defined in the SAP):

- Subjects with less than three vaccinations (Day 1, 29 and 57).
- Subjects who received the wrong study medication.
- Subjects who fulfilled exclusion criteria 2, 8, 9, 14.

These criteria for potential protocol violations are identified at the time of planning the study. However, during the course of the trial unforeseen events may occur or new scientific knowledge may become available, therefore final decisions on all protocol violations will be made on a case by case decision in a data review meeting. The PP population serves as primary analysis population for immunogenicity analysis.

Modified Intent-to-Treat (mITT) Population

The mITT population is defined to include all subjects enrolled who received at least one vaccination. Subjects will be analyzed according to the treatment group they had been allocated to, rather than by the actual treatment they received.

9.3 Immunogenicity Analysis

Immunogenicity analyses include the analysis of OspA serotype (ST1 to ST6) specific IgG levels by ELISA and by a supportive functional assay (e.g. growth inhibition, surface staining).

The primary immunogenicity analysis will compare the Geometric Mean Titers (GMTs) of serotype specific IgG against each OspA ST1 to ST6 as determined by ELISA in the PP population between treatment groups that are selected for the Main Study phase and between the VLA15 groups and the placebo group, respectively, on Day 85. GMTs and GMT ratios will be estimated by applying an analysis of variance (ANOVA) including the factor treatment group and study site. 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. In addition, sensitivity analyses (ANOVAs with factors study site, treatment group, study site*treatment group, age, and *B.b.* s.l serostatus at baseline) will be performed.

As secondary analysis, GMTs and GMT ratios will be analyzed by ELISA as outlined above on Day 1, 29, 57, 180, 236 and 365 as well as respective GMTs and GMT ratios as measured by functional antibody testing at Day 1, 85 and 365, and on Day 57, if deemed meaningful. Analyses will compare both dose groups against each other and against the placebo group.

Further secondary immunogenicity analyses will compare the following:

- The Geometric Mean of the fold rise as compared to baseline (GMFR) for IgG against each OspA ST 1-6 as determined by ELISA (ANOVA) and functional antibody testing.
- Seroconversion Rates against each OspA serotype separately; against all six OspA serotypes combined and against OspA ST1 and ST2 combined. Seroconversion rates will be compared using Fisher-Freeman-Halton tests, a significant overall test will be amended by pair-wise tests (Fisher's exact test).
    - For ELISA: SCR is defined as rate of subjects achieving a 4-fold increase in OspA serotype specific IgG over baseline
    - For functional antibody testing: SCR is defined as rate of subjects that convert from baseline seronegative to seropositive.
- GMTs, SCRs and GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1, 29, 57, 85, 180, 236, and Month 12, stratified by age group.

All immunogenicity analyses will be performed for the PP population. It will be described in the SAP which analysis will be repeated for the mITT population and which analyses will also be repeated stratified by baseline *B.b.* s.l. serostatus, region and by age. Study sites with low enrollment numbers per randomization stratum will be combined in these analyses, further details will be provided in the SAP.

9.4 Safety Analysis

All subjects entered into the study who received at least one vaccination (safety population) will be included in the safety analysis. Safety tabulations will generally be provided separately for solicited AEs and unsolicited AEs, and for both types of AEs combined. 95%

confidence intervals according to Altman will generally be provided for all AE rates. Further details in addition to the outline below will be provided in the SAP.

The number and percentage of subjects with any AE, any unsolicited AE, any related unsolicited AE, any SAEs, any AESI, any related SAEs, any medically attended AE and any AE leading to withdrawal from further treatment, up to Day 85 and up to Day 365, will be presented for each treatment group, overall and by system organ class/preferred term. Differences between both dose groups and the placebo group 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. In addition, the number and percentage of subjects with any AE, any unsolicited AE, any related unsolicited AE, any SAEs, any related SAEs, any AESI, any medically attended AE and any AE leading to withdrawal from further treatment will be presented grouped by onset between 1st and 2nd vaccination, 2nd and 3rd vaccination, and within 28 days after the 3rd vaccination.

The number and percentage of subjects with solicited local and systemic AEs within 7 days after each vaccination and within 7 days after any vaccination will be tabulated. Differences between the treatment groups will be assessed for significance using Fisher's exact test, a significant overall test will be amended by pair-wise tests. The occurrence of solicited local and systemic AEs will also be tabulated by Subject Diary day.

Changes in laboratory values from study entry to end of treatment/follow-up will be analyzed descriptively and will be part of the unsolicited AE evaluation only in case of clinically relevant deviations. The frequencies of subjects with laboratory assessments outside the normal range, and with abnormal laboratory parameters falling into the grade 0 vs. 1 through 4 will be calculated. The frequency of subjects with urinalysis results according to the test manufacturer's results categories will be calculated.
It will be defined in the SAP which safety analyses will also be repeated stratified by baseline *B.b.* s.l. serostatus, region and by age.

9.5 General considerations for the determination of the optimal dose

The following considerations for identification of the dose to be selected for further development are anticipated. However, these considerations may change with availability of new knowledge (e.g., new information from ongoing studies, progress made in functional assay set up etc.) or upon review of actual results. In general, exclusion or ranking of dose groups will be based on the combined results for both age groups (18-49 years and 50-65 years).

1. If all doses are similarly immunogenic, in general the lowest dose will be selected;

2. If one dose is significantly more immunogenic, it will be selected unless safety favors another dose;

3. If safety favors another dose than immunogenicity, a comprehensive review of all safety and immunogenicity parameters will be performed to select the dose for further development;

4. Safety measures will be interpreted in conjunction with immunogenicity data:

    - A significantly higher frequency of related SAEs will preclude a dose from further consideration;
    - A significantly lower frequency of related AESIs favors one group over the other;

- A significantly lower frequency of solicited AEs favors one group over the other;
- A significantly lower rate of severe, related AEs favors one group over the other

5. In general, immunogenicity data from Phase 1 revealed that there was a constant pattern with regards to immune responses to VLA15 in the six serotypes, i.e., dose groups that induced a high immune response against one serotype induced high immune response against all six serotypes and vice versa. Therefore, as a conservative approach, the immune response for serotype 1, which was the serotype against which VLA15 induced lowest immune responses in the Phase 1 study and which is the serotype that circulates almost exclusively in the U.S., will be compared with highest priority for determination of the optimal dose. In case no significant difference between dose groups is observed for serotype 1, immune responses for serotype 2, the most prevalent serotype in Europe, will be considered. Therefore, immunogenicity parameters in general will be interpreted with the following order of priority:

    - GMTs for IgG against OspA serotype 1, determined by ELISA, has first priority. Significantly higher GMTs favor one dose over the other.
    - GMTs for IgG against OspA serotype 2, determined by ELISA, has second priority. Significantly higher GMTs favor one dose over the other.
    - Measures from supportive functional assays (e.g. growth inhibition assays) will be factored in.

9.6 Determination of Sample Size

The sample size for the Run-in phase has been chosen to allow detection of common AEs with the three initial dose levels. 30 Subjects will provide 95 % confidence that an AE not seen in the Run-in phase would have a true incidence of below 10 %.

The overall group size for the two doses (VLA15 w/ alum 135 µg and VLA15 w/ alum 180 µg) evaluated in the Main Study phase has been selected to provide a sufficient safety database and for determining the optimal dose before advancing the vaccine candidate into Phase 3. Upon completion of the study, the total number of subjects exposed to the dose used for Phase 3 trials would be a minimum of approximately N=210. The database would thus allow 95 % confidence that a given reaction would not be observed at a higher than 1:(210/3) rate, i.e. 1.4%, if it is not observed in the trials preceding Phase 3.

With respect to the primary endpoint, GMTs for ST1-6 specific IgGs on Day 85: In the absence of an established protective titer, sample size calculation is based on somewhat arbitrary differences in GMTs between VLA15 treatment groups, in order to demonstrate which titer levels could be distinguished with the proposed sample size. Titers observed in Phase 1 were used as basis: In the 90 µg w/ alum group (i.e. the lowest dose group used in the present Phase 2 study), a GMT of 61.3 was observed for ST1 (i.e. the serotype with lowest titers in Phase 1) with a Standard Deviation (LOG10) of 0.51. A total of 189 subjects per group (assuming 10 % of the 210 subjects per treatment group are excluded from primary PP analysis) will provide 80 % power at a two-sided alpha level of 5 % to distinguish a GMT of 61.3 in one treatment group from a putative higher GMT of 86.1 in another dose group. An approximately 1.5 fold higher titer could thus be distinguished. A 1.5 fold difference in GMTs is often considered a relevant difference in vaccine studies, e.g. when setting non-inferiority boundaries.
The overall sample size of 120 subjects in the placebo group has been selected to allow for the internal validation of both safety and immunogenicity results.

9.7 Interim Analysis/ Final Analysis

An interim analysis on safety and immunogenicity data will be performed after all subjects have completed Visit 6 (i.e. Day 236, six months after the last vaccination). This interim analysis will cover safety and immunogenicity data up to Visit 4 (i.e. Day 85, four weeks after the last vaccination) as well as safety data up to Visit 6 (i.e. Day 236, six months after the last vaccination). Data from selected dose groups in the Main Study and data from respective dose groups from the Run-in phase will be pooled for this and all further analyses.

A final data analysis will be conducted once the last subject has completed the study, i.e. Visit 7 (Month 12).

10. DEVIATIONS FROM THE PROTOCOL

10.1 Relevant Protocol Deviations

All protocol deviations will be tracked, actions defined, as feasible, and reviewed in Data Review Meetings for the interim analysis and the final analysis for assessment of their influence on the quality of the study analysis.

10.2 Premature Subject Withdrawal from Study or Treatment

Subjects have the right to withdraw from the study at any time for any reason, without the need to justify. The investigator also has the right to prematurely terminate a subject's further participation in the study, e.g. in the case of non-compliance.

The investigations described for Early Termination, see Table of Events (Table 2 and Table 3), should be carried out and recorded at the time of the subject's withdrawal, including obtaining an explanation of why the subject is withdrawing, if possible. Subjects will not be replaced.

Additionally, a subject will be withdrawn from further vaccination if any of the following criteria are met:

10.2.1 Individual stopping criteria

The following criteria will lead to a subject withdrawal from further vaccinations:

a. If subject becomes pregnant (please refer to section 8.4.5 for pregnancy reporting procedures).

b. If a subject reports symptoms or if abnormal lab values are found, which are considered unacceptable by the subject or the investigator, he or she will be withdrawn from further treatment.

c. If a subject experiences an SAE with no likely alternative cause than the study vaccine (i.e. possibly or probably related).

d. Solicited local AE: Grade 3 or 4 injection site reaction that lasts longer than 3 days.

e. Solicited systemic AE: Grade 3 or 4 solicited systemic reaction that lasts longer than 3 days. However, the subject may receive further vaccinations if there is a more plausible alternative cause for the reaction.

f. Any acute systemic allergic reaction after administration of the vaccine within 14 days following study vaccine administration, with no likely alternative cause than the study vaccine.

g. If subject develops or is found to present one of the following exclusion criteria after enrolment: 1, 2, 4-6, 8-10, 15-17.

Subjects withdrawn from further vaccination should perform their remaining regular study visits as scheduled if there are no other reasons for premature withdrawal from the study.

10.3 Documentation of Premature Withdrawal

The reasons for premature withdrawal of a study subject from treatment should be documented in the eCRF as follows:

- Withdrawal due to meeting individual stopping criteria (identify the respective criteria and AE, if applicable)
- Consent withdrawal due to adverse event (identify the respective AE)
- Consent withdrawal not due to an adverse event
- Investigator/ sponsor recommended withdrawal (include reasons, e.g. AE, incompliance, exclusion criterion met/ developed...)

The reasons for premature withdrawal of a study subject from the study should be documented in the eCRF as follows:

- Consent withdrawal due to adverse event (identify the respective AE)
- Consent withdrawal not due to an adverse event
- Investigator/ sponsor recommended withdrawal (include reasons, e.g. AE, incompliance, exclusion criterion met/ developed...)
- Moved from study area
- Lost to follow up
- Death

10.4 Subsequent Therapy

Not applicable.

11. ETHICAL AND REGULATORY ASPECTS

11.1 Ethical/ Regulatory Framework

The study will be conducted in accordance with the protocol, the current Declaration of Helsinki, current ICH/GCP guidelines, and with the applicable regulatory requirements.

11.2 Institutional Review Board/ Independent Ethics Committee

Prior to study initiation, the investigator, sponsor or CRO will submit the protocol, ICF and further requested information to the appropriate IRBs/ IECs in accordance with local requirements. The site will not enroll subjects before approval has been obtained.

11.3 Subject Information and Informed Consent

It is the investigator's responsibility to obtain freely given written informed consent from the subject after adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study, and before the subject is exposed to any study-related procedures, including screening tests for eligibility.

The investigator will explain that the subjects are completely free to refuse to enter the study or to withdraw from it at any time, without any prejudice and need for justification. The subjects will be informed that representatives of the sponsor and health authority inspector may review their source records, and that these persons are bound by confidentiality obligations.

The subject will be given a copy or a second original of the ICF. An original of the signed and dated ICF must be retained in the site's records, and is subject to inspection by representatives of the sponsor or representatives from regulatory agencies.

12. QUALITY CONTROL AND QUALITY ASSURANCE

12.1 Source Data and Records

Source data are defined as all information related to clinical findings, observations or other activities in the study, written down in original records or certified copies of original records. The investigator will permit study-related monitoring, audits, IRB/IEC review and regulatory inspections, by providing direct access to source data/records. Source records should be preserved for the maximum period of time required by local regulations.

Source data entries must be made in accordance with local requirements. Signed and dated copies of the laboratory result reports have to be kept within the subject's source data file.

The following data may directly be recorded in the eCRF at study visits and the eCRF is regarded as source document:

- Ethnic Group
- Systolic and diastolic blood pressure, pulse rate, oral body temperature
- Result of urine pregnancy test

eCRFs will not be used as source data for any other variable.

12.2 Periodic Monitoring

A designated CRA will check electronic system data and source data at regular intervals throughout the study to verify completeness, accuracy and consistency of the data, protocol adherence, and adherence to GCP guidelines. The monitor will work according to the Monitoring Plan. The investigator will cooperate with the monitor to ensure that any discrepancies identified are resolved.

12.3 Audit and Inspection

Upon request, the investigator will make all study-related source data and records available to a qualified quality assurance auditor mandated by the sponsor or to regulatory inspectors. The main purposes of an audit or inspection are to confirm that the rights and welfare of the subjects have been adequately protected, and that all data relevant for the assessment of safety and efficiency of the investigational product have appropriately been reported to the sponsor.

12.4 Confidentiality of Subject's Data

The investigator will exercise all reasonable precautions within the constraints of the applicable regulatory requirements to maintain the confidentiality of subjects' identities. On exported electronic source data or any other documents submitted to the sponsor, subjects will only be identified by subject number. Documents not for submission to the sponsor, e.g. subject identification log and original ICF, will be maintained by the investigator in strict confidence.

13. DATA HANDLING AND RECORD KEEPING

13.1 Information of Investigators

An Investigator Brochure (IB) containing all important data relating to the safe use of the investigational product will be supplied to the investigator prior to study start.

The investigator will be kept informed on new relevant safety data as the study proceeds.

13.2 Electronic Case Report Forms (eCRFs)

13.2.1 eCRF entries

eCRF entries and corrections will only be performed by study site staff authorized by the investigator. Each user is informed of the clinical study's web-site internet address and is allocated to a user account with personal password to access the confidential web site. The personal password must be kept confidentially and must only be used by the person to whom it was assigned. For additional authorized users at the site, a new user account needs to be requested to ensure that each entry/ change can be allocated to the person who performed the entry/ change.

All visit data need to be recorded in the eCRF database as soon as possible after each study visit.

13.2.2 Changes to eCRF data

Corrections may be requested as follows:

- Investigators' responses are checked as they are entered and are rejected if they do not fulfill quality criteria. A message will specify the type of error or syntax error and assist in its correction.
- If required, the CRA can ask for information to be corrected during monitoring.
- Computerized data-check programs and manual checks will identify clinical data discrepancies for resolution. Corresponding queries will be created within the data capturing system and the site will be informed about new issues to be resolved on-line.

All discrepancies will be solved on-line directly by the investigator or by authorized staff.

Corrections of eCRF data may be performed by authorized staff only. The person performing the changes in the eCRF is required to electronically confirm the changes made.

13.2.3 eCRF entry validation

The principal investigator or the authorized delegate will thoroughly review the data on the eCRF, and will finally certify the contents of the eCRF by electronic signature after completion of each patient. If a correction is made to the eCRF data after the investigator's final approval, the certification must be repeated after the changes have been performed.

13.2.4 Data collection

All visits and assessments are entered into an interactive form. All eCRFs will be source document verified as detailed in the Monitoring Plan. Maintenance of the study database will be performed. Details to eCRF handling are provided in a study specific eCRF manual.

13.3 Coding of Adverse Events, Drugs and Diseases

After data entry AEs and Medical History will be coded according to MedDRA, latest version. Previous and Concomitant Medication and Vaccines will be coded according to WHO Drug Reference List and Anatomical Therapeutic Chemical (ATC) Classification System, latest version.

13.4 Investigator File

13.4.1 Maintenance

The investigator will be provided with an initial investigator file during the initiation visit. The investigator is responsible for maintaining all records up to date to enable the conduct of the study to be fully documented. The records should include the protocol, study approval letters, all original ICFs, drug dispensing and accountability logs and all relevant correspondence pertaining to the study.

13.4.2 Archiving and destruction

All study-related documents should be kept by the investigator for the maximum period of time required by local regulations. No study document should be destroyed without prior written agreement between the investigator and the sponsor. Should the investigator elect to assign the study documents to another party, or move them to another location, the sponsor must be notified.

13.5 Provision of Additional Information

On request, the investigator will supply the sponsor with additional data relating to the study or copies of relevant source records, duly anonymized. In case of particular issues or governmental queries, it is also necessary to have access to the complete study records, provided that the subject's confidentiality is protected in accordance with applicable regulations.

14. CHANGES IN THE CONDUCT OF THE STUDY

14.1 Protocol Amendments

Proposed amendments must be submitted to the appropriate CA and IRB/IEC in line with regulatory requirements. Amendments may be implemented only after CA and IRB/IEC approval has been obtained, if applicable. Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving CA and IRB/IEC approval. However, in this case, approval must be obtained as soon as possible after implementation.

14.2 Study Termination – Study Stopping Rules

The sponsor and an independent DSMB will monitor safety data at regular intervals to identify applicability of study stopping rule or identify any potential safety concern. The DSMB will ad hoc review all cases of SAEs.

The occurrence of the following criterion will lead to suspension of any further enrolment and suspension of any subsequent vaccination of subjects already enrolled, until available safety data has been reviewed by the DSMB and their recommendation is available whether or not to proceed with enrolment and vaccination:

- Two or more related SAEs with the same suspected underlying pathological mechanism, where relationship to VLA15 cannot be ruled out (i.e. judged as probably or possibly related to vaccination).

The DSMB can issue a recommendation to stop the study or to discontinue a treatment group during planned or ad-hoc DSMB meetings, e.g. in response to an excess rate of AEs or AESIs with the same suspected underlying pathological mechanism.

If a study stopping rule is met or the DSMB recommends halting the study for other reasons, the sponsor will notify the Competent Authorities, IRBs/ECs and Principal Investigators within 48 hours by phone or fax. Vaccination of subjects already enrolled in the study and restart of recruitment may only proceed after positive DSMB recommendation and Competent Authorities will be informed.

If the sponsor or the investigator decides to terminate the study before it is completed, they will notify each other in writing, stating the reasons for early termination. In terminating the study, the sponsor and the investigator will ensure that adequate consideration is given to the protection of the subjects' interests. The investigator, sponsor or CRO will notify the relevant CA or IRB/ IEC in writing in accordance with local requirements. Documentation will be submitted for filing in the Central and Investigator File and the Trial Master File.

15. REPORTING AND PUBLICATION

15.1 Clinical Study Report

An interim study report will be compiled by the sponsor or delegate in accordance with relevant guidelines and will contain all data from all subjects on safety and immunogenicity up to Day 85 (Visit 4) as well as all safety data from all subjects up to Day 236 (i.e. 6 months after the last vaccination). A final Clinical Study Report will be written after the final analysis, once all data from all subjects are analyzed. The study coordinating investigator will be asked to review and sign the final study reports.

15.2 Publication Policy

All results generated in this study will be considered to be strictly confidential. The investigator may not submit the results for publication or presentation without prior written permission of the sponsor. Authorship for any publication will be determined in mutual agreement. Within the scope of publication, co-authorship may be offered, at the sole discretion of the sponsor, on a case by case basis taking scientific contribution into consideration. This is according to uniform requirements for manuscripts submitted to biomedical journals proposed by the International Committee of Medical Journal Editors.

16. LIABILITIES AND INSURANCE

The sponsor will contract a clinical trial insurance.

The name, address and the insurance policy number will be given to the investigator. Moreover a copy of the insurance conditions will be filed on site.

The investigator is responsible for dispensing the investigational product according to this protocol, and for its secure storage and safe handling throughout the study.

17. APPENDIX 1

Immune-mediated and neuroinflammatory disorders as proposed by FDA for previous clinical programs [25]:

Gastrointestinal disorders
- Celiac disease
- Crohn's disease
- Ulcerative colitis
- Ulcerative proctitis

Liver disorders
- Autoimmune cholangitis
- Autoimmune hepatitis
- Primary biliary cirrhosis
- Primary sclerosing cholangitis

Metabolic diseases
- Addison's disease
- Autoimmune thyroiditis (including Hashimoto thyroiditis)
- Diabetes mellitus type 1
- Grave's or Basedow's disease

Musculoskeletal disorders
- Antisynthetase syndrome
- Dermatomyositis
- Juvenile chronic arthritis (including Still's disease)
- Mixed connective tissue disorder
- Polymyalgia rheumatic
- Polymyositis
- Psoriatic arthropathy
- Relapsing polychondritis
- Rheumatoid arthritis
- Scleroderma, including diffuse systemic form and CREST syndrome
- Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis
- Systemic lupus erythematosus
- Systemic sclerosis

Neuroinflammatory disorders
- Acute disseminated encephalomyelitis, including site specific variants: eg, noninfectious encephalitis, encephalomyelitis, myelitis, myeloradiculomyelitis
- Cranial nerve disorders, including paralyses/paresis (eg, Bell's palsy)
- Guillain-Barré syndrome, including Miller Fisher syndrome and other variants
- Immune-mediated peripheral neuropathies and plexopathies (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy)
- Multiple sclerosis
- Narcolepsy
- Optic neuritis
- Transverse Myelitis

Skin disorders
- Alopecia areata
- Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis)
- Cutaneous lupus erythematosus
- Erythema nodosum
- Morphoea
- Lichen planus
- Psoriasis
- Sweet's syndrome
- Vitiligo

Vasculitides
- Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis
- Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopie polynagiitis, Wegener's granulomatosis, Churg-Strauss syndrome (allergic granulomatous angiitis), Buerger's disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis

Others
- Antiphospholipid syndrome
- Autoimmune hemolytic anemia
- Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoprofilerative glomerulonephritis, and masangioproliferative glomerulonephritis
- Autoimmune myocarditis/cardiomyopathy
- Autoimmune thrombocytopenia
- Goodpasture syndrome
- Idiopathic pulmonary fibrosis
- Pernicious anemia
- Raynaud's phenomenon
- Sarcoidosis
- Sjögren's syndrome
- Stevens- Johnson syndrome
- Uveitis

18. APPENDIX 2

In case there is clinical suspicion for Lyme borreliosis or a LB-associated event according to the scripted safety assessment, investigators are advised to perform the following clinical workup:

A. <u>Travel history and physical examination, medical history</u>

1. Assess subjects' travel and exposure history

   If subject observed a tick bite, time/date of tick attachment and time/date of tick removal should be requested.

2. Perform physical exam: general appearance, skin, head/ eyes /ears/ nose/ throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, and neurological system, assess body temperature and vital signs. Especially assess for symptoms of fever, fatigue, headache, mild stiff neck, arthralgia, myalgia and thoroughly check skin for rash, including under (body) hair.
3. Assess medical history

B. <u>Subject presents with an Erythema Migrans (EM) rash - early localized disease (<30 days after tick bite):</u>

1. Document localization of rash
2. Perform a photograph of the EM rash for documentation. Only the affected area should be visible in the picture. Avoid full-face views or other personal identification in photographs to ensure the subject's anonymity.

<u>Characteristics of an EM rash</u> [10]

- Erythema migrans usually occurs 7 to 14 days (range 3 to 30 days) after tick detachment or tick removal.
- Starts from a macule or papule and expands over time to form red or bluish-red patch
- EM should be at least 5 cm in largest diameter and usually increases in size over time, reaching up to 30 cm.
- EM can be homogeneously erythematous or can have prominent central clearing: See examples of Erythema migrans rashes on

  <u>https://www.cdc.gov/lyme/signs_symptoms/index.html</u>

The clinical diagnosis of Lyme borreliosis through presentation of a distinctive Erythema migrans is done by visual inspection of the skin lesion without laboratory confirmation. Treatment of patients should be initiated according to standard of care [10,26].

In case there is diagnostic uncertainty and symptoms persist, acute-phase and convalescent-phase (i.e., 2 weeks after the acute phase) serum samples should be tested using 2-tier testing algorithm as recommended by the CDC [27].

C. Subject presents with signs / symptoms suggesting early disseminated disease (< 3 months) or Late disseminated disease (≥3 months)

- In case of clinical suspicion of early disseminated or late disseminated Lyme borreliosis serologic testing via a two-tier approach using a sensitive enzyme-linked immunosorbent assay (ELISA) and confirmation of positive or equivocal results by a standardized Western blot/ Immuno blot assay as recommended by the CDC [27] should be ordered. Additional work-up as depicted in Table 11 should be initiated.
- In case of clinical suspicion of early disseminated or late disseminated Lyme borreliosis, consider initiating treatment according to standard of care [10,26] and send patient for consultation with a specialist as appropriate.

Table 11 Early disseminated or late disseminated Lyme borreliosis

	Symptom	**Additional work up at study site**
Disseminated skin manifestations	Multiple EM skin lesions • might be <5 cm in diameter and may expand Borrelial Lymphocytom (rare) • solitary bluish-red swelling with diameter up to few cm • most commonly presents at ear lobe, ear helix, breast (on or near the nipple), or scrotum Acrodermatitis Chronica Atrophicans • develops several years after infection, mainly observed in Europe • lesions are characterized by a slight bluish-red discoloration and doughy swelling • lesions enlarge slowly over months to years, in association with resolution of the edema and development of skin atrophy	Perform photograph and document localization of EM rashes and/or borrelial lymphocytom. Only the affected area should be visible in the picture. Avoid full-face views or other personal identification in photographs to ensure the subject's anonymity. Send patient for consultation with infectious disease/ LB specialist as appropriate for further clinical work-up
Neurological symptoms	• episodes of dizziness or shortness of breath • nerve pain suggesting suspicion of: • inflammation of the brain and spinal cord • cranial nerve palsy • meningo-radiculitis	Send patient for consultation with infectious disease specialist/ neurologist as appropriate for further clinical work-up

	• meningitis • radiculopathy • encephalitis • myelitis • cerebral vasculitis • facial palsy	
Arthritis	e.g. • recurrent attacks or persisting objective joint swelling (synovitis) in one or a few large joints • intermittent pain in tendons, muscles, joints and bones	Send patient for consultation with infectious disease specialist /rheumatologist as appropriate for further clinical work-up
Cardiac symptoms (rare)	• heart palpitations or an irregular heart beat e.g. suspicion of • atrio-ventricular conduction disturbances • rhythm distrurbances • myocarditis	Perform ECG; Send patient for consultation with infectious disease specialist/ cardiologist as appropriate for further clinical work-up
Ocular manifestations (rare)	e.g. • conjunctivitis • uveitis • papillitis • episcleritis • keratitis	Send patient for consultation with infectious disease specialist/ ophthalmologist as appropriate for further clinical work-up

19. REFERENCES

1. Mead PS. Epidemiology of Lyme Disease. *Infect Dis Clin North Am.* 2015;29(2):187-210. doi:10.1016/j.idc.2015.02.010

2. *Consultation on Tick-Borne Diseases MEETING REPORT Expert Consultation on Tick-Borne Diseases with Emphasis on Lyme Borreliosis and Tick-Borne Encephalitis2.* https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/1102_MER_Tickborne_2010.pdf. Accessed February 1, 2019.

3. Rizzoli A, Hauffe HC, Carpi G, Vourc'h GI, Neteler M, Rosà R. Lyme borreliosis in Europe. *Eurosurveillance.* 2011;16(27):19906. doi:10.2807/ese.16.27.19906-en

4. Lindgren E, Jaenson TGT. *Lyme Borreliosis in Europe: Influences of Climate and Climate Change, Epidemiology, Ecology and Adaptation Measures.*; 2006. http://www.euro.who.int/pubrequest. Accessed February 1, 2019.

5. Hinckley AF, Connally NP, Meek JI, et al. Lyme Disease Testing by Large Commercial Laboratories in the United States. *Clin Infect Dis.* 2014;59(5):676-681. doi:10.1093/cid/ciu397

6. Nelson CA, Saha S, Kugeler KJ, et al. Incidence of Clinician-Diagnosed Lyme Disease, United States, 2005-2010. *Emerg Infect Dis.* 2015;21(9):1625-1631. doi:10.3201/eid2109.150417

7. Lyme Disease Data Tables: Historical Data | Lyme Disease | CDC. https://www.cdc.gov/lyme/stats/tables.html. Accessed January 25, 2019.

8. Pritt BS, Mead PS, Johnson DKH, et al. Identification of a novel pathogenic Borrelia species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study. *Lancet Infect Dis.* 2016;16(5):556-564. doi:10.1016/S1473-3099(15)00464-8

9. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. *Lancet.* 2012;379(9814):461-473. doi:10.1016/S0140-6736(11)60103-7

10. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The Clinical Assessment, Treatment, and Prevention of Lyme Disease, Human Granulocytic Anaplasmosis, and Babesiosis: Clinical Practice Guidelines by the Infectious Diseases Society of America. *Clin Infect Dis.* 2006;43(9):1089-1134. doi:10.1086/508667

11. Steere AC, Sikand VK, Meurice F, et al. Vaccination against Lyme Disease with Recombinant *Borrelia burgdorferi* Outer-Surface Lipoprotein A with Adjuvant. *N Engl J Med.* 1998;339(4):209-215. doi:10.1056/NEJM199807233390401

12. Sigal LH, Zahradnik JM, Lavin P, et al. A Vaccine Consisting of Recombinant *Borrelia burgdorferi* Outer-Surface Protein A to Prevent Lyme Disease. *N Engl J Med.* 1998;339(4):216-222. doi:10.1056/NEJM199807233390402

13. Steere AC, Drouin EE, Glickstein LJ. Relationship between Immunity to Borrelia burgdorferi Outer-surface Protein A (OspA) and Lyme Arthritis. *Clin Infect Dis.* 2011;52(suppl_3):s259-s265. doi:10.1093/cid/ciq117

14. Wressnigg N, Pöllabauer E-M, Aichinger G, et al. Safety and immunogenicity of a novel multivalent OspA vaccine against Lyme borreliosis in healthy adults: a double-blind, randomised, dose-escalation phase 1/2 trial. *Lancet Infect Dis.* 2013;13(8):680-689. doi:10.1016/S1473-3099(13)70110-5

15. Wressnigg N, Barrett PN, Pöllabauer E-M, et al. A Novel Multivalent OspA Vaccine against Lyme Borreliosis Is Safe and Immunogenic in an Adult Population Previously Infected with Borrelia burgdorferi Sensu Lato. Plotkin SA, ed. *Clin Vaccine Immunol.*

2014;21(11):1490-1499. doi:10.1128/CVI.00406-14

16. Hirschfeld M, Kirschning CJ, Schwandner R, et al. Cutting edge: inflammatory signaling by Borrelia burgdorferi lipoproteins is mediated by toll-like receptor 2. *J Immunol*. 1999;163(5):2382-2386. http://www.ncbi.nlm.nih.gov/pubmed/10452971. Accessed February 1, 2019.

17. CBER, FDA. *HIGHLIGHTS OF PRESCRIBING INFORMATION TRUMENBA*. https://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM421139.pdf. Accessed February 1, 2019.

18. FDA, Cber. *HIGHLIGHTS OF PRESCRIBING INFORMATION BEXSERO*. https://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM431447.pdf. Accessed February 1, 2019.

19. *Lyme Disease Vaccine (Recombinant OspA) LYMErix*. https://wayback.archive-it.org/7993/20170405164754/https://www.fda.gov/ohrms/dockets/ac/01/briefing/3680b2_03.pdf. Accessed February 1, 2019.

20. Lathrop SL, Ball R, Haber P, et al. Adverse event reports following vaccination for Lyme disease: December 1998-July 2000. *Vaccine*. 2002;20(11-12):1603-1608. http://www.ncbi.nlm.nih.gov/pubmed/11858868. Accessed February 1, 2019.

21. Marshall HS, Richmond PC, Nissen MD, et al. Safety and Immunogenicity of a Meningococcal B Bivalent rLP2086 Vaccine in Healthy Toddlers Aged 18 to 36 Months. *Pediatr Infect Dis J*. 2012;31(10):1. doi:10.1097/INF.0b013e31826327e4

22. Schoen RT, Sikand VK, Caldwell MC, et al. Safety and immunogenicity profile of a recombinant outer-surface protein a Lyme disease vaccine: Clinical trial of a 3-dose schedule at 0, 1, and 2 months. *Clin Ther*. 2000;22(3):315-325. doi:10.1016/S0149-2918(00)80035-1

23. *Recommendations Related to Contraception and Pregnancy Testing in Clinical Trials Introduction and Scope*. http://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf. Accessed February 1, 2019.

24. FDA, CDER, Cderuser. *Guidance for Industry E2F Development Safety Update Report.*; 2011. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htmand/orhttp://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm. Accessed February 1, 2019.

25. FDA, Cber. *Vaccines and Related Biological Products Advisory Committee Meeting.*; 2017. https://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/VaccinesandRelatedBiologicalProductsAdvisoryCommittee/UCM568492.pdf. Accessed February 1, 2019.

26. Stanek G, Fingerle V, Hunfeld K-P, et al. Lyme borreliosis: Clinical case definitions for diagnosis and management in Europe. *Clin Microbiol Infect*. 2011;17(1):69-79. doi:10.1111/j.1469-0691.2010.03175.x

27. Two-step Laboratory Testing Process | Lyme Disease | CDC. https://www.cdc.gov/lyme/diagnosistesting/labtest/twostep/index.html. Accessed February 1, 2019.