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CLINICAL STUDY PROTOCOL

DRUG SUBSTANCE(S)	VLA15
VERSION NO.	Final 8.0
STUDY CODE	VLA15-221
DATE	30-Mar-2022

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

Phase 2 Study

Study Protocol VLA15-221

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CLINICAL STUDY PROTOCOL SYNOPSIS

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

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 borreliosis. The vaccine targets the majority of *Borrelia* strains expressing clinically relevant OspA serotypes (STs) present in the U.S. (ST1) and Europe (ST1 to ST6). 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). VLA15 is used in a formulation that contains aluminum hydroxide adjuvant.

In this study, VLA15 will be applied at a dose of 180 µg total protein (i.e., 60 µg per fusion protein). This is planned to be the dose for advancing the vaccine candidate into Phase 3 and for licensure. The dose selection has been made based on safety and immunogenicity data from two previous Phase 2 studies (VLA15-201 and VLA15-202).

A new VLA15 presentation will be used in the VLA15-221 study. This presentation contains the same amounts of VLA15 (180 µg) and alum (0.5 mg) in a concentrated form (i.e., formulated in 0.5 mL instead of 1.0 mL of buffer). Hence, the antigen-alum ratio stays the same with the same concentration of buffer excipients.

All injections will be administered intramuscularly (I.M.).

COMPARATOR PRODUCT, dosage and mode of administration

Placebo: Phosphate Buffered Saline (PBS) solution, 0.5 mL
All injections will be administered intramuscularly (I.M.).

STUDY OBJECTIVES

Primary objectives:

Safety:

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

Immunogenicity:

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

Secondary objectives:

Safety:

- To assess the safety and tolerability profile of VLA15, applied in a three- or two dose primary immunization schedule (Month 0-2-6 or Month 0-6), in a healthy study population aged 5 to 65 years, up to one year after the last primary vaccination (Month 18).
- To assess the safety and tolerability profile of a booster dose of VLA15, applied one year after completion of the primary immunization schedule (i.e., Month 18), up to three years after the booster (i.e., Month 54).

Immunogenicity:

- To assess the immune response to VLA15, applied in a three- or two dose primary immunization schedule (Month 0-2-6 or Month 0-6), in a healthy study population aged 5 to 65 years, up to one year after the last primary vaccination (Month 18).
- To assess the immune response to a booster dose of VLA15, applied one year after completion of the primary immunization schedule (i.e., Month 18), up to three years after the booster (Month 54).

Exploratory objective:

- [redacted]

STUDY DESCRIPTION

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

Subject Enrollment into Part A (Main Study Phase):

Enrollment will be performed in an age-descending, staggered manner for the three age cohorts (see Figure 1).

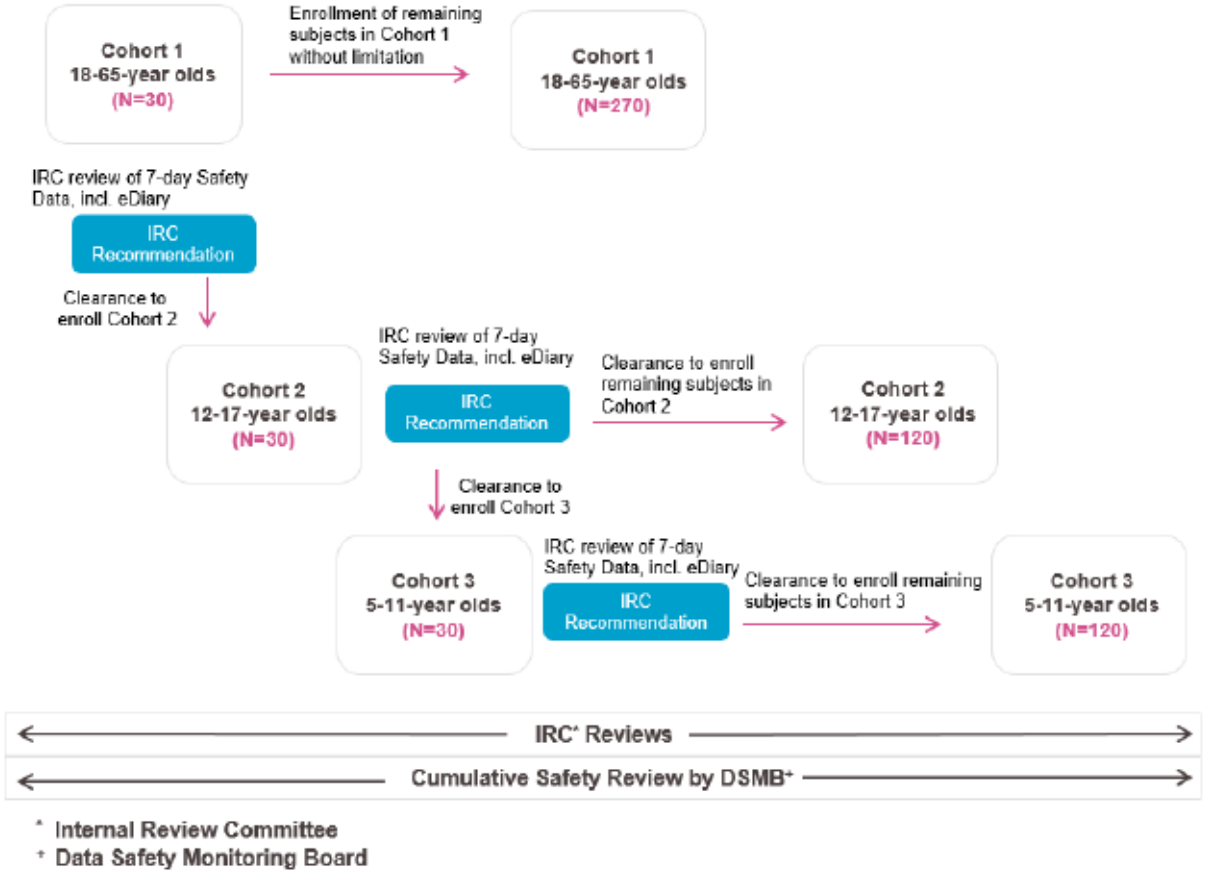

Figure 1 Subject enrollment process in Part A (Main Study Phase)

Enrollment Cohort 1 (18-65 years):

The enrollment will start with 30 sentinel adult subjects aged 18-65 years (Cohort 1). Subjects will be randomized 1:1:1 into one of three study groups to receive a vaccination of VLA15 (Groups 1 and 2) or placebo (Group 3). After these 30 sentinel subjects have received the first vaccination and have completed a 7-day post vaccination safety follow up visit (Visit 1A) including review of 7-day eDiary data, safety data will be reviewed in an unblinded manner by an independent Internal Review Committee (IRC). The IRC is composed of qualified personnel from Valneva's collaboration partner Pfizer. Based on this data, the IRC will make a recommendation on whether enrollment of the adolescent age cohort (12-17 years, Cohort 2) can be initiated. Enrollment of the remaining 270 adult subjects of Cohort 1 will continue without limitations during the IRC review.

Enrollment Cohort 2 (12-17 years):

Upon IRC clearance, 30 sentinel adolescent subjects aged 12-17 years (Cohort 2) will be enrolled. Subjects will be randomized 1:1:1 into one of the three study groups to receive a vaccination of VLA15 (Groups 1 and 2) or placebo (Group 3). Again, after these subjects have completed their safety follow up visit 7 days after the first vaccination (Visit 1A) including review of 7-day eDiary data, safety data will be reviewed by the IRC. The IRC will recommend whether enrollment of the youngest age cohort (5-11 years of age) can be initiated. In this age cohort, enrollment of the remaining 120 adolescent subjects will only be initiated after IRC clearance.

Enrollment Cohort 3 (5-11 years):

Upon IRC clearance, 30 sentinel subjects aged 5-11 years (Cohort 3) will be enrolled. Subjects will be randomized 1:1:1 into one of the three study groups to receive a vaccination of VLA15 (Groups 1 and 2) or placebo (Group 3). Again, after these 30 subjects have completed their safety follow up visit 7 days after the first vaccination (Visit 1A) including review of 7-day eDiary data, safety data will be reviewed by the IRC. Enrollment of the remaining 120 subjects in this age cohort will only be initiated after IRC clearance.

An external independent Data Safety Monitoring Board (DSMB) will review accruing safety data in an unblinded manner at regular intervals and might recommend adjusting, pausing or discontinuing the study at any time. Please refer to Section 8.6.2 for more details on the set-up and responsibilities of the DSMB.

Subject Enrollment into Part B (Booster Phase):

VLA15 Booster (Group 1 and 2)

- All eligible subjects within Group 1 and 2 will receive a VLA15 booster dose at Month 18.

Placebo (Group 3):

- All eligible subjects within Group 3 will receive Placebo at Month 18.

The study sponsor and study statisticians will be unblinded at the time of the primary endpoint analysis, i.e., prior to subject enrollment into Part B. Study participants and investigators will remain blinded throughout entire duration of study.

STUDY DESIGN

The study will be conducted in two study parts (Part A: Main Study Phase, Part B: Booster Phase). Please refer to Figure 2 for the study design.

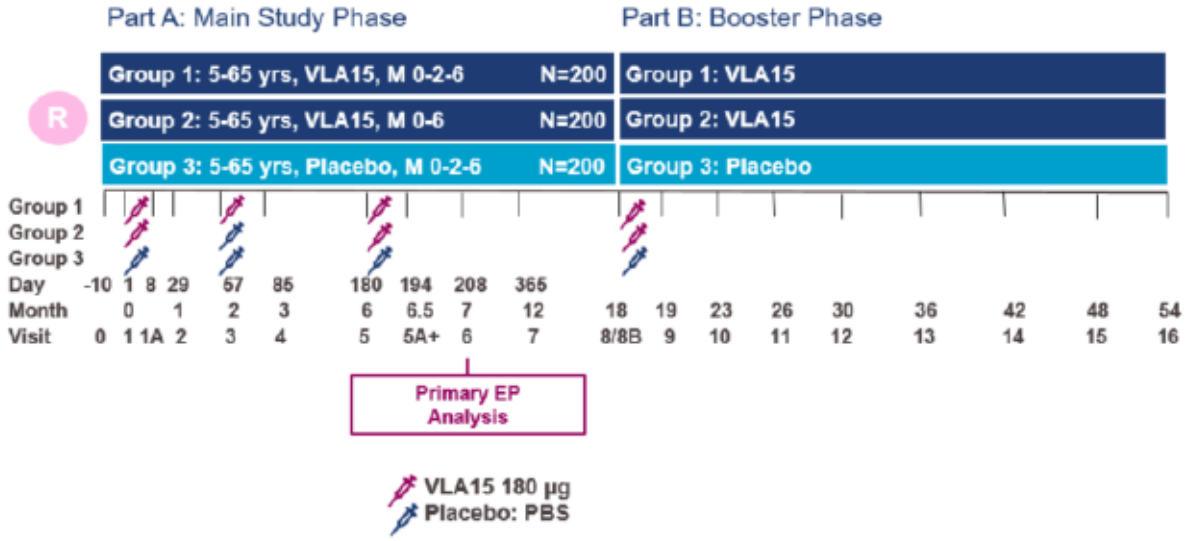

Figure 2 Study Design

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

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

In **Part B (Booster Phase)** all eligible subjects from Group 1 and 2 will receive a booster dose of VLA15 at Month 18. Placebo injections will be administered to Group 3 subjects.

All subjects involved in Part B will be followed-up for further 3 years (i.e., up to Month 54) with study visits at Months 19, 23, 26, 30, 36, 42, 48 and 54.

Table 1 provides an overview on study groups and treatments.

Table 1 Study Groups and Vaccinations

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

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

STUDY SPONSOR AND COLLABORATION PARTNERS

The development of VLA15 is a collaboration between Valneva and Pfizer. At study initiation, Valneva served as study sponsor. Upon IND transfer (mid November 2021), Pfizer takes over sponsorship, however some sponsor responsibilities are delegated to Valneva and CROs for the continuity of this study.

INVESTIGATOR AND SITES

Multicenter study in Lyme borreliosis endemic areas in the U.S., in total 14 active study sites.

STUDY START DATE

- Main Study Phase (Part A): March 2021

- Booster Phase (Part B): August 2022

STUDY DURATION

Study duration per subject will be a maximum of 19 months in the Main Study Phase and additional 37 months in the Booster Phase (i.e., Main Study Phase and Booster Phase participation results in an overall maximal duration of 56 months per subject). Overall study duration (i.e., First-Subject-In to Last-Subject-Out) is estimated to be 4 years and 11 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 approximately 600 healthy subjects aged 5 to 65 years will be enrolled in this study: 300 subjects aged 18-65 years, 150 subjects aged 12-17 years and 150 subjects aged 5-11 years.

CRITERIA FOR INCLUSION/EXCLUSION

Approximately 600 male or female subjects who meet 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:

1. Subject is aged 5 to 65 years* at the day of screening (Visit 0);
2. Subject is of good general health as determined by medical history, physical examination, and judgement of the investigator;
3. Parent(s)/legal representative(s) and subject who have an understanding of the study and its procedures as explained by the investigator and agree to its provisions:
   - For subjects aged 18-65 years†: Written informed consent prior to any study related procedures;
   - For subjects aged 5-17 years‡: Written informed consent by the subject's legal representative(s), according to local requirements, and written informed assent of the subject, if applicable, 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 according to following timelines:
      - Main Study Phase: duration of entire study
      - Booster Phase: until Month 23 (i.e., 5 months after booster dose)
5. Subject is willing and able to comply with scheduled visits, treatment plan, and other study procedures.
6. Subject is expected to be available for the duration of the study and can be contacted by telephone during study participation.

* From the 5th birthday until the last day before the 66th birthday
† From the 18th birthday until the last day before the 66th birthday
‡ From the 5th birthday until the last day before the 18th birthday

Exclusion criteria (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 Day 1/Visit 1;
2. Subject received previous vaccination against LB;
3. Subject had a tick bite within 4 weeks prior to Day 1/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 investigator's judgement, such as individuals with poorly controlled or unstable disease, ongoing suspected or active inflammation, or poor compliance with pharmacologic treatment;
5. Subject has a medical history of or currently has a neuro-inflammatory or autoimmune disease;
6. Subject has a known thrombocytopenia, bleeding disorder, or received anticoagulants in the 3 weeks prior to Day 1/Visit 1, contraindicating I.M. vaccination as judged by the investigator;
7. Subject has received an active or passive immunization within 4 weeks prior to Day 1/Visit 1;
8. Subject has received any other registered or non-registered medicinal product in another clinical trial within 4 weeks prior to vaccination at Day 1/Visit 1;
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 (e.g., radiation therapy, chemotherapy or glucocorticoid treatment) within 4 weeks prior to Day 1/Visit 1. Glucocorticoid 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 of unknown cause or severe allergic reactions of unknown cause. Subjects with known hypersensitivity or allergic reactions to one of the components of the vaccine are to be excluded;
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. Subjects with history of squamous cell or basal cell skin cancer which has been successfully treated by surgical excision and treatment is considered to have achieved cure may be enrolled. Subjects with a history of skin cancer must not be vaccinated at the previous tumor site;
12. Subject is pregnant (positive serum pregnancy test at Visit 0 or positive urine pregnancy test at Day 1/Visit 1), 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 during the course of the entire Main Study Phase;
13. Subject has donated or plans to donate blood or blood-derived products (e.g. plasma) within 4 weeks prior to Day 1/V1 or subject received blood or blood-derived products (e.g. plasma or immunoglobulins) within 3 months prior to vaccination at Day 1/Visit 1 or plans to donate or use blood or blood products within 4 weeks after vaccination at Day 1/Visit 1;

14. 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;
15. 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.

Exclusion criteria (Booster Phase):

Subjects who meet **ANY** of the following criteria are **NOT** eligible for participation in the Booster Phase:

1. Subject has developed a chronic illness related to Lyme borreliosis (LB) since Day 208/Visit 6 (i.e., Month 7) or an active symptomatic LB as suspected or diagnosed by a physician;
2. Subject has developed a clinically relevant disease (e.g., cardiovascular, respiratory, neurologic, psychiatric conditions, malignancy) which poses a risk for further 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;
3. Subject has developed a neuro-inflammatory or autoimmune disease since Day 180/Visit 5 (i.e., Month 6);
4. Subject has developed a thrombocytopenia, bleeding disorder, or received anticoagulants in the 3 weeks prior to Month 18/Visit 8, contraindicating I.M. vaccination as judged by the investigator;
5. Subject has developed a known or suspected defect of the immune system that would prevent an immune response to the vaccine, such as subjects with acquired immunodeficiency, including infection with human immunodeficiency virus (HIV), status post organ transplantation or immuno-suppressive therapy (e.g., radiation therapy, chemotherapy or glucocorticoid treatment) within 4 weeks prior to Month 18/Visit 8. Glucocorticoid 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;
6. Subject has experienced an anaphylaxis of unknown cause or severe allergic reactions of unknown cause or has developed hypersensitivity or allergic reactions to one of the components of the vaccine;
7. Subject is pregnant (positive urine pregnancy test), or is lactating at Month 18/Visit 8 or has plans to become pregnant before Month 23/Visit 10. Women of childbearing potential that are unwilling or unable to employ an adequate birth control measure up to Month 23/Visit 10;
8. Subject has donated or plans to donate blood or blood-derived products (e.g., plasma) within 4 weeks prior to Month 18/Visit 8 or received blood or blood-derived products (e.g. plasma or immunoglobulins) within 3 months prior to booster vaccination in this study or plans to donate or use blood or blood products within 4 weeks after the booster dose;
9. Subject has developed 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;
10. 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.

Booster Phase:

3. Subject has received an active or passive immunization within 4 weeks prior to Visit 8, except for influenza or pandemic vaccines which may be administered outside a 7-days interval before booster vaccination.

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 10 days after the screening visit). In case a first vaccination cannot be rescheduled within the specified time window (i.e., within 10 days after the screening visit), the subject might be invited for a rescreening.

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

   The rescheduled visit should be within the specified time window.

Forbidden concomitant therapies during study conduct are described in Section 6.2.2.

STUDY ENDPOINTS

Primary Endpoints:

Safety:

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

Immunogenicity:

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

Secondary Endpoints:

Safety:

+ Frequency of solicited local and solicited systemic AEs within 7 days after booster dose administration (Part B).
+ Frequency of SAEs during the entire study;
+ Frequency of AESIs during the entire study;

- Frequency of unsolicited AEs within 28 days after each vaccination;
- Frequency of SAEs, AESIs, unsolicited and solicited AEs stratified by age cohort.

Immunogenicity:

Part A: Main Study Phase:

- GMTs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at baseline (screening visit) and at Day 85, 180, 194*, 365 and Month 18;
- SCRs (Seroconversion Rate, defined as seroconversion from seronegative to seropositive or ≥four-fold increase in IgG titer compared to baseline if subject was tested OspA seropositive at baseline) for each OspA serotype specific IgG (ST1 to ST6), determined by an IgG binding assay, at Day 85, 180, 194*, 208, 365 and Month 18;
- GMFRs (Geometric Mean of the fold rise as compared to baseline) for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at Day 85 and 208;
- GMTs, SCRs and GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at specified time-points, stratified by age cohort.

Part B: Booster Phase:

- GMTs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at Month 18, 19, 23, 26, 30, 36, 42, 48 and 54;
- SCRs for each OspA serotype specific IgG (ST1 to ST6), determined by an IgG binding assay, at Month 18, 19, 23, 26, 30, 36, 42, 48 and 54;
- GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at Month 19;
- GMTs, SCRs and GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at specified time points, stratified by age cohort.

Exploratory Endpoints:

CCI [redacted]

[redacted]

[redacted]

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

CCI [redacted]

[redacted]

SAMPLE SIZE JUSTIFICATION

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

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

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

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

STATISTICAL METHODS

The primary immunogenicity analysis will be an overall and group-wise comparison of the OspA serotype specific IgG GMTs in the per-protocol analysis set between study groups at Day 208 (i.e. 28 days after the last vaccination) by ANOVA (factors treatment group, age group). In addition, GMTs and GMFRs (i.e., increase of IgG titers compared to respective titers at Day 1 (baseline)) against each OspA serotype ST1 to ST6 will be compared overall and pair-wise between study groups on specified time points. For Part B GMFRs at Months 19 will be calculated compared with respective titers at Day 208 (i.e., primary peak response) and Month 18 (pre-booster). SCRs will be compared overall and pair-wise between study groups by Fisher Freeman Halton test and Fisher exact test, respectively. Defined immunogenicity analyses will be repeated on the full analysis set, and will be repeated by baseline *B.b.* s.l. serostatus and age cohort.

All subjects entered into the study, who received at least one vaccination, will be included in the Safety Analysis Set (SAS) of Part A. The number and percentage of subjects with solicited local and solicited systemic AEs up to 7 days after each vaccination, after any active vaccination, and up to 7 days after any vaccination of the primary vaccination series (Month 0-2-6); as well as the number and percentage of subjects with unsolicited AEs, medically attended AEs, AESIs and SAEs will be presented for each study group overall and by body system/ preferred term. The same analyses will be performed for all subjects that received a booster vaccination (Booster Safety Analysis Set; BSAS) in Part B.
Differences between the study groups will be assessed for significance using Fisher's exact (Fisher Freeman Halton) test, whereby a significant overall test will be amended by pair-wise tests. Defined safety analyses will also be repeated stratified by baseline *B.b.* s.l. serostatus and age cohort.

DATA ANALYSES

Analysis 1 will include all safety and immunogenicity data from selected time points from Part A up to Day 208/ Month 7. This analysis will be done in four parts. Every age cohort will be analyzed separately as soon as data of this cohort is available (Analysis 1.1 (Cohort 1, aged 18-65 years), Analysis 1.2 (Cohort 2, aged 12-17 years), Analysis 1.3 (Cohort 3, aged 5-11 years)). Pooled data from all three cohorts will conclude Analysis 1.

Analysis 2 will include all safety and immunogenicity data from selected time points from Part A up to Month 12 (i.e., 6 months after the third immunization).

Analysis 3 will include all safety data and immunogenicity data from selected time points from Part A up to Month 18 and safety and immunogenicity data from Part B up to Month 19.

Analysis 4 will include safety and immunogenicity data of Part B up to Month 26.
Analysis 5 will include safety and immunogenicity data of Part B up to Month 30.
Analysis 6 will include safety and immunogenicity data of Part B up to Month 42.
Analysis 7 will include safety and immunogenicity data of Part B up to Month 54.

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

STUDY REPORTS

Clinical study report 1 will compile the data from Analysis 1.

Clinical study report 2 will include the data compiled in Analysis 2 and 3.

Clinical study report 3 will include the data compiled in Analysis 4.

Clinical study report 4 will include the data compiled in Analysis 5 and 6.

Clinical study report 5 will include the data compiled in Analysis 7.

SAFETY AND DATA MONITORING

All subjects will report all symptoms (solicited local and systemic AEs, unsolicited AEs) after each vaccination, as described below. The subjects / legal representative(s) will be asked to complete electronic Subject Diaries (eDiaries).

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 daily via an eDiary. Assessments by the subjects/ legal representative(s) will occur for a total of seven consecutive days, each day at the approximate same time, starting at the day of each vaccination. The subjects/ legal representative(s) 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), nausea, vomiting and fatigue.

Unsolicited Adverse Events

Unsolicited AEs will be captured in the eDiary until 28 days after each vaccination. The eDiary entries will be verified by the study clinician together with the subject/ legal representative(s) at the subject's next visit to the study site. Unsolicited AEs will be captured in the eCRF up to 28

days after each vaccination. Thereafter, reporting of AEs in the eCRF will be limited to AESIs (Adverse Events of Special Interest) and SAEs (Serious Adverse Events). AESIs and SAEs will be collected throughout the entire study period. SAEs need to be reported to Pfizer Safety and AESIs to CCI Safety Desk.

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:

- Erythema migrans: an 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 disturbances, myocarditis);
- Immune-mediated disorders as proposed by competent authorities for previous clinical programs (please refer to APPENDIX 1).

For avoidance of doubt, <u>solicited AEs do not qualify as AESI and should not be reported as such</u>.

As part of unsolicited AE assessments, at each study visit starting from Visit 1A, 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 asked 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. 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 CCI 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 CCI Safety Desk. In addition, the DSMB will regularly review accruing AEs and can recommend specialist work-up to the investigator 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.

INTERNAL REVIEW COMMITTEE (IRC)

As VLA15-221 is the first study with VLA15 enrolling a pediatric study population, rigorous safety monitoring precautions will be implemented to ensure well-being of all subjects. An independent Internal Review Committee (IRC) will be installed at the sponsor's collaboration partner Pfizer in order to review safety data of sentinel subjects in each age cohort prior to advancing the study into the next younger age cohort and prior to enrolling remaining subjects within each of the pediatric age cohorts (i.e. Cohort 2 and Cohort 3). The IRC will be composed of qualified personnel from Pfizer, and will include at least one pediatrician, statistician, vaccine safety expert and a senior member of the Pfizer Vaccine Research and Development organization. All IRC members will be independent from the VLA15-221 study team. During the staggered subject enrollment phase, the IRC will review unblinded safety data and will give recommendation on whether enrollment of the next younger age cohorts as well as enrollment of remaining subjects of the current age cohort can be initiated as described in Section 4.2.1 ("Subject enrollment"). A written IRC charter will be provided prior to the study start, including a detailed description of IRC set-up and processes.

DATA SAFETY MONITORING BOARD (DSMB)

An external independent Data Safety Monitoring Board (DSMB) comprising an experienced vaccinologist/pediatrician, a family practitioner/pediatrician, a rheumatologist and a neurologist, who are all experts in the field of Lyme borreliosis, will be installed. The DSMB will review accruing safety data in an unblinded manner at regular intervals throughout the study and might recommend adjusting, pausing or discontinuing the study at any time.

The DSMB will confirm diagnosis of potential LB cases based on medical records received from the investigator after clinical workup according to a standardized procedure on an ad-hoc basis. The DSMB will do an adjudication of AESIs and will assess whether cases were new in onset and whether there is any relationship to the study vaccine.

During vaccination periods (i.e. Day 1 to Day 180 in Part A; Month 18 in Part B), the DSMB will periodically review tables and listings of SAEs, Deaths, AESIs, medically attended AEs, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination on a regular basis in scheduled meetings and via remote safety data reviews. SAE reports will be provided to the DSMB up to one year after booster administration. AESIs will be reviewed by the DSMB on an ad-hoc basis up to one year after booster administration. Thereafter, SAEs and AESIs will be reviewed on an ad-hoc basis by Pfizer up to end of the study. A written DSMB charter will be provided including a detailed description of DSMB set-up and processes.

Table 2 TABLE OF EVENTS – Part A: MAIN STUDY PHASE

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

Table 3 TABLE OF EVENTS – Part B: BOOSTER PHASE

Visit	V8B (35)	V9	V10	V11	V12	V13	V14	V15	V16	Early Termination (36)
Month (M)	M18	M19 V8B + 28d	M23	M26	M30	M36	M42	M48	M54	<V16
Time windows (D)	V8 +14	+/- 4	+/- 14	+/- 14	+/-28	+/-28	+/-28	+/-28	+/-28	n/a
Visit type	In-person	In-person	In-person	In-person	In-person	In-person	In-person	In-person	In-person	In-person
Inclusion/Exclusion criteria (Booster Phase)	X									
Vaccination delay criteria	X									
Concomitant medications/ treatments incl. vaccinations	X (37)	X	X	X	X	X	X	X	X	X
Physical examination (9)	X									
Vital signs (10)	X									
Evaluation of oral body temperature	X (11)									
B.b. s.l. screening test [5.0 mL] (13) (38)					X		X		X	X
Urine Pregnancy test (15)	X (16)	X	X							X
Immunogenicity blood sample [12.0 mL] (17)		X	X	X	X	X	X	X	X	
Assay development sample [75.0 mL] (18)		X								
VACCINATION (20)	X									
Check for AEs following vaccination	X (21)									
Symptom-driven physical exam (22)		X	X	X	X	X	X	X	X	X
Inspection of injection site of previous vaccinations		X								X (25)
Explain eDiary (24)	X									
Review eDiary		X								X (26)
Check eDiary for SAEs/ AESIs	X	X	X	X	X	X	X	X	X	X
Scripted Safety Assessment	X	X	X	X	X	X	X	X	X	X
AE/ SAE/ AESI Assessment (27)	X	X	X	X	X	X	X	X	X	X
Blood Volume [mL]	0.0	12.0 (33); 87.0 (34)	12.0	12.0	17.0	12.0	17.0	12.0	17.0	5.0

(1) Re-screening of subjects is allowed once. Assessments, which have been performed during the first screening visit, remain valid for 14 days. In case re-screening occurs outside this time frame, already performed measures have to be repeated.
(2) At on-site Visits, subjects will be supplied with urine pregnancy test kits to ensure that subjects can test for pregnancy at home in case the COVID-19 pandemic prohibits in-person visits at subsequent scheduled visits (Visit 2, 4, 6, 7 and 8). Pregnancy test handling has to be explained to subjects by trained study site staff during Visit 1.
(3) Visit 1A is a safety follow-up visit to be performed 7 days after the first vaccination (i.e. on Day 8). It is intended to perform Visit 1A remotely via a phone/video call in adult subjects (aged 18-65 years) and preferably as an in-person visit in adolescent subjects (aged 12-17 years) and children (5-11 years). If the COVID-19 pandemic prohibits in-person visits, Visit 1A may also be conducted remotely via a phone/video call in the younger age groups. For details on differences in respective study event procedures during in-person or remote visits, please refer to Section 4.2.1.
(4) Visit should preferably be conducted as an in-person visit. If an in-person is not feasible due to COVID-19, e.g. travel restrictions, local recommendations, circumstances at the study site's location that prohibit an in-person visit, or if the PI believes that the subject's safety and well-being might be jeopardized with an in-person visit at the study site due to COVID-19, the visit should be conducted remotely. In case subject visit has to be performed remotely, a mobile nurse professional will come to the subject's home and take the immunogenicity sample within the specified time window. Review of subject eDiary safety data will be performed by the study site via phone/video call. For details on differences in respective study event procedures during in-person or remote visits, please refer to Section 4.2.4. In order to perform urine pregnancy test at home, subjects will be supplied with additional test kits at the last on-site Visit.
(5) Visit 5A will be performed in a subset of approximately 150 adult subjects only.
(6) Every effort should be made to have discontinued subjects complete the early termination visit. If the subject is unwilling to perform an ET visit, a phone-call should be made to follow-up on Adverse Events and Concomitant Medications/ Vaccinations. Note: If a subject presents at a regular study visit and informs the site that it will discontinue the study after this visit, the study visit will not be performed as an ET visit, but as a regular study visit including all events that are described for the respective study and ET visit.
(7) Informed consent/assent may be obtained within 10 days before Visit 1. For the Booster Phase, subjects are asked to reconfirm the consent/assent within 14 days before Visit 8B.
(8) Symptoms noted at Visit 1 (prior to first vaccination) are not considered AEs but will be recorded as medical history.
(9) Physical examination on the following body systems: general appearance (including assessment of body weight and height), skin, head/ eyes /ears/ nose/ throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, and neurological system. An ECG will be performed at Visit 0 only.
(10) Vital signs (Systolic and diastolic blood pressure and pulse rate while seated and at rest) to be measured prior to vaccination and in addition prior to discharge in case subject reports any complaints.
(11) To be performed prior to vaccination.
(12) The results of negative HIV tests that were performed up to 4 weeks before Visit 0 are acceptable (blood: HIV test 3.0 mL). Positive HIV test obtained by ELISA will have to be confirmed by a second method (e.g. Western Blot or PCR). HIV tests only in subjects aged ≥ 12 years.
(13) A commercially available Lyme borreliosis screening test will be performed (blood: 5.0 mL). Serum samples that are tested positive will have to be verified by a confirmatory immunoblot. Test result does not need to be available before randomization and remains valid for 4 weeks.
(14) In case an ET Visit is performed remotely, the collection of the Lyme borreliosis screening sample will be omitted.
(15) Female subjects aged ≥12 years and woman of childbearing potential. A woman is considered of childbearing potential if fertile and until becoming post-menopausal unless permanently sterile. A woman that is considered of non-childbearing potential must be e.g. surgically sterilized for at least 3 months prior to Visit 1 (e.g. by hysterectomy, bilateral salpingectomy, bilateral oophorectomy, transcervical sterilization), or postmenopausal for at least one year prior to Visit 1. For serum pregnancy test: tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable. Testing will only be performed in the 5 to 11 age cohort in female subjects after onset of menarche.
(16) At vaccination visits, all samples have to be obtained before vaccination. Pregnancy results must be available before vaccination.
(17) Blood will be collected for immunogenicity testing by an IgG binding assay CCI
(18) Assay development sera will be collected in adult subjects (age cohort 18 to 65 years) only and will be used for further development of clinical assays.
(19) To be performed by study staff otherwise not involved with study conduct to keep the study observer-blinded (i.e. un-blinded study staff)
(20) Preparation of vaccination must be done by designated unblinded staff members (4.2.12) only. Administration of the vaccine can be performed by blinded OR unblinded staff. Vaccination with the assigned treatment has to be administered into the deltoid of the non-dominant arm. Subjects should be observed for at least 30 minutes for treatment of any immediate reactions.
(21) If subject has any complaints after vaccination, a symptom-driven physical examination will be performed by the investigator prior to discharge.

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

SIGNATURE PAGE

Title of Clinical Trial:	**SAFETY AND IMMUNOGENICITY STUDY OF VLA15, A MULTIVALENT RECOMBINANT OSPA BASED VACCINE CANDIDATE AGAINST LYME BORRELIOSIS: A RANDOMIZED, CONTROLLED, OBSERVER-BLIND PHASE 2 STUDY IN A HEALTHY PEDIATRIC AND ADULT STUDY POPULATION**
Study Code:	VLA15-221
IND number:	CCI

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

PPD
Valneva Austria GmbH

Signature Date

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Signature Date

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LIST OF RESPONSIBLE PERSONNEL

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Valneva Austria GmbH
Phone: PPD
Mobile Phone: PPD
Fax: PPD
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Monitoring:

PPD
PPD
Phone: PPD
Fax: PPD

Serious Adverse Event (SAE) reporting by fax within 24 hours after discovery:

Pfizer Safety
SAE fax number: PPD

AESI reporting by email to:

PPD **Safety Desk**
PPD
Phone: PPD
AESI e-mail: PPD

Study Medical Monitor:

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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Event of Special Interest
Alum	$Al(OH)_3$, Aluminum Hydroxide
ANOVA	Analysis of variance
ATC	Anatomical Therapeutic Chemical
B.b. s.l.	*Borrelia burgdorferi* sensu lato
BFAS	Booster Full Analysis Set
BPPAS	Booster Per-Protocol Analysis Set
BSAS	Booster Safety Analysis Set
BSL2	Biological Safety Level 2
CA	Competent Authority
CI	Confidence Interval
CR	Clinically Relevant
CRA	Clinical Research Associate
CMO	Contract Manufacturing Organization
eCRF	Electronic Case Report Form
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
FAS	Full Analysis Set
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	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
i.e.	That Is
I.M.	Intramuscular
IgG	Immunoglobulin G
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
kDa	Kilo Dalton
IRC	Internal Review Board
ITT	Intent-to-Treat
LB	Lyme borreliosis
MedDRA	Medical Dictionary for Regulatory Activities
µg	Microgram
M	Month
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
PPAS	Per-Protocol Analysis Set

SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
CCI	[redacted]
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
yrs	Years

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 with a ≈62% rise in median Lyme disease diagnose incidences during 2010-2018 compared to 2005-2010[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. 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 kDa. 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]. 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 amino acids 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. Each protein is fused to an N-terminal lipid moiety which results in higher immunogenicity as shown in preclinical tests.

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 90 µg 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 demonstrated 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.

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

At the time of release date of this CSP Version 8.0, the clinical development program of VLA15 comprises one completed Phase 1 study (VLA15-101) and two previous Phase 2 studies (VLA15-201 and VLA15-202). Table 4 summarizes the VLA15 Clinical Development Program:

Table 4 Overview of the VLA15 Clinical Development Program

		Study Title	Vaccination Schedule (Month)	Number of Subjects	Randomization	Treatment	Study Region	Study Status
Phase 1	VLA15-101	Study assessing the safety, immunogenicity and dose response of VLA15, a new multivalent recombinant OspA vaccine candidate against Lyme borreliosis, in healthy adults aged below 40 years	0-1-2	179	1:1:1	12 µg w/ or w/o Alum 48 µg w/ or w/o Alum 90 µg w/ or w/o Alum	Non-endemic Europe & US	Completed
		Booster Extension of Study VLA15-101	13	64	1:1	48 µg w/ or w/o Alum 90 µg w/ or w/o Alum	Non-endemic Europe	Completed
Phase 2*	VLA15-201	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	0-1-2	Run-In Phase: 120 Main Study Phase: 452	1:1:1:1 2:2:1	(90 µg)† 135 µg 180 µg Placebo	Endemic Europe & US	Completed
	VLA15-202	Alternative schedule study for 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.	0-2-6	246	2:2:1	135 µg 180 µg Placebo	Endemic US	Completed
		Booster Extension of Study VLA15-202	18	58	2:1	180 µg Placebo		Ongoing - Vaccinations completed
	VLA15-221	Safety and immunogenicity study of VLA15, a multivalent recombinant OspA based vaccine candidate against Lyme Borreliosis: A randomized, controlled, observer-blind Phase 2 study in a healthy pediatric and adult study population	0-2-6 or 0-6	625	2:1	180 µg Placebo	Endemic US	Ongoing
		Booster Phase of Study VLA15-221	18	~600		180 µg Placebo		Planned Start: August 2022

* In Phase 2 only alum adjuvanted VLA15 presentations are applied.
† Run-In Phase only

1.4.3.1 Phase 1 Study VLA15-101

The first clinical study with VLA15 was conducted in 179 healthy adults aged 18 to <40 years in Belgium and in the US. As this Phase 1 study was the first-in-human study with VLA15, special safety measures were implemented and the first 24 subjects were vaccinated open-label and in a staggered dose escalation approach. Thereafter the study was conducted in an observer-blinded manner. The remaining 155 subjects were randomized into six study groups to receive either 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. The vaccinations were administered I.M. at 28 days apart (i.e. at Month 0-1-2).

Data from the primary vaccination series revealed that VLA15 was generally safe and well tolerated in all study groups with no associated safety concerns. In addition, this part of the study showed that VLA15 was immunogenic in all doses and formulations tested with adjuvanted formulations being more immunogenic than non-adjuvanted formulations of the same dose level.

Subjects that were enrolled in Belgium, who completed the primary immunization schedule (three vaccinations) and were assigned in the 48 µg or 90 µg dose groups, were asked to participate in a Booster Extension. In total, 64 subjects (out of 179 subjects from the Initial Study) participated in the Booster Extension and received a booster dose approximately one year after the first vaccination. The administration of a booster dose was also generally safe and well tolerated in all study groups. In addition, the booster vaccination resulted in a pronounced antibody titer increase for all doses and formulations tested and all for OspA serotypes (ST1-ST6).

Safety and immunogenicity results of the Phase 1 study VLA15-101 are described in more detail in the Clinical Study Report, dated 24 April 2019. Safety and Immunogenicity results of the Booster Extension of the VLA15-101 study are described in more detail in the Clinical Study Report, dated 05 December 2019. In addition, summarized safety and immunogenicity data of Phase 1 can be found in the current version of the Investigator's Brochure.

1.4.3.2 Phase 2 Studies VLA15-201 and VLA15-202

Two Phase 2 studies (VLA15-201 and VLA15-202) have been completed. Both studies are investigating higher VLA15 dose levels in adjuvanted forms as compared to Phase 1 as well as two different immunization schedules (Month 0-1-2 and Month 0-2-6). In both studies, subjects received in total three injections of either 135 µg VLA15, 180 µg VLA15 or placebo. Further on, eligible subjects receiving the VLA15 180 µg dose in study VLA15-202 were re-randomized to receive a booster dose of VLA15 180 µg or placebo at Month 18. A DSMB (same DSMB for both studies) has reviewed unblinded accruing safety information of both studies in parallel at regular intervals. Until now, the DSMB has not identified any safety concerns.

<u>VLA15-201 Phase 2 Clinical Study</u>

VLA15-201 was an observer-blinded, randomized, multicenter Phase 2 study. Main objective was to determine the optimal dose of VLA15 in healthy adults aged 18 to 65 years. The study started with a 120 subjects (18-40 years) Run-In Phase to evaluate safety of the higher dose levels of VLA15 w/ alum initially in a smaller population (90 µg: N=29; 135 µg: N=31; 180 µg: N= 30; Placebo: N=30). After review of safety data by an independent DSMB, 452 subjects (135 µg: N=183; 180 µg=175; Placebo: N=94) aged 18-65 years were enrolled in the Main Study Phase to receive according to the allocated study group three injections of either VLA15 or placebo at Month 0-1-2. Subjects were followed for safety and immunogenicity up to one year after administration of the first vaccination.

Overall, 517 of 572 subjects (90.4%) reported any solicited or unsolicited AE. Most AEs were mild to moderate in severity. A total of 18 subjects (3.1%) experienced severe related AEs, all

of them were solicited AEs and as such counted per definition as related: nine subjects (4.2%) in the 135 µg group and nine subjects (4.4%) in the 180 µg group. Solicited local AEs were reported by 89.7% (90 µg group), 93.0% (135 µg group) and 96.1% (180 µg group) of subjects, compared to 29.8% of subjects in the placebo group. The most common solicited local AEs were pain (84.6%, pooled VLA15 groups) and tenderness (92.2%, pooled VLA15 groups). Solicited systemic AEs were reported by 62.1% (90 µg group), 67.8% (135 µg group) and 71.7% (180 µg group) of subjects, compared to 42.7% of subjects in the placebo group. The most common solicited systemic AEs were headache (35.7%, pooled VLA15 groups), fatigue (33.9%, pooled VLA15 groups) and myalgia (47.8%, pooled VLA15 groups). In general, rates of solicited local and systemic AEs declined with subsequent vaccinations. An overview of solicited AEs is shown in Table 5 and Table 6.

No vaccine related severe unsolicited Adverse Event and no related Serious Adverse Event was reported. One case of osteoarthritis (135 µg group) was reported as AESI. This case was initially reported as suspected symptomatic borreliosis and assessed as possibly being related by the investigator. After detailed clinical workup including diagnostic serology testing and x-ray analysis by a rheumatologist, the DSMB concluded that this case is an osteoarthritis and not related to study vaccination. Diagnosis of osteoarthritis was agreed by the investigator. In addition, three cases (1.4%) of Lyme disease/Erythema migrans were reported in the 135 µg group and one case (0.5%) of Polyarthritis was reported in the 180 µg group as AESI. All of these were assessed as unrelated to study vaccine.

VLA15 was immunogenic at all dose levels tested and induced significant IgG antibody levels by Day 85 (i.e., 1 month after completion of the vaccination series). A dose response was observed, with lowest IgG titers being observed in the 90 µg dose group and highest titers being observed in the 180 µg dose group for all serotypes. At Day 85, in the 90 µg group, the GMTs ranged from 74.3 (ST1) to 267.4 (ST3). In the 135 µg group the GMTs reached levels of 101.9 (ST1) to 283.2 (ST3), whereas in the 180 µg group GMTs were 115.8 (ST1) to 308.6 (ST3). Differences in GMTs between VLA15 dose groups were not significant.

VLA15-202 Phase 2 Clinical Study

VLA15-202 is an observer-blinded, randomized, multicentre Phase 2 study. Main objective of this study is the investigation of the immune response of three primary vaccinations applied at an alternative immunization schedule, i.e., Month 0-2-6 (Main Study Phase). Further on, eligible subjects receiving the VLA15 180 µg dose were re-randomized to receive a booster dose of VLA15 180 µg or placebo at Month 18 (Booster Phase).

A total of 246 healthy adults aged 18-65 years were randomized 2:2:1 to receive three VLA15 vaccinations of either 135 µg w/ alum (N=97), 180 µg w/ alum (N=98) or placebo (N=51) at Month 0, 2 and 6. Thereof, 58 subjects from the VLA15 180 µg group were re-randomized to receive a booster vaccination of VLA15 180 µg (N=39) or placebo (N=19) at Month 18.

To date, safety and immunogenicity data up to Month 19 (i.e., 1 month after the booster vaccination) are available.

In the Main Study Phase, a total of 234 of 246 subjects (95.1%) reported any solicited or unsolicited AE up to Month 18 (i.e., one year after the last dose of the primary vaccination series). Most AEs were mild or moderate in severity. A total of 15 subjects (6.1%) experienced severe related AEs; 14 (5.7%) subjects experienced at least one severe solicited AEs of Grade 3, as such counted per definition as related: 6 subjects (6.2 %) in the 135 µg group, 7 subjects (7.1%) in the 180 µg group, and 1 subject (2.0 %) in the placebo group. One subject in the 135 µg group experienced a severe unsolicited event of Ventricular extrasystoles which was assessed as possibly related to study vaccine by the investigator. Subject had a history of benign premature ventricular contractions. The event occurred 13 days after the second vaccination and was treated with Propranolol. The event recovered after 39 days.

Solicited local AEs after the primary vaccination series were reported by 95.9% (135 µg group) and 98.0% (180 µg group) of subjects, compared to 45.1% of subjects in the placebo group. The most common solicited local AEs were pain (89.2%, pooled VLA15 groups) and tenderness (95.9%, pooled VLA15 groups). Solicited systemic AEs were reported by 78.4% (135 µg group) and 69.4% (180 µg group) of subjects, compared to 51.0% of subjects in the placebo group. The most common solicited systemic AEs were myalgia (54.9%, pooled VLA15 groups), headache (44.6%, pooled VLA15 groups) and fatigue (31.8%, pooled VLA15 groups). Rates of solicited local and systemic AEs declined after the first vaccination. Most solicited AEs were mild or moderate. An overview of solicited AEs after the primary vaccination series is shown in Table 5 and Table 6.

After administration of a booster dose of VLA15 180 µg at Month 18, 89.7% of subjects reported any solicited local AE: 63.2 % pain, 89.5% tenderness, 19.4% erythema, 7.9% swelling, 5.3% induration. Further on, 51.3% of subjects reported any solicited systemic AE: 21.1% headache, 2.7% flu like symptoms, 7.9% nausea, 2.7% vomiting, 18.4% fatigue, 10.5% arthralgia and 28.9% myalgia.

No related serious AE was reported up to Month 19. Two events were reported as AESI (Adverse Event of Special Interest): One case of Lyme disease (135 µg group): erythematous rash (approximately 2 cm), developed approximately 2 weeks after 1st vaccination. One case of ventricular extrasystoles (135 µg group) which was assessed as possibly related to study vaccine by the Investigator. The subject had a history of benign premature ventricular contractions. No other cases of predefined AESIs, e.g. arthritis, rheumatoid arthritis or facial paralysis, were observed.

VLA15 was immunogenic at both dose levels (135 µg, 180 µg) tested. GMTs at Day 208 (i.e., 1 months after the third vaccine dose) ranged from 278.5 (ST1) to 545.2 (ST2) in the 135 µg group and from 274.7 (ST1) to 596.8 (ST3) in the 180 µg group. There was a trend that the 180 µg dose induces a faster onset of immunity. Functionality of antibodies could be demonstrated using a CCI for all serotypes with GMTs ranging from 30.9 (ST5) to 1201.8 (ST3) in the 135 µg group and from 36.8 (ST5) to 1408.0 (ST3) in the 180 µg group at Day 208. Respective seroconversion rates (SCRs) ranged from 41.9% (ST5) to 94.8% (ST3) in the 135 µg group and from 44.7% (ST5) to 97.5% (ST3) in the 180 µg group. CCI At Month 19, one month after booster dose administration, GMTs increased by factor 2.9 (ST3) to 4.2 (ST1, ST4) as compared to Day 208 (peak levels after primary vaccination series) and ranged from 1253.6 (ST1) to 2160.4 (ST2) in the 180 µg with booster group.

Table 5 Solicited Local Adverse Events after any Vaccination (primary vaccination series) by Symptom, Safety Population

	VLA15-201 Study			VLA15-202 Study	
	90 µg w Alum N=29 n (%)	135 µg w Alum N=214 n (%)	180 µg w Alum N=205 n (%)	135 µg w Alum N=97 n (%)	180 µg w Alum N=98 n (%)
Solicited local AE after any vacc.	26 (89.7)[a]	199 (93.0)[a]	197 (96.1)[a]	93 (95.9)[c]	96 (98.0)[c]
Pain	22 (75.9)[a]	176 (82.2)[a]	181 (88.3)[a]	87 (89.7)[c]	87 (88.8)[c]
Tenderness	25 (86.2)[a]	194 (90.7)[a]	194 (94.6)[a]	92 (94.8)[c]	95 (96.9)[c]
Erythema	8 (27.6)[a]	68 (31.8)[a]	72 (35.1)[a]	28 (28.9)	41 (41.8)[d]
Swelling	4 (13.8)[a]	57 (26.6)[a]	68 (33.2)[a]	24 (24.7)[c]	32 (32.7)[c]
Induration	3 (10.3)[a,b]	64 (29.9)[a,b]	65 (31.7)[a,b]	27 (27.8)[c]	29 (29.6)[c]

n: number of subjects with event
N: number of subjects studied in treatment group
[a] *significant pairwise comparison Placebo vs. 90 µg, Placebo vs. 135 µg, Placebo vs. 180 µg*
[b] *significant pairwise comparison: 90 µg vs. 135 µg, 90 µg vs. 180 µg*
[c] *significant pairwise comparison Placebo vs. 135 µg, Placebo vs. 180 µg*
[d] *significant pairwise comparison Placebo vs. 180 µg*

Table 6 Solicited Systemic Adverse Events after any Vaccination (primary vaccination series) by Symptom, Safety Population

	VLA15-201 Study			VLA15-202 Study	
	90 µg w Alum N=29 n (%)	135 µg w Alum N=214 n (%)	180 µg w Alum N=205 n (%)	135 µg w Alum N=97 n (%)	180 µg w Alum N=98 n (%)
Solicited systemic AE after any vacc.	18 (62.1)	145 (67.8)[b]	147 (71.7)[b]	76 (78.4)[b]	68 (69.4)[b]
Headache	10 (34.5)	69 (32.2)	81 (39.5)	45 (46.4)	42 (42.9)
Fever	2 (6.9)	5 (2.3)	5 (2.4)	7 (7.2)	4 (4.1)
Flu like symptoms	3 (10.3)	23 (10.7)	33 (16.1)	17 (17.5)	7 (7.1)
Nausea	4 (13.8)	23 (10.7)	32 (15.6)	17 (17.5)	15 (15.3)
Vomiting	1 (3.4)	4 (1.9)	6 (2.9)	2 (2.1)	3 (3.1)
Fatigue	8 (27.6)	65 (30.4)	79 (38.5)[c]	30 (30.9)	32 (32.7)
Arthralgia	2 (6.9)	35 (16.4)	35 (17.1)	20 (20.6)	21 (21.4)
Myalgia	14 (48.3)[a]	103 (48.1)[a]	97 (47.3)[a]	53 (54.6)[b]	54 (55.1)[b]

n: number of subjects with event
N: number of subjects studied in treatment group

[a] *significant pairwise comparison Placebo vs 90 µg, Placebo vs 135 µg, Placebo vs 180 µg*
[b] *significant pairwise comparison Placebo vs 135 µg, Placebo vs 180 µg*
[c] *significant pairwise comparison: Placebo vs 180 µg*

In summary, the safety and immunogenicity data of Phase 1 and Phase 2 are encouraging, with a positive Benefit Risk profile at all doses. The AE profile of VLA15, including a booster dose, is comparable to licensed lipidated recombinant vaccines or lipid-containing formulations. Moreover, to date, an independent DSMB has not identified any safety concerns. Higher VLA15 doses and a broader immunization schedule investigated in the Phase 2 studies led to a further optimized immune response. In addition, administration of a booster dose led to a substantial anamnestic response. Available data support advancing the vaccine development with the 180 µg dose.

For more detailed information on available data, please refer to the current version of the Investigator's Brochure.

1.5 Study Rationale

Study VLA15-221 is conducted to compare two different vaccination schedules of VLA15 and to generate a sufficient safety database for advancing the vaccine candidate into Phase 3. VLA15-221 is the first study to involve children and adolescents.

Therefore, as a safety precaution, subject enrollment at study start of the Main Study Phase (Part A) will be performed in an age-descending, staggered manner (see Figure 1 and Section 4.2.1) with in total 90 sentinel subjects (30 subjects per age cohort). Staggered enrollment of younger age cohorts (12-17 years and 5-11 years, respectively) will only start upon review of 7-day safety data incl 7-day eDiary data of the previous age cohort and positive recommendation by an independent Internal Review Committee (IRC).

Overall, it is anticipated to enroll 600 subjects aged 5-65 years. In Part A subjects will be randomized 1:1:1 into three study groups to receive either VLA15 at Month 0-2-6 or Month 0-6, respectively, or placebo. According to their initial study group allocation subjects will receive a booster injection of either VLA15 (Group 1 and 2) or placebo (Group 3) at Month 18 in Part B.

VLA15 will be administered at a dose of 180 µg. This dose has been selected based on available safety and immunogenicity data from Phase 2 studies VLA15-201 and VLA15-202.

The VLA15 presentation used in this study will be in a more concentrated form as compared to the presentation used in previous studies. It contains the same amount of antigen and aluminum adjuvant, and the same concentration of other excipients as the 1 mL presentation used previously. Thus, 0.5 mL of the new presentation will contain 180 µg VLA15 and 0.5 mg of alum. This presentation is planned to be the final vaccine presentation to be used in Phase 3 and for product commercialization. Hence, there will be a total of approximately 400 subjects who will have received VLA15 in the final presentation before advancing the vaccine into Phase 3.

Immunogenicity assessment will be done using an IgG binding assay. CCI

1.6 Risk - Benefit Analysis

Risks:
The safety of OspA based vaccines has previously been shown[16,17]. A theoretical risk put forward for the previously licensed OspA vaccine LYMErix, 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.

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.

Safety data in humans are available from a Phase 1 first-in-human study (VLA15-101) and from two Phase 2 studies (VLA15-201 and VLA15-202). Most AEs were reported to be mild or moderate and comprised typical vaccine reactions: solicited injection site reactions (mainly injection site pain (67.0 %, 84.6%, 89.2% for VLA15-101, VLA15-201 pooled VLA15 groups and VLA15-202 pooled VLA15 groups, respectively) and injection site tenderness (84.4%, 92.2% pooled VLA15 groups and 95.9% pooled VLA15 groups, for VLA15-101, VLA15-201, VLA15-202 respectively)) or solicited systemic reactions (mainly headache (44.7%, 35.7% pooled VLA15 groups and 44.6% pooled VLA15 groups, for VLA15-101, VLA15-201, VLA15-202 respectively), fatigue (25.1%, 33.9% pooled VLA15 groups and 31.8% pooled VLA15 groups, for VLA15-101, VLA15-201, VLA15-202 respectively) and myalgia (25.1%, 47.8% pooled VLA15 groups and 54.9% pooled VLA15 groups, for VLA15-101, VLA15-201, VLA15-202 respectively)).

Reactogenicity after a booster dose was comparable to the primary series. Few severe related AEs were reported, all of them comprised solicited AEs, as such counted per definition as related. Also, rates of fever, a sign for inflammatory reactions, were low, ranging from 1.7% in VLA15-101 to 5.6% (pooled VLA15 groups) in VLA15-202. No related SAE was reported and no case of rheumatoid arthritis or other immune-mediated events was reported in any study. During vaccination periods of all studies, safety data have been reviewed regularly by an independent DSMB. The DSMB did not identify any safety concern.
Overall, the AE profile of VLA15 appeared comparable to licensed lipidated recombinant vaccines or lipid-containing formulations (e.g., Trumenba®[18] or Bexsero®[19]) that might be associated with an increased inflammatory response through interaction with Toll-like receptors[20].

To date, the overall safety profile of VLA15 including a booster dose is favorable. Available data support advancing the vaccine development with the 180 µg dose. For more detailed information on available data, please refer to the current version of the Investigator's Brochure.

In course of the VLA15-221 clinical study, subjects aged 5 to 65 years will be enrolled. The study will assess for the first time the safety and immunogenicity of VLA15 in children and adolescents. Children aged 5-15 years are one of the main target populations for a vaccine against Lyme borreliosis and therefore early inclusion of this age group into the clinical development of VLA15 is of importance. In order to ensure the safety in all age groups, a staggered enrollment process with limited recruitment speed is planned to establish initial safety data in adults, before initiating enrollment in adolescents and further on in children and prior to progressing to a larger study population.

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. In addition, possible triggering of an autoimmune disease in predisposed subjects cannot be excluded.

Benefits:
OspA ST1 based vaccines have been shown to be protective against LB in humans before and VLA15 was effective in animal models and immunogenic in humans. 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 addition, a portion of subjects will receive placebo injections and will therefore not benefit from the vaccinations.

In view of the positive safety profile observed so far 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

Safety:

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

Immunogenicity:

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

2.2 Secondary Objectives

Safety:

- To assess the safety and tolerability profile of VLA15, applied in a three- or two dose primary immunization schedule (Month 0-2-6 or Month 0-6), in a healthy study population aged 5 to 65 years, up to one year after the last primary vaccination (Month 18).
- To assess the safety and tolerability profile of a booster dose of VLA15, applied one year after completion of the primary immunization schedule (i.e. Month 18), up to three years after the booster (i.e. Month 54).

Immunogenicity:

- To assess the immune response to VLA15, applied in a three- or two dose primary immunization schedule (Month 0-2-6 or Month 0-6), in a healthy study population aged 5 to 65 years, up to one year after the last primary vaccination (Month 18).
- To assess the immune response to a booster dose of VLA15, applied one year after completion of the primary immunization schedule (i.e. Month 18), up to three years after the booster (Month 54).

2.3 Exploratory Objective:

- [redacted]

3 SELECTION OF STUDY POPULATION

CRITERIA FOR INCLUSION/EXCLUSION

Approximately 600 male or female subjects who meet 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:

1. Subject is aged 5 to 65 years* at the day of screening (Visit 0);
2. Subject is of good general health as determined by medical history, physical examination, and judgement of the investigator
3. Parent(s)/legal representative(s) and subject who have an understanding of the study and its procedures as explained by the investigator and agree to its provisions:
   - For subjects aged 18-65 years†: Written informed consent prior to any study related procedures;
   - For subjects aged 5-17 years‡: Written informed consent by the subject's legal representative(s), according to local requirements, and written informed assent of the subject, if applicable, 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 according to following timelines:
      - Main Study Phase: duration of entire study
      - Booster Phase: until Month 23 (i.e., 5 months after booster dose)
5. Subject is willing and able to comply with scheduled visits, treatment plan, and other study procedures.
6. Subject is expected to be available for the duration of the study and can be contacted by telephone during study participation.

* From the 5th birthday until the last day before the 66th birthday
† From the 18th birthday until the last day before the 66th birthday
‡ From the 5th birthday until the last day before the 18th birthday

Exclusion criteria (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 Day 1/Visit 1;
2. Subject received previous vaccination against LB;
3. Subject had a tick bite within 4 weeks prior to Day 1/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 investigator's judgement, such as individuals with poorly controlled or unstable disease, ongoing suspected or active inflammation, or poor compliance with pharmacologic treatment;
5. Subject has a medical history of or currently has a neuro-inflammatory or autoimmune disease;
6. Subject has a known thrombocytopenia, bleeding disorder, or received anticoagulants in the 3 weeks prior to Day 1/Visit 1, contraindicating I.M. vaccination as judged by the investigator;
7. Subject has received an active or passive immunization within 4 weeks prior to Day 1/Visit 1;
8. Subject has received any other registered or non-registered medicinal product in another clinical trial within 4 weeks prior to vaccination at Day 1/Visit 1;
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 (e.g., radiation therapy, chemotherapy or glucocorticoid treatment) within 4 weeks prior to Day 1/Visit 1. Glucocorticoid 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 of unknown cause or severe allergic reactions of unknown cause. Subjects with known hypersensitivity or allergic reactions to one of the components of the vaccine are to be excluded;
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. Subjects with history of squamous cell or basal cell skin cancer which has been successfully treated by surgical excision and treatment is considered to have achieved cure may be enrolled. Subjects with a history of skin cancer must not be vaccinated at the previous tumor site;
12. Subject is pregnant (positive serum pregnancy test at Visit 0 or positive urine pregnancy test at Day 1/Visit 1), 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 during the course of the entire Main Study Phase;
13. Subject has donated or plans to donate blood or blood-derived products (e.g., plasma) within 4 weeks prior to Day 1/V1 or subject received blood or blood-derived products (e.g., plasma or immunoglobulins) within 3 months prior to vaccination at Day 1/Visit 1 or plans to donate or use blood or blood products within 4 weeks after vaccination at Day 1/Visit 1;

14. 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;
15. 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.

Exclusion criteria (Booster Phase):

Subjects who meet **ANY** of the following criteria are **NOT** eligible for participation in the Booster Phase:

1. Subject has developed a chronic illness related to Lyme borreliosis (LB) since Day 208/Visit 6 (i.e., Month 7) or an active symptomatic LB as suspected or diagnosed by a physician;
2. Subject has developed a clinically relevant disease (e.g., cardiovascular, respiratory, neurologic, psychiatric conditions, malignancy) which poses a risk for further 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;
3. Subject has developed a neuro-inflammatory or autoimmune disease since Day 180/Visit 5 (i.e., Month 6);
4. Subject has developed a thrombocytopenia, bleeding disorder, or received anticoagulants in the 3 weeks prior to Month 18/Visit 8, contraindicating I.M. vaccination as judged by the investigator;
5. Subject has developed a known or suspected defect of the immune system that would prevent an immune response to the vaccine, such as subjects with acquired immunodeficiency, including infection with human immunodeficiency virus (HIV), status post organ transplantation or immuno-suppressive therapy (e.g., radiation therapy, chemotherapy or glucocorticoid treatment) within 4 weeks prior to Month 18/Visit 8. Glucocorticoid 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;
6. Subject has experienced an anaphylaxis of unknown cause or severe allergic reactions of unknown cause or has developed hypersensitivity or allergic reactions to one of the components of the vaccine;
7. Subject is pregnant (positive urine pregnancy test), or is lactating at Month 18/Visit 8 or has plans to become pregnant before Month 23/Visit 10. Women of childbearing potential that are unwilling or unable to employ an adequate birth control measure up to Month 23/Visit 10;
8. Subject has donated or plans to donate blood or blood-derived products (e.g., plasma) within 4 weeks prior to Month 18/Visit 8 or received blood or blood-derived products (e.g., plasma or immunoglobulins) within 3 months prior to booster vaccination in this study or plans to donate or use blood or blood products within 4 weeks after the booster dose;
9. Subject has developed 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;

10. 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.

Booster Phase:

3. Subject has received an active or passive immunization within 4 weeks prior to Visit 8, except for influenza or pandemic vaccines which may be administered outside a 7-days interval before booster vaccination.

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 10 days after the screening visit). In case a first vaccination cannot be rescheduled within the specified time window (i.e., within 10 days after the screening visit), the subject might be invited for a rescreening.

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

    The rescheduled visit should be within the specified time window.

    Forbidden concomitant therapies during study conduct are described in Section 6.2.2.

4 INVESTIGATIONAL PLAN

4.1 Study Endpoints

Primary Endpoints:

Safety:

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

Immunogenicity:

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

Secondary Endpoints:

Safety:

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

Immunogenicity:

Part A: Main Study Phase:

+ GMTs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at baseline (screening visit) and at Day 85, 180, 194*, 365 and Month 18;
+ SCRs (Seroconversion Rate, defined as seroconversion from seronegative to seropositive or ≥four-fold increase in IgG titer compared to baseline if subject was tested OspA seropositive at baseline) for each OspA serotype specific IgG (ST1 to ST6), determined by an IgG binding assay, at Day 85, 180, 194*, 208, 365 and Month 18;
+ GMFRs (Geometric Mean of the fold rise as compared to baseline) for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at Day 85 and 208;
+ GMTs, SCRs and GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at specified time-points, stratified by age cohort.

Part B: Booster Phase:

+ GMTs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at Month 18, 19, 23, 26, 30, 36, 42, 48 and 54;
+ SCRs for each OspA serotype specific IgG (ST1 to ST6), determined by an IgG binding assay, at Month 18, 19, 23, 26, 30, 36, 42, 48 and 54;
+ GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at Month 19;

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

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

CCI

4.2 Study Design and Plan

4.2.1 Study Description

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

Subject Enrollment into Part A (Main Study Phase):

Enrollment will be performed in an age-descending, staggered manner for the three age cohorts (see Figure 1).

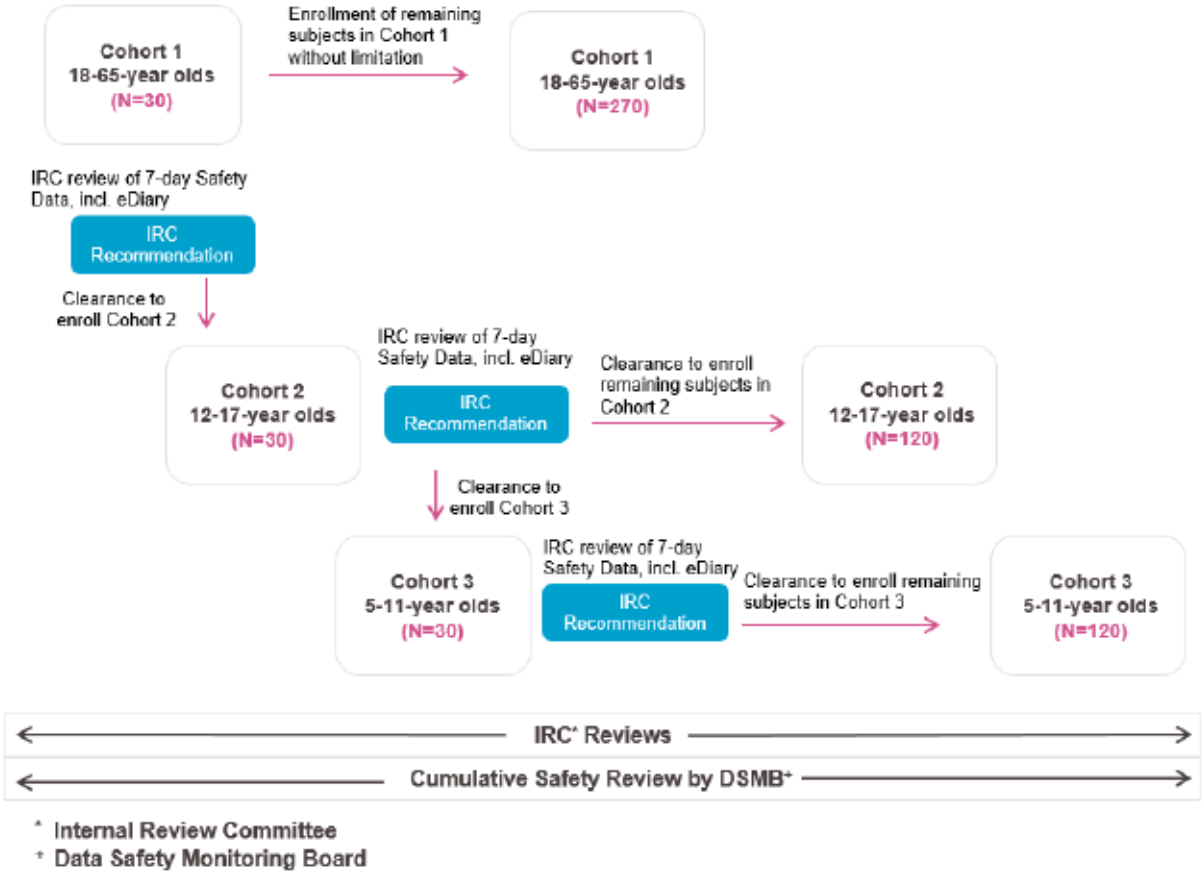

Figure 1 Subject enrollment process in Part A (Main Study Phase)

Enrollment Cohort 1 (18-65 years):

The enrollment will start with 30 sentinel adult subjects aged 18-65 years (Cohort 1). Subjects will be randomized 1:1:1 into one of three study groups to receive a vaccination of VLA15 (Groups 1 and 2) or placebo (Group 3). After these 30 sentinel subjects have received the first vaccination and have completed a 7-day post vaccination safety follow up visit (Visit 1A) including review of 7-day eDiary data, safety data will be reviewed in an unblinded manner by an independent Internal Review Committee (IRC). The IRC is composed of qualified personnel from Valneva's collaboration partner Pfizer. Based on this data, the IRC will make a recommendation on whether enrollment of the adolescent age cohort (12-17 years, Cohort 2) can be initiated. Enrollment of the remaining 270 adult subjects of Cohort 1 will continue without limitations during the IRC review.

Enrollment Cohort 2 (12-17 years):

Upon IRC clearance, 30 sentinel adolescent subjects aged 12-17 years (Cohort 2) will be enrolled. Subjects will be randomized 1:1:1 into one of the three study groups to receive a vaccination of VLA15 (Groups 1 and 2) or placebo (Group 3). Again, after these subjects have completed their safety follow up visit 7 days after the first vaccination (Visit 1A) including review of 7-day eDiary data, safety data will be reviewed by the IRC. The IRC will recommend whether enrollment of the youngest age cohort (5-11 years of age) can be initiated. In this age cohort, enrollment of the remaining 120 adolescent subjects will only be initiated after IRC clearance.

Enrollment Cohort 3 (5-11 years):

Upon IRC clearance, 30 sentinel subjects aged 5-11 years (Cohort 3) will be enrolled. Subjects will be randomized 1:1:1 into one of the three study groups to receive a vaccination of VLA15 (Groups 1 and 2) or placebo (Group 3). Again, after these 30 subjects have completed their safety follow up visit 7 days after the first vaccination (Visit 1A) including review of 7-day eDiary data, safety data will be reviewed by the IRC. Enrollment of the remaining 120 subjects in this age cohort will only be initiated after IRC clearance.

An external independent Data Safety Monitoring Board (DSMB) will review accruing safety data in an unblinded manner at regular intervals and might recommend adjusting, pausing or discontinuing the study at any time. Please refer to Section 8.6.2 for more details on the set-up and responsibilities of the DSMB.

Subject Enrollment into Part B (Booster Phase):

VLA15 Booster (Group 1 and 2)

- All eligible subjects within Group 1 and 2 will receive a VLA15 booster dose at Month 18.

Placebo (Group 3):

- All eligible subjects within Group 3 will receive Placebo at Month 18.

The study sponsor and study statisticians will be unblinded at the time of the primary endpoint analysis, i.e., prior to subject enrollment into Part B. Study participants and investigators will remain blinded throughout entire duration of study.

4.2.2 Study Design

The study will be conducted in two study parts (Part A: Main Study Phase, Part B: Booster Phase). Please refer to Figure 2 for the study design.

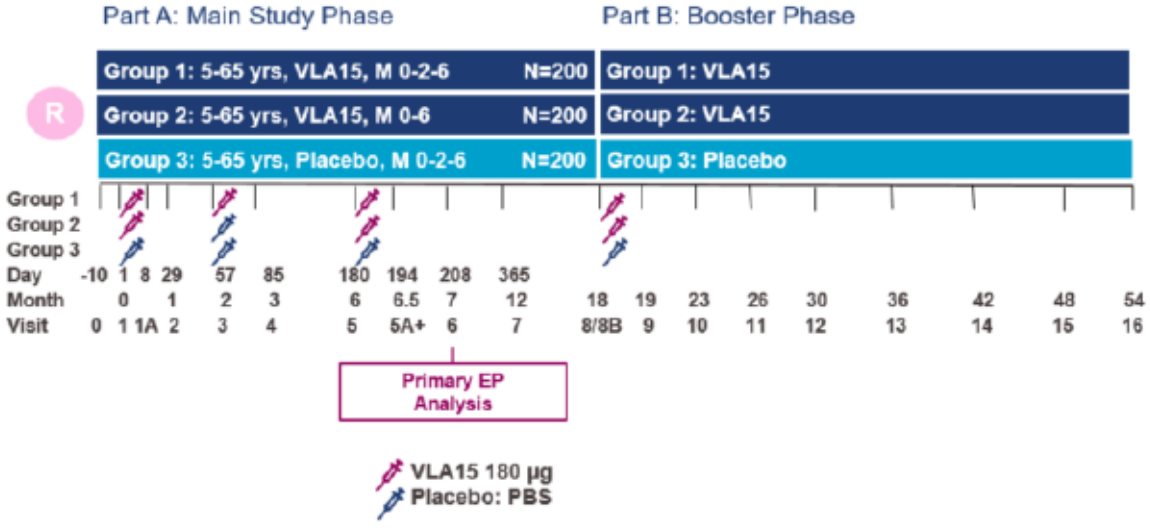

Figure 2 Study Design

In **Part A (Main Study Phase)** a total of approximately 600 subjects aged 5 to 65 years will be randomized 1:1:1 into three study groups: Group 1 (approximately 200 subjects) will receive three vaccinations of VLA15 at Month 0-2-6. Group 2 (approximately 200 subjects) will receive

two VLA15 vaccinations at Month 0-6 and a placebo injection at Month 2 in order to keep the blind. Group 3 (approximately 200 subjects) will receive three placebo injections at Month 0-2-6. Within each study group subjects will be enrolled 2:1:1 in three age cohorts (18-65 years, 12-17 years and 5-11 years).

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

In **Part B (Booster Phase)** all eligible subjects from Group 1 and 2 will receive a booster dose of VLA15 at Month 18. Placebo injections will be administered to Group 3 subjects.

All subjects involved in Part B will be followed-up for further 3 years (i.e., up to Month 54) with study visits at Months 19, 23, 26, 30, 36, 42, 48 and 54.

Table 1 provides an overview on study groups and treatments.

Table 1 Study Groups and Vaccinations

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

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

4.2.3 Discussion of study design

Rationale for dose/ presentations

The dose and formulation used in this study were decided based on data from Phase 1 and Phase 2 development with VLA15. Phase 1 data revealed that adjuvanted VLA15 formulations were more immunogenic compared with non-adjuvanted formulations with a comparable safety profile. Hence only adjuvanted formulations were tested in Phase 2. Initial data from the ongoing Phase 2 studies, where higher vaccine doses and a broader immunization schedule were investigated, revealed, that VLA15 demonstrated a good safety profile for all dose groups and schedules tested. Based on safety and immunogenicity results obtained from precedent Phase 2 studies, the 180 µg dose was selected to be used in this study. This is the highest dose investigated in the development program and is demonstrating a good safety profile and good immunogenicity. Sustained high levels of antibodies are of utmost importance for an

OspA based vaccine to confer protection. Hence, the 180 µg dose will be continued in further clinical development of this vaccine.
This dose 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]).

Rationale for immunization schedule

Initial data from study VLA15-202 have demonstrated that a Month 0-2-6 schedule induced 1.7 (ST6) to 2.4-fold (ST1) higher antibody levels in the 180 µg dose group, depending on the serotype, compared with levels obtained in study VLA15-201 using the 180 µg dose group in an immunization schedule of Month 0-1-2. Therefore, the Month 0-2-6 schedule will be used in further development as obtaining high circulating antibody levels is of utmost importance for an OspA based vaccine.
On the other hand, the magnitude and durability of the immune response may be determined by the spacing between the first and last dose of the primary series, with possibly limited additional benefit of a dose in between[22,23]. If both primary vaccination schedules (Month 0-6 or Month 0-2-6) are similarly immunogenic, in general the schedule with the lowest number of vaccinations will be selected for further clinical development. Hence, the primary vaccination series investigated in present study VLA15-221 will consist of either three VLA15 vaccinations at Month 0-2-6 or two VLA15 vaccinations at Month 0-6.

In Part B, subjects that received VLA15 vaccinations at Month 0-2-6 (Group 1) or Month 0-6 (Group 2), will receive a booster dose of VLA15 at Month 18. This will allow to assess the immunogenic effect of a booster after two or three primary vaccinations. The booster vaccination is anticipated to induce even higher antibody levels and improve antibody sustainability. The efficacy of previous OspA based Lyme vaccines LYMErix and ImuLyme was shown to be substantially increased after a booster vaccination administered prior to the second tick season characterized by a significant increase in anti-OspA antibodies, which is seen as a prerequisite for protection[11,12,24].

Data generated in the study group receiving placebo (Group 3) will serve to compare the safety and tolerability profile of the VLA15 booster dose with placebo.

Rationale for study population

The proposed study population consists of healthy subjects aged 5 to 65 years, who live in LB endemic regions in the US. Major exclusion criteria include chronic illness related to Lyme borreliosis (LB), an active symptomatic LB as suspected or diagnosed by a physician, history of a neuro-inflammatory or autoimmune disease, history of immunodeficiency or ongoing immunosuppressive therapy (incl. infection with HI virus), known history of anaphylaxis, pregnancy and lactation or any active or passive vaccination within 4 weeks prior to first study vaccination.

This study is the first study which includes a pediatric population. Early involvement of children is crucial for the development of a vaccine against Lyme borreliosis as children aged 5-15 years are one of the age groups at highest risk to get LB. Hence it is important to provide a vaccine for this age groups as early as possible.

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.)* will also be enrolled. As a previous Borrelia infection does not confer protection against new infection, this study population is also a target population for a Lyme vaccine and hence will be included in this Phase 2 study.

The rationale for selecting this study population is to evaluate the final vaccine dose and presentation and the most appropriate schedule in all anticipated target populations to establish efficacy of the LB vaccine in a Phase 3 study; which will include healthy subjects

aged ≥ 5 years who live in Lyme borreliosis endemic areas. The results of the present study VLA15-221 will be used as data basis for selecting the optimal primary immunization schedule (Month 0-2-6 or Month 0-6) of VLA15 for use in Phase 3 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 Competent Authority in an End-of-Phase 1 meeting on May 29, 2018 and from scientific advice procedures with EMA on 18 Oct 2018 and on 26 Mar 2020 has been taken into consideration in the design of the protocol.

4.2.4 Study events description

For an outline of procedures required at each visit, please refer to the Tables of Events (Table 2 for Part A, Table 3 for Part B, respectively).

Part A will start with a Screening Visit within 10 days prior to the first administration of the investigational medicinal product (IMP).

The experimental part involves following visits:

- Main Study Phase: nine to ten study visits (Days 1, 8*, 29, 57, 85, 180, 194†, 208, 365, 545)
- Booster Phase: nine study visits (Months 18, 19, 23, 26, 30, 36, 42, 48, 54)

Vaccinations will be performed at following time-points:

- Main Study Phase: Days 1-57-180 (Month 0-2-6)
- Booster Phase: Month 18

In order to observe immediate reactions, sentinel subjects of all age cohorts (18-65 years, 12-17 years and 5-11 years) will be observed for at least 30 minutes after each vaccination prior to discharge from study site.

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

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

<u>In the Main Study Phase (Part A) following procedures are performed for all subjects:</u>

Visit 0, Screening Visit (-10 to 0 days prior to Visit 1):

This visit is performed as an in-person visit at the study site.

Signed and dated informed consent/assent 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.

* Visit 1A (i.e. on Day 8) is to be performed as an in-person visit for subjects aged 5-17 years only. Due to the COVID-19 pandemic exceptions can be made according to the Table of Events (i.e. Table 2). For adults (18-65 years) this visit will routinely be performed via phone/video call.
† Visit 5A is to be performed by a subset of approximately 150 adult subjects only. Subjects will be asked prior to study start, if they would like to perform this additional visit.

- Perform physical examination (general appearance including assessment of body weight and height, skin, head/ eyes /ears/ nose/ throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, and neurological system), measure ECG and vital signs (systolic and diastolic blood pressure, pulse rate) in seated position and at rest, measure oral body temperature.
- Laboratory tests:
  - HIV Test: HIV testing will only be performed in subjects aged ≥12 years. The results of negative HIV tests that were performed up to 4 weeks before Visit 0 are acceptable. Positive HIV tests obtained by ELISA will have to be confirmed by a second method (e.g., Western blot or PCR).
  - *B.b.* s.l. screening Test: For serological screening of previous infection with Lyme borreliosis, a commercially available Lyme borreliosis screening test will be performed. Serum samples that are tested positive will have to be verified by a confirmatory immunoblot. Test result does not need to be available before randomization and remains valid for 4 weeks.
  - Serum Pregnancy Test: A serum pregnancy test has to be performed for each female subject aged ≥ 12 years and woman of childbearing potential*. If the test is positive, the subject must be excluded from the study.
- Collect immunogenicity sample: Blood will be collected for immunogenicity testing by an IgG binding assay CCI [redacted]

Visit 1, Day 1:

This visit is performed as an in-person visit at the study site.

- 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 are 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 female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4). Pregnancy test result must be obtained prior to vaccination.
- Collect assay development sample (prior to vaccination): Assay development sera will be collected from subjects in age group 18 to 65 years only.
- **Designated unblinded staff member only (Section 4.2.12):** Randomize subject to treatment group as described in Section 4.2.11. Prepare vaccination according to assigned treatment group. As this study is performed observer-blind, subjects and

* A woman is considered of childbearing potential if fertile and until becoming post-menopausal unless permanently sterile. A female 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.

study staff involved in general study conduct and safety assessments must not be informed about the treatment group allocation.

- Administration of vaccination according to assigned treatment group can be performed by **blinded OR unblinded staff**. Vaccine is to be administrated into deltoid of the non-dominant arm.
  - Blinded staff member: Observe subject for at least 30 minutes after vaccination for immediate treatment of possible AEs. Record any AEs and local and systemic tolerability following vaccination, if applicable.
  - If the subject has any complaints after vaccination, perform a symptom-driven physical examination and record vital signs prior to discharge. Only discharge subject if in the opinion of the investigator no further concerns exist.
- Explain eDiary: Instruct subject/ legal representative(s) how and when to complete the eDiary. Distribute thermometer and measuring tape.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g. red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).
- Supply subjects with urine pregnancy test kits to ensure that subjects can test for pregnancy at home in case the COVID-19 pandemic prohibits in-person visits at subsequent scheduled visits.

Visit 1A, Day 8 (+ 2 days):

This safety follow-up visit is to be performed remotely (phone/video call) for subjects aged 18-65 years. For subjects aged 5-17 years, Visit 1A will be performed as an in-person visit. If an in-person visit for adolescents or children cannot be done or is inacceptable due to COVID-19, e.g. travel restrictions, local recommendations, circumstances at the study site's location that prohibit an in-person visit, or if the PI believes that the subject's safety and well-being might be jeopardized with an in-person visit at the study site due to COVID-19, the visit should be conducted remotely (e.g., phone/ video call).

For in-person visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- In subjects aged 5-17 years:
  - Perform symptom-driven physical examination.
  - Inspect vaccination site for (ongoing) Adverse Events.
- Perform scripted safety assessment.
- Review eDiary: 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, any AESI or SAE since last study visit. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:

- any severe adverse event
- any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

For remote visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review eDiary: verify entries with subject. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI or SAE since last study visit.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

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

This visit should preferably be conducted as in-person visit. If an in-person visit cannot be done or is inacceptable due to COVID-19, e.g. travel restrictions, local recommendations, circumstances at the study site's location that prohibit an in-person visit, or if the PI believes that the subject's safety and well-being might be jeopardized with an in-person visit at the study site due to COVID-19, the visit should be conducted remotely (e.g., phone/ video call).

For in-person visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4).
- Inspect vaccination site for (ongoing) Adverse Events.
- Review eDiary: 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.
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI or SAE since last study visit. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

For remote visits:

- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4).
- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review eDiary: verify entries with subject. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI or SAE since last study visit.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

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

This visit is performed as an in-person visit at the study site.

- 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).
- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4). Pregnancy test result must be obtained prior to vaccination.
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI or SAE since last study visit.
- **Designated unblinded staff member only (Section 4.2.12):** Prepare vaccination according to assigned treatment group. As this study is performed observer-blind, subjects and study staff involved in general study conduct and safety assessments must not be informed about the treatment group allocation.
- Administration of vaccination according to assigned treatment group can be performed by **blinded OR unblinded staff**. Vaccine is to be administrated into deltoid of the non-dominant arm.
  - Blinded staff member: Observe subject for at least 30 minutes after vaccination for immediate treatment of possible AEs. Record any AEs and local and systemic tolerability following vaccination, if applicable.

- If the subject has any complaints after vaccination, perform a symptom-driven physical examination and record vital signs prior to discharge. Only discharge subject if in the opinion of the investigator no further concerns exist.

- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g. red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).
- Supply subjects with additional urine pregnancy test kits to ensure that subjects can test for pregnancy at home in case the COVID-19 pandemic prohibits in-person visits at subsequent scheduled visits.

Visit 4, Day 85/Month 3: Visit 3 + 28 days (+/- 4 days):

This visit should preferably be conducted as in-person visit. If an in-person visit cannot be done or is inacceptable due to COVID-19, e.g., travel restrictions, local recommendations, circumstances at the study site's location that prohibit an in-person visit, or if the PI believes that the subject's safety and well-being might be jeopardized with an in-person visit at the study site due to COVID-19, the visit should be conducted remotely (e.g., phone/ video call).

For in-person visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4).
- Collect immunogenicity and assay development sample: Assay development sera will be collected from subjects in age group 18 to 65 years only.
- Inspect vaccination site for (ongoing) Adverse Events.
- Review eDiary: 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.
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI or SAE since last study visit. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

For remote visits:

- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4).
- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review eDiary: verify entries with subject. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI or SAE since last study visit.
- The immunogenicity sample will be taken by a mobile nurse professional at the subject's home within the specified time window.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

Visit 5, Day 180/Month 6 (+4/-28 days):

This visit is performed as an in-person visit at the study site.

- 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).
- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4). Pregnancy test result must be obtained prior to vaccination.
- Collect immunogenicity sample before vaccination.
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI or SAE since last study visit.
- **Designated unblinded staff member only (Section 4.2.12):** Prepare vaccination according to assigned treatment group. As this study is performed observer-blind, subjects and study staff involved in general study conduct and safety assessments must not be informed about the treatment group allocation.
- Administration of vaccination according to assigned treatment group can be performed by **blinded OR unblinded staff**. Vaccine is to be administrated into deltoid of the non-dominant arm.

  - Blinded staff member: Observe subject for at least 30 minutes after vaccination for immediate treatment of possible AEs. Record any AEs and local and systemic tolerability following vaccination, if applicable.
  - If the subject has any complaints after vaccination, perform a symptom-driven physical examination and record vital signs prior to discharge. Only discharge subject if in the opinion of the investigator no further concerns exist.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).
- Supply subjects with additional urine pregnancy test kits to ensure that subjects can test for pregnancy at home in case the COVID-19 pandemic prohibits in-person visits at subsequent scheduled visits.

Visit 5A, Day 194/Month 6.5: Visit 5 + 14 days (+/-2 days):

Visit 5A will be performed in a subset of approximately 150 adult subjects. Subjects will be asked prior to study start, if they agree to perform this additional visit. This visit should preferably be conducted as in-person visit. If an in-person visit cannot be done or is inacceptable due to COVID-19, e.g., travel restrictions, local recommendations, circumstances at the study site's location that prohibit an in-person visit, or if the PI believes that the subject's safety and well-being might be jeopardized with an in-person visit at the study site due to COVID-19, the visit should be conducted remotely (e.g., phone/ video call).

<u>For in-person visits:</u>

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Collect immunogenicity sample.
- Inspect vaccination site for (ongoing) Adverse Events.
- Review eDiary: 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.
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI or SAE since last study visit. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

<u>For remote visits:</u>

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review eDiary: verify entries with subject. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI and any SAE since the last study visit.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).
- The immunogenicity sample will be taken by a mobile nurse professional at the subject's home within the specified time window.

Visit 6, Day 208/Month 7/ Visit 5 + 28 days (+2/- 4 days):

This visit should preferably be conducted as in-person visit. If an in-person visit cannot be done or is inacceptable due to COVID-19, e.g., travel restrictions, local recommendations, circumstances at the study site's location that prohibit an in-person visit, or if the PI believes that the subject's safety and well-being might be jeopardized with an in-person visit at the study site due to COVID-19, the visit should be conducted remotely (e.g., phone/ video call).

For in-person visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4).
- Collect immunogenicity and assay development sample. Assay development sera will be collected from subjects in age group 18 to 65 years only.
- Inspect vaccination site for (ongoing) Adverse Events.
- Review eDiary: 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.
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI or SAE since last study visit. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

For remote visits:

- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4).
- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review eDiary: verify entries with subject. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI and any SAE since the last study visit.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).
- The immunogenicity sample will be taken by a mobile nurse professional at the subject's home within the specified time window.

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

This visit should preferably be conducted as in-person visit. If an in-person visit cannot be done or is inacceptable due to COVID-19, e.g., travel restrictions, local recommendations, circumstances at the study site's location that prohibit an in-person visit, or if the PI believes that the subject's safety and well-being might be jeopardized with an in-person visit at the study site due to COVID-19, the visit should be conducted remotely (e.g., phone/ video call).

For in-person visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4).
- Collect immunogenicity sample;
- Perform scripted safety assessment.
- Record any AESI or SAE since last study visit.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

For remote visits:

- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4).
- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review eDiary: verify entries with subject. Instruct subject/ legal representative(s) to continue using the eDiary for documenting SAEs
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI and any SAE since the last study visit.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).
- The immunogenicity sample will be taken by a mobile nurse professional at the subject's home within the specified time window.

Visit 8, Day 545/Month 18 (+/- 14 days):

This visit should preferably be conducted as in-person visit. If an in-person visit cannot be done or is inacceptable due to COVID-19, e.g., travel restrictions, local recommendations, circumstances at the study site's location that prohibit an in-person visit, or if the PI believes that the subject's safety and well-being might be jeopardized with an in-person visit at the study site due to COVID-19, the visit should be conducted remotely (e.g., phone/ video call).

For in-person visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4). In case Visit 8 and Visit 8B are performed as a combined visit, the pregnancy test result must be obtained prior to vaccination.
  - *B.b.* s.l. screening test: For serological screening on previous infection with Lyme borreliosis, a commercially available Lyme borreliosis screening test will be performed. Serum samples that are tested positive will have to be verified by a confirmatory immunoblot.
- Collect immunogenicity sample: In case Visit 8 and Visit 8B are performed as a combined visit, the immunogenicity sample has to be drawn prior to vaccination.
- Perform scripted safety assessment.
- Record, any AESI and any SAE since the last study visit.

- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

For remote visits:

- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4).
- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review eDiary: verify entries with subject. For subjects who continue study, instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI and any SAE since the last study visit.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).
- The immunogenicity and *B.b.* s.l. screening sample will be taken by a mobile nurse professional at the subject's home within the specified time window.

ET Visit, Early Termination Visit before V8:

Subjects who terminate participation or who are withdrawn from the study prematurely 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 ET visit is prior to Visit 6, inspect vaccination site for (ongoing) Adverse Events.
- Review eDiary: 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, any AESI or SAE since last study visit.
- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4).
  - *B.b.* s.l. screening test: For serological screening on previous infection with Lyme borreliosis, a commercially available Lyme borreliosis screening test will be performed. Serum samples that are tested positive will have to be verified by a confirmatory immunoblot.

The reason for early termination should be clarified in as much detail as possible. If an AE is 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 or an in-person ET visit is not possible due to circumstances of the ongoing COVID-19 situation, a remote visit (e.g., phone/video 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 is 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 subject presents at a regular study visit within the acceptable time window and inform that he/she discontinues the study after this visit, the study visit is not performed as an ET visit, but is 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 is performed in addition for serological screening on infection with Lyme borreliosis.

In the Booster Phase (Part B) following procedures are performed for all subjects:

Visit 8B, Month 18 (Visit 8 + 14 days):

This visit is performed as an in-person visit at the study site. Visit 8 and Visit 8B should be combined in one visit if possible. The time window between Visit 8 and Visit 8B should be kept as short as possible (max. 14 days apart). If both visits are scheduled on the same day, all procedures from Visit 8 have to be performed prior to performing any procedures from Visit 8B.

- Reconfirm consent/assent
- Check booster 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.
- 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 female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4). Pregnancy test result must be obtained prior to vaccination.
- Perform scripted safety assessment.
- Record any AESI or SAE since last study visit
- **Designated unblinded staff member only (Section 4.2.12):** Prepare vaccination according to assigned treatment group. As this study is performed observer-blind, subjects and study staff involved in general study conduct and safety assessments must not be informed about the treatment group allocation.
- Administration of vaccination according to assigned treatment group can be performed by **blinded OR unblinded staff**. Vaccine is to be administrated into deltoid of the non-dominant arm.

- Blinded staff member: Observe subject after vaccination for immediate treatment of possible AEs. Record any AEs and local and systemic tolerability following vaccination, if applicable.
- If the subject has any complaints after vaccination, perform a symptom-driven physical examination and record vital signs prior to discharge. Only discharge subject if in the opinion of the investigator no further concerns exist.

- Explain eDiary: Instruct subject/ legal representative(s) how and when to complete the eDiary. Distribute thermometer and measuring tape.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g. red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

Visit 9, Month 19/V8B + 28 days (+/-4 days):

This visit is performed as an in-person visit at the study site.

- Document any changes to medical history and concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4). Collect immunogenicity and assay development sample. Assay development sera will be collected from subjects in age group 18 to 65 years only.
- Inspect vaccination site for (ongoing) Adverse Events.
- Review eDiary: 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.
- Perform scripted safety assessment.
- Record any unsolicited AEs, any AESI or SAE since last study visit. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.

Visit 10, Month 23 (+/-14 days):

This visit is performed as an in-person visit at the study site.

- Document any changes to medical history and concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4).

- Collect immunogenicity sample.
- Perform scripted safety assessment.
- Record any AESI or SAE since last study visit. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

Visit 11, Month 26 (+/-14 days):

This visit is performed as an in-person visit at the study site.

- Document any changes to medical history and concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Collect immunogenicity sample.
- Perform scripted safety assessment.
- Record any AESI or SAE since last study visit. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

Visit 12, Month 30 (+/-28 days):

This visit is performed as an in-person visit at the study site.

- Document any changes to medical history and concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Laboratory tests:
  - *B.b.* s.l. screening test: For serological screening on previous infection with Lyme borreliosis, a commercially available Lyme borreliosis screening test will be performed. Serum samples that are tested positive will have to be verified by a confirmatory immunoblot.
- Collect immunogenicity sample.
- Perform scripted safety assessment.
- Record any AESI or SAE since last study visit. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.

- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

Visit 13, Month 36 (+/-28 days):

This visit is performed as an in-person visit at the study site.

- Document any changes to medical history and concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Collect immunogenicity sample.
- Perform scripted safety assessment.
- Record any AESI or SAE since last study visit. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

Visit 14, Month 42 (+/-28 days):

This visit is performed as an in-person visit at the study site.

- Document any changes to medical history and concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Laboratory tests:
  - *B.b.* s.l. screening test: For serological screening on previous infection with Lyme borreliosis, a commercially available Lyme borreliosis screening test will be performed. Serum samples that are tested positive will have to be verified by a confirmatory immunoblot.
- Collect immunogenicity sample.
- Perform scripted safety assessment.
- Record any AESI or SAE since last study visit. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

Visit 15, Month 48 (+/-28 days):

This visit is performed as an in-person visit at the study site.

- Document any changes to medical history and concomitant medication/treatments including vaccination(s) since the previous study visit.
- Collect immunogenicity sample.
- Perform symptom-driven physical examination.
- Perform scripted safety assessment.
- Record any AESI or SAE since last study visit. Instruct subject/ legal representative(s) to continue using the eDiary for documenting AEs.
- Remind subject/ legal representative(s) to immediately inform the site in case subject experiences one of the following:
  - any severe adverse event
  - any symptoms suspicious for Lyme borreliosis or diagnosis of LB, e.g., red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).

Visit 16, Month 54 (+/-28 days):

This visit is performed as an in-person visit at the study site.

- Document any changes to medical history and concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Laboratory tests:
  - *B.b.* s.l. screening test: For serological screening on previous infection with Lyme borreliosis, a commercially available Lyme borreliosis screening test will be performed. Serum samples that are tested positive will have to be verified by a confirmatory immunoblot.
- Collect immunogenicity sample.
- Perform scripted safety assessment.
- Record any AESI or SAE since last study visit.

ET Visit, Early Termination Visit before V16:

Subjects who terminate participation or who are withdrawn from the study prematurely 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.
- Inspect vaccination site for (ongoing) Adverse Events.
- Perform scripted safety assessment.

- Review eDiary: 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, any AESI or SAE since last study visit.
- Laboratory tests:
  - Urine pregnancy test: In female subjects aged ≥ 12 years and women of childbearing potential (Section 6.4).
  - *B.b.* s.l. screening test: For serological screening on previous infection with Lyme borreliosis, a commercially available Lyme borreliosis screening test will be performed. Serum samples that are tested positive will have to be verified by a confirmatory immunoblot.

The reason for early termination should be clarified in as much detail as possible. If an AE is 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 or an in-person ET visit is not possible due to circumstances of the ongoing COVID-19 situation, a remote visit (e.g., phone/ video 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 is 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 subject presents at a regular study visit within the acceptable time window and inform that he/she discontinues the study after this visit, the study visit is not performed as an ET visit, but is performed and documented as a regular study visit including all events that are described for respective study visit. Additionally, a Lyme borreliosis screening test is performed as required for the ET visit.

Unscheduled Visits:

An unscheduled visit can be held at any time during the study if deemed necessary by the Investigator (e.g., follow-up on unexpected AEs, SAEs or AESIs) or requested by the DSMB. 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.

4.2.5 Number of subjects and study centres

A total of approximately 600 healthy subjects aged 5 to 65 years will be enrolled in this study: 300 subjects aged 18-65 years, 150 subjects aged 12-17 years and 150 subjects aged 5-11 years. Subjects will be recruited at in total 14 active study centers. Study sites will be located in Lyme borreliosis endemic areas in the U.S.

4.2.6 Timely conduct of the study

Study start for the Main Study Phase (Part A) was March 2021. Booster Phase will start in August 2022.

4.2.7 Study duration

Study duration per subject will be a maximum of 19 months in the Main Study Phase and additional 37 months in the Booster Phase (i.e., Main Study Phase and Booster Phase participation results in an overall maximal duration of 56 months per subject). Overall study duration (i.e., First-Subject-In to Last-Subject-Out) is estimated to be 4 years and 11 months. The end of the study is defined as the date of the last visit performed by the last subject.

4.2.8 Assignment of subjects to study groups

In the Main Study Phase (Part A) 600 subjects will be randomized 1:1:1 to receive VLA15 vaccinations at Month 0-2-6 (Group 1) or Month 0-6 (plus a placebo injection at Month 2, Group 2) or placebo injections at Month 0-2-6 (Group 3) according to the procedure described in Section 4.2.11. During the Booster Phase (Part B) Group 1 and Group 2 subjects will receive a VLA15 booster dose at Month 18. For comparison, Group 3 will receive an additional placebo injection at Month 18.

4.2.9 Subject Identification

At Visit 0 a 10-digit subject number will be assigned to each subject. The first five digits are the study identifier (15221 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.

4.2.10 Re-screening

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

4.2.11 Randomization

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

4.2.12 Blinding

The study will be an observer-blinded study, which will be conducted in a blinded manner for the study investigators, the sponsor including laboratory personnel, and the subjects. At the study site, only designated study staff who randomize subjects into study groups and perform preparation of the vaccine will be unblinded.
Administration of the vaccine can be performed by staff from either the blinded or unblinded study site team. An overview of persons who will be unblinded and blinded is provided below:

Unblinded:

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

Blinded:

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

Blinding process:
To ensure that study participants cannot tell the group they have been allocated to from the physical appearance 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 and its legal representative(s). Details on blinding will be provided in the study specific IMP manual.

Unblinding during the study:
The blind must not be broken for anyone involved in the study conduct. Unblinding of individual subjects 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 CCI /Pfizer Safety 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 study vaccines (SUSARs), to fulfil safety reporting requirements. Procedures are described in a safety management plan.
The study sponsor/ collaboration partner and study statisticians will be unblinded at the time of the primary endpoint analysis, i.e., Day 208 analysis, in each age cohort (i.e., Analysis 1.1, Analysis 1.2 and Analysis 1.3) after the respective database snapshot has been performed. All other study personnel including the investigators and other study staff involved in general study conduct and safety assessments as well as laboratory personnel who are performing analytical assays will remain blinded until study end.

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 (alum) as adjuvant. PBS will be used as placebo.

5.1 Description of IMP

5.1.1 VLA15 Presentation

In this study, a new VLA15 presentation will be used. Compared to the previous Phase 2 studies (VLA15-201 and VLA15-202), this presentation contains the same amounts of VLA15 (i.e. 180 µg) and alum (i.e. 0.5 mg) in a more concentrated form (i.e., formulated in 0.5 mL instead of 1.0 mL of buffer). Subjects will be vaccinated with 180 µg VLA15/dose. The injection volume is 0.5 mL.

The VLA15 presentation contains the three proteins Lip-D1B2B, Lip-D4Bva3B and Lip-D5B6B, which are formulated in a 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).
The 180 µg dose contains 360 µg/ mL total protein (i.e. 120 µg/mL for each protein).

After sterilizing filtration of the VLA15 solution, sterile aluminum hydroxide is added aseptically to a target concentration of 1.0 mg/mL aluminum. The VLA15 presentation for is filled into 2R Type I Plus® glass vials (0.65 mL filling volume per vial, resulting in 0.50 mL extractable volume) closed with 13 mm injection Flurotec® stoppers secured by crimp caps. The new

presentation is available in 2 mL glass vials in form of a white to off-white suspension with a protein content of 360 µg/mL (180 µg/ 0.5 mL).

The VLA15 presentation 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 months with data from ongoing stability program provided no significant trends in product quality are observed.

Table 7 Overview of components of 180 µg VLA15 presentation

Component	Amount
Lip-D1B2B	120 µg/mL
Lip-D4Bva3B	120 µg/mL
Lip-D5B6B	120 µg/mL
L-Methionine	10 mM
NaH_2PO_4 dihydrate	10 mM
NaCl	150 mM
Sucrose	5% (w/v)
Tween®20	0.05% (v/v)
Aluminum hydroxide, 0.30%	1.0 mg/mL

5.1.2 VLA Placebo

The VLA Placebo is a PBS buffer based on Dulbecco's PBS media presentation without Calcium and Magnesium. Subjects will receive placebo injections with a volume of 0.5 mL. VLA Placebo is filtered and filled in sterile 2R glass vials under constant stirring. The vials are 2R Type I Plus® glass vials closed with 13 mm injection Flurotec® secured by aluminum crimp caps.

The total filling volume of 0.6 mL will ensure an extractable volume of 0.5 mL. Only excipients of non-human and/or non-animal origin are used for VLA Placebo presentation. Storage of VLA Placebo should be done at +2-8°C.

The Placebo 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 months 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 VLA15 and placebo is performed by a qualified CMO. Labels will be written in accordance with local law.

5.3 Condition of storage of IMP

VLA15 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!

More details on packaging and labelling as well as storage of IMP will be provided in the study specific IMP manual.

6 TREATMENT OF SUBJECTS

6.1 Investigational Treatment

6.1.1 Dose and dosing schedule

Within the VLA15-221 study different vaccination schedules are applied:
Table 1 summarizes the treatment and vaccination schedule for each study group.

Table 1 Study Groups and Vaccinations

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

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

6.1.2 Preparation and method of administration

All IMPs are filled in glass vials with a minimum extractable volume of 0.5 mL.

Remove the vial from the refrigerator. Invert vial at least three times before drawing the respective injection volume into the syringe. DO NOT SHAKE!

Preparation of IMP needs to be done by designated unblinded staff members only in a separate room unobserved by the subject/legal representative(s) 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 after a maximum time period of one hour for removal of the vial from the fridge to administration. 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 injections of VLA15 or Placebo I.M. in the deltoid region of the non-dominant arm. Vaccinations should be administered in the contra-lateral arm e.g. in case subject has a history of skin cancer at the deltoid region of the non-dominant arm or if subject has a tattoo or scar that could prohibit evaluation of adverse reactions at the injection site.

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

6.1.3 Treatment duration

In the Main Study Phase (Part A) subjects will be vaccinated within a period of approximately 6 months (vaccinations at Months 0, 2 and 6). In the Booster Phase (Part B) subjects will receive an additional vaccination at Month 18.

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 being taken at 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 concomitant therapy

The following concomitant therapy is not allowed:

- Administration of anticoagulants within three weeks prior to each study vaccination contraindicating I.M. vaccination as judged by the investigator.
- Administration of any active or passive immunization 4 weeks before or after any study vaccination; except for influenza or pandemic vaccines which may be administered outside a 7-days interval before or after any study vaccination.
- Administration of any other registered or non-registered medicinal product in another clinical study throughout Month 19 in Part B.
- Administration of chronic (longer than 14 consecutive days) prednisone or equivalent ≥0.05 mg/kg/day. Topical and inhaled steroids are allowed.
- Donation of blood or blood-derived products (e.g., plasma) within 4 weeks prior to each study vaccination or administration of any blood or blood-derived products (e.g. plasma or immunoglobulins) within 3 months prior to each study vaccination and within 4 weeks after each study vaccination.

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 unblinded staff. This documentation will be available to the 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

Females 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 and until becoming post-menopausal unless permanently sterile. Females aged ≥ 12 years and women of childbearing potential must have a negative serum pregnancy test at Visit 0 (Screening Visit of Part A), a negative urine pregnancy test before each vaccination and should be practicing an acceptable method of birth control throughout the entire Main Study Phase (Part A). During Part A, urine pregnancy tests will be conducted in females aged ≥ 12 years and women of childbearing potential at defined study visits (see Table 2) and at the ET Visit (if applicable). To ensure that subjects can perform pregnancy testing at home in case visits are performed remotely instead of in-person due to the ongoing COVID-19 pandemic, subjects will be supplied with urine pregnancy test kits at on-site vaccination visits (Visit 1, 3 and 5).

During the Booster Phase (Part B) women of childbearing potential must have a negative urine pregnancy test before administration of the booster vaccination at Visit 8B and should be practicing an acceptable method of birth control until Month 23. During the Booster Phase urine pregnancy tests will be conducted at study visit 8B, 9 and 10 (see Table 3) and at the ET Visit (if applicable).

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 1 month prior to Visit 1.

Females without childbearing potential do not need to perform any birth control. A female 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[25], 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 8.4.4

7 ASSESSMENT OF IMMUNOGENICITY

7.1 Immunogenicity Measurements

Immunogenicity assessments measuring OspA serotype specific IgG by an IgG binding assay will be performed on samples collected at Visit 0 and Visit 4 to 8 of the Main Study Phase (Part A) and at Visit 9 to 16 during the Booster Phase (Part B).

CCI [redacted]

Assays will be performed at Valneva Austria GmbH Vienna, Department of Clinical Serology and at Pfizer, Pearl River, New York 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. Operators conducting the assays will be blinded to the samples.

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 binding assay

For the evaluation of immunogenicity, human sera will be analyzed for IgG against each OspA serotype (ST1 to ST6) separately by a quantitative 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. The ELISA method was limited validated for analysis of Phase 2 clinical samples.

Anti-OspA IgG against the six serotypes included in the VLA15 vaccine may also be assessed by a direct Luminex immunoassay (dLIA). This assay will be based on Luminex MagPlex® microspheres read off the xMAP® family of instruments. MagPlex® microspheres are superparamagnetic carboxylated xMAP microspheres that allow for potential assay multiplexing and automation. For this, microspheres will be coated with different OspAs and will be distinguished by their internal impregnated dyes. Bound anti-OspA IgG will be detected with a secondary antibody conjugated to a fluorophore and read by fluorescence excitation on a Luminex reader. Signals will be expressed as median fluorescent intensities and read against an arbitrarily assigned reference standard. The magnitude of the fluorescent signal is proportional to the amount of antigen-specific IgG present in the sample.

CCI [redacted]

CCI [redacted]

7.1.3 Additional Testing Procedures

Serum samples obtained in this study may, in addition to its use for assessment of OspA specific IgG antibodies CCI [redacted] also be used for further development of the vaccine, including other serological assays and for the development of proficiency panels and reference standards for the purpose of vaccine development.

8 ASSESSMENT OF SAFETY

For the exact time points of the safety assessment, please refer to Table 2 for the Main Study Phase and Table 3 for the Booster Phase.

AE occurrence frequencies will be used to evaluate the study objectives 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 (including assessment of body weight and height), skin, head/ eyes /ears/ nose/ throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, and neurological system.

A symptom-driven physical examination will be performed at all in-person 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 screening and before each vaccination, i.e. in the Main Study Phase at Visit 0, 1, 3 and 5 and in the Booster Phase at Visit 8B. Vital signs will be recorded in a seated position and at rest. At least 15 min should have passed before measurement of oral body temperature after consumption of hot or cold beverages or smoking, if applicable. An ECG will be done at Visit 0 of Part A.

8.3 Solicited AEs and Subject Diary

Subjects will record AEs for the first seven days after each vaccination (starting on the day of vaccination) in electronic Subject Diaries (eDiaries).
Solicited AEs (predefined) are listed in the eDiaries 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), 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, study physician and study participant should liaise immediately, independent of any scheduled study visit.

8.3.1 Collection, assessment and documentation of solicited AEs

Subjects/ legal representative(s) will be instructed to report solicited AEs via eDiaries. At vaccination visits V1 in the Main Study Phase (Part A) and at Visit V8B of the Booster Phase (Part B), the use of eDiaries will be explained to the subjects. Subjects/ legal representative(s) 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.

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 fever (oral body temperature ≥ 38.0 °C/100.4 °F) occurs, body temperature should be measured at least every 8 hours until it returns to normal (< 38.0 °C/100.4 °F). All body temperature measurements including the date and time should be recorded in the eDiary. In case of fever, the subject/ legal representative(s) should record all fever measurements in the subject eDiary including the first value that shows a return to normal body temperature. For other symptoms the maximum severity observed on a given day should be recorded. The subject/ legal representative(s) will note down information about the symptom by selecting the appropriate description. Additionally, the following information will be collected: symptoms present after Day 6 (yes/ no), last day of symptoms (date).

Subjects/ legal representative(s) and site staff should liaise immediately in case of symptoms suggesting possible Lyme borreliosis, severe solicited AEs or other severe symptoms as instructed in the eDiary.

The eDiary entries will be reviewed by a clinician together with the subject/ legal representative(s) 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 should not suggest answers when performing the eDiary verification with the subject/ legal representative(s) but can question and call the subject's attention to obviously wrong and misleading entries. If the subject comes to the conclusion that an eDiary question had been misunderstood or that an entry was made by mistake, the investigator will take this information into account when performing the severity assessment.

After eDiary verification, the clinician will perform a severity assessment on the basis of the information provided by the subject in the eDiary. If an AE existed over a period of days, only the maximum severity will be documented in the eCRF. The severity assessment will be based on the grading scale in Table 8 below. Severity categories in this table are NOT identical to the categories provided in the eDiary.

For solicited AEs, which are serious and/ or medically attended or solicited systemic Grade 3/4 reactions, 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 in the eDiary 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 8 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) and on Division of AIDS (Table for Grading the Severity of Adult and Pediatric Adverse Events; July 2017).

Table 8 Grading of Solicited Adverse Events

Vaccine specific Criteria	Age	Grade 1 mild	Grade 2 moderate	Grade 3 severe	Grade 4 potentially life-threatening
Injection Site Pain	5-65 years [A]	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	5-65 years [A]	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Injection Site Induration/ Hardening [1]	5-11 years [B]	1.0 - ≤ 2.5 cm (0.39-0,98 inch) in diameter	> 2.5 - 5.0 cm [2] in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	> 5 cm [2] OR ≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh) OR Ulceration OR Secondary infection OR Phlebitis	Potentially life-threatening consequences (e.g., Skin necrosis)
	12-65 years [A]	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
Injection Site Swelling [1]	5-11 years [B]	1.0 - ≤ 2.5 cm (0.39-0,98 inch) in diameter	> 2.5 - 5.0 cm [2] in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	> 5 cm [2] OR ≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh) OR Ulceration OR Secondary infection OR Phlebitis	Potentially life-threatening consequences (e.g., Skin necrosis)
	12-65 years [A]	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
Injection Site Erythema or Redness[1]	5-11 years [B]	1.0 - ≤ 2.5 cm (0.39-0,98 inch) in diameter	> 2.5 - 5.0 cm [2] in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	> 5 cm [2] OR ≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Potentially life-threatening consequences (e.g., Skin necrosis)
	12-65 years [A]	2.5–5 cm (0.98–1.96 inch)	5.1–10 cm 1.97–3.94 inch	>10 cm >3.94 inch	Necrosis or exfoliate dermatitis

Vaccine specific Criteria	Age	Grade 1 mild	Grade 2 moderate	Grade 3 severe	Grade 4 potentially life-threatening
Headache	5-65 years (A)	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	5-65 years (A)	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Arthralgia(3)	5-65 years (A)	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Fever(4) (°C)/(°F)	5-65 years (A)	38.0–38.4/ 100.4–101.1	38.5–38.9/ 101.2–102.0	39.0–40/ 102.1–104	>40/ >104
Nausea	5-65 years (A)	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	5-65 years (A)	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	5-65 years (A)	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

(A) Grading based on FDA Guidance on Toxicity Grading Scales Adults/ Adolescents[26]
(B) Grading based on Division of AIDS, Table for Grading the Severity of Adult and Pediatric Adverse Events[27]

(1) Injection Site Erythema or Redness should be evaluated and graded using the greatest single diameter or measured surface area
(2) Modified grading based on Division of AIDS; 5.0 cm cut-off will be included as moderate instead of severe
(3) Symptom not described in the FDA or Division of AIDS 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 exacerbations in intensity or frequency (worsening) of a pre-existing condition during or after the first vaccination represent AEs.

Study participants/ legal representative(s) 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 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.

8.4.1 Collection, follow-up and documentation of unsolicited AEs

Unsolicited AEs will be reported in the eCRF up to 28 days after each vaccination and will be documented in the eCRF within this respective timeframe. Thereafter, only SAEs and AESIs will be documented in the eCRF.

Investigators are not obligated to actively seek information on AEs or SAEs after the subject has concluded study participation. However, if the investigator learns of any SAE, including a death, at any time after a subject has completed the study, and he/she considers the event to be reasonably related to the study intervention, the investigator must promptly report the SAE to Pfizer Safety using the Vaccine SAE Report Form.

Collection:

- The eDiary is explained at V1 of the Main Study Phase and at V8B of the Booster Phase. The eDiary will contain sections for the assessment of unsolicited AEs. Unsolicited AEs will be captured in the eCRF up to 28 days after each vaccination. The subjects will be asked to complete the eDiary throughout the study. The eDiary entries will be verified by the study physician together with the subject/ legal representative(s) at the subject's next visit to the study site. Subjects will be encouraged to note down any either ongoing or new AEs plus the dates of onset/ resolution, whether medication was taken in response and whether medical care was sought.
- Subjects/ legal representative(s) will be instructed to immediately inform the site in case of an SAE or AESI.
- Additionally, the investigator should enquire about unsolicited AEs and should perform the scripted safety assessment during each study visit.
- Symptoms noted during the symptom-driven physical exams (unless already covered by respective solicited AE) constitute unsolicited AEs too.

Follow-up:

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 until the end of the study for the subject. SAEs ongoing at the time of last regular study visit for a subject (or ET Visit) will be followed until resolution or achievement of stable clinical conditions, latest until the global end of the study.

Documentation:

All unsolicited AEs with an onset date up to 28 days after each vaccination need to be documented in the respective AE section of the eCRF, regardless of their source (e.g. open question to subject, symptom-driven physical examination, unsolicited AEs noted in the eDiary). Thereafter, only SAEs and AESIs have to be documented in the eCRF.

Any symptom is regarded as separate AE. However, if the investigator considers several symptoms to be in the context of one underlying diagnosis, (s)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 evaluated and documented for each unsolicited AE: Causality (see Section 8.4.2.7), Severity (see Section 8.4.2.6), Outcome (see Section 8.4.2.8), Seriousness (see Section 8.4.2.2), Medically-attended (see Section 8.4.2.1), Action Taken to Treat AE and on IMP (see Section 8.4.2.9), 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 SAE reporting procedure

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 or she 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 within 24 hours after the investigator has become aware of it to Pfizer Safety.

Pfizer Safety

SAE Fax Number: [redacted]

In addition, expedited and periodic reporting to Competent Authorities and IRBs 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 Pfizer Safety and will be provided to the Medical Monitor, Valneva Austria GmbH and the independent DSMB.

8.4.2.4 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.[28]

8.4.2.5 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:

- Erythema migrans: an 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 disturbances, myocarditis);
- Immune-mediated disorders as proposed by competent authorities for previous clinical programs (please refer to APPENDIX 1).

For avoidance of doubt, <u>solicited AEs do not qualify as AESI and should not be reported as such</u>.

As part of unsolicited AE assessments, at each study visit starting from Visit 1A, 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 asked 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. 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 CCI 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 CCI Safety Desk. In addition, the DSMB will regularly review accruing AEs and can recommend specialist work-up to the investigator 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.

If an AESI meets the definition of an SAE the event must be submitted through the SAE reporting instructions in Section 8.4.2.3 using the Pfizer Vaccine SAE Report Form. In addition an AESI report has to be completed and send to CCI Safety Desk.

The AESI Report Form (for serious and non-serious AESIs) should be reported by the investigator by email to CCI Safety Desk:

CCI Safety Desk

E-mail: CCI

8.4.2.6 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 5.0, 2017 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.

8.4.2.7 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 vaccine. AEs with missing causality assessment will be regarded as related unless further specified. All other AEs will be considered as not related to study vaccine.

8.4.2.8 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.

8.4.2.9 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 has to 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

Unsolicited AEs will be captured in the eCRF until 28 days after each study vaccination. AESIs and SAEs will be captured throughout the entire study period.

8.4.4 Environmental Exposure, Exposure During Pregnancy or Breastfeeding and Occupational Exposure reporting procedures

Environmental exposure occurs when a person not enrolled in the study as a participant receives unplanned direct contact with or exposure to the study intervention. Such exposure may or may not lead to the occurrence of an AE or SAE. Persons at risk for environmental exposure include healthcare providers, family members, and others who may be exposed. An environmental exposure may include exposure during pregnancy, exposure during breastfeeding, and occupational exposure.
Any such exposure to the study intervention under study are reportable to Pfizer Safety within 24 hours of investigator awareness.

Females must not become pregnant during the entire Main Study Phase and until Month 23 in the Booster Phase. If a subject becomes pregnant during the study, she or the legal representative(s) must immediately inform the investigator. No further study vaccinations must be administered but the subject should attend all remaining visits as planned.

The investigator should report pregnancies to Pfizer Safety using the Pfizer Vaccine SAE Report Form and Exposure During Pregnancy (EDP) Supplemental Form. Reporting procedures are similar to SAE reporting procedures (contacts and processing), although a pregnancy per se 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 an Exposure During Pregnancy (EDP) supplemental form and a Pfizer Vaccine CT SAE Report Form have to be filled out.

8.4.4.1 Exposure During Pregnancy (EDP)

An EDP occurs if:

- A female participant is found to be pregnant while receiving or after discontinuing study intervention.

- A male participant who is receiving or has discontinued study intervention exposes a female partner prior to or around the time of conception.

- A female is found to be pregnant while being exposed or having been exposed to study intervention due to environmental exposure. Below are examples of environmental EDP:

  - A female family member or healthcare provider reports that she is pregnant after having been exposed to the study intervention by inhalation or skin contact.

  - A male family member or healthcare provider who has been exposed to the study intervention by inhalation of skin contact then exposes his female partner prior to or around the time of conception.

The investigator must report EDP to Pfizer Safety within 24 hours of the investigator's awareness, irrespective of whether an SAE has occurred. The initial information submitted should include the anticipated date of delivery (see below for information related to termination of pregnancy).

- If EDP occurs in a participant, the investigator must report this information to Pfizer Safety on the Pfizer Vaccine SAE Report Form and an EDP Supplemental Form, regardless of whether an SAE has occurred. Details of the pregnancy will be collected after the start of study intervention and until the participant's last visit in the study.

- If EDP occurs in the setting of environmental exposure, the investigator must report information to Pfizer Safety using the Pfizer Vaccine SAE Report Form and EDP Supplemental Form. Since the exposure information does not pertain to the participant enrolled in the study, the information is not recorded on a eCRF; however, a copy of the completed Pfizer Vaccine SAE Report Form is maintained in the investigator site file.

Follow-up is conducted to obtain general information on the pregnancy and its outcome for all EDP reports with an unknown outcome. The investigator will follow the pregnancy until completion (or until pregnancy termination) and notify Pfizer Safety of the outcome as a follow-up to the initial EDP Supplemental Form. In the case of a live birth, the structural integrity of the neonate can be assessed at the time of birth. In the event of a termination, the reason(s) for termination should be specified and, if clinically possible, the structural integrity of the terminated fetus should be assessed by gross visual inspection (unless preprocedure test findings are conclusive for a congenital anomaly and the findings are reported).

Abnormal pregnancy outcomes are considered SAEs. If the outcome of the pregnancy meets the criteria for an SAE (ie, ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly the investigator should follow the procedures for reporting SAEs. Additional information about pregnancy outcomes that are reported to Pfizer Safety as SAEs follows:

- Spontaneous abortion including miscarriage and missed abortion;

- Neonatal deaths that occur within 1 month of birth should be reported, without regard to causality, as SAEs. In addition, infant deaths after 1 month should be reported as SAEs when the investigator assesses the infant death as related or possibly related to exposure to the study intervention.

- Additional information regarding the EDP may be requested by the sponsor. Further follow-up of birth outcomes will be handled on a case-by-case basis (eg, follow-up on

preterm infants to identify developmental delays). In the case of paternal exposure, the investigator will provide the subject with the Pregnant Partner Release of Information Form to deliver to his partner. The investigator must document in the source documents that the subject was given the Pregnant Partner Release of Information Form to provide to his partner.

8.4.4.2 Exposure During Breastfeeding

An exposure during breastfeeding occurs if:

- A female participant is found to be breastfeeding while receiving or after discontinuing study intervention.

- A female is found to be breastfeeding while being exposed or having been exposed to study intervention (ie, environmental exposure). An example of environmental exposure during breastfeeding is a female family member or healthcare provider who reports that she is breastfeeding after having been exposed to the study intervention by inhalation or skin contact.

The investigator must report exposure during breastfeeding to Pfizer Safety within 24 hours of the investigator's awareness, irrespective of whether an SAE has occurred. The information must be reported using the Pfizer Vaccine SAE Report Form. When exposure during breastfeeding occurs in the setting of environmental exposure, the exposure information does not pertain to the participant enrolled in the study, so the information is not recorded on an eCRF. However, a copy of the completed Pfizer Vaccine SAE Report Form is maintained in the investigator site file.

An exposure during breastfeeding report is not created when a Pfizer drug specifically approved for use in breastfeeding women (e.g., vitamins) is administered in accord with authorized use. However, if the infant experiences an SAE associated with such a drug, the SAE is reported together with the exposure during breastfeeding.

8.4.4.3 Occupational Exposure

The investigator must report any instance of occupational exposure to Pfizer Safety within 24 hours of the investigator's awareness using the Pfizer Vaccine SAE Report Form, regardless of whether there is an associated SAE. Since the information about the occupational exposure does not pertain to a participant enrolled in the study, the information is not recorded on an eCRF; however, a copy of the completed Pfizer Vaccine SAE Report Form must be maintained in the investigator site file.

8.4.5 Medication Errors

Medication errors may result from the administration or consumption of the study intervention by the wrong participant, or at the wrong time, or at the wrong dosage strength.

Exposures to the study intervention under study may occur in clinical trial settings, such as medication errors.

Safety Event	Recorded on the eCRF	Reported on the CT SAE Report Form to Pfizer Safety Within 24 Hours of Awareness
Medication errors	All (regardless of whether associated with an AE)	Only if associated with an SAE

Medication errors include:

- Medication errors involving participant exposure to the study intervention;
- Potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the study participant.

Other examples include, but are not limited to:

- The administration of expired study intervention;
- The administration of an incorrect study intervention;
- The administration of an incorrect dosage;
- The administration of study intervention that has undergone temperature excursion from the specified storage range, unless it is determined by the sponsor that the study intervention under question is acceptable for use.

Such medication errors occurring to a study participant are to be reported as Protocol Deviation and captured on the medication error page of the eCRF, which is a specific version of the AE page.

In the event of a medication dosing error, the sponsor should be notified within 24 hours.

Whether or not the medication error is accompanied by an AE, as determined by the investigator, the medication error is captured as Protocol Deviation and recorded on the medication error page of the eCRF and, if applicable, any associated AE(s), serious and non-serious, are recorded on the AE page of the eCRF.

Medication errors should be reported to Pfizer Safety within 24 hours on a Pfizer Vaccine SAE Report Form **only when associated with an SAE**.

8.4.6 Lack of Efficacy

The investigator must report signs, symptoms, and/or clinical sequelae resulting from lack of efficacy. Lack of efficacy or failure of expected pharmacological action is reportable to Pfizer Safety **only if associated with an SAE**.

8.5 Laboratory Parameters

The following laboratory parameters will be assessed at time points specified below. Parameters will be analyzed on site or by local laboratories according to the applicable laboratory SOP:

- HIV test: for subjects aged ≥12 years, a positive HIV test obtained by ELISA will have to be confirmed by a second method [e.g. Western blotting or PCR]. Performed at following visits: Part A at Visit V0. [3.0 mL blood]
- Serum pregnancy test: for female subjects aged ≥ 12 years and women of childbearing potential. Refer to Section 6.4 for consideration of females as being of childbearing potential. Performed at following visits: Part A at Visit V0. [2.0 mL blood]

- Urine pregnancy test: for female subjects aged ≥ 12 years and women of childbearing potential. Refer to Section 6.4 for consideration of females as being of childbearing potential). Performed at following visits: Part A at Visit 1 and 2-8 and ET, if applicable; Part B at Visit 8B, 9, 10 and ET, if applicable.

The following laboratory parameter will be assessed at time points specified below and will be analyzed by a central laboratory specified by the sponsor:

- *Borrelia burgdorferi* s.l. screening by commercially available Lyme borreliosis screening test, verified by a confirmatory immunoblot for samples with positive result. Performed at following Visits: Part A at Visit 0, 8 and ET, if applicable; Part B at Visit 12, 14 and 16 and ET, if applicable. [5.0 mL blood]

- All laboratory assessments will be documented in the eCRF.

In case lab tests performed during the study as part of medical care reveal abnormal laboratory parameters that are clinically relevant in the opinion of the investigator, these need to be documented as unsolicited AEs. They should be assessed further as done for unsolicited AEs described in Section 8.4.2.

8.6 Safety Monitoring

8.6.1 Internal Review Committee (IRC)

As VLA15-221 is the first study with VLA15 enrolling a pediatric study population, rigorous safety monitoring precautions will be implemented to ensure well-being of all subjects. An independent Internal Review Committee (IRC) will be installed at the sponsor's collaboration partner Pfizer in order to review safety data of sentinel subjects in each age cohort prior to advancing the study into the next younger age cohort and prior to enrolling remaining subjects within each of the pediatric age cohorts (i.e. Cohort 2 and Cohort 3). The IRC will be composed of qualified personnel from Pfizer, and will include at least one pediatrician, statistician, vaccine safety expert and a senior member of the Pfizer Vaccine Research and Development organization. All IRC members will be independent from the VLA15-221 study team. During the staggered subject enrollment phase, the IRC will review unblinded safety data and will give recommendation on whether enrollment of the next younger age cohorts as well as enrollment of remaining subjects of the current age cohort can be initiated as described in Section 4.2.1 ("Subject enrollment"). A written IRC charter will be provided prior to the study start, including a detailed description of IRC set-up and processes.

8.6.2 Data Safety Monitoring Board (DSMB)

An external independent Data Safety Monitoring Board (DSMB) comprising an experienced vaccinologist/pediatrician, a family practitioner/pediatrician, a rheumatologist and a neurologist, who are all experts in the field of Lyme borreliosis, will be installed. The DSMB will review accruing safety data in an unblinded manner at regular intervals throughout the study and might recommend adjusting, pausing or discontinuing the study at any time.

The DSMB will ad-hoc confirm diagnosis of potential LB cases based on medical records received from the investigator after clinical workup according to a standardized procedure. The DSMB will do an adjudication of AESIs and will assess whether cases were new in onset and whether there is any relationship to the study vaccine.

During vaccination periods (i.e., Day 1 to Day 180 in Part A; Month 18 in Part B), the DSMB will periodically review tables and listings of SAEs, Deaths, AESIs, medically attended AEs, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination on a regular basis in scheduled meetings and via remote safety data reviews. SAE reports will be provided to the DSMB up to one year after booster administration. AESIs will be reviewed by the DSMB on an ad-hoc basis up to one year after booster administration. Thereafter, SAEs

and AESIs will be reviewed by Pfizer on an ad-hoc basis up to end of the study. A written DSMB charter will be provided including a detailed description of DSMB set-up and processes.

- Part A: During the vaccination period (i.e., Day 1 to Day 180), the DSMB will periodically review tables and listings of SAEs, Deaths, AESIs, medically attended AEs, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination e.g., on a bi-weekly basis in alternating DSMB meetings and remote reviews.
- Part B: During the vaccination period (i.e., ongoing Visits 8B/Month 18), the DSMB will remotely review tables and listings of SAEs, Deaths, AESIs, medically attended AEs, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination e.g. on a monthly basis. Schedules may be adjusted as described in the DSMB charter.
- Any case reports of SAEs will be provided to the DSMB up to one year after booster dose administration at Month 18.
- Any case reports of AESIs will be reviewed by the DSMB on an ad-hoc basis up to one year after booster dose administration at Month 18. The DSMB will verify diagnosis of potential LB cases (based on medical records), adjudicate all AESIs and assess whether cases are new in onset and causally linked to application of the study vaccine.
- Ad-hoc DSMB reviews will be initiated if at any time during study conduct enrollment is interrupted by a principal investigator, the sponsor or the medical monitor for any safety reasons, or if a pre-specified study stopping rule applies, as described in Section 14.2.

8.6.3 Sponsor

Until the last subject reached Day 180 of Part A or Month 18 of Part B, 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. SAEs and AESIs will be reviewed during the entire study period on an ad-hoc basis.

9 STATISTICAL METHODS AND SAMPLE SIZE

9.1 General Aspects

The data will be analyzed by CCI [redacted]. An SAP will be provided describing in more detail, how the study results will be evaluated.

Data will be summarized by study 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.

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 Sets

Safety Analysis Set (SAS)

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

Full Analysis Set (FAS)

The Full Analysis Set (FAS) is defined to include all subjects enrolled who received at least one vaccination. Subjects will be analyzed according to the study group they had been allocated to, rather than by the actual treatment they received.

Per-Protocol Analysis Set (PPAS)

The Per-Protocol Analysis Set (PPAS) will consist of the FAS population excluding subjects that meet one of the following criteria, which possibly have an impact on the immunogenicity read-out:

- Subjects with less than three primary vaccinations (Day 1, 57 and 180).
- Subjects who received the wrong study medication.
- Subjects who fulfilled exclusion criteria 2, 8, 9, 13.

These criteria for potential protocol violations are identified at the time of study planning. However, during the course of the study 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 blind data review meeting.

Booster Safety Analysis Set (BSAS)

All safety analyses of Part B will be based on the Booster Safety Analysis Set (BSAS), which is defined as all subjects enrolled in Part B who received the booster vaccination. The BSAS will be used for all baseline, safety and tolerability analyses including demographic data, local/systemic tolerability, laboratory data and (S)AEs and AESIs. All analyses based on the BSAS will be carried out using the actual treatment received.

Booster Full Analysis Set (BFAS)

The Booster Full Analysis Set (BFAS) is defined as all subjects who received the booster vaccination. Subjects will be analyzed according to the study group they had been allocated to, rather than by the actual treatment they received.

Booster Per-Protocol Analysis Set (BPPAS)

The Booster Per-Protocol Analysis Set (BPPAS) will consist of the BFAS excluding subjects that meet one of the following criteria, which possibly have an impact on the immunogenicity read-out:

- Subjects enrolled despite exclusion from the PPAS of the Main Study Phase
- Subjects who received the wrong booster medication
- Subjects who fulfilled the Booster Phase exclusion criteria 5 and 8

Further criteria may be defined in the SAP.

9.3 Immunogenicity Analysis

Immunogenicity analyses include the analysis of OspA serotype (ST1 to ST6) specific IgG levels by an IgG binding assay CCI [redacted].

Main Study Phase (Part A):

The primary immunogenicity analysis will compare the Geometric Mean Titers (GMTs) of serotype specific IgG against each OspA ST1 to ST6 between study groups in the PPAS on Day 208. GMTs and GMT ratios will be estimated by applying an analysis of variance (ANOVA) including the factor study group and age cohort. 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, study group, age cohort, study group*age cohort, and *B.b.* s.l. serostatus at baseline) will be performed.

As secondary analysis, GMTs and GMT ratios will be analyzed as outlined above at baseline (screening visit) and Day 85, 180, 194*, 208, 365 and Month 18 for the Main Study Phase; CCI [redacted]

Further secondary immunogenicity analyses will compare the following at time points outlined in the study endpoints:

- The Geometric Mean of the fold rise as compared to baseline (GMFR) for IgG against each OspA ST 1-6 as determined by an IgG binding assay, e.g. ELISA (ANOVA).
- Seroconversion Rates against each OspA serotype. 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:
        - For subjects that are seronegative at Visit 0 (baseline) the SCR is defined as a change from seronegative at Visit 0 to seropositive (i.e., antibody titer of ≥40 U/mL) at a certain time point.
        - For subjects that are seropositive at Visit 0 (baseline) the SCR is defined as a ≥ 4-fold rise in IgG antibody titer from Visit 0.
    - CCI [redacted]
- GMTs for IgG against each OspA serotype (ST1 to ST6), at specified time points, stratified by age group.
- Reverse cumulative distribution analyses of IgG responses will be provided for Day 0, 85, 180, 194†, 208, 365 and Month 18.

All immunogenicity analyses will be performed for the PPAS. It will be described in the SAP which analyses will be repeated for the FAS and which analyses will also be repeated by baseline *B.b.* s.l. serostatus and by age group.

* Visit 5A will be performed by a subset of approximately 150 adult subjects only.
† Visit 5A will be performed by a subset of approximately 150 adult subjects only.

Booster Phase (Part B)
Immunogenicity analyses include the analysis of OspA serotype (ST1 to ST6) specific IgG levels by an IgG binding assay CCI .

The primary immunogenicity analysis will provide 95% Confidence Intervals for Geometric Mean Titers (GMTs) of serotype specific IgG against each OspA ST1 to ST6 in the BPPAS population at Visit 10 (Month 19).
As secondary analysis, GMTs and 95% CI will be provided at Month 18, 23, 26, 30, 36, 42, 48 and 54 in the Booster Phase.

Further secondary immunogenicity analyses will provide the following:

- The Geometric Mean of the fold rise (GMFR) at Month 19 as compared to Visit 0 (baseline), Day 208 and Month 18 for IgG against each OspA ST 1-6 as determined by an IgG binding assay (ANOVA) CCI if applicable.
- Seroconversion Rates against each OspA serotype separately at all study visits.
- GMTs for IgG against each OspA serotype (ST1 to ST6), determined by an IgG binding assay, at specified time points, by age group.
- Reverse cumulative distribution analyses of IgG responses will be provided for Day 0, 85, 180, 194†, 208, 365 and Month 18, 19, 23, 26, 30, 36, 42, 48, and 54.

All immunogenicity analyses of the Booster Phase will be performed for the BPPAS. It will be described in the SAP whether any analyses will be repeated for the BFAS.

9.4 Safety Analysis

Main Study Phase (Part A):

All subjects entered into the study who received at least one vaccination (SAS) 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% CI 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 related AESIs, any medically attended AE and any AE leading to withdrawal from further treatment will be presented for each study group, overall and by system organ class/preferred term up to Day 208. The number and percentage of subjects with any SAE, any AESI, any related SAEs and any related AESIs will also be presented up to Month 18. Differences between study groups will be assessed for significance using Fisher's exact (Fisher-Freeman-Halton) test, whereby a significant overall test will be amended by pair-wise tests.

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 study groups will be assessed for significance using Fisher's exact test, a significant overall test will be amended by pair-wise tests.

It will be defined in the SAP which safety analyses will also be repeated by baseline *B.b.* s.l. serostatus and age group.

Booster Phase (Part B):
All subjects included in the Booster Phase who received a booster vaccination will be included in the booster safety analysis (BSAS). The number and percentage of subjects with solicited

local and systemic AEs up to 7 days after the booster vaccination, with any solicited or unsolicited AE, with any unsolicited AE, with any AESI, and with any SAE up to Visit 9 (Month 19) and with any AESI or SAE up to Visit 16 (Month 54) will be presented for each study group overall and by body system/preferred term. The safety analysis will be done in accordance to procedures used in Part A as outlined in Section 9.4.

9.5 General considerations for the determination of the optimal schedule

The following considerations for identification of the optimal primary vaccination schedule for further development are anticipated. However, these considerations may change with availability of new knowledge (e.g., new information from ongoing studies) or upon review of actual results. In general, exclusion or ranking of primary vaccination schedules will be based on the combined results for all age groups.

1. If both primary vaccination schedules (Month 0-6 or Month 0-2-6) are similarly immunogenic, in general the schedule with the lowest number of vaccinations will be selected;
2. In general, immunogenicity data generated to date indicate a consistent pattern with regards to immune responses to VLA15 in the six serotypes, i.e., 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 clinical data so far and which is the serotype that circulates almost exclusively in the U.S., will be compared with highest priority for determination of the optimal schedule. In case no significant difference between schedules 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 an IgG binding assay, has first priority. Significantly higher GMTs favor one schedule over the other.
    - GMTs for IgG against OspA serotype 2, determined by an IgG binding assay, has second priority. Significantly higher GMTs favor one dose over the other.

9.6 Determination of Sample Size

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

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

Titers observed in the primary endpoint immunogenicity analysis (i.e., Day 208; one month after third vaccination) of 86 subjects receiving 180 µg of VLA15 in an ongoing Phase 2 study

with VLA15 (VLA15-202) were used as basis: In the 180 µg group (i.e. the dose chosen for further development of VLA15), a GMT of 274.7 was observed for ST1 (i.e. the serotype with lowest titers) with a Standard Deviation (LOG10) of 0.53. A total of 180 subjects per group (assuming 10% of the 200 subjects per study group are excluded from the primary PP analysis) will provide 80% power at a two-sided alpha level of 5% to distinguish a GMT of 274.7 in one study group from a putative higher GMT of 394.3 in the other study group.

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

9.7 Data Analyses

Analysis 1 will include all safety and immunogenicity data from selected time points from Part A up to Day 208/ Month 7. This analysis will be done in four parts. Every age cohort will be analyzed separately as soon as data from each cohort is available (Analysis 1.1 (Cohort 1, aged 18-65 years), Analysis 1.2 (Cohort 2, aged 12-17 years), Analysis 1.3 (Cohort 3, aged 5-11 years)). Pooled data from all three cohorts will conclude Analysis 1.

Analysis 2 will include all safety and immunogenicity data from selected time points from Part A up to Month 12 (i.e., 6 months after the third immunization).

Analysis 3 will include all safety data and immunogenicity data from selected time points from Part A up to Month 18 and safety and immunogenicity data from Part B up to Month 19.

Analysis 4 will include safety and immunogenicity data of Part B up to Month 26.
Analysis 5 will include safety and immunogenicity data of Part B up to Month 30.
Analysis 6 will include safety and immunogenicity data of Part B up to Month 42.
Analysis 7 will include safety and immunogenicity data of Part B up to Month 54.

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

10 DEVIATIONS FROM THE PROTOCOL

10.1 Relevant Protocol Deviations

All protocol deviations will be tracked, actions defined, as feasible, and reviewed and assessed. Furthermore, Protocol Deviations will be assessed in a blinded Data Review Meeting before data analyses for their impact on the immunogenicity 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 (Main Study Phase Table 2 and Booster Phase 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:

1. If subject becomes pregnant (please refer to Section 8.4.4 for pregnancy reporting procedures).
2. If a subject reports symptoms which are considered unacceptable by the subject or the investigator, he or she will be withdrawn from further treatment.
3. If a subject experiences an SAE with no likely alternative cause than the study vaccine (i.e., possibly or probably related).
4. Solicited local AE: Grade 3 or 4 injection site reaction that lasts longer than 3 days.
5. 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.
6. 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.
7. If subject develops a malignancy during vaccination phase and receives radiation- or chemotherapy.
8. If subject develops or is found to violate one of the following in- or exclusion criteria of Main Study Phase after enrollment: inclusion criterion 3, exclusion criteria 1, 4-6, 10, 13-15.

If subject develops LB during course of the study and receives treatment for LB within 3 months prior to planned next vaccination, respective vaccination will not be performed.

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/assent withdrawal due to adverse event (identify the respective AE)
- Consent/assent 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/assent withdrawal due to adverse event (identify the respective AE)
- Consent/assent 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

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

11.3 Subject Information and Informed Consent/Assent

It is the investigator's responsibility to obtain freely given written informed consent/assent from the subject or the subject's legal representative(s), as required by local regulations, and the subject's written assent, depending on the subject's age and capability, after adequate explanation of the aims, methods, potential benefits and hazards of the study. Written informed consent/assent has to be obtained before the subject is exposed to any study-related procedures, including screening tests for eligibility.

For children below 7 years of age or according to local requirements, the investigator has to orally explain the aims of the study, its procedures, benefits and risks in an appropriate level.

For children aged 7 years and older or according to local requirements, assent has to be sought.

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's legal representative(s) and the subject, as applicable, will be given a copy of the signed informed consent/assent documentation. The original of the signed and dated informed consent/assent must be retained in the site's records, and is subject to inspection by representatives of the sponsor, or representatives from CAs.

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 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; a risk-based monitoring approach is planned for this study. 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/ assent form, will be maintained by the investigator in strict confidence.

13 DATA HANDLING AND RECORD KEEPING

13.1 Information for Investigators

An Investigator's 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 in line with regulatory requirements. Amendments may be implemented only after CA and IRB 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 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 occurrence of the following criterion will lead to suspension of any further enrollment 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 enrollment and vaccinations:

- 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 study 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 FDA, IRBs 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/ EC in writing in accordance with local requirements. Documentation will be submitted for filing in the Central and Investigator File and the Trial Master File.

14.3 Study Changes in Response to the COVID-19 Pandemic Situation

The study sponsor will continuously monitor and evaluate the development of the COVID-19 pandemic in the area of study sites to determine if any measures need to be implemented to mitigate undue risks to the subjects or in response to local governmental recommendations. Such measures may include, temporarily halting further recruitment, switching in-person visits to phone/video calls, or employing mobile teams to collect serum samples. Any measure would be communicated to the relevant CA and IRB.

15 REPORTING AND PUBLICATION

15.1 Clinical Study Report

In total 5 study reports with following content will be compiled:

Clinical study report 1 will compile the data from Analysis 1:

- All safety and immunogenicity data from selected time points from Part A up to Day 208/ Month 7

Clinical study report 2 will include the data compiled in Analysis 2 and 3:

- All safety and immunogenicity data from selected time points from Part A up to Month 12 (i.e., 6 months after the third immunization)
- All safety data and immunogenicity data from selected time points from Part A up to Month 18 and safety and immunogenicity data from Part B up to Month 19.

Clinical study report 3 will include the data compiled in Analysis 4:

- Safety and immunogenicity data of Part B up to Month 26

Clinical study report 4 will include the data compiled in Analysis 5 and 6:

- Safety and immunogenicity data of Part B up to Month 30
- Safety and immunogenicity data of Part B up to Month 42

Clinical study report 5 will include the data compiled in Analysis 7:

- Safety and immunogenicity data of Part B up to Month 54

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[29]:

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. Travel history and physical examination, medical history

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 (including assessment of body weight and height), skin, head/ eyes /ears/ nose/ throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, and neurological system, 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. Subject presents with an Erythema Migrans (EM) rash - early localized disease (<30 days after tick bite):

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.

Characteristics of an EM rash[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

  https://www.cdc.gov/lyme/signs_symptoms/index.html

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.[10,30]

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.[31]

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[31] should be ordered. Additional work-up as depicted in Table 9 should be initiated.

- In case of clinical suspicion of early disseminated or late disseminated Lyme borreliosis, consider initiating treatment according to standard of care[10,30] and send patient for consultation with a specialist as appropriate.

Table 9 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 • meningitis • radiculopathy • encephalitis • myelitis • cerebral vasculitis • facial palsy	Send patient for consultation with infectious disease specialist/ neurologist as appropriate for further clinical work-up
Arthritis	e.g.	Send patient for consultation with infectious disease

	Symptom	Additional work up at study site
	• recurrent attacks or persisting objective joint swelling (synovitis) in one or a few large joints • intermittent pain in tendons, muscles, joints and bones	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

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