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Clinical Study Protocol

DRUG SUBSTANCE(S)	VLA15
VERSION NO.	Final 6.0
STUDY CODE	VLA15-202
DATE	02-Nov-2021

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.

Phase 2 study

Study Protocol VLA15-202

IND number: CCI

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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.

INVESTIGATIONAL PRODUCT, dosage and mode of administration

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

Two dose levels of VLA15 are evaluated in this study. These dose levels were determined in the run-in phase of another Phase 2 study, VLA15-201: VLA15 135 µg w/ Alum and VLA15 180 µg w/ Alum. Vaccinations are administered intramuscular (I.M.) at three occasions on Day 1 (Month 0), Day 57 (Month 2) and Day 180 (Month 6) (Main Study Phase).

Subjects in the 180 µg dose groups, who completed their primary immunization schedule according to protocol (i.e., three vaccinations without relevant protocol deviations*), are included in a Booster Phase and will receive an additional vaccination administered I.M. at approximately 18 months after their first immunization (Visit 9, see **Table 1** for administration details).

COMPARATOR PRODUCT, dosage and mode of administration

Main Study Phase:

Placebo: Phosphate Buffered Saline (PBS) solution, 1 mL; I.M. vaccinations on Day 1 (Month 0), Day 57 (Month 2) and Day 180 (Month 6).

Booster Phase:

Placebo: Phosphate Buffered Saline (PBS) solution, 1 mL; I.M. vaccination at Month 18.

* A relevant Protocol Deviation (PD) is a PD with possible impact on the immunogenicity profile of the subject.

STUDY OBJECTIVES

Primary objective:

- To investigate the immune response to VLA15 when used in an alternative immunization schedule (i.e. Month 0-2-6) at Day 208 (Month 7, i.e. 1 month after the third immunization) in healthy adults aged 18 - 65 years.

Secondary objectives:

Immunogenicity:

- To investigate the immune response of VLA15 when used in an alternative immunization schedule (i.e. Month 0-2-6) in healthy adults aged 18 – 65 years up to Month 18 (i.e. 12 months after the third immunization).

- To investigate the immune response of a booster dose of VLA15 given approximately 18 months after the first vaccination in healthy adults up to Month 30 (i.e. twelve months after booster).

Safety:

- To investigate the safety profile of VLA15 when used in an alternative immunization schedule (i.e. Month 0-2-6) in healthy adults aged 18 – 65 years up to Month 18 (i.e. 12 months after the third immunization).

- To investigate the safety profile of a booster dose of VLA15 given approximately 18 months after the first vaccination in healthy adults up to Month 30 (i.e. twelve months after booster).

STUDY DESIGN

This is a randomized, observer-blind (subject, sponsor and investigator/site staff involved in clinical evaluation of subjects are blinded), placebo controlled, multicenter Phase 2 study (Figure 1).

A total of approximately 250 subjects were randomized and to be stratified by study site, age group and baseline *B.b.* s.l. serostatus 2:2:1 to receive 135 µg VLA15 w/ alum (approximately100 subjects), 180 µg VLA15 w/ alum (approximately 100 subjects), or placebo (approximately 50 subjects). Selection of the two VLA15 dose groups was performed in a parallel Phase 2 study (i.e. VLA15-201), that evaluated three treatment groups (90 µg, 135 µg and 180 µg of VLA15 w/ alum) in its run-in phase prior to starting study VLA15-202. Vaccinations were administered as intramuscular (I.M.) vaccinations on Day 1 (Month 0), Day 57 (Month 2) and Day 180 (Month 6). Dosing is adjusted by injection volume (see Table 1). Subjects in the 180 µg dose group, the dose selected for further development, who completed the primary immunization schedule (i.e., received vaccinations according to protocol, excluding subjects with relevant protocol deviations*), will be asked to participate in a Booster Phase to investigate the immunogenicity and safety of a booster dose of VLA15 administered approximately 18 months after the first immunization. In the Booster Phase subjects are randomized 2:1 to receive an additional 180 µg VLA15 vaccination or Placebo.

Two interim analyses on safety and immunogenicity data will be performed during the Main Study Phase. The first interim analysis was performed once all subjects have completed Visit

* A relevant Protocol Deviation (PD) is a PD with possible impact on the immunogenicity profile of the subject.

6 (i.e. Day 208/Month 7, four weeks after the last primary vaccination) covering safety and immunogenicity data of selected time points up to Visit 6 including the primary endpoint analysis. The second interim analysis will be performed once all subjects have completed Visit 7 (i.e. Day 365, six months after the last primary vaccination), covering in addition all safety data collected up to this time point. Final analysis of safety and immunogenicity data from the Main Study Phase will be performed once all subjects have completed the follow-up period up to Visit 8 (i.e. Day 545/Month 18, 12 months after the last primary vaccination).

In the Booster Phase three data analyses will be performed: A first analysis will be conducted after all subjects completed Visit 10 (Month 19). The second analysis will be performed once all subjects have completed Visit 11 (Month 24). A final analysis on safety and immunogenicity will be performed after the last subject has completed the last study visit at Month 30 (Visit 12) and will include all safety and immunogenicity data up to Month 30.

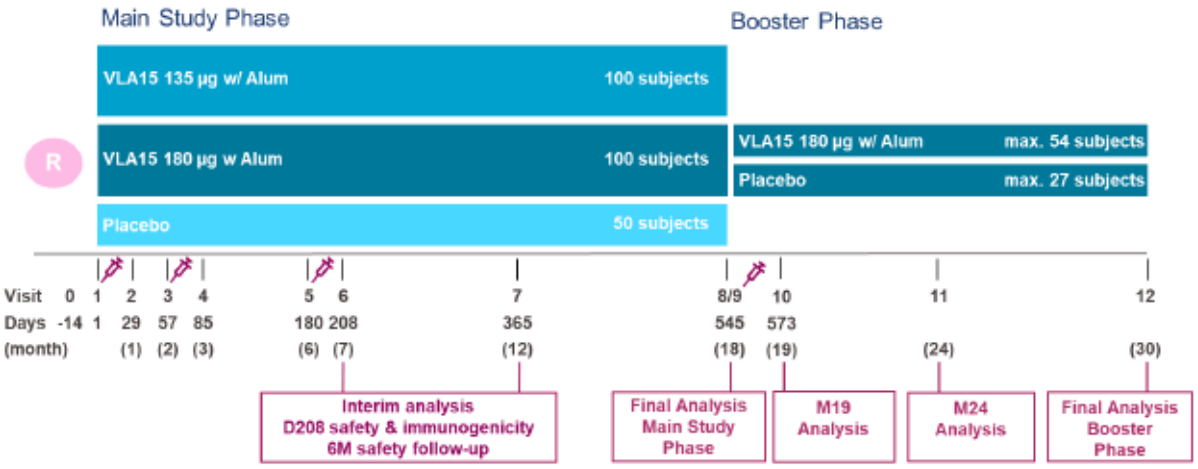

Figure 1 Study Design

Following VLA15 treatment groups were selected for study VLA15-202:

Table 1 Treatment Groups and Vaccinations Main Study Phase

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

Table 2 Treatment Groups and Vaccinations Booster Phase

Group	Treatment	Injection Volume (mL)	Month of Vaccination
180 µg w/ B[1]	VLA15 180 µg w/ alum	1.00	18
180 µg w/o B[1]	PBS	1.00	18

Note: Abbreviation of "180 µg w/ B" refer to treatment group receiving a booster vaccination with 180 µg of VLA15 at Visit 9, while "180 µg w/o B" refers to treatment group receiving a Placebo injection at Visit 9.

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, 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 five study centers.

START DATE

Main Study Phase: July 2019

Booster Phase: January 2021

STUDY DURATION

Study duration per subject in the Main Study Phase is approximately 20 months:

- Screening period: max. 21 days
- Treatment period: 6 months (+/- 7 days)
- Follow-up period: 12 months (+/- 28 days) after the third vaccination

Overall study duration of the Main Study Phase is estimated to be 22 months.

Study duration per subject in the Booster Phase is a maximum of approximately 13 months.

Study duration per subject in the Main Study Phase and Booster Phase together is estimated to be a maximum of approximately 33 months.
Overall study duration (i.e., First-Subject-In to Last-Subject Out/ end of Booster Phase) is estimated to be approximately 37 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 250 healthy subjects aged 18 to 65 years* were to be enrolled in this study. Subjects were enrolled in two age groups (18-49 years† and 50-65 years‡) in a ratio of approximately 2:1.

Target was to enroll approximately 10% or more of subjects that are baseline seropositive for *Borrelia burgdorferi sensu lato* (*B.b. s.l.*). This is aimed to be achieved through selection of endemic recruitment areas as well as database searches for *B.b. s.l.* seropositive subjects. In the Booster Phase of this study only subjects from the Main Study Phase, who completed the primary immunization schedule (i.e., received three vaccinations according to protocol, excluding subjects with relevant protocol deviations§) and who were randomized into the 180 µg dose groups will be asked to participate in the Booster Phase. In the Booster Phase subjects are randomized 2:1 to receive an additional 180 µg VLA15 vaccination or Placebo. Based on

* From the 18th birthday until the last day before the 66th birthday
† From the 18th birthday until the last day before the 50th birthday
‡ From the 50th birthday until the last day before the 66th birthday
§ A relevant Protocol Deviation (PD) is a PD with possible impact on the immunogenicity profile of the subject.

previously observed relevant protocol deviations and dropout rates, the number of participants are estimated to be a maximum of 81 subjects.

CRITERIA FOR INCLUSION/EXCLUSION

Main Study Phase

Approximately 250 male or female adults who meet the inclusion and exclusion criteria listed below were to 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 18 to 65 years* at the day of screening (Visit 0)
2. Subject is of good general health, including subjects with pharmacologically controlled chronic conditions;
3. Subject has an understanding of the study and its procedures, agrees to its provisions, and gives written informed consent prior to any study-related procedures;
4. If subject is of childbearing potential:
    a. Subject has a negative serum pregnancy test at screening (Visit 0);
    b. Subject agrees to employ adequate birth control measures for the duration of the study (please refer to section 6.4).

***Exclusion criteria*:**

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

1. Subject has a chronic illness related to Lyme borreliosis (LB), an active symptomatic LB as suspected or diagnosed by a physician, or received treatment for LB within the last 3 months prior to Visit 0;
2. Subject received previous vaccination against LB;
3. Subject had a tick bite within 4 weeks prior to Visit 1;
4. Subject has a medical history of or currently has a clinically relevant disease (e.g. cardiovascular, respiratory, neurologic, psychiatric conditions) which poses a risk for participation in the study, based on investigators judgement, such as individuals with poorly controlled or unstable disease, ongoing suspected or active inflammation, or poor compliance with pharmacologic treatment. Subjects with pharmacologically controlled conditions like osteoarthritis, depression, or asthma are eligible;
5. Subject has a medical history of or currently has a neuroinflammatory or autoimmune disease, including Guillain Barré Syndrome;
6. Subject has a known thrombocytopenia, bleeding disorder, or received anticoagulants in the 3 weeks prior to each study vaccination, contraindicating I.M. vaccination as judged by the investigator;
7. Subject has received an active or passive immunization within 28 days before or after any vaccination; except for influenza (seasonal or pandemic vaccines which may be administered outside a 7-days interval before or after any trial vaccination);
8. Subject has received any other non-registered medicinal product in another clinical trial within 28 days prior to VLA15 vaccination at Visit 1 (Day 1) and throughout the entire

* From the 18th birthday until the last day before the 66th birthday

study period or has received a registered medicinal product in another clinical trial within 28 days prior to VLA15 vaccination at Visit 1 (Day 1) and up to Day 208;

9. Subject has a known or suspected defect of the immune system that would prevent an immune response to the vaccine, such as subjects with congenital or acquired immunodeficiency, including infection with human immunodeficiency virus (HIV), status post organ transplantation or immuno-suppressive therapy within 30 days prior to Visit 1. Immuno-suppressive therapy is defined as administration of chronic (longer than 14 days) prednisone or equivalent ≥0.05 mg/kg/day. Topical and inhaled steroids are allowed;
10. Subject has a history of anaphylaxis or severe allergic reactions or a known hypersensitivity or allergic reactions to one of the components of the vaccine;
11. Subject had any malignancy in the past 5 years. If treatment for cancer was successfully completed more than 5 years ago and the malignancy is considered to be cured, the subject may be enrolled;
12. Subject had acute febrile infections within 10 days prior to first vaccination;
13. Subject is pregnant (positive serum pregnancy test at screening), has plans to become pregnant during the course of the study or is lactating at the time of enrollment. Women of childbearing potential that are unwilling or unable to employ an adequate birth control measure for the duration of the study.
14. Subject has donated blood or blood-derived products (e.g. plasma) within 30 days or received blood or blood-derived products (e.g. plasma) within 90 days prior to first vaccination in this study or plans to donate or use blood or blood products during the course of the study;
15. Subject has any condition that, in the opinion of the investigator, may compromise the subject's well-being, might interfere with evaluation of study endpoints, or would limit the subject's ability to complete the study;
16. Subject is committed to an institution (by virtue of an order issued either by the judicial or the administrative authorities);
17. Subject is in a dependent relationship with the sponsor, an investigator or other study team member, or the study center. Dependent relationships include close relatives and household members (i.e. children, partner/spouse, siblings, parents) as well as employees of the investigator or study center personnel.

Delay Criteria for Vaccination

Vaccination will be delayed if:

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

In addition, the following criteria must be met:

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

within the specified time window (i.e. within 21days after the screening visit), the subject might be invited for a rescreening.

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

   The rescheduled visit should be within the specified time window.

Booster Phase

Inclusion criteria:

Subjects must meet **ALL** of the following criteria to be eligible for the Booster Phase:

1. Randomization into 180 µg group in the Main Study Phase
2. No relevant protocol deviation* in the Main Study Phase, i.e., included in the PP population for the Day 208 interim analysis of the Main Study;
3. Subject is of good general health, including subjects with pharmacologically controlled chronic conditions;
4. Subject has an understanding of the study and its procedures, agrees to its provisions, and gives written informed consent prior to any study-related procedures;
5. If subject is of childbearing potential:
   a. Subject has a negative Urine pregnancy test before booster vaccination (Visit 9);
   b. Subject agrees to employ adequate birth control measures for the duration of the study (please refer to section 6.4).

Exclusion criteria:

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

1. Subject met an individual stopping criterion during the Main Study Phase
2. Subject has developed a chronic illness related to Lyme borreliosis (LB), an active symptomatic LB as suspected or diagnosed by a physician, or received treatment for LB within the last 3 months prior to Visit 9;
3. Subject has developed a clinically relevant disease (e.g. cardiovascular, respiratory, neurologic, psychiatric conditions) 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.
4. Subject has developed a neuroinflammatory or autoimmune disease, including Guillain Barré Syndrome;
5. Subject has developed an immunodeficiency, including known infection with human immunodeficiency virus (HIV), status post organ transplantation, or immuno-suppressive therapy within 30 days prior to Visit 9. 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 developed anaphylaxis or severe allergic reactions;
7. Subject has developed allergic reactions to one of the components of the vaccine;

* A relevant Protocol Deviation (PD) is a PD with possible impact on the immunogenicity profile of the subject.

8. Subject has developed a malignancy;
9. Subject has developed thrombocytopenia or received anticoagulants in the 3 weeks prior to the booster vaccination contraindicating I.M. vaccination as judged by the investigator;
10. Subject has received any other non-registered medicinal product in another clinical trial within 28 days prior to VLA15 booster vaccination at Visit 9 (Month 18) or plans to participate in another clinical trial with a non-registered medicinal product until Visit 11 (Month 24);
11. Subject is pregnant, or plans to become pregnant prior to Visit 11 (Month 24), or is lactating. Women of childbearing potential that are unwilling or unable to employ an adequate birth control measure for the duration of the study;
12. 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;
13. Subject has been committed to an institution (by virtue of an order issued either by the judicial or the administrative authorities);
14. 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 Booster Vaccination

Vaccination will be delayed if:

1. Subject has
    i. an acute illness with elevated body temperature (≥100.4 °F [38.0 °C]) within 3 days prior to the scheduled vaccination or
    ii. an acute illness, which in the opinion of the investigator could influence post-vaccination safety assessments

    Subjects may be rescheduled for vaccination at a later date provided that the illness has resolved;
2. Subject has received antipyretics within 4 hours prior to the scheduled time of vaccination.
3. Subject received seasonal influenza or pandemic vaccine within 7 days before planned booster vaccination; or an active or passive immunization within 28 days before planned booster vaccination.
4. Subject has donated blood or blood-derived products (e.g. plasma) within 30 days prior to booster vaccination;
5. Subject has received blood or blood-derived products within 90 days prior to booster vaccination

The rescheduled visit should be within the specified time window for Visit 9.

STUDY ENDPOINTS

Primary Endpoint

+ GMTs (Geometric Mean Titers) for IgG against each OspA serotype ST1 to ST6, determined by ELISA at Day 208 (Month 7).

Secondary Endpoints:

Immunogenicity:

Main Study Phase:

+ GMTs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1 (Month 0), 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 365 (Month 12), and 545 (Month 18).
+ SCRs (Seroconversion Rate, defined as rate of subjects that change from seronegative* at Visit 1 (baseline) to seropositive†, if seronegative at baseline, or that achieve a four-fold increase in IgG titer compared to baseline, if seropositive at Visit 1 for each OspA serotype specific IgG (ST1 to ST6), determined by ELISA, at Day 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 208 (Month 7), 365 (Month 12), and 545 (Month 18).
+ GMFR (Geometric Mean of the fold rise) as compared to baseline for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 208 (Month 7), 365 (Month 12), and 545 (Month 18).
+ GMTs, SCRs and GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1 (Month 0), 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 208 (Month 7), 365 (Month 12), and 545 (Month 18) stratified by age group.

Booster Phase (applicable for a subset of subjects participating in booster phase):

+ GMTs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1 (Month 0), 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 365 (Month 12), 545 (Month 18), Month 19, Month 24 and Month 30.
+ SCRs for each OspA serotype specific IgG (ST1 to ST6), determined by ELISA, at Day 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 208 (Month 7), 365 (Month 12), 545 (Month 18), Month 19, Month 24 and Month 30.
+ GMFR as compared to baseline for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 208 (Month 7), 365 (Month 12), 545 (Month 18), Month 19, Month 24 and Month 30.
+ GMFR as compared to Day 208 for IgG against each OspA serotype (ST1 to ST6), determined by ELISA at Month 19, Month 24 and Month 30;
+ GMFR as compared to Month 18 (pre-boost) for IgG against each OspA serotype (ST1 to ST6), determined by ELISA at Month 19, Month 24 and Month 30;
+ GMTs, SCRs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1 (Month 0), 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 208 (Month 7), 365 (Month 12), and 545 (Month 18), Month 19, Month 24 and Month 30, stratified by age group.

* An ELISA titer below 40 U/mL (i.e., the quantitation limit of the ELISA) is considered "OspA IgG seronegative". Values will be replaced by 20 U/mL.
† An ELISA ≥40 U/mL is considered "OspA IgG seropositive".

Safety:

Main Study Phase:

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

Booster Phase (applicable for a subset of subjects participating in booster phase):

- Frequency of SAEs during the entire Booster Phase;
- Frequency of related SAEs during the entire Booster Phase;
- Frequency of AESIs during the entire Booster Phase;
- Frequency of related AESIs during the entire Booster Phase;
- Frequency of unsolicited AEs (incl. clinically relevant laboratory parameters) up to Month 19;
- Frequency of related unsolicited AEs (incl. clinically relevant laboratory parameters) up to Month 19;
- Frequency of solicited local and solicited systemic AEs within 7 days after booster vaccination.
- Frequency of SAEs, AESIs, solicited and unsolicited AEs, stratified by age group.

SAMPLE SIZE JUSTIFICATION

The sample size in study VLA15-202 has been selected to provide a sufficient safety database and for determining the optimal dose (VLA15 w/ alum 135 µg or VLA15 w/ alum 180 µg) in the alternative schedule before advancing the vaccine candidate into Phase 3. Upon completion of the study, the total number of subjects exposed to the dose selected for further development would be approximately N=310, taking together both Phase 2 studies that are currently being conducted with VLA15 (VLA15-201 and VLA15-202). A total of N=100 subjects will have received the selected dose in the alternative immunization schedule Day 1-57-180 (Month 0-2-6). The database would thus allow 95 % confidence that a given reaction would not be observed at a higher rate than 1:(100/3) rate, i.e. 3 %, if it is not observed in this trial using selected dose and a vaccination schedule of Day 1-57-180 (Month 0-2-6).

With respect to the primary endpoint, GMTs for ST1-6 specific IgGs on Day 208: In the absence of an established protective titer and without an estimate for the GMTs with a longer immunization schedule, sample size calculation is based on somewhat arbitrary differences in GMTs between VLA15 treatment groups, in order to demonstrate which titer levels could be distinguished with the proposed sample size. Titers observed in Phase 1 using an

immunization schedule Day 1-29-57 were used as basis: In the 90 µg w/ alum group (i.e. the lowest possible dose group used in the present Phase 2 study), a GMT of 61.3 was observed for ST1 (i.e. the serotype with lowest titers in Phase 1) with a Standard Deviation (LOG10) of 0.51. A total of 100 randomized subjects (90 evaluable subjects assuming a 10% drop-out rate for Day 208) per group will provide 80% power at a two-sided alpha level of 5 % to distinguish a GMT of 61.3 in one treatment group from a putative higher GMT of 100.4 in another treatment group. An approximately 1.6 fold higher titer could thus be distinguished, which is considered a relevant difference.

The overall sample size of 50 subjects in the placebo group has been selected to allow for the internal validation of both safety and immunogenicity results.

For the Booster Phase of this study no formal sample size calculation has been performed and no minimal or maximal number of participants is defined. Based on previously observed relevant protocol deviations and dropout rates, the number of participants are estimated to be a maximum of 81 subjects.

STATISTICAL METHODS

The primary immunogenicity analysis will be an overall and group-wise comparison of the GMTs against each OspA ST1 to ST6 in the per-protocol (PP) population between treatment groups at Day 208 (i.e. 28 days after the last primary immunization with VLA15) by ANOVA (factors treatment group, study site). In addition, GMTs and GMFR's against each OspA serotype ST1 to ST6 will be compared overall and pair-wise between treatment groups on all time points. SCRs will be compared overall and pair-wise between treatment groups by Fisher Freeman Halton test and Fisher exact test, respectively.
Immunogenicity analysis of the booster phase will compare GMTs and GMFR's against each OspA ST1 to ST6 between VLA15 and placebo at Day 1, Day 208, Month 18, Month 19, Month 24 and Month 30 by ANOVA (factors treatment group, study site), in the booster per-protocol (Booster PP) population. GMFRs against each OspA serotype ST1 to ST6 at Month 19, Month 24 and Month 30 will be compared, whereby the increase in IgG titers at Month 19, Month 24 and Month 30 will be calculated compared to respective titers at Day 1 (baseline), Day 208 (post primary series) and Month 18 (pre-booster). SCRs will be compared between VLA15 and placebo by Fisher exact test.
Defined immunogenicity analyses will be repeated on the modified intent-to-treat (mITT) population, and will be repeated stratified by baseline *B.b.* s.l. serostatus and age.

All subjects entered into the study, who received at least one vaccination, will be included in the safety analysis. Subjects who received a booster vaccination will be included into the booster safety analysis. The number and percentage of subjects with solicited local and solicited systemic AEs up to 7 days after each and after any vaccination, and the number and percentage of subjects with unsolicited AEs, medically attended AEs, AESIs and SAEs will be presented for each treatment group overall and by body system/ preferred term. Differences between the treatment groups will be assessed for significance using Fisher's exact (Fisher Freeman Halton) test, whereby a significant overall test will be amended by pair-wise tests. Changes in laboratory values and the frequency of abnormal values will be analyzed descriptively. Defined safety analysis will also be repeated stratified by baseline *B.b. s.l.* serostatus and by age.

INTERIM/ FINAL ANALYSIS

Two interim analyses on safety and immunogenicity data will be performed during the Main Study Phase. The first interim analysis was performed once all subjects have completed Visit 6 (i.e. Day 208/Month 7, four weeks after the last primary vaccination) covering safety and immunogenicity data of selected time points up to Visit 6 including the primary endpoint analysis. The second interim analysis will be performed once all subjects have completed Visit

7 (i.e. Day 365, six months after the last primary vaccination), covering in addition all safety data collected up to this time point.

A final analysis of data from the Main Study Phase will be conducted once the last subject has completed the Main Study Phase, i.e. Visit 8 (Month 18).

In the Booster Phase three data analyses will be performed: A first analysis will be conducted after all subjects completed Visit 10 (Month 19). The second analysis will be performed once all subjects have completed Visit 11 (Month 24). A final analysis on safety and immunogenicity will be performed after the last subject has completed the last study visit at Month 30 (Visit 12) and will include all safety and immunogenicity data up to Month 30.

STUDY REPORTS

A final Clinical Study Report will be written once all data from all subjects up to Day 545 / Month 18 (Main Study Phase) are analyzed.

A Month 24 Addendum to the Clinical Study Report will be compiled containing data on safety and immunogenicity from all subjects included in the Booster Phase up to Month 24 (Visit 11). Further on, a Month 30 Addendum to the Clinical Study Report will contain all safety and immunogenicity data obtained up to Month 30.

SAFETY AND DATA MONITORING

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

Solicited Adverse Events

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

Solicited local Adverse Events (AEs)

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

Solicited systemic Adverse Events (AEs)

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

Unsolicited Adverse Events

Unsolicited AEs are captured in the Subject Diary or Memory Aid throughout the study period. The Subject Diary is verified by a study clinician together with the subject at the subject's next study site visit, prior to these data being entered into the eCRF and prior to the next vaccination (when applicable). Adverse Events of Special Interest (AESI) and SAEs (Serious Adverse Events) 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 are 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 constitute AESIs. In addition, the onset of any potentially autoimmune or neuro-inflammatory disorders 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. Furthermore, symptoms suggesting an LB-associated event and/ or onset of potentially autoimmune or neuro-inflammatory disorders with a potential relationship to the study vaccine receive special attention. Identification of such events from a pre-defined list of AESIs and symptoms suggesting a Borrelia infection are assessed in a guided approach as described below.

The following symptoms will receive particular consideration:

- Expanding red or bluish-red patch (≥5 cm in diameter) with or without central clearing, (i.e. Erythema Migrans);
- 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 FDA for previous clinical programs (please refer to APPENDIX 1).

As part of unsolicited AE assessments, at study Visits 1-12 and ET, if applicable, the investigator will be guided through a scripted safety assessment (i.e. questionnaire) to enquire about symptoms that are consistent with Lyme borreliosis, allowing the investigator to assess whether there is a clinical suspicion for infection with Borrelia or a LB-associated event. In addition, presence of or symptoms suggesting one of the other AESIs from the pre-defined list are determined by the investigator.

In case there is clinical suspicion for Lyme borreliosis or an LB-associated event with potential relationship to the study vaccine, investigators are advised to perform a clinical workup as described in Appendix 2, including specialist referral as needed. Subjects with suspected other AESIs (i.e. immune-mediated disorders) should also be referred to a respective clinical expert for full diagnostic work-up as needed. Retrospective investigation of a pre-vaccination sample may be considered for clinical work-up. The investigator requests 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 fills out the AESI Report Form with all available information, including information provided by the clinical expert, if applicable, and provides 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 with potential relationship to the study vaccine, 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 also provides the AESI Report Form together with available medical records to the DSMB through the CCI Safety Desk. In addition, the DSMB regularly reviews accruing AEs and can recommend to the investigator specialist work-up as needed for any case they consider potential AESIs or cases of LB. The DSMB does a final adjudication of all AESIs and assesses whether cases were new in onset and whether there is any relationship to application of the study vaccine. Narratives with detailed case descriptions are provided for all AESIs.

DATA SAFETY MONITORING BOARD (DSMB)

An independent DSMB, which will include Lyme borreliosis experts, is installed to review accruing safety information. During the Main Study Phase, this review was to be performed in parallel to Phase 2 trial VLA15-201.

Main Study Phase:
The DSMB will, if necessary, determine whether study or individual subject stopping rules have been met. The DSMB will perform ad hoc review of all cases of SAEs. In addition, the DSMB does a final adjudication of all AESIs, assesses whether cases were new in onset and whether there is any relationship to application of the study vaccine. While study vaccinations are ongoing, the DSMB will review listings of SAEs, Deaths, AESIs, medically attended AEs, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination on an approximately monthly basis in scheduled DSMB meetings. A written DSMB charter including a detailed description of DSMB set-up and processes is prepared.

Booster Phase:
The DSMB will continue the ad hoc review of all cases of SAEs and AESIs and will be available for ad hoc meetings, if needed. After IND transfer, SAEs will be reviewed by Pfizer Safety and the DSMB will receive copies of the SAE reports for review. AESIs will be reviewed by the DSMB until study end.

Table 3 TABLE OF EVENTS – Main Study Phase

Visit	V0	V1	V2	V3	V4	V5	V6	V7	V8	Early Termination
Timing Day (D) Month (M)	**D-21**	**D1 M0**	**D29 M1**	**D57 M2**	**D85 M3**	**D180 M6**	**D208 M7**	**D365 M12**	**D545 M18**	**before V8**
Time windows	**-21 to -1**	**0**	**+/- 4**	**+/- 4**	**+/- 4**	**+/- 7**	**+4/-10**	**+7/- 14**	**+/- 28**	**n/a**
Visit type	in-person	in-person	in-person	in-person	in-person	in-person	in-person or remotely (2)	in-person or remotely (2)	in-person or remotely (2)	in-person or remotely (2)
Informed consent (3)	X									
Inclusion/exclusion criteria	X	X (Review)								
Vaccination delay criteria		X		X		X				
Demographic data	X									
Medical history incl. vaccinations	X	X (4)								
Concomitant medications/ treatments incl. vaccinations	X	X	X	X	X	X	X	X	X	X
Physical examination (5), ECG	X									
Vital signs (6)	X	X		X		X				
Evaluation of oral body temperature	X	X (7)		X (7)		X (7)				
HIV test [3.5 mL] (8)	X (9)									
Bb s.l. screening test [4.0 mL] (10)	X (9)								X (11)	X (11)
Baseline serology Sample [5.0 mL] (12)	X (9)									
Serum Pregnancy test [3.5 mL] (13)	X (9)									
Urine Pregnancy test (13)		X (14)	X	X (14)	X	X (14)	X (11)	X (11)	X (11)	X (11)
Clinical chemistry [8.5 mL] (15)	X (9)		X	X (14)	X	X (14)	X (11)		X (11)	
Hematology [4.0 mL] (16)	X (9)		X	X (14)	X	X (14)	X (11)		X (11)	
Coagulation blood sample [4.5 mL] (17)	X (9)									
Urinalysis (18)	X		X	X (14)	X	X (14)	X (11)		X (11)	
Immunogenicity blood sample (19)		X (14) [54 mL]	X [27 mL]	X (14) [27 mL]	X [54 mL]	X (14) [27 mL]	X (11) [54 mL]	X (11), (20) [54 mL]	X (11), (20) [54 mL]	
Randomization (21)		X								
VACCINATION (22)		X		X		X				
Check for AEs following vaccination		X		X		X				
Symptom-driven physical exam (24)		X (25)	X	X (25)	X	X (25)	X (11)	X (11)	X (11)	X (11)
Inspection of injection site of previous vaccinations			X		X		X (23)			X (11), (28)
Distribute and explain Subject Diary (26)		X		X		X				
Review and collect Subject Diary			X		X		X (27)			X(27), (28)
Distribute and explain Memory Aid			X		X		X (11)	X (11)		
Review and collect Memory Aid				X		X		X (11), (27)	X (11), (27)	X(27), (28)

Visit	V0	V1	V2	V3	V4	V5	V6	V7	V8	Early Termination
Timing Day (D) Month (M)	D-21	D1 M0	D29 M1	D57 M2	D85 M3	D180 M6	D208 M7	D365 M12	D545 M18	before V8
Time windows	-21 to -1	0	+/- 4	+/- 4	+/- 4	+/- 7	+4/-10	+7/- 14	+/- 28	n/a
Visit type	in-person	in-person	in-person	in-person	in-person	in-person	in-person or remotely (2)	in-person or remotely (2)	in-person or remotely (2)	in-person or remotely (2)
AE/ SAE/ AESI Assessment (29)		X	X	X	X	X	X	X	X	X
Blood Volume [mL]	33 (13); 29,5 (30)	54.0	39.5	39.5	66.5	39.5	66.5	54.0	70.5	4.0

(1) Every effort should be made to have discontinued subjects complete the early termination visit. If the subject is unwilling to perform an ET visit or an in-person ET visit is not possible due to circumstances of the ongoing COVID-19 situation, a phone-call should be made to follow-up on Adverse Events and Concomitant Medications/ Vaccinations. Note: If a subject presents at a regular study visit and informs that it 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 the respective study visit; in addition. In this case, a Lyme borreliosis screening test is performed in addition.

(2) 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 (e.g., phone/ video call). In case subject visit is performed remotely and the COVID-19 situation allows coming to the site at a later time point, the immunogenicity sample should be taken during an unscheduled visit as early as possible within a maximum time window of 2 months after the remote study visit. For Visit 8, subjects should perform the unscheduled visit for immunogenicity blood within a maximum time window of 1 month after the remote study visit.

(3) Occurs before screening and prior to any study-related procedures.

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

(5) Physical examination includes, but is not limited to assessment of general appearance and skin, head/ eyes/ ears/ nose/ throat, cardiovascular system, respiratory system, abdominal and gastrointestinal system, musculoskeletal system, neurological system and lymph nodes. If applicable, physical examination as well as ECG performed within the study VLA15-201 is acceptable for study VLA15-202 if within the specified visit window.

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

(7) To be performed prior to vaccination.

(8) The results of negative HIV tests that are performed up to 30 days before Visit 0 are acceptable (blood: HIV test 3.5 mL). Positive HIV test obtained by ELISA has to be confirmed by a second method (e.g. Westernblot or PCR).

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

(10) A commercially available Lyme borreliosis screening test will be performed (blood: 4 mL). Serum samples that are tested positive have to be verified by a confirmatory immunoblot. LB test results need to be available before randomization and remain valid for 4 weeks.

(11) For in-person study visits only.

(12) A baseline serology sample is taken at the screening visit and might be used for retrospective work-up of suspected AESIs (e.g. analysis of Rheumatoid factor (RF), anti citrullinated protein antibodies (ACPA) etc., as appropriate). (blood: 5.0 mL). If applicable, a baseline serology sample collected at the study site within the study VLA15-201 is acceptable for study VLA15-202 if within the specified visit window.

(13) In women of childbearing potential. A woman is considered of childbearing potential if fertile, following menarche and until becoming post-menopausal unless permanently sterile. A woman who 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, trans cervical 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.

(14) At vaccination visits, all samples have to be obtained before vaccination. Pregnancy results and urinalysis must be available before vaccination.
(15) Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP (blood: 8.5 mL). Test results from this study visit do not need to be available before vaccination. Tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable.
(16) Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets (EDTA blood: 4 mL). Test results from this study visit do not need to be available before vaccination. Tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable.
(17) Prothrombin time, aPTT, fibrinogen (blood: 4.5 mL). Tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable.
(18) Standard urine dipstick: pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes. Tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable.
(19) Blood is collected for immunogenicity testing by ELISA and for supportive functional antibody analysis by serum bactericidal assay or animal protection models.
(20) In case subject visit is performed remotely, if the COVID-19 situation allows, the immunogenicity sample should be taken in an unscheduled visit as early as possible, within a maximum time window of 2 months after the remote study visit.
(21) To be performed by study staff otherwise not involved with study conduct to keep the study observer-blinded (i.e. unblinded study staff).
(22) Study vaccine has to be administered by study staff otherwise not involved with study conduct to keep the study observer-blinded. Subjects should be observed for at least 30 min after vaccination for treatment of any immediate reactions.
(23) In case subject visit is performed remotely ask subject to describe the appearance of the injection site, to understand whether there is any residual local reaction. If yes, document as AE.
(24) 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.
(25) If subject has any complaints after vaccination, a second symptom-driven physical examination will be performed by the investigator prior to discharge. Subject will only be discharged if in the opinion of the investigator no further concerns exist.
(26) At Visit 1, the subjects are provided with thermometer and measuring tapes. The subjects assess solicited local and systemic AEs themselves over a period of seven consecutive days after each vaccination.
(27) In case visit is performed remotely ask subject to read through diary/memory aid during call, or to mail or email pictures of the diary/memory aid entries to aid the discussion. Instruct subject to continue using the diary/memory aid for documenting AEs and to bring the diary/memory aid along for the next study visit, if applicable.
(28) Unreturned Subject Diaries/ Memory Aids should be collected at the Early Termination Visit. For Early Terminations prior to Visit 6, the previous injection site should be inspected.
(29) AEs, SAEs and AESIs are collected throughout study conduct. Symptoms noted at Visit 1 (prior to vaccination) are not considered adverse events but will be recorded as medical history.
(30) Women of non-childbearing potential and male subjects.

Table 4 TABLE OF EVENTS – Booster Phase

Visit	V9 (1)	V10	V11	V12	Early Termination (2)
Timing Month (M)	**M18**	**M19**	**M24**	**M30**	**n/a**
Timing	**Date of V8**	**Date of V9 +28 days**	**Date of V9 + 6 months**	**Date of V9 + 12 months**	**before V12**
Time windows	**+ max. 2 months**	**+/- 4 days**	**+ /- 14 days**	**+/- 28 days**	**n/a**
Visit type	In-person	In-person or remotely (3)	In-person or remotely (3)	In-person or remotely (3)	In-person or remotely (3)
Informed consent (4)	X				
Inclusion/exclusion criteria	X				
Vaccination delay criteria	X				
Concomitant medications/ treatments incl. vaccinations (5)	X	X	X	X	X
Vital signs (6)	X				
Evaluation of oral body temperature	X (7)				
Bb s.l. screening test [4.0 mL] (8)	X (9)			X	X
Urine Pregnancy test (11)	X (10)	X	X		X
Clinical chemistry [8.5 mL] (12)		X			
Hematology [4.0 mL] (13)		X			
Urinalysis (14)	X (10)	X			
Immunogenicity blood sample (15)	X (10) (9) [27 mL]	X [54 mL]	X [27 mL]	X [27 mL]	
Randomization (16)	X				
VACCINATION (17)	X				
Check for AEs following vaccination	X				
Symptom-driven physical exam (18)	X (19)	X	X	X	X
Inspection of injection site of previous vaccinations		X			X
Distribute and explain Subject Diary (20)	X				
Review and collect Subject Diary		X			X (21)
Distribute and explain Memory Aid		X	X		
Review and collect Memory Aid			X	X	X (21)
AE/ SAE/ AESI Assessment (22)	X	X	X	X	X
Blood Volume [mL]	31.0	66.5	27.0	31.0	4.0

(1) Visit 8 and Visit 9 should be combined in one visit if possible; otherwise the time between Visit 8 and Visit 9 should be kept as short as possible (preferably maximum of one month apart). If the COVID19 pandemic mandates, the subject has the possibility to perform the study visit within 2 months after Visit 8. If both visits are scheduled on the same day, all procedures from Visit 8 have to be performed prior to performing procedures from Visit 9.
(2) 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.
(3) 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 to collect the immunogenicity sample, to perform urine pregnancy testing and urinalysis, and to take a blood sample for assessment of safety lab parameters (i.e., clinical chemistry, hematology), as applicable. Review of subject diary safety data will be performed by the study site via phone/video call. In circumstances where collection of blood samples by the mobile nurse professional or processing of blood samples for safety labs is not feasible subjects will be asked to come to the study site for an unscheduled visit within 1 month after the remote visit to provide respective blood samples, as applicable.
(4) Occurs prior to any procedures of the booster phase.
(5) Any concomitant medications/ treatments or vaccinations should be documented.
(6) Vital signs (Systolic and diastolic blood pressure, pulse rate) to be measured prior to vaccination and in addition prior to discharge in case subject reports any complaints.
(7) To be performed prior to vaccination.
(8) A commercially available Lyme borreliosis screening test will be performed, results are not required for randomization (blood: 4 mL). Serum samples that are tested positive will have to be verified by a confirmatory immunoblot.
(9) Must only be taken if Visit 9 is later than 1 month after Visit 8, otherwise blood sample from Visit 8 will be used as pre-booster sample.
(10) Samples have to be obtained before vaccination. Urinalysis and pregnancy results must be available before vaccination.
(11) In women of childbearing potential. A woman is considered of childbearing potential if fertile, following menarche and until becoming post-menopausal unless permanently sterile. A woman that is considered of non-childbearing potential must be e.g. surgically sterilized for at least 3 months prior to Visit 9 (e.g. by hysterectomy, bilateral salpingectomy, bilateral oophorectomy, transcervical sterilization), or postmenopausal for at least one year prior to Visit 9.
(12) Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP (blood: 8.5 mL). Tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable.
(13) Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets (Ethylenediaminetetraacetic acid [EDTA] blood: 4 mL). Tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable.
(14) Standard urine dipstick: pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes. Tests that were performed in study laboratory within visit window and where results are available at study visit are acceptable.
(15) Blood is collected for immunogenicity testing by ELISA and for supportive functional antibody analysis by serum bactericidal assay or animal protection models.
(16) To be performed by study staff otherwise not involved with study conduct to keep the study observer-blinded (i.e. un-blinded study staff).
(17) Vaccination has to be administered by study staff otherwise not involved with study conduct to keep the study observer-blinded. Subjects enrolled in the Booster Phase should be observed for at least 30 min after vaccination for treatment of any immediate reactions.
(18) Body systems for which the subject reports any symptoms should be evaluated and relevant abnormal findings documented as AEs. At vaccination day the symptom-driven physical exam is to be performed before administration of the vaccination
(19) If subject has any complaints after vaccination, a second symptom-driven physical examination will be performed by the investigator prior to discharge
(20) At Visit 9, the subjects will also 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 booster vaccination
(21) Unreturned Subject Diaries/ Memory Aids should be collected at the Early Termination Visit. For Early Terminations prior to Visit 10, the previous injection site should be inspected.
(22) AEs, SAEs and AESIs will be collected throughout study conduct. All AEs will be reported via eCRF up to Visit 10. Thereafter, only SAEs and AESI will be reported via the eCRF.

SIGNATURE PAGE

Title of Clinical Trial:	**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.**
Study Code:	VLA15-202
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

PPD
Valneva Austria GmbH

Signature Date

PPD
Chief Medical Officer
Valneva Austria GmbH

Signature Date

LIST OF RESPONSIBLE PERSONNEL

Responsible Medical / Safety Officer:

PPD
Valneva Austria GmbH
PPD

Monitoring:

PPD

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

PPD

AESI reporting by email to:
CCI **Safety Desk:**
PPD

Study Medical Monitor:
PPD

Laboratories:

Immunogenicity Sample Logistics, Storage:

The contact details of the Central Lab responsible for sample logistics and storage will be maintained by the Sponsor and provided to the Investigator.

Immunological Assays:

Valneva Austria GmbH
Clinical Serology
PPD

IMP Logistics, Labelling and packaging:

PPD

Statistical Analysis:

PPD

Data Management:

PPD

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LIST OF ABBREVIATIONS

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

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

1. INTRODUCTION

1.1 Disease Background

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

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

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

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

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

1.2 Lyme borreliosis vaccines

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

In LYMErix, OspA was absorbed to aluminum hydroxide and demonstrated vaccine efficacy of 49 % in the first tick season (i.e. after 2 vaccine doses) and of 76 % in the second tick season

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

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

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

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

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

1.3 Vaccine Candidate VLA15

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

Further, the putative T cell epitope in OspA ST1 which presents homology to human leukocyte function-associated antigen-1 (hLFA-1) and previously claimed to induce antibiotic-refractory Lyme arthritis in a subset of naturally infected patients has been replaced with the corresponding sequence from OspA ST2. The design of the VLA15 vaccine has been chosen in order to induce a strong serotype specific immunity needed for protection against infection by *Borrelia* expressing OspA ST1 to ST6.

1.4 Previous Results

1.4.1 Repeat dose toxicology study in rabbits

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

1.4.2 *In vivo* efficacy studies in mice

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

VLA15 induced significant protection compared to placebo at a 3 µg dose against challenge with ticks harboring *B. burgdorferi* (ST1), *B. afzelii* (ST2) or *B. bavariensis* (ST4) which was equal or better than full length OspA with the corresponding serotype. For the *B. burgdorferi* (ST1) challenge, two different strains (Pra1 and Pra4) were used. Immunizing with 0.03 µg or 0.003 µg VLA15 provided significant protection in mice, when *B. afzelii* (ST2) infected ticks were used for challenge. When different doses (3 µg, 0.3 µg, 0.03 µg and 0.003 µg) of VLA15 were assessed for vaccine efficacy against a challenge with either *B. garinii* (ST5) or *B. garinii* (ST6), significant protection could be shown down to 0.03µg. Please refer to Table 5 for a summary of efficacy data in mice.

Table 5 Efficacy of VLA15 in two mouse challenge models using either ticks infected with B. burgdorferi (ST1), B. afzelii (ST2) or B. bavariensis (ST4) (tick challenge) or in vitro grown B. garinii (ST5 or ST6) (needle challenge) for challenge

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

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

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

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

1.4.3 Clinical studies with VLA15

Data from three clinical studies with VLA15 are available: Phase 1 (VLA15-101), as well as two ongoing Phase 2 studies (VLA15-201 and VLA15-202).

1.4.3.1 Phase 1 Study VLA15-101

VLA15-101 is an observer-blind, partially randomized, multi-center dose escalation Phase 1 study. In this first-in-human study a total of 179 subjects aged 18 to < 40 years were enrolled in six treatment groups (approximately 30 subjects per treatment group) to receive three I.M. vaccinations of VLA15 12 µg w/ Alum, VLA15 12 µg w/o Alum, VLA15 48 µg w/ Alum, VLA15 48 µg w/o Alum, VLA15 90 µg w/ Alum or VLA15 90 µg w/o Alum on Days 0, 28 and 56. Primary objective of this study was to assess the safety and tolerability of VLA15 on Day 84 (i.e. one month after the third vaccination). Secondary objectives include safety and immunogenicity of VLA15 until one year after the first vaccination. A subset of subjects (i.e, subjects from one study site that received one of the two higher dose groups (i.e. 48 µg or 90 µg) with or without adjuvant received a booster vaccination at approximately 13 months after the first immunization and were followed up for further six months for safety and immunogenicity. In total 64 subjects participated in the Booster Extension Phase.

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

After the primary vaccination series, 168 of 179 subjects (93.9%) reported any solicited or unsolicited AE up to Day 84. The majority of AEs were mild or moderate. A total of eight subjects (4.5 %) experienced severe related AEs; all of them were solicited AEs, as such counted by definition as related. No vaccine related SAE was reported. There was one death that was not related to the study vaccination and occurred 94 days after the subject received the third vaccination. The subject experienced an SAE of gunshot wound, which was medically attended and had a fatal outcome.Solicited local AEs were reported by 72.4 % (12 µg w/o alum group) to 96.7 % (90 µg w/o alum group) of subjects. Overall, solicited local AEs were significantly less common in the 12 µg groups compared with the 48 µg and 90 µg groups. No significant difference was observed between combined adjuvanted and combined non-adjuvanted treatment groups. The most common solicited local AEs were pain (67.0 %) and tenderness (84.4 %). Rates of solicited local AEs declined after the first vaccination.
Administration of a booster dose at approximately 13 months after the first primary vaccination was generally safe and well tolerated in all treatment groups. A total of 58 of 64 subjects (90.6%) reported any solicited or unsolicited AE up to 6 months after the booster dose. All related AEs were mild or moderate. Neither SAEs were reported during the Booster Extension Phase nor cases of arthritis, rheumatoid arthritis or facial paralysis. Adverse Events that received special consideration, were observed during the entire study duration.

VLA15 was immunogenic in all doses and formulations tested. Adjuvanted formulations were more immunogenic compared to respective non-adjuvanted formulations of the same dose level. A statistically significant dose-response was observed between the 12 µg dose groups and higher dose groups, but not between the 48 and 90 µg groups. The 90 µg w/ Alum group induced SCRs against individual OspA serotypes ranging between 71.4 % for ST1 and

96.4 % for ST2 at one month after the third immunization. There was a consistent pattern with regards to immune responses to VLA15 in the 6 serotypes, i.e., the 48 and 90 µg w/ Alum groups showed highest GMTs for all OspA serotypes. IgG antibody titers and SCRs were substantially higher after three immunizations (Day 84) compared to after two immunizations (Day 56) for all 6 OspA serotypes. As expected, antibodies declined to Day 365. A booster dose applied at approximately 1 year after the first vaccination was highly immunogenic for all doses and formulations tested and all OspA serotypes ST1 to ST6. Four weeks after the booster GMTs for all ST-specific IgGs increased to 2.7 (ST3) to 5.8 (ST1) of respective levels observed at peak levels of the primary vaccination series (Day 84).

Safety and Immunogenicity results of Phase 1 study VLA15-101 are described in more detail in the current version of the Investigator's Brochure.

1.4.3.2 Phase 2 Studies (VLA15-201 and VLA15-202)

Initial data from the two ongoing Phase 2 studies VLA15-201 and VLA15-202 is available.

VLA15-201 Phase 2 Clinical Study

VLA15-201 is an observer-blinded, randomized, multi-centre Phase 2 study. Main objective is to determine the optimal dose of VLA15 in healthy adults aged 18 to 65 years. In total, 572 subjects have been enrolled. Prior to start of the Main Study Phase of VLA15-201, a 120 subjects Run-In Phase was performed in order to evaluate safety of the higher VLA15 dose levels as compared doses used in Phase 1 prior to enrolling higher subject numbers.

In brief, in the Run-In Phase, 120 subjects aged 18-40 years were vaccinated three times at Month 0-1-2 with VLA15 w/ alum 90 µg (N=29), 135 µg (N=31) or 180 µg (N=30), or with placebo (N=30). After DSMB review of available safety data up to Day 85, the higher dose groups (135 µg and 180 µg of VLA15 w/ alum) were cleared for further development and the Main Study Phase was initiated. In the Main Study Phase a total of 452 subjects aged 18-65 years were randomized 2:2:1 to receive VLA15 135 µg (N=183), 180 µg w/ alum (N=175) or placebo (N=94) at Month 0-1-2. Subjects are followed for safety and immunogenicity up to one year after administration of the first vaccination.

To date, safety data up to Day 85 (i.e, 1 month after the third vaccination) and immunogenicity data from Day 1 and Day 85 are available.

Overall, 508 of 572 subjects (88.8%) reported any solicited or unsolicited AE up to Day 85. 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 w/alum group and nine subjects (4.4%) in the 180 µg group.

Solicited local AEs were reported by 89.7% (90 µg w/ alum group), 93.0% (135 µg w/ alum groups) and 96.1% (180 µg w/ alum group) of subjects, compared to 29.8% of subjects in the placebo group. The most common solicited local AEs were pain (68.4%) and tenderness (76.6%). Solicited systemic AEs were reported by 62.1% (90 µg w/ alum group), 67.8% (135 µg w/ alum groups) and 71.7% (180 µg w/ alum group) of subjects, compared to 42.7% of subjects in the placebo group. The most common solicited systemic AEs were headache (33.2%), fatigue (31.6%) and myalgia (41.1%). In general, rates of solicited local and systemic AEs declined with subsequent vaccinations. An overview of solicited AEs is shown in Table 6 and Table 7.

No vaccine related severe unsolicited Adverse Event and no related Serious Adverse Event was reported. One case of osteoarthritis (135 µg w/ alum 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, because no Lyme arthritis or other inflammatory rheumatic disease was observed. Diagnosis of osteoarthritis was agreed by the investigator.

VLA15 was immunogenic at all dose levels tested. A dose response was observed, with lowest IgG titers being observed in the 90 µg w/ alum dose group and highest titers being observed in the 180 w/ alum µg dose group for all serotypes. In the 90 w/ alum µg treatment group, the GMTs ranged from 74.3 (ST1) to 267.4 (ST3). In the 135 µg treatment group the GMTs reached levels of 101.1 (ST1) to 282.2 (ST3), whereas in the 180 µg w/ alum treatment group GMTs as high as 115.8 (ST1) to 308.6 (ST3) could be observed. Differences in GMTs between VLA15 treatment groups were not significant.

VLA15-202 Phase 2 Clinical Study

To date, safety data up to Day 208 (i.e, 1 month after the third vaccination) and immunogenicity data from Day 1, Day 85 and Day 208 from present study VLA15-202 are available.

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).

Overall, 232 of 246 subjects (94.3%) reported any solicited or unsolicited AE up to Day 208. Most AEs were mild or moderate in severity. A total of 15 subjects (6.1%) experienced severe related AEs; 14 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. Subject recovered after 39 days. No related serious AE was reported up to Day 208. One case of Lyme disease (135 µg group) was reported as AESI: 1 subject presented an erythematous rash (approximately 2 cm) approximately 2 weeks after the 1st vaccination. No other cases of predefined AESIs, e.g. arthritis, rheumatoid arthritis or facial paralysis, were observed.

Solicited local AEs 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 (72.8%) and tenderness (82.5%). 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 (48.0%), headache (41.5%) and fatigue (30.1%). Rates of solicited local and systemic AEs declined after the first vaccination. Most solicited AEs were mild or moderate.

An overview of solicited AEs is shown in Table 6 and Table 7.

VLA15 was immunogenic at both dose levels (135 µg, 180 µg) tested. GMTs at Day 85, as measured by ELISA, ranged from 64.1 (ST1) to 166.4 (ST3) in the 135 µg group and from 75.2 (ST1) to 217.7 (ST3) in the 180 µg group. By Day 208, they increased to 276.4 (ST1) to 539.0 (ST2) in the 135 µg group and to 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 Serum Bactericidal Assay for all serotypes with GMTs ranging from 31.4 (ST5) to 1200.8 (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 42.5% (ST5) to 94.7% (ST3) in the 135 µg group and from 44.7% (ST5) to 97.5% (ST3) in the 180 µg group. ELISA titer correlated significantly with SBA titer for all serotypes.

Table 6 Solicited Local Adverse Events After Any Vaccination 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]	31 (31.6)[c]
Induration	3 (10.3)[a,b]	64 (29.9)[a,b]	65 (31.7)[a,b]	26 (26.8)[c]	30 (30.6)[c]

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

Table 7 Solicited Systemic Adverse Events After Any Vaccination 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)	44 (45.4)	42 (42.9)
Fever	2 (6.9)	5 (2.3)	5 (2.4)	6 (6.2)	3 (3.1)
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)	14 (14.3)
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]	29 (29.9)	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
No significant differences
a significant pairwise comparison Placebo vs 90µg, Placebo vs 135µg, Placebo vs 180µg
b significant pairwise comparison Placebo vs 135µg, Placebo vs 180µg
c significant pairwise comparison: Placebo vs 180µg

Reactogenicity after the second and third dose were lower as compared to the first dose.

In summary, the safety and immunogenicity data of Phase 1 and Phase 2 so far are considered encouraging. The AE profile of VLA15 appears comparable to licensed lipidated recombinant vaccines or lipid-containing formulations. Moreover, to date, the independent DSMB has not identified any safety concerns and both Phase 2 studies (VLA15-201 and VLA15-202) are progressing as planned. Indeed, the broader immunization schedule investigated in the ongoing VLA15-202 Phase 2 study contributed to a further optimized immune response.

1.5 Study Rationale

This Phase 2 study VLA15-202 investigates an alternative, broader immunization schedule for the vaccine candidate VLA15. A broader immunization schedule might have a positive impact on the induction of antibodies, which are of utmost importance for OspA-based vaccines where high levels of circulating antibodies are a key factor.

VLA15-202 was initiated following the safety run-in phase of another Phase 2 study (VLA15-201) which assessed 3 doses of VLA15 w/ alum given at three occasions each one month apart (i.e. Month 0-1-2, same immunization schedule as used in the Phase 1 study VLA15-101). In the Run-In phase, a total of 120 healthy subjects aged 18 to 40 years received 90 µg (N=29) VLA15 w/ alum, 135µg VLA15 w/ alum (N=31), 180 µg VLA15 w/ alum (N=30) or placebo (N=30). Based on review of Day 85 safety data by an independent Data Safety Monitoring Board (DSMB), two VLA15 dose groups (135 µg and 180 µg VLA15 w/ alum) were selected for further investigation using schedule Month 0-1-2 in study VLA15-201. At the same time, this present Phase 2 study VLA15-202 was initiated with the two dose groups (135 µg and 180 µg VLA15 w/ alum) selected in VLA15-201 in order to evaluate in parallel an alternative immunization schedule of Month 0-2-6. Taken together, both studies will be used to conclude on the final dose and schedule for further development incl. Phase 3.
Since high levels of OspA antibodies are needed to confer protection with an OspA based vaccine, a booster dose of VLA15 is evaluated to further increase antibody levels. The booster phase of this study will generate data on immune response of a booster dose and antibody persistence data with and without a VLA15 booster dose administered at approximately 1 year after completion of the primary vaccination series to determine whether this is an appropriate time point for administration of the booster dose.
Immunogenicity assessment will be done using a validated serotype specific full-length OspA-ELISA for each serotype. A Serum Bactericidal Assay (SBA) has been developed to assess functionality of antibodies. This assay will be employed at selected time points.

1.6 Risk - Benefit Analysis

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

Safety data in humans are available from a Phase 1 first-in-human study (VLA15-101) and initial data from the ongoing Phase 2 studies are available: Day 85 data from Phase 2 study VLA15-201 and Day 208 data from Phase 2 study VLA15-202.

An independent DSMB has been installed for the purpose of reviewing accumulated safety data in all studies. To date, the DSMB has not identified any safety concerns.

Most AEs were reported to be mild or moderate and comprised typical vaccine reactions: solicited injection site reactions (mainly injection site pain (67.0 %, 68.4%, 72.8% for VLA15-101, VLA15-201, VLA15-202 respectively) and injection site tenderness (84.4% 76.6%, 82.5% for VLA15-101, VLA15-201, VLA15-202 respectively)) or solicited systemic reactions (mainly headache (44.7% 33.2%, 41.5% for VLA15-101, VLA15-201, VLA15-202 respectively), excessive fatigue (25.1%, 31.6%, 30.1% for VLA15-101, VLA15-201, VLA15-202 respectively) and myalgia (25.1%, 41.1%, 48.0%)).

Overall, in all three clinical trials the AE profile of VLA15 appeared comparable to other lipidated recombinant vaccines that might be associated with an increased inflammatory response through interaction with Toll-like receptors [16], for example when compared with the licensed vaccine Trumenba®: For example, in the Phase 2 study VLA15-201, rates of pain in the individual dose groups ranged from 75.9% to 81.8%, from 41.4% to 74.2% and from 55.6% and 63.8% after the first, second and third vaccination with VLA15, respectively. In the Phase 2 study VLA15-202, rates of pain in the individual dose groups ranged from 73.2% to 78.4 %, from 68.1% to 72.8 %, and from 67.4 to 71.1% after the first, second and third vaccination with VLA15, respectively. In comparison, respective rates after each vaccination for Trumenba® (Meningococcal Group B vaccine containing lipidated proteins) were 84.2%, 79.3% and 80.4%, after the first, second and third vaccination [17]. Low rates of fever also appeared to be comparable, ranging from 0% to 3.6% in VLA15-201, 0% to 3.4% in VLA-202 compared with 1.2% to 2.4% for Trumenba®. across all three vaccinations.

In VLA15-201 and VLA15-202, VLA15 is also tested in an adult population that was previously infected with *Borrelia Burgdorferi* sensu lato (*B.B.* s.l.) and has shown a comparable safety profile in this subset of subjects as well. The safety of OspA based vaccines has previously been shown with LYMErix [18, 19] and a similar multivalent OspA based vaccine [15]. In addition, a theoretical risk put forward for a previously licensed OspA vaccine, although never proven, has been eliminated in the design of VLA15: the putative T cell epitope in OspA ST1 presenting homology to human leukocyte function-associated antigen-1 (hLFA-1) and claimed to induce antibiotic-refractory Lyme arthritis in a subset of naturally infected patients, has been eliminated through the replacement by corresponding sequence from OspA ST2.

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

Overall, the safety profile for VLA15 to date is favorable. The safety data support further development with the highest dose tested (180 µg w/ alum), extending the primary immunization schedule with a booster immunization approximately 1 year after the primary vaccination in other to further improve immunogenicity and antibody persistence.

Benefits:

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

In view of the positive safety profile with VLA15 so far and the usually limited risks associated with vaccinations, the risk benefit ratio for VLA15 is assessed to be positive.

2. STUDY OBJECTIVES

2.1 Primary Objective

- To investigate the immune response to VLA15 when used in an alternative immunization schedule (i.e. Month 0-2-6) at Day 208 (Month 7, i.e. 1 month after the third immunization) in healthy adults aged 18 - 65 years.

2.2 Secondary Objectives

Immunogenicity:

- To investigate the immune response of VLA15 when used in an alternative immunization schedule (i.e. Month 0-2-6) in healthy adults aged 18 – 65 years up to Month 18 (i.e. 12 months after the third immunization).

- To investigate the immune response of a booster dose of VLA15 applied approximately 18 months after the first vaccination in healthy adults up to Month 30 (i.e. twelve months after booster).

Safety:

- To investigate the safety profile of VLA15 when used in an alternative immunization schedule (i.e. Month 0-2-6) in healthy adults aged 18 – 65 years up to Month 18 (i.e. 12 months after the third immunization).

- To investigate the safety profile of a booster dose of VLA15 given approximately 18 months after the first vaccination in healthy adults up to Month 30 (i.e. twelve months after booster).

3. SELECTION OF STUDY POPULATION

CRITERIA FOR INCLUSION/EXCLUSION

Main Study Phase:
Approximately 250 male or female adults who meet the inclusion and exclusion criteria listed below were to 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 18 to 65 years* at the day of screening (Visit 0)
2. Subject is of good general health, including subjects with pharmacologically controlled chronic conditions;
3. Subject has an understanding of the study and its procedures, agrees to its provisions, and gives written informed consent prior to any study-related procedures;
4. If subject is of childbearing potential:
    a. Subject has a negative serum pregnancy test at screening (Visit 0);
    b. Subject agrees to employ adequate birth control measures for the duration of the study (please refer to section 6.4).

* From the 18th birthday until the last day before the 66th birthday

Exclusion criteria:

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

1. Subject has a chronic illness related to Lyme borreliosis (LB), an active symptomatic LB as suspected or diagnosed by a physician, or received treatment for LB within the last 3 months prior to Visit 0;
2. Subject received previous vaccination against LB;
3. Subject had a tick bite within 4 weeks prior to Visit 1;
4. Subject has a medical history of or currently has a clinically relevant disease (e.g. cardiovascular, respiratory, neurologic, psychiatric conditions) which poses a risk for participation in the study, based on investigators judgement, such as individuals with poorly controlled or unstable disease, ongoing suspected or active inflammation, or poor compliance with pharmacologic treatment. Subjects with pharmacologically controlled conditions like osteoarthritis, depression, or asthma are eligible;
5. Subject has a medical history of or currently has a neuroinflammatory or autoimmune disease, including Guillain Barré Syndrome;
6. Subject has a known thrombocytopenia, bleeding disorder, or received anticoagulants in the three weeks prior to each study vaccination, contraindicating I.M. vaccination as judged by the investigator;
7. Subject has received an active or passive immunization within 28 days before or after any vaccination; except for influenza (seasonal or pandemic) vaccines which may be administered outside a 7-days interval before or after any trial vaccination;
8. Subject has received any other non-registered medicinal product in another clinical trial within 28 days prior to VLA15 vaccination at Visit 1 (Day 1) and throughout the entire study period or has received a registered medicinal product in another clinical trial within 28 days prior to VLA15 vaccination at Visit 1 (Day 1) and up to Day 208;
9. Subject has a known or suspected defect of the immune system that would prevent an immune response to the vaccine, such as subjects with congenital or acquired immunodeficiency, including infection with human immunodeficiency virus (HIV), status post organ transplantation or immuno-suppressive therapy within 30 days prior to Visit 1. Immuno-suppressive therapy is defined as administration of chronic (longer than 14 days) prednisone or equivalent ≥0.05 mg/kg/day. Topical and inhaled steroids are allowed;
10. Subject has a history of anaphylaxis or severe allergic reactions or a known hypersensitivity or allergic reactions to one of the components of the vaccine;
11. Subject had any malignancy in the past 5 years. If treatment for cancer was successfully completed more than 5 years ago and the malignancy is considered to be cured, the subject may be enrolled;
12. Subject had acute febrile infections within 10 days prior to first vaccination;
13. Subject is pregnant (positive serum pregnancy test at screening), has plans to become pregnant during the course of the study or is lactating at the time of enrollment. Women of childbearing potential that are unwilling or unable to employ an adequate birth control measure for the duration of the study.
14. Subject has donated blood or blood-derived products (e.g. plasma) within 30 days or received blood or blood-derived products (e.g. plasma) within 90 days prior to first vaccination in this study or plans to donate or use blood or blood products during the course of the study;

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

Delay Criteria for Vaccination

Vaccination was delayed if:

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

In addition, the following criteria must be met:

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

   The rescheduled visit should be within the specified time window.

Booster Phase

Inclusion criteria:

Subjects must meet **ALL** of the following criteria to be eligible for the Booster Phase:

1. Randomization into 180 µg group in the Main Study Phase
2. No relevant protocol deviation in the Main Study Phase*, i.e., included in the PP population for the Day 208 interim analysis of the Main study;
3. Subject is of good general health, including subjects with pharmacologically controlled chronic conditions;
4. Subject has an understanding of the study and its procedures, agrees to its provisions, and gives written informed consent prior to any study-related procedures;
5. If subject is of childbearing potential:
   a. Subject has a negative Urine pregnancy test before booster vaccination (Visit 9);

* A relevant Protocol Deviation (PD) is a PD with possible impact on the immunogenicity profile of the subject.

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

Exclusion criteria:

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

1. Subject met an individual stopping criterion during the Main Study Phase
2. Subject has developed a chronic illness related to Lyme borreliosis (LB), an active symptomatic LB as suspected or diagnosed by a physician, or received treatment for LB within the last 3 months prior to Visit 9;
3. Subject has developed a clinically relevant disease (e.g. cardiovascular, respiratory, neurologic, psychiatric conditions) 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.
4. Subject has developed a neuroinflammatory or autoimmune disease, including Guillain Barré Syndrome;
5. Subject has developed an immunodeficiency, including known infection with human immunodeficiency virus (HIV), status post organ transplantation, or immuno-suppressive therapy within 30 days prior to Visit 9. 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 developed anaphylaxis or severe allergic reactions;
7. Subject has developed allergic reactions to one of the components of the vaccine;
8. Subject has developed a malignancy;
9. Subject has developed thrombocytopenia or received anticoagulants in the 3 weeks prior to the booster vaccination contraindicating I.M. vaccination as judged by the investigator;
10. Subject has received any other non-registered medicinal product in another clinical trial within 28 days prior to VLA15 booster vaccination at Visit 9 (Month 18) or plans to participate in another clinical trial with a non-registered medicinal product until Visit 11 (Month 24);
11. Subject is pregnant, or plans to become pregnant prior to Visit 11 (Month 24), or is lactating. Women of childbearing potential that are unwilling or unable to employ an adequate birth control measure for the duration of the study;
12. 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;
13. Subject has been committed to an institution (by virtue of an order issued either by the judicial or the administrative authorities);
14. 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 Booster Vaccination

Vaccination will be delayed if:

1. Subject has
    i. an acute illness with elevated body temperature (≥100.4 °F [38.0 °C]) within 3 days prior to the scheduled vaccination or
    ii. an acute illness, which in the opinion of the investigator could influence post-vaccination safety assessments

    Subjects may be rescheduled for vaccination at a later date provided that the illness has resolved;
2. Subject has received antipyretics within 4 hours prior to the scheduled time of vaccination.
3. Subject received seasonal influenza or pandemic vaccine within 7 days before planned booster vaccination; or an active or passive immunization within 28 days before planned booster vaccination.
4. Subject has donated blood or blood-derived products (e.g. plasma) within 30 days prior to booster vaccination;
5. Subject has received blood or blood-derived products within 90 days prior to booster vaccination

The rescheduled visit should be within the specified time window for Visit 9.

4. INVESTIGATIONAL PLAN

4.1 Study Endpoints

Primary Endpoint:

+ GMTs (Geometric Mean Titers) for IgG against each OspA serotype ST1 to ST6, determined by ELISA at Day 208 (Month 7).

Secondary Endpoints:

Immunogenicity:

Main Study Phase:

+ GMTs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1 (Month 0), 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 365 (Month 12), and 545 (Month 18).
+ SCRs (Seroconversion Rate, defined as rate of subjects that change from seronegative* at Visit 1 (baseline) to seropositive†, if seronegative at baseline, or that achieve a four-fold increase in IgG titer compared to baseline, if seropositive at Visit 1 for each OspA serotype specific IgG (ST1 to ST6), determined by ELISA, at Day 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 208 (Month 7), 365 (Month 12), and 545 (Month 18).
+ GMFR (Geometric Mean of the fold rise) as compared to baseline for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 208 (Month 7), 365 (Month 12), and 545 (Month 18).
+ GMTs, SCRs and GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1 (Month 0), 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 208 (Month 7), 365 (Month 12), and 545 (Month 18) stratified by age group.

Booster Phase (applicable for a subset of subjects participating in booster phase):

+ GMTs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1 (Month 0), 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 365 (Month 12), 545 (Month 18), Month 19, Month 24 and Month 30.
+ SCRs for each OspA serotype specific IgG (ST1 to ST6), determined by ELISA, at Day 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 208 (Month 7), 365 (Month 12), 545 (Month 18), Month 19, Month 24 and Month 30.
+ GMFR as compared to baseline for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 208 (Month 7), 365 (Month 12), 545 (Month 18), Month 19, Month 24 and Month 30.
+ GMFR as compared to Day 208 for IgG against each OspA serotype (ST1 to ST6), determined by ELISA at Month 19, Month 24 and Month 30;
+ GMFR as compared to Month 18 (pre-boost) for IgG against each OspA serotype (ST1 to ST6), determined by ELISA at Month 19, Month 24 and Month 30;
+ GMTs, SCRs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1 (Month 0), 29 (Month 1), 57 (Month 2), 85 (Month 3), 180 (Month 6), 208

* An ELISA titer below 40 U/mL (i.e., the quantitation limit of the ELISA) is considered "OspA IgG seronegative". Values will be replaced by 20 U/mL.
† An ELISA ≥40 U/mL is considered "OspA IgG seropositive".

(Month 7), 365 (Month 12), and 545 (Month 18), Month 19, Month 24 and Month 30, stratified by age group.

Safety:

Main Study Phase:

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

Booster Phase (applicable for a subset of subjects participating in booster phase):

+ Frequency of SAEs during the entire Booster Phase;
+ Frequency of related SAEs during the entire Booster Phase;
+ Frequency of AESIs during the entire Booster Phase;
+ Frequency of related AESIs during the entire Booster Phase;
+ Frequency of unsolicited AEs (incl. clinically relevant laboratory parameters) up to Month 19;
+ Frequency of related unsolicited AEs (incl. clinically relevant laboratory parameters) up to Month 19;
+ Frequency of solicited local and solicited systemic AEs within 7 days after booster vaccination.
+ Frequency of SAEs, AESIs, solicited and unsolicited AEs, stratified by age group.

4.2 Overall Study Design and Plan

4.2.1 Overall study design

This is a randomized, observer-blind (subject, sponsor and investigator/site staff involved in clinical evaluation of subjects are blinded), placebo controlled, multicenter Phase 2 study (Figure 2).

A total of approximately 250 subjects were randomized stratified by study site, age group and baseline *B.b.* s.l. serostatus 2:2:1 to receive 135 µg VLA15 w/ alum (approximately 100 subjects), 180 µg VLA15 w/ alum (approximately 100 subjects), or placebo (approximately 50 subjects). Selection of the two VLA15 dose groups was performed in a parallel Phase 2 study (i.e. VLA15-201), that evaluated three treatment groups (90 µg, 135 µg and 180 µg of VLA15 w/ alum) in its run-in phase prior to starting study VLA15-202. Vaccinations were administered as intramuscular (I.M.) vaccinations on Day 1 (Month 0), Day 57 (Month 2) and Day 180 (Month

6). Dosing is adjusted by injection volume (see Table 1). Subjects in the 180 µg dose group, the dose selected for further development, who completed the primary immunization schedule (i.e., received three vaccinations according to protocol, excluding subjects with relevant protocol deviations*), will be asked to participate in a Booster Phase to investigate the immunogenicity and safety of a booster dose of VLA15 administered approximately 18 months after the first immunization. In the Booster Phase subjects are randomized 2:1 to receive an additional 180 µg VLA15 vaccination or Placebo.

Two interim analyses on safety and immunogenicity data will be performed during the Main Study Phase. The first interim analysis was performed once all subjects have completed Visit 6 (i.e. Day 208/Month 7, four weeks after the last primary vaccination) covering safety and immunogenicity data of selected time points up to Visit 6 including the primary endpoint analysis. The second interim analysis will be performed once all subjects have completed Visit 7 (i.e. Day 365, six months after the last vaccination), covering in addition all safety data collected up to this time point. Final analysis of safety and immunogenicity data from the Main Study Phase will be performed once all subjects have completed the follow-up period up to Visit 8 (i.e. Day 545/Month 18, 12 months after the last vaccination).

In the Booster Phase three data analyses will be performed: A first analysis will be conducted after all subjects completed Visit 10 (Month 19). The second analysis will be performed once all subjects have completed Visit 11 (Month 24). A final analysis on safety and immunogenicity will be performed after the last subject has completed the last study visit at Month 30 (Visit 12) and will include all safety and immunogenicity data up to Month 30.

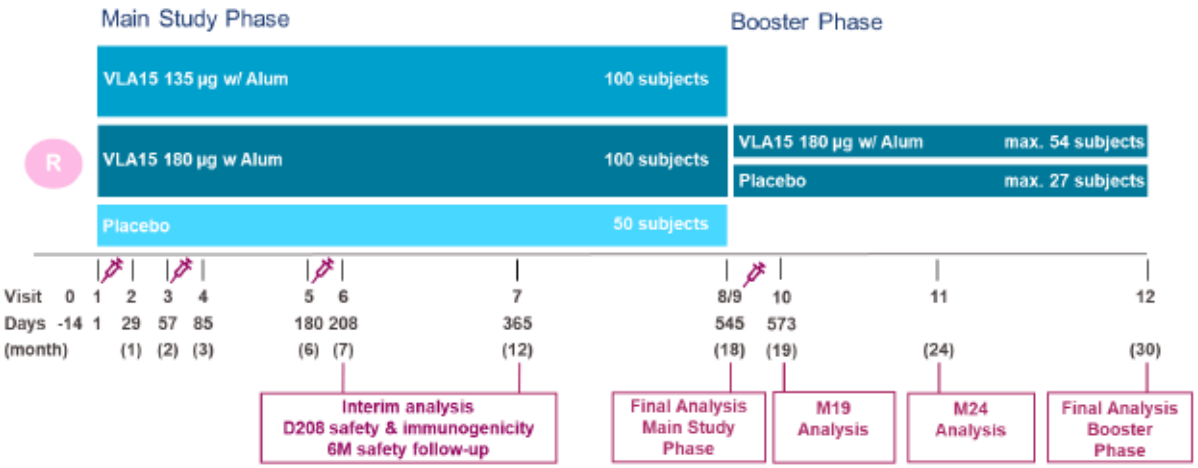

Figure 2 Study Design

Following VLA15 treatment groups were selected for study VLA15-202:

Table 1 Treatment Groups and Vaccinations Main Study Phase

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

* A relevant Protocol Deviation (PD) is a PD with possible impact on the immunogenicity profile of the subject.

Table 2 Treatment Groups and Vaccinations Booster Phase

Group	Treatment	Injection Volume (mL)	Month of Vaccination
180 µg w/ B[1]	VLA15 180 µg w/ alum	1.00	18
180 µg w/o B[1]	PBS	1.00	18

Note: Abbreviation of "180 µg w/ B" refer to treatment group receiving a booster vaccination with VLA15 at Visit 9, while "180 µg w/o B" refers to a treatment group receiving a Placebo injection at Visit 9.

SUBJECT ENROLLMENT

Enrollment in the Main Study Phase was performed without restrictions of recruitment pace. Eligible subjects for the Booster Phase will be enrolled without recruitment pace as well.

4.2.2 Discussion of study design

Rationale for dose/formulation:

The dose groups and formulation used in the present Phase 2 study have been selected based on data from the precedent Phase 1 study VLA15-101. This study revealed that the adjuvanted VLA15 vaccine formulation groups induce better immune response compared to the non-adjuvanted formulation, while safety did not appear to differ between the two formulations. As circulating antibody levels are of utmost importance and a boosting effect upon natural infection cannot be expected for an OspA based vaccine, further dose increase compared to Phase 1 aiming to induce higher immune response is considered. Two higher dose groups of VLA15 w/ alum of 135 µg and 180 µg were investigated in addition to 90 µg (the highest dose group used in Phase 1) in a safety run-in phase in a first Phase 2 study VLA15-201, which started prior to the present study. After review of safety data from the run-in phase at one month after the last immunization by an independent DSMB, the two highest safe dose groups (VLA15 w/ alum 135 µg and 180 µg) were selected to be further investigated in the main study phase of VLA15-201 using an immunization schedule of Month 0-1-2. In parallel, the present Phase 2 study VLA15-202 was initiated in order to investigate the same VLA15 dose groups and formulation as used in the main study phase of VLA15-201 in a broader immunization schedule of Month 0-2-6. A maximum protein dose of 180 µg/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 [20]).

Based on safety and immunogenicity results obtained from the Day 208 interim analysis of this study, the 180 µg dose was selected for further development. 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 treatment group will be continued in the booster phase and in further clinical development of this vaccine.

Doses are adjusted per volume. VLA15 treatment groups with alum content and volume administered are depicted in Table 8 and were selected after DSMB review of safety data from the VLA15-201 run-in phase.

Table 8 Amount of alum and antigen per treatment group

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

Rationale for immunization schedule

The proposed immunization schedule for VLA15 in this study consists of three primary immunizations administered at Month 0, Month 2 and Month 6. In the Phase 1 study as well as in the first Phase 2 study conducted with VLA15, an immunization schedule of three vaccinations one month apart were applied. In general, applying broader immunization schedules is anticipated to result in higher immune responses. This has also been observed in studies using the previously licensed OspA based vaccine LYMERix: In two different clinical studies evaluating alternative immunization schedules for LYMERix primary immunization schedules of Month 0-1-2 and Month 0-1-6 were applied in comparison to the licensed primary schedule of two immunizations one month apart (Month 0-1). Data reveal that the broader immunization schedule of Month 0-1-6 results in 1.5-fold higher GMTs as compared to schedule Month 0-1-2 at one month after the last vaccination (GMT 7205 EU/mL versus 4842 EU/mL, respectively) [21][22]. Initial data of the ongoing Phase 2 studies with VLA15 also demonstrated that a broader immunization schedule of Month 0-2-6 (as applied in present study VLA15-202) led to a 1.7-fold to 2.7-fold increase in peak antibody levels at one month after the third vaccination, depending on serotype, compared with GMTs obtained with the same dose groups (VLA15 135 µg, 180 µg) using a Month 0-1-2 schedule in study VLA15-202. As high circulating antibody levels are of utmost importance for an OspA based vaccine an immunization schedule of Month 0-2-6 is investigated in this study. The efficacy of 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][21]. A booster dose of VLA15 was also highly immunogenic in Phase 1. In order to keep high levels of circulating antibodies for the subsequent tick season, a booster vaccination approximately 18 months after the first immunization will be investigated in subjects that received the 180 µg dose, the dose selected for further development of the VLA15 vaccine, in the Main Study Phase. For comparison of safety and immunogenicity, 1/3 of these subjects will receive a placebo vaccination. Antibody persistence in all subjects will be followed for further 12 months.

Rationale for study population

The proposed study population consists of subjects aged 18 to 65 years* of age of good general health, including subjects with pharmacologically controlled chronic conditions who live in LB endemic regions. Major exclusion criteria include chronic illness related to Lyme borreliosis (LB), an active symptomatic LB as suspected or diagnosed by a physician, treatment for LB within the last three months prior to Visit 0, a tick bite within four weeks prior to Visit 1, history of a neuroinflammatory or autoimmune disease, history of immunodeficiency or ongoing immunosuppressive therapy, known history of anaphylaxis, pregnancy and lactation or any active or passive vaccination within 28 days prior to first study vaccination and during treatment phase. Subjects tested positive for HIV at screening are excluded from the study due to the possible impact of HIV infection on the immunogenicity of the study vaccine. Subjects with a

* From the 18th birthday until the last day before the 66th birthday

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.)* were also enrolled.

The rationale for selecting this study population is to evaluate the most appropriate dose in one of the anticipated target populations for a LB vaccine: adults from 18 to 65 years of age who live in Lyme disease endemic areas. A considerable number of people living in Lyme disease endemic areas were previously infected with Borrelia and are hence seropositive for *Borrelia burgdorferi sensu lato (B.b. s.l.)* antibodies. 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 is be included in this Phase 2 study.

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

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

4.2.3 Study events description

For an outline of procedures required at each visit, please refer to the Tables of Events (Main Study Phase: Table 3, Booster Phase: Table 4).

The study consists of a Screening Visit within 21 days before the first administration of the investigational medicinal product (IMP), eight study visits (Days 1, 29, 57, 85, 180, 208, 365 and 545) in the Main Study Phase and four study visits (Month 18, 19, 24 and 30) in the Booster Phase. Vaccinations will be performed on Days 1, 57 and 180/ Month 0, 2, 6 and at Month 18 for subjects enrolled in the Booster Phase.

Subjects will be observed for 30 min after each vaccination before discharge from study site.

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

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

<u>The following procedures are performed for all subjects:</u>

Visit 0, Screening Visit (1 to 21 days prior to Visit 1):

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

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

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

performed within the study VLA15-201 is acceptable for study VLA15-202 if within the specified visit window.

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

Visit 1, Day 1:

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:

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

† A woman that is considered of non-childbearing potential must be e.g. surgically sterilized for at least 3 months prior to Visit 1 (e.g. by hysterectomy, bilateral salpingectomy, bilateral oophorectomy, transcervical sterilization), or postmenopausal for at least one year prior to Visit 1.

- Urine pregnancy test: In women of childbearing potential. Pregnancy test result must be obtained prior to vaccination.

- Collect immunogenicity blood sample (before vaccination).
- **Designated unblinded staff member only:** Randomize subject to treatment group as described in section 4.2.9. As this study is performed observer-blind, subjects must not be informed about the treatment group they have been allocated to.
- **Designated unblinded staff member only:** Prepare and administer first vaccination according to assigned treatment group into deltoid of the non-dominant arm.
  - Blinded staff member takes over subject: Observe subject for 30 min after vaccination for immediate treatment of possible AEs.
- Record any AEs and local and systemic tolerability following vaccination, if applicable.
- Distribute Subject Diary, thermometer and measuring device: Instruct subject how and when to complete the diary. Subject is also instructed to immediately inform the site in case they experience any severe solicited AEs (see Section 8.3.2 for severity grading of AE) or other severe symptoms or if subject has any suspicion of having Lyme borreliosis.
- 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.

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

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

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

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).
- Review and collect Memory Aid, including verification of entries with the subject.
- Record any unsolicited AEs since the last study visit.
- Laboratory tests (all samples have to be obtained before vaccination, pregnancy results and urinalysis must be available before vaccination):
  - Urine pregnancy test: In women of childbearing potential. Pregnancy test result must be obtained prior to vaccination.
  - Clinical Chemistry: Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP.
  - Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
  - Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.
- Collect immunogenicity blood sample (before vaccination).
- **Designated unblinded staff member only:** Prepare and administer second vaccination according to assigned treatment group into deltoid of the non-dominant arm.
  - Blinded staff member takes subject over: Observe subject for 30 min after vaccination for immediate treatment of possible AEs.
- Record any AEs and local and systemic tolerability following vaccination, if applicable.
- Distribute Subject Diary, instruct subject how and when to complete the diary. Instruct subject also to immediately inform the site in case the subject experiences any severe solicited AEs (see section 8.3.2 for severity grading of AE) or other severe symptoms or if subject has any suspicion of having Lyme borreliosis.
- If the subject has any complaints after vaccination, perform a symptom-driven physical examination and record vital signs prior to discharge. Only discharge a subject if in the opinion of the investigator no further concerns exist.

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

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

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Perform symptom-driven physical examination.
- Inspect vaccination site from second vaccination for (ongoing) Adverse Events.
- Review and collect the Subject Diary, including verification of entries with the subject. Clinician to re-assess severity of reported solicited local and systemic AEs. In addition re-

assess causality for any reported medically attended or severe solicited local and systemic AEs.

- Record any unsolicited AEs since the last study visit.
- Distribute and explain Memory Aid to document unsolicited Adverse Events. Instruct subject to immediately inform the site in case the subject experiences any severe symptoms or if subject has any suspicion of having Lyme borreliosis.
- Laboratory tests:
  - Urine pregnancy test: In women of childbearing potential.
  - Clinical Chemistry: Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP.
  - Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
  - Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.
- Collect immunogenicity blood sample.

Visit 5, Day 180/ Month 6 (+/- 7 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).
- Review and collect Memory Aid, including verification of entries with the subject.
- Record any unsolicited AEs since the last study visit.
- Laboratory tests (all samples have to be obtained before vaccination, pregnancy results and urinalysis must be available before vaccination):
  - Urine pregnancy test: In women of childbearing potential. Pregnancy test result must be obtained prior to vaccination.
  - Clinical Chemistry: Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP.
  - Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
  - Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.
- Collect immunogenicity blood sample (before vaccination).
- **Designated unblinded staff member only:** Prepare and administer third vaccination according to assigned treatment group into deltoid of the non-dominant arm.
  - Blinded staff member takes subject over: Observe subject for 30 min after vaccination for immediate treatment of possible AEs.
- Record any AEs and local and systemic tolerability following vaccination, if applicable.

- Distribute Subject Diary, instruct subject how and when to complete the diary. Instruct subject also to immediately inform the site in case the subject experiences any severe solicited AEs (see section 8.3.2 for severity grading of AE) or other severe symptoms or if subject has any suspicion of having Lyme borreliosis.
- 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.

Visit 6, Day 208/ Month 7 (+/- 10 days):

This visit should preferably be conducted as in-person visit. If an in-person visit cannot be executed 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.
- Inspect vaccination site from third vaccination for (ongoing) Adverse Events.
- Review and collect the Subject Diary, including verification of entries with the subject. Clinician to re-assess severity of reported solicited local and systemic AEs. In addition re-assess causality for any reported medically attended or severe solicited local and systemic AEs.
- Record any unsolicited AEs since the last study visit.
- Distribute and explain Memory Aid to document unsolicited Adverse Events. Instruct subject to immediately inform the site in case the subject experiences any severe symptoms or if subject has any suspicion of having Lyme borreliosis.
- Laboratory tests:
  - Urine pregnancy test: In women of childbearing potential.
  - Clinical Chemistry: Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP.
  - Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
  - Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.
- Collect immunogenicity blood sample.

For remote visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Inspection of vaccination site from third vaccination: Ask subject to describe the appearance of the injection site, to understand whether there is any residual local reaction. If yes, document as AE.

- Review of Subject Diary: Ask subject to read through diary, or to mail or email pictures of the diary entries upfront to aid the discussion. Instruct subject to continue using the diary for documenting AEs and to bring the diary along for the next in-person subject visit.
- Instruct subject to immediately inform the site in case subject experiences one of the following:
    - any severe adverse event
    - any symptoms suspicion or diagnosis of Lyme borreliosis, e.g. red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).
- Perform scripted safety assessment. Record any unsolicited AEs since the last study visit.

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

This 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 (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.
- Review and collect Diary (if not returned yet due to previous visits performed remotely)/ Memory Aid, including verification of entries with the subject.
- Record any unsolicited AEs since the last study visit.
- Distribute and explain Memory Aid to document unsolicited Adverse Events. Instruct subject to immediately inform the site in case the subject experiences any severe symptoms or if subject has any suspicion of having Lyme borreliosis.
- Laboratory tests:
    - Urine pregnancy test: In women of childbearing potential.
- Collect immunogenicity blood sample.

For remote visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review of Memory Aid: Ask subject to read through memory aid/ AE sections of subject diary if previous visit was conducted as remotely, or to mail or email pictures of the memory/diary entries upfront to aid the discussion. Instruct subject to continue using the memory aid/diary for documenting AEs and to bring the memory aid/diary along for the next study visit.
- Instruct subject to immediately inform the site in case subject experiences one of the following:
    - any severe adverse event
    - any symptoms suspicion or diagnosis of Lyme borreliosis, e.g. red or bluish-red patch (≥5 cm, indicate location), or joint swelling (indicate which joint).
- Perform scripted safety assessment. Record any unsolicited AEs since the last study visit.

In case the subject visit is performed remotely and the COVID-19 situation allows coming to the site at a later time point, the immunogenicity sample should be taken during an unscheduled visit as early as possible within a maximum time window of 2 months after the V7 remote study visit.

Visit 8, Day 545/ Month 18 (+/- 28 days):

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

For remote visits

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review of Memory Aid: Ask subject to read through memory aid/ AE sections of subject diary if previous visits were conducted as remote study visits, or to mail or email pictures of the memory/diary entries upfront to aid the discussion.
- Perform scripted safety assessment. Record any unsolicited AEs since the last study visit.
- Inform subjects of the possibility of an unscheduled visit within 1 month after the remote visit for immunogenicity sample collection, if the COVID19 situation allows.
- If the COVID-19 situation allows coming to the site for an unscheduled visit within 1 month after the remote visit: take immunogenicity sample.

The following procedures are performed for subjects enrolled in the Booster Phase:

Visit 9, Month 18 (Date of Visit 8 (+ 2 max. months)):

Visit 8 and Visit 9 should be combined in one visit if possible; otherwise the time between Visit 8 and Visit 9 should be kept as short as possible (preferably maximum of one month apart). If the COVID19 pandemic mandates, the subject has the possibility to perform the study visit within 2 months after Visit 8. If both visits are scheduled on the same day, all procedures from Visit 8 have to be performed prior to performing procedures from Visit 9.

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

- Check of inclusion and exclusion criteria.
- Check vaccination delay criteria.
- Document any changes to concomitant medication/treatments including vaccination(s) since the Visit 8.
- 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 (samples to be taken prior to vaccination):
  - Lyme borreliosis screening test (only to be performed if Visit 9 is later than 1 month after Visit 8): 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 be verified by a confirmatory immunoblot.
  - Urine pregnancy test: In women of childbearing potential.
  - Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.
- Collect immunogenicity blood sample (before vaccination, only to be performed if Visit 9 is later than 1 month after Visit 8).
- Record any unsolicited AEs since the last study visit.
- **Designated unblinded staff member only:** Randomize subject to treatment group as described in section 4.2.9. As this study is performed observer-blind, subjects must not be informed about the treatment group they have been allocated to.
- **Designated unblinded staff member only:** Prepare and administer booster vaccination according to assigned treatment group into deltoid of the non-dominant arm.
- Blinded staff member takes over subject and:
  - Observe subject for 30 min after vaccination for immediate treatment of possible AEs.
- Record any AEs and local and systemic tolerability following vaccination, if applicable.
- Distribute Subject Diary, thermometer and measuring device: Instruct subject how and when to complete the diary.
- Remind subject 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).

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

Visit 10, Month 19 (Date of V9 + 28 days (+/- 4 days)):

For in-person visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review and collect Subject Diary, including verification of entries with the subject. Clinician to re-assess causality for any reported medically attended or severe solicited local and systemic AEs.
- Record any unsolicited AEs since the last study visit.
- Perform symptom-driven physical examination.
- Inspect vaccination site from Booster vaccination for (ongoing) Adverse Events.
- Laboratory tests:
  - Urine pregnancy test: In women of childbearing potential.
  - Clinical Chemistry: Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP.
  - Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
  - Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.
- Collect immunogenicity blood sample.
- Distribute and explain Memory Aid to document unsolicited Adverse Events.
- Remind subject 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.
- Inspection of vaccination site from booster vaccination: Ask subject to describe the appearance of the injection site, to understand whether there is any residual local reaction. If yes, document as AE.
- Review of subject diary: Ask subject to read through subject diary or to mail or email pictures of the diary entries upfront to aid the discussion. Instruct subject to continue using the diary for documenting AEs and to bring the diary along for the next study visit.
- Remind subject 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).

- Perform scripted safety assessment. Record any unsolicited AEs since the last study visit.
- Procedures performed by a mobile nurse professional:
  - Laboratory tests:
    - Urine pregnancy test: In women of childbearing potential.
    - Collect blood sample for Clinical Chemistry: Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin, CRP.
    - Collect blood sample for Hematology: Hemoglobin, hematocrit, erythrocyte count, white blood count, platelets.
    - Urinalysis (standard urine dipstick): pH, specific gravity, leucocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes.
  - Collect immunogenicity sample
  - In circumstances where collection of blood samples by the mobile nurse professional or processing of blood samples for safety labs is not feasible, subjects will be asked to come to the study site for an unscheduled visit within 1 month after the remote visit to provide respective blood samples, as applicable.

Visit 11, Month 24 (Date of V9 + 6 months (+/- 14 days)):

For in-person visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review and collect Memory Aid / Subject Diary, including verification of entries with the subject.
- Record any unsolicited AEs since the last study visit.
- Perform symptom-driven physical examination.
- Laboratory tests:
  - Urine pregnancy test: In women of childbearing potential.
- Collect immunogenicity blood sample.
- Distribute and explain Memory Aid to document unsolicited Adverse Events.
- Remind subject 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 of memory aid/subject diary: Ask subject to read through memory aid/diary or to mail or email pictures of the memory/diary entries upfront to aid the discussion. Instruct subject to continue using the memory aid/subject diary for documenting AEs and to bring the memory aid/subject diary along for the next study visit.
- Remind subject 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).
- Perform scripted safety assessment. Record any unsolicited AEs since the last study visit.
- Procedures performed by a mobile nurse professional:
    - Laboratory tests:
        - Urine pregnancy test: In women of childbearing potential.
    - Collect immunogenicity sample

Visit 12, Month 30 (Date of V9 + 12 months (+/- 28 days):

For in-person visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review and collect Memory Aid / Subject Diary, including verification of entries with the subject.
- Record any unsolicited AEs since the last study visit.
- Perform symptom-driven physical examination.
- Laboratory tests:
    - Lyme borreliosis 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 be verified by a confirmatory immunoblot.
- Collect immunogenicity blood sample

For remote visits:

- Document any changes to concomitant medication/treatments including vaccination(s) since the previous study visit.
- Review of memory aid/diary: Ask subject to read through memory aid/diary or to mail or email pictures of the memory aid/diary upfront to aid the discussion.
- Perform scripted safety assessment. Record any unsolicited AEs since the last study visit.
- Procedures performed by a mobile nurse professional:
    - Collect immunogenicity sample

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. For subjects who perform Visit 10/Month 19 remotely and in circumstances where collection of blood samples by the mobile nurse professional or processing of blood samples for safety labs is not feasible, subjects will be asked to come to the study site for an unscheduled visit within 1 month after the remote visit to provide respective blood sample, as applicable.

Early Termination (before Visit 12):

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 Early Termination visit is before Visit 6 in the Main Study Phase or before Visit 10 in the Booster Phase, inspect vaccination site from previous vaccination for (ongoing) Adverse Events.
- Review and collect unreturned subject diaries or Memory Aids, including verification of entries with the subject. Study physician to re-assess causality for any reported medically attended or severe solicited local and systemic AEs.
- Record any unsolicited AEs since the last study visit.
- Laboratory tests:
  - Lyme borreliosis screening test: For serological screening on infection with Lyme borreliosis, a commercially available Lyme borreliosis screening test is performed. Serum samples that are tested positive are verified by a confirmatory immunoblot.
  - Urine pregnancy test: In women of childbearing potential.

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.

4.2.4 Number of subjects and study centres

Overall, a total of approximately 250 healthy subjects aged 18 to 65 years* were to be enrolled into the Main Study Phase. Subjects were to be enrolled in two age groups (18-49 years† and 50-65 years‡) in a ratio of approximately 2:1. In the Booster Phase eligible subjects from the Main Study Phase from the 180 µg treatment group will be continued. No minimal or maximal number of subjects is defined for the Booster Phase. Based on previously observed relevant protocol deviations and dropout rates, the number of participants are estimated to be a maximum of 81 subjects.
Five study centers in Lyme disease endemic areas in the US were selected to participate in this study. The enrollment was stopped as soon as the required number of subjects was reached.

4.2.5 Timely conduct of the study

Study start of the Main Study Phase was July 2019. Study start of the Booster Phase is expected in January 2021.

4.2.6 Study duration

Study duration per subject in the Main Study Phase is approximately 20 months:

- Screening period: max. 21 days
- Treatment period: 6 months (+/- 7 days)
- Follow-up period: 12 months (+/- 28 days) after the third vaccination

Overall study duration of the Main Study Phase is estimated to be 22 months.

Study duration per subject in the Booster Phase is a maximum of approximately 13 months.

Study duration per subject in the Main Study Phase and Booster Phase together is estimated to be a maximum of approximately 33 months.
Overall study duration (i.e., First-Subject-In to Last-Subject Out/ end of Booster Phase) is estimated to be approximately 37 months.

The end of the study is defined as the date of the last visit performed by the last subject.

4.2.7 Assignment of subjects to treatment groups

A total of 250 subjects were to be randomized to receive 135 µg VLA15 w/ alum, 180 µg VLA15 w/ alum or placebo according to the procedure described in section 4.2.9.

4.2.8 Subject Identification

At Visit 0 a 10-digit subject number was assigned to each subject. The first five digits are the study identifier (15202 for this study). The sixth and seventh digit is the site identification number (e.g. 01). The last three digits are assigned in ascending order as the subjects are screened.

4.2.9 Randomization

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

* From the 18th birthday until the last day before the 66th birthday
† From the 18th birthday until the last day before the 50th birthday
‡ From the 50th birthday until the last day before the 66th birthday

All eligible subjects enrolled in the Booster Phase will be randomized 2:1 to receive an additional VLA15 180 µg vaccination or Placebo according to a randomization list created by a statistician.

4.2.10 Blinding

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

Unblinded:

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

Blinded:

- Study participants
- Investigators and other study staff involved in general study conduct and safety assessments.
- All other CRAs (responsible for monitoring study data apart from IMP handling/drug accountability).
- All other sponsor/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 and the content of the syringe, preparation of IMP must be done by unblinded staff members only in a separate room, unobserved by blinded staff members and the subject. After drawing the respective injection volume into syringes and visual check, the syringe content is masked by covering the syringe with a white, non-see-through adhesive label wrapped around the syringe.

Unblinding during the study:
The blind must not be broken for anyone involved in the trial conduct. Unblinding of individual cases can be performed by the investigator in case of emergency and if knowledge of the treatment assignment is mandatory for emergency treatment. Designated personnel at the 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 trial vaccines (SUSARs), to fulfil safety reporting requirements. Procedures are described in a safety management plan. The same blinding/ emergency unblinding procedures as in the Main Study Phase will be applied during the Booster Phase.

The study sponsor/collaboration partner and trial statisticians will be unblinded at the time of the Day 208 Interim Analysis (primary endpoint analysis), after the respective database

snapshot has been performed. All other study personnel including investigators and other study staff involved in general study conduct and safety assessments as well as laboratory personnel who are performing analytical assays remain blinded and will remain blinded until start of Month 18 visits (Visit 8). As only subjects randomized in the 180 µg dose group during the Main Study Phase will be included in the Booster Phase, partial unblinding of the study site staff and subjects will occur, as subjects that will be asked to continue the study will know that they had been randomized to the VLA15 180 µg group in the Main Study Phase. Investigators will be informed shortly prior to the start of Visit 8 which subjects they should ask to continue in the study via a list of subjects that will be provided by Data Management. Investigators and other site staff will therefore be partially unblinded shortly prior to Visit 8. Subjects who are not being invited will not be able to ascertain if they have been enrolled to the VLA15 135 µg group or the placebo group. Booster subjects, sponsor and investigator/site staff involved in clinical evaluation of subjects will however be blinded for the Booster Phase, i.e., they will not know if subjects that continue the study in the Booster Phase will receive VLA15 180 µg or Placebo.

In the Booster Phase the study sponsor/collaboration partner and trial statisticians will be unblinded at the time of the Month 19 analysis, after the respective database snapshot has been performed.

5. INVESTIGATIONAL MEDICINAL PRODUCTS

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

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

5.1 Description of IMP

5.1.1 VLA15 Drug Product w/ Alum

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

After sterilizing filtration of the VLA15 solution, sterile aluminum hydroxide is added aseptically to a target concentration of 0.5 mg/mL aluminum. The VLA15 Drug Product w/ Alum is filled into 2R Type I Plus® glass vials (1.2 mL filling volume per vial, resulting in 1.0 mL extractable volume) closed with 13 mm injection Flurotec® stoppers secured by crimp caps.

VLA15 w/ Alum is available as a white to off-white suspension of 180 µg/mL protein in a 2 mL glass vial. VLA15 w /Alum should be stored at +2-to +8°C and a retest date of 24 months from date of production is assigned.

This retest date is extended to 36 and further 48 months with data from ongoing stability program provided no significant trends in product quality are observed.

The VLA15 w/ Alum IMP is used for vaccinations in all VLA15 groups that are used in this study: 135 µg w/ Alum and 180 µg w/ Alum.

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

The following two dose groups are used in the present study. Dose selection was performed following a safety run-in phase in a previously started first Phase 2 study VLA15-201.

Table 9 VLA15 Treatment dose with protein content, injection volumes and alum amount

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

5.1.2 VLA Placebo

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

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

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

For the time being, a retest date of 24 months is assigned for VLA Placebo, based on stability data of previous VLA Placebo batches.
This retest date is extended to 36, and further to 48 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 both IMPs (VLA15 w/ Alum and Placebo) is performed by PPD Labels are written in accordance to local law.

5.3 Condition of storage of IMP

VLA15 w/ Alum and Placebo are to 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 are to be used.

DO NOT FREEZE THE VACCINE!

6. TREATMENT OF SUBJECTS

6.1 Investigational Treatment

6.1.1 Dose and dosing schedule

VLA15 is administered at two doses: 135 µg VLA15 w/ Alum, and 180 µg VLA15 w/ Alum. Dose is adjusted by injection volume (see Table 1, Table 2). Placebo is administered as a 1 mL injection. During the Main Study Phase each subject was to receive three I.M. vaccinations on Days 1, 57 and 180. During the Booster Phase subjects from the VLA15 180 µg groups are randomized 2:1 to receive an additional VLA15 vaccination of 180 µg or Placebo.

Table 1 Treatment Groups and Vaccinations Main Study Phase

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

Table 2 Treatment Groups and Vaccinations of Booster Phase

Group	Treatment	Injection Volume (mL)	Month of Vaccination
180 µg w/ B[1]	VLA15 180 µg w/ alum	1.00	18
180 µg w/o B[1]	PBS	1.00	18

Note : Abbreviation of "180 µg w/ B" refers to treatment group receiving a booster vaccination with VLA15 at Visit 9, while "180 µg w/o B" refers to treatment group receiving a Placebo injection at Visit 9.

6.1.2 Preparation and method of administration

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

Remove the vial from the refrigerator. Invert vial at least three times to provide a homogenous turbid suspension (VLA15 w/ Alum) before drawing the respective injection volume into the syringe. Refer to Table 1 (Main Study Phase) or Table 2 (Booster Phase) for volumes to be drawn. DO NOT SHAKE!

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

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

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

A study specific IMP manual with further details on IMP handling has been provided.

Following treatments were selected for investigation in the present study. Dose is adjusted by volume:

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

Main Study Phase:

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

Booster Phase:

- Subjects allocated to the **VLA15 180 µg w/ B group** receive **1.00 mL (1000 µl)** of VLA15 w/ alum I.M. in the deltoid region of the upper non-dominant arm.
- Subjects allocated to the **VLA15 180 µg w/o B group** receive **1.00 mL (1000 µl)** of Placebo I.M. in the deltoid region of the upper non-dominant arm.

6.1.3 Treatment duration

In the Main Study Phase each subject was to be vaccinated within a period of approximately 180 days (vaccinations on Days 1, 57 and 180).
Subjects participating in the Booster Phase will receive an additional vaccination administered I.M. at approximately 18 months after their first immunization.

6.2 Prior and Concomitant Therapy

6.2.1 Permitted prior and concomitant therapy

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

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

6.2.2 Forbidden prior and concomitant therapy

The following prior and concomitant therapy is not allowed:

- Treatment for Lyme borreliosis (LB) within the last three months prior to Visit 0 and Visit 9 (Booster Phase).
- Previous vaccination against LB prior to enrollment into the Main Study Phase.
- Administration of anticoagulants within the three weeks prior to each study vaccination, contraindicating I.M. vaccination as judged by the investigator.
- Administration of any other non-registered medicinal product within 28 days prior to VLA15 vaccination at Visit 1 (Day 1) and up to Visit 11. Administration of any registered medicinal product in another clinical trial within 28 days prior to VLA15 vaccination at Visit 1 (Day 1) and up to Visit 6 of the Main Study Phase and Visit 11 (Month 24) of the Booster Phase.

- Administration of any active or passive immunization 28 days before and after any VLA15 vaccination; except for influenza (seasonal) or pandemic vaccines which may be administered outside a 7-day interval before or after any trial vaccination.
- Administration of chronic (longer than 14 consecutive days) prednisone or equivalent ≥0.05 mg/kg/day. Topical and inhaled steroids are allowed.
- Administration of any blood or blood-derived product within 90 days before Visit 0 (Screening Visit) and Visit 9 and during the entire study period.

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

All forbidden concomitant medications are reflected in exclusion criteria.

6.3 Treatment Compliance

6.3.1 Drug dispensing and accountability

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

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

6.4 Pregnancy testing and birth control

Women of childbearing potential with a negative pregnancy test and the use of adequate birth control before (as defined below) and during conduct of the study until Visit 11 (Month 24) are eligible for inclusion into the study. Negative urine pregnancy results must be available before each study vaccination. Urine pregnancy tests will be conducted at Visit 1-11 and at the ET Visit (if applicable).

A woman is considered of childbearing potential if fertile, following menarche and until becoming post-menopausal unless permanently sterile. Women of childbearing potential must have a negative serum pregnancy test at Visit 0 (Screening Visit), a negative urine pregnancy test before each vaccination and should be practicing an acceptable method of birth control until Visit 11 (Month 24). Urine pregnancy tests are conducted in women of childbearing potential at Visit 1-11 and at the ET Visit (if applicable).

Women of childbearing potential are required to practice birth control throughout the entire Main Study Phase and up to Visit 11 (Month 24) of the Booster Phase. An acceptable method of birth control is defined as those, which result in a low failure rate (i.e. less than 1 % per year) when used consistently and correctly. Such methods include combined (estrogen and progesterone containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal or transdermal), progesterone-only hormonal contraception associated with inhibition of ovulation (oral, injectable or implantable), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), vasectomized partner, sexual abstinence or same sex relationships. Hormonal contraception associated with inhibition of ovulation need to be in place at least for 30 days prior to Visit 1.

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

7. ASSESSMENT OF IMMUNOGENICITY

7.1 Immunogenicity Measurements

Immunogenicity assessments measuring OspA serotype specific by IgG ELISA are performed on samples collected at Visits 1-12, or at respective unscheduled visits after Visit 7 or 8, if one or more of these visits are performed remotely (e.g., phone/ video calls).

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

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

7.1.1 OspA serotype specific IgG antibodies (ELISA)

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

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 and serum bactericidal activity, also be used for further development of the vaccine, including other experimental set-ups and methods.

8. ASSESSMENT OF SAFETY

Please refer to Table 3 (Table of Events – Main Study Phase) and Table 4 (Table of Events – Booster Phase) for exact time points.

AE occurrence rates/ frequencies are used to evaluate the secondary study objectives on safety.

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

The following safety measures are taken:

8.1 Physical Examination

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

A symptom-driven physical examination is performed at each in-person study visit except the Screening Visit (Visit 0), i.e. only in case a symptom is reported by the subject, a system-based assessment is 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, is recorded as an AE unless it occurred prior to vaccination at Visit 1. Symptoms noted during the symptom-driven physical examination at Visit 1 (prior to vaccination) are not considered AEs but are recorded as medical history.

8.2 Vital Signs, ECG and Oral Body Temperature

Vital signs (i.e. systolic and diastolic blood pressure, pulse rate) and oral body temperature will be measured at Visits 0, 1, 3, 5 and 9 (for subjects enrolled in the booster phase). Vital signs will be recorded in a seated position and at rest. At Visit 1, 3, 5, and 9 (if applicable) vital signs and oral body temperature will be measured prior to vaccination. An ECG is performed at Visit 0.

8.3 Solicited AEs and Subject Diary

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

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

Solicited local AEs are the following injection site reactions: pain, tenderness, induration/ hardening, swelling and erythema/ redness.

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

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

8.3.1 Collection, assessment and documentation of solicited AEs

At each vaccination visit, a Subject Diary is distributed and explained to the subject. Subjects are trained to assess presence of solicited AEs and impact on daily activities; to measure the size of the affected area with a measuring tape (for symptoms induration/ hardening, swelling

and erythema/ redness), and instructed to take oral temperature once every day. Unsolicited AEs may be entered into a free text field in the Subject Diary to aid memorizing the AEs.

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

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

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

For Visit 6-8, Visit 10-12, in case visit is performed remotely, subject should be asked to read through the diary/memory aid, or to mail or email pictures of the diary/memory aid entries to aid the discussion. Subject should be instructed to continue using the diary/memory aid for documenting AEs and to bring the diary/memory aid along for the next study visit, if applicable.

After Subject Diary verification, the clinician performs a severity assessment on the basis of the information provided by the subject in the Subject Diary. The severity assessment is based on the grading scale in Table 10 below. Severity categories in this table are NOT identical to the categories provided in the Subject Diary. The investigator or authorized delegate enters diary data and his severity assessment into the eCRF.

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

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

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

8.3.2 Severity of solicited AEs

Severity grading of solicited AEs by the investigator is performed according to Table 10 below. Criteria are based on the FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Subjects Enrolled in Preventive Vaccine Clinical Trials (2007), where possible. When symptoms are below the limit for Grade 1, they are captured as "below grade 1" in the eCRF.

Table 10 Grading of Solicited Adverse Events

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

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

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

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

8.4 Unsolicited AEs

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

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

Unsolicited local and systemic AEs are defined as follows:

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

8.4.1 Collection, assessment and documentation of unsolicited AEs

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

Clinically relevant laboratory parameter changes constitute unsolicited AEs, too, unless they are considered a symptom of an underlying AE or part of a syndrome that is reported as AE (e.g. presence of blood cells in urine in a person diagnosed with urinary tract infection). In addition, symptoms noted during the symptom-driven physical exams (unless already covered by respective solicited AE) constitute unsolicited AEs. At study Visits 2, 4, 6, 7, 10 and 11 when no vaccination is given and hence no Subject Diary is issued, Memory Aids are handed out to

subjects to note down any either ongoing or new AEs and dates of onset/ resolution, whether medication was taken in response and whether medical advice was sought.

For Visit 6, 7, 10 and 11 in case visit is performed remotely, subject should be instructed to continue using the diary/memory aid distributed at the last study visit for documenting AEs and to bring the diary/memory aid along for the next study visit, if applicable.

The investigator follows-up each AE until it is resolved or until the medical condition of the subject is stable. All relevant follow-up information is reported until the end of the study for the subject. SAEs ongoing at the time of Visit 8 (or ET Visit) of the Main Study Phase or until Visit 12 of the Booster Phase are followed until resolution or achievement of stable clinical conditions, latest until the global end of the study.

Unsolicited AEs need to be assessed for causality and graded for severity by the investigator, using a grading of mild, moderate, severe or life-threatening. For the severity grading of unsolicited AEs, see section 8.4.2.5.

All unsolicited AEs reported during the Main Study Phase and up to Visit 10 of the Booster Phase need to be documented in the respective AE section of the eCRF during every study visit (Visit 1-10, or ET visit if applicable), regardless of their source (e.g. open question to subject, lab parameters from Visit 2-10, symptom-driven physical examination, unsolicited AEs noted in the Subject Diary/ Memory Aid). Any unsolicited AEs reported during the Booster Phase after Visit 10 will remain in the source data, but will not be documented in the respective AE section of the eCRF.

Any systemic symptom is regarded as separate AE. However, if the investigator considers several systemic symptoms to be in the context of one underlying diagnosis, (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 is documented for each unsolicited AE: Causality, Severity, Outcome, Seriousness, Medically-attended, Action Taken to Treat AE, Action Taken on IMP Start and Stop Dates.

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.

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 classifies the SAEs as either expected or unexpected.

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

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

8.4.2.3 Definition of Adverse Events of Special Interest (AESIs)

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

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

Subjects are 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 receives therefore particular attention and are captured as AESIs as well. Furthermore, symptoms suggesting an LB-associated event and/ or onset of potentially autoimmune or neuro-inflammatory disorders with a potential relationship to the study vaccine receive special attention. Identification of such events from a pre-defined list of AESIs and symptoms suggesting a Borrelia infection are assessed in a guided approach as described below.

The following symptoms will receive particular consideration:

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

As part of unsolicited AE assessments, at study Visits 1-12 and ET, if applicable, the investigator is 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 are determined by the investigator.

In case there is clinical suspicion for Lyme borreliosis or an LB-associated event with potential relationship to the study vaccine, investigators are advised to perform a clinical workup as described in Appendix 2, including specialist referral as needed. Subjects with suspected other AESIs (i.e. immune-mediated disorders) should also be referred to a respective clinical expert for full diagnostic work-up as needed. Retrospective investigation of a pre-vaccination sample may be considered for clinical work-up. The investigator requests 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 fills out the AESI Report Form with all available information, including information provided by the clinical expert, if applicable, and provides 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 with potential relationship to the study vaccine, the DSMB confirms 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 also provides the AESI Report Form together with available medical records to the DSMB through the CCI n Safety Desk. In addition, the DSMB regularly reviews accruing AEs and can recommend to the investigator specialist work-up as needed for any case they consider potential AESIs or cases of LB. The DSMB does a final adjudication of all AESIs and assesses whether cases were new in onset and whether there is any relationship to application of the study vaccine. Narratives with detailed case descriptions are 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 8.4.4 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:

8.4.2.5 Severity of unsolicited AEs

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

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

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

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

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

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

8.4.2.6 Causality assessment

Probable: A reaction that follows a reasonable temporal sequence from administration of the investigational medicinal product; or that follows a known or expected response pattern to the suspected

treatment; or that is confirmed by stopping or reducing the dosage of the treatment; and that could not reasonably be explained by known characteristics of the subject's clinical state.

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

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

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

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

8.4.2.7 Outcome

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

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

8.4.2.8 Actions taken

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

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

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

In the case of a severe anaphylactic reaction subjects are promptly 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

a) in general	b) on the investigational product
Unknown	Third dose not administered
Withdrawn from study	

8.4.3 Timeframe for reporting of unsolicited AEs

All AEs occurring during the study after administration of the first vaccination up to Visit 10 must be recorded in the eCRF. SAEs must be reported to the sponsor in expedited manner as outlined below.

8.4.4 SAE reporting procedures

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

Medical or diagnostic procedures due to an underlying disease or symptom are not considered an AE but a consequent measure following an AE. A correct SAE report therefore has 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.

PPD

In addition, expedited and periodic reporting to the Competent Authority and IRB(s) is 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 requirements, International Conference on harmonization (ICH) GCP, national law 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 are 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.

8.4.5 Pregnancy reporting procedures

Women must not become pregnant during the entire Main Study Phase and up to Visit 11 (i.e., Month 24, 6 months after the booster dose, if applicable). If a subject becomes pregnant during the study, she must immediately inform the investigator. No further study vaccinations must be administered and the subject should attend all remaining visits as planned.

Reporting requirements start with the first vaccination until Visit 12 (or ET Visit, if applicable). All pregnancies are followed up for three months after delivery or termination of the pregnancy. Any effect on either mother or fetus should be determined. A pregnancy which led to a

congenital anomaly/ birth defect must be followed-up by the investigator longer or until resolution which will be decided on individual basis and in accordance with the sponsor.

The investigator should report pregnancies to CCI Safety Desk within 24 hours of being notified using the Pregnancy Report Form. A pregnancy is not considered an SAE.

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

8.5 Laboratory Parameters

The following laboratory parameters are assessed at time points specified in the Table of Events Table 3 (Main Study Phase) and Table 4 (Booster Phase). Parameters are analyzed by local laboratories according to the applicable laboratory SOP:

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

The following parameter is assessed at time points specified in the Table of Events (Table 3 and Table 4) and is analyzed by a local laboratory or a central laboratory as specified by the sponsor.

- *Borrelia burgdorferi* s.l. screening by commercially available Lyme borreliosis screening test , at Visit 0, Visit 8, 9, 12 and ET, if applicable, and in cases where subject informs at a regular visit that it discontinues the study after this visit. Serum samples that are tested positive are verified by a confirmatory immunoblot.

An amount of 3.5 mL is withdrawn for the HIV screening sample and a blood sample of 4.0 mL is taken for the *B.b.* s.l. screening test. For the baseline serology sample, 5.0 mL are taken, and 3.5 mL are taken for the serum pregnancy test. An amount of 8.5 mL is taken for clinical chemistry testing, 4.0 mL for hematology testing and further 4.5 mL for testing of coagulation factors at screening. The maximum total blood volume including blood samples for immunogenicity testing is 70.5 mL (Visit 8) (as depicted in Table 2).

- All laboratory assessments and the clinical relevance of abnormal values are documented in the eCRF.

- Abnormal laboratory assessments that are clinically relevant (in the opinion of the investigator) need to be documented as unsolicited AEs and assessed further for severity according to Table 11, for causality and other assessments done for unsolicited AE. Laboratory assessments, for which no grading is described in Table 11, are graded as described in section 8.4.2.4.
- Abnormal laboratory assessments that are considered a symptom of an underlying AE or part of a syndrome that is reported as AE (e.g. presence of blood cells in urine in a person diagnosed with urinary tract infection) do NOT additionally need to be documented as unsolicited AE, but a respective comment should be added to that AE.
- For statistical analysis, laboratory assessments are graded according to the grading scale provided in Table 11. The grading scale is based on the FDA Guidance for Industry, Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (September 2007), where applicable.

Table 11 Grading Scale for Abnormal Laboratory Assessments

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

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

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

8.6 Safety Monitoring

8.6.1 Data Safety Monitoring Board (DSMB)

An independent DSMB was installed to review accruing safety information from this trial in parallel to Phase 2 trial VLA15-201 and VLA15-221, and if necessary, to determine whether study or individual subject stopping rules are met. The DSMB reviews all cases of SAEs on an ad-hoc-basis during the main study phase. In addition, the DSMB adjudicates potential cases of LB and AESIs and determines whether cases were new in onset and whether there is any relationship to application of the study vaccine. During the Main Study Phase while study vaccinations are ongoing, the DSMB reviewed listings of SAEs, deaths, AESIs, medically attended AEs, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination on an approximately monthly basis in scheduled DSMB meetings. During the Booster Phase the DSMB will review all cases of SAEs and AESIs and will be available for ad hoc meetings if requested. After the IND transfer, SAEs will be reviewed by Pfizer Safety and the DSMB will receive copies of the SAE reports for review. AESIs will be reviewed by the DSMB until study end.

A written DSMB charter including a detailed description of DSMB set-up and processes is prepared.

The DSMB reviews the following data:

- Any case reports of SAEs are provided to the DSMB until study end.
- At scheduled meetings, the DSMB reviews listings and summary tabulations of SAEs, AESIs, deaths, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination.
- Ad-hoc DSMB reviews are initiated if at any time during study conduct enrollment is interrupted by a principal investigator, the sponsor or the medical monitor at the CRO for any safety reasons, or if a pre-specified study stopping rule applies, as described in section 14.2.

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

8.6.2 Sponsor

Until the last subject of the Main Study Phase reaches Day 180 and during ongoing vaccinations in the Booster Phase, available safety data is reviewed by the sponsor on a regular basis to identify any potential safety concerns and applicability of study stopping rule as described in section 14.2.

8.6.3 Investigator

To ensure information exchange on safety across sites during recruitment and treatment phases, investigators are provided safety listings once a week until the last subject reached Day 180 in the Main Study Phase and while vaccinations are ongoing in the Booster Phase. These listings present information on all grade 3 and 4 solicited and unsolicited adverse events, SAEs and SUSARs reported in the safety database.

9. STATISTICAL METHODS AND SAMPLE SIZE

9.1 General Aspects

The data will be analyzed by a CRO. An SAP will be provided describing in more detail how the study results will be evaluated.

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

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

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

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

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

9.2 Analysis Populations

Safety Population

The safety population includes all subjects who entered into the study and received at least one vaccination. The safety population will be used for all safety and tolerability analyses up to Visit 8 (i.e., Main Study Phase), including demographic data, local/systemic tolerability, laboratory data, (S)AEs and AESIs. All analysis based on the safety population will be carried out using the actual treatment received.

Per-Protocol (PP) Population

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

- Subjects with less than three vaccinations (Day 1, 57 and 180).
- Subjects who received the wrong study medication.
- Subjects who fulfilled exclusion criteria 2, 8, 9, 14.

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

Modified Intent-to-Treat (mITT) Population

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

Booster Safety Population

All safety analyses of the Booster Phase will be based on the booster safety population, which is defined as all subjects who received the booster vaccination. The booster safety population will be used for all safety and tolerability analyses including demographic data, vital signs, local/systemic tolerability, laboratory data, (S)AEs and AESIs. All analyses based on the safety booster population will be carried out using the actual treatment received.

Booster Per-Protocol (PP) Population

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

- Subjects enrolled into the Booster Phase despite exclusion from the PP Population of the Main Study Phase;
- Subjects who received the wrong booster vaccination;
- Subjects who fulfilled the booster exclusion criterion 5;
- Subjects with a forbidden prior or concomitant therapy will be reviewed on a case by case basis

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

Booster modified Intent-to-Treat (mITT) Population

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

9.3 Immunogenicity Analysis

Immunogenicity analyses include the analysis of OspA serotype (ST1 to ST6) specific IgG levels by ELISA and by a serum bactericidal assay (SBA).

Main Study Phase:

The primary immunogenicity analysis will compare the Geometric Mean Titers (GMTs) of serotype specific IgG against each OspA ST1 to ST6 as determined by ELISA in the PP population between the treatment groups VLA15 w/ alum 135 µg and VLA15 w/ alum 180 µg and between the VLA15 treatment groups and the placebo group, respectively, on Day 208. GMTs and GMT ratios will be estimated by applying an analysis of variance (ANOVA) including the factor treatment group and study site. This will be done using log10 transformed data and taking the anti-log of the resulting point estimates for the least squares means, least squares means differences and the corresponding 95% CIs. Tukey's HSD test will be applied for pair-wise comparisons. In addition, sensitivity analyses (ANOVAs with factors study site, treatment group, study site*treatment group, age, and *B.b.* s.l serostatus at baseline) will be performed.

As secondary analysis, GMTs and GMT ratios will be analyzed by ELISA as outlined above on Day 1, 29, 57, 85, 180, 208, 365 and 545. Analyses will compare both dose groups against each other and against the placebo group.

Further secondary immunogenicity analyses will compare the following:

- The Geometric Mean of the fold rise as compared to baseline (GMFR) for IgG against each OspA ST 1-6 as determined by ELISA (ANOVA).
- Seroconversion Rates against each OspA serotype separately; against all six OspA serotypes combined and against OspA ST1 and ST2 combined. Seroconversion rates will be compared using Fisher-Freeman-Halton tests, a significant overall test will be amended by pair-wise tests (Fisher's exact test).
    - For ELISA: SCR is defined as rate of subjects that change from seronegative* at Visit 1 (baseline) to seropositive†, if seronegative at baseline, or that achieve a four-fold increase in IgG titer compared to baseline, if seropositive at Visit 1
    - For functional antibody testing (SBA): SCR is defined as rate of subjects that change from seronegative to seropositive CCI [redacted] or as a > 4-fold rise in IgG antibody titer from Visit 1 for subjects that are seropositive at Visit 1 (baseline).

- GMTs, SCRs and GMFRs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at Day 1, 29, 57, 85, 180, 208, 365 and 545, stratified by age group.

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

Booster Phase:

The primary immunogenicity analysis will provide 95 % Confidence Intervals for Geometric Mean Titers (GMTs) of serotype specific IgG against each OspA ST1 to ST6 as determined by ELISA in the Booster PP population at Visit 10 (Month 19). GMTs and GMT ratios will be estimated by applying an analysis of variance (ANOVA) including the factor treatment group and study site. This will be done using log10 transformed data and taking the anti-log of the resulting point estimates for the least squares means, least squares means differences and the corresponding 95 % CIs. In addition, sensitivity analyses (ANOVAs with factors study site, treatment group, study site*treatment group, age, and *B.b.* s.l serostatus at baseline) will be performed.

As secondary analysis, GMTs and 95%CI will be provided at Month 18 (Visit 9), Month 24 (Visit 11), and Month 30 (Visit 12). Analyses will compare the VLA15 group against the placebo group.

Further secondary immunogenicity analyses will provide the following:

- The Geometric Mean of the fold rise (GMFR) at Visit 10 (Month 19) as compared to Visit 1 (baseline), Visit 6 (Day 208, peak titer after primary immunization series) and Visit 9 (pre-boost) for IgG against each OspA ST 1-6 as determined by ELISA (ANOVA) and SBA, if applicable.
- Seroconversion Rates against each OspA serotype separately at all study visits (ELISA and SBA).
- GMTs for IgG against each OspA serotype (ST1 to ST6), determined by ELISA, at specified time points, by age group.

* An ELISA titer below 40 U/mL (i.e., the quantitation limit of the ELISA) is considered "OspA IgG seronegative". Values will be replaced by 20 U/mL.
† An ELISA ≥40 U/mL is considered "OspA IgG seropositive.

All immunogenicity analyses of the Booster Phase will be performed for the Booster PP Population. It will be described in the SAP whether any analyses will be repeated for the Booster mITT.

9.4 Safety Analysis

Main Study Phase:

All subjects entered into the study who received at least one vaccination (safety population) will be included in the safety analysis. Safety tabulations will generally be provided separately for solicited AEs and unsolicited AEs, and for both types of AEs combined. 95% confidence intervals according to Altman will generally be provided for all AE rates. Further details in addition to the outline below will be provided in the SAP.

The number and percentage of subjects with any AE, any unsolicited AE, any related unsolicited AE, any SAEs, any AESI, any related SAEs, any medically attended AE and any AE leading to withdrawal from further treatment, up to Day 208 and up to Day 545, will be presented for each treatment group, overall and by system organ class/preferred term. Differences between both dose (VLA15 135 µg w/ alum and VLA15 180 w/ alum) groups and the placebo group will be assessed for significance using Fisher's exact (Fisher-Freeman-Halton) test, whereby a significant overall test will be amended by pair-wise tests. In addition, the number and percentage of subjects with any AE, any unsolicited AE, any related unsolicited AE, any SAEs, any related SAEs, any AESI, any medically attended AE and any AE leading to withdrawal from further treatment will be presented grouped by onset within 28 days after the first, the second or the third vaccination.

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

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

Booster Phase:

All subjects included in the Booster Phase who received a booster vaccination will be included in the booster safety analysis (Booster Safety Population). 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, AESI, and SAE up to Visit 10 (Month 19) and with any AESI or SAE up to Visit 12 (Month 30) will be presented for each treatment group overall and by body system/preferred term. The safety analysis will be done in accordance to procedures used in the Main Study Phase.

9.5 General considerations for the determination of the optimal dose

Based on safety and immunogenicity results obtained from the Day 208 interim analysis of this study, the 180 µg dose was selected for further development. 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 treatment group will be continued in further clinical development of this vaccine.

9.6 Determination of Sample Size

The group size for the two doses (VLA15 w/ alum 135 µg and VLA15 w/ alum 180 µg) evaluated in the Main Study Phase of study VLA15-202 has been selected to provide a sufficient safety database and for determining the optimal dose before advancing the vaccine candidate into Phase 3. Upon completion of the study, the total number of subjects exposed to the dose used for Phase 3 trials would be approximately N=310, taken together both Phase 2 studies that are performed with VLA15 (VLA15-201 and VLA15-202). A total of N=100 subjects will have received the selected dose for Phase 3 in the alternative immunization schedule Day 1-57-180 (Month 0-2-6). The database would thus allow 95 % confidence that a given reaction would not be observed at a higher rate than 1:(100/3) rate, i.e. 3. %, if it is not observed in this trial using selected dose and a vaccination schedule of Day 1-57-180 (Month 0-2-6).

With respect to the primary endpoint, GMTs for ST1-6 specific IgGs on Day 208: In the absence of an established protective titer and without an estimate for the GMTs with a longer immunization schedule, sample size calculation is based on somewhat arbitrary differences in GMTs between groups, in order to demonstrate which titer levels could be distinguished with the proposed sample size. Titers observed in Phase 1 using an immunization schedule Day 1-29-57 were used as basis: In the 90 µg dose group (i.e. the lowest dose group that was used in the run in phase of the parallel Phase 2 study VLA15-201, which was performed prior to study start of present VLA15-202 study and where dose selection for the present study was done), a GMT of 61.3 was observed for ST1 (i.e. the serotype with lowest titers in Phase 1) with a Standard Deviation (LOG10) of 0.51. A total of 100 randomized subjects (90 evaluable subjects assuming a 10% drop-out rate for Day 208) per group will provide 80% power at a two-sided alpha level of 5 % to distinguish a GMT of 61.3 in one treatment group from a putative higher GMT of 100.4 in another treatment group. An approximately 1.5 fold higher titer could thus be distinguished. A 1.5 fold difference in GMTs is often considered a relevant difference in vaccine studies, e.g. when setting non-inferiority boundaries.
The overall sample size of 50 subjects in the placebo group has been selected to allow for the internal validation of both safety and immunogenicity results.
For the Booster Phase of this study no formal sample size calculation has been performed and no minimal or maximal number of participants is defined. Based on previously observed relevant protocol deviations and dropout rates, the number of participants are estimated to be a maximum of 81 subjects.

9.7 Data Analyses

Two interim analyses on safety and immunogenicity data will be performed during the Main Study Phase. The first interim analysis was performed once all subjects have completed Visit 6 (i.e. Day 208/Month 7, four weeks after the last primary vaccination) covering safety and immunogenicity data up to Visit 6 including the primary endpoint analysis. The second interim analysis will be performed once all subjects have completed Visit 7 (i.e. Day 365, six months after the last vaccination), covering in addition all safety data collected up to this time point). Final analysis of safety and immunogenicity data from the Main Study Phase will be performed once all subjects have completed the follow-up period up to Visit 8 (i.e. Day 545/Month 18, 12 months after the last vaccination).

In the Booster Phase three data analyses will be performed: A first analysis will be conducted after all subjects completed Visit 10 (Month 19). The second analysis will be performed once all subjects have completed Visit 11 (Month 24). A final analysis on safety and immunogenicity will be performed after the last subject has completed the last study visit at Month 30 (Visit 12) and will include all safety and immunogenicity data up to Month 30.

10. DEVIATIONS FROM THE PROTOCOL

10.1 Relevant Protocol Deviations*

All protocol deviations are tracked, actions defined, as feasible, and reviewed in Data Review Meetings for the data analyses for assessment of their influence on the quality of the study analysis.

10.2 Premature Subject Withdrawal from Study or Treatment

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

The investigations described for Early Termination, see Table of Events (Table 3 and Table 4), 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 are not replaced.

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

10.2.1 Individual stopping criteria

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

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

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

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

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

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

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

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

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

10.3 Documentation of Premature Withdrawal

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

* A relevant Protocol Deviation (PD) is a PD with possible impact on the immunogenicity profile of the subject.

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

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

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

10.4 Subsequent Therapy

Not applicable.

11. ETHICAL AND REGULATORY ASPECTS

11.1 Ethical/ Regulatory Framework

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

11.2 Institutional Review Board/ Independent Ethics Committee

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

11.3 Subject Information and Informed Consent

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

The investigator explains 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 are 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 is given a photocopy or a second original of the ICF. An original of the signed and dated ICF must be retained in the site's records, and is subject to inspection by representatives of the sponsor or representatives from regulatory agencies.
Accordingly, the investigator has the responsibility to obtain freely given informed consent from subjects that are asked to participate in the Booster Phase prior to any study related procedures of the Booster Phase.

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 permits study-related monitoring, audits, IRB/IEC review and regulatory inspections, by providing direct access to source data/records. Source records should be preserved for the maximum period of time required by local regulations.

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

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

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

eCRFs are not used as source data for any other variable.

12.2 Periodic Monitoring

A designated CRA checks 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 works according to the Monitoring Plan. The investigator cooperates with the monitor to ensure that any discrepancies identified are resolved.

12.3 Audit and Inspection

Upon request, the investigator makes 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 been appropriately reported to the sponsor.

12.4 Confidentiality of Subject's Data

The investigator exercises 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 are only identified by subject number. Documents not for submission to the sponsor, e.g. subject identification log and original ICF, are maintained by the investigator in strict confidence.

13. DATA HANDLING AND RECORD KEEPING

13.1 Information of Investigators

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

The investigator is 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 are only 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. Any unsolicited AEs reported during the Booster Phase after Visit 10 will remain in the source data, but will not be documented in the respective AE section of the eCRF. Thereafter, only SAEs and AESI will be reported via the eCRF.

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 identify clinical data discrepancies for resolution. Corresponding queries are created within the data capturing system and the site is informed about new issues to be resolved on-line.

All discrepancies are 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 thoroughly reviews the data on the eCRF, and finally certifies the contents of the eCRF by electronic signature after completion of each subject. 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 are source document verified as detailed in the Monitoring Plan. Maintenance of the study database is 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 are 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 is 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 supplies the sponsor with additional data relating to the study or copies of relevant source records, duly anonymized. In case of particular issues or governmental queries, it is also necessary to have access to the complete study records, provided that the subject's confidentiality is protected in accordance with applicable regulations.

14. CHANGES IN THE CONDUCT OF THE STUDY

14.1 Protocol Amendments

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

14.2 Study Termination – Study Stopping Rules

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

The occurrence of the following criterion leads 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 vaccination:

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

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

If a study stopping rule is met or the DSMB recommends halting the study for other reasons, the sponsor will notify the Competent Authorities, IRBs/ECs and Principal Investigators within 48 hours by phone, email 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 notify each other in writing, stating the reasons for early termination. In terminating the study, the sponsor and the investigator ensure that adequate consideration is given to the protection of the subjects' interests. The investigator, sponsor or CRO will notify the relevant CA or IRB/ IEC in writing in accordance with local requirements. Documentation is submitted for filing in the Central and Investigator File and the Trial Master File.

15. REPORTING AND PUBLICATION

15.1 Clinical Study Report

A final Clinical Study Report will be written once all data from all subjects up to Day 545 / Month 18 (Main Study Phase) are analyzed.

A Month 24 Addendum to the Clinical Study Report will be compiled containing data on safety and immunogenicity from all subjects included in the Booster Phase up to Month 24 (Visit 11). Further on, a Month 30 Addendum to the Clinical Study Report will contain all safety and immunogenicity data obtained up to Month 30.

15.2 Publication Policy

All results generated in this study are 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 contracts a clinical trial insurance.

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

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

17. APPENDIX 1

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

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

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

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

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

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

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

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

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

18. APPENDIX 2

In case a Lyme borreliosis is suspected by the investigator or in case of a potential LB-associated event according to the scripted safety assessment with a potential relationship to the study vaccine, 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, 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],[26].

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

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

- 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 Immuno blot assay as recommended by the CDC [27] should be ordered. Additional work-up as depicted in Table 12 should be initiated.
- In case of clinical suspicion of early disseminated or late disseminated Lyme borreliosis, consider initiating treatment according to standard of care [10],[26] and send patient for consultation with a specialist as appropriate.

Table 12 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	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.
	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	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

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

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