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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 III efficacy study for Pfizer and Valneva's investigational Lyme disease vaccine, VLA15. VLA15 phase III will be conducted in the USA 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 general practice (GP)/primary care sites, allowing for phase III site vetting and better characterisation 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 to December 2022 and will be summarised with counts (n), percentages (%) and associated 95% CIs. 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 2 years after initial diagnosis, while patients remain symptomatic.

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

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study uses consistent Lyme disease (LD) case definitions to establish comparative LD incidence from high-incidence areas across six European countries.
⇒ LD surveillance is conducted in clearly defined populations (ie, 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 LD.
⇒ 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.
⇒ Preseason baseline serology specimens will not be available to assess for seroconversion, therefore asymptomatic LD 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*. 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 six-valent vaccine (VLA15) for the prevention of LD caused by *Borrelia* strains expressing OspA ST 1–6 by active immunisation.

This prospective epidemiology study will collect key information to support VLA15's phase III efficacy study. While two successful phase III 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 the USA, VLA15 includes expanded serotype coverage, so its phase III efficacy study will be conducted in both the USA and Europe. In the USA, 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 III efficacy trial sites that would have access to a population with high annual incidence of acute LD from which to enrol phase III study participants. On this basis, this active, prospective surveillance study will identify discrete general practice (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 III sites and better characterisation 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 intracountry 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. Burden of Lyme disease (BOLD) active surveillance-based incidence estimates from GP/primary care-based sites in endemic regions will allow for better characterisation of LD burden in high-incidence regions of six 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 post-treatment Lyme disease (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 6 months after treatment for LD. There is a broad range from 5% to 20% of patients that continue to suffer from persistent symptoms not meeting PTLD case definition for months to years postantibiotic treatment.[12 13] Given the heterogeneity and lack of consensus of the existing literature, PTLD is poorly characterised in terms of the size of the patient group, severity and duration of symptoms, impact on quality of life and healthcare utilisation. Thus, BOLD aims to assess the incidence, severity and duration of persistent symptoms (including PTLD) by clinical manifestation (erythema migrans vs 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.

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: the 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 (online supplemental figure). In a second phase, enrolment ends but LD surveillance continues. Enrolled suspected LD cases are followed up to 2 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 III efficacy trial. Feasibility questionnaires and pretrial assessments

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 III 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 III 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 III 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 III 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 III efficacy trial sites for the VLA15 vaccine who have conditions that would exclude their participation in the proposed phase III 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 III 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 (eg, time outdoors, pets, personal protective behaviours, occupational and leisure exposures) and potential phase III trial exclusion criteria among practice panel patients of all ages, races and ethnicities without current LD at potential phase III 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 III 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 hospitalisation 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 QoL.	► Scores of physical, mental functions and QoL measured by SF-36, degree of pain, severity of pain of different body parts and degree of fatigue and its specific impact measured by FSS and SF-MPQ.
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 LD cases, by clinical manifestation (erythema migrans vs disseminated LD), that subsequently develop persistent symptoms, including PTLD.	► Proportion of treated LD cases by clinical manifestation (erythema migrans vs 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 (SF-MPQ and the pain subscale of the Medical Outcomes SF-36); fatigue severity (FSS); cognitive impairment (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 QoL between suspected LD cases in comparison with age-matched controls.	► Symptom severity by subgroup (PTLD cases compared with 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 hospitalisation, and mean length of stay.

BOLD, Burden of Lyme disease; CFQ, Cognitive Failures Questionnaire; FSS, Fatigue Severity Scale; GP, general practice; LD, Lyme disease; OspA, outer surface protein A; PTLD, post-treatment Lyme disease; QoL, quality of life; SF-36, 36-Item Short Form Survey; SF-MPQ, Short Form McGill Pain Questionnaire.

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 pretrial assessments (based on requirements for feasible phase III 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: the 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 six countries, with five in Germany, three in the Czech Republic, three in Poland, two in Slovakia and one 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 (online supplemental figure). While most LD is diagnosed in the primary care setting, investigators seek to identify LD events from other settings (eg, hospital, emergency department) via their routine methods for tracking the healthcare contacts of practice panel patients. Medical records are searched for any keywords, for example, International Statistical Classification of Diseases (ICD) codes as well as diagnoses/terms that are used for LD locally as part of daily weekday surveillance (online 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, standardised 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 (figure 1). During this period, the screening log also includes information relating to patient consent and enrolment.

Eligibility

LD case participants' 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.

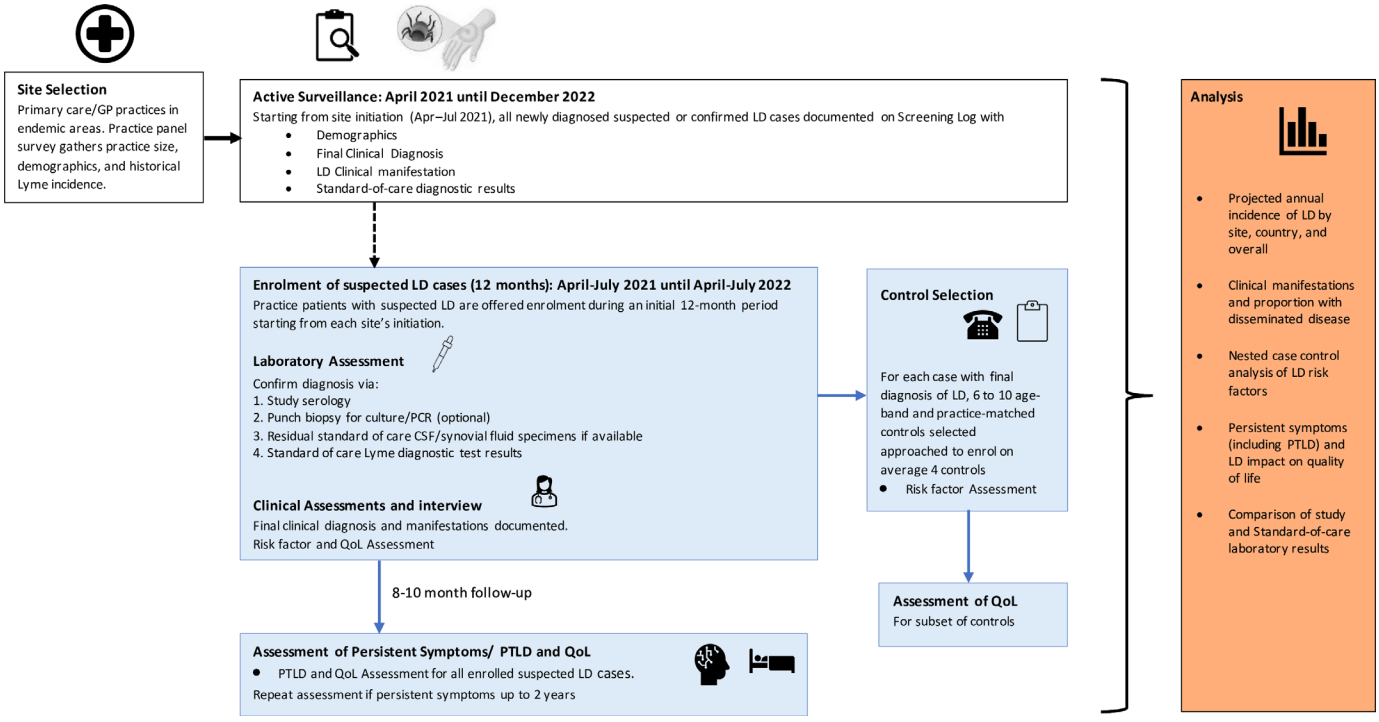

Figure 1 Design of burden of Lyme disease study. CSF, cerebrospinal fluid; GP, general practice; LD, Lyme disease; PTLD, post-treatment Lyme disease; QoL, quality of life.

Control participants' 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 participants' 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 an 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 (eg, 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.

Study-specific procedures and laboratory testing

Collected serological samples are tested at Pfizer laboratories using Pfizer's modified two-tiered testing 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 LD. Skin biopsies for *Borrelia* culture and qPCR are performed on participants >1 year of age who have an 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 characterised 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 cerebrospinal fluid (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, New York, USA). 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: 36-Item Short Form Survey (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 standardised 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 reassess LD impact on participant-reported physical and mental functions and quality of life, as measured by SF-36, degree of fatigue measured by the FSS, degree of pain measured by the Short Form McGill Pain Questionnaire and degree of cognitive difficulties measured by the CFQ.

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

Controls

To obtain incidence estimates by LD risk factors and proposed phase III exclusion criteria, information on unaffected controls is obtained to allow for a nested case–control analysis. Adjusted ORs 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 (eg, time outdoors, pets, personal protective behaviours, occupational and leisure exposures), history for tick-borne disease including LD, tick-borne encephalitis (TBE), tick bite prophylaxis and known tick bites, interest in investigational LD vaccine study participation and assessment of meeting potential phase III exclusion criteria (table 2). If the proportion of potential control participants declining participation is higher than anticipated, the number of potential controls approached

Table 2 Burden of Lyme disease study-specific procedures

LD case participants						Controls	
Procedure/assessment	Visit 1 Day 1*	Visit 2 Day 28	Visit 3 Month 10†	Visit 4 Month 16–18‡	Visit 5 Month 22–24‡	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 2 mm skin punch biopsies of any LD-related rash	X						
Chart review to collect details of current illness and SOC physical exam findings	X	X	X	X	X		
Collect prespecified medical history of clinical significance including past LD diagnoses	X	X	X	X	X	X	X
Collect SOC LD diagnostic laboratory testing results	X	X	X	X	X		
Collect LD treatment and healthcare resource utilisation, 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 information			X				X
Collect health survey outcome information							X
Assess adverse events (2 hours after blood draw and 24 hours after skin punch biopsy) and research related injuries	X	X	X	X	X		
Assess interest in participation in follow-up studies, and the potential for the participant to meet phase III exclusion criteria	X					X	

*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.
†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-month 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.
‡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 reassessed.
LD, Lyme disease; PTLD, post-treatment Lyme disease; SOC, standard of care.

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 reconsented 16–18 months later to collect health survey outcome information. This includes an SF-36, degree of fatigue measured by FSS, degree of cognitive difficulties measured by the CFQ and degree of pain measured by the Short Form McGill Pain Questionnaire as well as to assess prespecified 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 5000, we expect approximately 500 participants with suspected/confirmed LD to be enrolled across 20 sites. Among those, we expect approximately 75% to have erythema migrans (EM) or other rash and 30% of those to consent to skin punch biopsies, thus overall ~113 participants will have ~226 punch biopsy specimens.

In total, 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 2025 controls enrolled.

Data analysis

Analysis of endpoints

For proportion endpoints, data will be summarised with counts (n), percentages (%) and associated 95% CIs, which will be calculated using the Clopper-Pearson method. The frequency of research related injuries and adverse events (AEs) following study procedures will be tabulated. The results for cases and control participants will be presented separately. For the primary endpoint, data will be summarised 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 1 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 III exclusion criteria and history of LD. Using these adjusted ORs, 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 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.

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 IDSA as clinical symptoms persisting at least 6 months after LD treatment.[10] The case definition for PTLD is described in table 4.

Persistent symptoms of LD

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 standardised 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.

- The patient had a final diagnosis of LD and completed treatment with an appropriate antibiotic regimen with resolution or stabilisation of objective manifestations of LD.
- The patient suffers from debilitating (results in substantial reduction in activities) symptoms of fatigue, generalised musculoskeletal pain or cognitive difficulties having onset within 6 months after completing therapy and lasting for at least 6 months after onset.

Table 3 LD case definitions

Presentation	Sign/symptom (detailed definition)	Diagnostic(s)*
Erythema migrans†	► Characteristic red or bluish-red patch, with or without central clearing‡ (Lesion should be photographed.)§	► Positive IgG/IgM on serum antibody testing. ► Positive PCR of Bbsl result from skin biopsy. ► Positive culture of Bbsl from skin biopsy.
Borrelial lymphocytoma†	► Painless bluish red nodule or plaque, usually on ear lobe, ear helix, nipple or scrotum (Nodule/plaque should be photographed.)§	► Positive IgG/IgM on serum antibody testing. ► Positive PCR of Bbsl result from biopsy. ► Positive culture of Bbsl from biopsy.
Acrodermatitis chronica atrophicans†	► 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.)§	► Positive IgG/IgM on serum antibody testing. ► Positive PCR of Bbsl result from biopsy. ► Positive culture of Bbsl from biopsy.
Lyme neuroborreliosis†	► Meningoradiculitis (Bannwarth syndrome), facial palsy, meningitis, encephalomyelitis or cerebral vasculitis (Clinical manifestation (eg, facial palsy) should be photographed if applicable.)§	► Intrathecal IgM and/or IgG antibodies. ► Positive intrathecal anti-Borrelia antibody index (CSF vs serum) reflecting intrathecal antibody production. ► Positive PCR of Bbsl result from cerebrospinal fluid. ► Positive culture of Bbsl from cerebrospinal fluid.
Lyme carditis†	► Acute onset of high degree atrioventricular conduction disturbances, rhythm disturbances, myocarditis or pancarditis	► Positive IgG/IgM on serum antibody testing.
Lyme arthritis†	► Marked swelling in one or few large joints, most often the knee. (Clinical manifestation (eg, swollen joint) should be photographed.)§	► Positive IgG/IgM on serum antibody testing. ► Positive PCR of Bbsl result from synovial fluid or tissue. ► Positive culture of Bbsl from synovial fluid or tissue.
LD ocular manifestations†	► Conjunctivitis, uveitis, papillitis, episcleritis or keratitis	► Positive IgG/IgM on serum antibody testing. ► Positive PCR of Bbsl result from ocular fluid. ► Positive culture of Bbsl from ocular fluid.

*Laboratory confirmation will primarily come from dedicated specimens collected specifically for the study (ie, serum and skin biopsy specimens). At least one positive diagnostic test result is required for laboratory confirmation.
†These clinical manifestations categories are disseminated Lyme manifestations. EM will only be considered disseminated if there are multiple EM lesions.
‡EM cases can be diagnosed on the basis of clinical symptoms alone.
§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.
Bbsl, Borrelia burgdorferi sensu lato; CSF, cerebrospinal fluid; EM, erythema migrans; IgG, immunoglobulin G; IgM, immunoglobulin M; LD, Lyme disease.

► 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 and 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

Table 4 PTLD case definition

Presentation	Sign/symptom (detailed definition)	Evaluations(s)
Post-treatment Lyme disease	► Prior documented case of clinically confirmed LD as per definitions in table 3. ► Treatment with accepted antibiotic regimen with resolution or stabilisation of objective manifestations of LD. ► Fatigue, musculoskeletal pain and cognitive difficulties that begin within 6 months and last for 6 months after completion of antibiotic therapy. ► Subjective symptoms are so severe that result in a substantial reduction in activities. ► No other comorbidities that can explain illness.	► Final diagnosis of LD (clinical diagnosis only or laboratory confirmed). ► 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 afore-mentioned questionnaires.[16] ► 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]

CFQ, Cognitive Failures Questionnaire; FSS, Fatigue Severity Scale; LD, Lyme disease; SF-36, 36-Item Short Form Survey.

Human Participants (Council for International Organizations of Medical Sciences, 2002), International Council for Harmonisation Good Clinical Practice (ICH GCP) Guideline and the Declaration of Helsinki. The ethics committees that approved this study are as follows:
Ethical commission of Institute for Clinical and Experimental Medicine and 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's 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 AEs (SAEs) and non-serious 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 on 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, exposure during pregnancy is reported within 24 hours of investigator awareness.

Dissemination plan

This study has been approved by all sites' local ethics committees in participating countries. The 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.

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Competing interests EB, AL-B, AP, JHS, KH, LRH, BDG and KEA are employees of Pfizer 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.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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