A Phase 3 randomized, double-blind, placebo-controlled study of SHB004 (10% topical azithromycin) administered locally twice daily for three consecutive days for the prevention of Borreliosis in subjects bitten by a tick.

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8044 Zürich, Switzerland

**Clinical Research Organization:** PAREXEL International GmbH  
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14059 Berlin, Germany

**Sponsor Protocol No.:** A2301  
**EudraCT No.:** 2011-000117-39  
**Study Drug Name:** SHB004 (topical azithromycin)  
**Development Phase:** 3  
**Date of Protocol:** January 27, 2013  
Version 4 (Incorporating changes from Amendment 3)  
**Date of Previous Protocol:** October 15, 2012

*The study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP), with the Declaration of Helsinki¹, and with other applicable regulatory requirements.*
AMENDMENT 03

Amendment Rationale

*Definition of a treatment failure*

The protocol stated, that the analysis of treatment failures based on seroconversion should be according to MIQ 12 (MiQ12 (2000). Lyme-Borreliose by B. Wilske, L. Zöller, V. Brade, H. Eiffert, U. B. Göbel, G. Stanek). However, the MIQ was not correctly reflected in the previous version and this is now corrected accordingly. The previous protocol failed to count IgM based seroconversion as Treatment Failure (TF) and this is now corrected.

*IDMC*

In the previous version of the protocol, the IDMC was responsible to decide on doubtful cases of treatment failures (TF) and agree if the doubtful case is to be counted as TF or not. This is only detailed in this protocol to ensure, that the current MIQ (see above) is reflected. The protocol is now particularly pointing the IDMC’s attention to cases within which serological results switch from “negative” (deemed as ‘negative’ by the MIQ; Visit 1) to “borderline” (deemed as ‘questionable’ by the MIQ; Visit 4).

*Futility boundary*

A stricter futility boundary was introduced (futility stop in case of a 1-sided p-value ≥ 0.1587, compared to ≥ 0.5 in protocol version 3), and this boundary is now considered as binding.

*Analysis set*

The mITT set was changed. The previous protocol used the serostatus of the index tick to define the mITT set. The new mITT set introduced here is as the ITT set but TF defined on IgM only seroconversion or IgG only seroconversion (e.g. not in combination with an EM) are excluded. Changes to the PP set became necessary accordingly and the *Borrelia* status of the index tick is now integrated within this analysis set.

*Statistics*

It has been detailed, at which point the second interim analysis analysis is to be conducted. Details on the asymptotic distribution of the test statistic have been added.

*Determination of sample size*

A larger treatment effect is assumed in the sample size calculation (relative risk of 0.4, corresponding to treatment failures rates of 3.1% for placebo and 1.24% for SHB004). Following BfArM advice, we did not change the alpha-spending function.

*Changes to the Protocol*

Changes to the ‘final protocol including amendment 2’ (version as of October 15, 2012) are shown by strike-through black font for deletions and red and bold font for insertions.

Changes due to grammatical or typographical corrections or format changes are not shown.
AMENDMENT 02

Amendment Rationale

Endpoints:

Change Number 1:

The definition of the primary endpoint was clarified (following the BfArM Stellungnahme of 25.07.2012): In the previous version it was mentioned, that subjects developing an erythema migrans (EM) are counted as treatment failures in the efficacy analysis as are those who develop a seroconversion (IgG; following the BfArM Stellungnahme of 25.03.2010). These events (EM and IgG seroconversion) are now addressed under the umbrella term “treatment failure”.

Change Number 2:

Following the BfArM Stellungnahme of 25.03.2010, we introduce a new secondary efficacy endpoint based on seroconversion as measured by (i) IgM or (ii) IgM and IgG or (iii) IgM and IgG and appearance of EM in baseline-seronegative subjects (see change number 4 below).

Change Number 3:

It is detailed, that the efficacy analysis is only conducted on IgM and / or IgG baseline-seronegative subjects (following the BfArM Stellungnahme of 25.03.2010).

Change Number 4:

In Chapter 8.5.2, the secondary efficacy analyses were reformulated as follows:

Secondary efficacy analysis 1 will be performed for all treated subjects (see change number 27 for this newly introduced analysis set), the modified ITT and PP sets (see section 8.1.3 for definition of analysis sets) on the primary efficacy endpoint (rate of treatment failures at Day 57) and will include the same analyses as for the primary efficacy analysis (see section 8.5.1).

Secondary efficacy analysis 2 will check for the reduction in the rate of seroconversion as demonstrated by (i) IgM seroconversion only, (ii) IgM and IgG seroconversion and (iii) IgM and IgG seroconversion and appearance of EM in the All Treated Subjects, ITT, modified ITT and PP set, respectively. The testing procedure will be the same as for the primary efficacy endpoint (see section 8.5.1). Note that (i), (ii) and (iii) are secondary alternatives for the definition of treatment failures.

Exclusion criteria:

Change Number 5:

The previous version did not allow entry for subjects with a skin reaction at the tick bite scored at 2 or higher than 2 according to the scoring table provided in Appendix 1. However, after having enrolled approximately 300 subjects in 2011, we recognized, that this criterion prevented several subjects from participation, as their skin reaction to the tick bite scored ‘2’. In the (unchanged) risk-benefit assessment we outlined, that subjects with a skin reaction grading ‘3’ or worse should not receive further treatment. Therefore, we believe it is justified to enroll subjects presenting at baseline with a score of ‘2’ or less. Furthermore, this better represents the true population (including those having a hypersensitivity reaction to the tick bite which is
unrelated to a possible Borreliosis) having been exposed to a tick bite while still being within the boundaries of the unchanged risk-benefit assessment of amendment 1 and the original, non-amended protocol.

**Statistical section:**

**Change Number 6:**

Various text changes were necessary in Chapter 8.1.2 after we improved the specification of the analysis sets in Chapter 8.1.3. The improved definition of the analysis sets is also now visually detailed in a consort flow diagram as part of the newly introduced Appendix 2 at the end of the protocol.

**Change Number 7:**

Missing data conventions were updated in section 8.2 with respect to the primary endpoint. Subjects with missing serological test result at Day 57 and not having an erythema migrans (EM) will be excluded from the ITT population. The exclusion of these subjects is justified as follows:

When there are subjects with missing serologic test results at Day 57 and not having an EM there are three possible strategies:

1. to be regarded as no treatment failure – this would favor the test substance;
2. to be regarded as treatment failure – this would not favor the test substance;
3. to be excluded from the ITT population.

Given the small number of expected treatment failures (3.1% placebo vs. 1.5% verum, see section 8.8), treating such cases as treatment failures (strategy 2) will cause great loss of power and may introduce over-estimation of treatment failures. In addition, under study conditions there is a known tendency to have less treatment failure rates than in real life. Also, the pre-interim assessment of approximately 300 subjects revealed that only a small number of subjects (1%) would have to be excluded from the ITT population, as these subjects did not appear at Day 57. Therefore, in this particular situation, approach 3 is seen as most appropriate. Please note, that events collected before the subjects withdrew (e.g. an EM) are included into the ITT or modified ITT analysis. This approach is following the BfArM “Stellungnahme” of July 25, 2012.

Baseline seronegative subjects who recognize an additional tick bite during the study are excluded from the ITT population. If these subjects develop an EM before the additional tick bite, the EM is counted as treatment failure (TF). These subjects are excluded, for the fact that they cannot receive treatment for the additional tick bite, which has to be considered a study artificiality.

(i) This protocol does not allow the treatment of additional tick bites. However, in “normal” life, these tick bites would be treated. Therefore, we have a study artificiality and the ITT population must be adapted, such that the “normal” population is reflected. Consequently, we exclude subjects bitten by an additional tick.

(ii) As we cannot know if the subject received placebo or verum for the initial (index) bite, we are unable to allocate the respective treatment to the additional bite. If we had decided to treat the additional tick bite in a blinded fashion, there would have been a 1:1 chance that the subject
received the same or the other treatment as compared to the index bite – again, if treatments wouldn’t have been identical, we could not have assigned the subjects to a respective treatment and the subject would have been lost for the efficacy analysis. Therefore and within the logic of this trial, the subject with the additional tick bite must be excluded from the ITT.

(iii) Another reason is of immunological consideration. Typically, a seroconversion takes up to 6-8 weeks after exposure. If the additional tick bite occurs sometime within the study, there may not be sufficient time for seroconversion – therefore, the primary endpoint is directly affected by such subjects. Hence, such subjects must be excluded from the ITT set. Approximately 10% of subjects experienced an additional tick bite.

Change Number 8:
In chapter 8.5.1.2, a Wald type test statistic was specified replacing the Fisher’s exact test which was mentioned in the previous version. Also, the Farrington-Manning test for repeated confidence intervals was mentioned. Various text changes had to be done, accordingly.

Change Number 9:
The study design was reformulated to point out that the power of the testing procedure depends mainly on the expected overall number of treatment failures (TF). Thus, for a relative risk of 0.484 (corresponding to assumed rates of 3.1% for placebo and 1.5% for SHB004) an expected number of about 76 treatment failures is needed at the last stage to have an overall power of 80%. Therefore, an interim analysis is now planned after a certain number of treatment failures have been observed (e.g. a first interim analysis is planned to be conducted after 25 ± 20% treatment failures have been observed).

See sections 8.8 for details.

Change Number 10:
Chapter 8.8 Determination of Sample Size was thoroughly reviewed. Based on the (blinded) read-out from these approximately 300 subjects randomized in 2011, we received initial information on the true rate of ticks carrying *Borrelia* s.l., and concerning the serology of subjects at baseline. As the true rates were different from those originally assumed based on literature information and as detailed in the previous version of this protocol, numbers were corrected from previously 800 subjects per group or 200 subjects having been exposed to bite from a positively tested tick. Also, since the power of the testing procedure depends mainly on the expected overall number of treatment failures (TF) the interim analyses will be done after a certain number of treatment failures have been observed.

The following table shows an overview of the intended numbers of treatment failures per stage and the corresponding number of patients for treatment failure rates of 3.1% (placebo) and 1.5% (SHB004), assuming that 10% of the randomized patients have to be excluded from the ITT population due to additional tick bites or missing Day 57 results:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Cumulative number of TF in ITT set</th>
<th>Cumulative patient numbers for TF rates 3.1% (placebo), 1.5% (SHB004)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
The following pre-interim, blinded baseline information was collected from the analysis of the samples collected from the approximately 300 subjects in 2011. These rates were now used for the computation of estimated treatment failures and corresponding sample sizes:

- 16.7% of subjects in the ITT population have been bitten by a positive tick (tick is carrying *Borrelia s.l*);
- 3.8% of the ITT population, placebo group will be treatment failures at Day 57 (numbers derived from community screenings – equals overall percentage of seroconversion within any subject bitten by any tick (regardless if carrying *Borrelia* or not)). This data could not be obtained from the pre-interim analysis as the study is still blinded. In our study we are now assuming 3.1%. Likely, subjects participating in the trial are better informed and taken care of as compared to community screenings and, therefore, we slightly adapt our numbers, accordingly.
- 20.0% of the seropositive subjects at Day 57 already presented seropositive at baseline;
- SHB004 will be reducing the rate of treatment failures by 50% or more as compared to placebo;
- The rates of subjects with treatment failures are assumed to be 3.1% for placebo and 1.5% for verum. This data could not be obtained from the pre-interim analysis as the study is still blinded.

See section 8.8 for further details.

**Change Number 11:**

Related to the concern of preserving the overall significance level ($\alpha = 0.025$), it was mentioned that:

The type-1 error to be spent at each stage will be computed with an alpha-spending function, which approximately produces the Pocock stopping boundaries (~2.289 for the test statistic, 0.011 for the 1-sided p-value), if the interim analyses are done after one third and two thirds of the total number of treatment failures.

See section 8.7 for details.

**Change Number 12:**

A short description of the analysis at interim was given in section 8.7 as follows:

- Primary and secondary efficacy analyses will be performed on data as detailed in section 8.5). If differences between the two treatment groups suggest that criteria for efficacy or futility may be fulfilled, the Independent Data Monitoring Committee (IDMC) may ask for the study to stop.
- Otherwise: the study continues at the next stage as planned by the group sequential design.

Change Number 13:

The previous version referred to a Data Safety Monitoring Board which is now referred to as an Independent Data Monitoring Committee (IDMC). This resulted in various small text changes. Furthermore, the role of the IDMC (see section 9.9) has been described in more detail. Specifically, the IDMC is requested – before unblinding - to decide on doubtful cases of treatment failures (TF) and agree if the doubtful case is to be counted as TF or not. If necessary, the IDMC may consult the respective Investigator to clarify possibly open questions. This is to be done before unblinding. No other changes have been introduced regarding the role of the IDMC and as compared to the previous version of this protocol.

Other changes:

Change Number 14:

The site of the tick bite is now advised to be marked with a skin marker rather than a conventional ball pen.

Change Number 15:

The physical examination is now detailed as being preferentially (and not mandatory be) done by the same physician. Physical examinations are routine and it is standard that these are not necessarily conducted by the same person. A 2nd pregnancy test at EOS is mandatory for Austrian sites (not in Germany) according to Austrian law (AMG-Novelle BGBl. Nr.35/2004, and referring guidance “Richtlinie Schwangerschaftstest bei AMG-Studien” Version 1.0 6.4.2006, Forum Österreichische Ethikommissionen). Therefore, Austrian sites must perform the additional pregnancy tests. This regulation does not apply to German sites.

Change Number 16:

The use of term ‘*Borrelia burgdorferi*’ was incorrect at some locations in the protocol when in fact ‘*Borrelia s.l.*’ was meant. This has been corrected.

Change Number 17:

The number of centers was adapted to 20 to 35.

Change Number 18:

The role of the Sponsor and the designee were detailed.

Change Number 19:

The previous protocol contained a mistake. It was stated, that subjects experiencing an EM are withdrawn from the study. This is now changed and such patients are counted as treatment failure in the efficacy analysis (if seronegative at baseline). As outlined in the previous version of the protocol (amendment 1), these patients will immediately receive proper treatment at the discretion of the respective physician and, therefore, the risk benefit assessment of the study is unaffected as the clinical proceedings have not changed as compared to the previous version of the protocol.

Change Number 20:

Subjects who develop a skin reaction graded at least as 3 according to Appendix I are now not withdrawn (as was true for the previous version of this protocol) but kept in
the study according to the regular schedule – treatment of the index site with the study medication (SHB004 or Placebo) must be stopped immediately (if applicable) and appropriate treatment may commence immediately according to the physician’s discretion (e.g. with dermal corticosteroids). Therefore, the risk benefit assessment of the study is unaffected as the clinical proceedings have not changed as compared to the previous version of the protocol.

**Change Number 21:**

The requirement that subjects must return to the study center if suspicious events have been collected during the telephone follow up calls is now better reflected in this amendment and as compared to the previous version. Only the wording was affected by this amendment, not the content and as compared to the previous version.

**Change Number 22:**

In case an EM is observed, this EM should now be documented photographically if possible.

**Change Number 23:**

Details on stopping criteria for futility/efficacy were added in section 8.7.

**Change Number 24:**

Details on the test statistic were added in section 8.7.

**Change Number 25:**

In section 8.5.1.1 the testing hypotheses were more clearly formulated as follows:

- **Null hypothesis** $H_0$: The proportion of treatment failures in the SHB004 group $\pi_1$ is larger than or equal to the corresponding proportion $\pi_2$ in the placebo group, i.e. the relative risk of SHB004 versus placebo is $\geq 1$:
  
  $$H_0: \frac{\pi_1}{\pi_2} \geq 1$$

- **Alternative hypothesis** $H_A$: The proportion of treatment failures in the SHB004 group $\pi_1$ is smaller than the corresponding proportion $\pi_2$ in the placebo group, i.e. the relative risk of SHB004 versus placebo is smaller than 1:
  
  $$H_A: \frac{\pi_1}{\pi_2} < 1$$

The overall significance level is $\alpha = 0.025$, 1-sided. The study is powered at 80% to detect a relative risk of 0.484, corresponding to a 51.6% improvement of SHB004 group versus placebo group (e.g. treatment failure proportions of 3.1% for placebo and 1.5% for SHB004). See section 8.8 for details of the sample size calculation.

**Change Number 26:**

Synopsis, Chapter 8 and sections 2.1, 3.2, 4.4, 9.9 were updated according to the statistical changes from sections 8.5, 8.7, 8.8.

**Change Number 27:**

BfArM advised in its “Schriftliche Stellungnahme” as of July 25, 2012 to include a new analysis set for the sensitivity analysis and following the discussion to exclude subjects who prematurely finish the study from the ITT analysis (see Change Number
5). Following this advice, we integrated a new analysis set referred to as ‘All Treated Subjects Set’. This set is identical to the safety analysis set (SAF), but analyzing subjects according to their randomized treatment. Drop-outs are counted as TF. Subjects with additional tick bites are counted as TF providing they develop an EM before the additional tick bite (see Change Number 5).

**Changes to the Protocol**

Changes to the ‘final protocol including amendment 1’ (version as of June 8, 2011) are shown by strike-through black font for deletions and red and bold font for insertions. Changes due to grammatical or typographical corrections or format changes are not shown.
AMENDMENT 01

Amendment Rationale

Some aspects regarding time-windows were detailed:

- Widening the time-window for the second administration of the study drug on Day 1 from ±2 hours to ±6 hours and clarification of days for administrations. Broadening the time window for the second administration on Day 1 will allow subjects to adopt a morning and evening administration frequency independently of the time of first administration at the study center. Based on Phase 1/2 study results, this shift in the administration time window will have no clinical impact.

- Widening the time-window for Visit 4 (last visit and collection of the second blood samples) from +7 days to +14 days (as IgG antibody conversion is of interest and since these antibodies have a long persistence, this window expansion will not impact the validity of the study but will facilitate the study conduct, e.g. in vacation time periods).

Some inclusion and exclusion criteria were detailed:

- It is sufficient to collect and handover only parts of the tick (e.g. hypostom) rather than the entire tick (inclusion criterion #3).

- Only subjects with known active infectious mononucleosis are excluded, not those reporting a past infection (exclusion criterion #4).

- In addition to Lyme disease during the previous 12 months, a positive test for antibodies against B. burgdorferi as assessed within the last two years prior to enrollment was added as exclusion criterion. The purpose of this was to exclude subjects for whom a seroconversion was expected at baseline, and who therefore cannot be included in the efficacy analysis (exclusion criterion #7).

- Exclusion criterion #10 was reworded to history of “one or more” tick bites (instead of “tick bites”) within the last 60 days (to clarify that just one tick bite in the history was sufficient to exclude the subject).

We detailed, that subjects must be withdrawn if they develop Lyme Disease / Borreliosis. Furthermore, it is detailed, that patients receive the necessary therapy in case they develop Lyme Disease / Borreliosis.

The statistical aspects of the study were detailed to fully comply with the confirmatory character of this phase III study. A detailed description of the adaptive interim assessment was added including the statistical means controlling the type I error. The sample size was re-calculated according to the three stage group sequential design (two interim analyses and one final analysis).

Changes to the Protocol

Changes to the final protocol, dated 01 April 2011, are shown by strike-through black font for deletions and red and bold font for insertions.
Changes due to grammatical or typographical corrections or format changes are not shown.
SIGNATURE PAGE

Declaration of Sponsor or Responsible Medical Officer

Title: A Phase 3 randomized, double-blind, placebo-controlled study of SHB004 (10% topical azithromycin) administered locally twice daily for three consecutive days for the prevention of Borreliosis in subjects bitten by a tick.

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki, [as amended in 2008, http://www.wma.net/en/30publications/10policies/b3/index.html], and the guidelines on Good Clinical Practice (GCP).

____________________________________  ____________________
Name: Dr Gustave Huber              Date
Title: CEO Ixodes
Institution: Ixodes AG
Declaration of the National Coordinating Investigator

Title: A Phase 3 randomized, double-blind, placebo-controlled study of SHB004 (10% topical azithromycin) administered locally twice daily for three consecutive days for the prevention of Borreliosis in subjects bitten by a tick.

This study protocol was subjected to critical review and has been approved by the Sponsor. The information it contains is consistent with the current risk/benefit evaluation of the investigational product as well as with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki, [as amended in 2008, http://www.wma.net/en/30publications/10policies/b3/index.html] and the guidelines on GCP.

National Coordinating Investigator

_______________________________________      ______________________
Signature                                      Date

____________________________________________
Name (block letters)

____________________________________________
Title (block letters)

____________________________________________
Institution (block letters)

____________________________________________
Phone number
Declaration of the Investigator

Title: A Phase 3 randomized, double-blind, placebo-controlled study of SHB004 (10% topical azithromycin) administered locally twice daily for three consecutive days for the prevention of Borreliosis in subjects bitten by a tick.

All documentation for this study that is supplied to me and that has not been previously published will be kept in the strictest confidence. This documentation includes this study protocol, Investigator's Brochure, electronic data capture (EDC) system/electronic CRF (eCRF), and other scientific data.

The study will not be commenced without the prior written approval of a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). No changes will be made to the study protocol without the prior written approval of the Sponsor and the IRB or IEC, except where necessary to eliminate an immediate hazard to the subjects.

I have read and understood and agree to abide by all the conditions and instructions contained in this protocol.

Responsible Investigator of the local study center

________________________________________________________________________
Signature Date

________________________________________________________________________
Name (block letters)

________________________________________________________________________
Title (block letters)

________________________________________________________________________
Institution (block letters)

________________________________________________________________________
Phone number
PROTOCOL SYNOPSIS

Title
A Phase 3 randomized, double-blind, placebo-controlled study of SHB004 (10% topical azithromycin) administered locally twice daily for three consecutive days for the prevention of Borreliosis in subjects bitten by a tick.

Sponsor Study No.
A2301

Phase
III

Sponsor
IXODES AG
Susenbergstrasse 107
8044 Zürich, Switzerland

Study Center(s)
This study is planned to involve 20 to 35 study centers based in Germany and Austria.

Objective(s)
Primary objective:
- To demonstrate a reduction in the rate of treatment failure at Day 57 in the ITT set by at least 50% in response to SHB004 (10% topical azithromycin) administered locally within four calendar days after the tick bite had been first noticed, twice daily (BID) for three consecutive days, as compared to placebo.

Secondary objectives:
- Efficacy: Secondary objective 1 is as the primary objective for the All Treated Subjects A-C set, and modified ITT set. Secondary objective 2 is as the primary objective in the ITT set and isolated IgM (isolated defined as no other criterion present in parallel to the IgM seroconversion, leading to the definition of a Treatment Failure (TF) such as an EM) are not counted as TF. Secondary objective 3 is as the primary objective in the ITT set and isolated IgG are not counted as TF. Exploratory objective is as the primary objective but for the PP set.
- Safety: To demonstrate the local safety and tolerability of SHB004 (10% topical azithromycin) administered locally BID for three consecutive days.

Design
This study is a Phase 3, randomized, parallel-group, double-blind, placebo-controlled study of SHB004 (10% topical azithromycin) versus placebo in male or female subjects (aged ≥18 years and <80 years) bitten by a tick. SHB004 or placebo will be administered locally BID for three consecutive days at the site of tick bite, starting at the latest on the 4th calendar day from the day the tick bite was first noticed. Subjects will attend the study center twice: at Day 1 for screening/baseline (informed consent, tick collection, screening assessments and first study drug administration) and at Day 57 (+14 days) for last assessments and controls. Between these 2 visits, subjects will be contacted twice (Days 7 and 30) by phone for follow-up information.

Duration
Subjects are expected to participate in the study for 57 days (up to 71 days in case the +14 days time window is taken).
Treatment

Subjects will randomly be assigned to one of the following treatment groups in a 1:1 ratio:

**SHB004:**
SHB004 gel (10% azithromycin); administered topically to the site of the tick bite (dermal route); one drop at each administration, left as is for 30 minutes to allow it to dry; BID (once in the morning and once in the evening, 12 hours apart; time window of ±2 hours; only for the second study drug administration, a time window of ±6 hours is allowed [i.e. for the first application done by subject alone]) for three consecutive days. In total, 6 administrations are required.

**Placebo:**
Placebo gel matching SHB004 in color and aspect but without active investigational product; administered topically (dermal route) to the site of the tick bite; one drop at each administration, left as is for 30 minutes to allow it to dry; BID (once in the morning and once in the evening, 12 hours apart; time window of ±2 hours; only for the second study drug administration, a time window of ±6 hours is allowed [i.e. for the first application done by subject alone]) for three consecutive days. In total, 6 administrations are required.

Number of Subjects

According to a three stage group sequential design (two interim analyses and one final analysis), the study is powered at 80% to detect a total of 76 treatment failures corresponding to a total expected sample size for the intention to treat (ITT) population of about 3300 subjects (1100 subjects at each stage).

A first interim analysis is planned to be conducted after 25 ± 20% treatment failures have been observed. If required, a second interim analysis will be performed when about 51 ± 20% treatment failures have been observed.

Population

The study population will consist of male and non-pregnant or non-breast feeding female subjects aged ≥18 years and < 80 years, bitten by a tick (single bite), who have such tick or parts of the tick (e.g. hypostom) collected, and who are able to receive the first treatment administration at the latest on the 4th calendar day from the day the tick bite was first noticed.

Main reasons for exclusion from the study are: subjects whose tick bite site was already treated with a topical formulation of antibiotics; subjects presenting with multiple tick bites or with a history of tick bite within 60 days prior to randomization (except for the current tick bite qualifying for this study); subjects unable to spot the site of the index tick bite at screening/baseline visit; subjects who received systemic antibiotics within 10 days prior to enrollment; who receive systemic steroids at time of screening (or received systemic steroids within 30 days before enrollment); who received immunomodulatory drugs or cytostatics (definition of such drugs detailed in section 4.2) within 30 days before enrollment; who have a history of Borrelia s.l. within the last 24 months (if the last test before enrollment did demonstrate a negative antibody titer, the subject may be included); who have known allergic reactions to macrolide antibiotics; who present other significant medical conditions preventing participation (autoimmune disease, active infectious mononucleosis, history or clinical signs of syphilis, active herpes virus infection, immunosuppressive therapy, collagen vascular or immunodeficiency disease, concurrent tick-borne diseases [e.g. babesiosis or ehrlichiosis] or any other drug allergy or medical condition which at the investigator’s opinion places the subject at unacceptable risk).
Criteria for Evaluation of Efficacy

- Rate of treatment failure at Day 57 (with an allowed time-window of +14 days) in the ITT set; primary objective.
- As for the primary objective for all treated subject A-C sets, modified ITT and PP sets (sensitivity analysis); secondary objective 1.
- As for the primary objective but isolated IgM are not counted as TF; secondary objective 2.
- As for the primary objective but isolated IgG are not counted as TF; secondary objective 3.
- As for the primary objective but in the PP set; exploratory objective 3.

Criteria for Evaluation of Safety

- Safety and tolerability
  - Adverse events (AEs) and serious AEs (SAEs) (skin, and other)
Treatment difference in preventing an infection with *Borrelia s.l.* as measured by treatment failure at Day 57 will be determined by an analysis of the proportions (Wald type test statistic, overall 1-sided $\alpha = 0.025$, power = 80%, Pocock efficacy boundaries, futility stop in case of a 1-sided p-value $\geq 0.1587$) and repeated confidence intervals for the relative risk (based on Farrington-Manning test). Primary efficacy will be confirmed if the group sequential testing procedure results in rejection of the null hypothesis at any of the three stages, i.e. the proportion of treatment failures in the SHB004 group is significantly smaller than the corresponding proportion in the placebo group (at a significance level computed using an alpha-spending function).

A first interim analysis is planned to be conducted after $25 \pm 20\%$ treatment failures have been observed. Also, due to the seasonal aspect of the study, it is expected to perform the first interim analysis by the end of the second season of the study.

An Independent Data Monitoring Committee (IDMC) will decide on the continuation or stopping of the study after first interim analysis results become available.

Efficacy variables will be listed and summarized by treatment group using descriptive statistics. Demographic and safety variables will be listed and summarized by treatment group using frequency tables and descriptive statistics, as appropriate.

### Sample size considerations and calculation

This study has been designed to show superior efficacy of SHB004 compared to placebo with respect to treatment failure rate at Day 57. The following assumptions were made for the calculation of the sample size:

- 16.7% of subjects in the ITT population are bitten by a positive tick (tick is carrying *Borrelia s.l.*).
- 3.8% of the ITT population, placebo group will be treatment failures at Day 57 (numbers derived from community screenings – equals overall percentage of seroconversion within any subject bitten by any tick [regardless if carrying *Borrelia* or not]). This data could not be obtained from the pre-interim analysis as the study is still blinded. In our study we are now assuming 3.1%. Likely, subjects participating in the trial are better informed and taken care of as compared to community screenings and, therefore, we slightly adapt our numbers, accordingly.
- 20.0% of the seropositive subjects at Day 57 already presented seropositive at baseline.
- SHB004 will be reducing the rate of treatment failure by 60% or more as compared to placebo.
- The rates of subjects with treatment failure are assumed to be 3.1% for placebo and 1.24% for verum.

The following hypotheses have been formulated based on the above given assumptions:

**Null hypothesis $H_0$:**

The proportion of treatment failures in the SHB004 group $\pi_1$ is larger than or equal to the corresponding proportion $\pi_2$ in the placebo group, i.e. the relative risk of SHB004 versus placebo is $\geq 1$:

$H_0: \frac{\pi_1}{\pi_2} \geq 1$

**Alternative hypothesis $H_A$:**

The proportion of treatment failures in the SHB004 group $\pi_1$ is smaller than the corresponding proportion $\pi_2$ in the placebo group, i.e. the relative risk of SHB004 versus placebo is smaller than 1:

$H_A: \frac{\pi_1}{\pi_2} < 1$
A three stage group sequential design will be applied with two interim analyses and one final analysis (Wald type test statistic, overall 1-sided \( \alpha = 0.025 \), Pocock efficacy boundaries, futility stop in case of a 1-sided p-value \( \geq 0.1587 \)). The study is powered at 80% to detect a relative risk of 0.4, corresponding to at least 60% improvement of SHB004 group versus placebo group. A \( \geq 50\% \) cut-off follows regular authorities’ advices.

The overall type-1 error (\( \alpha = 0.025 \), 1-sided) will be controlled using an alpha-spending function (Pocock boundaries).

The power of the testing procedure depends mainly on the expected overall number of treatment failures (TF) and the relative risk. For a relative risk of 0.4 (corresponding to assumed rates of 3.1% for placebo and 1.24% for SHB004) an expected number of about 52 treatment failures is needed at the last stage to have an overall power of 80%.

The following table shows an overview of the intended numbers of treatment failures per stage and the corresponding number of patients for treatment failure rates of 3.1% (placebo) and 1.24% (SHB004), assuming that 10% of the randomized patients have to be excluded from the ITT population due to second tick bites or missing Day 57 results.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Cumulative expected number of TF in ITT set</th>
<th>Cumulative patient numbers for TF rates 3.1% (placebo), 1.24% (SHB004)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ITT population</td>
<td>Randomized</td>
</tr>
<tr>
<td>1</td>
<td>( \approx 25 )</td>
<td>( \approx 1152 )</td>
</tr>
<tr>
<td>2</td>
<td>( \approx 37 )</td>
<td>( \approx 1706 )</td>
</tr>
<tr>
<td>3</td>
<td>( \approx 52 )</td>
<td>( \approx 2396 )</td>
</tr>
</tbody>
</table>

The power of the 3-stage design (section 8.8) is 82% for expected cumulative treatment failure numbers of 25, 37 and 52 at stages 1, 2 and 3 and a relative risk of 0.4: If the expected number of treatment failures at stage 1 is 22 instead, then the power is 80%. Since stage 1 is almost complete and preliminary blinded stage 1 data suggest around 20 to 25 treatment failures, the study was planned to have 80% power for 22 expected treatment failures at stage 1, which is achieved when 15 additional treatment failures can be expected for each of the subsequent 2 stages, resulting in cumulative numbers of 22, 37 and 52.

<table>
<thead>
<tr>
<th>Relative risk</th>
<th>Cumulative expected number of TF</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage 1</td>
<td>Stage 2</td>
</tr>
<tr>
<td>0.4</td>
<td>25</td>
<td>37</td>
</tr>
<tr>
<td>0.4</td>
<td>22</td>
<td>37</td>
</tr>
</tbody>
</table>

A first interim analysis is planned to be conducted after 25 \( \pm 20\% \) treatment failures have been observed. Also, due to the seasonal aspect of the study, it is expected to perform the first interim analysis by the end of the second season of the study. The second and third analyses are planned after 37 \( \pm 20\% \) and 52 \( \pm 20\% \) treatment failures. Since the already available preliminary stage 1 data suggests an actual number of treatment failures slightly below 25, an expected number of 22 was assumed for the power calculation.

Due to logistic reasons it will not be possible to reach the numbers of treatment failures exactly. The correspondence between the expected number of treatment failures and the patient numbers depends on the unknown overall treatment failure rate.
The Independent Data Monitoring Committee (IDMC) will have to decide whether the study should be continued or not owing to the results of the first or second interim analysis. The IDMC will decide on doubtful cases of treatment failures (TF) and agree if the doubtful case is to be counted as TF or not. If necessary, the IDMC may request additional information from the respective Investigator to clarify possibly open questions. This is to be done before unblinding. The IDMC is particularly discussing cases within which “negative” serological results against IgM and / or IgG antibodies for Borrelia burgdorferi s.l. were obtained for Visit 1 and “borderline” results were obtained for Visit 4. According to the current MIQ, these results allow no final conclusion if antibodies are present in the respective serum samples.

If changes to the protocol are recommended by the IDMC, the Sponsor will communicate changes to the protocol by means of a protocol amendment.

A first interim analysis is planned to be conducted after a total of 25 ± 20% treatment failures have been observed. Also, due to the seasonal aspect of the study, it is expected to perform the first interim analysis by the end of the second season of the study.

A second interim analysis is planned to be conducted after a total of 37 ± 20% treatment failures have been observed. Also, due to the seasonal aspect of the study, it is expected to perform the second interim analysis by the end of a recruitment season.

At each stage the type-1 error to be spent will be computed using an alpha-spending function, which approximately produces the Pocock stopping boundaries $b_k$, where $k = 1, 2, 3$ is the analysis stage.

The stopping criteria for futility or efficacy are summarized as follows:

**Stage 1** (interim analysis)
- End of the study for demonstrated futility (accept $H_0$), if the proportion of treatment failures in the SHB004-group is $\geq$ the corresponding proportion in the placebo group, i.e. if the 1-sided p-value $p_1$ is $\geq 0.1587$.
- End of the study for demonstrated efficacy (reject $H_0$), if the 1-sided p-value does not exceed the corresponding boundary, i.e. $p_1 \leq b_1$.

Otherwise continue with Stage 2.

**Stage 2** (interim analysis)
- End of the study for demonstrated futility (accept $H_0$), if the proportion of treatment failures in the SHB004-group is $\geq$ the corresponding proportion in the placebo group, i.e. if the 1-sided p-value $p_2$ is $\geq 0.1587$.
- End of the study for demonstrated efficacy (reject $H_0$), if the 1-sided p-value does not exceed the corresponding boundary, i.e. $p_2 \leq b_2$.

Otherwise continue with Stage 3 (final analysis).

**Stage 3** (final analysis)
- End of the study for demonstrated futility (accept $H_0$), if the 1-sided p-value is larger than the corresponding boundary, i.e. $p_3 > b_3$.
- End of the study for demonstrated efficacy (reject $H_0$), if the 1-sided p-value does not exceed the corresponding boundary, i.e. $p_3 \leq b_3$. 
These stopping criteria along with other statistical considerations (e.g. conditional power, sensitivity analyses in other populations, repeated confidence intervals) are going to be used by the IDMC as a guideline for taking decisions.

The futility boundaries are binding in the sense that the study will be stopped, if the futility stopping criterion is met, i.e. if $p_k$ is $\geq 0.1587$ at an interim analysis.

The efficacy boundaries are binding in the sense that the study can only be stopped, if the stopping criterion is met at one of the stages, i.e. $p_k \leq b_k$ for at least one stage $k$.

The study biostatistician(s) will analyse the data according to the statistical analysis plan (SAP) and hand in the interim results to the IDMC in order to support the safety and efficacy review meetings. The independent statistician of the IDMC will analyse the data using a trial monitoring guideline and will provide data to the committee at least 5 days prior to a scheduled meeting.

| Schedule of Procedures | See Table 1 |
### Table 1: Schedule of Procedures

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Screening/ Baseline Visit</th>
<th>Telephone Follow-up 1 *</th>
<th>Telephone Follow-up 2 *</th>
<th>End of Study/ Withdrawal Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Visit number</strong></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td><strong>Day (± or + allowed time window)</strong></td>
<td>1</td>
<td>7 (±3 days)</td>
<td>30 (±5 days)</td>
<td>57 (+14 days)</td>
</tr>
<tr>
<td>Informed consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical history and current medical conditions a</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demographic details</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inclusion / exclusion criteria</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical examination</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine pregnancy test b</td>
<td>X</td>
<td></td>
<td>(X) b</td>
<td></td>
</tr>
<tr>
<td>Collect the tick or parts of the tick (e.g. hypostom only) and record site if tick bite in eCRF</td>
<td>X i i i</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mark site of tick bite k</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood sample for seroconversion test a b</td>
<td>X</td>
<td></td>
<td>X a</td>
<td></td>
</tr>
<tr>
<td>Randomization</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First treatment administration, and instructions to subject c</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dispensing of study drug</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First phone call to the electronic diary (eDiary) b</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collection of study drug</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Review of subject’s eDiary d</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Telephone Follow-up to check:</strong></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Appearance of signs and symptoms of Borreliosis (EM and/or other) i</td>
<td>X i i</td>
<td>X i i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Adverse events e and local tolerability f</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Any additional tick bite</td>
<td>X i i</td>
<td>X i i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Concomitant medications (including antibiotic) g</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>At site visits:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Appearance of signs and symptoms of Borreliosis (EM and/or other) i</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>- Adverse events e and local tolerability i</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>- Any additional tick bite</td>
<td>X i i</td>
<td>X i i</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Previous and concomitant medications g</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>- Control, that samples for shipment to central laboratory have been collected and are adequately stored</td>
<td>X b t s</td>
<td></td>
<td></td>
<td>X b s</td>
</tr>
</tbody>
</table>

*IVRS (interactive voice response system)
a Analyzed at central laboratory (see sections 6.1.2, 6.5 and 6.6 for details). End of study seroconversion sample should not be obtained before Day 57.

b For women of childbearing potential a 2nd pregnancy test as required by Health Authorities in Austria does not constitute a protocol violation and is mandatory for Austrian sites.

c First administration by the physician or other medically-trained person (e.g. nurse), who will also instruct the subject regarding the proper administration technique and mode. This first administration will be performed after the baseline skin tolerance assessment (see footnote f).

Subsequent study drug administrations will be done by the subject at home, following the detailed instructions provided, and subject will document these by means of a call to the eDiary (see footnote o).

See section 5.2 for details on the procedure of administration, dosing schedule (At marked site, one drop of gel is applied and let as it is to dry for 30 minutes, BID once in the morning and once in the evening, 12 hours apart; time window of ±2 hours; only for the second study drug administration, a time window of ±6 hours is allowed [i.e. for the first application done by subject alone] for three consecutive days. In total, 6 administrations are required. If only one administration is possible on Day 1 due to constraints of time windows, then the second administration will be done the morning of Day 2; finally the sixth and latest administration will be done in the morning of Day 4.) or conduct in case the drop is inadvertently wiped out. For study drugs storage condition instructions for sites and for subjects see section 6.2.3.

d Review of the eDiary will include the assessment of regular topical administration.

e Adverse events (AE): all AEs will be reported in the adverse event eCRF from time of signature of informed contents until Visit 4. Serious adverse events will be reported from time of informed consent signature until Visit 4, or occurring up to 4 weeks after the last study drug administration (whichever is longer). (See section 6.2.1 for more details about AE/Serious AE reporting and follow-up)

f Skin tolerance will be assessed using the modified CPMP-SWP 2145/00 score (see APPENDIX (APPENDIX 1)). If possible skin reactions are assumed after the telephone calls at Day 7 and Day 30, subjects should be invited to the study center for diagnosis.

g Medication taken prior to first dosing: All prescription medications taken within one month, and over-the-counter drugs (including vitamins) taken within 14 days prior to baseline and throughout the study must be recorded on the Concomitant Medications/ Non-Drug Therapies page of the eCRF. This information on previous medication is only to be collected at baseline / Visit 1.

Concomitant medications/ Significant non-drug therapies: must be recorded from time of first administration of study drug until Study Completion.

h t = tick, s = blood/serum sample

i In case of additional tick bite after enrollment in the study, the subject will be asked to collect it and bring it back to the clinical site at the next study visit. The investigator or other medically trained personnel will confirm the subject’s suspicion, that the collected specimen is indeed a tick, report this and take care of having this tick destroyed (see section 6.1.5) (The subject has to be instructed that he/she must not apply any study drug gel on this new tick bite.)

k See section 5.2 for more details on how to mark the site of tick bite.

l When checking with the subjects for signs and symptoms of Borrelia, the appearance of EM should specifically be controlled (see section 6.2.3 for EM definition criteria). In case of suspected Borrelia the patient is to be treated as outlined in section 5.7.3. At the same time the patient has to be asked whether he has experienced another tick bite (for procedure go to section 6.1.4). In any case, EM should be documented photographically if possible and pictures should show a ruler for size comparison.

m In case the question by phone about signs and symptoms of Borrelia raises the possibility of appearance of EM, the subject must be instructed to come to the site as soon as possible for a mandatory additional clinical visit, so that the investigator can control/validate the appearance of EM, other signs and symptoms of Borrelia and initiate an antibiotic treatment against Borrelia at the Investigator’s discretion (see section 6.2.3 for EM definition criteria).

n Within the last 2 years before screening (see section 6.3.2).

o The first phone call to record the first gel application in the eDiary system using the IVRS will be done by the subject with the help of the investigator. For the next study drug applications, the subjects will need to call the IVRS themselves from home. Separate instructions will be provided on the use of IVRS by the investigator and subjects.

p Up to 5 years after the study completion, the remaining blood samples may be used to perform additional antibody measurements (see section 6.6.2).
*Telephone follow-ups: In case it is medically required (e.g. medical emergency, skin tolerance issues, signs and symptoms, suspected appearance of EM, the investigator considers this necessary, etc), telephone follow-ups will lead to additional clinical visits at the investigational site as soon as possible (see section 7.2.6).

Investigators will report subject information in the eCRF and subject medical files throughout the study.
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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE      Adverse event
ASR     All subjects randomized
ATC     Anatomical Therapeutic Chemical [Classification System]
BfArM   German Federal Institute for Drugs and Medical Devices
BID, bid Twice daily
Borrelia Borrelia burgdorferi sensu lato
s.l.    s.l.
CPMP    Committee for Proprietary Medicinal Products
CTCAE   Common Terminology Criteria for Adverse Events
DMF     Drug Master Files
DNA     Deoxyribonucleic Acid
DRL     Drug Reference List
IDMC    Independent Data Monitoring Committee
eCRF    Electronic case report form
eDiary  Electronic diary
EDC     Electronic data capture
ELISA   Enzyme-Linked ImmunoSorbent Assay
EM      Erythema migrans
EMA     European Medicines Agency
EU      European Union
FDA     United States of America Food and Drug Administration
GCP     Good Clinical Practice
GMP     Good Manufacturing Practice
H₀      Null Hypothesis
Hₐ      Alternative Hypothesis
HIV     Human immunodeficiency virus
IB      Investigator’s Brochure
ICH     International Conference on Harmonization
IEC     Independent Ethics Committee
IgG     Immunoglobulins of G subclass
IgM     Immunoglobulins of M subclass
IRB     Institutional Review Board
ITT     Intent to treat; intention to treat
IVRS  Interactive Voice Response System
MedDRA  Medical Dictionary for Regulatory Activities
MIC  Minimal inhibitory concentration
MITT  Modified Intention to treat
MTD  Maximum tolerated dose
NCI  National Cancer Institute
OD  Once daily
OspA and OspC  Outer surface protein A and protein C from the Lyme disease spirochete, Borrelia burgdorferi
PCR  Polymerase chain reaction
Ph. Eur.  European Pharmacopoeia
PP  Per Protocol
SAE  Serious adverse event
SAF  Safety Analysis Set
SAP  Statistical analysis plan
SUSAR  Suspected unexpected serious adverse reaction
SWP  Safety Working Party
TEAE  Treatment-emergent adverse event
TF  Treatment Failure
USP  United States Pharmacopeia
WHO  World Health Organization
1 INTRODUCTION

1.1 Background

1.1.1 Disease and Treatment

Lyme borreliosis — the most common arthropod-borne infection in Europe and the United States of America — is a complex multisystem disorder caused by *Borrelia burgdorferi* sensu lato (*Borrelia burgdorferi* s.l), a group of genetically diverse spirochetes. The principal vectors of these spirochetes are ticks belonging to the genus *Ixodes* [2]. Depending on regions, countries, seasons, and age of the tick (adult or nymph) the rate of infection of ticks varies significantly. A Swedish study showed that 33% of the adult ticks and 14% of the nymph were infected by *Borrelia* (overall prevalence 19%) [3]. In a German study rates of tick infections were higher: 40% in adults, 30% in nymphs and 35% overall [2]; whereas in a study conducted in the Luxembourg the overall *Borrelia burgdorferi* s.l infection rate observed was lower: 11.3% [4]. A meta-analysis investigating the literature on the prevalence of *Borrelia burgdorferi* s.l genospecies in *Ixodes ricinus* ticks in Europe reported overall infection rates of 18.6% in adults and 10.1% in nymphs [5].

After a tick bite, *Borrelia burgdorferi* s.l transiently remain localized in the skin at the site of the tick bite. Proteins present in the tick’s saliva adsorb to the bacteria, thus reducing bacteria’s immunogenicity, impairing the recognition of the bacteria by the host’s innate immune system and protecting the bacteria upon entry in the skin. After several days, *Borrelia* disseminate from the skin into the body, potentially causing if untreated the symptoms of the Lyme disease: carditis, arthritis or central nervous system disorders. The 3 stages of the Lyme disease are: [6]

- Stage 1: within 3 weeks, erythema migrans (EM) and generalized flu-like symptoms;
- Stage 2: after months/years, Lyme arthritis, cardiac symptoms, central nervous system involvement (5% of cases) and cranial nerve deficits;
- Stage 3: after months/years, chronic inflammation of the joints, fibromyalgia, and polyneuropathy.

*Treatments with curative intent:*

When first symptoms occur (leading symptoms is generally an EM), an oral antibiotic therapy is given for several weeks. However, up to 50% of the patients are reported to not develop an EM and are consequently often not diagnosed and, therefore, left untreated. Antibiotic therapy brings about immediate symptomatic improvement in borreliosis. However, the antibiotic treatments may be associated with adverse effects. Also, antibiotics administered orally may not reach sufficient concentrations in the organ tissues to eliminate *Borrelia*, and some bacteria survive [7]. In addition, if oral therapy is initiated too late, clinical symptoms can repeatedly flare-up months and even years later [8]. With repeated therapy, symptoms tend to be recurrent, often in 4-weekly cycles. In these cases, cure is unlikely and continuous administration of antibiotics is required if tolerated by the patient [9].

In cases at stages 2 or 3, intravenous antibiotics are administered for up to several weeks with often unsatisfactory outcome (e.g. recurrent flares cannot be prevented).
Another hypothesis links *Borrelia* infections (e.g. in the joints) to *Borrelia*-induced autoimmune diseases, which might explain why some patients have a disease becoming refractory to antibiotic treatments [10]. This hypothesis further fosters the need to prevent the dissemination of *Borrelia* from the site of tick bite.

**Preventive treatments:**

Preventive oral antibiotic treatment after tick bite has been used in endemic regions (once a day up to a week). Nadelmann *et al.* (2001) [11] is the only clinical study to demonstrate a significant effect (p=0.045) of antibiotic prophylaxis after treatment with a single dose of 200 mg of doxycycline. About a half of the patients receiving doxycycline reported gastrointestinal problems. Data from a meta-analysis suggested that the preventive treatment was fairly ineffective with only one case of Lyme disease estimated to be prevented by treating 50 patients in highly endemic areas [12]. Oral antibiotic treatment comes with safety risks (e.g. rash, nausea, or rarely anaphylaxis), potential development of resistances for treatment of other diseases, and is particularly problematic in children or pregnant women (e.g. in case where doxycycline is used). Due to these reasons, a prophylactic oral treatment is not recommended by treatment guidelines [9].

1.1.2 *Choice of the Antibiotic for a Topical Treatment*

Various antibiotics can be effectively used in the treatment of the early stages of Lyme disease (such as amoxicillin, cefuroxime axetil, or doxycycline), but there is currently no universally accepted antibiotic to treat Lyme borreliosis [13]. The treatment of Lyme disease with any antibiotic is associated with insufficient or transient response, or even lack of response in 50% of cases when initiated at late-stage infections [14, 15, 16] and in 10% of cases when the treatment is initiated in early phases of Lyme borreliosis [17, 18, 19]. Any treatment of symptomatic Lyme borreliosis using antibiotics is, therefore, associated with a possible risk of insufficient response, with likely causes being persistence of *Borrelia* in tissues into which only insufficient concentrations of antibiotics can be achieved or maintained, reported intracellular localization (escape of *Borrelia*), and the ability to build up a biofilm [20].

The lack of agreement in the literature concerning the *in-vitro* susceptibility of *Borrelia burgdorferi* to macrolide antibiotics such as erythromycin [21, 22, 23] and the efficacy of macrolides in the treatment of Lyme disease [24, 25] is at least in part the result of the difficulty of culture of spirochetes, the lack of standardized methods to define for this pathogen the minimal inhibitory concentration (MIC), and the transformation of the bacteria in culture that may result in changes of susceptibility to antibiotics (e.g. changes in plasmid number, growth rate, and infectivity) [26]. Similarly, resistance of some *Borrelia burgdorferi* strains to erythromycin has already been established in spite of the sensitivity to erythromycin *in-vitro* [26].

The current recommendation of the German Robert Koch Institute is to start a treatment at the most early stage possible, after an erythema has been observed [27]. The therapy of choice is oral tetracyclines or amoxicillin with azithromycin being an alternative in case of contraindications to the afore-mentioned antibiotics. Antibiotics such as doxycycline are relatively contraindicated in some patient groups such as children below 8 years, pregnant women, and those who are intolerant to the drug. Alternative oral antibiotics with clinical efficacy in the Lyme disease that can be
administered in such patient groups include amoxicillin, cefuroxime axetil, and —more important in the context of this study— azithromycin [9].

1.1.3 SHB004 Azithromycin Topical Treatment

SHB004 is an azithromycin topical gel formulation developed by IXODES AG.

A SHB001 formulation has been used for Phase 1 and 2 studies and is now replaced by SHB004 for this Phase 3 study. The difference between SHB001 and SHB004 is the replacement of a well known excipient in SHB001 by an also well known, similar acrylic polymer of pharmaceutical quality grade (USP) in SHB004. No changes in the tolerability or properties are thus expected. See the latest investigator’s brochure for more details about the differences between the 2 formulations and section 5.1 for the composition of SHB004.

The active substance of SHB004 —azithromycin— is widely used to treat or prevent bacterial infections and is commonly administered as tablets or oral suspension. Azithromycin is also available in an ophthalmic solution formulation for the indication of conjunctivitis.

Azithromycin is an active pharmaceutical ingredient of choice, since it can be used in a broad patient population and is a currently recommended antibiotic in the treatment of early stages of Lyme Borreliosis. The topical formulation of azithromycin extrapolates the German Robert Koch Institute recommendation (see section 1.1.2), by aiming at the development of a drug formulation which justifies the treatment of any tick bite immediately after removal of the tick. This approach allows to potentially shift current treatment recommendations by allowing immediate treatment instead of waiting for the development of signs such as an erythema adjacent to the tick bite before an oral therapy commences. In spite of the fact, that no systemic drug availability was recorded upon topical administration of SHB001 [28] (formulation similar to SHB004), azithromycin was selected as the active ingredient to open this treatment option to children in the future (children are not included in this study) and, in addition, due to known allergic skin reactions or phototoxicity problems associated with many other antibiotics other than azithromycin.

Also, azithromycin is expected to penetrate easily into skin tissues due to its high lipid solubility, facilitated by the excipient / penetration enhancer Miglyol™ 812. It possesses a long half-life in tissues (approximately 68 hours), and effectively targets both, the extra-as well as the intracellular compartments of the skin. Therefore, if used shortly after a tick bite has been identified, it should be possible to eradicate *Borrelia burgdorferi* from their site of entry in the skin, thereby preventing the spread of *Borrelia burgdorferi* and any consecutive systemic infestation.

The pathogenesis of the Borreliosis in its early stages is associated largely with the presence of viable bacteria at the site of tick bite/entry and inflammation [29]. Using SHB004 azithromycin topical formulation *Borrelia s.l* are exposed to a high concentration of antibiotics for a sustained period and thereby eliminated as demonstrated in preclinical studies (see in section 1.1.4). IXODES AG topical approach allows treatment with a high concentration but —as the azithromycin is not systematically available— unlikely to be associated with any systemic side effects. SHB004 therefore has favorable properties to allow it to be used repeatedly after every tick bite. In contrast, current oral or even intravenous antibiotic therapy cannot be justified after every tick bite due to a negative risk-benefit assessment and the
disease may have become refractory to treatment by then. In summary, currently recommended treatments are started only after appearance of symptoms, but by then a cure can no longer be guaranteed.

1.1.4 Pre-clinical Studies with SHB001 Azithromycin Topical Treatment

**Efficacy**

The efficacy was proven in three experiments using a murine model with local application of SHB001 (20%, 10%, 5%, 4% and 0%) after infection by ticks as well as after the infection caused by needle inoculation with *Borrelia burgdorferi* (see Table 2 and Table 3). None of the 47 animals treated with active-ingredient containing SHB001 right after and 3 days after the infection, were infected at the days 7, 14 and 56. In the meantime, all 15 out of 17 control/placebo animals in contact with *Borrelia burgdorferi* were found to be infected.

**Table 2: Overview of Efficacy Studies with SHB001 after Exposure of Mice to Ticks**

<table>
<thead>
<tr>
<th>Report</th>
<th>Treatment Dosage</th>
<th>Length of exposure</th>
<th>Total no. of animals</th>
<th>Animals exposed to infected ticks</th>
<th>Animals infected at Days 14 and 28</th>
<th>Adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>University Leipzig</td>
<td>SHB001 20% 200 mg</td>
<td>Once a day during three consecutive days</td>
<td>9 mice</td>
<td>6</td>
<td>0</td>
<td>3 animals died following anesthesia</td>
</tr>
<tr>
<td>IZI 5.6.08</td>
<td>No treatment</td>
<td></td>
<td>8 mice</td>
<td>7</td>
<td>4</td>
<td>2 animals died following anesthesia</td>
</tr>
<tr>
<td>University Leipzig</td>
<td>SHB001 10% 200 mg</td>
<td>Once a day during three consecutive days</td>
<td>9 mice</td>
<td>5</td>
<td>0</td>
<td>Slight inflammation</td>
</tr>
<tr>
<td>IZI 2.12.08</td>
<td>SHB001 4% 200 mg</td>
<td>Once a day during three consecutive days</td>
<td>9 mice</td>
<td>5</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>SHB001 0% 200 mg</td>
<td>Once a day during three consecutive days</td>
<td>9 mice</td>
<td>3</td>
<td>3</td>
<td>None</td>
</tr>
</tbody>
</table>

The 1st experiment (5.6.08) compared 20% azithromycin versus no treatment:

- Treated group: infected mice. Topical application of SHB001 20% gel on three consecutive days, starting five days after the tick bite. No infection detected in the treated animals (no evidence of specific antibodies at Day 57 or of pathogen deoxyribonucleic acid (DNA) at Days 14 and 28). A transient inflammatory reaction was observed in all animals following application of the gel.
- Control group: infected mice. These animals were not treated and all of them showed signs of disease.
- Three (3) animals from the treatment group and 2 animals from the control group died following (and causally linked to) repeated anesthesia.
The 2\textsuperscript{nd} experiment (2.12.08) was a repetition of the 1\textsuperscript{st} experiment with lower concentrations of active ingredient (4\% and 10\%), in order to reduce the inflammatory reaction observed with the formulation containing 20\% and the protocol for anesthesia was adapted using less anesthetic.

- Treated group: infected mice. Topical application of SHB001 4\% or 10\% gel on three consecutive days, starting five days after the tick bite. No infection detected in the treated animals (no evidence of specific antibodies at Day 57 or pathogen DNA at Days 15 and 18). A mild inflammatory reaction was seen following application of the 10\% gel. None of the animals treated with the 4\% formulation showed this inflammatory reaction.

- Control group: infected mice. All animals were given placebo (identical formulation but without active substance) and those with infected ticks showed signs of disease.

Following a request of German Federal Institute for Drugs and Medical Devices (BfArM) a murine model using intradermal infection by needle injection was set up. Animals were infected with 10,000 or 100,000 *Borrelia burgdorferi* organisms and treated either with SHB001 (5\%) or placebo. The infection status of animals was measured using cell lysate Enzyme-Linked ImmunoSorbent Assay (ELISA), C6-peptide specific ELISA and Western Blot. All 14 animals that received the 5\% azithromycin formulation were negative for an infection with *Borrelia burgdorferi* at Day 56, independently of the infection doses. All 14 control mice that received placebo formulation were positive for the infection (see Table 3).

### Table 3: Efficacy Studies with SHB001 in a Murine Model after Infection with *Borrelia burgdorferi* by Needle Injection

<table>
<thead>
<tr>
<th>Report</th>
<th>Treatment Dosage</th>
<th>Length of exposure</th>
<th>Infection status after inoculation with 10'000 <em>Borrelia burgdorferi</em></th>
<th>Infection status after inoculation with 100'000 <em>Borrelia burgdorferi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Animals inoculated</td>
<td>Animals infected</td>
</tr>
<tr>
<td>LMU &amp; IZI 17.9.09</td>
<td>SHB001 5% 200 mg</td>
<td>Once a day during three consecutive days</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>1\textsuperscript{st} working package</td>
<td>SHB001 0% 200 mg</td>
<td>Once a day during three consecutive days</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

Based on these observations, we decided to use 50,000 bacteria per mouse as an infection dose for future experiments, within which we investigated the effects of SHB001 5\% treatments at different time points in comparison to placebo treatment (LMU & IZI, 17.9.09 working package 2, see Table 4). The results indicate that SHB001 5\% treatment starting after 1 hour or 3 days after infection clears the infection. Only specific antibodies were detectable in 6 animals that received treatment from day 3 post needle inoculation. These antibodies were mainly directed against outer surface protein A and protein C (OspA and OspC) from the Lyme disease spirochete, *Borrelia burgdorferi*. Therefore, it is suggested that they were
caused by the inoculation of culture derived spirochetes, between the time point of inoculation and first treatment 3 days later.

In a following experiment, the concentration of azithromycin was determined in murine skin biopsies after the application of SHB001 5% by using agar diffusion assay (report LMU & IZI, 17.9.09, working package 3). The antibiotic concentration in murine skin was between 2,000 and 1,000,000-fold higher than the MIC (0.015 mg/mL) already 3 hours after application and remained at these levels for 24 hours.

Table 4: Effect of SHB001 5% at Different Time Points of Treatment, Compared to Placebo

<table>
<thead>
<tr>
<th>Treatment Dosage</th>
<th>Length of exposure</th>
<th>Start of treatment post inoculation (days)</th>
<th>Number positive OspA PCR (skin biopsies) day</th>
<th>Culture</th>
<th>Elisa</th>
<th>Western blot</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHB001 0% 200 mg</td>
<td>Once a day during three consecutive days</td>
<td>1</td>
<td>10/10 5/10 2/10</td>
<td>1/10</td>
<td>9/10</td>
<td>9/10</td>
<td>9/10</td>
</tr>
<tr>
<td>SHB001 5% 200 mg</td>
<td>Once a day during three consecutive days</td>
<td>1</td>
<td>0/10 0/10 0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0/10 0/10 0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>2/10 0/10 1/10</td>
<td>0/10</td>
<td>7/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

PCR = polymerase chain reaction

Safety

The local safety evaluations, local tolerability studies in rabbits (5% and 10%, applied daily for four weeks), sensitizing potential (with photosensitivity) in guinea-pigs and skin sensitization tests in guinea pigs (according to Magnusson and Kligman [30]), have successfully been completed. SHB001 topical formulation (10%) did not reveal any photosensitizing properties. Following twice daily topical application on the intact and abraded skin of rabbits on 28 consecutive days, very slight to severe erythema were observed. However, the effects were most pronounced for the 10% formulation and least pronounced for the placebo, possibly caused by test item deposits. There was no difference between the intact and abraded skin. SHB001 topical formulation (10%) was found to be not sensitizing to guinea pigs in a test model according to Magnusson and Kligman. In addition, the phototoxicity of SHB004-Topical Formulation 10% was tested in vitro in cultured Balb/C 3T3 cells and no toxicity was found.

1.1.5 Clinical Studies with SHB001 Azithromycin Topical Treatment

The clinical studies have been designed according to scientific advice given by BfArM (see Table 5 for a summary).

Phase 1 (local tolerability in humans) has successfully been completed with 8 subjects. Based on the (i) low rate of Adverse Events observed, (ii) the absence of any significant changes in safety laboratory parameters and (iii) the systemic
unavailability of azithromycin as assessed in serum, SHB001 is considered to be safe and very well tolerated. No allergic reactions have been observed [28].

The Phase 2 study had the objective to select a dose based on the concentration profile of azithromycin in the skin (above the MIC of *Borrelia burgdorferi* for 50% of the time span of three days). No safety concern was reported [28] (during the entire study no serious adverse events and no unexpected adverse drug reactions occurred). The objective of the study to reach or exceed the MIC for two consecutive measurements (i.e. two consecutive days) was reached for both treatment schemes (once or twice a day [BID]) by a large margin. In summary, for SHB001, azithromycin 10% BID treatment, the MIC was reached and exceeded in all skin compartments of all volunteers at all three measurement time points, i.e. after 1, 2 and 3 days of treatment. The median of azithromycin concentrations in all compartments was at least 20-fold higher than the MIC, and the 25th percentile value was at least 5-fold over the MIC [28].

Also for SHB001, azithromycin 10% once daily (OD) treatment, the MIC was reached and exceeded in all skin compartments of all volunteers after 2 and 3 days of treatment. The median of azithromycin concentrations in all compartments was at least 7-fold higher than the MIC, and the 25th percentile value was at least 3.5-fold over the MIC.

<table>
<thead>
<tr>
<th>Table 5: Summary of the Clinical Development Programme</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Status</strong></td>
</tr>
<tr>
<td>Phase 1 IXO-01 Completed</td>
</tr>
<tr>
<td>Phase 2 IXO-01 Completed</td>
</tr>
<tr>
<td>Phase 1 (A2102) Completed</td>
</tr>
</tbody>
</table>

**MTD = maximum tolerated dose**

Further details on the formulation, pre-clinical, and clinical development phases of the azithromycin topical formulation can be found in the Investigator's Brochure [31], which contains comprehensive information on the investigational product.
See section 3.1 for details concerning the design of the current study and section 3.3 for justification of the design of this study.

1.2 Rationale

The purpose of this study is to demonstrate that, in subjects bitten by a tick, SHB004 is effective in treating an infection with *Borrelia s.l.* bacteria and is preventing Lyme Borreliosis.

The formulation strength, dose and administration schedule of SHB004 have been chosen in view of the pre-clinical and previous Phase 1/2 studies in healthy volunteers where the maximum tolerated dose has been determined as a gel containing 10% of azithromycin applied to the skin BID.

The derived dose of one drop of SHB004 gel planned to be applied to the skin was chosen to achieve antibiotic levels well in excess of *Borrelia s.l.* MIC for azithromycin [28]. Combined with animal model data it is thus expected that SHB004 will eradicate *Borrelia s.l.* infection before systemic infection can be established.

A dose of one drop of SHB004 gel is planned to be applied to the skin. From a safety perspective and under the assumption that the whole dose is taken up (which is unlikely and therefore, this assessment is extremely conservative), this corresponds to only 20 mg of the active ingredient in the concentration used (10% azithromycin). If SHB004 is applied twice a day for 3 days, this would result in a maximum exposure of 120 mg azithromycin. This is less than ¼ of the 500 mg dose for a single oral tablet (indication in adults being 1,500 mg to be administered as 500 mg per day for three consecutive days [32]).

During this study, one interim analysis will be conducted (or even two, if required). The interim analyses are required to assess the true rate of seroconversion (IgG and/or IgM) and/ or appearance of erythema migrans per geographic region and to address the unpredictable annual fluctuation in the number of ticks carrying *Borrelia*.

1.3 Risk-Benefit Assessment

The appearance of EM triggers the use of oral antibiotics according to current treatment recommendations. This study strictly follows this treatment guideline and any subject developing EM or other symptom of Lyme disease will receive appropriate treatment according to local guidelines. However, the appearance of EM occurs in only about 60% to 90% of cases who develop a clinically manifested Borreliosis. Therefore, Borreliosis remains undetected in many patients, if the appearance is taken as the only leading symptom (which is typically the case). Therefore, subjects who are randomized to SHB004 treatment in this study potentially have an additional benefit as it has been demonstrated that treatment with SHB001 leads to bactericidal antibiotic concentrations in the skin [28].

Azithromycin – the active pharmaceutical ingredient in SHB004 and SHB001 – has side effects related to the systemic use (other than dermal reactions) and high serum concentrations (gastrointestinal: pseudomembranous colitis, diarrhea, nausea, abdominal pain, vomiting). A suspected interference with birth control pills has been reported. Due to the inability to trace azithromycin in plasma of subjects who participated in the IXODES AG topical formulation Phase 1 study (systemic absorption was not observed) and by virtue of the low amount of total azithromycin
applied to the skin (e.g. a few milligrams in the SHB004 gel formulation compared to 500 mg in the oral tablet), IXODES AG does not believe that special precautions are required regarding the side effects or potential interference with contraception, as observed for oral azithromycin.

The summaries of product characteristics for systemic formulations of azithromycin mention several interactions with other medicinal products. However, previous studies with SHB001 showed no detectable plasma concentrations of azithromycin, such interactions are thus unlikely with the topical formulation used for this study.

However, serious allergic reactions, nervousness, dermatologic reactions, and fatalities have been reported for the systemic formulations. Particularly allergic reactions and dermatologic reactions may occur due to local treatment with SHB004 although the formulation has been well tolerated in the Phase 1 study. IXODES AG topical approach allows treatment with a high concentration locally in skin but, as the azithromycin is not systematically available, it is likely that no systemic side effects will be observed. Subjects will be questioned during screening for a potential known sensitivity to azithromycin and will be excluded in case such sensitivity is reported. Furthermore, clinical centers will be prepared for the treatment of possible skin reactions (e.g. dermal corticosteroids). In the case of skin irritations characterized by a score of 3 or higher, no further treatment can be given (score is modified from the advise in European Medicines Agency [EMA] Note for Guidance on dermatologic tolerance testing [Committee for Proprietary Medicinal Products - Safety Working Party: CPMP-SWP 2145/00; preclinical guidance] and according to an established procedure with a slightly modified proposal of the Standardization Group of the European Society of Contact Dermatitis [see APPENDIX 1 of this protocol]. This score was used in the Phase I study).

Based on the toxicological profile of azithromycin and the good preclinical and clinical tolerance results obtained so far with SHB001, there are no relevant safety issues to be expected with SHB004 in this study.

The available information suggests that the present study has a favorable risk-benefit ratio.
2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to demonstrate a reduction in the rate of treatment failure within the ITT set at Day 57 by at least 50% in response to SHB004 (10% topical azithromycin) administered locally within four calendar days after the tick bite had been first noticed, BID for three consecutive days, as compared to placebo. Treatment failure is defined as seroconversion (IgM and / or IgG) and / or appearance of EM throughout the study in baseline-seronegative (IgM and / or IgG) subjects. Subjects experiencing an additional tick bite are not counted as treatment failure unless they experience an EM occurring before the additional tick bite.

2.2 Secondary Objectives

2.2.1 Secondary Efficacy Objectives

Secondary objective 1 is as the primary objective for the All Treated Subjects A-C set, and modified ITT set.
Secondary objective 2 is as the primary objective in the ITT set and isolated IgM are not counted as Treatment Failure (TF).
Secondary objective 3 is as the primary objective in the ITT set and isolated IgG are not counted as TF.
Exploratory objective is as the primary objective but for the PP set.

2.2.2 Secondary Safety Objective

To demonstrate the local safety and tolerability of SHB004 (10% topical azithromycin) administered locally BID for three consecutive days.
OVERALL DESIGN AND PLAN OF THE STUDY

3.1 Overview

This study is a Phase 3, randomized, parallel-group, double-blind, placebo-controlled study of SHB004 (10% topical azithromycin) versus placebo in male or female subjects (aged ≥ 18 years and < 80 years) bitten by a tick. SHB004 or placebo gel will be administered locally BID for three consecutive days at the site of tick bite, starting at the latest on the 4th calendar day from the day the tick bite was first noticed. Subjects are expected to participate in the study for 57 days (+14 days allowed). The subjects will attend the study center twice: at Day 1 for screening/baseline (informed consent, tick collection, screening assessments and first study drug administration) and at Day 57 (+14 days allowed) for last assessments and controls. Between these 2 visits, subjects will be contacted twice (Days 7 and 30) by phone for follow-up information. See section 5 for more details about treatment administered; see Table 1 and section 7 for more details of assessments that will be performed during the study.

Figure 1 below summarizes the study design.

Figure 1: Study Design

During this study, a first interim analysis is planned to be conducted after a total of 25 ± 20% treatment failures have been observed (see section 8.7 for details).

If required, a second interim analysis is planned to be performed after a total of 37 ± 20% treatment failures have been observed. The interim analyses are required to assess the true rate of seroconversion (IgG and/or IgM) and appearance of erythema migrans per geographic region and to address the unpredictable annual and quarterly fluctuation in the number of ticks carrying Borrelia s.l.

Based on the interim analyses results, an Independent Data Monitoring Committee (IDMC) will decide on the continuation or stopping of the study for demonstrated efficacy or futility. If changes to the protocol are recommended by the IDMC, the
Sponsor will communicate changes to the protocol by means of a protocol amendment.

See section 9.9 for more details on the roles of the IDMC.

See section 4.3 for more details about subject withdrawal and replacement, and section 9.11 for premature termination of the study.

Any subject developing EM or other symptom of Lyme disease will receive appropriate treatment according to local guidelines and at the treating physician’s discretion.

### 3.2 Criteria for Evaluation of the Study

#### 3.2.1 Primary Efficacy Criterion

The primary evaluation criterion for this study is for efficacy and is the rate of treatment failure at Day 57 (with an allowed time-window of +14 days) as determined in the ITT set. Treatment failure is defined as seroconversion (IgM and/or IgG) and/or appearance of EM throughout the study in baseline-seronegative (IgM and IgG) subjects. Baseline-seronegative subjects experiencing an additional tick bite are not counted as treatment failure unless they experience an EM occurring before the additional tick bite.

*As a consequence, End of study seroconversion sample should not be obtained before Day 57.*

#### 3.2.2 Secondary Efficacy Criterion

Secondary objective 1 is as the primary objective for the All Treated Subjects A-C set, and modified ITT set.

Secondary objective 2 is as the primary objective in the ITT set and isolated IgM are not counted as Treatment Failure (TF).

Secondary objective 3 is as the primary objective in the ITT set and isolated IgG are not counted as TF.

#### 3.2.3 Exploratory Efficacy Criterion

Exploratory objective is as the primary objective but for the PP set.

#### 3.2.4 Secondary Safety Criterion

The secondary evaluation criteria for this study are for safety and tolerability and will be recorded throughout the study.

They will include:

- Adverse events (AEs) and serious AEs (SAEs) (skin and other).
3.3 Justification of the Study Design

This clinical study is to be conducted as a double-blind, randomized, placebo-controlled, multicenter study. This standard and generally accepted study design allows elucidating differences in efficacy, safety and tolerability of the different treatment groups of topical gels in the present study. Clinical assessments, or treatment compliance are known to be possibly influenced by the knowledge of the treatment received. The study will thus be double-blinded to provide an unbiased comparison of the two treatment groups. This study will also be randomized to avoid any selection bias. The inclusion of a placebo control arm in this study will help to put into context the AEs reported in the SHB004 10% treatment arm. The use of a placebo at the very early stage of tick bite was considered ethical in this pathology of Lyme disease where guidelines recommend treatment only at appearance of symptoms, i.e. noticeably later in the disease development.

The subject will recognize the tick within one’s skin and this self diagnosis is confirmed by the medical personnel in the clinical center, as the subjects have to bring the tick with them in order to satisfy the study entry criteria. The type of lesions to be included in this study is considered uncomplicated and susceptible for topical antibiotic treatment. Given the uncomplicated nature of the incidental tick bite and the preventative concept of the study treatment, self administration of the study drug gels at the tick bite site is a reasonable intervention, after the first administration by the investigator or designated personnel and proper instructions are given to the subject. Hence, the self-administration will be followed by means of a subject electronic diary (eDiary).

The primary efficacy endpoint of treatment failure at Day 57 was chosen as 56 days are medically recognized as sufficient time for a seroconversion in Lyme disease for both, IgM and IgG antibodies (see also the draft Guidance for Industry about Lyme Disease treatment development from the United States of America Food and Drug Administration (FDA) [33]). Subjects are excluded from the efficacy analysis if they are IgM and / or IgG seropositive at baseline as these subjects cannot seroconvert. EM occurring throughout the study in baseline-seronegative subjects are counted as treatment failure regardless of serostatus.

Baseline-seronegative subjects who recognize an additional tick bite during the study are excluded from the ITT population. However, if these subjects develop an EM before the additional tick bite, the EM is counted as treatment failure (TF). These subjects are excluded, for the fact that they cannot receive treatment for the additional tick bite, which has to be considered a study artificiality.

(i) This protocol does not allow the treatment of additional tick bites. However, in “normal” life, these tick bites would be treated. Therefore, we have a study artificiality and the ITT population must be adapted, such that the “normal” population is reflected. Consequently, we exclude subjects bitten by an additional tick.

(ii) As we cannot know if the subject received placebo or verum for the initial (index) bite, we are unable to allocate the respective treatment to the additional bite. If we had decided to treat the additional tick bite in a blinded fashion, there would have been a 1:1 chance that the subject received the same or the other treatment as compared to the index bite – again, if treatments wouldn’t have been identical, we could not have
assigned the subjects to a respective treatment and the subject would have been lost for the efficacy analysis. Therefore and within the logic of this trial, the subject with the additional tick bite must be excluded from the ITT.

(iii) Another reason is of immunological consideration. Typically, a seroconversion takes up to 6-8 weeks after exposure. If the additional tick bite occurs sometime within the study, there may not be sufficient time for seroconversion – therefore, the primary endpoint is directly affected by such subjects. Hence, such subjects must be excluded from the ITT set. Approximately 10% of subjects experienced an additional tick bite.

The subjects will be instructed to contact the investigator immediately whenever they feel it necessary to consult the investigator. Moreover, the investigator will be allowed to schedule additional visits as necessary, discontinue study drug if indicated and to treat the subject according to the best medical practice.

For more details on the choice of the antibiotic and the topical formulation of SHB004, see sections 1.1.2 and 1.1.3.

For more details see also section 1.2 for study rationale and section 1.3 for risk-benefit assessment.

With respect to the primary endpoint (see section 2.1), subjects with missing serologic test result at Day 57 and not having an EM (see section 6.2.3) will be excluded from the ITT population. When there are subjects with missing serologic test results at Day 57 and not having an EM there are three possible strategies:

1. to be regarded as no treatment failure – this would favor the test substance;
2. to be regarded as treatment failure – this would not favor the test substance;
3. to be excluded from the ITT population.

Given the small number of expected treatment failures (3.1% placebo vs. 1.24% verum, see section 8.8), treating such cases as treatment failures (strategy 2) will cause great loss of power and may introduce over-estimation of treatment failures. In addition, under study conditions there is a known tendency to have less treatment failure rates than in real life. Also, the pre-interim assessment of approximately 300 subjects revealed that only a small number of subjects (1%) would have to be excluded from the ITT population, as these subjects did not appear at Day 57. Therefore, in this particular situation, approach 3 is seen as most appropriate. Please note, that events collected before the subjects withdrew (e.g. an EM) are included into the ITT or modified ITT analysis. This approach is following the BfArM “Stellungnahme” of July 25, 2012.
4 STUDY POPULATION

The study population will consist of subjects bitten by a tick (single bite) and able to receive the first treatment administration at the latest on the 4th calendar day from the day the tick bite was first noticed. Subjects must be able to provide written consent and meet all the inclusion criteria and none of the exclusion criteria.

4.1 Inclusion Criteria

Subjects will be entered into this study only if they meet all of the following criteria:

1. Signed written informed consent by the subject before any study procedure is performed.
2. Males or females aged ≥ 18 years and < 80 years at screening.
3. Subjects bitten by one tick (single bite), who have extracted the tick from the skin and collected such tick or parts of the tick (e.g. hypostom), or have the tick or parts of the tick still attached to the skin, and are willing to hand it over.
4. Subjects able to receive the first study treatment administration at the latest on the 4th calendar day from the day the tick bite was first noticed (note: the day the tick bite was first noticed being counted as the first calendar day).
5. Are neither pregnant, nor breast-feeding and do not plan to become pregnant during the study. Females with childbearing potential must undergo a urine pregnancy test at screening. Please note that female subjects who have had a hysterectomy or are postmenopausal for longer than 2 years are not considered as being of childbearing potential. Pregnant or breast feeding females, or women planning to become pregnant during the study cannot participate.

4.2 Exclusion Criteria

Subjects will be entered into this study only if they meet none of the following criteria:

1. Subjects who have treated the site of the index tick bite with a topical formulation of an antibiotic other than SHB004 gel. Administration of a topical antibiotic other than SHB004 outside the area of tick bite is allowed (e.g. ophthalmic or otic applications, etc).
2. Subjects who received parenteral or oral antibiotic treatment within 10 days prior to enrollment.
3. Subjects who have a skin score according to Appendix 1 of this protocol grading 3 or worse at baseline.
4. Subjects with a history of allergic reaction or hypersensitivity to macrolide antibiotics (e.g. erythromycin, azithromycin) characterized e.g. by rash, itching, difficulty in breathing or anaphylaxis.
5. Subjects with a history of autoimmune diseases (e.g. rheumatoid arthritis or lupus erythematosus), history or clinical signs of syphilis, active herpes virus infection, subjects under immunosuppressive therapy (prescription drugs only), collagen...
vascular or immunodeficiency disease, or with a known active infectious mononucleosis (please note, that subjects reporting a past infection are not excluded, only those reporting a current infection).

6. Concurrent systemic steroid therapy. Inhaled steroids are allowed. Topical steroids are allowed when applied at least 10 cm apart from the index tick bite site.

7. Treatment with systemic steroids, other immunomodulatory drugs, or cytostatics within 30 days before enrollment.

8. History of Borrelia / Lyme disease during the previous 12 months or positive test for antibodies against Borrelia s.l. (seroconverted) as assessed within the last two years prior to enrollment (only the most recent antibody test has to be taken into account; if this latest test is negative the subject is not to be excluded for this reason).

9. Subjects presenting with multiple tick bites at screening/baseline visit.

10. Subjects unable to spot the site of the index tick bite at screening/baseline visit.

11. Subjects who have a history of one or more tick bites within 60 days prior to randomization (except for the current tick bite qualifying for this study).

12. Concurrent tick-borne diseases, such as babesiosis or ehrlichiosis.

13. Any other drug allergy or condition or significant medical problem which in the opinion of the investigator places the subject at unacceptable risk or does not allow the subject to follow study procedures as planned.

14. Have received treatment with any other investigational drug, and/or have participated in another clinical study within 30 days before screening.

15. Are pregnant or a nursing mother.

16. Have a history of, or known current problems with drug or alcohol abuse. (Subject with a history of abuse [drug and/or alcohol], but who have been observing a strict abstinence for at least 1 year will be allowed to participate).

17. Have a history or suspicion of unreliability, poor cooperation or non-compliance with medical treatment.

18. Inability to follow the procedures of the study, e.g. due to language problems, psychological disorders, dementia or confused state of the subject.

19. Have previously been enrolled in this study.
4.3 **Subject Withdrawal and Replacement**

Subjects are free to withdraw from the entire study including follow-up at any time without penalty and for any reason without prejudice to his or her future medical care. Subject takes medications / drugs that are not approved by the investigator or that are not allowed during the study (see section 5.7.2);

- New diseases occur that could influence the effectiveness of the study treatment;
- Intolerable AEs;
- At the discretion of the investigator;
- At the request of the subject;
- Subject lost to follow-up (in case the subject cannot be reached, all effort should be taken by the medical center to contact the subject);
- Protocol violation (after discussion with study Medical Monitor to determine if there are safety concerns related to protocol violation);
- Blind is broken.

A subject who is withdrawn from the study should, as far as possible, continue the follow-up as described in Table 1: telephone follow-up 1 (Day 7), telephone follow-up 2 (Day 30), and end of study/withdrawal visit (Day 57), as appropriate depending on when the subject is withdrawn.

If it is not possible to continue as per the study follow-up scheme (subject has withdrawn his/her consent for the whole study participation, is lost to follow-up, etc), the subject must perform, whenever possible, a complete and final examination (end of study/withdrawal visit as per Table 1).

In case the subject has an ongoing AE/SAE or an SAE is discovered up to 4 weeks after last study drug administration or at Visit 4 (whichever is longer), please see section 6.2.1 for more details on reporting/follow-up requirements.

In all cases, the reason(s) for withdrawal, and the primary reason, must be recorded on the electronic case report form (eCRF).

A subject may also be withdrawn from study drug and/or study by the Sponsor, Regulatory Authorities, or Independent Ethics Committees (IECs)/Institutional Review Boards (IRBs).

Subjects will also be withdrawn if the entire study is terminated prematurely as described in section 9.11. Subjects withdrawn entirely from the study will not be replaced.
Subjects will not receive further study drug (but are not withdrawn and consequently stay in the study) under the following circumstances:

- Pregnancy (see section 6.2.1.8);
- Case of skin irritations characterized by a score of 3 or worse than 3, subjects receive no further study treatment if applicable and treatment of the skin reaction should commence at the physician’s discretion (score see APPENDIX 1APPENDIX 4).
- Subjects who develop Lyme Disease as diagnosed by the Investigator (e.g. as diagnosed based on the appearance of an Erythema migrans - see section 6.2.3. for more signs and symptoms of Borreliosis/Lyme Disease). Patients who develop Lyme Disease must receive necessary antibiotic treatment according to the discretion of the Investigator.

4.4 Planned Sample Size and Number of Study Centers

According to a three stage group sequential design (two interim analyses and one final analysis), the study is powered at 80% for a relative risk of 0.4 and a total of 76 treatment failures corresponding to a total expected sample size for the intent-to-treat (ITT) population of about 2400 subjects.

A first interim analysis is planned to be conducted after 25 ± 20% treatment failures have been observed to assess the regional rate of seroconversion (IgG and/or IgM) and appearance of erythema migrans and (possibly) to reassess the numbers of subjects given above.

If required, a second interim analysis will be performed at the end of a season when about 37 ± 20% treatment failures have been observed.

The study is planned to be performed at 20 to 35 centers in Germany and Austria.

See section 8.8 for the determination of the sample size.

4.5 Subject Identification and Randomization

4.5.1 Subject Identification

Subjects will be allocated a unique 8-digit subject number (subject identification number) which will include the country number (2 digits), the site number (3 digits), as well as a consecutive individual number (3 digits). The subject number will be assigned on enrolment (i.e. provision of written informed consent) in chronological order of screening and will be used throughout the study. If a subject is not subsequently randomized, his or her screening number will not be reallocated. Each screened subject will therefore have a unique identifier.

4.5.2 Randomization Scheme

Subjects will be randomized on a 1:1 basis to SHB004 or Placebo.

Randomization will be stratified by center, and a sequence of randomization numbers will be assigned to each study center.

See section 5.4 for details on blinding and breaking the blind.
4.5.3 Allocation/Randomization of Subjects to Treatment

Randomization of subjects to treatment will occur at the screening/baseline visit (Visit 1) after all screening procedures have been performed and eligibility for the study confirmed. Each enrolled subject receives a unique subject identification number (see section 4.5.1). Randomized subjects who terminate their study participation for any reason, regardless of whether study drug was taken or not, will retain their randomization number.

For the randomization of subjects, the investigator will use a centralized Interactive Voice Response System (IVRS). Details can be found in the study file. IVRS will assign subjects to a treatment group based on the pre-defined randomization list prepared by the study biostatistician.
5 STUDY DRUG

5.1 Identity

5.1.1 SHB004

Name of product: SHB004
Active compound: Azithromycin
Inactive compound Carbopol 20/20, Klucel MFTM, Miglyol™ 812, Ethanol.
Chemical formula: C_{38}H_{72}N_{2}O_{12}
CAS: 83905-01-5
ATC code: J01FA10
Pharmacotherapeutic group: Antimicrobial agent, macrolide
Anticipated clinical use: Prevention of infection of Borrelia s.l. in early stages of infection after tick bite
Presentation: Dermal formulation / homogenous colorless gel, 2 mL tube.
Administration route: Dermal
Dosage: 10%
Dosing schedule: BID
Duration of treatment: 3 days
Batch No.: Will be provided in the clinical study report
Expire date: Will be specified prior to study start and documented in a note to file.
Storage conditions: In a refrigerator between 2 to 8°C at study site and subject’s home. Transport between study site and subject’s home can be at room temperature.
Manufacturer: The SBH004 finished product is manufactured according to current Good Manufacturing Practice (GMP) at the following facility: Grünenthal Pharma AG Postfach 67 CH-8756 Mitlödi, Switzerland
The SHB004 dermal formulation will appear as a homogenous colorless gel in a 10% azithromycin dose. The gel will be applied on the skin at the site of tick bite BID on three consecutive days (for details see section 5.2).

Stability at 2-8°C is indicated as on label.

For more details about the difference between the formulation used for previous studies (SHB001) and SHB004 that is administered in this study, see the latest Investigator’s Brochure.

5.1.2 Placebo

Name of product: Placebo
Active compound: No active compound
Inactive compound Carbopol 20/20, Klucel MFTM, Triethanolamine, Ethanol.
Presentation: Dermal formulation / homogenous colorless gel, 2 mL tube.
Administration route: Dermal
Dosage: 0%
Dosing schedule: BID
Duration of treatment: 3 days
Batch No.: Will be provided in the clinical study report
Expire date: Will be specified prior to study start and documented in a note to file.
Storage conditions: In a refrigerator between 2 to 8°C at study site and subject’s home. Transport between study site and subject’s home can be at room temperature.
Manufacturer: The placebo formulation is manufactured according to current GMP at the following facility:
Grünenthal Pharma AG
Postfach 67
CH-8756 Mitlödi, Switzerland

The placebo will have the same appearance as SHB004. It will be applied to the skin at the site of tick bite BID on three consecutive days (for details see section 5.2).

The constituents of the placebo formulation slightly differ from the constituents of the SHB004 azithromycin-containing formulation, but the only clinically relevant change is the absence of azithromycin.

For more details about the difference between the formulations that is administered in this study (SHB004) and the placebo formulation, see the latest Investigator’s Brochure.
5.2 Administration

The investigator, a physician, or medically-trained person will mark – at the screening/baseline visit – the site of the initial tick bite using a skin marker pen. The marking of the site will consist of a circle with a radius of approximately 1 cm, with the tick bite centered in this circle. Such mark will help the investigator/designee, and later on the subject, to apply the formulation to the correct spot.

SHB004 and matching placebo are provided as gels. SHB004 or placebo will be applied topically - as a drop, which is not distributed over the skin and left as is - to the identified tick bite area (it has been marked at the screening/baseline visit, as described above) twice a day (once in the morning and once in the evening, 12 hours apart; time window of ±2 hours; only for the second study drug administration, a time window of ± 6 hours is allowed [i.e. for the first application done by subject alone]), for 3 consecutive days. In total, 6 administrations are required. If only one administration is possible on Day 1 due to constraints of time windows, then the second administration will be done the morning of Day 2; finally the sixth and latest administration will be done in the morning of Day 4.

The first gel application will be performed at the study site by the investigator or designee the day of randomization (screening/baseline visit [Visit 1]). One drop of gel, covering an area of approximately 1 cm in diameter that will be centered in the targeted/marketed site, will be applied as a drop, which will not be distributed over the skin but left as is. The drop should not be touched, particularly not rubbed (it is essential, that the drop remains liquid as otherwise penetration of azithromycin ceases prematurely). The treated surface will be allowed to dry for 30 minutes. If the formulation is wiped away before the 30-minute drying time was reached, then this must be documented in the eCRF for the first application and in the subject’s eDiary for further applications. During the time of drying, the subject is advised to remain sitting or lying (at the study site for the first administration and at home for the following ones) with the treated area uncovered. Care should be taken to prevent the test product from dribbling outside the original application site while it is still runny. For that, subjects should be allowed to rest for 10 minutes in a position, which prevents the dripping off the administration site. Subjects with hairy area of tick bite will be shaved in the defined treatment sites (dry shaving, done by study staff at study center). After the 30 minutes of drying, the treated area may be covered with a dressing if desired. In case the subject prematurely wipes away the formulation (i.e. before 30 min has passed), the subject is allowed to apply another drop to the skin site, but this must be recorded in the subject’s eDiary / eCRF (as applicable).

Proper gel application will be taught to each subject and instructions on how to use the IVRS to enter information in the eDiary – where subjects will need to record every application – will be delivered at Visit 1. No dose is to be missed.

No further study drug should be applied in cases outlined in section 4.3.
5.3 Packaging, Labeling and Storage

IXODES AG will supply the SHB004 and placebo dermal formulations.

Study drugs will be packaged by Grünenthal Pharma AG according to all local legal requirements. The 2 mL tubes of study drugs and outer package will be labeled in accordance with applicable regulatory requirements and sent to the study site.

Upon receipt of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be documented in the study files. The investigator must notify the study Sponsor of any damaged or unusable study treatments that were supplied to the investigator’s site.

Regular study drug reconciliation will be performed to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team.

All drug supplies must be stored in accordance with the manufacturer’s instructions in a refrigerator at 2 to 8° C (at study site). In case the investigator / study staff suspects that site’s refrigerator had periods where this range was not adhered to, the Sponsor must be contacted to investigate the need for a re-supply of study drugs.

When study drug is delivered to subjects, they will be instructed to keep it in a secured place, out of reach of children, and in a refrigerator at 2 to 8° C. The transport between the study site and the subject’s home can be at room temperature. In case a subject is not storing the drug properly, a remark should be put into the subject’s notes so that this information is available in files. Storage other than within the subject’s refrigerator does not constitute a protocol violation.

At the study site the investigator is responsible for assuring that SHB004 and placebo are stored according to the recommended conditions, protected from exposure to any environmental changes and are locked, so that only the investigator and specifically designated other persons can have access.

5.4 Blinding and Breaking the Blind

The study will be performed in a double-blind manner. All study drugs will be supplied in identical tubes and will be similar in color, smell, and appearance, thereby enabling double-blind conditions.

The study blind should not be broken except in a medical emergency (where knowledge of the study drug received would affect the treatment of the emergency) or regulatory requirement (e.g., for SAEs or death).

The blind must only be broken following discussion on a case-by-case basis, at the discretion of the Medical Monitor.

If the blind is broken, the date, time, and reason must be recorded in the subject’s eCRF, and any associated AE report.

The investigator should notify the Medical Monitor prior to contacting IVRS. All calls resulting in an unblinding event will be recorded and reported by the IVRS to the Medical Monitor and the Sponsor.
If an investigator, site personnel performing assessments, or subject, is unblinded, the
subject must be listed as having had a major protocol deviation. Subjects in whom
study drug was unblinded will have to be discontinued from study treatment.
However, subjects should undergo the follow-up as planned in the study.

Serious unexpected suspected adverse reactions (SUSARs), which are subject to
expedited reporting, should be unblinded before submission to the Regulatory
Authorities.

The overall randomization code will be broken only for reporting purposes. This will
occur once all final clinical data have been entered onto the database and all data
queries have been resolved, and the assignment of subjects to the analysis sets has
been completed.

5.5 Drug Accountability

Dispensation of study drug and accountability

SHB004 dermal formulation and placebo have been manufactured by the Grünenthal
Pharma AG and supplied by IXODES AG.

The study drug will be directly sent to the study site. The study staff has to sign the
“study supply form” confirming the delivery of the study drug. Receipt and return of
all supplies will be documented on the “Drug Distribution and Receipt Form”.

SHB004 and placebo dermal formulation supplies must be kept in a secure limited
access storage area under the recommended storage conditions (at study site and
subject’s home; in a refrigerator between 2 to 8°C; transport between the study site
and the subject’s home can be at room temperature).

At study sites, the investigational product will only be handled by trained study staff.
It will be handed out to the participating subjects as appropriate during the
screening/baseline visit (Visit 1) when the subject has been randomized and
instructed on how to use and handle it. The subjects will be instructed to store the
study drug tubes at home in the refrigerator, separated from food, and out of reach of
children.

The subject will receive, when given the study drug, instructions on how to use the
IVRS to enter information in the eDiary, in which they must record every study drug
application, and they will be instructed to return any unused, partially or fully used
study drug at the end of the study (Visit 4).

The investigator must maintain accurate and adequate records including dates, lot
number, quantities received and individual usage of the SHB004 and placebo dermal
formulations.

The investigator is responsible for maintaining accurate study drug accountability
records throughout the study.

Each dispensing of study drug by the investigator will be documented in the eCRF.

Return or destruction of study drug

After completion of the study, the remaining study drugs at the study site will be
counted and documented.
There will be a final reconciliation of drug shipped, drug consumed, and drug remaining. The results of this inventory will be recorded on the “Study Return Form” and be signed and dated by the investigator.

Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug.

The investigator must return unused supplies to the Sponsor giving an exact amount of usage in a study whether completed or terminated. The investigator must verify that all unused or partially used investigational product supplies have been returned to the Sponsor and that no remaining supplies are in the investigator's possession.

Unused drugs should be returned to IXODES AG.

5.6 Compliance

Subjects will be instructed to record each application of study drug in the eDiar by calling the IVRS to give corresponding date and hour, and to bring their study drug supplies back to the study site at the end of study (end of study/withdrawal visit [Visit 4]). The number and condition of dispensed/returned tubes will be recorded in the eCRF. Subject compliance will be assessed using eDiar data as well as drug accountability information from the eCRF.

See sections 8.1.3 and 8.4 for the impact of compliance on the data analyses.

5.7 Previous and Concomitant Medications

5.7.1 Medications Recorded

Any medication the subject takes other than the study drug, including herbal and other non-traditional remedies, over-the-counter drugs (including vitamins) or significant non-drug therapies, is considered a concomitant medication. All concomitant medications must be recorded in the eCRF. The following information must be recorded in the eCRF for each concomitant medication: generic name, route of administration, start date, stop date, dosage, and indication. Any changes in the dosage or regimen of a concomitant medication must be recorded in the eCRF.

At screening (for up to first dosing), subjects will be asked what prescribed medications they have taken during the last month, and what herbal or other non-traditional remedies, over-the-counter drugs (including vitamins) or significant non-drug therapies they have taken during the last 14 days. At each subsequent study visit (i.e. from first administration of study drug until study completion [Visit 4]), subjects will be asked what concomitant medications they are currently taking and information on previous medication must not be requested again.

5.7.2 Prohibited Medications

The following medications are prohibited during the study:

- Immunomodulatory drugs (cyclosporine, tacrolimus, sirolimus, immunomodulatory antibodies (e.g. antibodies against cytokines), or immunostimulating therapy using cytokines (interleukins, interferon, tumor necrosis factor, colony stimulating factor);
- Cytostatics;
- Systemic steroids. Inhaled steroids are allowed. Topical steroids are allowed when applied at least 10 cm apart from the index tick bite site other than when treatment is indicated as a result of skin reactions at the index site in which case topical treatment is allowed at the discretion of the treating physician (this does not constitute a protocol violation but must be recorded in the eCRF).
- Any kind of systemic antibiotics or antibiotics applied topically to the site of the tick bite. Local treatments in areas other than site of tick bite (e.g. ophthalmic or otic applications) are not restricted. For use of antibiotics in case an EM occurs, please see section 5.7.3 (this does not constitute a protocol violation but must be recorded in the eCRF);

Systemic steroids, other immunomodulatory drugs, and cytostatics are also prohibited within 30 days prior to enrollment.

Parenteral or oral antibiotic treatments are also prohibited within 10 days prior to enrollment and for the study duration (except if required for Borreliosis treatment).

See also section 4.2 for details of exclusion criteria related to prohibited medications.

5.7.3 Allowed Concomitant Medications

All medications –other than those described in section 5.7.2– necessary for the well-being of the subject are permitted, but have to be accurately recorded in the eCRF by the investigator.

Immunostimulatory drugs are allowed if Echinacea extracts, inosin derivates or ergamisol but should be mentioned in the CRF

See section 5.7.2 for details on exceptions to forbidden medications.

Subjects will be instructed to contact the clinical center in case of skin reactions at the site of administration or in case of signs and symptoms of Borreliosis.

Upon observation of an EM (see section 6.2.3), subjects are included as treatment failures in the efficacy analysis and will receive antibiotics according to local treatment guidelines at the discretion of the treating physician. A blood sample may be collected at the discretion of the treating physician but must not be sent to the central laboratory. Instead, the treating physician should follow his/her own judgment regarding the treatment to give according to his/her diagnosis. See section 8.1.2 for more details on the consequences in terms of protocol deviation.
6 VARIABLES AND METHODS OF ASSESSMENT

6.1 Efficacy Variables

6.1.1 Definition of treatment failure
A treatment failure is defined as seroconversion (IgM and / or IgG) and / or appearance of EM throughout the study in baseline-seronegative (IgM and IgG) subjects. Baseline-seronegative subjects experiencing an additional tick bite are not counted as treatment failure in the ITT population unless they experience an EM occurring before the additional tick bite.

6.1.2 Seroconversion of Subjects
An ELISA search test is performed followed by a confirmative line blot test. The ELISA tests are performed for IgM and IgG. Decision taking is as follows:
1. ELISA: If positive or borderline, perform Line Blot.
2. ELISA: If negative, do not perform Line Blot and categorize as negative.
3. Line Blot: If positive or borderline, categorize as positive.
4. Line Blot: If negative, categorize as negative

Test details are provided in APPENDIX 3. The presence of IgG or IgM against *Borrelia* s.l will be assessed by the central laboratory (see the List of Study Personnel) as advised by guidelines [33,34]). These tests will be performed on the
blood collected at the screening/baseline visit and at the Day 57 visit. At each
timepoint, 5 mL of blood will be drawn for the given sample as well as an additional
5 mL of blood as a back-up for this sample. This will lead to a total of 20 mL of
blood for the whole study duration for this test.

In case a clinical presentation of Borreliosis is suspected, an additional blood sample
for diagnosis confirmation might be required (see sections 5.7.3 and 6.2.3), for a total
of about 10 extra mL (depending on local practices). Collection of this sample is at
the discretion of the physician and in case he/she intends to corroborate his/her
diagnosis by a serum test. Note, this sample is not to be shipped to the central
laboratory; instead the physician is advised to proceed according to what he/she
deems necessary for a confirmation of his/her diagnosis.

See the Investigator Laboratory Manual for more details about sample collection,
processing, storage and shipment to central laboratory.

The test kits are detailed in Appendix 3. Alternatively, other blots which are CE
marked can be used.

Seroconversion will be assessed based on the comparison of the serostatus at Visit 1
(screening/baseline visit) and at Visit 4 (Day 57 +14 day time window allowed, but
not before Day 57) (see Table 1 for more details).

Subjects being seropositive for IgM and/or IgG at baseline are regarded as baseline-
seropositive. Seroconversion is determined on IgG status (following BfArM
Stellungnahme as of 25.3.2010) as primary efficacy criterion.

See sections 6.5 and 6.6 for more details on how the blood samples will be used
during and after the study.

6.1.3 Infection Status of the Tick to Borrelia s.l

The site of tick bite is recorded in the eCRF. Ticks or parts of the tick (e.g. hypostom)
collected at screening/baseline (Visit 1) will be sent to the central laboratory for
determination whether the tick is carrying Borrelia s.l. (a differentiation of Borrelia
burgdorferi sensu stricto, Borrelia garinii or Borrelia afzelii is possible), in triplicate.
The quantification will be done against a standard curve titrated with defined amounts
of the respective strain DNA.

See the Investigator Laboratory Manual for more details about the initial tick
collection, processing, storage and shipment to central laboratory.

Ticks collected in case of additional tick bites occurring after enrollment in the study
are not to be sent to the central laboratory. The medical personnel is to confirm the
subject’s assessment, that the specimen he/she collected is indeed a tick
(morphological assessment). See section 6.1.5 for more details.

6.1.4 Appearance of Erythema Migrans

The appearance of EM throughout the study is counted as treatment failure (TF) in
baseline-seronegative subjects. See section 6.2.3 for more details on definition of EM.
The patient must be asked whether he has experienced another tick bite and this must
be documented in the relevant pages of the eCRF (see section 6.1.5 for procedure).
6.1.5 Appearance of Additional Tick Bites during the Study

In case of additional tick bite(s) after enrollment in the study, the subject will be asked to collect it/them (in a plastic bag or another appropriate tightly closing container) and bring it/them back to the clinical site at the next study visit. If the tick has not been attached to subject’s skin, he/she does not need to collect it (because that tick has not yet bitten the subject). At home, the subject can store the new tick(s) at room temperature up to the next study visit. In case a new tick bite is noticed during the course of the study, the subject has to be instructed that he/she must not apply any study drug gel on it (even if there was some gel left in the tube after the normal 3 days of application at the start of the study). At visit 4, the investigator or other medically trained personnel will confirm the subject’s suspicion, that the newly collected specimen(s) is (are) indeed a tick(s) (morphological assessment), and thus that the subject has been exposed to a new tick bite. The investigator or designee will then take care of the destruction of the tick (the new tick(s) will not be analyzed by the central laboratory and thus will not be kept).

Finally the investigator will report the relevant information concerning the confirmed new tick bite(s) in the eCRF and the site of the new tick bite.

6.2 Safety Variables

6.2.1 Adverse Events

See section 9.4 for more details on the coding of AEs.

6.2.1.1 Collection of Adverse Events

It is the responsibility of the investigator to collect all AEs (both serious and non-serious) derived by spontaneous, unsolicited reports of subjects, by observation and by routine open questionings e.g., "How have you felt since I last saw you?"

6.2.1.2 Definitions

An AE is any untoward medical occurrence that occurs in a clinical investigation subject administered a pharmaceutical product, and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the product.

All AEs, including intercurrent illnesses, occurring during the study will be documented in the eCRF. Concomitant illnesses, which existed before entry into the study, will not be considered AEs unless they worsen during the treatment period. All AEs, regardless of the source of identification (e.g., physical examination, laboratory assessment, reported by subject), must be documented.

An abnormal test finding will be classified as an AE if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms;
- The test finding necessitates additional diagnostic evaluations or medical / surgical intervention; including significant additional
concomitant drug treatment or other therapy;
Note: Simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an AE.

- The test finding leads to a change in study dosing or discontinuation of study treatment or subject participation in the clinical study.

All AEs will be reported in the adverse event eCRF from time of signature of informed contents until Study Completion (Visit 4).

A treatment-emergent AE (TEAE) will be defined as an AE that begins or that worsens in severity after at least one dose of study drug has been administered and up to Study Completion (Visit 4).

Pre-existing conditions will be recorded in the eCRF on the Medical History / Current Medical Conditions or appropriate page.

Surgeries or other invasive procedures that had already been planned prior to the start of the study do not have to be documented as AEs. These planned procedures will be recorded in the eCRF by the investigator at the baseline visit. It is not important if the condition was known before enrollment, only if the procedure was planned before.

6.2.1.3 Assessment of Adverse Events

Each AE will be assessed by the investigator with regard to the following categories.

6.2.1.3.1 Seriousness

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

- Results in death;
- Is life-threatening;
  This means that the subject is at risk of death at the time of the event; it does not mean that the event hypothetically might have caused death if it were more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability or incapacity;
- Is a congenital anomaly or birth defect;
- Is an important medical event(s) that may not be immediately life-threatening or result in death or hospitalization but that may jeopardize the subject or require intervention to prevent one of the above outcomes. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Medical and scientific judgment should be exercised in deciding whether a case is serious and whether expedited reporting is appropriate.

If there is any doubt about whether or not an AE has to be considered serious, the Medical Monitor should be contacted.
6.2.1.3.2 Intensity

Investigators should assess the severity of AEs according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) (available at http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf) [35] and record it in the eCRF. This full reference document provides detailed event-specific intensity criteria for most of the common AEs.

See also section 6.2.2 for more detail on the assessment and grading of skin tolerance.

In general, CTCAE v4.03 (dated 14 June 2010) Severity Grades are:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated;

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL) (Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.);

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL (Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.);

Grade 4: Life-threatening consequences; urgent intervention indicated;

Grade 5: Death related to AE.

6.2.1.3.3 Causality

The investigator will – for all AEs – assess the causality / relationship between the study drug and the AE and record that assessment in the eCRF.

The causal relationship of the AE to study drug will be described in terms of:

- **Probable**: the AE:
  - Follows a reasonable temporal sequence from administration of the study drug.
  - Could not be reasonably explained by the subject’s clinical state, environmental or toxic factors or other therapies administrated to the subject.
  - Disappears or decreases on cessation or reduction in dose of the study drug (dechallenge).
  - Follows a known pattern of response to the study drug.
  - Reappears or worsens upon rechallenge.
**Possible:** the AE:
- Follows a reasonable temporal sequence from administration of the study drug.
- Could be reasonably explained by the subject’s clinical state, environmental or toxic factors or other therapies administrated to the subject.
- Follows a known pattern of response to the study drug.

**Unlikely:** the AE
- Does not follow a reasonable temporal sequence from administration of the study drug.
- Could be reasonably explained by the subject’s clinical state, environmental or toxic factors or other therapies administrated to the subject.
- Does not follow a known pattern of response to the study drug.
- Does not reappear or worsen upon rechallenge.

**Not related:**
- The AE does not meet the above criteria.
- There is sufficient information that the etiology of the AE is not related to the study drug.

### 6.2.1.4 Recording Adverse Events

AEs will be reported from time of informed consent signature until Visit 4. SAEs will be reported from time of informed consent signature until Visit 4, or occurring up to 4 weeks after the last study drug administration (whichever is longer).

In case the subject cannot be reached, every effort should be made by the medical center to contact the subject.

All AEs, regardless of the relationship to study drug, will be recorded in the eCRF. All AE reports should contain a brief description of the event, date of onset, date of resolution, intensity, treatment required, relationship to study drug, action taken with the study drug, outcome, and whether the event is classified as serious.

### 6.2.1.5 Reporting Serious Adverse Events

All SAEs that occur during the period of observation from time of informed consent signature until Visit 4 or occurring up to 4 weeks after the last study drug administration (whichever is longer), whether considered to be associated with the study drug or not, must be reported within 24 hours by telephone or fax to the Sponsor or delegate using the numbers in the List of Study Personnel (and in the box below).

The minimum information required for an initial report is:
- Name of person sending the report (i.e., name, address of investigator);
- Subject identification (screening/randomization number, initials, NOT subject name);
- Protocol number;
• SAE term;
• Causality assessment, if possible.

However, as far as possible all points on the SAE form should be covered in the initial report, and the completed SAE form itself must be faxed to the Sponsor or delegate. The original SAE form must then be sent by mail to the Sponsor or delegate. In addition, the event must be documented in the eCRF.

After receipt of the initial report, the Sponsor or delegate will review the information and, if necessary, contact the investigator, to obtain further information for assessment of the event. The Sponsor will be responsible for all information processing and reporting according to local legal requirements. Where necessary, investigators will inform Regulatory Authorities in their own countries within the appropriate timeframes (e.g. to IEC / IRB and any other relevant authorities).

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PAREXEL Medical Department - Paris
SAE Fax: + 33 1 44 90 32 75 (24-hour service)
Medical hotline: + 33 1 44 90 32 90
Medical Monitor:
Dr Claire Nguyen
Tel: + 33 1 44 90 35 71
In case Dr Nguyen can not be reached please contact:
Dr Eric Zafarana
Tel: + 33 1 44 90 32 90

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6.2.1.6 Follow-up of Adverse Events

All AEs experienced by a subject, irrespective of the suspected causality, will be monitored until the AE has resolved, any abnormal laboratory values linked to the AE have returned to baseline or stabilized at a level acceptable to the investigator and Medical Monitor, until there is a satisfactory explanation for the changes observed, until the subject is lost to follow-up, or until the subject has died. If required, the subject will be asked to return to the study site for follow-up investigations, even after Visit 4.

For any AEs the outcome "unknown" is not acceptable, except if attempts to collect the information have been made and documented. In case of subjects lost to follow-up, efforts should be made and documented to contact the subject to encourage him / her to continue study participation as scheduled. In case of minor AEs a telephone call to the subject may be acceptable.
Follow-up information on the outcome will be recorded on the respective AE page in the eCRF. All other information has to be documented in the source documents. Source data has to be available upon request.

6.2.1.7 Suspected Unexpected Serious Adverse Reactions

Any adverse event that is serious, associated with the use of the study drug, and unexpected (suspected unexpected serious adverse reaction [SUSAR]) has additional reporting requirements, as described below.

- If the SUSAR is fatal or life-threatening, Regulatory Authorities and IECs will be notified within 7 calendar days after the Sponsor learns of the event. Additional follow-up (cause of death, autopsy report, and hospital report) information should be reported within an additional 8 days (15 days total).

- If the SUSAR is not fatal or life-threatening, Regulatory Authorities and IECs will be notified within 15 calendar days after the Sponsor learns of the event.

The Sponsor will notify the investigators in a timely fashion of relevant information about SUSARs that could adversely affect the safety of subjects. Follow-up information may be submitted if necessary.

The Sponsor will also provide annual safety updates to the Regulatory Authorities and IECs responsible for the study. These updates will include a concise critical summary of the safety profile of the drug studied, information on SUSARs and other relevant safety findings.

6.2.1.8 Pregnancy

The Sponsor has a responsibility to monitor the outcome of pregnancies where there has been maternal exposure to the study drug.

Pregnancy alone is not regarded as an AE unless there is a suspicion that the study drug may have interfered with the effectiveness of a contraceptive medication. However, AEs related to a pregnancy have to be reported like any other AEs. Pregnancy should be confirmed by a reliable laboratory test.

Elective abortions without complications should not be handled as AEs, unless they were therapeutic abortions (see below). Hospitalization for normal delivery of a healthy newborn should not be considered a SAE.

All pregnancies occurring during the study (i.e. from the signature of informed consent up to Visit 4 –and up to 30 days after discontinuation of study drug in case of a premature withdrawal– have to be reported to the Sponsor within one working day of the investigational site’s knowledge of the pregnancy.

Pregnant subjects must be immediately withdrawn from study drug but attempts should be made to complete study follow-up as detailed in Table 1. The investigator must follow up and document the course and the outcome of all pregnancies even if the subject was discontinued from the study or if the study has finished.
All outcomes of pregnancy must be reported by the investigator to Sponsor on the pregnancy outcome report form within 30 days after he or she has gained knowledge of the normal delivery or elective abortion.

Any SAE that occurs during pregnancy (including SAEs occurring after last administration of study drug) must be recorded on the SAE report form (e.g., maternal serious complications, spontaneous or therapeutic abortion, ectopic pregnancy, stillbirth, neonatal death, congenital anomaly, or birth defect) and reported within 24 hours in accordance with the procedure for reporting SAEs.

### 6.2.2 Skin Tolerance

Acute skin tolerance will be assessed and documented before the first application of study drug (screening/baseline; Visit 1). In addition, it will be recognized during telephone follow-ups and in case of recognition confirmed by the physician (Visits 2 and 3). In this case, the subject will return to the study center and the reaction will be graded as in Appendix 1. At the end of study/withdrawal visit (Visit 4) (see Table 1) an additional assessment will be done. Symptoms should be graded according to APPENDIX 1 and described (such as redness, swelling, itching, or tingling) and be documented in the eCRF as adverse event.

An attempt should be made so that the acute tolerance assessment will be done every time by the same investigator in order to avoid subjective interpretations of the skin status by different persons. This acute tolerance assessment is based on the EMEA Note for Guidance on dermatologic tolerance testing (CPMP-SWP 2145/00 [36]). This is a preclinical guideline which can nevertheless serve as the basis for determinations in human. Scoring and grading of skin reactions will be done according to an established procedure and will be documented based on the slightly modified proposal of the Standardization Group of the European Society of Contact Dermatitis, as presented in APPENDIX 1.

During baseline assessment (evaluation before first administration of study drug), subjects with up to grade 2 (inclusive) are included (this is a reaction due to the tick bite and not to the study drug as at this time the study drug has not been applied yet). During telephone follow-ups, the investigator will question the subject about such signs and in case of doubt, the subject will be asked to come to the study center as soon as possible for a full assessment and corrective actions concerning subject’s participation to be taken if appropriate.

In case a subject experiences skin irritations characterized by a score of 3 or higher than 3, no further study treatment can be given (if applicable; see section 4.3) and treatment of the irritation may commence at the discretion of the treating physician – such subjects are not withdrawn from the study.

### 6.2.3 Signs and Symptoms of Borreliosis

The appearance of signs and symptoms of Borreliosis will be checked in accordance with the Schedule of Procedures (Table 1). EM should be documented if possible photographically and pictures should show a ruler for size comparison.

Depending on the stage of disease, the symptoms of Borreliosis might change from one subject to another. These can include, but are not limited to: EM, Lyme arthritis, cardiac symptoms, central nervous system involvement, cranial nerve deficits,
chronic inflammation of the joints, fibromyalgia, or polyneuropathy (see section 1.1.1, but also local and applicable Lyme disease guidelines).

- An erythema migrans corresponds to the primary phase of the disease. It consists of an erythematous annular macular, with several centimeters in diameter, displays a centrifugal growth and can appear several days to several weeks after the tick bite. The presence of an erythema migrans confirms the diagnosis and, therefore, serology is typically not indicated.

- Neurological features consisting of meningo-radiculitis (presenting as cranial nerve palsy(s) and / or nerve root pain, isolated meningitis, meningomyelitis or meningo-encephalitis. Typically, a lumbar puncture is performed to detect a lymphocytic meningitis.

- Rheumatological features consisting of isolated monoarthritis or oligoarthritis, almost always involving the knee.

**Definition of erythema migrans (EM):**

EM is defined with subjects having an expanding erythematous skin lesion with several centimeters in diameter. Annular erythematous lesions occurring within hours after a tick bite represent hypersensitivity reactions and do not qualify as EM [33]. EM is considered “related” to the tick bite if occurring within 30 days (±5 days) after the baseline visit and if occurring approximately within 30 cm distance of the index tick bite.

The presence of signs and symptoms of Borreliosis other than EM will only be checked during this study to detect any case of early/developing Borreliosis. In such cases where a disease is suspected, see section 5.7.3 for details on appropriate actions to be taken (blood sample can be collected for diagnosis at the discretion of the investigator but is to not be sent to the central laboratory, and possible treatment).

If during the telephone follow-up visits (Visits 2 and 3) the question by phone about signs and symptoms of Borreliosis raises the possibility of appearance of EM, the subject must be instructed to come to the site as soon as possible for a mandatory additional clinical visit, so that the investigator can control the appearance of EM (see paragraphs above for EM criteria), and curative treatment started if appropriate (see section 5.7.3).

**6.2.4 Urine Pregnancy Test**

For female subjects of child-bearing potential, a urine (dipstick) pregnancy test will be performed at Visit 1 (screening/baseline visit).

Please note that, as per inclusion criteria, female subjects who have had a hysterectomy or are postmenopausal for longer than 2 years are not considered as being of childbearing potential. Sites in Austria must perform an addition test at EOS by law (not relevant for German sites).

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1“Related EM” is a term relevant for the PP set only (for sets, see section 8.1.3)
6.2.5 Physical Examinations

Physical examinations will be performed at baseline, in accordance with the Schedule of Procedures (Table 1).

The examination should be based on the following body systems: general appearance, head (ear, nose, and throat), eyes, neck and thyroid, cardiovascular, respiratory, abdomen, urogenital, musculoskeletal, neurological, lymph nodes, skin. Preferentially, the assessment should be done by the same physician throughout the study for each patient but a different physician may carry out the physical examination in case of logistical challenges.

All abnormal findings must be recorded in the eCRF.

6.3 Demographics and Baseline Characteristics

Demographics and Baseline Characteristics consist of those variables that are assessed only at screening/baseline.

6.3.1 Subject Demography

Subject demography consists of:

- Birth date;
- Race and ethnicity;
- Height;
- Sex.

6.3.2 Medical History and Current Medical Conditions

For the documentation of the medical history, any previous and concomitant diseases/conditions within the last 2 years before screening will be documented. The current medical conditions will also be rechecked at Visit 4.

The medical history and current medical conditions will be obtained by interviewing the subject or by inspecting his/her medical records.

For coding of medical history and current medical conditions, see section 9.4.

6.3.3 Previous and Concomitant Medications

Previous and concomitant medication will be documented as described in section 5.7.

6.4 Other Variables

Not applicable.

6.5 Blood Sampling

Blood sampling will follow the details given in section 6.1.2 and in the Investigator Laboratory Manual (collection, processing, storage, shipment, etc) for samples required to assess seroconversion. In case the Investigator decides to collect another sample than those collected at Visit 1 and Visit 4, that sample will be analyzed by
local laboratories, and the procedures to collect, store, and analyze them will follow local practices.

During the time of their participation in this study, and if no additional/optional samples are required, subjects will be drawn a total of approximately 20 mL of blood for assessment of seroconversion.

See section 6.1.2 for more details about the efficacy-related blood samples.

6.6 Use of Blood Samples

6.6.1 During the Study

During the study, the blood samples will be used to perform tests for Borreliosis infection diagnosis and seroconversion determinations.

6.6.2 After the Study

After the study completion, Ixodes AG will be allowed to use the remaining blood samples to perform additional antibody measurements using the appropriate method(s).

These additional tests will possibly be performed within the 5 years after study completion. After 5 years, the remaining samples will be destroyed.

Of note, no genetic testing will be performed on any study sample, either during or after the study.

If a subject refuses to have his/her blood samples used after end of study, he/she will nonetheless be allowed to enter the study.
7 STUDY CONDUCT

7.1 Schedule of Procedures
See Table 4 in the synopsis for the schedule of procedures.

7.2 Procedures by Visit
All times should be recorded using the 24-hour clock (e.g., 23:20, not 11:20 pm).
See the corresponding subsections of section 6 for more details on the assessments listed in subsections below.

7.2.1 Screening/Baseline (Visit 1; Day 1)
Interested persons will be invited to the study site for discussing the study in detail in a medical education interview and to receive the information sheet, which contains all study details. Any question regarding the subject information or the study will be answered. Those inclusion / exclusion criteria, which can be assessed by questionnaire, will be checked.

Potential study subjects will have enough time to reconsider their participation in the study. If they wish, an appointment for another screening/baseline visit will be arranged. However, it should be kept in mind that, as per inclusion criteria, the subject has to start treatment within four calendar days after the tick bite was first noticed.

During the screening/baseline visit, the following assessments will be performed:

- Obtain written informed consent;
- Record medical history and current medical conditions;
- Verify conformance with entry criteria;
- Record demographic details;
- Perform a physical examination;
- Collect the tick or parts of the tick (e.g. if hypostom only is within skin, collect that hypostom), which will be sent to the central laboratory to determine the infection status of the tick;
- Check for the appearance of signs and symptoms of Borreliosis (EM and others, see section 6.2.3);
- Check if any additional tick bite is present (see section 6.1.5);

Note: In case of additional tick bite after enrollment in the study, the subject will be asked to collect it (in a plastic bag or another appropriate tightly closing container) and bring it back to the clinical site at the next study visit. The investigator or other medically trained personnel will confirm the subject’s suspicion, that the collected specimen is indeed a tick, take care of the destruction of the new tick and report the relevant information in the eCRF. (The subject has to be instructed that he/she must not apply any study drug gel on this new tick bite).
• Mark the site of tick bite using a skin marker pen (see section 5.2 for details on the marking procedure);
• Record previous and concomitant medications (see section 6.3.3);
• Collect an urine sample and have a urine pregnancy test performed for women of childbearing potential (see section 6.2.4);
• Randomization;
• Draw the blood for serostatus testing (see section 6.1.2);
• Record baseline local tolerability before the first application of study drug (see section 6.2.2);
• First study drug administration will be performed by the investigator or a designate (see section 5.2);
• Give the subject instructions on study drug administration (details on proper gel application, time to let it dry, frequency, etc.) (see section 5.2);
• Give the subject the separate documents and instructions on how to use IVRS to enter information in the eDiary (subjects will also be told to bring back all used/unused study drug when coming back to the site for Visit 4);
• Record AEs/SAEs (see section 6.2.1);
• Ship the blood sample for baseline seroconversion testing and the tick to central laboratory. See the Investigator Laboratory Manual for more details about sample/initial-tick collection, processing, storage and shipment to central laboratory.

During the next days, the subject should self-administer the remaining doses of study drug BID as detailed in section 5.2 and record these administrations in the eDiary using the IVRS.

No further study drug should be applied in cases outlined in section 4.3.

7.2.2 Self-administration at home by subjects (after Visit 1 and before Visit 2; Day 1 to Day 3 [or Day 4 in some cases])

After Visit 1 and before the first Telephone Follow-up 1, the subject will self-administer the study drug BID:
• once in the morning and once in the evening,
• 12 hours apart;
• time window of ±2 hours; only for the second study drug administration, a time window of ± 6 hours is allowed [i.e. for the first application done by subject alone])
• for three consecutive days.
• In total, 6 administrations are required.
• If only one administration is possible on Day 1 due to constraints of time windows, then the second administration will be done the
morning of Day 2. As a consequence, for these subjects the sixth and latest administration will be done in the morning of Day 4.

- The subject will record the corresponding days and times of application in the eDiary using the IVRS.

### 7.2.3 Telephone Follow-up 1 (Visit 2; Day 7 ±3 days)

At Day 7, the investigator will contact the subject by phone to check the following with him/her and record the corresponding answers in the eCRF:

- Ask the subject for any concomitant medications intake with emphasis on any prohibited medications (e.g. antibiotics);
- Ask the subject for any recent history of additional tick bite (since study enrollment) (see section 6.1.5);
  
  Note: In case of additional tick bite after enrollment in the study, the subject will be asked to collect it (in a plastic bag or another appropriate tightly closing container) and bring it back to the investigator at the next study visit. The investigator or other medically trained personnel will confirm the subject’s suspicion, that the collected specimen is indeed a tick, take care of the destruction of the new tick and report the relevant information in the eCRF. (The subject has to be instructed that he/she must not apply any study drug gel on this new tick bite.)
- Ask the subject for any AEs/SAEs since last visit (see section 6.2.1);
- Ask the subject for any signs of local application site skin intolerance (see section 6.2.2);
- Ask the subject for the appearance of any signs and symptoms of Borreliosis (EM and others, see section 6.2.3).

In case the subject cannot be reached, every effort should be made by the study center to contact the subject within the allowed time window.

In case of local skin intolerance at the index site (or in case of any doubt), of signs and symptoms of Borreliosis, or if the investigator deems it necessary, the subject will be asked to come to the study center as soon as possible for appropriate actions to be taken (see sections 6.2.2, 5.7.3, and 7.2.6 and section 4.3).

In case EM is suspected, the subject will be required to come to the study center as soon as possible for a mandatory additional visit, so that the investigator can control/validate the appearance of EM and for appropriate actions to be taken (see sections 5.7.3, and 7.2.6 for action and visits, and section 6.2.3 for EM definition criteria). EM should be documented photographically if possible and pictures should show a ruler for size comparison.

### 7.2.4 Telephone Follow-up 2 (Visit 3; Day 30 ±5 days)

At Day 30, the investigator will contact the subject by phone to check the following with him/her and record the corresponding answers in the eCRF:

- Ask the subject for any concomitant medications intake with emphasis on any prohibited medications (e.g. antibiotics);
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- Ask the subject for any recent history of additional tick bite (since the last telephone follow-up) (see section 6.1.5);

  *Note: In case of additional tick bite after enrollment in the study, the subject will be asked to collect it (in a plastic bag or another appropriate tightly closing container) and bring it back to the investigator at the next study visit. The investigator or other medically trained personnel will confirm the subject’s suspicion, that the collected specimen is indeed a tick, take care of the destruction of the new tick and report the relevant information in the eCRF. (The subject has to be instructed that he/she must not apply any study drug gel on this new tick bite.)*

- Ask the subject for any AEs/SAEs since last visit (see section 6.2.1);

- Ask the subject for any signs of local application site skin intolerance (see section 6.2.2);

- Ask the subject for the appearance of any signs and symptoms of Borreliosis (EM and others, see section 6.2.3).

In case the subject cannot be reached, every effort should be made by the study center to contact the subject within the allowed time window.

In case of local skin intolerance (or in case of any doubt), of signs and symptoms of Borreliosis, or if the investigator deems it necessary, the subject will be asked to come to the study center as soon as possible for appropriate actions to be taken (see sections 6.2.2, 5.7.3, and 7.2.6).

In case EM is suspected, the subject will be required to come to the study center as soon as possible for a mandatory additional visit, so that the investigator can control/validate the appearance of EM and for appropriate actions to be taken (see sections 5.7.3, and 7.2.6 for action and visits, and section 6.2.3 for EM definition criteria). EM should be documented photographically if possible and pictures should show a ruler for size comparison.

### 7.2.5 End of Study/Withdrawal Visit (Visit 4; Day 57 +14 days, or at withdrawal)

During the end of study/withdrawal visit, the following assessments will be performed:

- Record medical history and current medical conditions;

- Draw the blood for seroconversion testing (see section 6.1.2);

- Collect the used/unused study drug that subject was instructed to bring back for this visit;

- Review of the eDiary (including the assessment of regular topical administration);

- Check for the appearance of signs and symptoms of Borreliosis (EM and others, see section 6.2.3);

- Record AEs/SAEs (see section 6.2.1);

- Record the local tolerability (see section 6.2.2);
- Ask the subject for any recent history of additional tick bite (since study enrollment) (see section 6.1.5);

  Note: In case of additional tick bite after enrollment in the study, the subject will be asked to collect it (in a plastic bag or another appropriate tightly closing container) and bring it back to the investigator at the next study visit (as far as possible). The investigator or other medically trained personnel will confirm the subject’s suspicion, that the collected specimen is indeed a tick, take care of the destruction of the new tick and report the relevant information in the eCRF. (The subject has to be instructed that he/she must not apply any study drug gel on this new tick bite.)

- Record previous and concomitant medications (see section 6.3.3);
- Ship the blood sample for seroconversion testing to central laboratory.

See the Investigator Laboratory Manual for more details about sample collection, processing, storage and shipment to central laboratory.

Subjects who discontinue or are discontinued early from the study must, as far as possible, attend a withdrawal visit with the observations and procedures performed as scheduled for this Visit 4.

See section 9.11 in case of premature termination of the study, as some subject must also perform an anticipated Visit 4.

This visit should take place as soon as possible after the subject stops taking study drug if the subject is withdrawn from the whole study. If the subject stops taking study drug but agrees to continue the study follow-up, he/she should continue the follow-up as described in Table 1: telephone follow-up 1 (Day 7), telephone follow-up 2 (Day 30), and end of study/withdrawal visit (Day 57), as applicable.

### 7.2.6 Optional Visits

Optional, unscheduled visits may be carried out at any time during the study in the case of a medical emergency, when a subject would like to report a medical problem in context with the study, or when the investigator considers this necessary for the subject’s well being.

If the subject shows a reaction in the treated skin area, he or she should return to the study center as soon as possible to be examined by the investigator. In case of any skin irritations following dermal administration of study drugs with a score ≥3 (see APPENDIX 1 APPENDIX 1), the treatment of the subjects with the study drug should be discontinued (see section 6.2.2). Additional treatment with e.g. topical corticosteroids of the irritated skin may be done as deemed necessary by the investigator.

If the subject presents signs and symptoms of Borreliosis he/she will come to the study center as soon as possible to be examined by the investigator and will receive antibiotics according to local treatment guidelines (see section 5.7.3) and at the discretion of the treating physician.

If during the telephone follow-up visits (Visits 2 and 3) the question by phone about signs and symptoms of Borreliosis raises the possibility of appearance of EM, the subject must be instructed to come to the site as soon as possible for a mandatory
additional clinical visit, so that the investigator can control/validate the appearance of EM (see section 6.2.3 for EM criteria). EM must be documented photographically and pictures should show a ruler for size comparison.
8 STATISTICAL METHODS

The statistical considerations summarized in this section outline the plan for data analysis of this study.

Before interim analysis/unblinding/database lock, a separate statistical analysis plan (SAP) will be finalized, providing detailed methods for the analyses outlined below. Any deviations from the planned analyses will be described and justified in the final integrated study report.

8.1 Study Subjects

8.1.1 Disposition of Subjects

The number and percentage of subjects entering and completing each phase of the study will be presented, summarized by treatment. Reasons for withdrawal pre- and post-randomization will also be summarized.

The disposition of subjects will also include information on the number and percentage of subjects who:

- completed study drug and follow-up (i.e. up to Visit 4);
- withdrew from study drug but completed follow-up (i.e. up to Visit 4);
- withdrew from study drug and from follow-up (i.e. up to Visit 4).

Listings of subjects who withdrew and corresponding reasons will be provided.

8.1.2 Protocol Deviations

Deviations from the protocol including violations of inclusion/exclusion criteria will be assessed as “minor” or “major” in cooperation with the Sponsor. Major deviations from the protocol will lead to the exclusion of a subject from the per protocol (PP) analysis (see section 8.1.3 for details on analysis sets). Deviations will be defined prior to study database unblinding.

Subjects who develop Borreliosis / Lyme Disease as diagnosed by the Investigator (e.g. erythema migrans (EM) – see section 6.2.3), will receive antibiotics according to local treatment guidelines and at the discretion of the treating physician. Baseline-seronegative cases in which an EM was recorded will be regarded as treatment failures (TF) independent of blood testing at Day 57 in the ITT set; in baseline-seronegative subjects experiencing an additional tick bite, EM are counted as TF if occurring before the additional tick bite but not if occurring thereafter in the ITT set. Subjects who develop a skin reaction of grade 3 or higher (see Appendix I for score) will not receive further study drug and this irritation will be treated at the discretion of the treating physician. The use of topical corticosteroids for such treatment does not constitute a protocol violation. Even though the treatment is incomplete, these subjects with skin reaction of grade 3 or higher are not regarded as TF but are excluded from the PP set.

Baseline-seronegative subjects who report another tick bite at any time during the study must be excluded from the intention to treat (ITT), modified ITT (MITT) and per protocol (PP) analysis (see section 8.1.3 for details on analysis sets). If these
baseline-seronegative subjects develop an EM before the additional tick bite is recognized, this EM is counted as TF.

Subjects who fail to complete at least 67% (can fail to take 2 out of 6 times at any time) of the study drug course or who fail to administer the formulation for 2 consecutive time points are excluded from the PP analysis. Subjects who receive less than 4 or more than 7 administrations will be excluded from the PP population.

### 8.1.3 Analysis Sets

<table>
<thead>
<tr>
<th>Set Description</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All Subjects Randomized Set (ASR):</strong></td>
<td>All subjects meeting the inclusion / exclusion criteria and who will be randomly assigned to one of the two study treatments.</td>
</tr>
<tr>
<td><strong>Safety Analysis Set (SAF):</strong></td>
<td>All randomized subjects who received at least one dose of study treatment. Subjects will be included in the analysis according to the treatment received.</td>
</tr>
<tr>
<td><strong>All Treated Subjects Set A:</strong></td>
<td>Same as SAF, but analyzing subjects according to their randomized treatment. Used for sensitivity efficacy analysis. Count drop-outs as TF. Count subjects with additional tick bites as TF providing they develop an EM or a seroconversion.</td>
</tr>
<tr>
<td><strong>All Treated Subjects Set B:</strong></td>
<td>Same as SAF Set A, but drop-outs are not counted as TF.</td>
</tr>
<tr>
<td><strong>All Treated Subjects Set C:</strong></td>
<td>Same as SAF Set B, but subjects with an additional tick bite are not counted as TF. EM occurring before the additional tick bite has been noticed are counted as treatment failure in baseline-seronegative subjects.</td>
</tr>
<tr>
<td><strong>Intention to treat Set (ITT):</strong></td>
<td>Same as SAF set but including only baseline-seronegative subjects who have completed the study (completed defined as having a test result from Day 57 sample available) and who have no additional tick bite after the index tick bite during the study. With respect to the primary endpoint (see section 2.1), subjects with missing serologic test result at Day 57 and not having an EM (see section 6.2.3) will be excluded from the ITT population. Baseline-seronegative subjects (IgM and / or IgG) having an additional tick bite and not having an EM before the additional tick bite has been noticed will be also excluded from the ITT. Subjects will be included in the analysis according to the randomized treatment.</td>
</tr>
<tr>
<td><strong>Modified ITT Set (MITT):</strong></td>
<td>Same as ITT but TF defined based on isolated IgM seroconversion or isolated IgG seroconversion are not counted as TF.</td>
</tr>
<tr>
<td><strong>Per Protocol Set (PP):</strong></td>
<td>Same as MITT but including only subjects bitten by a tick carrying <em>Borrelia s.l.</em> and including only subjects who are compliant with the study protocol (see below).</td>
</tr>
</tbody>
</table>
Subjects will be included in the analysis according to the randomized treatment.

Subjects presenting any of the following will be excluded from the PP set:

- seropositive at baseline
- failure to meet the inclusion and exclusion criteria
- skin reaction grading 3 or worse (according to APPENDIX I)
- experience an EM not related to the index site (being defined as an observation of the center of an EM with a distance of at least approximately 30 cm from the index bite).
- have an EM beyond day 30 (±5 days) after enrollment.
- have an EM which is not at least several cm long in one direction.
- major protocol violations as determined by the investigator / medical review (performed at a blind data review meeting prior to database lock).
- non-compliance for study drug (to be reviewed at the blind data review meeting prior to database lock).

The minimum number of doses required to be considered for the PP population will be 4 (this means subjects can miss two doses unless those doses are missed consecutively; if missed consecutively then subject is excluded from the PP population). The maximum number of doses received will be 7 in order to remain in the PP population.

The primary efficacy analysis will be based on the ITT set. Further efficacy analyses (sensitivity analyses) will also be done using all treated subject sets A–C, the modified ITT and PP sets. All safety analyses will be based upon the SAF set. Demographic and baseline characteristics will be evaluated for the ITT, all treated subject sets A–C, mITT and the PP sets. If one or more subjects received incorrect study drug, these data will also be presented for the safety set. A summary of the analysis sets is provided by the consort flow diagram (see Appendix 2).
8.2 General Considerations

The present Phase III study is planned as a three stage group sequential design (Pocock boundaries) with two interim analyses and one final analysis; see section 8.7 for details about the interim analyses.

All statistical tests will be one-sided and will be performed at the levels of significance specified for each stage of the design; see sections 8.7, 9.9 for details.

Continuous data will be summarized by treatment group using descriptive statistics (number, mean, median, standard deviation [SD], minimum, and maximum).

Categorical data will be summarized by treatment group using frequency tables (frequencies and percents).

Analysis and data conventions:

Definition of baseline

The baseline assessment will be the latest, valid pre-dose assessment available.

Visit windows

Assessments taken outside of protocol allowable windows will be displayed according to the eCRF assessment recorded by the investigator.

Unscheduled assessments

Extra assessments (laboratory data or vital signs associated with non-protocol clinical visits or obtained in the course of investigating or managing adverse events) will be included in listings, but not summaries. It is noted that invalid laboratory data may not be used (from hemolyzed samples, mishandled samples, quantity not sufficient, or other conditions that would render values invalid).

Missing data conventions

Unless stated otherwise, data will not be imputed for safety or efficacy analyses.

With respect to the primary endpoint (see section 2.1), subjects with missing serologic test result at Day 57 and not having an EM (see section 6.2.3) will be excluded from the ITT population.

Baseline seronegative subjects who recognize an additional tick bite during the study are excluded from the ITT population. If these subjects develop an EM before the additional tick bite, the EM is counted as treatment failure (TF). These subjects are excluded, for the fact that they cannot receive treatment for the additional tick bite, which has to be considered a study artificiality.

(i) This protocol does not allow the treatment of additional tick bites. However, in “normal” life, these tick bites would be treated. Therefore, we have a study artificiality and the ITT population must be adapted, such that the “normal” population is reflected. Consequently, we exclude subjects bitten by an additional tick.

(ii) As we cannot know if the subject received placebo or verum for the initial (index) bite, we are unable to allocate the respective treatment to the additional bite. If we had decided to treat the additional tick bite in a blinded fashion, there would have been a 1:1 chance that the subject received the same or the other treatment as compared to the index bite – again, if treatments wouldn’t have been identical, we could not have
assigned the subjects to a respective treatment and the subject would have been lost for the efficacy analysis. Therefore and within the logic of this trial, the subject with the additional tick bite must be excluded from the ITT.

(iii) Another reason is of immunological consideration. Typically, a seroconversion takes up to 6-8 weeks after exposure. If the additional tick bite occurs sometime within the study, there may not be sufficient time for seroconversion – therefore, the primary endpoint is directly affected by such subjects. Hence, such subjects must be excluded from the ITT set. Approximately 10% of subjects experienced an additional tick bite.

All efforts will be put into collecting data as complete as possible, e.g. including repeated attempts to contact the patients by telephone or through mail. One should try as much as possible to schedule a last visit at Day 57 for a serologic test and EM check-up, even for subjects who might decide to leave the study earlier for various reasons. So far, the unblinded, pre-interim assessment of 300 subjects showed that only about 1% of the subjects would have to be excluded from the ITT set due to missing serologic test results from Day 57 and another 10% due to additional tick bite.

8.3 Demographics, Medical History, Baseline Characteristics, and Concomitant Medications

Demographic data, medical history, concomitant disease, and concomitant medication will be summarized by means of descriptive statistics (n, mean, SD, median, minimum and maximum) or frequency tables, overall and stratified by treatment.

The medications will be coded using the Anatomical Therapeutic Chemical [Classification System] (ATC) drug classification system (see section 9.4). The determination of whether a medication is prior study or concomitant will be following the timeframe given in section 5.7.1. Prior medications continuing during the study will be labeled accordingly in the listings.

8.4 Treatment Compliance

Treatment compliance will be assessed by means of the data obtained in the eCRF and in the subject’s eDiaries.

Subject’s eDiary data will be listed by treatment group for all subjects.

Treatment compliance will be summarized by means of descriptive statistics (n, mean, SD, median, minimum, and maximum) and/or frequency tables, stratified by treatment group.

The number of administrations, their time of administration (morning, evening), the number of missed administration, the number/percentage of subjects with all required administrations, the number/percentage of subjects with less than 4 administrations, the number/percentage of subjects with more than 7 administrations will be reported by treatment group and overall.
8.5 Efficacy Analyses

8.5.1 Primary Efficacy Analysis

8.5.1.1 Hypothesis to be tested

This study has been designed as a three stage group sequential design (with two interim analyses and one final analysis) to show superior efficacy of SHB004 compared to placebo with respect to treatment failure at Day 57 (see section 3.2.1 for details).

The following hypotheses have been formulated based on the above given assumptions:

- **Null hypothesis** $H_0$: The proportion of treatment failures in the SHB004 group $\pi_1$ is larger than or equal to the corresponding proportion $\pi_2$ in the placebo group, i.e. the relative risk of SHB004 versus placebo is $\geq 1$:
  
  $$H_0: \frac{\pi_1}{\pi_2} \geq 1$$

- **Alternative hypothesis** $H_A$: The proportion of treatment failures in the SHB004 group $\pi_1$ is smaller than the corresponding proportion $\pi_2$ in the placebo group, i.e. the relative risk of SHB004 versus placebo is smaller than 1:

  $$H_A: \frac{\pi_1}{\pi_2} < 1$$

The overall significance level is $\alpha = 0.025$, 1-sided. The study is powered at 80% to detect a relative risk of 0.4, corresponding to a 60% improvement of SHB004 group versus placebo group (e.g. treatment failure proportions of 3.1% for placebo and 1.24% for SHB004). See section 8.8 for details of the sample size calculation.

8.5.1.2 Statistical Methods

The primary efficacy analysis will be performed for the ITT set.

At each stage, treatment difference between SHB004 and placebo in preventing an infection with *Borrelia s.l.* as measured by the treatment failures at Day 57 will be determined by an analysis of the proportions and corresponding repeated confidence intervals for the ratio of the treatment failure proportions.

A Wald type test statistic will be used (difference in observed proportions, divided by the estimated standard error, using the pooled variance estimator based on the observed overall treatment failure rate), so that the square of the test statistic will be identical with the usual Chi-Square test for 2 x 2 tables.

Repeated confidence intervals for the relative risk $\frac{\pi_1}{\pi_2}$ will be calculated based on the Farrington-Manning test.

Primary efficacy will be confirmed if the group-sequential testing procedure results in rejection of the null hypothesis at any of the three stages.

For the statistical details of the group sequential design see sections 8.7, 8.8.

Efficacy variables will also be listed and summarized by treatment group using descriptive statistics.
8.5.2 Secondary and Exploratory Efficacy Analyses

Secondary objective 1 is as the primary objective for the All Treated Subjects A-C set, and modified ITT set (see section 8.1.3 for definition of analysis sets) on the primary efficacy endpoint (rate of treatment failures at Day 57) and will include the same analyses as for the primary efficacy analysis (see section 8.5.1).

Secondary objective 2 is as the primary objective in the ITT set and isolated IgM are not counted as Treatment Failure (TF) (see section 8.1.3 for definition of analysis sets) on the primary efficacy endpoint (rate of treatment failures at Day 57) and will include the same analyses as for the primary efficacy analysis (see section 8.5.1).

Secondary objective 3 is as the primary objective in the ITT set and isolated IgG are not counted as TF (see section 8.1.3 for definition of analysis sets) on the primary efficacy endpoint (rate of treatment failures at Day 57) and will include the same analyses as for the primary efficacy analysis (see section 8.5.1).

The Exploratory objective is as the primary objective but for the PP set and will include the same analyses as for the primary efficacy analysis (see section 8.5.1).

Subjects experiencing an additional tick bite are not counted as treatment failure in the ITT set unless they experience an EM occurring before the additional tick bite and will include the same analyses as for the primary efficacy analysis (see section 8.5.1).

The term “treatment failure” is reserved for the primary endpoint in this protocol (IgM and / or IgG seroconversion and / or EM) and must be used in analogy in section 8.5.1. for the modified approach as foreseen in the secondary efficacy analysis.

Efficacy variables will also be listed and summarized by treatment group using descriptive statistics.

8.6 Safety Analyses

8.6.1 Adverse Events

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary and will be classified by system organ classes and preferred terms (see section 9.4). In the event that intensity or causality of an AE to the study drug is missing, a worst-case scenario will prevail (severe in intensity or probably related). Only treatment-emergent AEs (TEAE, AEs that occur after the first study drug administration, that were not pre-existing or that increased in intensity) will be included in the summary tables. Counting will be performed by subjects and events, separately. Subjects experiencing the same event more than once will have that event counted once within the corresponding system organ class and with a unique preferred term. All AEs will be included in the data listings. Listings of SAEs, of AEs leading to study drug withdrawal and listing of AEs leading to death will be provided.

The following will be summarized by treatment group for all TEAEs in the safety population:

- Number and percentage of subjects with at least one TEAE;
- Number and percentage of subjects with TEAEs by intensity (CTCAE grade);
- Number and percentage of subjects with TEAE by seriousness (serious/non-serious);
- Number and percentage of subjects with TEAE leading to death;
- Number and percentage of subjects with TEAE leading to withdrawal (of study drug);
- Number and percentage of subjects with TEAEs by causality (not assessable/unclassifiable, not related, unlikely, possible, and probable);
- Total number of TEAEs;
- Total number of TEAEs by intensity;
- Total number of TEAE by seriousness;
- Total number of TEAEs by causality;
- Total number of deaths.

TEAEs with a positive causal relationship are defined as those that were recorded on the eCRF as having a “probable” or “possible” causal relationship.

The following TEAE will be classified by treatment group according to system organ class and preferred term for the safety population:

- All TEAEs;
- TEAE by intensity;
- TEAE by seriousness;
- TEAEs by causality;
- TEAEs leading to withdrawal (of study drug);
- TEAEs leading to death.

8.6.2 Skin Tolerance

Skin tolerance will be graded using the procedure described in APPENDIX 1.

In the event that the score of an event of skin intolerance is missing, it will be considered as “unknown” for the analyses. Counting will be performed by subjects and skin intolerance events, separately. Subjects experiencing the same skin intolerance event more than once will have that event counted once. All skin intolerance events will be included in the data listings.

The following will be summarized by treatment group for the safety population:

- Number and percentage of subjects with at least one skin intolerance event;
- Number and percentage of subjects with skin intolerance events by intensity (score);
- Total number of skin intolerance events;
- Total number of skin intolerance events by intensity.
8.6.3 Signs and Symptoms of Borreliosis

Subjects with any sign or symptom of Borreliosis and the details of symptoms (see section 6.2.3) will be listed including flags for subjects with an EM.

The number of subjects with any sign or symptom of Borreliosis and the details of symptoms (if enough cases in each sign/symptom are reported) will be summarized by treatment group at each visit for the safety population.

The number of subjects with EM will be presented in a frequency table. EM should be documented if possible photographically and pictures should show a ruler for size comparison.

Listings will also be provided summarizing the subjects with an EM by treatment group and overall.

8.6.4 Additional tick bite

Additional tick bite data will be listed.

8.6.5 Urine Pregnancy Test

Urine pregnancy test data will be listed.

8.6.6 Physical Examinations

Baseline physical examination details will be summarized by treatment group for the safety population. By-subject results will also be listed.

8.7 Interim Analyses

As mentioned in section 8.2, the study is planned as a three stage group sequential design with two interim analyses and one final analysis ([37]).

Both early stopping for efficacy and early stopping for futility will be possible. The efficacy stopping boundaries will be determined according to the Lan / DeMets and Pocock procedure, to allow a large probability of stopping for efficacy already after stage 1.

As described in section 8.8, the power of the testing procedure depends mainly on the expected overall number of treatment failures.

A first interim analysis after an expected overall number of about 22 treatment failures, a second interim analysis after an expected overall number of about 37 treatment failures and a final analysis after an expected overall number of about 52 treatment failures will ensure an overall power of about 80% to detect a reduction of 60% in the treatment failure rates (relative risk = 0.4, e.g. treatment failure proportions of 3.1% for placebo and 1.24% for SHB004).

It will not be possible to stop recruitment for the interim/final analysis exactly after a given number of expected or observed treatment failures. The true overall proportion of treatment failures is not known and due to the duration of the follow-up (about 2 months) and time needed for laboratory analyses and data cleaning thereafter, it will not be possible to have interim analyses exactly after 22 or 37 observed treatment failures.
Due to logistic reasons it would also be preferable to conduct an interim analysis at the end of a season.

Therefore the actually observed numbers of treatment failures at the interim/final analyses will deviate from the numbers of 22, 37 and 52. An information based alpha-spending approach will be used to control the overall one-sided type-1 error of 2.5% in this situation. The cumulative number of observed treatment failures will be used as a measure of the information already available. The percentage of treatment failures already observed (76 = 100%) will be used as information fraction to recalculate the efficacy stopping boundaries.

Since a maximum number of 76 treatment failures was intended in the previous protocol version 3, and since stage 1 is already almost finalized (≈20 to 25 treatment failures), the alpha spending function defined in protocol version 3 will not be changed, to ensure that the overall 2.5% alpha level is still attained. Therefore the information fraction will still be computed as a fraction of 76, although the intended number of TF at the end of stage 3 is only 52. The following test statistic will be used to monitor the results at the 3 stages:

\[
Z_k = \frac{\hat{\pi}_{ik} - \hat{\pi}_{2k}}{\sqrt{\hat{\pi}_k (1 - \hat{\pi}_k) \left( \frac{1}{n_{ik}} + \frac{1}{n_{2k}} \right)}}
\]

where \( k = 1, 2, 3 \) is the analysis stage, \( \hat{\pi}_{ik} = F_{ik} / n_{ik} \) and \( \hat{\pi}_{2k} = F_{2k} / n_{2k} \) are the observed proportions of treatment failures in the SHB004, respectively the placebo group, \( F_{ik} \) and \( F_{2k} \) are the observed numbers of treatment failures, \( n_{ik} \) and \( n_{2k} \) are the observed subject numbers in each treatment group at stage \( k \) and

\[
\hat{\pi}_k = F_k / n_k = (F_{ik} + F_{2k}) / (n_{ik} + n_{2k}).
\]

is the overall treatment failure rate at stage \( k \), using for all numbers the cumulative data up to and including stage \( k \).

Since the rates are small and the subject numbers in the two groups are approximately equal, the above test statistics are approximately equal to the test statistics based on the unpooled variance estimate, which have the canonical joint distribution (Jennison, Turnbull) under both the null and the alternative hypothesis with information levels proportional to the numbers of subjects and using the rate difference as parameter for the treatment effect (EAST 5.4 User's Manual, Section A.1.2 - Distribution Theory, Binomial Data). Using \( \pi = (\pi_1 + \pi_2) / 2 \) for the overall rate, the expected value and covariances of the canonical joint distribution of the test statistics can be rewritten as

\[
E(Z_k) \approx \frac{\pi_1 - \pi_2}{\sqrt{4\pi(1-\pi)} / n_k} = 0 \sqrt{I_k}, \quad \text{Cov}(Z_j, Z_k) = \frac{I_j}{I_k} \text{ for } j < k
\]

with parameter \( \theta = \frac{\rho - 1}{\rho + 1} \) depending only on the relative risk \( \rho = \pi_1 / \pi_2 \), and with information levels \( I_k = \frac{E(F_k)}{1 - \pi} \) proportional to the expected number of treatment failures.
failures. Therefore a group sequential design can be based on the expected number of
treatment failures, using the relative risk as parameter for the treatment effect.

One-sided p-values $p_k$ for the test statistics will be calculated based on the standard
normal distribution:

$$ p_k = \phi(Z_k), $$

(e.g. $\phi(-1.96)=0.025$).

The type-1 error to be spent at each stage will be computed with an alpha-spending
function, which approximately produces the Pocock stopping boundaries ($-2.289$ for
the test statistic, $0.011$ for the 1-sided p-value), if the interim analyses are done after
one third and two thirds of the total number of treatment failures. This alpha-spending
function is defined as follows:

$$ g(0) = 0, $$
$$ g(1/3) = 0.011, $$
$$ g(2/3) = 0.019, $$
$$ g(1) = 0.025, $$

using linear interpolation between the above values. The value $g(t)$ is the cumulative
type-1 error probability, which will be spent when $100 \times t \%$ of the total number of
the originally intended 76 treatment failures have been observed.

Corresponding stopping boundaries $b_1$, $b_2$ and $b_3$ for the one-sided p-values, which
maintain the overall 1-sided 2.5% level will be calculated with a group-sequential
software like EAST (EAST®, Cytel Statistical Software & Services, Cambridge,
MA, 2007).

E.g. for interim and final analyses after 22, 37 and 52 treatment failures, the
recomputed stopping boundaries would be as follows:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Cumulative type-1 error</th>
<th>Efficacy Stopping Boundaries for test statistics $Z_k$</th>
<th>for p-values $p_k$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$g(22/76) = 0.0096$</td>
<td>$-2.343$</td>
<td>$b_1 = 0.0096$</td>
</tr>
<tr>
<td>2</td>
<td>$g(37/76) = 0.0147$</td>
<td>$-2.407$</td>
<td>$b_2 = 0.0080$</td>
</tr>
<tr>
<td>3</td>
<td>0.0250</td>
<td>$-2.140$</td>
<td>$b_3 = 0.0162$</td>
</tr>
</tbody>
</table>

At stage 3 the full remaining alpha will be spent, even if less than 76 treatment failures are
observed. The computation of the efficacy stopping boundaries will not consider the futility
boundaries, i.e. the calculated efficacy boundaries will be the same as in a design without
futility stopping.

A first interim analysis is planned to be conducted after a total of $25 \pm 20\%$ treatment
failures have been observed. Also, due to the seasonal aspect of the study, it is
expected to perform the first interim analysis by the end of the second season of the
study.

Depending on the effectively found rates and results at the first interim analysis, the
Independent Data Monitoring Committee (IDMC) will advise for continuation or
stopping of the study, see section 9.9 for details on the responsibilities of IDMC.
A brief overview of the statistical analysis at the interim analysis is given below:

- Primary and secondary efficacy analyses will be performed on data as detailed in section 8.5. If differences between the two treatment groups suggest that criteria for efficacy or futility may be fulfilled, the Independent Data Monitoring Committee (IDMC) may ask for the study to stop.
- Otherwise the study continues at the next stage as planned by the group sequential design.

**Stopping criteria for futility/efficacy**

According to the three stage group sequential design, the stopping criteria for futility/efficacy are:

**Stage 1** (interim analysis)

- End of the study for demonstrated futility (accept $H_0$), if the proportion of treatment failures in the SHB004-group is larger than or equal to the corresponding proportion in the placebo group, i.e. if the 1-sided p-value $p_1$ is $\geq 0.1587$ (or equivalently $Z_1 \geq -1$).
- End of the study for demonstrated efficacy (reject $H_0$), if the 1-sided p-value does not exceed the corresponding boundary, i.e. $p_1 \leq b_1$.

Otherwise continue with Stage 2.

**Stage 2** (interim analysis)

- End of the study for demonstrated futility (accept $H_0$), if the proportion of treatment failures in the SHB004-group is larger than or equal to the corresponding proportion in the placebo group, i.e. if the 1-sided p-value $p_2$ is $\geq 0.1587$ (or equivalently $Z_2 \geq -1$).
- End of the study for demonstrated efficacy (reject $H_0$), if the 1-sided p-value does not exceed the corresponding boundary, i.e. $p_2 \leq b_2$.

Otherwise continue with Stage 3 (final analysis).

**Stage 3** (final analysis)

- End of the study for demonstrated futility (accept $H_0$), if the 1-sided p-value is larger than the corresponding boundary, i.e. $p_3 > b_3$.
- End of the study for demonstrated efficacy (reject $H_0$), if the 1-sided p-value does not exceed the corresponding boundary, i.e. $p_3 \leq b_3$.

These stopping criteria along with other statistical considerations (e.g. conditional power, sensitivity analyses in other populations, confidence intervals) are going to be used by the IDMC as a guideline for taking decisions.

The futility boundaries are binding in the sense that the study will be stopped, if the futility stopping criterion is met, i.e. if $p_k \geq 0.1587$ at an interim analysis.

The efficacy boundaries are binding in the sense that the study can only be stopped, if the stopping criterion is met at one of the stages, i.e. $p_k \leq b_k$ for at least one stage $k$. Otherwise the overall type-1 error of 2.5% will not be maintained.

See section 9.9 for more details on the role and responsibilities of the IDMC.
Repeated confidence intervals

Repeated confidence intervals for the relative risk will be computed based on the Farrington-Manning test ([38]).

This is a test of the null hypothesis $\pi_1/\pi_2 = \rho_0$ versus the 1-sided alternative $\pi_1/\pi_2 < \rho_0$. For $\rho_0 = 1$ the test statistic is equivalent to the primary test statistic and thus to the usual Chi-Square test for 2 x 2 tables. The 1-sided 97.5% repeated confidence interval for the relative risk will be determined as the set of all values $\rho_0$, for which the null hypothesis cannot be rejected at level $b_k$:

$$RCI_k = \{ \rho_0 | 1\text{-sided } p\text{-value of Farrington-Manning test} > b_k \},$$

where $b_k$ is the same rejection boundary as used for the primary test procedure.

These intervals will be consistent with the test decision of the group sequential testing procedure. The repeated confidence interval at stage $k$ will include the relative risk of 1 (no treatment effect), if and only if the group sequential test cannot reject the null hypothesis at stage $k$. Conversely, when the group sequential test rejects $H_0$ at stage $k$, then the corresponding repeated confidence interval will exclude the relative risk of 1.

Further details of the statistical analysis will be specified in the statistical analysis plan (SAP).

An independent team will be appointed to prepare the results of the unblinded interim analyses that will be presented to the IDMC.

8.8 Determination of Sample Size

In protocol version 3 it was calculated that for a relative risk of 0.484 an expected number of 76 treatment failures at the last stage is needed for a power of 80% for the 3-stage Pocock design with futility stop in case of $Z_k \geq 0$ (result not favoring SHB004) at both interim analyses. The details are repeated in the first part of this section.

The current protocol version uses stricter futility boundaries, stopping already in case of $Z_k \geq -1$ (or equivalently a 1-sided p-value $\geq 0.1587$), assumes a larger treatment effect (relative risk of 0.4) and plans an expected number of 52 treatment failures at stage 3, calculating information fractions still based on 76 events (i.e. no change in the alpha-spending function). The power resulting from these new assumptions is assessed via simulation and described in the second part of this section.

Sample size calculation from protocol version 3(futility stop, if $Z_k \geq 0$):

The number of subjects to be recruited was determined based on the rates assumed for the primary objective of the study (see section 2.1).

Due to delayed study start in 2011 (first recruitment season), only approximately 300 evaluable subjects were accrued in the first recruitment season. The blinded, pre-interim assessment of baseline data of the 300 subjects revealed changes of the assumed rates in the Version 1.2 of the study protocol (incorporating changes from amendment 1). Based on this, the estimated sample size was adjusted accordingly as outlined below:

- 16.7% of subjects in the ITT population are bitten by a positive tick (tick is carrying *Borrelia s.l.*);
3.8% of the ITT population, placebo group will be treatment failures at Day 57 (numbers derived from community screenings – equals overall percentage of seroconversion within any subject bitten by any tick (regardless if the tick is carrying Borrelia or not)). This data could not be obtained from the pre-interim analysis as the study is still blinded; In our study we are now assuming 3.1%. Likely, subjects participating in the trial are better informed and taken care of as compared to community screenings and, therefore, we slightly adapt our numbers, accordingly.

20.0% of the seropositive subjects at Day 57 already presented seropositive at baseline;

SHB004 will be reducing the rate of treatment failures by 50% or more as compared to placebo;

The rates of subjects with treatment failures are assumed to be 3.1% for placebo and 1.5% for verum (i.e. relative risk of 0.484).

With these rates, the sample sizes were newly adjusted using a similar design as in the Version 1.2 of the study protocol (incorporating changes from amendment 1), more precisely, a three stage group sequential design with two interim analyses and one final analysis (Wald type test statistic, overall 1-sided $\alpha = 0.025$, power = 80%, Pocock efficacy boundaries as described in section 8.7, non-binding futility stop when results do not favor SHB004, i.e. in case of $Z_k \geq 0$ or a 1-sided p-value $\geq 0.5$.

For the sample size calculation it is assumed that the interim analyses will be done after one third and two thirds of the total sample size.

The maximum sample size needed in the group-sequential design is the number $n_3$ of patients at the third and final analysis. This maximum sample size is larger than the corresponding fixed sample size in a design without interim analyses. It is

$$n_3 = n_{fix} \times \text{Inflation factor}$$

where the inflation factor depends on the chosen group sequential design (number of interim analyses, stopping boundaries), and the chosen values for $\alpha$ and the power.

For the considered 3-stage Pocock design with efficacy boundaries as described in section 8.7 and non-binding futility stop in case of $Z_k \geq 0$ (1-sided p-value $\geq 0.5$), EAST computes an inflation factor of 1.199.

The fixed sample size of the Chi-Square test for 80% power at the 1-sided 2.5% level for treatment failure proportions $\pi_1$ and $\pi_2$ and equal group sizes is

$$n_{fix} = \frac{\phi^{-1}(0.975)\sqrt{4\pi(1-\pi) + \phi^{-1}(0.8)\sqrt{2\pi(1-\pi_1) + 2\pi_2(1-\pi_2)}}^2}{(\pi_1 - \pi_2)^2}$$

where $\pi$ denotes the average of the two proportions $\pi_1$ and $\pi_2$. Since these proportions are small, the two terms within the square roots are approximately identical and the formula can be simplified.

The maximum group sequential sample size is then

$$n_3 = 4\pi(1-\pi)\left[\frac{\phi^{-1}(0.975) + \phi^{-1}(0.8)}{(\pi_1 - \pi_2)^2}\right]^2 \times \text{InflationFactor}$$
so that

\[ n_3 = \frac{37.64 \pi (1-\pi)}{(\pi_1 - \pi_2)^2} = \frac{9.41 (1-\pi)}{\theta^2}. \]

where

\[ \theta = \frac{\pi_1 - \pi_2}{(\pi_1 + \pi_2)} = \frac{\pi_1 / \pi_2 - 1}{\pi_1 / \pi_2 + 1}. \]

The value \( \theta \) depends only on the relative risk, but the number \( n_3 \) of patients needed for 80% power depends also on the overall treatment failure rate \( \pi \), with larger patient numbers needed for smaller rates.

The number of patients \( n_3 \) is proportional to the expected number of treatment failures \( E(F_3) \), \( E(F_3) = n_3 \pi \), so that the sample size formula can be rewritten as

\[ E(F_3) = \frac{9.41 (1-\pi)}{\theta^2}. \]

This gives the expected number of treatment failures at stage 3, which is needed for 80% power. Since the rates are small, the term \( (1-\pi) \) will be close to 1. Therefore the expected number of treatment failures needed for 80% power depends essentially only on the relative risk and only to a negligible extent on the overall treatment failure rate.

The following table shows the expected number of treatment failures needed for 80% power for different values of the relative risk and the overall treatment failure rate:

<table>
<thead>
<tr>
<th>Relative risk ( \rho )</th>
<th>Overall TF Rate ( \pi )</th>
<th>Expected number of TF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.025</td>
<td>75.976.3</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>76.7</td>
</tr>
<tr>
<td></td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>0.484</td>
<td>0.025</td>
<td>63.8</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>64.1</td>
</tr>
<tr>
<td></td>
<td>0.015</td>
<td>64.4</td>
</tr>
<tr>
<td>0.45</td>
<td>0.025</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>50.2</td>
</tr>
<tr>
<td></td>
<td>0.015</td>
<td>50.5</td>
</tr>
<tr>
<td>0.40</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.015</td>
<td></td>
</tr>
</tbody>
</table>

**Power with new futility bounds (stop, if \( Z_k \geq -1 \) or 1-sided p-value \( \geq 0.1587 \)):**

The following results were assessed via simulation of random numbers from the binomial distribution (using 1000000 simulation runs for each scenario).

<table>
<thead>
<tr>
<th>Relative risk ( \rho )</th>
<th>Expected cumulative number of TF Rate ( \pi )</th>
<th>Overall TF Rate ( \pi )</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>25 37 52</td>
<td>0.025</td>
<td>82%</td>
</tr>
</tbody>
</table>
Using alpha-spending function as detailed in section 8.7, with information fractions based on 76. If <76 TF are observed at stage 3, the remaining alpha is completely spent at stage 3.

For a relative risk of 0.4 (corresponding to assumed rates of e.g. 3.1% for placebo and 1.24% for SHB004) the 3-stage design (with stopping rules as specified in section 8.7) has a power of 80% for 22 expected treatment failures in stage 1 and 15 expected treatment failures in each of the two subsequent stages, corresponding to cumulative numbers of 22, 37 and 52 treatment failures.

The next table shows the intended numbers of treatment failures per stage and the corresponding number of patients for treatment failure rates of 3.1% and 1.24%, assuming that 10% of the randomized patients have to be excluded from the ITT population due to second tick bites or missing Day 57 results.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Cumulative number of TF in ITT set</th>
<th>Cumulative patient numbers for TF rates 3.1%, 1.24%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ITT population (10% of randomized patients excluded)</td>
</tr>
<tr>
<td>1</td>
<td>≈22</td>
<td>≈1014</td>
</tr>
<tr>
<td>2</td>
<td>≈37</td>
<td>≈1706</td>
</tr>
<tr>
<td>3</td>
<td>≈52</td>
<td>≈2396</td>
</tr>
</tbody>
</table>

These numbers will be revised after stage 1, taking into account the actually observed overall treatment failure rate and the actual percentage of randomized patients excluded from the ITT population at stage 1.
9 ETHICAL, LEGAL, AND ADMINISTRATIVE ASPECTS

9.1 Data Quality Assurance

The Sponsor or Sponsor’s designee will conduct a site visit to verify the qualifications of each investigator, inspect the site facilities, and inform the investigator of responsibilities and the procedures for ensuring adequate and correct documentation.

The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study participant. All information recorded on the eCRF for this study must be consistent with the subjects’ source documentation (i.e. medical records).

9.1.1 Database Management and Quality Control

All data generated by the site personnel will be captured electronically at each study center using eCRFs. Data from external sources (such as laboratory data) will be imported into the database. Once the eCRF clinical data have been submitted to the central server at the independent data centre, corrections to the data fields will be captured in an audit trail. The reason for change, the name of the person who performed the change, together with the time and date will be logged to provide an audit trail.

If additional corrections are needed, the responsible monitor or data manager will raise a query in the electronic data capture (EDC) application. The appropriate staff at the study site will answer queries sent to the investigator. The name of the staff member responding to the query, and time and date stamp will be captured to provide an audit trail. Once all source data verification is complete and all queries are closed, the monitor will freeze the eCRF page.

The specific procedures to be used for data entry and query resolution using the EDC system/eCRF will be provided to study sites in a training manual. In addition, site personnel will receive training on the EDC system/eCRF.

9.2 Case Report Forms and Source Documentation

All data obtained during this study should be entered in the eCRFs promptly. All source documents from which eCRF entries are derived should be placed in the subject’s medical records. Measurements for which source documents are usually available include laboratory assessments or other local medical investigations.

Source data are the original records of all variables collected for all the study. They include, but are not limited to:

- Signed informed consent;
- Laboratory parameters;
- Individual subject clinical notes;
- Details concerning inclusion and exclusion criteria;
- Hospital charts or pharmacy records and any other similar reports and records of any procedure performed in accordance with the protocol.
Data that will be entered directly into the eCRF (i.e., for which there is no prior written or electronic record of data) are considered to be source data.

The original eCRF entries for each subject may be checked against source documents at the study site by the Sponsor or Sponsor’s delegate site monitor.

After review by the site monitor, completed eCRF entries will be uploaded and forwarded to Sponsor or delegate. Instances of missing or uninterpretable data will be discussed with the investigator for resolution.

The specific procedures to be used for data entry and query resolution using the eCRF will be provided to study sites in a training manual. In addition, site personnel will receive training on the eCRF.

9.2.1 Data Collection

The investigators (and appropriately authorized staff) will be given access to an online web-based EDC system which is 21 CFR Part 11 compliant. This system is specifically designed for the collection of the clinical data in electronic format. Access and right to the EDC system will be carefully controlled and configured according to each individual’s role throughout the study. In general, only the investigator and authorized staff will be able to enter data and make corrections in the eCRFs.

The eCRF should be completed for each subject included in the study and should reflect the latest observations on the subjects participating in the study. Therefore, the eCRFs are to be completed as soon as possible during or immediately after the subject’s visit or assessment. The investigator must verify that all data entries in the eCRF are accurate and correct. If some assessments cannot be done, or if certain information is unavailable, not applicable or unknown, the investigator should indicate this in the eCRF.

Computerized data-check programs and manual checks will identify any clinical data discrepancies for resolution. Corresponding queries will be loaded into the system and the site will be informed about new issues to be resolved on-line. All discrepancies will be solved on-line directly by the investigator or by authorized staff. Off-line edit checks will be done to examine relationships over time and across panels to facilitate quality data.

After completion, the investigator will be required to electronically sign off the clinical data.

Data about all study drug dispensed to the subject and any dosage changes will be tracked on the eCRF.
9.3 Access to Source Data

During the study, a monitor will make site visits to review protocol compliance, compare eCRF entries and individual subject’s medical records, assess drug accountability, and ensure that the study is being conducted according to pertinent regulatory requirements. eCRF entries will be verified with source documentation. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

Checking of the eCRF entries for completeness and clarity, and cross-checking with source documents, will be required to monitor the progress of the study. Moreover, Regulatory Authorities of certain countries, IRBs, IECs, and/or the Sponsor’s Clinical Quality Assurance Group may wish to carry out such source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical confidentiality. The investigator assures the Sponsor or delegates of the necessary support at all times.

9.4 Data Processing

All data will be entered by site personnel into the EDC system/eCRF (as detailed in section 9.2.1).

The data-review and data-handling document, to be developed during the initiation phase of the study, will include specifications for consistency and plausibility checks on data and will also include data-handling rules for obvious data errors. Query/correction sheets for unresolved queries will be sent to the study monitors for resolution with the investigator. The database will be updated on the basis of signed corrections.

Previous and concomitant medications will be coded using the World Health Organization (WHO) Drug Reference List (DRL), which employs the ATC classification system. Medical history/current medical conditions and adverse events will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

Previous and concomitant diseases as well as AEs will be coded with Medical Dictionary for Regulatory Activities (MedDRA).

The versions of the coding dictionaries will be provided in the Clinical Study Report.

9.5 Archiving Study Records

According to International Conference on Harmonization (ICH) guidelines, essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. However, these documents should be retained for a longer period if required by the applicable legal requirements. For example, these documents should be retained for at least 10 years after the regular end or a premature termination of the respective study (VKlin Art. 25).
9.6 Good Clinical Practice

The procedures set out in this study protocol are designed to ensure that the Sponsor and investigator abide by the principles of the Good Clinical Practice guidelines of the International Conference on Harmonization (ICH) (CPMP/ICH/135/95), and of the Declaration of Helsinki (2008) [1]. The study also will be carried out in keeping with local legal requirements.

The investigator will ensure that the study described in this protocol will be carried out to the highest standards of medical and clinical research practice. The investigator will also ensure that all those involved in the conduct of the study, such as co-investigators, pharmacists and study nurses, are provided with the copies of the protocol and safety information before study start, and are fully familiar with it and qualified for their role.

The investigator will inform all personnel involved in the study about the specific study issues by explaining the details regarding all routines involved and handing out the study protocol.

9.7 Informed Consent

Before each subject is admitted to the study, informed consent will be obtained from the subject according to the regulatory and legal requirements of the participating country. This consent form must be dated and retained by the investigator as part of the study records. The investigator will not undertake any investigation specifically required only for the clinical study until valid consent has been obtained. The terms of the consent and when it was obtained must also be documented in the eCRF.

If a protocol amendment is required, the informed consent form may need to be revised to reflect the changes to the protocol. If the consent form is revised, it must be reviewed and approved by the appropriate IEC/IRB, and signed by all subjects subsequently enrolled in the study as well as those currently enrolled in the study.

9.8 Protocol Approval and Amendment

Before the start of the study, the study protocol and/or other relevant documents will be approved by the IEC/IRB/Competent Authorities, in accordance with local legal requirements. The Sponsor must ensure that all ethical and legal requirements have been met before the first subject is enrolled in the study.

This protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel, and receive IRB/IEC/Competent Authority approval prior to implementation (if appropriate).

Administrative changes (not affecting the subject benefit/risk ratio) may be made without the need for a formal amendment. All amendments will be distributed to all protocol recipients, with appropriate instructions.
9.9 Independent Data Monitoring Committee

An Independent Data Monitoring Committee (IDMC) will be appointed for this study. The IDMC is independent and operating under full confidentiality and bound to take decisions as outlined below. No member of the IDMC or the IDMC itself will communicate any aspects other than what is detailed below. The IDMC will have at least 3 members as follows:

- 2 independent physicians,
- An independent statistician.

The IDMC will have to decide whether the study should be continued or not owing to the results of the unblinded interim analyses. The IDMC will decide on doubtful cases of treatment failures (TF) and agree if the doubtful case is to be counted as TF or not. The IDMC is particularly discussing cases within which “negative” serological results against IgM and/or IgG antibodies for Borrelia burgdorferi s.l. were obtained for Visit 1 and “borderline” results were obtained for Visit 4. According to the current MIQ, these results allow no final conclusion if antibodies are present in the respective serum samples. If necessary, the IDMC may request additional information from the respective Investigator to clarify possibly open questions. This is to be done before unblinding.

If changes to the protocol are recommended by the IDMC, the Sponsor will communicate changes to the protocol by means of a protocol amendment.

A first interim analysis is planned to be conducted after a total of 25 ± 20% treatment failures have been observed. Also, due to the seasonal aspect of the study, it is expected to perform the first interim analysis by the end of the second season of the study.

At each stage the type-1 error to be spent will be computed using an alpha-spending function, which approximately produces the Pocock stopping boundaries \( b_k \), where \( k = 1, 2, 3 \) is the analysis stage (see section 8.7 for details).

The stopping criteria for futility or efficacy are summarized as follows:

**Stage 1** (interim analysis)

- End of the study for demonstrated futility (accept \( H_0 \)), if the proportion of treatment failures in the SHB004-group is larger than or equal to the corresponding proportion in the placebo group, i.e. if the 1-sided \( p \)-value \( p_1 \) is \( \geq 0.1587 \).

- End of the study for demonstrated efficacy (reject \( H_0 \)), if the 1-sided \( p \)-value does not exceed the corresponding boundary, i.e. \( p_1 \leq b_1 \).

Otherwise continue with Stage 2.

**Stage 2** (interim analysis)

- End of the study for demonstrated futility (accept \( H_0 \)), if the proportion of treatment failures in the SHB004-group is larger than or equal to the corresponding proportion in the placebo group, i.e. if the 1-sided \( p \)-value \( p_2 \) is \( \geq 0.1587 \).

- End of the study for demonstrated efficacy (reject \( H_0 \)), if the 1-sided \( p \)-value does not exceed the corresponding boundary, i.e. \( p_2 \leq b_2 \).
Otherwise continue with Stage 3 (final analysis).

**Stage 3** (final analysis)

- End of the study for demonstrated futility (accept $H_0$), if the 1-sided p-value is larger than the corresponding boundary, i.e. $p_3 > b_3$.
- End of the study for demonstrated efficacy (reject $H_0$), if the 1-sided p-value does not exceed the corresponding boundary, i.e. $p_3 \leq b_3$.

These stopping criteria along with other statistical considerations (e.g. conditional power, sensitivity analyses in other populations, repeated confidence intervals) are going to be used by the IDMC as a guideline for taking decisions.

The futility boundaries are binding in the sense that the study will be stopped, if the futility stopping criterion is met, i.e. if $p_k \geq 0.1587$ at an interim analysis.

The efficacy boundaries are binding in the sense that the study can only be stopped, if the stopping criterion is met at one of the stages, i.e. $p_k \leq b_k$ for at least one stage $k$. Otherwise the overall type-1 error of 2.5% will not be maintained.

The repeated confidence intervals for the relative risk will be consistent with the test decision of the group sequential design. Thus, the repeated confidence interval at stage $k$ will include the relative risk of 1 (no treatment effect) if and only if the group sequential test cannot reject the null hypothesis at stage $k$. Conversely, when the group sequential test rejects $H_0$ at stage $k$, then the corresponding repeated confidence interval will exclude the relative risk of 1.

For further statistical details see section 8.7.

The study biostatistician(s) will analyze the data according to the statistical analysis plan (SAP) and hand in the interim results to the IDMC in order to support the safety and efficacy review meetings. The independent statistician of the IDMC will analyze the data using a trial monitoring guideline and will provide data to the committee at least 5 days prior to a scheduled meeting.

### 9.10 Duration of the Study

For an individual subject, the maximum duration of the study for each subject will be up to 71 days (including the day of screening/baseline, 3 days of treatment and up to the end of study/withdrawal visit [at Day 57 with a +14 days window]). This is provided that visits are as planned in Table 1, i.e. without any delay caused by allowed time-windows, or additional optional visits are required (e.g. for follow-up of an AE/SAEs or other reason [see section 7.2.6]). In those cases, the subject might participate a few more days.

The study will close when all subjects have completed the end of study/withdrawal visit (Visit 4) (or optional visit if any is required after the final Visit 4).
9.11 Premature Termination of the Study

If the investigator, the Sponsor, or the Medical Monitor becomes aware of conditions or events that suggest a possible hazard to subjects if the study continues, the study may be terminated after appropriate consultation between the relevant parties. The study may also be terminated early at the Sponsor’s discretion in the absence of such a finding.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the study;
- Failure to enroll subjects at an acceptable rate;
- A decision on the part of the Sponsor to suspend or discontinue development of the drug.

In case of premature termination of the study, subjects who received at least one dose of study drug will have a withdrawal visit and procedures scheduled for Visit 4 (see section 7.2.5).

9.12 Confidentiality

All study findings, data and documents will be regarded as confidential. The investigator and members of his/her research team must not disclose such information without prior written approval from the Sponsor.

The anonymity of participating subjects must be maintained. Subjects will be identified on eCRF and other documents submitted to Sponsor or delegate by their subject number, initials and/or birth date (depending on local legal requirements), not by name. Documents not to be submitted to Sponsor or delegate that identify the subject (e.g., the signed informed consent) must be maintained in confidence by the investigator.

The data collected in the context of this clinical study are subject to the applicable local data protection regulations.

9.13 Other Ethical and Regulatory Issues

If a significant safety issue is identified, either from an individual case report or review of aggregate data, then the Sponsor will issue prompt notification to all parties – Regulatory Authorities, investigators, and IRB/IECs.

A significant safety issue is one that has a significant impact on the course of the clinical study or program (including the potential for suspension of the development program or amendments to protocols) or warrants immediate update of informed consent.

9.14 Liability and Insurance

The Sponsor will take out reasonable third-party liability insurance cover in accordance with all local legal requirements. The civil liability of the investigator, the persons instructed by him or her and the hospital, practice, or institute in which they are employed and the liability of the Sponsor with respect to financial loss due to...
personal injury and other damage that may arise as a result of the carrying out of this study are governed by the applicable law.

The Sponsor will arrange for subjects participating in this study to be insured against financial loss due to personal injury caused by the pharmaceutical products being tested or by medical steps taken in the course of the study.

9.15 Publication Policy

By signing the study protocol, the investigator agrees with the use of results of the study for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, Regulatory Authorities will be notified of the investigator's name, address, qualifications and extent of involvement.

An investigator shall not publish any data (poster, abstract, paper, etc.) without having consulted with the Sponsor in advance. Details are provided in a separate document.
10 REFERENCE LIST


28 Clinical Study Report of January 21, 2010; Clinical Study Report I XO-01, “A Phase I/II, single centre, randomized, investigator blinded, placebo-controlled ascending dose study to assess the local safety and the skin and plasma concentration of azithromycin dermal formulation during repeated applications on the skin of healthy volunteers”.
34 National Committee for Clinical Laboratory Standards, Western Blot Assay for Antibodies to Borrelia Burgdorferi; Proposed Guideline, Draft, NCCLS Document M34-P, NCCLS, Wayne, PA.


11 APPENDICES

APPENDIX 1 Skin Tolerance Score

Score modified from the advice in European Medicines Agency (EMA) Note for Guidance on dermatologic tolerance testing (Committee for Proprietary Medicinal Products: CPMP SWP 2145/00; preclinical guidance [36]) and according to an established procedure with a slightly modified proposal of the Standardization Group of the European Society of Contact Dermatitis (Chart 3).

<table>
<thead>
<tr>
<th>Score</th>
<th>Qualification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Negative</td>
<td>No reaction</td>
</tr>
<tr>
<td>1</td>
<td>Doubtful</td>
<td>Very weak / slight erythema or scaling (spotty or diffuse)</td>
</tr>
<tr>
<td>2</td>
<td>Weak</td>
<td>Weak / slight erythema, scaling, edema or roughness</td>
</tr>
<tr>
<td>3</td>
<td>Moderate</td>
<td>Moderate erythema, scaling, edema or roughness or weak / slight erosions, vesicles or fissures</td>
</tr>
<tr>
<td>4</td>
<td>Strong</td>
<td>Strong erythema, scaling, edema or roughness, or clear erosions, vesicles or fissures</td>
</tr>
<tr>
<td>5</td>
<td>Very strong / caustic</td>
<td>As 4, with necrotic areas</td>
</tr>
</tbody>
</table>
APPENDIX 2 Consort flow diagram

Patient screened, n=…

All subjects randomized (ASR), n=…

Safety analysis set *(SAF), n=…

Intention to treat analysis set (ITT), n=…

Modified intention to treat analysis set (MITT), n=…

Per protocol analysis set (PP), n=…

Screening failures, n=…

Subject did not take any study medication

Subject is baseline-seropositive (IgM and/or IgG)
Subject has not completed the study **
Subject was bitten by two or more ticks after the index bite***

Treatment failures defined as such based on isolated IgM or IgG seroconversion

Index tick is not carrying *Borrelia s.l.*
Major protocol deviations as defined in the SAP

*Three more sets are defined (and not outlined in this chart) for sensitivity efficacy analysis: All Treated Subjects Set A: Same as SAF, but analyzing subjects according to their randomized treatment. Count drop-outs as TF. Count subjects with additional tick bites as TF providing they develop an EM or a seroconversion. All Treated Subjects Set B is identical to All Treated Subjects Set A but drop-outs are not counted as TF. All Treated Subjects Set C is identical to All Treated Subjects Set B but subjects with an additional tick bite are not counted as TF.

**These subjects are excluded given the small number of expected treatment failures (3.1% placebo vs. 1.5% verum, see section 8.8). Treating such cases as treatment failures would cause great loss of power and may introduce over-estimation of treatment failures. However, events collected throughout the presence of those subjects who did not complete the study are recorded and are part of the ITT and modified ITT analysis (e.g. occurrence of an EM before the subject withdrew would be counted as a treatment failure). See section 3.3 for details.

***Baseline-seronegative subjects bitten additionally by a tick are excluded as this represents a study artificiality. This protocol prohibits the treatment of additional tick bites as we cannot determine the nature of the initial treatment, placebo or verum and, therefore, would not know what to administer to the additional tick bite without unblinding. EM occurring before the additional tick bite has been noticed are counted as treatment failure in baseline-seronegative subjects. See section 3.3 for details.
APPENDIX 3: Serum analytics

IgM Enzyme-Linked Immuno Sorbent Assay (Borrelia afzelii IgM ELISA IgM Testkit)

Method: B. afzelii + IgM ELISA IgM Testkit, Virotech
Use: Screen / search test to detect *B. burgdorferi sensu lato* specific antibodies (IgM) in human serum.
Source document: SOP-GLP-0036-01 (Fraunhofer-Gesellschaft, Institut für Zelltherapie und Immunologie IZI Leipzig)
Analysis: According to SOP-GLP-0036-01, dimensionless ‘virotech units (VE)’ are calculated. These VE are used to decide as outlined below:

<table>
<thead>
<tr>
<th>VE</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;9,0</td>
<td>Negative</td>
</tr>
<tr>
<td>9,0 – 11,0</td>
<td>Borderline</td>
</tr>
<tr>
<td>&gt;11,0</td>
<td>Positive</td>
</tr>
</tbody>
</table>

IgG ELISA (B. afzelii + VlsE IgG Europe ELISA IgG Testkit)

Method: B. afzelii + VlsE IgG Europe IgG ELISA
Use: Screen / search test to detect *B. burgdorferi sensu lato* specific antibodies (IgG) in human serum.
Analysis: According to SOP-GLP-0035-01, dimensionless ‘virotech units (VE)’ are calculated. These VE are used to decide as outlined below:

<table>
<thead>
<tr>
<th>VE</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;9,0</td>
<td>Negative</td>
</tr>
<tr>
<td>9,0 – 11,0</td>
<td>Borderline</td>
</tr>
<tr>
<td>&gt;11,0</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Line Blot for IgM (Borrelia Europe LINE IgG / IgM Line Immunoblot) and IgG (Borrelia Europe plus TpN17 LINE IgG Line Immunoblot)

Method: Borrelia Europe plus TpN17 LINE
Use: Screen / search test to detect *B. burgdorferi sensu lato* specific antibodies (IgG) in human serum.
Analysis:
For IgG: According to SOP-GLP-0033-03, the following analysis is conducted for IgG:

| Negative  | No band ≥ cut off band |
| Borderline| One band ≥ cut off band|
| Positive  | Two or more bands ≥ cut off band |

For IgM: According to SOP-GLP-0033-03, the following analysis is conducted for IgM:

| Negative  | No band ≥ cut off band |
| Borderline| One band of BmpA (p39), DpA-Mix, VlsE-Mix ≥ cut off band |
| Positive  | Two or more bands ≥ cut off band or isolated OspC (p23) band ≥ cut off band |