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Title Page

TITLE

Burden of Lyme Disease (BOLD) Prospective Incidence Epidemiology Study Methods: Conducting Active Surveillance in Endemic Areas of Six European countries

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ABSTRACT

Introduction:

This study is being conducted to inform the Phase 3 efficacy study for Pfizer and Valneva's investigational Lyme Disease Vaccine, VLA15. Previous Lyme vaccine efficacy studies exclusively involved US sites. VLA15 Phase 3 will be conducted in US and Europe due to the vaccine's expanded serotype coverage and the public health burden of LD in Europe. In Europe the existence and location of study sites that have access to populations with high LD annual incidence is uncertain. This active, prospective surveillance study assesses annual LD incidence of GP/primary care clinical trial sites in high-incidence regions. This will allow for Phase 3 site vetting and better characterization of LD burden in selected regions for study size calculations.

Methods and analysis:

The Burden of Lyme disease (BOLD) study will assess LD incidence at 15 GP/primary care practices in endemic areas of 6 European countries from Spring 2021 until December 2022. Suspected LD cases identified from each site's practice panel are documented on Screening Logs. Clinical diagnoses are recorded, alongside LD clinical manifestations and standard of care Lyme diagnostic results. In the initial 12-month enrolment phase, suspected LD cases are offered enrolment. Participants undergo interview and clinical assessments to establish medical history, final clinical diagnosis, clinical manifestations, and impact of LD on quality of life. Study specific procedures include LD serology, skin punch biopsies and photographs of Lyme manifestations. For every enrolled participant diagnosed with LD, 6-10 age-matched controls are randomly selected and offered enrolment for an embedded LD risk factor analysis. Persistent symptoms or post-treatment Lyme Disease will be assessed at follow-up visits up to two years after initial diagnosis while patients remain symptomatic.

Ethics and dissemination:

This study has been approved by all sites' local ethics committees. Results will be presented at conferences and published in peer-reviewed journals.

STRENGTHS AND LIMITATIONS

- The quality and quantity of LD incidence data from European countries varies due to consensus case definitions not being consistently used and differing reporting procedures. This study uses consistent LD case definitions to establish comparative LD incidence from high-incidence areas across 6 European countries.
- This study conducts LD surveillance in clearly defined populations (i.e., the practice panel of a primary care provider), which will be used as the denominator for LD incidence calculations, allowing for accurate calculation of incidence rates.
- The study will follow enrolled LD cases post antibiotic treatment to assess the proportion of patients that have persistent symptoms or Post Treatment Lyme Disease (PTLD).
- The study can only capture LD diagnoses that the study site staff are aware of, and as such may miss some or all events only treated outside of the practice due to travel or other reasons depending on the completeness of practices' routine systems to identify such events.

- Pre-season baseline serology specimens will not be available from the study population to assess for seroconversion across the Lyme season and, on this basis, asymptomatic Lyme disease infections will also not be captured.

INTRODUCTION

Lyme Disease (LD) is the most frequent tick-borne disease in the moderate climates of the northern hemisphere.[1] LD is caused by infection with *Borrelia burgdorferi sensu lato (B.b.s.l.).* There are 18 documented *Borrelia* genospecies, but only a subset has been associated with human disease.[2] Serotypes (ST) are determined by outer surface protein A (OspA) types. In North America, almost all LD (>98%) is due to *B. burgdorferi senso stricto* (ST1*)*, with minor contribution from *B. mayonii* (1-2%). In Europe, *B. afzelii* (ST2) and *B. garinii* (ST3,5,6) are predominant, but *B. burgdorferi* s.s. (ST1) and *B. bavariensis* (ST4) are also documented.[3] To address the high burden of LD, Pfizer and Valneva are jointly developing a 6-valent vaccine (VLA15) for the prevention of LD caused by *Borrelia* strains expressing outer surface protein A (OspA) ST 1-6 by active immunization.

This prospective epidemiology study will collect key information to support VLA15's Phase 3 efficacy study. While two successful Phase 3 efficacy studies have been previously conducted for other investigational Lyme vaccines, they exclusively involved US sites because those vaccines included Serotype 1 only.[4,5] Due to high medical need in Europe and US, VLA15 includes expanded serotype coverage, so its Phase 3 efficacy study will be conducted in both the US and Europe. In the US, it is established that at least a 1% annual incidence of acute LD is present in high-risk areas.[3] However, due to less developed LD surveillance in Europe, uncertainty exists regarding the existence and location of potential Phase 3 efficacy trial sites that would have access to a population with high annual incidence of acute LD from which to enrol Phase 3 study participants. On this basis, this active, prospective surveillance study will identify discrete GP/primary care practice-based sites in potential high-incidence geographical regions and assess their annual LD incidence. This will allow for vetting of potential Phase 3 sites and better characterization of the burden of LD in the region for use in study size calculations.

BOLD study sites are embedded in GP/primary care practices to provide accurate denominators for LD incidence measurements (i.e., the practice panel is a clearly delineated source population) and comprehensive event capture for the incidence numerator (i.e., primary care physicians should be aware of Lyme events among their patients diagnosed at their practice sites as well as in other clinical settings such as urgent care centers and hospitals). To estimate LD incidence by LD risk factors, 6-10 randomly selected potential control participants from the site's practice panel will be approached for participation for each enrolled case with newly diagnosed LD to create a comparison group for a nested case control analysis.

BOLD will also deliver epidemiologic data on high-risk geographic areas for uses beyond the Phase 3 trial. The quality and quantity of LD incidence data from European countries varies due to consensus case definition not being consistently used and differing reporting procedures.(Burn L, Tran TMP, Pilz A, et al. Incidence of Lyme Borreliosis in Europe from National Surveillance Systems (2005-2020). *Vector Borne Zoonotic Dis* 2022 submitted) Annual LD incidence is reported as up to 632 per 100,000 population in Sweden,[6] and the population-weighted LD incidence in western Europe has been estimated at 22 cases per 100,000 person-years among all ages.[7] However, these composite estimates and national incidence estimates are limited by under-reporting and marked intra-country

regional variation.(Burn L, Vyse A, Pilz A, et al. Incidence of Lyme Borreliosis in Europe, A Systematic Literature Review (2005-2020). *Vector Borne Zoonotic Dis* 2022 submitted) For these reasons, it is difficult to compare incidence among different sites in Europe, either across or within countries, and true LD incidence is not well understood. BOLD's active-surveillance-based incidence estimates from GP/primary care-based sites in endemic regions will allow for better characterization of LD burden in high-incidence regions of 6 European countries.

The BOLD study will also characterize the frequency and type of persistent Lyme Disease symptoms. Following antibiotic treatment for LD, a proportion of patients continue to have persistent subjective symptoms, a subset of which will meet the case definition for PTLD.[8,9] In 2006 guidelines from the Infectious Disease Society of America (IDSA) created a working definition for PTLD with clinical symptoms persisting at least six months after treatment for LD. There is a broad range from 5-20% of patients that continue to suffer from persistent symptoms not meeting PTLD case definition for months to years post-antibiotic treatment.[10,11] Given the heterogeneity of existing literature, PTLD is poorly characterized in terms of the size of the patient group, severity of symptoms, duration of symptoms, impact on quality of life, and health care utilization. Thus, BOLD aims to assess the incidence, severity, and duration of persistent symptoms (including PTLD) by clinical manifestation (Erythema migrans versus disseminated LD), as well as the quality of life and health resource use associated with persistent symptoms (including PTLD) among suspected enrolled LD cases. The study also aims to assess the impact of LD on quality of life by comparing suspected LD cases with persistent symptoms (including PTLD cases) with age-matched controls to support future cost-effectiveness analysis.

OBJECTIVES AND ENDPOINTS

Objectives and endpoints are classified into primary, secondary, exploratory in Table 1. Those for the assessment of persistent symptoms of LD, see Table 2.

1 *Table 1 – BOLD Objectives and Endpoints*

Primary Objective:	**Primary Endpoint:**
• To assess LD annual incidence rate in persons of all ages, races, and ethnicities at potential Phase 3 efficacy trial sites for the VLA15 vaccine in geographic regions that are endemic for LD, overall and by site.	• The annual incidence rate of newly diagnosed LD in persons of all ages, races, and ethnicities who are patients of the study sites' GP/primary care practice, overall and by site.
Secondary Objectives:	**Secondary Endpoints:**
• To assess LD annual incidence rate in persons of all ages, races, and ethnicities at potential Phase 3 efficacy trial sites for the VLA15 vaccine in geographic regions that are endemic for LD, by age, month of diagnosis, and LD risk factor.	• The annual incidence rate of newly diagnosed LD in persons of all ages, races, and ethnicities at study sites by age, month of diagnosis, and LD risk factor.
• Describe the *Borrelia* genospecies/OspA serotype distribution of LD in persons of all ages, races, and ethnicities at potential Phase 3 efficacy trial sites for the VLA15 vaccine in geographic regions that are endemic for LD.	• Proportion for each Borrelia genospecies/OspA serotypes of LD among participants with available genospecies/OspA serotype results.
• Describe the proportion of LD cases by clinical manifestation category among persons of all ages, races, and ethnicities at potential Phase 3 efficacy trial sites for the VLA15 vaccine in geographic regions that are endemic for LD, by specific clinical manifestation category (ie, erythema migrans, neuroborreliosis, Lyme arthritis, Lyme carditis, borrelial lymphocytoma, acrodermatitis chronica atrophicans, LD ocular manifestations) and for all disseminated disease combined.	• Proportion of newly diagnosed LD cases by specific clinical manifestation category (ie, erythema migrans, neuroborreliosis, Lyme arthritis, Lyme carditis, borrelial lymphocytoma, acrodermatitis chronica atrophicans, LD ocular manifestations) and for all disseminated disease combined.
• To estimate the proportion of persons of all ages with newly diagnosed LD at potential Phase 3 efficacy trial sites for the VLA15 vaccine who have conditions that would exclude their participation in the proposed Phase 3 efficacy trial sites based on potential exclusion criteria (eg, immunosuppression), overall, by site, by age group, by season, and by exclusion criteria.	• The proportion of participants among persons of all ages, races, and ethnicities with newly diagnosed LD who have conditions that would exclude their participation from the proposed Phase 3 efficacy trial sites based on potential exclusion criteria (eg, immunosuppression), overall, by site, by age group, by season, and by exclusion criteria.
Exploratory Objectives:	**Exploratory Endpoints:**
• Describe the prevalence of LD risk factors and potential Phase 3 trial exclusion criteria among practice panel patients of all ages, races, and ethnicities without current LD at potential Phase 3 efficacy trial sites for the VLA15 vaccine, overall and by site.	• Proportions of site practice panel patients of all ages, races, and ethnicities without current LD with key characteristics, (eg, self-reported specific LD risk factors and conditions that would exclude their participation from the potential Phase 3

	efficacy trial), overall, by age group, and by site.
• Describe signs and symptoms of LD and patient treatment journey for LD under current standard of care.	• Time from symptom onset to diagnosis, duration of symptoms, treatment duration and type, number and type of medical visits and therapeutic procedures, and frequency of hospitalization and mean length of stay.
• Describe LD diagnostic testing practices under current standard of care.	• Proportion of participants with standard of care LD diagnostic testing, overall and by type.
• Estimate the ratio of LD incidence based on LD surveillance to LD incidence measured by this study by region and country.	• Ratio of LD incidence from local LD surveillance system (in regions where available) to incidence of LD cases at study site(s) in that region.
• To describe possible LD events with standard of care LD diagnosis without established LD clinical manifestations (ie, erythema migrans, neuroborreliosis, Lyme arthritis, Lyme carditis, borrelial lymphocytoma, acrodermatitis chronica atrophicans, LD ocular manifestations).	• For standard of care LD diagnoses without established LD clinical manifestations, frequency and duration of symptoms experienced, frequency of physical exam findings by type, and LD diagnostic testing results by type of test.
• To describe LD impact on participants' mental and physical functions and quality of life.	• Scores of physical, mental functions and quality of life measured by 36-Item Short Form Survey (SF-36), degree of pain, severity of pain of different body parts, and degree of fatigue and its specific impact measured by Fatigue Severity Scale (FSS) and Short Form McGill Pain Questionnaire

Table 2 - Objectives for the assessment of persistent symptoms of LD including PTLD

Objectives for assessment of persistent symptoms of LD including PTLD:	Endpoints for assessment of persistent symptoms of LD including PTLD:
• To assess the proportion of suspected Lyme disease (LD) cases, by clinical manifestation (Erythema migrans versus disseminated LD), that subsequently develop persistent symptoms, including PTLD	• Proportion of treated LD cases by clinical manifestation (Erythema migrans versus disseminated LD that subsequently develop persistent symptoms, including PTLD
• To assess the severity of persistent symptoms (including PTLD) by clinical manifestation (Erythema migrans and disseminated LD) among suspected LD cases	• Severity of persistent symptoms (including PTLD) by clinical manifestation (Erythema migrans and disseminated LD) among suspected LD cases: Pain severity (Short Form McGill Pain Questionnaire [SF-MPQ], and the pain subscale of the Medical Outcomes Survey Short Form-36 [SF-36]); Fatigue Severity (Fatigue Severity Scale [FSS]); Cognitive impairment (Cognitive Failures Questionnaire [CFQ]).
• To compare the severity of symptoms among PTLD cases to those of patients with persistent symptoms that do not meet PTLD case definition	• Duration of persistent symptoms (including PTLD) by clinical manifestation (Erythema migrans and disseminated LD)
• To assess the impact of persistent symptoms (including PTLD) on health-related quality of life (QoL) between suspected LD cases in comparison with age-matched controls	• Symptom severity by subgroup (PTLD cases compared to treated LD cases with symptoms not meeting PTLD case definition, and participants with other non LD diagnosis. SF-36, SF-MPQ, FSS and CFQ subscale scores and summary scores.
• To assess the health resource use associated with persistent symptoms (including PTLD) among suspected LD cases	• Treatment duration and type, number and type of medical visits and therapeutic procedures, and frequency of hospitalization, and mean length of stay.

METHODS AND ANALYSIS

Study Design

This prospective, epidemiological study uses active surveillance to measure the annual LD incidence of newly diagnosed LD at 15 GP/primary care practices in 6 European countries: Czech Republic, Germany, Poland, Slovenia, Slovakia, and Sweden. A nested case control analysis is embedded within the study to assess LD risk factors. The BOLD study was initiated in April 2021 and LD surveillance will continue through to the end of 2022. There is an initial 12-month study enrolment phase starting from the sites' activation where suspected LD cases identified are offered enrolment within the study (Fig.1). In a second phase, enrolment ends but LD case surveillance continues. Enrolled suspected LD cases are followed up to two years after enrolment to assess any persistent symptoms, and the impact of LD on quality of life by comparing suspected LD cases with persistent symptoms (including PTLD cases) with age-matched controls.

Site Selection

Study sites are embedded in general practices/primary care practices and the "practice panel" will serve as the denominator for the incidence estimate. A practice panel is defined as all persons of any age enrolled in the primary care practice for routine outpatient care (registered to GP practice or healthcare contact with the practice in the last 2 years). Selected study sites needed to have the clinical research infrastructure to conduct a vaccine clinical trial so that they can potentially serve as study sites for the VLA15 Phase 3 efficacy trial. Based on published LD incidence and incidence maps, over 250 sites across 11 European countries were reviewed and contacted. Feasibility questionnaires and pre-trial assessments were conducted at potential study sites to ascertain practice panel size and annual LD incidence in the previous 12-month period. Potential sites were not selected if annual LD incidence was less than 0.5% according to the pre-trial assessments, the site was not a primary care clinic, or the site's research infrastructure was inadequate. Subsequently, BOLD was able to select 20 GP/primary care practices in 6 European countries: Czech Republic, Germany, Poland, Slovenia, Slovakia, and Sweden. Of these 20 sites, all were initiated and 5 were subsequently closed – largely for operational issues. Fifteen sites remain active across these 6 countries.

Active Surveillance

The study's primary focus is measuring the Lyme disease incidence starting from the sites' activation in April–July 2021 and continuing until the end of 2022 through active surveillance of all suspected LD cases (Fig. 1). While most LD is diagnosed in the primary care setting, investigators seek to identify LD events from other settings (e.g., hospital, emergency department) via their routine methods for tracking the healthcare contacts of practice panel patients. Medical records are also searched for any diagnoses/terms e.g., ICD codes (International Statistical Classification of Diseases) that are used for LD locally as part of daily weekday surveillance (Table 3). Each site maintains a Screening Log to support complete identification of possible LD events. This is documented weekly by site personnel with information including demographic, LD diagnosis and manifestations and Standard of Care (SOC) laboratory data, if applicable. The first 12-months of the active surveillance period starting from each sites' activation is an initial enrolment phase when all suspected LD cases identified are offered enrolment within the study (Fig. 2). During this period, the Screening Log also includes information relating to patient consent and enrolment.

Table 3 Lyme Disease and related diagnoses with relevant International Classification of Diseases (ICD; procedure codes used in medical billing) codes if available.

Condition	ICD9 Code	ICD10 Code
Lyme disease	088.81	A69.2
Lyme borreliosis	NA	NA
Erythema migrans	NA	NA
Acrodermatitis atrophicans chronica	701.8	L90.4
Lyme arthritis	NA	A69.23, M01.2
Lyme neuroborreliosis	NA	A69.22
Borrelial lymphocytoma	NA	NA
Lyme carditis	NA	NA
Lyme-related intraocular inflammation	NA	NA
Bell's Palsy	351.0	G51.0
Various neuritis, neuropathy, and nerve disorders	351.8-357.89	G51.8-G61.89

NA = not available

Eligibility

LD case participant Inclusion Criteria

During the 12-month enrolment period, patients must meet all of the following criteria to be enrolled in the consented portion of the study:

1. Member of participating patient practice.
2. Suspected or confirmed newly diagnosed LD during enrolment period regardless of timing of infection.
3. Evidence of a personally signed and dated informed consent and assent (when age-appropriate and per local requirements) document indicating that the patient (or a legally acceptable representative) has been informed of all pertinent aspects of the study and that they agree to participate.

There are no exclusion criteria for the LD case participants.

Control Participant Inclusion Criteria

Control participants must meet all of the following inclusion criteria to be eligible for inclusion in the study:

1. Member of participating patient practice at time of associated case diagnosis.

2. Evidence of informed consent and assent (when age-appropriate and per local requirements) indicating that the patient (or a legally acceptable representative) has been informed of all pertinent aspects of the study and that they agree to participate.

Control Participant Exclusion Criteria

Control participants meeting any of the following criteria will not be included in the study:

1. Active Lyme Disease in last 90 days.

Study Visits

Study specific procedures are performed at up to five visits (Table 4).

Table 4 – BOLD Study Specific Procedures

	LD Case Participants					Controls	
Procedure/ Assessment	**Visit 1 Day 1[a]**	**Visit 2 Day 28**	**Visit 3 Month 10[b]**	**Visit 4 Month 16-18[c]**	**Visit 5 Month 22-24[c]**	**Contact 1 Day 1**	**Contact 2 Month 16-18**
Screening, Demographics, and Informed Consent/assent	X		X	X	X	X	X
Confirm eligibility (inclusion criteria)	X					X	
Patient (or parent/legal guardian) interview, including symptoms and LD risk factors	X	X	X	X	X	X	
Study blood sample for Lyme serology, and scavenge residual SOC cerebral spinal fluid and synovial fluid specimens if available	X	X	X				
Photograph of LD manifestations, and two 2mm skin punch biopsies of any LD-related rash	X						
Chart review to collect details of current illness, and standard of care (SOC) physical exam findings	X	X	X	X	X		
Collect pre-specified medical history of clinical significance including past LD diagnoses	X	X	X			X	X
Collect SOC LD diagnostic laboratory testing results	X	X	X	X	X		
Collect LD treatment and healthcare utilization, and LD event outcome	X	X	X	X	X		
Record clinical diagnosis and LD manifestation categories experienced based on clinical assessment	X	X	X				
Record clinical assessment of persistent symptoms/PTLD			X	X	X		
Collect Charlson Comorbidity Index (CCI) information			X				X
Collect health survey outcome information							X
Assess Adverse Events (AEs) 2 hours after blood draw and 24 hours after skin punch biopsy	X	X	X	X	X	X	
Assess interest in participation in follow-up studies, and the potential for the participant to meet Phase 3 exclusion criteria	X	X	X			X	

a. If the participant is ≥21 days after LD diagnosis at Visit 1, then Visit 2 data collection will be performed at Visit 1 and no separate Visit 2 will be performed.
b. Visit 3 will take place approximately 9 – 10 months after Visit 2. The latter part of the visit window could be extended up to 12 months after Visit 2 if the participant's persistent symptoms have not reached a 6-months duration after the completion of antibiotic therapy. Participants who did not have a separate Visit 2 will have Visit 3 approximately 9-10 months after Visit 1.
c. Participants who had any persistent symptoms (including PTLD) documented at Visit 3 will be invited for long term follow-up at approximately 6-8 months (Visit 4) and 12-14 months (Visit 5) after Visit 3. Participants are interviewed, have medical record review performed, and LD event outcome will be re-assessed.

Study Specific Procedures and Laboratory Testing

Collected serological samples are tested at Pfizer laboratories using Pfizer's modified two-tiered testing (PMTTT) which consists of two separate Lyme Diagnostic immunoassays. Serum samples with positivity in the tier 1 test (BioRad Lyme Total assay) are then tested in the second test (Zeus Lyme Total assay). A sample must be positive in both tests to be considered diagnostically positive for Lyme Disease. Skin biopsies for *Borrelia* culture and qPCR are performed on participants > 1 year of age who have a LD-related rash and consent to the procedure. Punch biopsy specimens are assessed, for positivity, by a Borrelia 16S qPCR assay and microbiological positivity for the presence of *Borrelia* spirochete through darkfield microscopy and further characterized to genospecies and OspA serotype by sequencing. SOC Lyme diagnostic laboratory results are collected including but not limited to serology with ELISA and/or immunoblots, culture and PCR for *Borrelia* from specimen, histology and neurological, dermatological and/or rheumatological assessments. If CSF and/or synovial fluid samples are obtained from a participant as a part of SOC testing, site staff should request that the laboratory retain any residual sample after SOC testing. These samples may be analysed for antibodies against different borrelial antigens by various immunoassay techniques or for the presence of borrelial molecules by different biochemical techniques, immunoassays and/or nucleic acid sequences by PCR. Specimen processing and testing will be conducted at designated central laboratories and/or Pfizer (401 N Middletown Rd, Pearl River, NY 10965, United States). Photograph (s) of Lyme manifestations will be obtained and used to support Lyme diagnosis.

Participants' mental and physical functions and quality of life are measured by SF-36 standard form, degree of pain, severity of pain of different body parts, and degree of fatigue and its specific impact measured by the Short Form McGill Pain Questionnaire and the Fatigue Severity Scale (FSS), respectively. At Visit 3 (9-10 months after Visit 2), a questionnaire on neurocognitive dysfunction (Cognitive Failures Questionnaire; CFQ) is added for assessment of persistent symptoms. Persistent symptoms and PTLD are evaluated by standardized questionnaires and by patient (or parent(s)/legal guardian(s)) interview at Visit 3. In addition, the investigator performs a clinical assessment to determine if the patient meets PTLD criteria.

At Visits 4 and 5, participants with a final diagnosis of LD who had any persistent symptoms documented at the previous visit (Visit 3 or Visit 4, respectively) are asked to return for participant interview, medical record review and to re-assess LD impact on participant-reported physical and mental functions and quality of life, as measured by 36-Item Short Form Survey (SF-36), degree of fatigue measured by Fatigue Severity Scale (FSS), degree of pain measured by the Short Form McGill Pain Questionnaire, and degree of cognitive difficulties measured by the Cognitive Failures Questionnaire (CFQ).

Participants may decline study-specific procedures, however, remain enrolled to allow for complete tracking of all clinical diagnoses of LD and capture of standard of care diagnosis data.

Controls

To obtain incidence estimates by LD risk factors and proposed Phase 3 exclusion criteria, information on unaffected controls is obtained to allow for a nested case control analysis. Adjusted odds ratios for key characteristics obtained from this analysis and estimated LD incidence will be used to calculate incidence estimates for these characteristics. To achieve this, for each enrolled participant with final LD diagnosis, six practice panel patients without current LD are approached regarding

enrolling as control participants to collect the following information: demographic information (age, sex), risk factors for LD (e.g. time outdoors, pets, personal protective behaviours, occupational and leisure exposures), past history for tick-borne disease including LD, TBE, tick bite prophylaxis and known tick bites, interest in participating in an investigational LD vaccine study, and assessment of meeting potential Phase 3 exclusion criteria (Table 4). If the proportion of potential control participants declining participation is higher than anticipated, the number of potential controls approached will be increased to 10 so approximately 4 control participants are enrolled per LD event. Control selection, consent and enrolment is tracked on the Screening Log.

To assess the impact of LD on quality of life, for each enrolled LD case participant with a final diagnosis of LD, one of the age-matched control participants who had Contact 1 performed is re-consented 16-18 months later to administer 36-Item Short Form Survey (SF-36), degree of fatigue measured by Fatigue Severity Scale (FSS), degree of cognitive difficulties measured by the Cognitive Failures Questionnaire (CFQ), and degree of pain measured by the Short Form McGill Pain Questionnaire as well as to assess pre-specified medical history and comorbidities.

Control participants may complete interview questions via telephone or other remote means, or via in person visit.

Sample Size Estimates

Study size is based on feasibility, not on hypothesis testing as this is a descriptive study. It is expected that approximately 0.5% of practice panel participants per year will be newly diagnosed with LD. Approximately 80% of potentially eligible participants are expected to meet inclusion criteria and agree to enrol. We estimate that on average approximately 25% more participants with suspected LD events will need to be enrolled to identify all events with a final LD diagnosis. Assuming an average practice size of 5,000, we expect approximately 500 participants with suspected/confirmed LD to be enrolled across 20 sites. Among those, we expect approximately 75% to have EM or other rash and 30% of those to consent to skin punch biopsies, thus overall ~113 participants will have ~226 punch biopsy specimens.

6-10 potential control participants are approached for each enrolled participant with a final clinical diagnosis of LD, with approximately 75% (4.5 controls per case) expected to enrol . If 500 participants are enrolled, approximately 90% of these will have final clinical diagnosis of LD (no laboratory confirmation required), yielding an estimate of 2,025 controls enrolled.

Data Analysis

Analysis of Endpoints

For proportion endpoints, data will be summarized with counts (n), percentages (%), and associated 95% confidence interval (CI)s, which will be calculated using the Clopper-Pearson method. Frequency of Research Related Injuries (RRIs) and adverse events (AEs) following study procedures will be tabulated. Results for cases and control participants will be presented separately. For the primary endpoint, data will be summarized overall, by site, by country and by province. The population denominator will be based on the size of the primary care practices' patient panels. All suspected LD cases with final clinical diagnosis of LD will be included in incidence estimates and the contribution of each case type will be completely delineated. The numerator will be the number of newly identified clinically diagnosed LD cases (to be captured from electronic Case Report Form and

Screening Log) occurring in the active surveillance period from sites' activation to the end of 2022. The annual incidence will first be calculated as a fraction (numerator ÷ denominator) and then expressed as a rate per 100,000 population by multiplying the fraction by 100,000. The incidence will be calculated for 2021, 2022, 2021-2022, and for one year following the surveillance start date of each site. When estimating the incidence for 2021, where surveillance is conducted for less than the full LD surveillance year, an adjustment will be used to account for the proportion of the surveillance year when surveillance was not conducted. The adjustment will be based on the proportion of clinically diagnosed LD cases reported by each participating site in 2019 and 2020 by month during the time period when there was no surveillance. Annual incidence estimates by age group, sex, month of diagnosis will also be calculated using administrative information from the practice to estimate size of these subpopulations (ie, subgroup denominators).

Nested Case Control Analysis

In the nested case control analysis, multivariate conditional logistic regression (and/or other multivariate analysis approach) will be used to calculate the adjusted odds ratios (and/or other measure of effect size) for LD risk factors, Phase 3 exclusion criteria, and history of LD. Using these adjusted odds ratios, estimated annual LD incidence obtained from the practice panel from LD surveillance, and distributions of specific risk factors in the LD case group; incidences for each specific characteristic can be calculated.

LD Surveillance Case Definitions

Participants are considered to have a final diagnosis of LD if they have any manifestation of LD described in Table 5 with the associated laboratory confirmation. All presentations listed in Table 5 are considered disseminated LD except EM (unless multiple EM lesions are present). These definitions are derived from consensus case definitions developed by EUCALB (European Union Concerted Action on Lyme Disease).[2] Laboratory confirmation primarily comes from dedicated specimens collected specifically for the study.

Table 5 LD Case Definitions

Presentation	Sign/Symptom (Detailed Definition)	Diagnostic(s)[a]
Erythema Migrans[b]	• Characteristic red or bluish-red patch, with or without central clearing (Lesion should be photographed.)[c]	• Positive IgG/IgM on serum antibody testing OR • Positive PCR of Bbsl result from skin biopsy OR • Positive Culture of Bbsl from skin biopsy
Borrelial Lymphocytoma[b]	• Painless bluish red nodule or plaque, usually on ear lobe, ear helix, nipple, or scrotum (Nodule/plaque should be photographed.)[c]	• Positive IgG/IgM on serum antibody testing OR • Positive PCR of Bbsl result from biopsy OR • Positive Culture of BbsI from biopsy
Acrodermatitis Chronica Atrophicans[b]	• Long-standing red or bluish-red lesions, usually on the extensor surfaces of extremities. Initially doughy swelling. Possible skin induration and fibroid nodules over bony prominences. (Nodule/plaque/lesion should be photographed.)[c]	• Positive IgG/IgM on serum antibody testing OR • Positive PCR of Bbsl result from biopsy OR • Positive Culture of BbsI from biopsy
Lyme Neuroborreliosis[b]	• Meningo-radiculitis (Bannwarth syndrome), facial palsy, meningitis, encephalomyelitis, OR cerebral vasculitis (Clinical manifestation [eg, facial palsy] should be photographed if applicable.)[c]	• Intrathecal IgM and/or IgG antibodies OR • Positive intrathecal anti-Borrelia antibody index (CSF vs Serum) reflecting intrathecal antibody production OR • Positive PCR of Bbsl result from cerebrospinal fluid OR • Positive Culture of BbsI from cerebrospinal fluid

Presentation	Sign/Symptom (Detailed Definition)	Diagnostic(s)[a]
Lyme Carditis[b]	• Acute onset of high degree atrioventricular conduction disturbances, rhythm disturbances, myocarditis, OR pancarditis	• Positive IgG/IgM on serum antibody testing
Lyme Arthritis[b]	• Marked swelling in one or few large joints, most often the knee. (Clinical manifestation (eg, swollen joint) should be photographed.)[c]	• Positive IgG/IgM on serum antibody testing OR • Positive PCR of Bbsl result from synovial fluid or tissue OR • Positive Culture of BbsI from synovial fluid or tissue
LD Ocular Manifestations[b]	• Conjunctivitis, uveitis, papillitis, episcleritis, OR keratitis	• Positive IgG/IgM on serum antibody testing OR • Positive PCR of Bbsl result from ocular fluid OR • Positive Culture of BbsI from ocular fluid

a. *Laboratory confirmation will primarily come from dedicated specimens collected specifically for the study (ie, serum and skin biopsy specimens)*

b. *These clinical manifestations categories are disseminated Lyme manifestations. EM will only be considered disseminated if there are multiple EM lesions.*

c. *Photos will be taken in a manner as to not identify the participant and may be declined at the time of the procedure by the participant.*

Persistent symptoms and PTLD Case Definition

Participants are considered to have PTLD at Visit 3 (9-10 months after Visit 2) if they continue to have persistent symptoms of LD and meet the case definition defined by the Infectious Disease Society of America (IDSA) as clinical symptoms persisting at least six months after LD treatment.[9] The case definition for PTLD is described in Table 6.

Table 6 PTLD Case Definition

Presentation	Sign/Symptom (Detailed Definition)	Evaluations(s)
Post-treatment Lyme Disease	• Prior documented case of clinically confirmed Lyme Disease as per definitions on Table 5. • Treatment with accepted antibiotic regimen with resolution or stabilization of objective manifestations of LD	• Final diagnosis of LD (clinical diagnosis only or laboratory-confirmed)
	• Fatigue, musculoskeletal pain, and cognitive difficulties that begin within 6 months and last for 6 months after completion of antibiotic therapy	• Participants with questionnaire scores exceeding the cut-off scores for fatigue (FSS mean score of 4 or higher), pain (SF-36, pain subscale, score 55 or lower),[12] or neurocognitive functioning (CFQ score 44 or higher), that begin within 6 months and last for 6 months after completion of antibiotic therapy, as assessed by aforementioned questionnaires.[13]
	• Subjective symptoms are so severe that result in substantial reduction in activities • No other co-morbidities that can explain illness	• Subjective symptoms result in reduction of activities as assessed by either the "limitations in physical activities" subscale (score 55 or lower) of the SF-36.[12]

Persistent Symptoms of Lyme Disease

The incidence and severity of persistent symptoms (including PTLD) by clinical manifestation, and quality of life and health resource use associated with persistent symptoms among suspected (including confirmed) enrolled LD cases are assessed. This is evaluated at Visit 3 by standardized questionnaires and by patient (or parent(s)/legal guardian(s)) interview. In addition, the investigator performs a clinical assessment at Visit 3 to determine if the patient meets the following PTLD criteria.

- Patient had a final diagnosis of Lyme disease and completed treatment with an appropriate antibiotic regimen with resolution or stabilization of objective manifestations of Lyme disease.

- Patient suffers from debilitating (results in substantial reduction in activities) symptoms of fatigue, generalized musculoskeletal pain, or cognitive difficulties having onset within 6 months after completing therapy and lasting for at least 6 months after onset.

- No concurrent comorbidities can otherwise explain the patient's subjective symptoms.

At Visits 4 and 5, participants with a final diagnosis of LD who had any persistent symptoms documented at the previous visit (Visit 3 or Visit 4, respectively) are asked to return for participant interview, medical record review and to re-assess LD impact on participant-reported physical and mental functions and quality of life.

Patient and Public Involvement

No patients were involved.

ETHICS AND DISSEMINATION

The study is conducted in accordance with the protocol, legal and regulatory requirements, and the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Participants (Council for International Organizations of Medical Sciences [CIOMS], 2002), ICH GCP, and the Declaration of Helsinki.

Consent and assent

The Informed Consent Documents (/assent documents) and any participant recruitment materials follow ICH GCP, local regulatory requirements, and legal requirements, including applicable privacy laws. The investigator, or a person designated by the investigator, obtains written informed consent from each participant (or the participant's legally acceptable representative, parent[s], or legal guardian and the participant's assent, when applicable) before any study-specific activity is performed. Participants may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety, behavioural, or administrative reasons. If the participant withdraws from the study and withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data is collected.

Confidentiality

Measures are taken to ensure protection of participant personal data. Participant names or other directly identifiable data on any sponsor forms, reports, publications, or in any other disclosures, are omitted, except where required by applicable laws. Participant names are removed and replaced by a single, specific numerical code.

Adverse Events (AEs)

All Serious Adverse Events (SAEs) and nonserious AEs that are directly observed and/or spontaneously reported by the participant during the active collection period (2 hours after blood sample collection and 24 hours after skin punch biopsy collection) or outside the active collection period if related to a study procedure are recorded in the CRF. Any SAE that an investigator suspects may be related to any Pfizer product used by the participant under routine care during and outside the active collection period is reported immediately upon awareness, and under no circumstance exceeding 24 hours. All processes comply with country specific regulatory requirements relating to safety reporting to the regulatory authority, IRBs/ECs, and investigators. Reporting of exposure to any Pfizer product during pregnancy or breast feeding applies throughout the active collection period; when required, is reported within 24 hours of investigator awareness.

Dissemination plan

This study has been approved by all sites' local ethics committees in participating countries. Results from this study will be published in peer-reviewed international journals and presented at relevant national and international conferences. Pfizer supports publication by a Principal Investigator (PI) of the results of the study based on information collected or generated by the PI, however, the first manuscript will be a joint publication covering all sites.

AUTHORS' CONTRIBUTIONS:

The study concept and design was developed by EB, AP, JS, BG, NB, ALB, KA and BP. The protocol manuscript was drafted by MB and critically reviewed by EB, KH, ALB, AP, BG, KA and JS.

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COMPETING INTERESTS' STATEMENT.

BB, ALB, AP, EB, JS, KH, LH, BG and KA are employees of Pfizer Inc., and may own Pfizer stock. NB is an employee of Valneva Austria GmbH. BP and MB were University students on placement at Pfizer UK during the BOLD Study.

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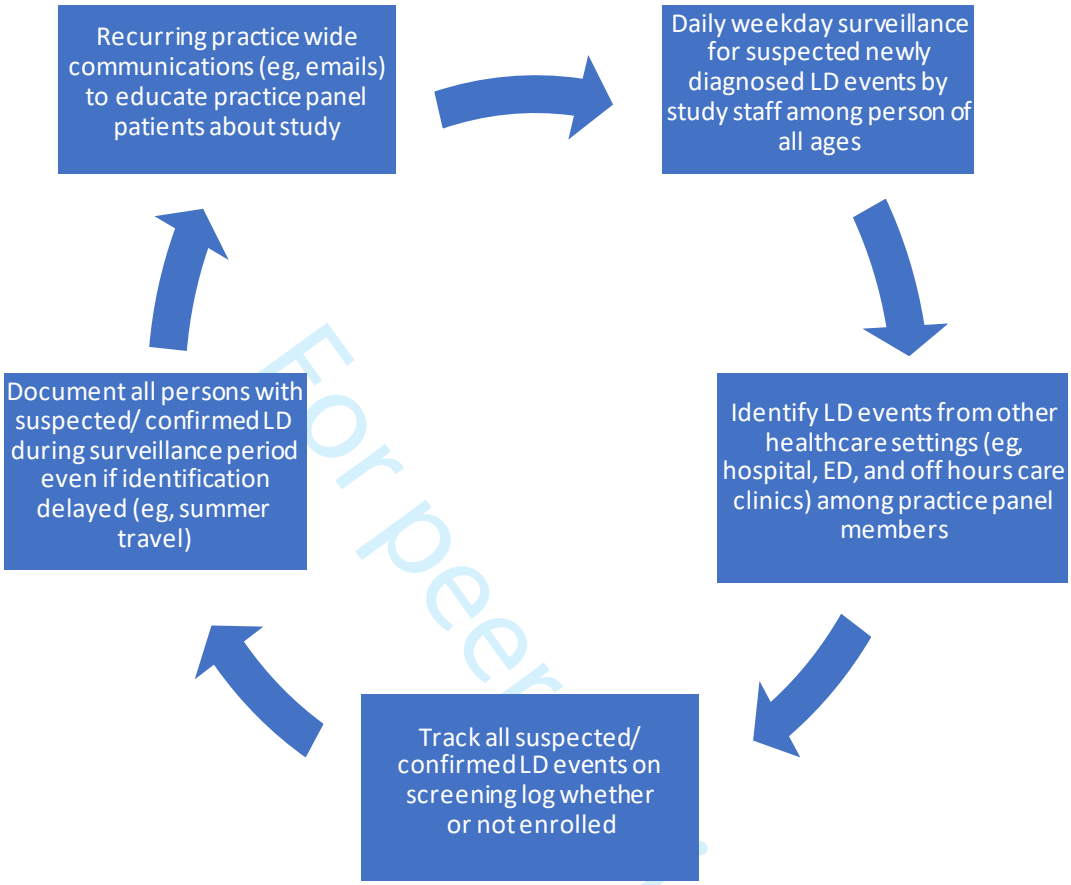

Figure 1 – Active Surveillance Process within GP practice panels

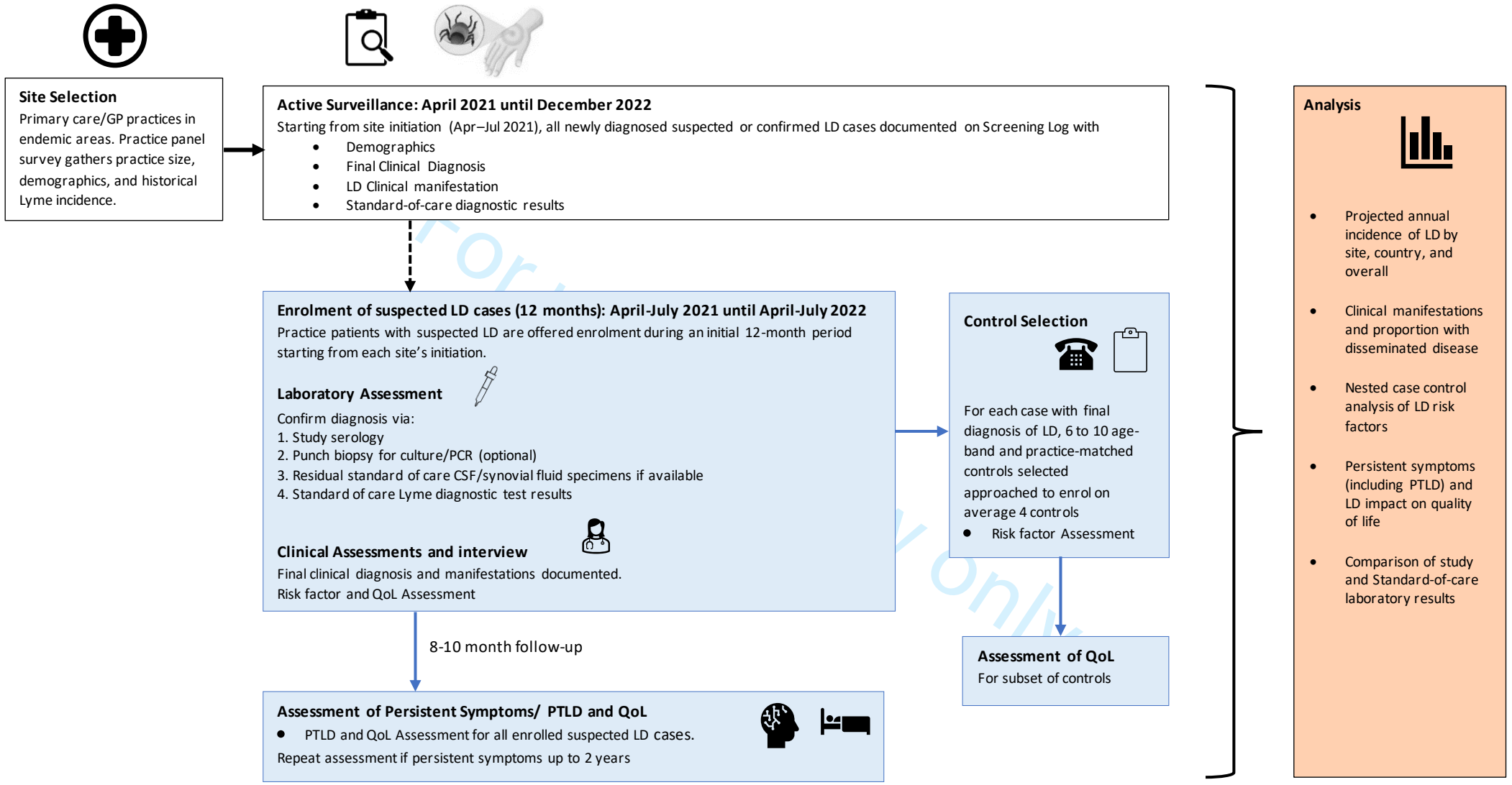

Figure 2 - Design of BOLD Study

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A Prospective Incidence Epidemiology Study Protocol: Conducting Active Surveillance to assess the Burden of Lyme disease (BOLD) in Primary Care Practices in Endemic Areas of Six European countries

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Title Page

TITLE

A Prospective Incidence Epidemiology Study Protocol: Conducting Active Surveillance to assess the Burden of Lyme disease (BOLD) in Primary Care Practices in Endemic Areas of Six European countries

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ABSTRACT

Introduction:

Lyme Disease (LD) is the most frequent tick-borne disease in the moderate climates of Europe. This study will inform the Phase 3 efficacy study for Pfizer and Valneva's investigational Lyme Disease Vaccine, VLA15. VLA15 Phase 3 will be conducted in US and Europe due to the vaccine's serotype coverage and public health burden of LD. In Europe the existence and location of sites that have access to populations with high LD annual incidence is uncertain. This active, prospective surveillance study assesses annual LD incidence at GP/primary care sites, allowing for Phase 3 site vetting and better characterization of LD burden in selected regions for study size calculations.

Methods and analysis:

This Burden of Lyme disease (BOLD) study will assess LD incidence overall and by site at 15 GP/primary care practices in endemic areas of 6 European countries from Spring 2021 until December 2022 and will be summarized with counts (n), percentages (%), and associated 95% confidence intervals. Suspected LD cases identified from site's practice panels are documented on Screening Logs, where clinical LD manifestations, diagnoses, and standard of care diagnostic results are recorded. In the initial 12-month enrolment phase, suspected LD cases are offered enrolment. Participants undergo interview and clinical assessments to establish medical history, final clinical diagnosis, clinical manifestations, and quality of life impact. Study specific procedures include LD serology, skin punch biopsies and Lyme manifestation photographs. For every enrolled participant diagnosed with LD, 6-10 age-matched controls are randomly selected and offered enrolment for an embedded LD risk factor analysis. Persistent symptoms or post-treatment LD will be assessed at follow-up visits up to two years after initial diagnosis while patients remain symptomatic.

Ethics and dissemination:

This study has been approved by all sites' local ethics committees. Results will be presented at conferences and published in peer-reviewed journals.

STRENGTHS AND LIMITATIONS

- This study uses consistent LD case definitions to establish comparative LD incidence from high-incidence areas across 6 European countries.
- LD surveillance is conducted in clearly defined populations (i.e., the practice panel of a primary care provider), allowing for accurate calculation of LD incidence rates.
- The study will follow enrolled LD cases post antibiotic treatment to assess persistent symptoms or Post Treatment Lyme Disease (PTLD).
- The study can only capture LD diagnoses that study site staff are aware of, and thus may miss some or all events only treated outside of the practice due to travel or other reasons.
- Pre-season baseline serology specimens will not be available to assess for seroconversion, therefore asymptomatic Lyme disease infections will not be captured.

INTRODUCTION

Lyme Disease (LD) is the most frequent tick-borne disease in the moderate climates of the northern hemisphere.[1] LD is caused by infection with *Borrelia burgdorferi sensu lato (B.b.s.l.).* There are 18 documented *Borrelia* genospecies, but only a subset has been associated with human disease.[2]

Serotypes (ST) are determined by outer surface protein A (OspA) types. In North America, almost all LD (>98%) is due to *B. burgdorferi senso stricto* (ST1*)*, with minor contribution from *B. mayonii* (1-2%). In Europe, *B. afzelii* (ST2) and *B. garinii* (ST3,5,6) are predominant, but *B. burgdorferi* s.s. (ST1) and *B. bavariensis* (ST4) are also documented.[3] To address the high burden of LD, Pfizer and Valneva are jointly developing a 6-valent vaccine (VLA15) for the prevention of LD caused by *Borrelia* strains expressing outer surface protein A (OspA) ST 1-6 by active immunization.

This prospective epidemiology study will collect key information to support VLA15's Phase 3 efficacy study. While two successful Phase 3 efficacy studies have been previously conducted for other investigational Lyme vaccines, they exclusively involved US sites because those vaccines included Serotype 1 only. [4,5] Due to high medical need in Europe and US, VLA15 includes expanded serotype coverage, so its Phase 3 efficacy study will be conducted in both the US and Europe. In the US, it is established that at least a 1% annual incidence of acute LD is present in high-risk areas.[3] However, due to the heterogeneity of LD surveillance in Europe, uncertainty exists regarding the existence and location of potential Phase 3 efficacy trial sites that would have access to a population with high annual incidence of acute LD from which to enrol Phase 3 study participants. On this basis, this active, prospective surveillance study will identify discrete GP/primary care practice-based sites in potential high-incidence geographical regions and assess their annual LD incidence. This will allow for vetting of potential Phase 3 sites and better characterization of the burden of LD in the region for use in study size calculations.

The quality and quantity of LD incidence data from European countries varies due to consensus case definition not being consistently used and differing reporting procedures.[6] However, with the European Commission adoption of a consensus case definition for Lyme neuroborreliosis in 2018, progress has been made. Annual LD incidence is reported as up to 632 per 100,000 population in Sweden,[7] and the population-weighted incidence in western Europe has been estimated at 22 cases per 100,000 person-years among all ages.[8] However, these composite estimates and national incidence estimates are limited by under-reporting and marked intra-country regional variation.[9] It is therefore difficult to compare incidence among different sites in Europe, either across or within countries, and true LD incidence is not well understood. BOLD's active-surveillance-based incidence estimates from GP/primary care-based sites in endemic regions will allow for better characterization of LD burden in high-incidence regions of 6 European countries.

Following antibiotic treatment for LD, a proportion of patients continue to have persistent symptoms, a subset of which will meet the case definition for PTLD.[10,11] In 2006 guidelines from the Infectious Disease Society of America (IDSA) created a working definition for PTLD with clinical symptoms persisting at least six months after treatment for LD. There is a broad range from 5-20% of patients that continue to suffer from persistent symptoms not meeting PTLD case definition for months to years post-antibiotic treatment.[12,13] Given the heterogeneity and lack of consensus of existing literature, PTLD is poorly characterized in terms of the size of the patient group, severity and duration of symptoms, impact on quality of life, and health care utilization. Thus, BOLD aims to assess the incidence, severity, and duration of persistent symptoms (including PTLD) by clinical manifestation (Erythema migrans versus disseminated LD), as well as the quality of life and health resource use associated with persistent symptoms (including PTLD) among suspected enrolled LD cases. The study also aims to assess the impact of LD on quality of life by comparing suspected LD

cases with persistent symptoms (including PTLD) with age-matched controls to support future cost-effectiveness analysis.

OBJECTIVES AND ENDPOINTS

Objectives and endpoints are classified into primary, secondary, exploratory, and assessment of persistent symptoms of LD including PTLD in Table 1.

Table 1 – BOLD Objectives and Endpoints

Primary Objective:	**Primary Endpoint:**
• To assess LD annual incidence rate in persons of all ages, races, and ethnicities at potential Phase 3 efficacy trial sites for the VLA15 vaccine in geographic regions that are endemic for LD, overall and by site.	• The annual incidence rate of newly diagnosed LD in persons of all ages, races, and ethnicities who are patients of the study sites' GP/primary care practice, overall and by site.
Secondary Objectives:	**Secondary Endpoints:**
• To assess LD annual incidence rate in persons of all ages, races, and ethnicities at potential Phase 3 efficacy trial sites for the VLA15 vaccine in geographic regions that are endemic for LD, by age, month of diagnosis, and LD risk factor.	• The annual incidence rate of newly diagnosed LD in persons of all ages, races, and ethnicities at study sites by age, month of diagnosis, and LD risk factor.
• Describe the *Borrelia* genospecies/OspA serotype distribution of LD in persons of all ages, races, and ethnicities at potential Phase 3 efficacy trial sites for the VLA15 vaccine in geographic regions that are endemic for LD.	• Proportion for each Borrelia genospecies/OspA serotypes of LD among participants with available genospecies/OspA serotype results.
• Describe the proportion of LD cases by clinical manifestation category among persons of all ages, races, and ethnicities at potential Phase 3 efficacy trial sites for the VLA15 vaccine in geographic regions that are endemic for LD, by specific clinical manifestation category (ie, erythema migrans, neuroborreliosis, Lyme arthritis, Lyme carditis, borrelial lymphocytoma, acrodermatitis chronica atrophicans, LD ocular manifestations) and for all disseminated disease combined.	• Proportion of newly diagnosed LD cases by specific clinical manifestation category (ie, erythema migrans, neuroborreliosis, Lyme arthritis, Lyme carditis, borrelial lymphocytoma, acrodermatitis chronica atrophicans, LD ocular manifestations) and for all disseminated disease combined.
• To estimate the proportion of persons of all ages with newly diagnosed LD at potential Phase 3 efficacy trial sites for the VLA15 vaccine who have conditions that would exclude their participation in the proposed Phase 3 efficacy trial sites based on potential exclusion criteria (eg, immunosuppression), overall, by site, by age group, by season, and by exclusion criteria.	• The proportion of participants among persons of all ages, races, and ethnicities with newly diagnosed LD who have conditions that would exclude their participation from the proposed Phase 3 efficacy trial sites based on potential exclusion criteria (eg, immunosuppression), overall, by site, by age group, by season, and by exclusion criteria.
Exploratory Objectives:	**Exploratory Endpoints:**
• Describe the prevalence of LD risk factors (e.g., time outdoors, pets, personal protective behaviors, occupational and leisure exposures) and potential Phase 3 trial exclusion criteria among practice panel patients of all ages, races, and ethnicities without current LD at potential Phase 3 efficacy trial sites for the VLA15 vaccine, overall and by site.	• Proportions of site practice panel patients of all ages, races, and ethnicities without current LD with key characteristics, (eg, self-reported specific LD risk factors and conditions that would exclude their participation from the potential Phase 3 efficacy trial), overall, by age group, and by site.
• Describe signs and symptoms of LD and patient treatment journey for LD under current standard of care.	• Time from symptom onset to diagnosis, duration of symptoms, treatment duration and type, number and type of medical visits and therapeutic procedures, and frequency of hospitalization and mean length of stay.

• Describe LD diagnostic testing practices under current standard of care. • Estimate the ratio of LD incidence based on LD surveillance to LD incidence measured by this study by region and country. • To describe possible LD events with standard of care LD diagnosis without established LD clinical manifestations (ie, erythema migrans, neuroborreliosis, Lyme arthritis, Lyme carditis, borrelial lymphocytoma, acrodermatitis chronica atrophicans, LD ocular manifestations). • To describe LD impact on participants' mental and physical functions and quality of life.	• Proportion of participants with standard of care LD diagnostic testing, overall and by type. • Ratio of LD incidence from local LD surveillance system (in regions where available) to incidence of LD cases at study site(s) in that region. • For standard of care LD diagnoses without established LD clinical manifestations, frequency and duration of symptoms experienced, frequency of physical exam findings by type, and LD diagnostic testing results by type of test. • Scores of physical, mental functions and quality of life measured by 36-Item Short Form Survey (SF-36), degree of pain, severity of pain of different body parts, and degree of fatigue and its specific impact measured by Fatigue Severity Scale (FSS) and Short Form McGill Pain Questionnaire
Objectives for assessment of persistent symptoms of LD including PTLD:	**Endpoints for assessment of persistent symptoms of LD including PTLD:**
• To assess the proportion of suspected Lyme disease (LD) cases, by clinical manifestation (Erythema migrans versus disseminated LD), that subsequently develop persistent symptoms, including PTLD • To assess the severity of persistent symptoms (including PTLD) by clinical manifestation (Erythema migrans and disseminated LD) among suspected LD cases • To compare the severity of symptoms among PTLD cases to those of patients with persistent symptoms that do not meet PTLD case definition • To assess the impact of persistent symptoms (including PTLD) on health-related quality of life (QoL) between suspected LD cases in comparison with age-matched controls • To assess the health resource use associated with persistent symptoms (including PTLD) among suspected LD cases	• Proportion of treated LD cases by clinical manifestation (Erythema migrans versus disseminated LD that subsequently develop persistent symptoms, including PTLD • Severity of persistent symptoms (including PTLD) by clinical manifestation (Erythema migrans and disseminated LD) among suspected LD cases: Pain severity (Short Form McGill Pain Questionnaire [SF-MPQ], and the pain subscale of the Medical Outcomes Survey Short Form-36 [SF-36]); Fatigue Severity (Fatigue Severity Scale [FSS]); Cognitive impairment (Cognitive Failures Questionnaire [CFQ]). • Duration of persistent symptoms (including PTLD) by clinical manifestation (Erythema migrans and disseminated LD) • Symptom severity by subgroup (PTLD cases compared to treated LD cases with symptoms not meeting PTLD case definition, and participants with other non LD diagnosis. SF-36, SF-MPQ, FSS and CFQ subscale scores and summary scores. • Treatment duration and type, number and type of medical visits and therapeutic procedures, and frequency of hospitalization, and mean length of stay.

1

METHODS AND ANALYSIS

Study Design

This prospective, epidemiological study uses active surveillance to measure the annual LD incidence of newly diagnosed LD at 15 GP/primary care practices in 6 European countries: Czech Republic, Germany, Poland, Slovenia, Slovakia, and Sweden. A nested case control analysis is embedded within the study to assess LD risk factors. The BOLD study was initiated in April 2021 and LD surveillance will continue through to the end of 2022. There is an initial 12-month study enrolment phase starting from the sites' activation where suspected LD cases identified are offered study enrolment (Supplemental Figure). In a second phase, enrolment ends but LD surveillance continues. Enrolled suspected LD cases are followed up to two years after enrolment to assess any persistent symptoms, and the impact of LD on quality of life by comparing suspected LD cases with persistent symptoms (including PTLD cases) with age-matched controls.

Site Selection

Study sites are embedded in GP/primary care practices and the "practice panel" will serve as the denominator for the incidence estimate. A practice panel is defined as all persons of any age enrolled in the primary care practice for routine outpatient care (registered to GP practice or healthcare contact with the practice in the last 2 years). All European countries were considered for this study but based on a review of literature and surveillance data, feasibility efforts were only conducted in 11 countries with over 250 sites reviewed and contacted. Selected study sites needed to have the clinical research infrastructure to conduct a vaccine clinical trial to potentially serve as study sites for the VLA15 Phase 3 efficacy trial. Feasibility questionnaires and pre-trial assessments were conducted at potential study sites to ascertain practice panel size and annual LD incidence in the previous 12-month period. Sites were not selected if annual LD incidence was less than 0.5% according to the pre-trial assessments (based on requirements for feasible Phase 3 efficacy trial sample size), the site was not a primary care clinic, or the site's research infrastructure was inadequate. Subsequently, BOLD was able to select 20 GP/primary care practices in 6 European countries: Czech Republic, Germany, Poland, Slovenia, Slovakia, and Sweden. Of these 20 sites, all were initiated and 5 were subsequently closed – largely for operational issues. Fifteen sites remain active across these 6 countries, with 5 in Germany, 3 in Czech Republic, 3 in Poland, 2 in Slovakia, and 1 each in Sweden and Slovenia.

Active Surveillance

The study's primary focus is measuring LD incidence starting from the sites' activation in April–July 2021 and continuing until the end of 2022 through active surveillance of all suspected LD cases (Supplemental Figure). While most LD is diagnosed in the primary care setting, investigators seek to identify LD events from other settings (e.g., hospital, emergency department) via their routine methods for tracking the healthcare contacts of practice panel patients. Medical records are searched for any key words e.g., ICD codes (International Statistical Classification of Diseases) as well as diagnoses/terms that are used for LD locally as part of daily weekday surveillance (Supplemental Table). Each site maintains a Screening Log to support complete identification of possible LD events. This is documented weekly by site personnel with information including demographic, LD diagnosis and manifestations and Standard of Care (SOC) laboratory data, if applicable. Additionally, standardized training regarding screening and diagnosis of LD based on established clinical best practices was provided to site personnel. The first 12-months of the active surveillance period,

starting from each sites' activation, is an enrolment phase when all suspected LD cases identified are offered study enrolment (Fig. 1). During this period, the Screening Log also includes information relating to patient consent and enrolment.

Eligibility

LD case participant Inclusion Criteria

During the 12-month enrolment period, patients must meet all of the following criteria to be enrolled in the consented portion of the study:

1. Member of participating patient practice.
2. Suspected or confirmed newly diagnosed LD during enrolment period regardless of timing of infection.
3. Evidence of a personally signed and dated informed consent and assent (when age-appropriate and per local requirements) document indicating that the patient (or a legally acceptable representative) has been informed of all pertinent aspects of the study in an age-appropriate manner and that they agree to participate.

There are no exclusion criteria for the LD case participants.

Control Participant Inclusion Criteria

Control participants must meet all of the following inclusion criteria to be eligible for inclusion in the study:

1. Member of participating patient practice at time of associated case diagnosis.
2. Evidence of informed consent and assent (when age-appropriate and per local requirements) indicating that the patient (or a legally acceptable representative) has been informed of all pertinent aspects of the study and that they agree to participate.

Control Participant Exclusion Criteria

Control participants meeting any of the following criteria will not be included in the study:

1. Active Lyme Disease in last 90 days

Controls were selected as soon as feasible after enrolment of the related LD case and those who later became a LD case were retained as a control if there were no LD associated symptoms at the time of control enrolment or other evidence of infection (e.g., serological seroconversion).

Study Visits

Study specific procedures are performed at up to five visits for LD cases (Table 2). Visits 4-5 are for participants with a final diagnosis of LD who had any persistent symptoms documented at the previous visit. Controls are seen at Contact 1, and a selection of controls will have Contact 2.

Table 2 – BOLD Study Specific Procedures

	LD Case Participants					Controls	
Procedure/ Assessment	**Visit 1 Day 1[a]**	**Visit 2 Day 28**	**Visit 3 Month 10[b]**	**Visit 4 Month 16-18[c]**	**Visit 5 Month 22-24[c]**	**Contact 1 Day 1**	**Contact 2 Month 16-18**
Screening, Demographics, and Informed Consent/assent	X		X	X	X	X	X
Confirm eligibility (inclusion criteria)	X					X	
Patient (or parent/legal guardian) interview, including symptoms and LD risk factors	X	X	X	X	X	X	
Study blood sample for Lyme serology, and scavenge residual SOC cerebral spinal fluid and synovial fluid specimens if available	X	X	X				
Photograph of LD manifestations, and two 2mm skin punch biopsies of any LD-related rash	X						
Chart review to collect details of current illness, and standard of care (SOC) physical exam findings	X	X	X	X	X		
Collect pre-specified medical history of clinical significance including past LD diagnoses	X	X	X			X	X
Collect SOC LD diagnostic laboratory testing results	X	X	X	X	X		
Collect LD treatment and healthcare resource utilization, and LD event outcome	X	X	X	X	X		
Record clinical diagnosis and LD manifestation categories experienced based on clinical assessment	X	X	X				
Record clinical assessment of persistent symptoms/PTLD			X	X	X		
Collect Charlson Comorbidity Index (CCI) information			X				X
Collect health survey outcome information							X
Assess Adverse Events (AEs) 2 hours after blood draw and 24 hours after skin punch biopsy	X	X	X	X	X		
Assess interest in participation in follow-up studies, and the potential for the participant to meet Phase 3 exclusion criteria	X	X	X			X	

a. If the participant is ≥21 days after LD diagnosis at Visit 1, then Visit 2 data collection will be performed at Visit 1 and no separate Visit 2 will be performed.
b. Visit 3 will take place approximately 9 – 10 months after Visit 2. The latter part of the visit window could be extended up to 12 months after Visit 2 if the participant's persistent symptoms have not reached a 6-months duration after the completion of antibiotic therapy. Participants who did not have a separate Visit 2 will have Visit 3 approximately 9-10 months after Visit 1.
c. Participants who had any persistent symptoms (including PTLD) documented at Visit 3 will be invited for long term follow-up at approximately 6-8 months (Visit 4) and 12-14 months (Visit 5) after Visit 3. Participants are interviewed, have medical record review performed, and LD event outcome will be re-assessed.

Study Specific Procedures and Laboratory Testing

Collected serological samples are tested at Pfizer laboratories using Pfizer's modified two-tiered testing (PMTTT) which consists of two separate Lyme Diagnostic immunoassays. Serum samples with positivity in the tier 1 test (BioRad Lyme Total assay) are then tested in the second test (Zeus Lyme Total assay). A sample must be positive in both tests to be considered diagnostically positive for Lyme Disease. Skin biopsies for *Borrelia* culture and qPCR are performed on participants > 1 year of age who have a LD-related rash and consent to the procedure. Punch biopsy specimens are assessed, for positivity, by a Borrelia 16S qPCR assay and microbiological positivity for the presence of *Borrelia* spirochete through darkfield microscopy and further characterized to genospecies and OspA serotype by sequencing. SOC Lyme diagnostic laboratory results are collected including but not limited to serology with ELISA and/or immunoblots, culture and PCR for *Borrelia* from specimen, histology and neurological, dermatological and/or rheumatological assessments. If CSF and/or synovial fluid samples are obtained from a participant for SOC testing, site staff should request that the laboratory retain any residual sample after SOC testing. These samples may be analysed for antibodies against different borrelial antigens by various immunoassay techniques or for the presence of borrelial molecules by different biochemical techniques, immunoassays and/or nucleic acid sequences by PCR. Specimen processing and testing will be conducted at designated central laboratories and/or Pfizer (401 N Middletown Rd, Pearl River, NY 10965, United States). Photograph (s) of Lyme manifestations will be obtained and used to support Lyme diagnosis.

Participants' LD event outcome, including mental and physical functions and quality of life are measured by self-completed/assisted surveys including: SF-36 standard form, degree of pain, severity of pain of different body parts, and degree of fatigue and its specific impact measured by the Short Form McGill Pain Questionnaire and the Fatigue Severity Scale (FSS), respectively. At Visit 3 (9-10 months after Visit 2), a questionnaire on neurocognitive dysfunction (Cognitive Failures Questionnaire; CFQ) is added for assessment of persistent symptoms. Persistent symptoms and PTLD are evaluated by standardized questionnaires and by patient (or parent(s)/legal guardian(s)) interview at Visit 3. In addition, the investigator performs a clinical assessment to determine if the patient meets PTLD criteria.

At Visits 4 and 5, participants with a final diagnosis of LD who had any persistent symptoms documented at the previous visit (Visit 3 or Visit 4, respectively) are asked to return for participant interview, medical record review and to re-assess LD impact on participant-reported physical and mental functions and quality of life, as measured by 36-Item Short Form Survey (SF-36), degree of fatigue measured by Fatigue Severity Scale (FSS), degree of pain measured by the Short Form McGill Pain Questionnaire, and degree of cognitive difficulties measured by the Cognitive Failures Questionnaire (CFQ).

Participants may decline study-specific procedures and remain enrolled, allowing for complete tracking of all LD clinical diagnoses and capture of standard of care diagnosis data.

Controls

To obtain incidence estimates by LD risk factors and proposed Phase 3 exclusion criteria, information on unaffected controls is obtained to allow for a nested case control analysis. Adjusted odds ratios for key characteristics obtained from this analysis and estimated LD incidence will be used to calculate incidence estimates for these characteristics. To achieve this, for each enrolled participant

with final LD diagnosis, six practice panel patients without current LD are approached regarding enrolling as control participants to collect the following information: demographic information (age, sex), risk factors for LD (e.g. time outdoors, pets, personal protective behaviours, occupational and leisure exposures), past history for tick-borne disease including LD, TBE, tick bite prophylaxis and known tick bites, interest in investigational LD vaccine study participation, and assessment of meeting potential Phase 3 exclusion criteria (Table 2). If the proportion of potential control participants declining participation is higher than anticipated, the number of potential controls approached will be increased to 10 so approximately 4 control participants are enrolled per LD event. The Screening Log tracks control selection, consent and enrolment.

To assess the impact of LD on quality of life, for each enrolled LD case participant with a final diagnosis of LD, one of the age-matched control participants who had Contact 1 performed is re-consented 16-18 months later to collect health survey outcome information. This includes a 36-Item Short Form Survey (SF-36), degree of fatigue measured by Fatigue Severity Scale (FSS), degree of cognitive difficulties measured by the Cognitive Failures Questionnaire (CFQ), and degree of pain measured by the Short Form McGill Pain Questionnaire as well as to assess pre-specified medical history and comorbidities.

Control participants may complete interview questions via telephone, other remote means, or in person visit.

Sample Size Estimates

Study size is based on feasibility, not on hypothesis testing as this is a descriptive study. It is expected that approximately 0.5% of practice panel participants per year will be newly diagnosed with LD. Approximately 80% of potentially eligible participants are expected to meet inclusion criteria and agree to enrol. We estimate that on average approximately 25% more participants with suspected LD events will need to be enrolled to identify all events with a final LD diagnosis. Assuming an average practice size of 5,000, we expect approximately 500 participants with suspected/confirmed LD to be enrolled across 20 sites. Among those, we expect approximately 75% to have EM or other rash and 30% of those to consent to skin punch biopsies, thus overall ~113 participants will have ~226 punch biopsy specimens.

6-10 potential control participants are approached for each enrolled participant with a final clinical diagnosis of LD, with approximately 75% (4.5 controls per case) expected to enrol. If 500 participants are enrolled, approximately 90% of these will have final clinical diagnosis of LD (no laboratory confirmation required), yielding an estimate of 2,025 controls enrolled.

Data Analysis

Analysis of Endpoints

For proportion endpoints, data will be summarized with counts (n), percentages (%), and associated 95% confidence interval (CI)s, which will be calculated using the Clopper-Pearson method. Frequency of Research Related Injuries (RRIs) and adverse events (AEs) following study procedures will be tabulated. Results for cases and control participants will be presented separately. For the primary endpoint, data will be summarized overall, by site, by country and by province. The population denominator will be based on the size of the primary care practices' patient panels. All suspected LD cases with final clinical diagnosis of LD will be included in incidence estimates and the

contribution of each case type will be completely delineated. The numerator will be the number of newly identified clinically diagnosed LD cases (to be captured from electronic Case Report Form and Screening Log) occurring in the active surveillance period for each site. The annual incidence will first be calculated as a fraction (numerator ÷ denominator) and then expressed as a rate per 100,000 population by multiplying the fraction by 100,000. The incidence will be calculated for 2021, 2022, 2021-2022, and for one year following the surveillance start date of each site. When estimating the incidence for 2021, where surveillance is conducted for less than the full LD surveillance year, an adjustment will be used to account for the proportion of the surveillance year when surveillance was not conducted. The adjustment will be based on the proportion of clinically diagnosed LD cases reported by each participating site in 2019 and 2020 by month during the time period when there was no surveillance. Annual incidence estimates by age group, sex, month of diagnosis will also be calculated using administrative information from the practice to estimate these subpopulation sizes (ie, subgroup denominators). We do not plan to conduct subgroup analyses by race or ethnicity due to sample size limitations.

Nested Case Control Analysis

In the nested case control analysis, multivariate conditional logistic regression (and/or other multivariate analysis approach) will be used to calculate the adjusted odds ratios (and/or other measure of effect size) for LD risk factors, Phase 3 exclusion criteria, and history of LD. Using these adjusted odds ratios, estimated annual LD incidence obtained from the practice panel from LD surveillance, and distributions of specific risk factors in the LD case group; incidences for each specific characteristic can be calculated.

LD Surveillance Case Definitions

Suspected LD cases are assigned a final diagnosis based on clinical assessment and LD clinical manifestations will be recorded in line with the categories in Table 3. All presentations listed in Table 3 are considered disseminated LD except EM (unless multiple EM lesions are present). These definitions are derived from consensus case definitions originally developed by EUCALB (European Union Concerted Action on Lyme Disease) in 1996, and subsequently updated in 2011.[2,14] Laboratory confirmation primarily comes from at least one dedicated specimen collected specifically for the study.

1 *Table 3 LD Case Definitions*

Presentation	Sign/Symptom (Detailed Definition)	Diagnostic(s)[a]
Erythema Migrans[b]	• Characteristic red or bluish-red patch, with or without central clearing[d] (Lesion should be photographed.)[c]	• Positive IgG/IgM on serum antibody testing OR • Positive PCR of Bbsl result from skin biopsy OR • Positive Culture of Bbsl from skin biopsy
Borrelial Lymphocytoma[b]	• Painless bluish red nodule or plaque, usually on ear lobe, ear helix, nipple, or scrotum (Nodule/plaque should be photographed.)[c]	• Positive IgG/IgM on serum antibody testing OR • Positive PCR of Bbsl result from biopsy OR • Positive Culture of BbsI from biopsy
Acrodermatitis Chronica Atrophicans[b]	• Long-standing red or bluish-red lesions, usually on the extensor surfaces of extremities. Initially doughy swelling. Possible skin induration and fibroid nodules over bony prominences. (Nodule/plaque/lesion should be photographed.)[c]	• Positive IgG/IgM on serum antibody testing OR • Positive PCR of Bbsl result from biopsy OR • Positive Culture of BbsI from biopsy
Lyme Neuroborreliosis[b]	• Meningo-radiculitis (Bannwarth syndrome), facial palsy, meningitis, encephalomyelitis, OR cerebral vasculitis (Clinical manifestation [eg, facial palsy] should be photographed if applicable.)[c]	• Intrathecal IgM and/or IgG antibodies OR • Positive intrathecal anti-Borrelia antibody index (CSF vs Serum) reflecting intrathecal antibody production OR • Positive PCR of Bbsl result from cerebrospinal fluid OR • Positive Culture of BbsI from cerebrospinal fluid

Presentation	Sign/Symptom (Detailed Definition)	Diagnostic(s)[a]
Lyme Carditis[b]	• Acute onset of high degree atrioventricular conduction disturbances, rhythm disturbances, myocarditis, OR pancarditis	• Positive IgG/IgM on serum antibody testing
Lyme Arthritis[b]	• Marked swelling in one or few large joints, most often the knee. (Clinical manifestation (eg, swollen joint) should be photographed.)[c]	• Positive IgG/IgM on serum antibody testing OR • Positive PCR of Bbsl result from synovial fluid or tissue OR • Positive Culture of BbsI from synovial fluid or tissue
LD Ocular Manifestations[b]	• Conjunctivitis, uveitis, papillitis, episcleritis, OR keratitis	• Positive IgG/IgM on serum antibody testing OR • Positive PCR of Bbsl result from ocular fluid OR • Positive Culture of BbsI from ocular fluid

a. *Laboratory confirmation will primarily come from dedicated specimens collected specifically for the study (ie, serum and skin biopsy specimens)*

b. *These clinical manifestations categories are disseminated Lyme manifestations. EM will only be considered disseminated if there are multiple EM lesions.*

c. *Photos will be taken in a manner as to not identify the participant and may be declined at the time of the procedure by the participant.*

d. *EM cases can be diagnosed on the basis of clinical symptoms alone.*

Persistent symptoms and PTLD Case Definition

Participants are considered to have PTLD at Visit 3 (9-10 months after Visit 2) if they continue to have persistent symptoms of LD and meet the case definition defined by the Infectious Disease Society of America (IDSA) as clinical symptoms persisting at least six months after LD treatment.[10] The case definition for PTLD is described in Table 4.

Table 4 PTLD Case Definition

Presentation	Sign/Symptom (Detailed Definition)	Evaluations(s)
Post-treatment Lyme Disease	• Prior documented case of clinically confirmed Lyme Disease as per definitions in Table 3. • Treatment with accepted antibiotic regimen with resolution or stabilization of objective manifestations of LD	• Final diagnosis of LD (clinical diagnosis only or laboratory-confirmed)
	• Fatigue, musculoskeletal pain, and cognitive difficulties that begin within 6 months and last for 6 months after completion of antibiotic therapy	• Participants with questionnaire scores exceeding the cut-off scores for fatigue (FSS mean score of 4 or higher), pain (SF-36, pain subscale, score 55 or lower),[15] or neurocognitive functioning (CFQ score 44 or higher), that begin within 6 months and last for 6 months after completion of antibiotic therapy, as assessed by aforementioned questionnaires.[16]
	• Subjective symptoms are so severe that result in substantial reduction in activities • No other co-morbidities that can explain illness	• Subjective symptoms result in reduction of activities as assessed by either the "limitations in physical activities" subscale (score 55 or lower) of the SF-36.[15]

Persistent Symptoms of Lyme Disease

The incidence and severity of persistent symptoms (including PTLD) by clinical manifestation, and quality of life and health resource use associated with persistent symptoms among suspected (including confirmed) enrolled LD cases are assessed. This is evaluated at Visit 3 by standardized questionnaires and by patient (or parent(s)/legal guardian(s)) interview. In addition, the investigator performs a clinical assessment at Visit 3 to determine if the patient meets the following PTLD criteria.

- Patient had a final diagnosis of Lyme disease and completed treatment with an appropriate antibiotic regimen with resolution or stabilization of objective manifestations of Lyme disease.

- Patient suffers from debilitating (results in substantial reduction in activities) symptoms of fatigue, generalized musculoskeletal pain, or cognitive difficulties having onset within 6 months after completing therapy and lasting for at least 6 months after onset.

- No concurrent comorbidities can otherwise explain the patient's subjective symptoms.

At Visits 4 and 5, participants with a final diagnosis of LD who had any persistent symptoms documented at the previous visit (Visit 3 or Visit 4, respectively) are asked to return for participant interview, medical record review and to re-assess LD impact on participant-reported physical and mental functions and quality of life.

Patient and Public Involvement

No patients were involved.

ETHICS AND DISSEMINATION

The study is conducted in accordance with the protocol, legal and regulatory requirements, and the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Participants (Council for International Organizations of Medical Sciences [CIOMS], 2002), ICH GCP, and the Declaration of Helsinki.

The ethics committees that approved this study are as follows:

Ethical commission of IKEM and FTN, Faculty of Thomayer Hospital (Prague, Czechia)

Ethics Committee at the State Medical Association of Hesse (Frankfurt, Germany), Schleswig-Holstein (Bad Segeberg, Germany), and Saxony (Dresden, Germany)

Bioethics Committee at the Lublin Medical Chamber (Lublin, Poland), Medical University of Bialystok and Regional Bialystok Medical Chamber (Bialystok, Poland)

Ethical Commission of the Trencin Self-governing region (Trencin, Slovakia)

The Commission of the Republic of Slovenia for Medical Ethics (Ljubljana, Slovenia)

The Ethics Review Authority Box 2110 (Uppsala, Sweden)

Consent and assent

The Informed Consent Documents (/assent documents) and any participant recruitment materials follow ICH GCP, local regulatory requirements, and legal requirements, including applicable privacy laws. The investigator, or a person designated by the investigator, obtains written informed consent from each participant (or the participant's legally acceptable representative, parent[s], or legal guardian and the participant's assent, when applicable) before any study-specific activity is performed. Participants may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety, behavioural, or administrative reasons. If the participant withdraws from the study and withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data is collected.

Confidentiality

Measures are taken to ensure protection of participant personal data. Participant names or other directly identifiable data on any sponsor forms, reports, publications, or in any other disclosures, are

omitted, except where required by applicable laws. Participant names are removed and replaced by a single, specific numerical code.

Adverse Events (AEs)

All Serious Adverse Events (SAEs) and nonserious AEs that are directly observed and/or spontaneously reported by the participant during the active collection period (2 hours after blood sample collection and 24 hours after skin punch biopsy collection) or outside the active collection period if related to a study procedure are recorded in the CRF. Any SAE that an investigator suspects may be related to any Pfizer product used by the participant under routine care during and outside the active collection period is reported immediately upon awareness, and under no circumstance exceeding 24 hours. All processes comply with country specific regulatory requirements relating to safety reporting to the regulatory authority, IRBs/ECs, and investigators. Reporting of exposure to any Pfizer product during pregnancy or breast feeding applies throughout the active collection period; when required, is reported within 24 hours of investigator awareness.

Dissemination plan

This study has been approved by all sites' local ethics committees in participating countries. Results from this study will be published in peer-reviewed international journals and presented at relevant national and international conferences. Pfizer supports publication by a Principal Investigator (PI) of the results of the study based on information collected or generated by the PI, however, the first manuscript will be a joint publication covering all sites.

AUTHORS' CONTRIBUTIONS:

The study concept and design was developed by EB, AP, JS, BG, NB, ALB, KA and BP. The original protocol was written by EB and amended by ALB and MD. The protocol manuscript was drafted by MB and critically reviewed by EB, AP, ALB, LH, JS, KH, MD, NB, BP, KA and BG.

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COMPETING INTERESTS' STATEMENT.

EB, ALB, AP, EB, JS, KH, LH, BG and KA are employees of Pfizer Inc., and may own Pfizer stock. NB is an employee of Valneva Austria GmbH. BP and MB were University students on placement at Pfizer UK during the BOLD Study.

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FIGURE LEGEND:

Figure 1 – Design of BOLD Study.

SUPPLEMENTAL TABLES AND FIGURES:

Supplemental Table - Lyme Disease and related diagnoses with relevant International Classification of Diseases (ICD; procedure codes used in medical billing) codes if available.

Supplemental Figure – Active Surveillance Process within GP practice panels.

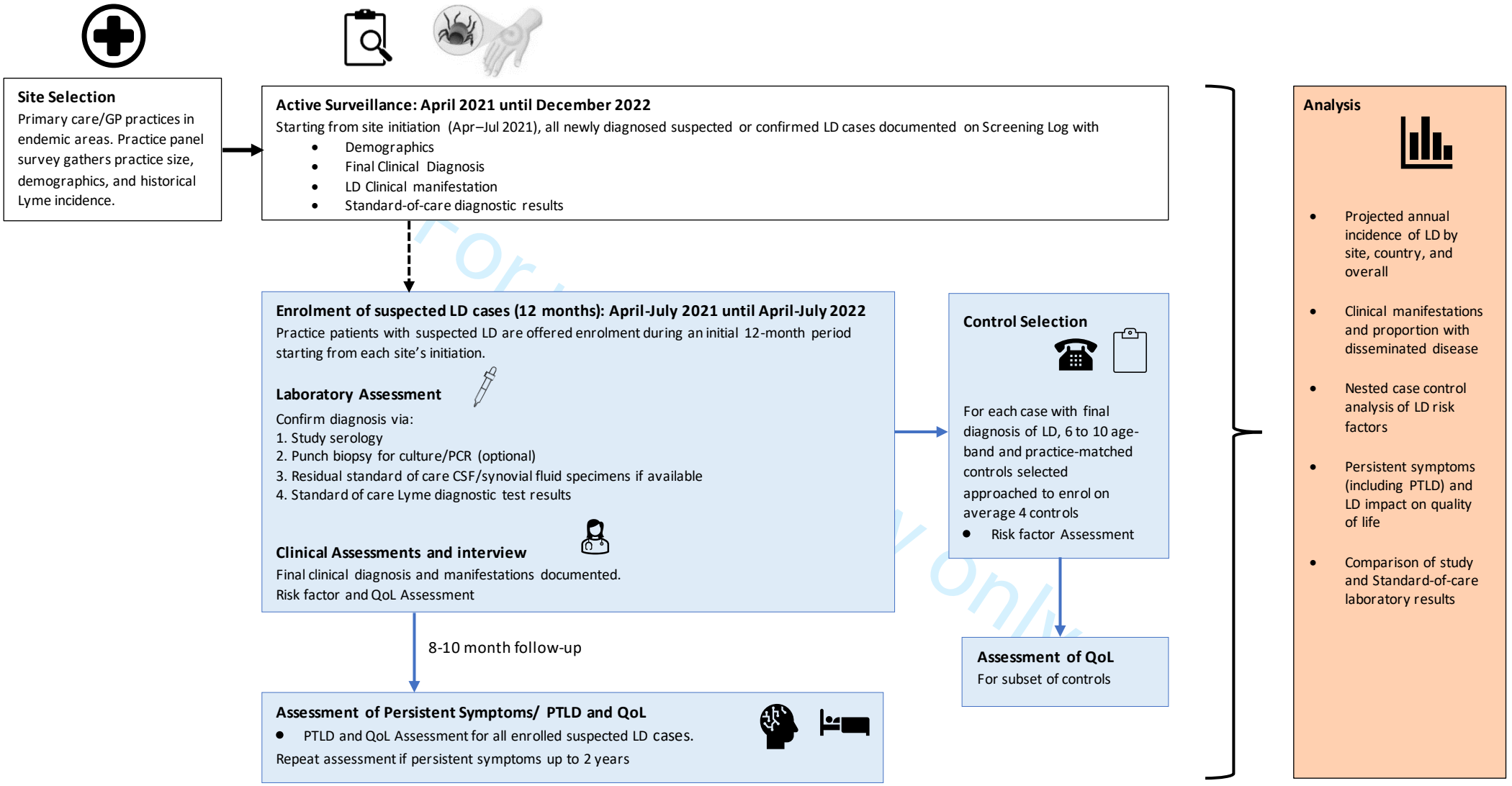
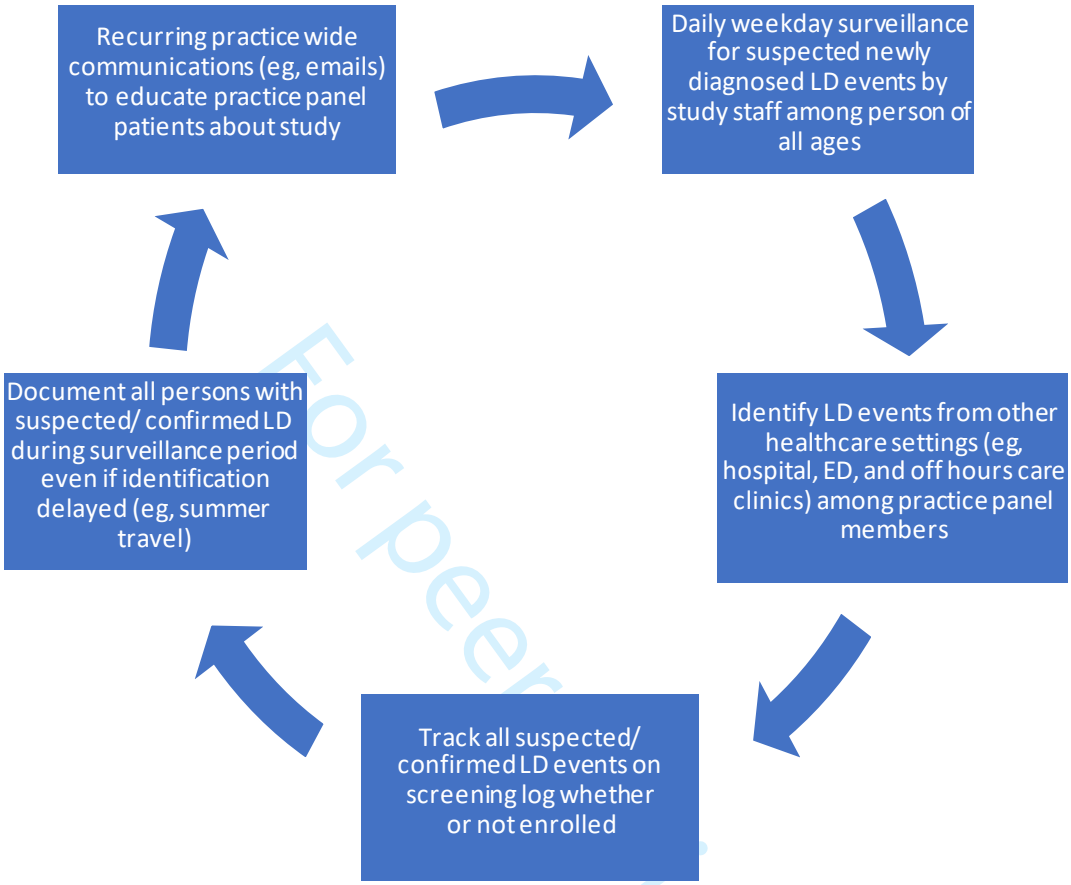


Figure 1 - Design of BOLD Study



Supplemental Figure – Active Surveillance Process within GP practice panels

Supplemental Table - Lyme Disease and related diagnoses with relevant International Classification of Diseases (ICD; procedure codes used in medical billing) codes if available.

Condition	ICD9 Code	ICD10 Code
Lyme disease	088.81	A69.2
Lyme borreliosis	NA	NA
Erythema migrans	NA	NA
Acrodermatitis atrophicans chronica	701.8	L90.4
Lyme arthritis	NA	A69.23, M01.2
Lyme neuroborreliosis	NA	A69.22
Borrelial lymphocytoma	NA	NA
Lyme carditis	NA	NA
Lyme-related intraocular inflammation	NA	NA
Bell's Palsy	351.0	G51.0
Various neuritis, neuropathy, and nerve disorders	351.8-357.89	G51.8-G61.89

NA = not available

a. *Records are searched using diagnoses/key words from the Table above as well as any diagnoses/terms that are used for LD locally.*